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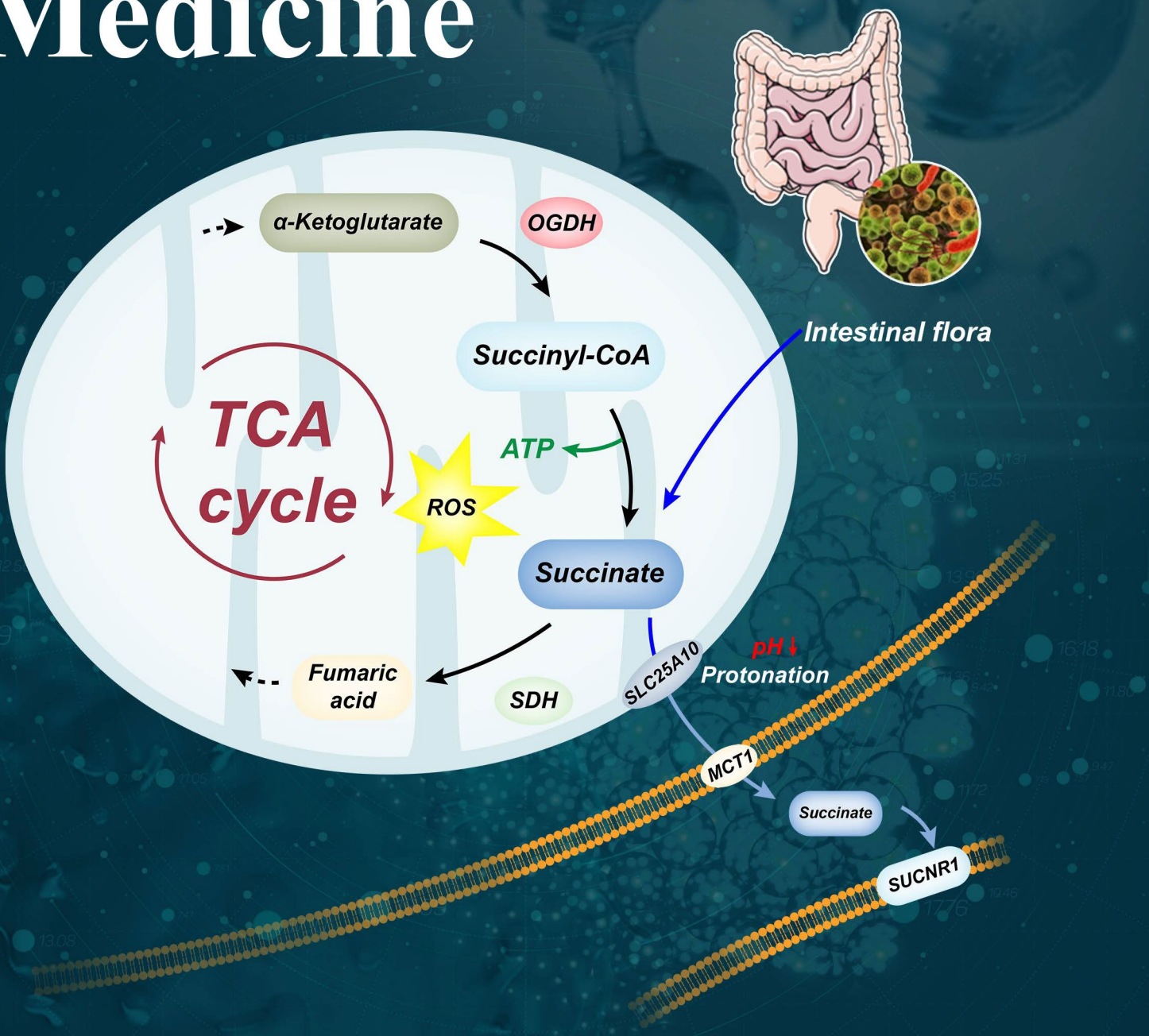

Succinate metabolism in cardiovascular diseases

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EDITORIAL

Inaugural editorial: A new platform dedicated to promote bench-to-bedside translation

Lemin Zheng†*

The Institute of Cardiovascular Sciences, Peking University, Beijing, China

We are pleased to introduce you to the inaugural volume of *Global Translational Medicine*, a new journal under AccScience Publishing (Singapore) Pte. Ltd. *Global Translational Medicine* is a quarterly journal that focuses on medicine, biological sciences, and biomaterials engineering. *Global Translational Medicine* provides a platform to fill the gaps in preclinical and inter-disciplinary research, to promote clinical translation of scientific research results, and to contribute to the conception of new and improved preventive measures as well as diagnostic and therapeutic techniques of diseases.

Translational medicine is a branch of medical research which aims to bridge basic research and clinical application. The main focus of *Global Translational Medicine* is to break down the barriers between basic research and clinical application, such as drug development, biomarker assay, and so on, paving a new way to translational medicine. The high incidence of cardiovascular and cerebrovascular diseases as well as tumors brought a huge burden to many families and the society. The pathology of these diseases involves both genetic and environmental risk factors, while the traditional methods are unable to meet the needs of prevention, diagnosis, treatments, and prognosis assessment of these diseases. The development of multiomics technology brings new chances to translational medicine. There may be two points that will contribute to the success of translational medicine, one is using the methods of multiomics to explore new targets and drugs, and the other is optimizing preclinical models.

The most downloaded articles in the first two issues of *Global Translation Medicine* are related to genomics, metabolomics, and preclinical animal models in cardiovascular disease (CVD) and idiopathic pulmonary fibrosis (IPF).

The first article[1] reviewed the genetic and non-genetic risk factors of IPF. IPF is the most common form of fibrosis of internal organs. The etiology and pathogenesis of IPF are still not well understood. However, a growing line of evidence shows that both genetic and non-genetic factors contribute to IPF development. The release of pro-inflammatory cytokines activates the immune cells. The enhanced synthesis of interleukins and cytokines, especially transforming growth factor β1, leads to the proliferation of fibroblasts, increased extracellular matrix formation, and epithelial-mesenchymal transformation of the lung tissue. These pathological changes could lead to fibrosis. Polymorphisms of genes responsible for the function of mucociliary clearance (MUC5B), telomerases (TERT, TERC), as well as signaling pathway-related genes, such as Sonic hedgehog, Wnt, and some other genes, are also risk factors for IPF development. Epigenetic regulatory mechanisms, such as methylation and acetylation of DNA and histones, may also influence the development and progression of this disease. At present, the role of non-coding RNAs, particularly long non-coding RNAs (lncRNA) in the development of fibrotic processes, is actively studied. LncRNA is an RNA that

†Professor Lemin Zheng the Editor-in-Chief of *Global Translational Medicine*.

***Corresponding author:**
Lemin Zheng
(zhengl@bjmu.edu.cn)

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is longer than 200 base pairs and does not code for any proteins. LncRNAs perform various functions in the cell, from nuclear compartmentation to epigenetic regulation of gene expression and post-translational modification of proteins. This article sheds light on the mechanisms of the pathogenesis of IPF and suggests potential therapeutic targets for IPF.

The second article[2] is about the application of metabolomics in CVD. Succinate is generally considered an important intermediate product of the tricarboxylic acid cycle. Mounting studies have shown that succinate is related to the pathophysiology of CVD, such as atherosclerosis, acute aortic dissection, hypertension, myocardial ischemia-reperfusion injury, and heart failure. It may represent a potential target or biomarker for CVD. It has been demonstrated that succinate not only participates in various energy metabolic pathways but also plays an important role in various pathophysiological activities as a signaling molecule. Given the significance of metabolites in CVD, it is important to focus on the metabolic regulation mechanism of succinate in CVD. This article outlines the latest evidence pointing to the potential role of succinate in CVD, along with its mechanisms, and updates the current understanding on the role of succinate in CVD, which is of great significance not only for clinical diagnosis and treatment but also for basic research.

The third article[3] introduces two preclinical murine models used in the basic research of atherosclerosis. Atherosclerosis is a leading cause of morbidity and mortality in many countries. Mice are the most frequently used animal model to study the pathogenesis and molecular mechanisms of atherosclerosis. En face analyses of the aorta and cross-sections of the aortic root are the two common modes for quantifying the severity of atherosclerosis in mice. This article outlines the pros and cons of these two methods and provides suggestions to optimize the quantification of atherosclerosis, thereby enhancing rigor and reproducibility in preclinical research, which means a lot to the further translation to clinic.

We hope that our readers could be inspired by the articles published in the inaugural volume of *Global Translational Medicine*. Moreover, we sincerely welcome submissions from our readers to publish new research, insights, and novel results in the forthcoming issues of *Global Translational Medicine*.

Conflict of interest

The author declares no conflict of interest.

References

1. Zulkarneev SR, Zulkarneev RH, Korytina GF, *et al*., 2022, Genetic and non-genetic risk factors of idiopathic pulmonary fibrosis: A review. *Glob Transl Med*, 1: 107.

   https://doi.org/10.36922/gtm.v1i2.107

2. Shan W, Cui H, Xu Y, *et al*., 2022, Succinate metabolism in cardiovascular diseases. *Glob Transl Med*, 1: 160.

   https://doi.org/10.36922/gtm.v1i2.160

3. Chen H, Howatt DA, Franklin MK, *et al*., 2002, A mini-review on quantification of atherosclerosis in hypercholesterolemic mice. *Glob Transl Med*, 1: 72.

   https://doi.org/10.36922/gtm.v1i1.76

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ORIGINAL RESEARCH ARTICLE

Effect of leptin on aortic dissection

Ling Chen[1,2], Yang Xi[1,2], Fan Xu[1,2], and Liangwan Chen[1,2]*

[1]Department of Cardiac Surgery, Union Hospital, Fujian Medical University, Fuzhou, Fujian 350001, People's Republic of China

[2]Fujian Key Laboratory of Cardio-Thoracic Surgery (Fujian Medical University), Fuzhou, Fujian 350001, People's Republic of China

Abstract

The most important clinical features of aortic dissection (AD) are its acute onset, rapid progress, and high fatality rate. The exact pathogenesis of AD is unclear, and the focus of current research on the mechanism of AD has been primarily on hypertension and changes in metalloproteinases, among which leptin plays an important role. The purpose of this study is to evaluate the effect of leptin on AD. We conducted a computerized literature search on animal studies related to leptin and dissecting aortic aneurysm in PubMed, EMBASE, Cochrane Library, MEDLINE, and other databases from their inception to present. Meta-analysis was conducted to compare the changes in aortic diameter, aortic dilatation, and the incidence of AD in mice under the local intervention of leptin or leptin antagonist (LepA). A total of four studies were included, involving five batches of animal experiments. According to the results of the meta-analysis, the increase in local leptin content led to the enlargement of aortic diameter (relative risk [RR] = 0.18; 95% confidence interval [CI]: 0.09 – 0.27; $P < 0.0001$) and increased aortic dilatation (RR = 0.11; 95% CI: 0.01 – 0.22; $P < 0.0001$). This meta-analysis showed that local leptin administration increased the aortic diameter and aortic dilatation. However, due to high heterogeneity between the results, it is difficult to draw a clear conclusion on the effect of leptin on AD.

Keywords: Leptin; Aortic dissection; Leptin antagonist

***Corresponding author:**
Liangwan Chen
(chengliangwan@tom.com)

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1. Introduction

Aortic dissection (AD) is an acute cardiovascular disease that is caused by high-speed blood flow impacting the aortic intima, resulting in a break or tear in the middle layer of the aortic wall, and separating the inner and outer layers, thus forming a true lumen (the original aortic cavity) and a false lumen (a gap formed between the inner and outer layers by the tearing of the middle layer). AD patients rely on the outer structure of the torn aortic wall to withstand the aortic pressure that should be borne by the entire aortic wall. This outer structure is vulnerable to rupture, which may lead to death within a short period of time. If a patient is not diagnosed and treated in time, the mortality rate within 1 week is about 37%, while the mortality rate within 2 weeks is as high as 75%[1,2]. There are about 12 new cases of AD in developed countries per million people per year[1-3], and up to date, no large-scale epidemiological studies have been reported in China. With the improvement of living standards and the progress of diagnostic technology, the number of AD patients in China is increasing at an explosive rate[4].

Stanford classification divides AD into two types. In type A, the rupture of intima may be located in the ascending aorta, aortic arch, or proximal descending aorta. The scope of dissection involves the ascending aorta, the aortic arch, descending aorta, and even the abdominal aorta. In type B, the rupture of intima is often located in the proximal descending aorta. The scope of dissecting aneurysm is limited to the descending aorta or extends into the abdominal aorta; it does not affect the ascending aorta. The most important clinical features of Stanford type A dissection are its acute onset and rapid progress. In clinical practice, we have found that the early diagnosis rate of AD is low, and some patients die of an aortic rupture before a definite diagnosis is made. Emergency surgery is the only effective treatment for acute Stanford type A dissection. Although the surgical technology for acute Stanford type A dissection has made significant advancements in recent years with the continuous progress of science and technology, such as the three-branch vascular stent technology used in aortic arch reconstruction and other technologies[5] that improve the safety of AD surgery and the prognosis of patients, the difficulty of surgery still poses a challenge, and the risk of mortality is still high. Usually, only large, well-equipped heart centers are able to carry out such emergency operations. The majority of patients need to be transferred to large heart centers for surgical treatment after a concrete diagnosis is made, among which some patients may die during the transfer. Therefore, it is of great significance to explore the molecular mechanism of AD and find potential therapeutic targets for the prevention and treatment of this life-threatening disease.

The exact pathogenesis of AD is unclear. At present, it is generally believed that AD is formed on the basis of the internal causes of the arterial wall itself in addition to a series of external factors, such as hypertension, trauma, and so on. AD often occurs in patients with idiopathic degeneration of the middle layers of the aorta and certain hereditary diseases, such as Marfan syndrome, Ehlers-Danlos syndrome, Turner syndrome, and other connective tissue disorders. This is due to the internal factor of weak aortic wall in these patients. In the process of AD formation, the external factors that cause tearing of the intima and promote high-speed blood flow into the middle layer play important roles. These external factors include arterial blood pressure, wall shear stress, and hemodynamic status[6,7]. However, AD is not caused merely by the stimulation of these external factors; instead, the primary cause of AD is the weakness of the aortic wall itself. The weakness of the aortic wall has been relentlessly investigated. Some studies have pointed out that the weakness of the aortic wall may be closely related to abnormal glucose and lipid metabolism[8-11], which are the main types of metabolic syndrome (MetS). MetS is characterized by abdominal obesity and abnormal metabolism of lipids, glucose, and carbohydrate, which may cause a series of cardiovascular and cerebrovascular diseases[12].

In recent years, research on the mechanism of AD has mainly focused on hypertension and changes in metalloproteinases, among which leptin plays an important role. Leptin is a hormone that is mainly secreted by adipocytes and involved in the control of food intake through its action on the hypothalamus, leading to the suppression of appetite[13]. Further research on leptin has found that the hormone not only has a variety of endocrine functions but also participates in immune and inflammatory responses, hematopoiesis, angiogenesis, reproduction, gene expression of cell cycle regulation, and regulation of cell matrix remodeling[8,9,14]. Leptin also participates in the decomposition of the extracellular matrix (ECM), promotes angiogenesis, and improves the mitotic activity of vascular endothelial cells by regulating the expression of matrix metalloprotein (MMP)-9 and tissue inhibitors of metalloproteinases (TIMPs)[15]. This suggests that leptin may be involved in the occurrence of AD. However, only a number of clinical trials have studied the role of leptin in AD thus far. Therefore, the role that leptin plays in AD patients remains unclear.

Several research groups all over the world have adopted animal models to study the impact of leptin on the occurrence and development of AD. The results of these pre-clinical studies are often derived from a relatively small sample, and there is no objective or quantitative way to systematically evaluate and summarize all the studies. Therefore, we present a systematic review and meta-analysis of leptin in AD animal models to clarify the relationship between leptin and AD.

2. Materials and method

2.1. Materials

We conducted a computerized literature search on animal studies related to leptin and AD in PubMed, EMBASE, Cochrane Library, MEDLINE, and other databases from their inception to present. The keywords used were "Leptin," "Obese Protein," "Obese Gene Product, "Gene Product, Obese," "Ob Gene Product," "Gene Product, Ob," "Ob Protein," "Aneurysm," "Aneurysms," "Fusiform Aneurysm," "Aneurysm, Fusiform," "Aneurysms, Fusiform," "Fusiform Aneurysms," and "Saccular Aneurysm."

The titles and abstracts of the identified studies were scanned to exclude any study that was irrelevant. The full texts of the remaining articles were read to determine

whether they contained information on the topic of interest. The reference lists of the articles with relevant information were reviewed to identify citations to other studies on the same topic.

2.2. Inclusion and exclusion criteria

2.2.1. Inclusion criteria

To prevent bias, the inclusion criteria were as follows: (i) The effect of leptin on AD tested in animal models; (ii) sufficient data, such as the increase in artery diameter and the comparison with animals receiving leptin or leptin antagonist (LepA); and (iii) original data, independent of other studies.

2.2.2. Exclusion criteria

The pre-defined exclusion criteria were as follows: (i) Specific article types including case reports, abstracts, reviews, editorials, and clinical trials; (ii) outcome variables that were not caused by leptin or LepA; (iii) literature without full text or available review and main outcome indicators; and (iv) repetitive publications.

2.2.3. Data extraction and quality assessment

The Chinese and English literature retrieved, according to the retrieval strategy, were screened by two researchers independently. The screening process was as follows: First, literature that were repeatedly searched were excluded; second, titles and abstracts were read to eliminate irrelevant studies; full texts were read to identify studies that met the criteria, and data extraction was carried out; cross-checks were carried out among researchers, and if there were any disagreements, a third researcher was consulted.

The following research design details were extracted from each study: (i) Year of publication, first author's name, and experimental model; (ii) individual data of each animal, including number, species, gender, *etc*.; (iii) treatment information, including treatment time, route of administration, and dosage; and (iv) result measurement and evaluation time. For results that were obtained from animal studies at different time points, we extracted the data at the time before killing. For data that were missing or presented graphically, they were measured using a digital scale software. In addition, we attempted to contact the author for more information or calculated on our own (if any); otherwise, we excluded it. For each comparison or each treatment and control group, we extracted data for the mean and its standard deviation. The time of the lesion was set to zero and the administration time was expressed relative to this. All data were extracted independently by two participants.

Cochrane Handbook for Systematic Reviews of Interventions 4.2.6 (Higgins, 2006)[16] was used as a quality assessment tool in this study. The Cochrane Quality Rating Form contains a total of seven items, with each item given 1 point for "low risk" and 0 point for "high risk." Independent evaluations were made by the two researchers and were then integrated. If there were any disagreements, another evaluation was made by the third researcher.

2.3. Statistical analysis

We separately pooled relative risk (RR) estimates from each study for each outcome using random effects meta-analysis. The statistical heterogeneity of the RRs was evaluated using the $\chi 2$ test, with significance set at $P < 0.01$, and the I^2 statistic was calculated. To evaluate whether there was publication bias in the included articles, we used R software to draw funnel plots for qualitative analysis and Egger's test for quantitative analysis. If $P < 0.05$, it indicated that there was publication bias. Low, moderate, and high degrees of heterogeneity corresponded to I^2 values of 25%, 50%, and 75%, respectively. Sensitivity analyses were conducted to evaluate whether the results could have been markedly affected by a single study. All data (except age) were expressed by $\bar{x} \pm s$.

3. Results

3.1. Search results

The references (n = 72) were retrieved by the original search strategy or manual searches. The abstracts were reviewed, and eight articles were selected for full-text evaluation after excluding repetitive literature and preliminary screening. After applying the inclusion and exclusion criteria, four articles were included. The flowchart of the study inclusion process is shown in Figure 1.

3.2. Research quality assessment results

The Cochrane scoring system was used to evaluate the quality of the included literature. The results showed that the lowest score was 6, while the highest was 10, with an average of 4.25 ± 0.96, which was in the upper-middle level (Figure 1).

3.3. Meta-analysis of studies on aortic diameter

The changes in mouse aortic diameter were reported in four studies (inclusive of five animal experiments). The mice in the experimental group of three animal experiments were intervened with leptin. It is worth noting that the results of two of these studies showed that there was a statistical difference in the enlargement of aortic diameter compared with the blank control group. However, one study reached the opposite conclusion, in which the diameter of the mouse aorta was smaller than that of the control group. The mice in the experimental group of the other two

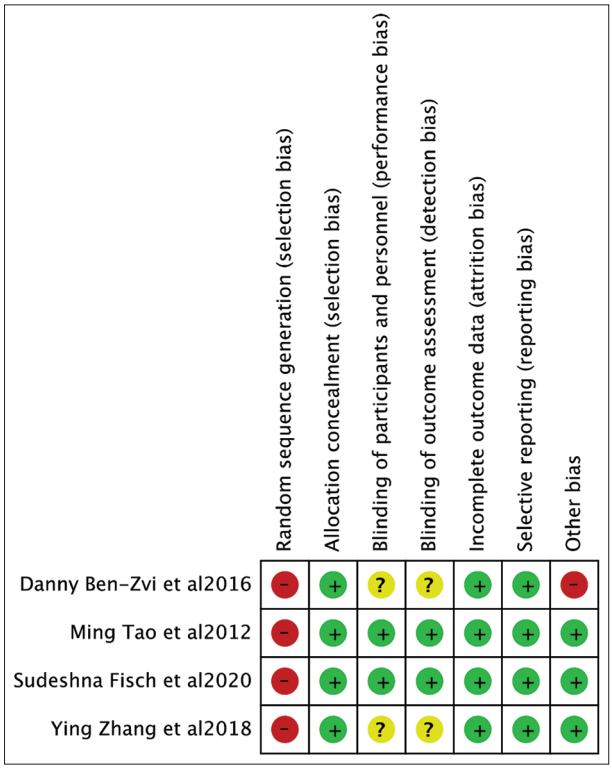

Figure 1. Risk of bias summary: Review authors' judgments about each risk of bias item for each included study.

animal experiments were intervened with LepA, and the results showed that there was a statistical difference in the reduction of aortic diameter compared with the control group. The meta-analysis of all four studies (inclusive of five animal experiments) showed that an increase in local leptin content had a statistically significant effect on the enlargement of aortic diameter, with a combined RR of 0.18 (95% CI: 0.09 – 0.27; P = 0.0001; random effects model) and statistical heterogeneity (P < 0.0001; I^2 = 96.9%). The increase in local leptin content promoted the enlargement of mouse aortic diameter. The forest plot is shown in Figure 2.

3.4. Meta-analysis of studies on aortic dilatation

The changes in aortic dilatation in mice were reported in four studies (inclusive of five animal experiments). The mice in the experimental group of three animal experiments were intervened with leptin. It is worth noting that the results of two of these studies showed that there was a statistical difference in the increased aortic dilatation compared with the blank control group. However, one study reached the opposite conclusion, wherein the aortic dilatation of the experimental mice was lesser than that of the control group. The mice in the experimental group of the other two animal experiments were intervened with LepA, and the results showed that there was a statistical difference in the reduction of aortic dilatation compared with the control group. The meta-analysis of all four studies (including five animal experiments) showed that the increase in local leptin content had a statistically significant effect on aortic dilatation, with a combined RR of 0.11 (95% CI: 0.01 – 0.22; P = 0.0001; random effects model) and statistical heterogeneity (P < 0.0001; I^2 = 95.7%). The increase in local leptin content promoted the dilation of mouse aorta. The forest plot is shown in Figure 3.

3.5. Publication bias results

First, a funnel chart was used to conduct a qualitative analysis of publication bias (sensitivity analysis based on the difference in artery diameter) for the included literature, showing that the distribution was asymmetric. For further verification, Egger's test was conducted, and the results showed that there was no publication bias, P = 0.988 (>0.05) (Figure 4 and Figure S1).

3.6. Sensitivity analysis

Due to the differences in the quality and sample size of the studies, the heterogeneity among the studies was significant. A sensitivity analysis was conducted to verify the reliability of the data. The difference in arterial diameter between the leptin enhanced group and the leptin weakened group was used for sensitivity analysis. The results showed that the study conducted by Ying *et al.*[17] was the most important factor affecting the effect scale and the main reason for the existence of heterogeneity (Figure 5).

4. Discussion

Aortic vascular remodeling is one of the key factors in the pathogenesis of AD, and the severity of the disease is closely related to the abnormality of glucose and lipid metabolism. The main physiological and pathological changes in AD include the degeneration of the aortic middle layer, the imbalance of ECM synthesis and degradation, the rupture of elastic fibers, the deposition of collagen fibers, and the transformation of cell phenotype[9]. The ECM is a dynamic network structure, mainly composed of a series of biological macromolecules, such as collagen, elastin, proteoglycan, and structural glycoprotein. Collagen and elastin are the main components of the aortic wall, accounting for 50% of the dry weight of normal arteries. They play an important role in maintaining the integrity of the aorta and withstanding the stress of blood flow on the wall[10]. A large number of studies have shown that in patients with AD, the arterial wall is thinner, elastic protein fragments can be seen in the middle layer, elastic protein and collagen are significantly reduced, elastic fibers (composed of elastic protein and tropocollagen)

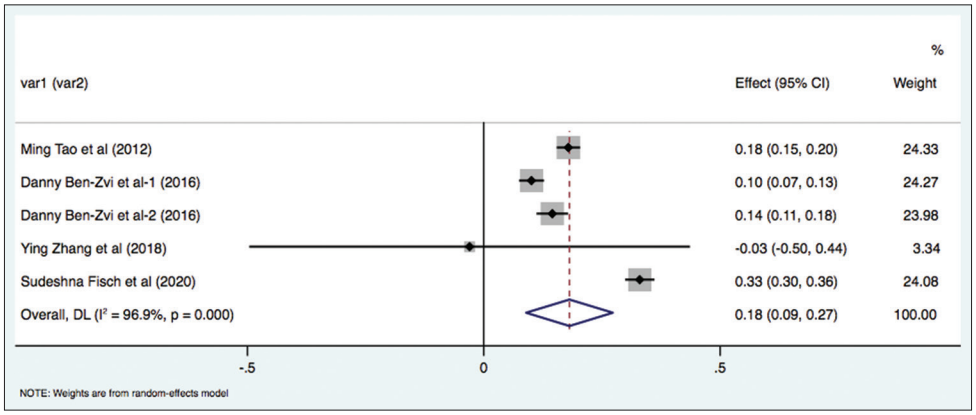

Figure 2. Forest diagram of the effect of leptin on aortic diameter.

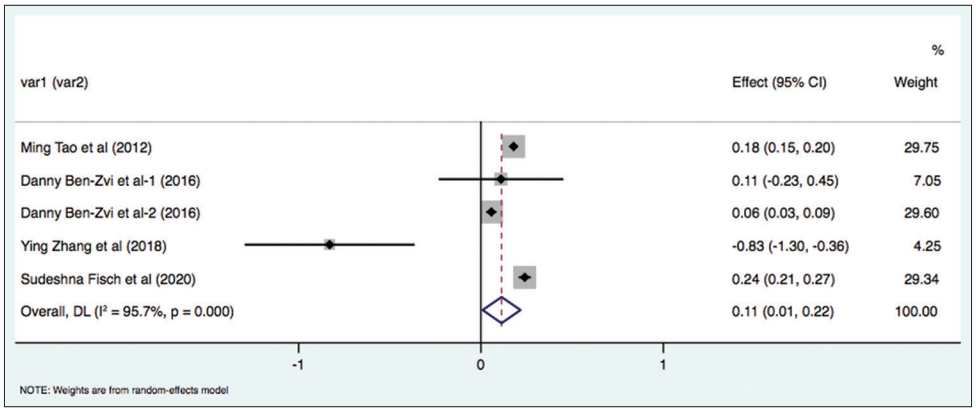

Figure 3. Forest diagram of the effect of leptin on aortic dilatation.

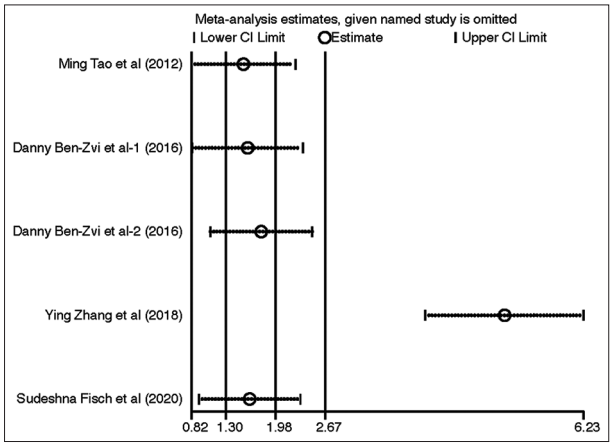

Figure 4. Results of sensitivity analysis (leptin enhanced group and leptin weakened group).

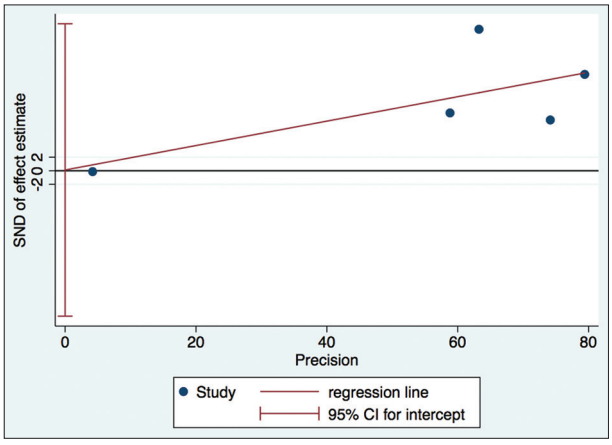

Figure 5. Egger's test plot.

are arranged in a disorderly fashion, and type IV collagen defects can be observed in the basement membrane of smooth muscle cells (SMCs), along with other pathological changes[9-11]. These pathological changes will increase the compliance of the aortic wall; a high compliance can cause degeneration of SMCs in the middle layer, further lead to elastic fiber defect, and eventually result in the degradation of the basement membrane, thus providing a pathological basis for the occurrence of AD[9].

Abnormal glucose and lipid metabolism are mainly characterized by abdominal obesity and metabolic disorders of lipid, glucose, and carbohydrate, which can cause

serious cardiovascular and cerebrovascular diseases[6]. In a study, abundant inflammatory cytokines were detected in adipocytes, and adipose tissue was found to have an effect on the oxidative stress reaction in AD patients by releasing large amounts of polypeptides and cytokines into the blood circulation, thus inducing the occurrence and development of various diseases[15]. A number of studies have also pointed out that lipid metabolism is involved in aortic vascular remodeling and further confirmed that obesity has a strong correlation with the occurrence and development of abdominal aortic aneurysm and AD[18-20]. Relevant research has confirmed this hypothesis laterally. Studies have found that insulin resistance (5.5% ≤ HbA1c ≤ 6.5%), caused by abnormal glucose metabolism, had significantly increased the cardiovascular incidence rate in this population[21,22]. The studies have also pointed out that insulin resistance is the main feature of pre-diabetes, which can cause hyperlipidemia syndrome with elevated plasma triglyceride (TG), reduced human low-density lipoprotein (HLDL), and elevated very low-density lipoprotein (VLDL). Obesity is one of the main risk factors for insulin resistance[19]. The inflammatory reaction caused by excessive fat accumulation and abnormal lipid metabolism may eventually lead to insulin resistance. It is evident that there is a close relationship between lipid and glucose metabolism.

Relevant studies have shown that glucose and lipid metabolism play an important role in the phenotypic transformation of SMCs[22-24]. The phenotype of aortic SMCs in AD patients changes from contractile to secretory, which is accompanied by increased glycolytic flux, decreased glucose oxidation, and increased cholesterol[19,24,25]. Moreover, the increase in insulin resistance and lactate dehydrogenase A (LDHA) levels can induce the phenotypic transformation of SMCs and promote the occurrence of AD. However, the study also found that the occurrence and development of AD are greatly inhibited by drug intervention or the inhibition of these processes[20,26].

In addition, abnormal glucose and lipid metabolism also affect a variety of physiological processes, including autophagy, inflammatory reaction, and vascular fibrosis, all of which play an important role in the progression of AD. The abnormal metabolism of glucose and lipid plays different roles in different stages of arterial dissection. Hyperglycemia in patients (diabetes), to a certain extent, provides the energy source for blood vessels. In some ways, this is beneficial to the stability of the disease, but long-term hyperglycemia promotes vascular inflammation and fibrosis and ultimately leads to vascular abnormalities. The abnormality of glucose and lipid metabolism will inevitably lead to a series of changes in cell function, which will have an irreversible impact on cell metabolism, cell self-renewal, and cell life cycle[27-33]. For example, in AD, the expression of autophagy in the middle layer was found to be significantly higher than that in the control group, thus suggesting the overactivation of autophagy in AD[27]. Studies have confirmed that MYH11, a myosin marker of SMCs, leads to autophagic conversion through the ubiquitin-proteasome system after being stimulated by the external environment[28,29]. In a study, adult mice that lack autophagy died of hypoglycemia 24 h after starvation, indicating that autophagy plays a key role in glucose homeostasis[30]. In addition, studies have shown that the phenotypic transformation of SMCs, induced by PDGF, can be inhibited by inhibiting autophagy levels[27]. Moreover, glucose is known to regulate the autophagy level in organisms by controlling glucagon/insulin secretion[31]. The inflammatory reaction and vascular fibrosis that are induced by abnormal glucose and lipid metabolism can promote the occurrence and development of AD. It has been found that macrophages can infiltrate the aortic wall and release matrix metalloproteinases that degrade the elastic fibers of the aortic wall, which, in turn, decreases the elasticity of the vascular wall. Eventually, the middle layer of the aortic wall degenerates and loses its elasticity, causing vascular tears under the stimulation of hypertension[32]. In view of the excessive fibrotic and brittle nature of the aortic wall as a result of the stimulation of inflammatory factors, the aortic wall is unable to withstand the shear force generated by blood pressure, which eventually causes dissection[33].

Leptin is closely related to glucose and lipid metabolism. Leptin plays an important role in the regulation of glucose and lipid metabolism, energy metabolism, reproductive development, and immune regulation by acting on the central nervous system and peripheral tissues. Leptin is an independent predictor of carotid intima-media thickness (cIMT) in obese patients[34]. Similar associations have been found in healthy male and female individuals[35], obese children[36], and psoriasis patients[37,38]. In addition, the presence of carotid plaque is associated with hyperleptinemia in patients with systemic lupus erythematosus (SLE)[39]. With regard to the severity of carotid artery disease, high leptin concentration has been found to be associated with plaque instability characteristics in carotid endarterectomy patients[40]. Furthermore, the overexpression of leptin receptor gene (*LEPR*) has been observed in advanced carotid atherosclerosis[41]. The previous studies, however, have reported that leptin concentration in symptomatic carotid artery disease patients was lower than that in asymptomatic patients[42]. In a rat carotid artery injury model, genistein (an isoflavone) has been found to

reduce leptin-induced neointima formation[43]. Overall, hyperleptinemia is associated with increased cIMT and carotid plaque instability.

Elevated leptin level is related to the development of insulin resistance and type 2 diabetes mellitus (T2DM)[44]. In T2DM, the relationship between high leptin concentration and increased cardiovascular risk, microvascular complications, and cardiac autonomic dysfunction has been reported[45-48]. Other studies have also reported that the concentration of leptin is associated with the occurrence and severity of asymptomatic myocardial infarction (MI) and carotid atherosclerosis (assessed by cIMT) in T2DM patients[49,50]. In addition, obesity, hypertension, MetS, and endothelial dysfunction have been found to be more common in T2DM patients with elevated leptin levels[51-53]. In both T2DM patients and healthy individuals, leptin decreases following an oral fat-tolerance meal[54]. Other than that, certain leptin gene polymorphisms have been found to be associated with the presence of T2DM[55-58]. It has been reported that leptin replacement therapy can improve muscle and liver insulin resistance in patients with lipodystrophy as well as inhibit liver gluconeogenesis, fat decomposition, and fasting hyperglycemia in animal diabetes models[59].

Leptin may affect cardiac remodeling, metabolism, and systolic function[60]. According to a study, the soluble leptin receptor (LepR) and leptin content in epicardial adipocytes are 56.9% and 28.6% higher, respectively, than those in subcutaneous adipocytes[61]. In patients with coronary heart disease (CHD), leptin levels have been found to be positively correlated with myeloperoxidase and C-reactive protein (an inflammatory marker) concentrations as well as the increase in factor VII activity[62-64]. There is also increased expression of leptin gene in the epicardium, pericardium, and subcutaneous adipose tissue of CHD patients with MetS[65]. It has been previously reported that leptin enhances platelet activation in CHD patients by promoting bone differentiation[66] and calcification of vascular cells *in vitro*[67]. In addition, leptin may directly affect coronary artery endothelial cells by increasing the expression of tissue factors and cell adhesion molecules[68]. Leptin can also increase insulin resistance in patients with CHD[69,70]. Statins, on the other hand, can reduce the concentration of leptin in patients with CHD[71,72]. Future research should clarify if this induction is related to the atherosclerotic protective properties of statins. In addition to statins, several other drugs, including hypoglycemic, antihypertensive, and anti-obesity drugs, have also shown effects on leptin levels[73,74]. Leptin may be a target drug candidate for therapeutic intervention. Hyperleptinemia, in general, is related to the existence and severity of CHD and heart failure. Statins and other drugs may reduce leptin concentration. Therefore, in patients with CHD and heart failure, the choice of leptin-lowering therapy may help reduce their cardiovascular risk.

Leptin can stimulate atherosclerosis, inflammatory responses, oxidative stress, and thrombosis, thereby promoting endothelial dysfunction, arterial stiffness, and the development of atherosclerotic plaques[75-77]. In addition, leptin regulates bone homeostasis, reproduction, and angiogenesis[78]. At present, there are many ongoing studies based on the physiological effects of leptin. Research has shown that leptin can regulate vascular remodeling *in vivo*[79] and that the increase in leptin levels can significantly promote the growth of lesions after experimental vascular injury in mice[67,80]. Leptin can also enhance platelet aggregation and stabilize arterial thrombosis, thereby increasing the possibility that elevated leptin levels in obese people may directly lead to an increased risk of cardiovascular disease associated with obesity[81-86]. However, the evidence for any potential association between leptin and AD is limited, thus requiring further studies.

In our study, two pre-clinical studies were conducted on 88 animals that met the inclusion criteria. The results showed that leptin had a significant effect on the enlargement of aortic diameter and the increase in aortic wall compliance at the animal level. In animal models, the local application of leptin is sufficient to induce regional degeneration of ECM, thus increasing the risk of dissection. The local inhibition of leptin activity on the aorta may weaken the progression of AD and its related heart diseases to some extent. Based on its protective properties, we expect a positive response in local aneurysms when local LepA is used in various aneurysms (i.e., aortic, peripheral, and visceral lesions). It is evident that there is obvious heterogeneity in the research. Through sensitivity analysis, we found that the combined effect value reversed after excluding the study conducted by Ying *et al.*[17], suggesting that this study is the reason for the heterogeneity in the meta-analysis. According to the study conducted by Ying *et al.*[17], the reason for the contrasting experimental results may be related to the difference in drug dosage and the mechanism of action. Through meta-regression, we found that the mode of administration (local sustained-release and intraperitoneal injection), reagent dosage, and mouse type were not the reasons for heterogeneity. Leptin is known to play a key role in regulating energy balance and controlling body weight. Once it is released into the circulation, it may exert central and peripheral effects by combining with LepR, found in many tissues, thus leading to the activation of several main signal transduction pathways[7,8,12,87]. We speculate that

intraperitoneal leptin injection may activate other signaling pathways, and leptin, as an upstream factor of the signaling pathway, may affect the occurrence of dissection in other ways. In a health study involving 12,203 men (screened by ultrasound; 875 aneurysms ≥ 30 mm), aged 65 – 83 years, it was found that there is no association between serum leptin levels and AD[88]. However, it is still unknown whether there is a causal relationship between the systemic absorption of leptin and the formation of dissecting aneurysms.

The limitations of this study include the following: (i) There are some differences in the detection of indicators considering that each research institute is located in a different region; in addition, data transformation was carried out in this study for systematic analysis since most studies reported in median and percentiles; (ii) there are inevitable differences in the level of experimental conditions in different places, which has a certain impact on the effect of the experiment.

5. Conclusion

Based on current fundamental research and the results of this study, we have reason to believe that the local synthesis of leptin can enlarge the aortic diameter and increase the dilation of the aortic wall. Although it is still impossible to ascertain the fact that it can promote the formation of dissection due to the lack of evidence, it can be concluded that the increase in dilation of the aortic wall can, to a certain extent, promote the formation of dissection, especially in AD patients with underlying diseases, such as atherosclerosis, and that any event that results in vascular wall instability will eventually lead to the occurrence of AD. In conclusion, this study provides evidence that leptin is a risk factor for AD.

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Conflict of interest

The paper has no financial interest in any individual or organization; it does not infringe on the intellectual property rights of others. The manuscript and images are original and have not been published before. All authors have no conflicts of interest or financial ties to disclose.

Author contributions

Conceptualization: Ling Chen and Liangwan Chen
Methodology: Ling Chen and Liangwan Chen
Writing – original draft: Ling Chen and YangXi
Writing – review & editing: Ling Chen, YangXi, and Fan Xu
All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data generated or analyzed during this study are included in this published article.

References

1. Bossone E, LaBounty TM, Eagle KA. 2018, Acute aortic syndromes: diagnosis and management, an update. *Eur Heart J*, 39: 739–749d.

   https://doi.org/10.1093/eurheartj/ehx319

2. Zindovic I, Gudbjartsson T, Ahlsson A, *et al*., 2019, Malperfusion in acute type A aortic dissection: An update from the Nordic Consortium for Acute Type A Aortic Dissection. *J Thorac Cardiovasc Surg*, 157: 1324–1333.e6.

   https://doi.org/10.1016/j.jtcvs.2018.10.134

3. Criado FJ. 2011, Aortic dissection: A 250-year perspective. *Tex Heart Inst J*, 38: 694–700.

4. Ma LY, Wang Z, Fan J, *et al*., 2022, China Cardiovascular Health and Disease Report 2021 on the prevalence and prevention of hypertension in China. Chin J General Pract, 25: 6.

5. Chen LW, Wu XJ, Dai XF, *et al*., 2016, Repair of acute type A aortic dissection with ascending aorta replacement combined with open fenestrated stent graft placement. *Ann Thorac Surg*, 101: 644–649.

   https://doi.org/10.1016/j.athoracsur.2015.07.060

6. Di S, Wang Y, Han L, *et al*., 2019, The intervention effect of traditional Chinese medicine on the intestinal flora and its metabolites in glycolipid metabolic disorders. *Evid Based Complement Altern Med*, 2019: 2958920.

   https://doi.org/10.1155/2019/2958920

7. Saxena NK, Vertino PM, Anania FA, *et al*., 2007, Leptin-induced growth stimulation of breast cancer cells involves recruitment of histone acetyltransferases and mediator complex to CYCLIN D1 promoter via activation of Stat3. *J Biol Chem*, 282: 13316–13325.

   https://doi.org/10.1074/jbc.M609798200

8. Otero M, Lago R, Lago F, *et al*., 2005, Signalling pathway involved in nitric oxide synthase Type II activation in chondrocytes: Synergistic effect of leptin with interleukin-1.

Arthritis Res Ther, 7: R581–R591.

https://doi.org/10.1186/ar1708

9. Maguire EM, Pearce SW, Xiao R, *et al*., 2019, Matrix metalloproteinase in abdominal aortic aneurysm and aortic dissection. *Pharmaceuticals (Basel)*, 12: 371–394.

https://doi.org/10.3390/ph12030118

10. Mimler T, Nebert C, Eichmair E, *et al*., 2019, Extracellular matrix in ascending aortic aneurysms and dissections-what we learn from decellularization and scanning electron microscopy. *PLoS One*, 14: e0213794.

https://doi.org/10.1371/journal.pone.0213794

11. Zhang L, Wang C, Xi Z, *et al*., 2018, Mercaptoethanol protects the aorta from dissection by inhibiting oxidative stress, inflammation, and extracellular matrix degeneration in a mouse model. *Med Sci Monit*, 24: 1802–1812.

https://doi.org/10.12659/msm.905151

12. Vuolteenaho K, Koskinen A, Kukkonen M, *et al*., 2009, Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage--mediator role of NO in leptin-induced PGE2, IL-6, and IL-8 production. *Mediators Inflamm*, 2009: 345838. https:/doi.org/10.1155/2009/345838

13. Mechanick JI, Zhao S, Garvey WT. 2018, Leptin, an adipokine with central importance in the global obesity problem. *Global Heart*, 13: 113–127.

https://doi.org/10.1016/j.gheart.2017.10.003

14. Park HY, Kwon HM, Lim HJ, *et al*., 2001, Potential role of leptin in angiogenesis: Leptin induces endothelial cell proliferation and expression of matrix metalloproteinases *in vivo* and *in vitro*. *Exp Mol Med*, 33: 95–102.

https://doi.org/10.1038/emm.2001.17

15. Cronin O, Walker PJ, Golledge J. 2013, The association of obesity with abdominal aortic aneurysm presence and growth. *Atherosclerosis*, 226: 321–327.

https://doi.org/10.1016/j.atherosclerosis.2012.10.041

16. Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions 4.2.6. In: The Cochrane Library, Issue 3, 2005. Chichester, UK: John Wiley & Sons, Ltd.

17. Zhan Y, Yuan H, Bu P, *et al*., 2018, Recombinant leptin attenuates abdominal aortic aneurysm formation in angiotensin II-infused apolipoprotein E-deficient mice. *Biochem Biophys Res Commun*, 503: 1450–1456.

https://doi.org/10.1016/j.bbrc.2018.07.062

18. Wu Z, Wang Z, Wu H, *et al*., 2020, Obesity is a risk factor for preoperative hypoxemia in Stanford A acute aortic dissection. *Medicine (Baltimore)*, 99: e19186.

https://doi.org/10.1097/MD.0000000000019186

19. Ellmark SH, Dusting GJ, Mark NT, *et al*., 2005, The contribution of Nox4 to NADPH oxidase activity in mouse vascular smooth muscle. *Cardiovasc Res*, 65: 495–504.

https://doi.org/10.1016/j.cardiores.2004.10.026

20. Zheng XL, Yuan SG, Peng DQ. 2007, Phenotype-specific inhibition of the vascular smooth muscle cell cycle by high glucose treatment. *Diabetologia*, 50(4): 881–890.

https://doi.org/10.1007/s00125-006-0543-6

21. Werle M, Kreuzer J, Höfele J, *et al*., 2005, Metabolic control analysis of the Warburg-effect in proliferating vascular smooth muscle cells. *J Biomed Sci*, 12: 827–834.

https://doi.org/10.1007/s11373-005-9010-5

22. Zheng H, Qiu Z, Chai T, *et al*., 2022, Insulin resistance promotes the formation of aortic dissection by inducing the phenotypic switch of vascular smooth muscle cells. *Front Cardiovasc Med*, 8: 732122.

https://doi.org/10.3389/fcvm.2021.732122

23. Yang L, Gao L, Nickel T, *et al*., 2017, Lactate promotes synthetic phenotype in vascular smooth muscle cells. *Circ Res*, 121: 1251–1262.

https://doi.org/10.1161/CIRCRESAHA.117.311819

24. Dusserre E, Bourdillon MC, Ciavatti M, *et al*., 1993, Lipid biosynthesis in cultured arterial smooth muscle cells is related to their phenotype. *Lipids*, 28: 589–592.

https://doi.org/10.1007/BF02536051

25. Salabei JK, Hill BG. 2013, Mitochondrial fission induced by platelet-derived growth factor regulates vascular smooth muscle cell bioenergetics and cell proliferation. *Redox Biol*, 1: 542–551.

https://doi.org/10.1016/j.redox.2013.10.011

26. Wu X, Ye J, Cai W, *et al*., 2022, LDHA mediated degradation of extracellular matrix is a potential target for the treatment of aortic dissection. *Pharmacol Res*, 176: 106051.

https://doi.org/10.1016/j.phrs.2021.106051

27. Wang Y, Zhao ZM, Zhang GX, *et al*., 2016, Dynamic autophagic activity affected the development of thoracic aortic dissection by regulating functional properties of smooth muscle cells. *Biochem Biophys Res Commun*, 479: 358–364.

https://doi.org/10.1016/j.bbrc.2016.09.080

28. Landsverk ML, Li S, Hutagalung AH, *et al*., 2007, The UNC-45 chaperone mediates sarcomere assembly through myosin degradation in *Caenorhabditis elegans*. *J Cell Biol*, 177: 205–210.

https://doi.org/10.1083/jcb.200607084

29. Bhatnagar S, Mittal A, Gupta SK, *et al*., 2011, TWEAK causes myotube atrophy through coordinated activation of ubiquitin-proteasome system, autophagy, and caspases. *J Cell Physiol*, 227: 1042–1051.

https://doi.org/10.1002/jcp.22821

30. Karsli-Uzunbas G, Guo JY, Price S, *et al*., 2014, Autophagy is required for glucose homeostasis and lung tumor maintenance. *Cancer Discov*, 4: 914–927.

https://doi.org/10.1158/2159-8290.CD-14-0363

31. Zhang S, Ye J, Dong G. 2010, Neuroprotective effect of baicalein on hydrogen peroxide-mediated oxidative stress and mitochondrial dysfunction in PC12 cells. *J Mol Neurosci*, 40: 311–320.

https://doi.org/10.1007/s12031-009-9285-5

32. Aliberti G, Proietta M, Tabacco F, *et al*., 2010, Inflammation and immune response in acute aortic dissection. *Ann Med*, 42: 622–629.

https://doi.org/10.3109/07853890.2010.518156

33. Bersi MR, Khosravi R, Wujciak AJ, *et al*., 2017, Differential cell-matrix mechanoadaptations and inflammation drive regional propensities to aortic fibrosis, aneurysm or dissection in hypertension. *J R Soc Interface*, 14: 20170327.

https:/doi.org/10.1098/rsif.2017.0327

34. Csongrádi É, Káplár M, Nagy B Jr., *et al*., 2017, Adipokines as atherothrombotic risk factors in obese subjects: Associations with haemostatic markers and common carotid wall thickness. *Nutr Metab Cardiovasc Dis*, 27: 571–580.

https://doi.org/10.1016/j.numecd.2017.02.007

35. Ciccone M, Vettor R, Pannacciulli N, *et al*., 2001, Plasma leptin is independently associated with the intima-media thickness of the common carotid artery. *Int J Obes Relat Metab Disord*, 25: 805–810.

https://doi.org/10.1038/sj.ijo.0801623

36. Stroescu R, Bizerea T, Doro G, *et al*., 2017, Correlation between adipokines and carotid intima media thickness in a group of obese Romanian children: Is small for gestational age status an independent factor for cardiovascular risk? *Arch Endocrinol Metab*, 61: 14–20.

https://doi.org/10.1590/2359-3997000000201

37. Robati RM, Partovi-Kia M, Haghighatkhah HR, *et al*., 2014, Increased serum leptin and resistin levels and increased carotid intima-media wall thickness in patients with psoriasis: Is psoriasis associated with atherosclerosis? *J Am Acad Dermatol*, 71: 642–648.

https://doi.org/10.1016/j.jaad.2014.06.006

38. Asha K, Sharma SB, Singal A, *et al*., 2014, Association of carotid intima-media thickness with leptin and apoliprotein B/apoliprotein A-I ratio reveals imminent predictors of subclinical atherosclerosis in psoriasis patients. *Acta Med (Hradec Kralove)*, 57: 21–27.

https://doi.org/10.14712/18059694.2014.4

39. McMahon M, Skaggs BJ, Grossman JM, *et al*., 2014, A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus. *Arthritis Rheumatol*, 66: 130–139.

https://doi.org/10.1002/art.38204

40. Gasbarrino K, Mantzoros C, Gorgui J, *et al*., 2016, Circulating chemerin is associated with carotid plaque instabaltty, whereas resistin is related to cerebrovascular symptomatology. *Arterioscler Thromb Vasc Biol*, 36: 1670–1678.

https://doi.org/10.1161/ATVBAHA.115.306741

41. Schneiderman J, Simon AJ, Schroeter MR, *et al*., 2008, Leptin receptor is elevated in carotid plaques from neurologically symptomatic patients and positively correlated with augmented macrophage density. *J Vasc Surg*, 48: 1146–1155.

https://doi.org/10.1016/j.jvs.2008.06.054

42. Bountouris I, Paraskevas Kl, Koutouzis M, *et al*., 2009, Serum leptin levels in patients undergoing carotid endarterectomy: A pilot study. *Angilogy*, 60: 698–704.

https://doi.org/10.1177/0003319709350133

43. Tsai YC, Leu SY, Peng YJ, *et al*., 2017, Genistein suppresses leptin-induced proliferation and migration of vascular smooth muscle cells and neointima formation. *J Cell Mol Med*, 21: 422–431.

https://doi.org/10.1111/jcmm.12986

44. Andrade-Oliveira V, Camara NO, Moraes-Vieira PM. 2015, Adipokines as drug targets in diabetes and underlying disturbances. *J Diabetes Res*, 2015: 681612.

https://doi.org/10.1155/2015/681612

45. Kurajoh M, Koyama H, Kadoya M, *et al*., 2015, Plasma leptin level is associated with cardiac autonomic dysfunction in patients with Type 2 diabetes: HSCAA study. *Cardiovasc Diabetol*, 14: 117.

https://doi.org/10.1186/s12933-015-0280-6

46. Vavruch C, Lanne T, Fredrikson M, *et al*., 2015, Serum leptin levels are independently related to the incidence of ischemic heart disease in a prospective study of patients with Type 2 diabetes. *Cardiovasc Diabetol*, 14: 62.

https://doi.org/10.1186/s12933-015-0208-1

47. Rodriguez AJ, Nunes VD, Mastronardi CA, *et al*., 2016, Association between circulating adipocytokine concentrations and microvascular complications in patients with Type 2 diabetes mellitus: A systematic review and meta-analysis of controlled cross-sectional studies. *J Diabetes Complications*, 30: 357–367.

https://doi.org/10.1016/j.jdiacomp.2015.11.004

48. Katsiki N, Yovos JG, Gotzamani-Psarrakou A, Karamitsos DT. Adipokines and vascular risk in Type 2 diabetes mellitus. *Angiology*, 62: 601–604.

https://doi.org/10.1177/0003319711409201

49. Guzel S, Seven A, Kocaoglu A, *et al*., 2013, Osteoprotegerin,

leptin and IL6: Association with silent myocardial ischemia in Type 2 diabetes mellitus. *Diab Vasc Dis Res*, 10: 25–31.

https://doi.org/10.1177/1479164112440815

50. Yamazaki Y, Emoto M, Morioka T, *et al*., 2013, Clinical impact of the leptin to soluble leptin receptor ratio on subclinical carotid atherosclerosis in patients with Type 2 diabetes. *J Atheroscler Thromb*, 20: 186–194.

https://doi.org/10.5551/jat.14662

51. Liu X, Li X, Li C, *et al*., 2019, Study on regulation of adipokines on body fat distribution and its correlation with metabolic syndrome in Type 2 diabetes mellitus. *Minerva Endocrinol*, 44: 259–263.

https://doi.org/10.23736/s0391-1977.17.02773-0

52. Asakawa H, Tokunaga K, Kawakami F. 2001, Relationship of leptin level with metabolic disorders and hypertension in Japanese Type 2 diabetes mellitus patients. *J Diabetes Complications*, 15: 57–62.

https://doi.org/10.1016/s1056-8727(00)00145-8

53. Morioka T, Emoto M, Yamazaki Y, *et al*., 2014, Leptin is associated with vascular endothelial function in overweight patients with Type 2 diabetes. *Cardiovasc Diabetol*, 13: 10.

https://doi.org/10.1186/1475-2840-13-10

54. Iraklianou S, Melidonis A, Tournis S, *et al*., 2001, Postprandial leptin responses after an oral fat tolerance test: Differences in Type 2 diabetes. *Diabetes Care*, 24: 1299–1301.

https://doi.org/10.2337/diacare.24.7.1299-a

55. Zhang L, Qin Y, Liang D, *et al*., 2018, Association of polymorphisms in LEPR with Type 2 diabetes and related metabolic traits in a Chinese population. *Lipids Health Dis,* 17: 2.

https://doi.org/10.1186/s12944-017-0644-x

56. Motawi T, Salman T, Shaker O, *et al*., 2015, Association of polymorphism in adiponectin (+45 T/G) and leptin (-2548 G/A) genes with Type 2 diabetes mellitus in male Egyptians. *Arch Med Sci*, 11: 937–944.

https://doi.org/10.5114/aoms.2015.54848

57. Su S, Zhang C, Zhang F, *et al*., 2016, The association between leptin receptor gene polymorphisms and Type 2 diabetes mellitus: A systematic review and meta-analysis. *Diabetes Res Clin Pract*, 121: 49–58.

https://doi.org/10.1016/j.diabres.2016.08.008

58. Yang MM, Wang J, Fan JJ, *et al*., 2016, Variations in the obesity gene "LEPR" contribute to risk of Type 2 diabetes mellitus: Evidence from a meta-analysis. *J Diabetes Res*, 2016: 5412084.

https://doi.org/10.1155/2016/5412084

59. Perry RJ, Petersen KF, Shulman GI. 2016, Pleotropic effects of leptin to reverse insulin resistance and diabetic ketoacidosis. *Diabetologia*, 59: 933–937.

https://doi.org/10.1007/s00125-016-3909-4

60. Feijoo-Bandin S, Portoles M, Rosello-Lleti E, *et al*., 2015, 20 years of leptin: Role of leptin in cardiomyocyte physiology and physiopathology. *Life Sci,* 140: 10–18.

https://doi.org/10.1016/j.lfs.2015.02.016

61. Gruzdeva OV, Akbasheva OE, Dyleva YA, *et al*., 2017, Adipokine and cytokine profiles of epicardial and subcutaneous adipose tissue in patients with coronary heart disease. *Bull Exp Biol Med,* 163: 608–611.

https://doi.org/10.1007/s10517-017-3860-5

62. Basati G, Razavi AE, Abdi S, *et al*., Association between adipokine and myeloperoxidase levels in patients with coronary artery disease. *Acta Med Iran*, 53: 25–29.

63. Lodh M, Goswami B, Parida A, *et al*., 2012, Assessment of serum leptin, pregnancy-associated plasma protein A and CRP levels as indicators of plaque vulnerability in patients with acute coronary syndrome. *Cardiovasc J Afr*, 23: 330–335.

https://doi.org/10.5830/CVJA-2012-008

64. Karatela RA, Sainani GS. 2010, Interrelationships of factor VII activity and plasma leptin with insulin resistance in coronary heart disease. *Atherosclerosis,* 209: 235–240.

https://doi.org/10.1016/j.atherosclerosis.2009.08.043

65. Gormez S, Demirkan A, Atalar F, *et al*., 2011, Adipose tissue gene expression of adiponectin, tumor necrosis factor-α and leptin in metabolic syndrome patients with coronary artery disease. *Intern Med,* 50: 805–810.

https://doi.org/10.2169/internalmedicine.50.4753

66. Bigalke B, Stellos K, Geisler T, *et al*., 2010, High plasma levels of adipocytokines are associated with platelet activation in patients with coronary artery disease. *Platelets,* 21: 11–19.

https://doi.org/10.3109/09537100903377584

67. Parhami F, Tintut Y, Ballard A, *et al*., 2001, Leptin enhances the calcification of vascular cells: Artery wall as a target of leptin. *Circ Res,* 88: 954–960.

https://doi.org/10.1161/hh0901.090975

68. Cirillo P, Angri V, De Rosa S, *et al*., 2010, Proatherothrombotic effects of leptin in human coronary endothelial cells. *Thromb Haemost*, 103: 1065–1075.

https://doi.org/10.1160/TH09-06-0392

69. Ikmal SI, Huri H, Vethakkan SR, *et al*., 2013, Potential biomarkers of insulin resistance and atherosclerosis in Type 2 diabetes mellitus patients with coronary artery disease. *Int J Endocrinol*, 2013: 698567.

https://doi.org/10.1155/2013/698567

70. Sainani GS, Karatela RA. 2009, Plasma leptin in insulin-resistant and insulin-nonresistant coronary artery disease and its association with cardio-metabolic risk factors among

Asian Indians. *Metab Syndr Relat Disord*, 7: 335–340.

https://doi.org/10.1089/met.2008.0097

71. Takahashi Y, Satoh M, Tabuchi T, *et al*., 2012, Prospective, randomized, single-blind comparison of effects of 6 months' treatment with atorvastatin versus pravastatin on leptin and angiogenic factors in patients with coronary artery disease. *Heart Vessels,* 27: 337–343.

https://doi.org/10.1007/s00380-011-0156-y

72. Sun YM, Li J, Luan Y, *et al*., 2010, Effect of statin therapy on leptin levels in patients with coronary heart disease. *Peptides*, 31: 1205–1207.

https://doi.org/10.1016/j.peptides.2010.03.023

73. Katsiki N, Mikhailidis DP, Gotzamani-Psarrakou A, *et al*., 2011, Effect of various treatments on leptin, adiponectin, ghrelin and neuropeptide Y in patients with Type 2 diabetes mellitus. *Expert Opin Ther Targets*, 15: 401–420.

https://doi.org/10.1517/14728222.2011.553609

74. Katsiki N, Mikhailidis DP, Gotzamani-Psarrakou A, *et al*., 2011, Effects of improving glycemic control with insulin on leptin, adiponectin, ghrelin and neuropeptide Y levels in patients with Type 2 diabetes mellitus: A pilot study. *Open Cardiovasc Med J*, 5: 136–147.

https://doi.org/10.2174/1874192401105010136

75. Pérez-Pérez A, Vilariño-García T, Fernández-Riejos P, *et al*., 2017, Role of leptin as a link between metabolism and the immune system. *Cytokine Growth Factor Rev*, 35: 71–84.

https://doi.org/10.1016/j.cytogfr.2017.03.001

76. Liberale L, Bonaventura A, Vecchiè A, *et al*., 2017, The role of adipocytokines in coronary atherosclerosis. *Curr Atheroscler Rep*, 19: 10.

https://doi.org/10.1007/s11883-017-0644-3

77. Beltowski J. 2006, Leptin and atherosclerosis. *Atherosclerosis*, 189: 47–60.

https://doi.org/10.1016/j.atherosclerosis.2006.03.003

78. Tahergorabi Z, Khazaei M. 2015, Leptin and its cardiovascular effects: Focus on angiogenesis. *Adv Biomed Res*, 4: 79.

https://doi.org/10.4103/2277-9175.156526

79. Shäfer K, Halle M, Goeschen C, *et al*., 2004, Leptin promotes vascular remodeling and neointimal growth in mice. *Arterioscler Thromb Vasc Biol*, 24: 112–117.

https://doi.org/10.1161/01.ATV.0000105904.02142.e7

80. Bodary PF, Gu S, Shen Y, *et al*., 2005, Recombinant leptin promotes atherosclerosis and thrombosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*, 25: e119–e122.

https://doi.org/10.1161/01.ATV.0000173306.47722.ec

81. Reilly MP, Iqbal N, Schutta M, *et al*., 2004, Plasma leptin levels are associated with coronary atherosclerosis in Type 2 diabetes. *J Clin Endocrinol Metab*, 89: 3872–3878.

82. Wallace AM, McMahon AD, Packard CJ, *et al*., 2001, Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation*, 104: 3052–3056.

https://doi.org/10.1161/hc5001.101061

83. Konstantinides S, Schafer K, Koschnick S, *et al*., 2001, Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. *J Clin Invest*, 108: 1533–1540.

https://doi.org/10.1172/JCI13143

84. Loffreda S, Yang SQ, Lin HZ, *et al*., 1998, Leptin regulates proinflammatory responses. *FASEB J*, 12: 57–65.

85. Yamagishi SI, Edelstein D, Du XL, *et al*., 2001, Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. *J Biol Chem*, 276: 25096–25100.

https://doi.org/10.1074/jbc.M007383200

86. Oda A, Taniguchi T, Yokoyama M. 2001, Leptin stimulates rat aortic smooth muscle cell proliferation and migration. *Kobe J Med Sci*, 47: 141–150.

87. Cassano S, Pucino V, La Rocca C, *et al*., 2014, Leptin modulates autophagy in human CD4+CD25-conventional T cells. *Metabolism*, 63: 1272–1279.

https://doi.org/10.1016/j.metabol.2014.06.010

88. Golledge J, Clancy P, Jamrozik K, *et al*., 2007, Obesity, adipokines, and abdominal aortic aneurysm: Health in men study. *Circulation*, 116: 2275–2279.

https://doi.org/10.1161/CIRCULATIONAHA.107.717926

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ORIGINAL RESEARCH ARTICLE

Association of insulin-like growth factor-1 and insulin-like growth factor-binding protein-3 with estimated glomerular filtration rate in patients with type 2 diabetes mellitus

Sheng Jiang†* and Jing Yang†

State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia, Department of Endocrinology, The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830 017, China

Abstract

Type 2 diabetes mellitus patients often suffer from kidney damage, which is more serious than in ordinary people. The insulin-like growth factor (IGF) system has synergistic effects with other hormonal axes and has an essential role in glucose metabolism and type 2 diabetes. The study aimed to observe the association of IGF-1 and IGF factor-binding protein-3 (IGFBP-3) with estimated glomerular filtration rate (eGFR) in patients with type 2 diabetes mellitus. We recruited 521 patients with type 2 diabetes from the Endocrinology Department of the First Affiliated Hospital of Xinjiang Medical University from March 1, 2021, to December 20, 2021. The clinical data we collected were analyzed to determine the association of IGF-1 and IGFBP-3 with eGFR in patients with type 2 diabetes. Spearman correlation analysis showed that eGFR was positively correlated with IGF-1 and IGFBP-3 in all subjects ($P = 0.044$ and $P = 0.004$, respectively). We developed a linear regression model. In the multiple linear regression model, serum IGF-1 and IGFBP-3 were positively correlated with eGFR ($\beta = 0.03$, 95% CI = 0.01 – 0.06; $P = 0.009$ and $\beta = 1.29$, 95% CI = 0.09 – 2.49; $P = 0.035$). The results of the correlations were further validated. This preliminary study demonstrated positive associations of serum IGF-1 and IGFBP-3 levels with eGFR in patients with type 2 diabetes.

Keywords: Estimated glomerular filtration rate; Insulin-like growth factor-binding protein-3; Insulin-like growth factor-1; Kidney function; Type 2 diabetes mellitus

1. Introduction

Diabetes mellitus, which is a serious global public health problem, is either caused by impaired insulin secretion or disturbed insulin effect, or usually both[1]. More than 90% of diabetic patients are classified under type 2 diabetes mellitus, which is characterized by insulin resistance with or without deficient insulin secretion[2]. It is well known that diabetes is a risk factor for kidney disease, which can lead to proteinuria, decreased renal function, and further development of diabetic nephropathy.

Approximately half of the patients with type 2 diabetes mellitus will develop chronic kidney disease (CKD) as the disease progresses and manifest increased urinary albumin

†These authors contributed equally to this work.

***Corresponding author:**
Sheng Jiang
(xjyj0611@126.com)

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excretion[3]. However, this view has been challenged, because many patients with type 2 diabetes mellitus suffer from a decline in glomerular filtration rate (GFR) in the absence of proteinuria, as confirmed by Kramer *et al.*[4], Garg *et al.*[5] and MacIsaac *et al.*[6] Therefore, in many cases, CKD is defined as a continuous decrease in GFR.

Insulin-like growth factors (IGFs) are proteins with multiple functions, including stimulation of cell proliferation, inhibition of apoptosis, and enhancement of cell motility as well as the regulation of cell differentiation and transformation[7]. Among the IGFs, the IGF-1 gene encodes a small protein made up of 70 amino acid residues. IGF-1 is an anabolic hormone and a primary mediator for growth hormone (GH)-related signaling pathways[8]. It also plays a versatile role in regulating many cellular processes, such as cellular metabolism, growth, proliferation, and apoptosis in multiple organs, and promoting the growth, development, and maintenance of cells[9]. The kidney can produce and release IGF-1 and is also the target organ of the GH/IGF-1 axis. Free IGF-1 in the blood is biologically active; more than 90% of IGF-1 binds to the IGF factor-binding protein-3 (IGFBP-3) in plasma. Therefore, IGFBP-3 is the primary regulator of the biological activity of plasma IGF-1[10-12]. In the literature, we can find that the molar ratio of IGF-1/IGFBP-3 is commonly used to reflect the bioactivity of IGF-1[13,14].

A prospective study observed that the serum IGFBP-3 levels were positively correlated with the risk of type 2 diabetes independent of IGF-1 levels[15]. IGF-1 and IGFBP-3 not only are correlated to diabetes but also have specific effects on the kidneys. It has been shown that serum IGF-1 levels are related to GFR[16]. Furthermore, the administration of intravenous recombinant human IGF-1 (rhIGF-1) stimulates the kidney in healthy subjects by increasing GFR and renal plasma flows[17,18]. Nevertheless, few studies have explored the level of IGF-1 in patients with type 2 diabetes and its relationship with kidney disease.

This cross-sectional study aimed to assess the association of IGF-1 and IGFBP-3 with indicators of renal function.

2. Materials and methods

2.1. Patients and study design

We reviewed the records of 614 patients who visited and completed type 2 diabetes screening at the Endocrinology Department of the First Affiliated Hospital of Xinjiang Medical University from March 1, 2021, to December 20, 2021. The exclusion criteria are as follows: (i) Patients whose data were incomplete or missing (n = 40); (ii) patients with other types of diabetes (n = 17); (iii) patients with diabetic ketoacidosis (n = 15); (iv) patients with diabetic foot (n = 8); and (v) patients with impaired glucose tolerance (n = 13). Finally, complete data from 521 patients were analyzed (Figure 1). This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

The diagnostic criteria for type 2 diabetes are based on the 1999 World Health Organization criteria: (i) Typical symptoms of diabetes (polydipsia, polyuria, polyphagia, and weight loss); and (ii) random venous plasma blood glucose ≥ 11.1 mmol/L (200 mg/dL) or fasting blood glucose ≥ 7.0 mmol/L (126 mg/dL) or oral glucose tolerance test (OGTT) 2h glucose in venous plasma (for those without diabetic symptoms, repeat the test on another day) ≥ 11.1 mmol/L (200 ng/dL).

2.2. Data collection

Clinical variables that were reviewed from electronic medical records include age, sex, body mass index (BMI), and blood pressure. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as body weight (kg) divided by the square of height (m). Readings of blood pressure (BP) were obtained using a mercury sphygmomanometer from the left arm of patients in supine position, after 5 min of quiet rest. Values were calculated as the average of the last two of three consecutive measurements obtained at 3-min intervals.

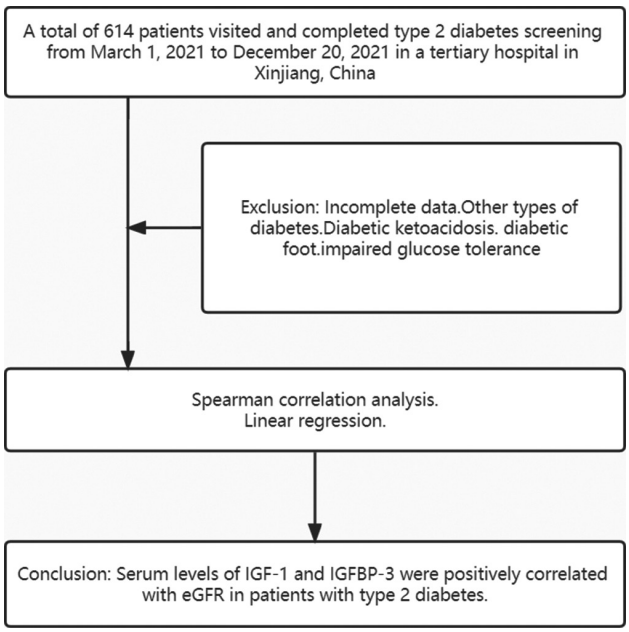

Figure 1. Flow chart of this study.
The relationship between insulin-like growth factor (IGF)-1, IGF factor-binding protein-3, and estimated glomerular filtration rate was clarified by correlation analysis and linear regression.

2.3. Biochemical indicators

Biochemical parameters of the present study include plasma glucose, glycated hemoglobin (HbA1c), serum creatinine, blood urea nitrogen (BUN), and serum cholesterol (total cholesterol [TC]), triglycerides (TG), alanine aminotransferase (ALT), and aspartate transaminase (AST), as determined by Fully Automatic Biochemistry Analyzer (Beckman Coulter).

2.4. Analytical determinations

Serum IGF-1 and IGFBP-3 levels were measured by the chemiluminescence immunometric method (Siemens Healthcare Diagnostics Products Ltd, United Kingdom). Serum creatinine concentration was determined using the Jaffé method. Low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) were measured by the direct method; TC was determined by enzymatic method; TG was determined by glycerol-3-phosphate (GPO)-peroxidase (POD) method; fasting plasma glucose (FPG) was determined by hexokinase method; and AST and ALT were determined by colorimetry (Fully Automatic Biochemistry Analyzer, Beckman Coulter, Inc., USA).

2.5. Calculations

GFR is estimated using the simplified Modification of Diet in Renal Disease (MDRD) formula:

Estimated glomerular filtration rate (eGFR) (mL/min/1.73 m^2) = 186.3 × serum creatinine (mg/dL)$^{-1.154}$ × age (years)$^{-0.203}$ × (0.742 for women)[19,20]

2.6. Statistical analysis

Statistical analysis was performed using a statistical computer program for the R (version 4.1.1) project. All the statistical tests were considered significant at the 0.05 probability level. Continuous variables are expressed as mean ± standard deviation (SD) or the median with the interquartile range. We used Shapiro–Wilk tests and Q-Q plot tests to verify the normality of the distribution of continuous variables. For the data of interest in this study, it has no impact on the normality of the distribution. Spearman's rank correlation coefficient was performed to analyze the correlation of IGF-1 and IGFBP-3 with other continuous variables of interest. Then, the univariate and multivariate linear regression analyses were performed to determine the association of circulating IGF-1 and other variables with eGFR in all study subjects. For a standardized coefficient (*B*), we estimated two-tailed probability values and the 95% confidence interval (95% CI). All P-values are two-tailed, and values of less than 0.05 are considered statistically significant.

3. Results

The study population comprised 317 men and 204 women. The mean age of the patients was 56.84 ± 12.38 years. The mean ± SD of IGF-1 was 152.46 ± 57.08 ng/mL, the mean ± SD of IGFBP-3 was 4.03 ± 1.22 μg/mL, and the mean ± SD of eGFR was 94.13 ± 21.08 ml/min/1.73 m^2. Baseline characteristics for the entire cohort are presented in Table 1.

All study subjects were divided into five groups according to eGFR. One-way analysis of variance (ANOVA) was performed for each group, and differences

Table 1. Characteristics of patients

Characteristic	N=521 (mean±SD)
Age (years)	56.84±12.38
Sex (female, %)	204 (39.16%)
SBP (mmHg)	129.18±17.17
DBP (mmHg)	78.54±10.98
Height (cm)	167.69±8.3
Weight (kg)	73.03±12.81
BMI (kg/m^2)	26.02±4.62
IGF-1 (ng/mL)	152.46±57.08
IGFBP-3 (μg/mL)	4.03±1.22
FBG (mmol/L)	7.93±2.67
2H-OGTT (mmol/L)	16.69±4.48
HbA1c (%)	8.75±2.21
eGFR (ml/min/1.73 m^2)	94.13±21.08
Cr (μmol/L)	71.71±24.94
BUN (mmol/L)	5.83±2.13
ALT (U/L)	25.16±31.76
AST (U/L)	21.26±18.48
TG (mmol/L)	2.15±1.97
TC (mmol/L)	4.19±1.21
LDL-c (mmol/L)	2.71±0.96
HDL-c (mmol/L)	0.98±0.27
24H-UP (g/24 h)	0.32±0.99
24H-UM (mg/24 h)	88.89±278.15
UAER (μg/min)	61.73±193.16

Data are presented as mean±standard deviation or as number (percentage) for sex.
Abbreviations: SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, IGF-1: Insulin-like growth factor 1, IGFBP-3: Insulin-like growth factor-binding protein 3, FPG: Fasting plasma glucose, 2H-OGTT: 2 h-oral glucose tolerance test, HbA1c: Glycated hemoglobin, eGFR: estimated Glomerular Filtration Rate, Cr: Creatinine, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TC: Total cholesterol, TG: Triglyceride, LDL-c: Low-density lipoprotein cholesterol, HDL-c: High-density lipoprotein cholesterol, 24H-UP: 24-h urinary protein, 24H-UM: 24-h urinary microalbumin, UAER: Urinary albumin excretion rate.

Table 2. Comparison of clinical data between groups stratified by glomerular filtration rate

Parameters	Glomerular filtration rate					*P*
	≥ 120	90 – 119	61 – 89	31 – 60	≤30	
NO	28	312	146	30	5	
Age (years)	35.86±10.53	54.14±10.23	63.84±9.61	69.77±10.77	60.60±12.58	<0.001
Diabetes duration (years)	6.93±7.22	7.93±6.80	10.13±7.49	11.22±9.80	13.60±4.39	0.003
Height (cm)	171.86±6.73	168.44±8.23	165.96±8.13	164.83±8.80	165.60±9.32	<0.001
Weight (kg)	79.86±15.33	73.40±13.16	71.65±11.10	69.45±11.23	73.40±19.89	0.015
BMI (kg/m^2)	27±4.64	26.01±5.11	25.94±3.44	25.50±3.88	26.80±7.69	0.769
SBP (mmHg)	126.93±16.48	128.40±17.69	129.21±15.37	137.43±18.77	140.00±17.58	0.039
DBP (mmHg)	83.61±9.43	79.15±11.39	76.29±9.63	78.80±11.34	75.80±17.41	0.009
IGF-1 (ng/mL)	147.84±39.56	155.06±57.02	148.13±61.25	147.63±53.92	171.80±33.68	0.653
IGFBP-3 (μg/mL)	4.54±1.20	4.06±1.18	3.87±1.24	3.85±1.45	4.64±0.67	0.048
FBG (mmol/L)	9.93±2.82	7.89±2.74	7.64±2.42	8.13±2.43	7.22±1.81	0.001
2H-OGTT (mmol/L)	18.78±3.83	16.56±4.38	16.72±4.83	16.37±3.93	14.49±4.22	0.101
HbA1c (%)	10.46±2.71	8.68±2.16	8.60±2.14	8.60±1.94	8.12±2.09	<0.001
eGFR (ml/min/1.73 m^2)	132.62±17.78	103.43±7.74	78.68±8.30	48.80±9.04	21.47±7.35	<0.001
Cr (μmol/L)	48.89±9.94	63.30±12.31	79.75±12.33	120.22±28.23	192.75±99.69	<0.001
BUN (mmol/L)	4±1.22	5.38±1.37	6.22±2.01	8.85±2.80	14.03±5.91	<0.001
ALT (U/L)	30.44±22.62	24.83±19.32	26.30±51.81	19.89±9.47	14.33±11.11	0.663
AST (U/L)	23.45±19.2	20.24±11.77	23.45±28.96	20.27±6.89	14.26±5.73	0.38
TG (mmol/L)	3.42±3.49	2.06±1.86	2.07±1.69	2.92±2.02	2.28±2.21	0.011
TC (mmol/L)	4.75±1.58	4.15±1.12	4.18±1.25	4.09±1.48	3.90±0.87	0.147
LDL-c (mmol/L)	2.89±0.99	2.70±0.91	2.73±1.01	2.58±1.07	2.00±0.97	0.354
HDL-c (mmol/L)	0.92±0.37	0.99±0.26	0.97±0.24	0.89±0.19	1.14±0.75	0.175
24H-UP (g/24h)	0.12±0.09	0.19±0.49	0.32±0.91	1.53±2.54	2.78±3.45	<0.001
24H-UM (mg/24h)	23.29±41.66	48.35±142.91	95.28±332.05	417.95±547.73	824.55±798.41	<0.001
UAER (μg/min)	16.17±28.93	33.58±99.24	66.17±230.59	290.24±380.37	572.60±554.45	<0.001

Abbreviations: SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, IGF-1: Insulin-like growth factor 1, IGFBP-3: Insulin-like growth factor-binding protein 3, FPG: Fasting plasma glucose, 2H-OGTT: 2h-oral glucose tolerance test, HbA1c: Glycated hemoglobin, eGFR: estimated glomerular filtration rate, Cr: Creatinine, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TC: Total cholesterol, TG: Triglyceride, LDL-c: Low-density lipoprotein cholesterol, HDL-c: High-density lipoprotein cholesterol, 24H-UP: 24-h urinary protein, 24H-UM: 24-h urinary microalbumin, UAER: Urinary albumin excretion rate

were found in age, duration of diabetes, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), IGFBP-3, FBG, HbA1c, BUN, TG, 24-h urine protein, and urinary albumin (Table 2).

In this study, the Spearman's rank correlation coefficients among IGF-1and IGFBP-3 with the above-mentioned parameters are illustrated in Table 3. Through Spearman correlation analysis, we found that there was a significantly positive correlation between IGF-1 and IGFBP-3 ($P < 0.001$). IGF-1 and IGFBP-3 were positively correlated with eGFR. A correlation heat map was created based on the results of the correlation analysis (Figure 2).

As presented in Table 4, eGFR was negatively correlated with age, diabetes course, SBP, BUN, serum creatinine, 24-h urine protein, 24-h urinary microalbumin and urinary albumin excretion rate, and positively correlated with FPG, HbA1c, IGF-1, and IGFBP-3 in univariate linear regression analysis.

Model I was developed after screening variables by the Akaike information criterion (AIC was used as a measure of goodness of fit for each model; all combinations of variables were tested, and the best model has the lowest AIC) based on univariate linear regression analysis, and we developed Model II by replacing the independent variable IGF-1 in Model I with IGFBP-3. In Model I, IGF-1 ($\beta = 0.03$, 95% CI = 0.01 – 0.06) was significantly associated

Table 3. Spearman's correlation analysis among IGF-1and IGFBP-3 with other parameters

Variable	IGF-1	IGFBP-3
Age (years)	−0.258 (<0.001)	−0.401 (<0.001)
Duration of diabetes (years)	0.007 (0.882)	−0.019 (0.657)
SBP (mmHg)	−0.013 (0.764)	−0.011 (0.792)
DBP (mmHg)	0.068 (0.119)	0.093 (0.033)
Height (cm)	0.104 (0.017)	0.046 (0.291)
Weight (kg)	0.027 (0.533)	0.047 (0.275)
BMI (kg/m^2)	−0.055 (0.204)	0.002 (0.957)
Glucose metabolism		
FBG (mmol/L)	0.000 (0.992)	0.061 (0.163)
2H-OGTT (mmol/L)	−0.021 (0.631)	−0.005 (0.916)
HbA1c (%)	0.015 (0.719)	0.055 (0.206)
Kidney function		
eGFR (ml/min/1.73 m2)	0.088 (0.044)	0.126 (0.004)
Cr (μmol/L]	0.056 (0.204)	0.002 (0.968)
BUN (mmol/L)	0.072 (0.101)	−0.019 (0.665)
Liver function		
ALT (U/L)	−0.076 (0.082)	−0.026 (0.558)
AST (U/L)	−0.123 (0.005)	−0.081 (0.062)
Blood lipids		
TG (mmol/L)	0.022 (0.615)	0.261 (0.001)
TC (mmol/L)	0.068 (0.122)	0.311 (<0.001)
LDL-c (mmol/L)	0.057 (0.191)	0.238 (<0.001)
HDL-c (mmol/L)	0.064 (0.436)	0.081 (0.301)
Urine protein		
24H-UP (g/24 h)	0.004 (0.926)	−0.019 (0.775)
24H-UM (mg/24 h)	0.016 (0.709)	0.013 (0.775)
UAER (μg/min)	0.016 (0.709)	0.013 (0.775)
IGFs		
IGF-1 (ng/mL)	-	0.550 (<0.001)
IGFBP-3 (μg/mL)	0.550 (<0.001)	-

Data are given as *r* (*P*), *r*: Spearman's correlation coefficient. Statistical correlation is significant at $P<0.05$. Abbreviations: SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, FPG: Fasting plasma glucose, 2H-OGTT: 2h-oral glucose tolerance test, HbA1c: Glycated hemoglobin, eGFR: estimated glomerular filtration rate, Cr: Creatinine, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TG: Triglyceride, TC: Total cholesterol, LDL-c: Low-density lipoprotein cholesterol, HDL-c: High-density lipoprotein cholesterol, 24H-UP: 24-h urinary protein, 24H-UM: 24-h urinary microalbumin, UAER: Urinary albumin excretion rate, IGF-1: Insulin-like growth factor 1, IGFBP-3: Insulin-like growth factor-binding protein 3.

with eGFR. In Model II, there was no difference in the correlation between each independent variable and eGFR despite the replacement with IGFBP-3. After adjusting for

Table 4. Univariate linear regression analysis of the correlation between eGFR and various parameters in patients with type 2 diabetes mellitus

Characteristic	β	95% CI	*P*
Age (years)	−1.06	−1.17, −0.94	<0.001
Diabetes duration (years)	−0.46	−0.71, −0.21	<0.001
SBP (mmHg)	−0.21	−0.32, −0.11	<0.001
DBP (mmHg)	−0.16	0.00, 0.32	0.057
Height (cm)	0.49	0.27, 0.70	<0.001
Weight (kg)	0.18	0.04, 0.32	0.013
BMI (kg/m^2)	0.03	−0.37, 0.42	0.892
IGF-1 (ng/mL)	0.03	0.01, 0.06	0.044
IGFBP-3 (μg/mL)	2.18	0.71, 3.66	0.004
FPG (mmol/L)	1.05	0.37, 1.72	0.002
2H-OGTT (mmol/L)	0.38	−0.02, 0.78	0.063
HbA1c (%)	1.47	0.66, 2.28	<0.001
Cr (μmol/L)	−0.61	−0.66, −0.56	<0.001
BUN (mmol/L)	−5.32	−6.04, −4.60	<0.001
ALT (U/L)	0.04	−0.02, 0.09	0.222
AST (U/L)	−0.01	−0.11, 0.09	0.816
TG (mmol/L)	0.73	−0.19, 1.65	0.122
TC (mmol/L)	1.26	−0.24, 2.76	0.099
LDL-c (mmol/L)	1.16	−0.74, 3.06	0.231
HDL-c (mmol/L)	0.09	−6.73, 6.91	0.979
24H-UP (g/24 h)	−6.68	−8.43, −4.94	<0.001
24H-UM (mg/24 h)	−0.03	−0.03, −0.02	<0.001
UAER (μg/min)	−0.04	−0.05, −0.02	<0.001

Abbreviations: β: Regression coefficient, CI: Confidence interval, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, IGF-1: Insulin-like growth factor 1, IGFBP-3: Insulin-like growth factor-binding protein 3, FPG: Fasting plasma glucose, 2H-OGTT: 2h-oral glucose tolerance test, HbA1c: Glycated hemoglobin, eGFR: estimated glomerular filtration rate, Cr: Creatinine, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TC: Total cholesterol, TG: Triglyceride, LDL-c: Low-density lipoprotein cholesterol, HDL-c: High-density lipoprotein cholesterol, 24H-UP: 24-h urinary protein, 24H-UM: 24-h urinary microalbumin, UAER: Urinary albumin excretion rate

IGF-1, IGFBP-3 (β = 1.29, 95% CI = 0.09 – 2.49) remained positively correlated with eGFR. In multiple linear regression models, diabetes duration was not associated with eGFR. SBP and BUN were negatively correlated with eGFR, while DBP and FBG were positively correlated with eGFR (Table 5).

4. Discussion

The patients with type 2 diabetes mellitus are a high-risk group of renal insufficiency. It is crucial for

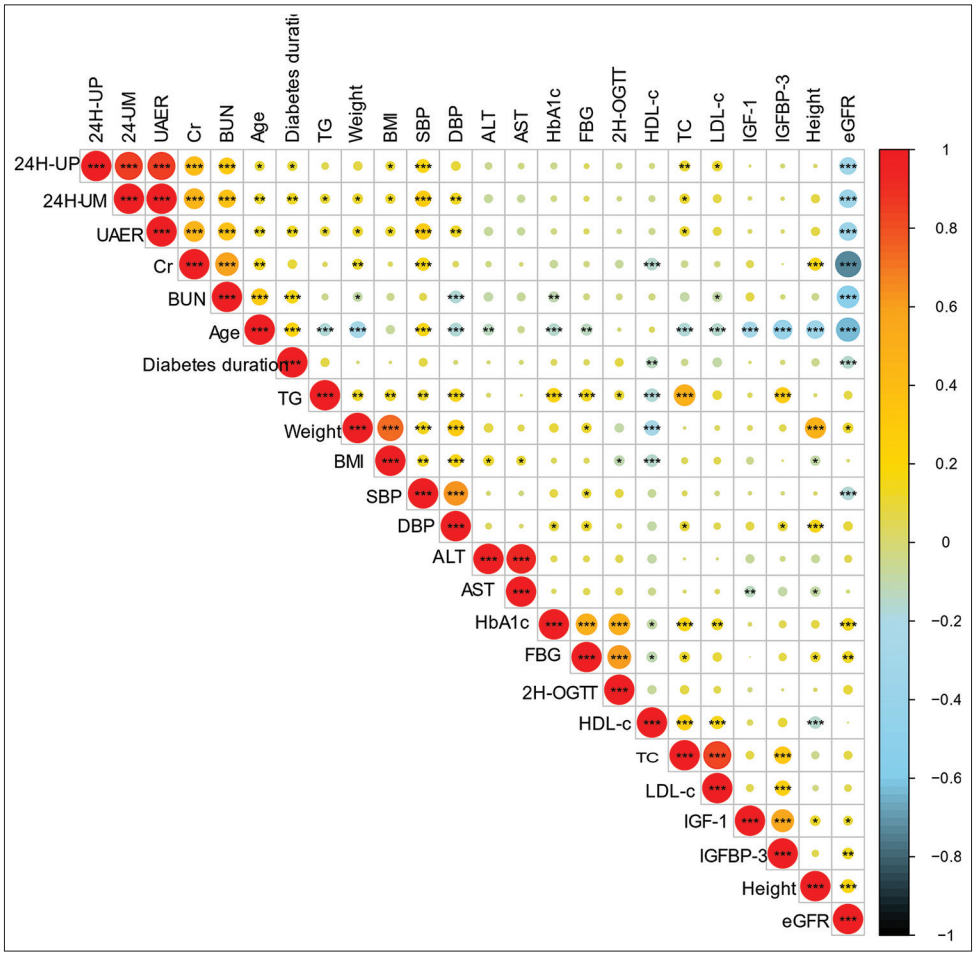

Figure 2. Correlation map reporting Spearman's correlation values for each comparison.
The bar on the right of the map indicates the color legend of the Spearman's correlation values calculated for each couple of samples in the matrix. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 5. Multiple linear regression analysis of the correlation between eGFR and various parameters in patients with type 2 diabetes mellitus

Factors included	Model I		Model II	
	β (95% CI)	*P*	β (95% CI)	*P*
Diabetes duration (years)	−0.15 (−0.35, −0.04)	0.128	−0.15 (−0.35, 0.05)	0.137
SBP (mmHg)	−0.25 (−0.35, −0.04)	<0.001	−0.25 (−0.36, −0.14)	<0.001
DBP (mmHg)	0.25 (0.08, 0.42)	0.005	0.25 (0.08, 0.43)	0.004
FBG (mmol/L)	1.01 (0.48, 1.55)	<0.001	0.98 (0.44, 1.52)	<0.001
BUN (mmol/L)	−4.37 (−5.12, −3.63)	<0.001	−4.29 (−5.03, −3.54)	<0.001
ALT (U/L)	0.23 (0.11, 0.35)	<0.001	0.23 (0.10, 0.35)	<0.001
AST (U/L)	−0.43 (−0.64, −0.21)	<0.001	−0.43(−0.64, −0.22)	<0.001
UAER (μg/min)	−0.02 (−0.03, −0.01)	<0.001	−0.02 (−0.03, −0.01)	<0.001
IGF-1 (ng/mL)	0.03 (0.01, 0.06)	0.009	-	-
IGFBP-3 (μg/mL]	-	-	1.29 (0.09, 2.49)	0.035

B: Regression coefficient, CI: Confidence interval, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BUN: Blood urea nitrogen, TC: Total cholesterol, LDL-c: Low-density lipoprotein, HbA1c: Glycated hemoglobin, FPG: Fasting plasma glucose, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, UAER: Urinary albumin excretion rate, IGF-1: Insulin-like growth factor-1, IGFBP-3: Insulin-like growth factor-binding protein-3

clinicians to focus on the effects of IGF-1 and IGFBP-3 on renal function in patients with type 2 diabetes and to understand the association of IGF-1 and IGFBP-3 with eGFR, which might be important for renal function assessment and early diagnosis. By observing the indicators of all patients, serum IGF-1 and IGFBP-3 were positively correlated with eGFR. This suggests that serum IGF-1 and IGFBP-3 play a key role in renal function.

The relationship between serum IGF-1 and GFR is unclear. So far, only one study has been conducted to investigate the correlation between serum IGF-1, IGFBP-3, and GFR in patients with type 2 diabetes, showing that the levels of these two proteins are not related to GFR in all patients[21]. In addition, another study found that serum IGF-1 reduction was associated with lower eGFR in insulin-resistant obese patients[22]. On the contrary, in 4028 (2048 women) subjects between the age of 20 and 81 years, IGF-1 was inversely correlated with BMI, presence of diabetes, and GFR[23,24]. Hence, the association between IGF-1 and eGFR in patients with type 2 diabetes is not yet understood. In this study, we studied 521 Chinese patients with type 2 diabetes. Spearman correlation analysis found that both serum IGF-1 and IGFBP-3 are positively linked with eGFR. Then, we established the linear regression model of IGF-1and the linear regression model of IGFBP-3. Interestingly, IGF-1and IGFBP-3 were still related to eGFR. In summary, we speculate that serum IGF-1and IGFBP-3 may be factors that affect the level of eGFR in type 2 diabetes.

This study shows that IGF-1 and IGFBP-3 are positively correlated with eGFR. The physiological link between IGF-1 levels and renal disease in type 2 diabetes is not fully understood; however, it is generally believed that the GH/IGF-1 axis affects renal function[25,26].

IGF-1 promotes the division of mesangial cell in glomeruli[27], and it can inhibit the apoptosis of mesangial and podocyte cells[28]. IGF-1 may increase glomerular perfusion by reducing the resistance of the arterioles[29,30]. It is worth noting that micro-puncture studies have also shown that IGF-1 increases single nephron GFR and blood flow by expanding the ultrafiltration coefficient and reducing the resistance of the efferent arterioles[26]. Furthermore, IGF-1 can increase extracellular volume and plasma volume[31,32], which also helps increase glomerular filtration. In most patients with decreased renal function, the expression of growth hormone receptor and IGF-1 gene in the kidney is diminished, which is a cause of reduced GFR[33,34].

Similarly, Jorgensen *et al.*[35] found that the reduction of renal plasma flow and glomerular filtration was related to the lack of IGF-1 and growth hormone. The role of IGF-1 in a high glucose environment induces mesangial cells to produce nitric oxide, which, further, leads to changes in renal hemodynamics. In addition, IGF-1 can interact with the renin-angiotensin system to cause changes in glomerular hemodynamics[36,37].

There are inconsistent results reported on the relationship between serum IGF-1, IGFBP-3, and diabetes risk. In a study that included normoglycemic patients between the ages of 45 and 65 years, it was observed that serum IGF-1 was associated with a reduced risk of type 2 diabetes after a glucose tolerance test[38]. In contrast, it has also been found that serum IGF-1or IGFBP-3 was not associated with diabetes risk[39]. In our study, serum IGF-1 and IGFBP-3 levels were not correlated with FBG, 2-h OGTT and HbA1c, which is consistent with previous reports[40,41]. However, in univariate regression analysis, FPG and HbA1c were significantly positively correlated with eGFR.

In comparison, only FPG was statistically significant in multivariate regression analysis. Weil *et al.* found that GFR was positively associated with fasting glucose and glycated hemoglobin in patients with type 2 diabetes[42]. Hyperglycemia may cause hyperfiltration in diabetic patients when they do not develop the end-stage renal disease in the early stages of diabetes.

Dyslipidemia is the basis of cardiovascular disease. The concentration of serum HDL-c is inversely correlated with the risk of coronary heart disease[43,44]. Each 1 mg/dL increase in HDL-c reduces the risk of coronary heart disease by approximately 2 – 3%[45]. It was previously reported that IGF-1 is a protective factor for coronary heart disease in patients with type 2 diabetes[46].

Song *et al.*[47] showed that serum IGF-1 was positively correlated with HDL-c. Our study showed a positive correlation between serum IGFBP-3 and TG, TC, and LDL-c in correlation analysis. However, no correlation was found in linear regression. Further studies need to confirm the role of serum IGFBP-3 on lipid metabolism in type 2 diabetic patients.

Although there are overlaps in the current findings with previous epidemiological and laboratory data, the present study has several limitations that must be considered in the interpretation of its findings. First, the small sample size does not allow for a comprehensive assessment of the entire population, and the findings may be biased. This bias may be reflected in the correlation between IGF-1 and eGFR, and large-scale population data are needed to confirm our results in the future. Furthermore, nutrition is an essential factor in the regulation of IGF-1. Another limitation of this study may be the lack of data concerning nutritional status[48]. In addition, we need to include more patients with eGFR of < 60 ml/min/1.73 m^2, and then conduct a

longitudinal subgroup analysis to clarify further the trend of IGF-1 changes in patients with severe renal impairment.

5. Conclusions

Serum IGF-1 and IGFBP-3 were positively correlated with eGFR in patients with T2DM. Although there has been an increased understanding of the function of IGF-1and IGFBP-3, there are still many unanswered questions about their effects on renal function in patients with T2DM. Therefore, whether serum IGF-1 can be used for the measurement and assessment of renal function requires further evaluation in prospective large-scale studies.

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Conflict of interest

The authors report no conflicts of interest in this work.

Author contributions

Conceptualization: Sheng Jiang
Formal analysis: Sheng Jiang, Jing Yang
Investigation: Jing Yang
Writing – original draft: Sheng Jiang, Jing Yang
Writing – review & editing: Jing Yang

Ethics approval and consent to participate

The study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was reviewed and approved by the Human Research Ethics Committee of the Affiliated Hospital of Xinjiang Medical University (K202205-11), and informed consent was obtained from all patients with type 2 diabetes mellitus before their participation.

Consent for publication

Not applicable.

Availability of data

The data used to support the findings of this study are available from the corresponding author on request.

References

1. Petersmann A, Muller-Wieland D, Muller UA, *et al*., 2019, Definition, classification and diagnosis of diabetes mellitus. *Exp Clin Endocrinol Diabetes*, 127: S1–S7.

   https://doi.org/10.1055/a-1018-9078

2. Compston J, 2018, Type 2 diabetes mellitus and bone. *J Intern Med*, 283: 140–153.

   https://doi.org/10.1111/joim.12725

3. Thomas MC, Brownlee M, Susztak K, *et al*., 2015, Diabetic kidney disease. *Nat Rev Dis Primers*, 1: 15018.

   https://doi.org/10.1038/nrdp.2015.18

4. Kramer HJ, Nguyen QD, Curhan G, *et al*., 2003, Renal insufficiency in the absence of albuminuria and retinopathy among adults with Type 2 diabetes mellitus. *JAMA*, 289: 3273–3277.

   https://doi.org/10.1001/jama.289.24.3273.

5. Garg AX, Kiberd BA, Clark WF, *et al*., 2002, Albuminuria and renal insufficiency prevalence guides population screening: Results from the NHANES III. *Kidney Int*, 61: 2165–2175.

   https://doi.org/10.1046/j.1523-1755.2002.00356.x

6. MacIsaac RJ, Tsalamandris C, Panagiotopoulos S, *et al*., 2007, Nonalbuminuric renal insufficiency in Type 2 diabetes. *Diabetes Care*, 27: 195–200.

   https://doi.org/10.2337/diacare.27.1.195

7. Rahmani J, Montesanto A, Giovannucci E, *et al*., 2022, Association between IGF-1 levels ranges and all-cause mortality: A meta-analysis. *Aging Cell*, 21: e13540.

   https://doi.org/10.1111/acel.13540

8. Liu H, Gu H, Kutbi EH, *et al*., 2021, Association of IGF-1 and IGFBP-3 levels with gastric cancer: A systematic review and meta-analysis. *Int J Clin Pract*, 75: e14764.

   https://doi.org/10.1111/ijcp.14764

9. Qiang J, Kai L, Lulu H, *et al*., 2020, The effect of resistance training on serum insulin-like growth factor 1(IGF-1): A systematic review and meta-analysis. *Complement Ther Med*, 50: 102360.

   https://doi.org/10.1016/j.ctim.2020.102360

10. Aguirre GA, Gonzalez-Guerra JL, Espinosa L, *et al*., 2018, Insulin-like growth factor 1 in the cardiovascular system. *Rev Physiol Biochem Pharmacol*, 175: 1–45.

    https://doi.org/10.1007/112_2017_8

11. Nambam B, Schatz D, 2018, Growth hormone and insulin-like growth factor-I axis in Type 1 diabetes. *Growth Horm IGF Res*, 38: 49–52.

    https://doi.org/10.1016/j.ghir.2017.12.005

12. Akcali A, Bal B, Erbagci B, 2017, Circulating IGF-1,

IGFB-3, GH and TSH levels in multiple sclerosis and their relationship with treatment. *Neurol Res*, 39: 606–611.

https://doi.org/10.1080/01616412.2017.1321711

13. Gatti R, De Palo E F, Antonelli G, *et al*., 2012, IGF-I/IGFBP system: Metabolism outline and physical exercise. *J Endocrinol Invest*, 35: 699–707.

https://doi.org/10.3275/8456

14. Ranke MB, 2015, Insulin-like growth factor binding-protein-3 (IGFBP-3). *Best Pract Res Clin Endocrinol Metab*, 29: 701–711.

https://doi.org/10.1016/j.beem.2015.06.003

15. Drogan D, Schulze MB, Boeing H, *et al*., 2016, Insulin-like growth factor 1 and insulin-like growth factor-binding Protein 3 in relation to the risk of Type 2 diabetes mellitus: Results from the EPIC-potsdam study. *Am J Epidemiol*, 183: 553–560.

https://doi.org/10.1093/aje/kwv188

16. Petta S, Camma C, Cabibi D, *et al*., 2011, Hyperuricemia is associated with histological liver damage in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*, 34: 757–766.

https://doi.org/10.1111/j.1365-2036.2011.04788.x

17. Giordano M, DeFronzo RA, 1995, Acute effect of human recombinant insulin-like growth factor I on renal function in humans. *Nephron*, 71: 10–15.

https://doi.org/10.1159/000188667

18. Hammerman MR, Miller SB, 1993, The growth hormone insulin-like growth factor axis in kidney revisited. *Am J Physiol*, 265: F1–F14.

https://doi.org/10.1152/ajprenal.1993.265.1.F1

19. Jalalonmuhali M, Lim SK, Shah MN, *et al*., 2017, MDRD vs. CKD-EPI in comparison to (51)Chromium EDTA: A cross sectional study of Malaysian CKD cohort. *BMC Nephrol*, 18: 363.

https://doi.org/10.1186/s12882-017-0776-2

20. Porrini E, Ruggenenti P, Luis-Lima S, *et al*., 2019, Estimated GFR: Time for a critical appraisal. *Nat Rev Nephrol*, 15: 177–19.

https://doi.org/10.1038/s41581-018-0080-9

21. NeamTu M C, Avramescu ET, Marcu IR, *et al*., 2017, The correlation between insulin-like growth factor with glycemic control, glomerular filtration rate, blood pressure, hematological changes or body mass index in patients with Type 2 diabetes mellitus. *Rom J Morphol Embryol*, 58: 857–861.

22. Perticone F, Maio R, Sciacqua A, *et al*., 2009, Insulin-like growth factor-1 and glomerular filtration rate in hypertensive patients. *J Hypertens*, 27: 613–617.

https://doi.org/10.1097/hjh.0b013e32831fda24

23. Lam CS, Chen MH, Lacey SM, *et al*., 2010, Circulating insulin-like growth factor-1 and its binding protein-3: Metabolic and genetic correlates in the community. *Arterioscler Thromb Vasc Biol*, 30: 1479–1484.

https://doi.org/10.1161/ATVBAHA.110.203943

24. Dittmann K, Wallaschofski H, Rettig R, *et al*., 2012, Association between serum insulin-like growth factor I or IGF-binding protein 3 and estimated glomerular filtration rate: Results of a population-based sample. *BMC Nephrol*, 13: 169.

https://doi.org/10.1186/1471-2369-13-169

25. Frystyk J, Ivarsen P, Skjaerbaek C, *et al*., 1999, Serum-free insulin-like growth factor I correlates with clearance in patients with chronic renal failure. *Kidney Int*, 56: 2076–2084.

https://doi.org/10.1046/j.1523-1755.1999.00798.x

26. Bach LA, Hale LJ, 2015, Insulin-like growth factors and kidney disease. *Am J Kidney Dis*, 65: 327–336.

https://doi.org/10.1053/j.ajkd.2014.05.024

27. Feld SM, Hirschberg R, Artishevsky A, *et al*., 1995, Insulin-like growth factor I induces mesangial proliferation and increases mRNA and secretion of collagen. *Kidney Int*, 48: 45–51.

https://doi.org/10.1038/ki.1995.265

28. Vasylyeva TL, Chen X, Ferry RJ Jr, 2005, Insulin-like growth factor binding protein-3 mediates cytokine-induced mesangial cell apoptosis. *Growth Horm IGF Res*, 15: 207–214.

https://doi.org/10.1016/j.ghir.2005.02.008

29. Guler HP, Eckardt KU, Zapf J, *et al*., 1989, Insulin-like growth factor I increase glomerular filtration rate and renal plasma flow in man. *Acta Endocrinol* (*Copenh*), 121: 101–106.

https://doi.org/10.1530/acta.0.1210101

30. Hirschberg R, Kopple JD, 1989, Evidence that insulin-like growth factor I increases renal plasma flow and glomerular filtration rate in fasted rats. *J Clin Invest*, 83: 326–330.

https://doi.org/10.1172/JCI113878

31. Moller J, 2003, Effects of growth hormone on fluid homeostasis. Clinical and experimental aspects. *Growth Horm IGF Res*, 13: 55–74.

https://doi.org/10.1016/s1096-6374(03)00011-x

32. Kamenicky P, Mazziotti G, Lombes M, *et al*., 2014, Growth hormone, insulin-like growth factor-1, and the kidney: Pathophysiological and clinical implications. *Endocr Rev*, 35: 234–281.

https://doi.org/10.1210/er.2013-1071

33. Amit T, Youdim MB, Hochberg Z, 2000, Clinical review 112: Does serum growth hormone (GH) binding protein reflect human GH receptor function? *J Clin Endocrinol Metab*, 85: 927–932.

https://doi.org/10.1210/jcem.85.3.6461

34. Kratzsch J, Keliner K, Zilkens T, *et al*., 1996, Growth hormone-binding protein related immunoreactivity is regulated by the degree of insulinopenia in diabetes mellitus. *Clin Endocrinol* (*Oxf*), 44: 673–678.

https://doi.org/10.1046/j.1365-2265.1996.672494.x

35. Jorgensen JO, Pedersen SA, Thuesen L, *et al*., 1989, Beneficial effects of growth hormone treatment in GH-deficient adults. *Lancet*, 1: 1221–1225.

https://doi.org/10.1016/s0140-6736(89)92328-3

36. Miyauchi S, Miyake T, Miyazaki M, *et al*., 2019, Insulin-like growth factor-1 is inversely associated with liver fibrotic markers in patients with Type 2 diabetes mellitus. *J Diabetes Investig*, 10: 1083–1091.

https://doi.org/10.1111/jdi.13000

37. Maximus PS, 2019, Insulin like growth factor 1 is linked to higher cardiovascular risk score in adults with Type 2 diabetes mellitus and chronic kidney disease. *Diabetes Metab Syndr*, 13: 2613–2618.

https://doi.org/10.1016/j.dsx.2019.07.008

38. Sandhu MS, Heald AH, Gibson JM, *et al*., 2002, Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: A prospective observational study. *Lancet*, 359: 1740–1745.

https://doi.org/10.1016/S0140-6736(02)08655-5

39. Simila ME, Kontto JP, Virtamo J, *et al*., 2019, Insulin-like growth factor I, binding proteins-1 and-3, risk of Type 2 diabetes and macronutrient intakes in men. *Br J Nutr*, 121: 938–944.

https://doi.org/10.1017/S0007114519000321

40. Aleidi SM, Shayeb E, Bzour J, *et al*., 2019, Serum level of insulin-like growth factor-I in Type 2 diabetic patients: Impact of obesity. *Horm Mol Biol Clin Investig*, 39: 20190015.

https://doi.org/10.1515/hmbci-2019-0015

41. Suda K, Matsumoto R, Fukuoka H, *et al*., 2016, The influence of Type 2 diabetes on serum GH and IGF-I levels in hospitalized Japanese patients. *Growth Horm IGF Res*, 29: 4–10.

https://doi.org/10.1016/j.ghir.2016.03.002

42. Weil EJ, Kobes S, Jones LI, *et al*., 2019, Glycemia affects glomerular filtration rate in people with Type 2 diabetes. *BMC Nephrol*, 20: 397.

43. Barter P, Gotto AM, LaRosa JC, *et al*., 2007, HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med*, 357: 1301–1310.

https://doi.org/10.1056/NEJMoa064278

44. Kosmas CE, Martinez I, Sourlas A, *et al*., 2018, High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs Context*, 7: 212525.

https://doi.org/10.7573/dic.212525

45. Gordon DJ, Probstfield JL, Garrison RJ, *et al*., 1989, High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation*, 79: 8–15.

https://doi.org/10.1161/01.cir.79.1.8

46. Teppala S, Shankar A, 2010, Association between serum IGF-1 and diabetes among U.S. adults. *Diabetes Care*, 33: 2257–2259.

https://doi.org/10.2337/dc10-0770

47. Song X, Teng J, Wang A, *et al*., 2016, Positive correlation between serum IGF-1 and HDL-C in Type 2 diabetes mellitus. *Diabetes Res Clin Pract*, 118: 44–49.

https://doi.org/10.1016/j.diabres.2016.04.056

48. Wang X, Tian F, Sun H, *et al*., 2019, Insulin-like growth factor-1 as a nutritional monitoring factor in patients with chronic intestinal failure. *Clin Nutr*, 38: 1737–1744.

https://doi.org/10.1016/j.clnu.2018.07.031

ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

Comparative proteomic analysis of hearts from mice with high-fat diet-induced metabolic cardiomyopathy

Zongzhe Jiang[1†], Mingyang Pang[2†], and Wei Huang[2,3,4*]

[1]Luzhou Key Laboratory of Cardiovascular and Metabolic Diseases, Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China

[2]Department of Cardiology, Chinese PLA Central War Command General Hospital, Wuhan, 430 010, China

[3]Division of Cardiology, Department of Internal Medicine, and Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiological Disorders, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430 010, China

[4]Gene Therapy Center and the Institute of Hypertension, Huazhong University of Science and Technology, Wuhan, 430 010, China

Abstract

In patients with obesity or type 2 diabetes, the accumulation of lipotoxic by-products in cardiomyocytes leads to apoptosis and contractile dysfunction, eventually resulting in metabolic cardiomyopathy (MC). However, the underlying mechanisms remain unclear. Inb this study, a comparative proteome analysis was conducted to evaluate the differentially expressed proteins (DEPs) in the hearts of normal mice on standard diet (control group) and of high-fat diet (HFD)-induced MC mice (HFD group). We identified 90 DEPs unique to the control group and 18 DEPs unique to the HFD group. In 90 DEPs unique to the control group, only 74 DEPs were annotated in the gene ontology (GO) database. These annotated DEPs are involved in 114 biological processes, 68 molecular functions, and 174 cellular components. In 18 DEPs unique to the HFD group, only 14 DEPs were annotated in the GO database. These annotated DEPs are involved in 24 biological processes, 22 molecular functions, and six cellular components. Protein levels of two fatty acid metabolism-related enzymes, carnitine palmitoyltransferase 1B (CPT1B) and acetyl-CoA acyltransferase 2 (ACAA2), in the hearts of the mice in control group and HFD group were analyzed by immunostaining and Western blot. The results showed that the protein levels of CPT1B and ACAA2 were elevated in hearts of the mice in HFD group, which were consistent with the proteomic analysis. Our results reveal the differentially expressed proteome related to the progression of MC, providing a series of potential therapeutic targets for MC.

Keywords: High-fat diet; Metabolic cardiomyopathy; Proteome analysis

1. Introduction

According to the report of The International Diabetes Federation, about 500 million people will become obese and insulin-resistant, while more than 500 million people

†These authors contributed equally to this work.

***Corresponding author:**
Wei Huang
(huangwei0521@126.com)

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will be affected by type 2 diabetes by the year 2040[1]. An increasing number of evidence indicates that patients who suffer from glucometabolic perturbations are more susceptible to metabolic cardiomyopathy (MC), which is independent to the presence of hypertension, coronary artery disease, and other comorbidities[2,3]. Over 80% of patients who suffered from heart failure are either obese or diabetic[4,5]. Notably, the prevalence of MC is expected to rise in the next decades. In patients with glucometabolic perturbations, the accumulation of lipotoxic by-products in cardiac myocyte could lead to myocyte apoptosis and contractile dysfunction, eventually resulting in MC. Detrimental effects on cardiomyocytes in patients with obesity and type 2 diabetes include changes in tissue metabolism, substrate utilization, inflammation, and oxidative stress. These effects are thought to induce heart failure[6-9]. However, the underlying mechanisms remain unclear and therapies of MC are yet to be developed.[2]

The molecular mechanisms underlying MC are mainly based on metabolic dysregulation, inflammation, fibrosis, oxidative stress, and apoptosis. Although free fatty acids are the preferred energy substrate for cardiac cells, alternative fuel sources, such as glucose, lactate, or ketone bodies, are also ultilized by the heart to balance the energy supply and by-products overproduction. In obese or diabetic people, mitochondrial fatty acid β-oxidation is increased due to the hyperglycemia and insulin resistance, which is regulated by the PPAR family-mediated transcriptional machinery. For example, the down-regulation of GLUT4 reduces the uptake of glucose in the cardic cells[10]. Peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, estrogen-related receptor alpha, NFE2 like BZIP transcription factor 2 (NFE2L2), or nuclear respiratory factor 1 (NRF1) and nuclear factor erythroid 2-related factor 2 (NRF2), which are up-regulated in obesity and diabetes, could promote fatty acid oxidation and shut down glucose oxidation[11,12]. It is well known that the increased fatty acid oxidation will lead to lipotoxicity, which subsequently activates the proinflammatory transcription factor such as nuclear factor kappa B (NF-κB). NF-κB could increase the downstream targets, such as activator protein 1, nuclear factor of activated T-cells, or NF-κB itself, which carry out numerous autocrine activities, including the secretion of cytokines and chemokines[13]. Increased inflammation finally impairs myocardial tissues and causes cardiac remodeling by interstitial fibrosis. In addition, increase fatty acid β-oxidation-induced oxidative stress stimulates the proinflammatory transcription factors and the activation of mitogen-activated protein kinase, involving the proapoptotic c-Jun N-terminal kinase and p38, which promotes cell death in MC[14].

In the present study, we aimed to identify the key differentially expressed proteins (DEPs) and pathways involved in high-fat diet (HFD)-induced MC by analyzing the proteome of hearts from the indicated mice. We compared the DEPs between samples from control and HFD groups. Interestingly, we identified 90 proteins which were only detected in the control group and 18 proteins which were only detected in the HFD group. In these DEPs, most of them belong to the metabolic related process. Our results revealed the differentially expressed proteome related to the progression of MC, which could be a potential therapeutic target for MC.

2. Materials and methods

2.1. Mice

Eight-week-old male wild-type mice (Chongqing Tengxin Biotechnology Co., Ltd) were fed a standard diet and HFD (60% kcal fat; Research Diets, New Brunswick, NJ, USA), respectively, for 5 months in a specific pathogen-free environment provided by the Experimental Animal Center of Southwest Medical University. The mice were separated into two groups, the control group and the HFD group. The mice were sacrificed to collect the hearts, and all the heart tissue were immediately labeled and stored at −80°C until protein extraction. Animal protocols were approved by Institutional Animals Ethics Committees of Southwest Medical University (Approval No. 20220225-014).

2.2. Preparation of samples for liquid chromatography-tandem mass spectrometry (LC-MS/MS)

To prepare samples for LC-MS/MS, we performed an in-solution digestion by trypsin; the protocol is as follows: (i) 8 M urea was added to 300 μg lysates; (ii) proteins were reduced with 5 mM dithiothreitol and incubated for 45 min at 56°C to reduce disulfide bonds; (iii) mixture was cooled to room temperature and alkylated with iodoacetamide to a final concentration of 20 mM; (iv) the mixture was incubated for 30 min in the dark at room temperature; (v) the mixture was diluted 8-fold with 1 M urea using 10 mM triethylammonium bicarbonate and subsequently digested using 1:20 (w/w) trypsin at 37°C overnight; and (vi) Oasis HLB Cartridge 30 mg (Waters Corporation, Milford, MA, USA) was used to desalt the tryptic digests, and it was lyophilized for the subsequent MS/MS analysis.

2.3. Data processing and parameters

LC-MS/MS was used for analysis for the six sets of digested peptides. The dried peptides were re-dissolved in 30 μL 0.1% formic acid in UHQ water. The nano-LC-MS experiments were performed using AB Sciex 5600+ mass spectrometer. The sample was applied onto a high-performance liquid

chromatography system (Sciex Co.). The peptides were concentrated on a 1.0-cm precolumn (75-μm inner diameter, 360-μm outer diameter, C18 5 μm, Sciex). The peptides were eluted from the precolumn using a gradient from 100% phase A (0.1% fatty acid aqueous solution) to 45% phase B (0.1% fatty acid, 100% acetonitrile) in 75 min at 300 nL/min directly onto an 15-cm analytical column (75-μm inner diameter, 360-μm outer diameter, ReproSil-Pur C18 3 μm, Sciex). The instrument was operated in a data-dependent mode automatically. Three biological replicates were prepared for each sample using a described parameters (2500V+).

2.4. Data processing and assembly

Raw files from LC-MS were searched using the Mascot search engine human database 3.78 for data processing. Parameters were adjusted for: (i) trypsin digestion, with two maximum missed cleavage points permitted; (ii) length of the digested peptide: 6 – 25; (iii) precursor mass tolerance of 10 ppm and fragment mass tolerance adjusted to 0.2 Da; (iv) dynamic variation oxidation of methionine: static modification carbamidomethyl of cysteine was selected; and (v) false discovery rate: < 1. The level of peptide confidence for the data filter was adjusted to "high."

2.5. Analysis of DEPs by GO and Kyoto Encyclopedia of Genes and Genomes (KEGG)

Gene ontology (GO) analysis was used to evaluate the biological function of DEPs. Pathways analysis was carried out to further evaluate metabolic or signal transduction pathways using the online PANTHER tools (version 15.0) (http://pantherdb.org/invalid Request.jsp).

2.6. Immunostaining

The myocardial tissues were fixed with 4% paraformaldehyde, and then dehydrated and embedded in paraffin. The hearts were cut into slices with a thickness of 4 μm and incubated overnight in a thermostat at 37°C. Then, the slices were put into xylene and alcohol (in a gradient of concentration) for dewaxing. After that, the slices were blocked with 5% bovine serum albumin. After being gently washed with phosphate-buffered saline, the cells were incubated with the primary antibodies, including carnitine palmitoyltransferase 1B (CPT1B) (1:100) and acetyl-CoA acyltransferase 2 (ACAA2) (1:100), overnight at 4°C, and then incubated with secondary antibodies conjugated with cyanine dye 3 (Cy3) for 1 h at room temperature. 4',6-diamidino-2-phenylindole (DAPI) was used for nuclear staining. Finally, the cells were observed under a confocal microscope (Leica, Germany).

2.7. Western blot

Total proteins from cells or tissue were lysed using RIPA buffer, and the protein concentration in the cell lysates was assayed by a protein assay dye reagent concentrate (Bio-Rad, USA). Samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes (pore size 0.45 μm). After being blocked with 5% bovine serum albumin for 1 h, the membranes were incubated with primary antibodies, including CPT1B (1:1000), ACAA2 (1:1000), and tubulin (1:5000) at 4°C overnight. After being washed with TBST, the membranes were incubated with secondary antibodies for 1 h at room temperature. Finally, the protein bands were visualized by enhanced chemiluminescence kit (Santa Cruz, Texas, USA).

3. Results

3.1. HFD-induced diabetic cardiomyopathy

Given that over 80% of patients with MC are either obese or diabetic, we employed diet-induced obesity to investigate the differential expressed proteins in the heart tissues between normal heart and mice with MC, which was induced by HFD. The experimental procedure is shown in Figure 1A.

In line with other reports,[15] the body weight of the mice in the HFD group almost doubled, and their

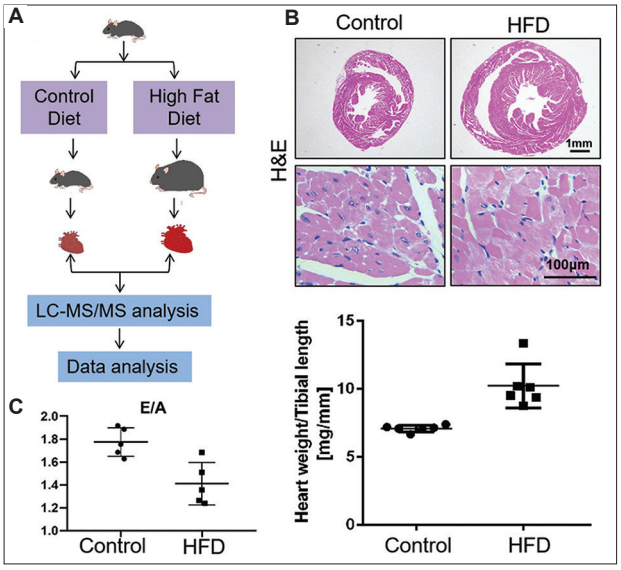

Figure 1. High-fat diet (HFD)-induced diabetic cardiomyopathy. (A) Experimental procedure chart for this study. (B) Immunohistochemistry showed an increase of heart volume, cardiac myocyte hypertrophy and an increase of heart weight in HFD-fed mice. (C) Echocardiographic measurement of the diastolic function in standard diet-fed mice or HFD-fed mice.

blood glucose levels also increased after being fed 60% kcal HFD for 5 months. Furthermore, the HFD group with hypertrophic cardiomyocytes had larger and heavier hearts than the control group (Figure 1B). Echocardiographic measurement showed that the diastolic function of hearts from HFD-fed mice were impaired (Figure 1C).

3.2. Identification of DEPs

An online tool Venny (Version 2.1) was used to identify DEPs in the hearts of standard diet-fed mice (the control group) and HFD-fed mice (the HFD group). Venn diagrams of data from the control group showed the overlap of identified proteins expressed in Control-1, Control-2, and Control-3. A total of 228 proteins were present in the triplicated control sample (Figure 2A). Venn diagrams of data from the HFD group showed the overlap of identified proteins expressed in HFD-1, HFD-2, and HFD-3. A total of 156 proteins were present in triplicated HFD samples (Figure 2B). A total of 138 common proteins were present in both control and HFD groups in the 3 sets of overlap. A total of 90 proteins were detected in the control group but not in the HFD group. However, 18 proteins were only detected in the HFD group but not in the control group (Figure 2C).

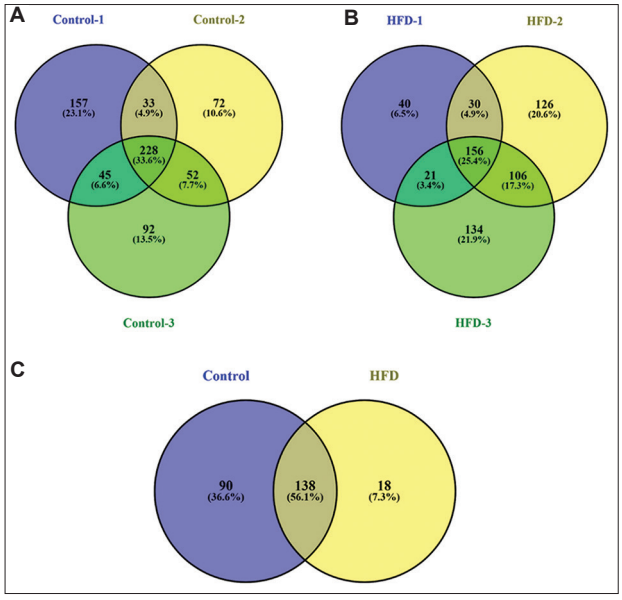

Figure 2. Differentially expressed proteins identified in hearts from standard diet-fed mice and high-fat diet (HFD)-fed mice. (A) Venn diagrams show the overlap of identified proteins in triplicate, which were expressed in Control-1, Control-2 and Control-3. A total of 228 proteins were present in triplicated control sample. (B) Venn diagrams show the overlap of identified proteins in triplicate, which were expressed in HFD-1, HFD-2 and HFD-3. A total of 156 proteins were present in triplicated HFD samples. (C) A total of 138 common proteins were present in both control group and HFD groups of the three sets of overlap; 90 proteins were unique to the control group and 18 proteins were unique to the HFD group.

3.3. GO analysis of control group-specific DEPs

To explore the function of DEPs in the heart tissue of wild-type and diabetic mice, we performed GO enrichment analysis for these two groups in the Blast2go (Version 2.5) program and the GO database. As shown in Figure 3, only 74 DEPs were annotated in the GO database among 90 HFD group-specific DEPs. These annotated DEPs are involved in 114 biological processes (Figure 3A), 68 molecular functions (Figure 3B), and 174 cellular components (Figure 3C). In the biological process, 42 DEPs are involved in the clusters of cellular process, and 28 DEPs are involved in the metabolic process (that account for 70/74 in total). The results indicate that HFD reduces proteins associated with the metabolic process in cardiac myocytes.

3.4. KEGG analysis of control group-specific DEPs

KEGG is an encyclopedia of genes and genomes that can be used to analyze gene function. It provides a valuable classification for understanding the complex biological functions of genes and genome[16]. Therefore, we graphically presented the results of the KEGG annotation analysis to analyze the distribution of DEPs in various pathways. As shown in Figure 4, these annotated DEPs are involved in 51 protein classes (Figure 4A) and 33 pathways (Figure 4B). Furthermore, most DEPs (26 proteins) belong to the cluster of metabolite interconversion enzymes. In the KEGG pathway, the clusters of "cytoskeletal regulation by Rho GTPase," "glycolysis," and "gonadotropin-releasing hormone receptor pathway" were the top three pathways that include 3 DEPs, respectively.

3.5. GO analysis of HFD group-specific DEPs

As shown in Figure 3, only 14 DEPs are annotated in the GO database in the 18 HFD group-specific DEPs. These annotated DEPs are involved in 24 biological processes (Figure 5A), 22 molecular functions (Figure 5B), and six cellular components (Figure 5C). In the biological process, 20 DEPs are involved in the clusters of cellular processes and 11 DEPs are involved in the metabolic process. The result is consistent with that in control group-specific DEPs, indicating that HFD activates proteins associated with the metabolic process in cardiomyocytes.

3.6. KEGG analysis of HFD group-specific DEPs

As shown in Figure 6, these annotated DEPs are involved in nine protein classes (Figure 6A) and seven pathways

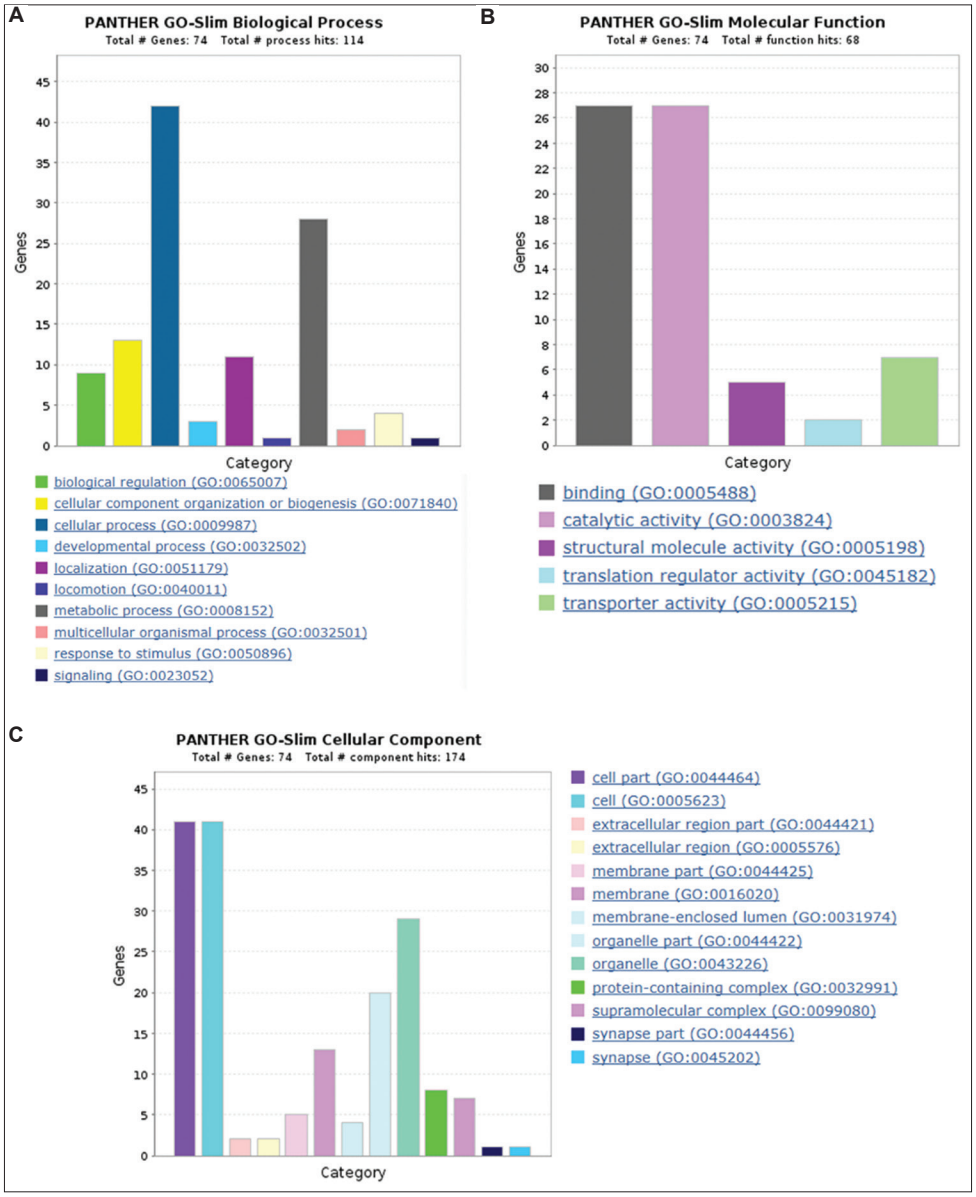

Figure 3. Gene ontology analysis of control group-specific differentially expressed proteins. Annotated differentially expressed proteins were distributed in 114 biological processes (A), 68 molecular functions (B), and 174 cellular components (C).

(Figure 6B). Most DEPs are involved in the cluster of metabolite interconversion enzymes (five proteins). The result is consistent with that in control group-specific DEPs. In the KEGG pathway, 7 DEPs were distributed in seven pathways.

3.7. Elevation of fatty acid metabolism-related enzymes in the hearts of HFD-fed mice

Since the cluster of metabolite interconversion enzymes has the most DEPs (5 proteins) and fatty acid metabolism plays critical roles in the MC, protein levels of two fatty acid metabolism-related DEPs, CPT1B and ACAA2, were analyzed by immunostaining and Western blot. The levels of both CPT1B and ACAA2 increased in the hearts of HFD-fed mice (Figures 7 and 8). The result is consistent with the previous proteomic analysis.

4. Discussion

Heart failure is the leading cause of MC[17]. Many complex molecular mechanisms are reportedly play a role in the pathogenic process of MC. Despite that, the changes of proteome in MC remain unknown. In this study, we used LC-MS/MS to observe the significant difference in proteome between standard diet-fed

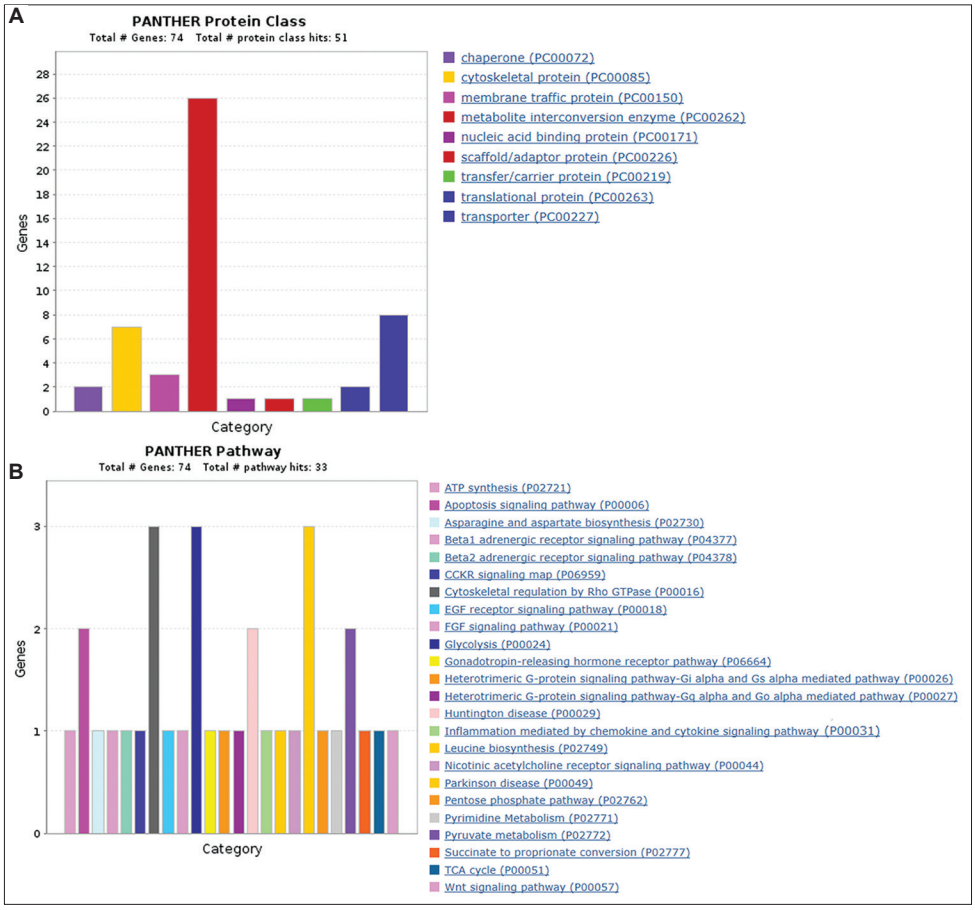

Figure 4. Kyoto Encyclopedia of Genes and Genomes analysis of control group-specific differentially expressed proteins. Annotated differentially expressed proteins were distributed in 51 protein classes (A) and 33 pathways (B).

mice and HFD-fed mice. The KEGG analysis of DEGs between standard diet-fed mice and HFD-fed mice showed that, irrespective of control group and HFD group, metabolite interconversion enzyme is the most significant protein. CPT1B and ACAA2 were, further, identified using Western blot and immunostaining. The results demonstrate that the altered expression of metabolite interconversion enzymes is related to MC. As the key enzymes in this pathway, CPT1B and ACAA2 may be potential targets for the treatment of MC.

In the GO enrichment analysis, we found the largest cluster changes in cellular and metabolic processes in both control or HFD group-specific DEPs (Figure 3A and 5A). Among differentially expressed genes (DEGs) involved in cellular and metabolic processes, two succinate dehydrogenase (SDH) family members[18], SDHA and SDHB, led to different expression levels in control and HFD group-specific DEPs. We detected SDHA only in the heart tissue from HFD-fed mice, whereas the SDHB was observed only in the heart tissue from standard diet-fed mice. The mutation of the *SDHA* gene is related to paraganglioma[19]. The *SDHB* mutation can result in pheochromocytoma/paraganglioma syndrome type 4[20]. Renal cell carcinoma is a metabolic disease caused by several genes, including *SDH*. The SDH participates in essential cellular processes regulating cell response to sense iron, oxygen, energy, and nutrients[21]. Therefore, SDHA and SDHB might play different roles in the progression of MC. We also found that peroxiredoxin V (PRDX5) was absent in the HFD group, in addition to mitochondria-related proteins. PRDX5 is a member of the family of mammalian proteins that neutralize reactive oxygen species[22], which plays a protective role during the early period of small-for-size syndrome in liver transplantation in rats[23]. The results suggest that the HFD may lead to the deficiency of protective factors, such as PRDX5, in heart tissue.

The expression data from the KEGG database were analyzed to further identify the differential pathways.

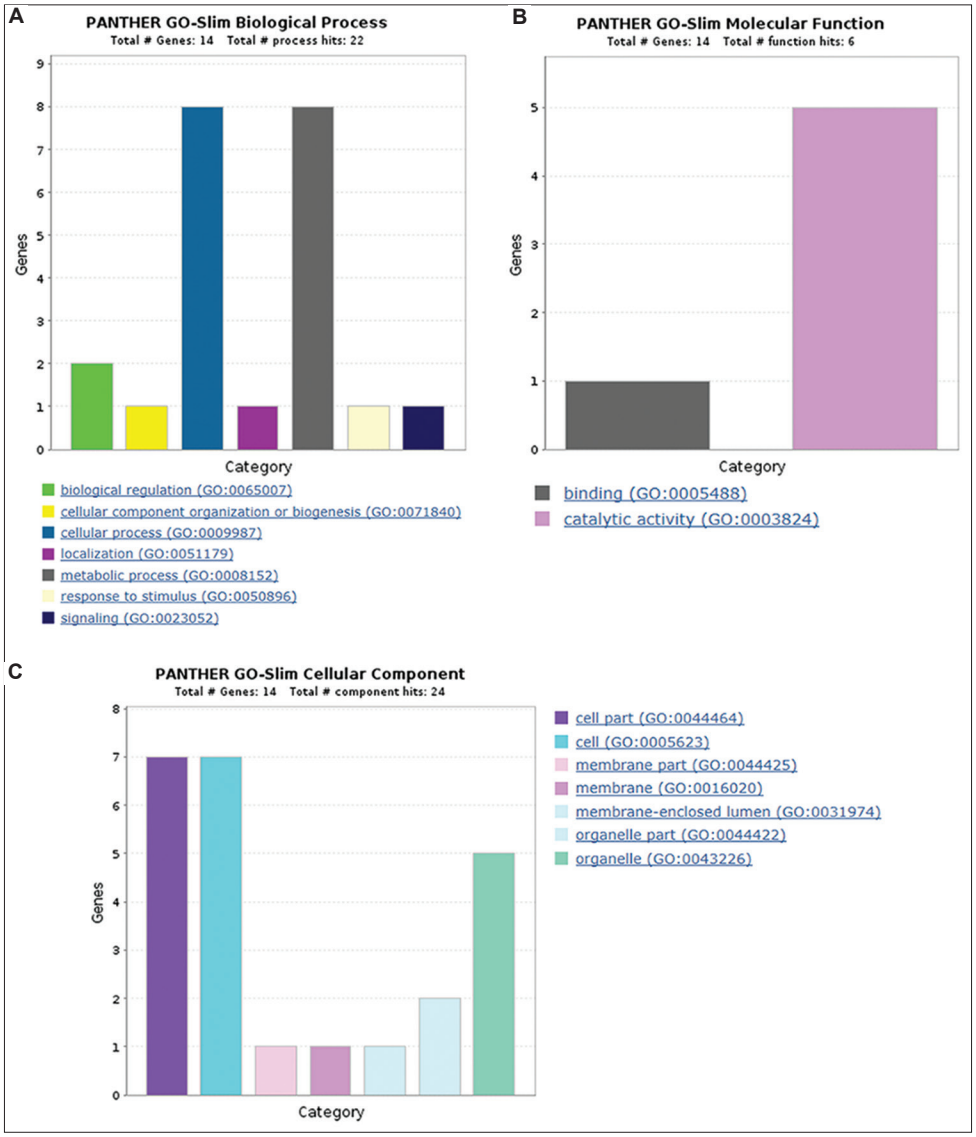

Figure 5. Gene ontology analysis of high-fat diet group-specific differentially expressed proteins. Annotated differentially expressed proteins were distributed in 24 biological processes (A), 22 molecular functions (B), and six cellular components (C).

We found that the "metabolite interconversion enzyme" pathway was the most significant in control or HFD group-specific DEPs (Figure 4A and 6A). In addition, two fatty acid metabolism-related DEPs, CPT1B and ACAA2, were only detected in HFD group-specific DEPs by Western blot and immunostaining. CPT1b is a fatty acid metabolism factor that regulates cardiac hypertrophy[24]. The deficiency of CPT1B can aggravate cardiac hypertrophy in lipotoxic cardiomyopathy caused by pressure overload[25]. In this study, the protein level of CPT1B increased in the hearts of HFD-fed mice, indicating that the CPT1B adaptively increased under lipotoxicity. ACAA2, an enzyme of the thiolase family, is involved in mitochondrial fatty acid elongation and degradation by catalyzing the last step of the respective β-oxidation pathway[26]. Overexpression of ACAA2 and HSD17B12 can inhibit triglyceride production and cell proliferation and induce apoptosis in mammary epithelial cells. In this study, we found that the protein level of ACAA2 increased in the hearts of HFD-fed mice, indicating that the lipotoxicity induced ACAA2 to promote apoptosis.

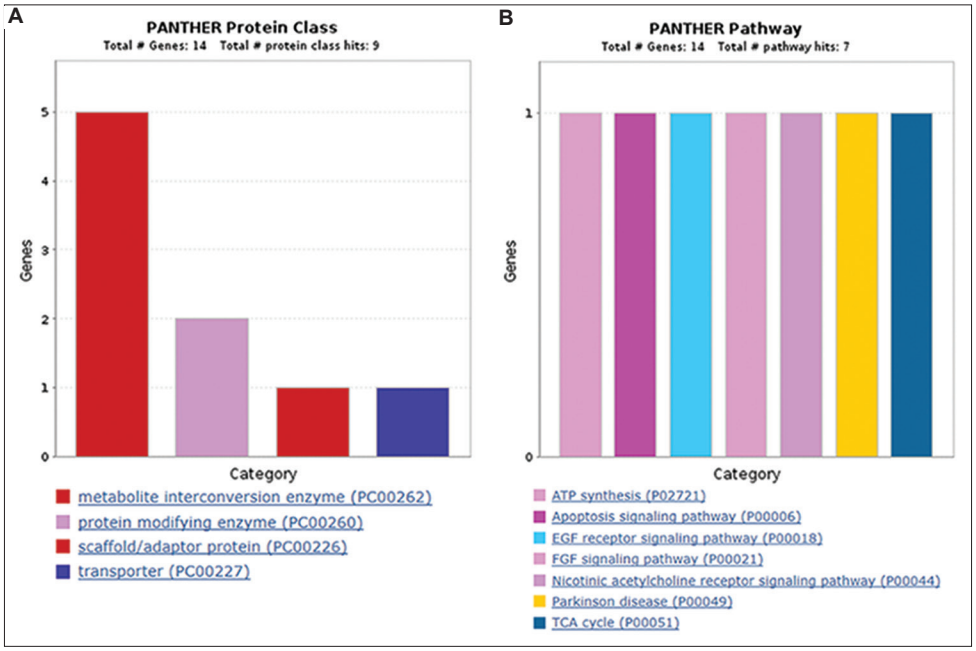

Figure 6. Kyoto Encyclopedia of Genes and Genomes analysis of high-fat diet group-specific differentially expressed proteins. Annotated differentially expressed proteins were distributed in nine protein classes (A) and seven pathways (B).

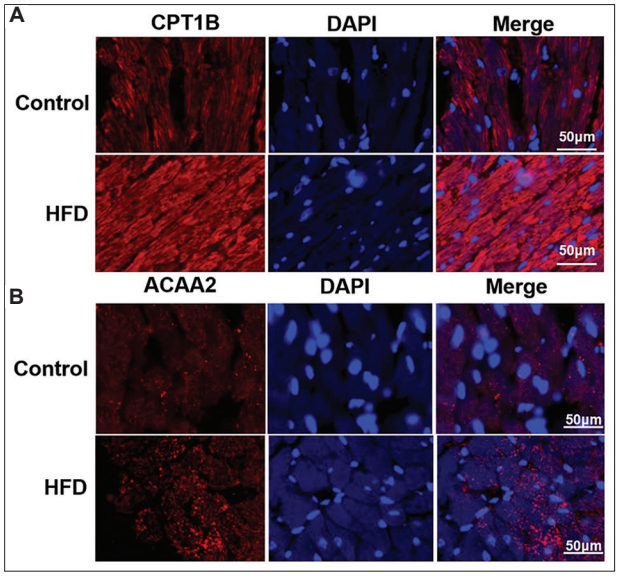

Figure 7. Immunostaining of CPT1B and ACAA2. CPT1B (A) and ACAA2 (B) in cardiomyocytes from indicated mice were detected by double labeling immunofluorescence. CPT1B and ACAA2 were labeled by corresponding antibodies (red) and DAPI labeled nucleus (blue).

5. Conclusion

Following the comparison of DEPs between samples from the control and HFD groups, we revealed novel DEPs between normal and MC mice. This finding provided essential clues for understanding the specific pathogenetic mechanisms and potential treatment alternatives for MC. The specific molecular mechanism of metabolic dysfunction in MC needs to be further studied.

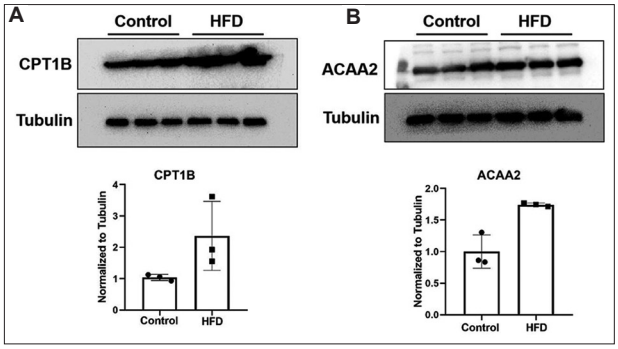

Figure 8. Western blot analysis of CPT1B and ACAA2. CPT1B (A) and ACAA2 (B) in cardiomyocytes from indicated mice were detected by Western blot. CPT1B and ACAA2 were incubated with corresponding antibodies and tubulin, which was used as reference. Quantification was performed by imageJ.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author contributions

Conceptualization: Wei Huang
Investigation: Zongzhe Jiang, Mingyang Pang
Formal analysis: Wei Huang
Writing-original draft: Zongzhe Jiang, Mingyang Pang
Writing-review and editing: Huang Wei

Ethics approval and consent to participate

Animal experiments were approved by the Institutional Animals Ethics Committees of Southwest Medical University (Approval No. 20220225-014). Consent to participate is not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets used and analyzed in present study can be obtained from the corresponding author on reasonable request.

References

1. Ogurtsova K, Da Rocha Fernandes JD, Huang Y, *et al.*, 2017, IDF diabetes atlas: global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res. Clin. Pract*, 128: 40–50.

   https://doi.org/10.1016/j.diabres.2017.03.024

2. Nishida K, Otsu K, 2017, Inflammation and metabolic cardiomyopathy. *Cardiovasc Res*, 113: 389–398.

   https://doi.org/10.1093/cvr/cvx012

3. Maack C, Murphy E, 2017, Metabolic cardiomyopathies—fighting the next epidemic. *Cardiovasc Res*, 113: 367–369.

   https://doi.org/10.1093/cvr/cvx022

4. Obokata M, Reddy YNV, Pislaru SV, *et al.*, 2017, Evidence supporting the existence of a distinct obese phenotype of heart failure with preserved ejection fraction., *Circulation*, 136: 6–19.

   https://doi.org/10.1161/CIRCULATIONAHA.116.026807

5. Ponikowski P, Voors AA, Anker SD, *et al.*, 2016, 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*, 37: 2129–2200.

   https://doi.org/10.1093/eurheartj/ehw128

6. Isfort M, Stevens SC, Schaffer S, *et al.*, 2014, Metabolic dysfunction in dia betic cardiomyopathy. *Heart Fail Rev*, 19: 35–48.

   https://doi.org/10.1007/s10741-013-9377-8

7. Seferovic PM, Paulus WJ, 2015, Clinical diabetic cardiomyopathy: A two-faced disease with restrictive and dilated phenotypes. *Eur Heart J*, 36: 1718–1727, 1727a–1727c.

   https://doi.org/10.1093/eurheartj/ehv134

8. Authors/Task Force Members, Ryden L, Grant PJ, *et al.*, 2013, ESC guidelines on diabetes, prediabetes, and cardiovascular diseases developed in collaboration with the EASD: the task force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European association for the study of Diabetes (EASD). *Eur Heart J*, 34: 3035–3087.

   https://doi.org/10.1093/eurheartj/eht108

9. Schulze PC, Drosatos K, Goldberg IJ, 2016, Lipid use and misuse by the heart. *Circ Res*, 118: 1736–1751.

   https://doi.org/10.1161/CIRCRESAHA.116.306842

10. Gibbs EM, Stock JL, McCoid SC, *et al.*, 1995, Glycemic improvement in diabetic db/db mice by overexpression of the human insulinregulatable glucose transporter (GLUT4). *J Clin Invest*, 95: 1512–1218.

    https://doi.org/10.1172/JCI117823

11. Niture SK, Khatri R, Jaiswal AK, 2014, Regulation of Nrf2-an update. *Free Radic Biol Med*, 66: 36–44.

    https://doi.org/10.1016/j.freeradbiomed.2013.02.008

12. Tan Y, Ichikawa T, Li J, *et al.*, 2011, Diabetic downregulation of Nrf2 activity via ERK contributes to oxidative stress-induced insulin resistance in cardiac cells *in vitro* and *in vivo*. *Diabetes*, 60: 625–633.

    https://doi.org/10.2337/db10-1164

13. Jia G, DeMarco VG, Sowers JR, 2016, Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol*, 12: 144–53.

    https://doi.org/10.1038/nrendo.2015.216

14. Wang Y, Zhou S, Sun W, *et al.*, 2014, Inhibition of JNK by novel curcumin analog C66 prevents diabetic cardiomyopathy with a preservation of cardiac metallothionein expression. Am J Physiol Endocrinol Metab, 306: E1239–E1247.

    https://doi.org/10.1152/ajpendo.00629.2013

15. Winzell MS, Ahren B, 2004, The high-fat diet-fed mouse: A model for studying mecha-nisms and treatment of impaired glucose tolerance and Type 2 diabetes. *Diabetes*, 53: S215–S219.

https://doi.org/10.2337/diabetes.53.suppl_3.s215

16. Kanehisa M, Furumichi M, Tanabe M, *et al*., 2017, KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res*, 45(D1): D353–D61.

https://doi.org/10.1093/nar/gkw1092

17. Costantino S, Akhmedov A, Melina G, *et al*., 2019, Obesity-induced activation of JunD promotes myocardial lipid accumulation and metabolic cardiomyopathy. *Eur Heart J*, 40: 997–1008.

https://doi.org/10.1093/eurheartj/ehy903

18. Bardella C, Pollard P, Tomlinson I, 2011, SDH mutations in cancer. *Biochim Biophys Acta*, 1807: 1432–1443.

https://doi.org/10.1016/j.bbabio.2011.07.003

19. Burnichon N, Briere JJ, Libe R, *et al*., 2010, Tissier, Sdha is a tumor suppressor gene causing paraganglioma. *Human Mol Genet*, 19: 3011–3020.

https://doi.org/10.1093/hmg/ddq206

20. Gill AJ, Pachter NS, Chou A, *et al*., 2011, Tucker, renal tumors associated with germline sdhb mutation show distinctive morphology. *Am J Surg Pathol*, 35: 1578–1585.

https://doi.org/10.1097/PAS.0b013e318227e7f4

21. Ricketts CJ, Shuch B, Vocke CD, *et al*., 2012, Middelton, succinate dehydrogenase kidney cancer: an aggressive example of the warburg effffect in cancer. *J Urol*, 188: 2063–2071.

https://doi.org/10.1016/j.juro.2012.08.030

22. Kropotov A, Usmanova N, Serikov V, *et al*., 2007, Mitochondrial targeting of human peroxiredoxin V protein and regulation of PRDX5 gene expression by nuclear transcription factors controlling biogenesis of mitochondria. *FEBS J*, 274: 5804–5814.

https://doi.org/10.1111/j.1742-4658.2007.06103.x

23. Wu J, Tang Q, Shen J, *et al*., 2010, Comparative proteome profile during the early period of small-for-size liver transplantation in rats revealed the protective role of Prdx5. *J Hepatol*, 53: 73–83.

https://doi.org/10.1016/j.jhep.2010.01.032

24. Sun Y, Fan W, Xue R, 2020, Transcribed ultraconserved regions, Uc.323, ameliorates cardiac hypertrophy by regulating the transcription of CPT1b (Carnitine Palmitoyl transferase 1b). *Hypertension*, 75: 79–90.

https://doi.org/10.1161/HYPERTENSIONAHA.119.13173

25. He L, Kim T, Long Q, *et al*., 2012, Carnitine palmitoyltransferase-1b deficiency aggravates pressure overload-induced cardiac hypertrophy caused by lipotoxicity. *Circulation*, 126: 1705–1716.

https://doi.org/10.1161/CIRCULATIONAHA.111.075978

26. Miltiadou D, Hager-Theodorides AL, Symeou S, *et al*., 2017, Variants in the 3' untranslated region of the ovine acetyl-coenzyme a acyltransferase 2 gene are associated with dairy traits and exhibit differential allelic expression. *J Dairy Sci*, 100: 6285–6297.

https://doi.org/10.3168/jds.2016-12326

ACCSCIENCE PUBLISHING

Global Translational Medicine

ORIGINAL RESEARCH ARTICLE

The status of compensated cirrhosis might be negatively associated with the tumor size in patients with hepatitis B virus-related hepatocellular carcinoma

Yanna Liu[1†], Xiangjun Qian[2†], Congying Wu[3], Weidong Pan[2], Jingmin Zhao[4], Xiangmei Chen[1*], and Fengmin Lu[1,5*]

[1]Department of Microbiology & Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, Peking University, Beijing 100191, China

[2]Department of Pancreatic-Hepatobiliary Surgery, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, China

[3]Institute of Systems Biomedicine, Beijing Key Laboratory of Tumor Systems Biology, School of Basic Medical Sciences, Peking University Health Science Center, Peking University, Beijing 100191, China

[4]Department of Pathology and Hepatology, Fifth Medical Center of Chinese PLA General Hospital, Beijing 100039, China

[5]Department of Epidemiology and Biostatistics, College of Public Health, Zhengzhou University, Zhengzhou 450001, China

[†]These authors contributed equally to this work.

Corresponding authors:
Fengmin Lu
(lu.fengmin@hsc.pku.edu.cn)

Xiangmei Chen
(xm_chen6176@bjmu.edu.cn)

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Abstract

Liver cirrhosis has been a well-known risk factor for the development of hepatocellular carcinoma (HCC). However, this view has recently been challenged. This study aimed to investigate the potential association of cirrhosis with hepatitis B virus (HBV)-related HCC. In this study, two independent multicenter clinical cohorts that included 1,431 HCC patients with chronic HBV infection were retrospectively studied. The first cohort consisted of 334 HCC patients undergoing curative resection and cirrhosis, who have been pathologically diagnosed. The second cohort consisted of 1,087 HCC patients, who have been diagnosed for the presence of cirrhosis based on clinical evidence. Patients of each cohort were further divided into different subgroups according to the presence of cirrhosis and the severity of the cirrhosis. In both cohorts, patients with cirrhosis had smaller tumor size compared to those without cirrhosis ($P < 0.05$) and a relatively lower proportion of large tumor, defined as tumor size > 5 cm in diameter ($P < 0.05$). Patients with decompensated cirrhosis had the highest rate of vascular invasion and/or extrahepatic metastases compared with compensated cirrhosis and non-cirrhosis (53.0% vs. 24.8% vs. 26.9%, $P < 0.001$). In the first cohort, globulin (odds ratio [OR] = 1.096, $P = 0.001$) and vascular invasion (OR = 4.013, $P = 0.013$) were independent risk predictors of HCC tumor size >5 cm, while cirrhosis stage Laennec 4B/C was a protective factor (OR = 0.372, $P = 0.002$). Similar results were observed in the second cohort. In conclusion, this study implied that HCC patients with compensated cirrhosis tend to harbor smaller tumor, but severe cirrhosis favors tumor vascular invasion and metastasis.

Keywords: Liver cirrhosis; Hepatocellular carcinoma; Hepatitis B virus; Neoplasm metastasis; Vascular invasion

1. Introduction

Hepatocellular carcinoma (HCC) is a primary liver cancer with an estimated 906,000 newly diagnosed cases and 830,000 deaths yearly, and is ranked the top six most common cancers and the third leading cause of cancer death worldwide in 2020[1]. Liver fibrosis is strongly associated with HCC, while 90% of HCC cases arising in cirrhotic livers[2]. For hepatitis B and C virus infection, the presence of fibrosis and cirrhosis has been identified as risk factors for HCC; and the cancer risk is positively correlated with the fibrosis severity[3,4]. HCC development has also been shown to be linked to alcoholic cirrhosis, nonalcoholic steatohepatitis, and hemochromatosis, with a yearly HCC incidence of 1.7% and 2.6% in alcoholic cirrhosis and non-alcoholic steatohepatitis cirrhosis, respectively[5]. It has been reported that cirrhosis, as a late-stage form of fibrosis, contributes to an over 30-fold increase in HCC risk[6]. Approximately 80% of hepatitis B and C patients presented with HCC are already cirrhotic[7], and liver cirrhosis occurs in about 80% of HCC patients, indicating that liver cirrhosis is the major risk factor for the development of HCC[8].

Recently, there has been emerging opinions suggesting that regenerative nodules (RNs) and fibrosis either exert physical forces to spatially restrict malignant hepatocytes or activate immunosurveillance to suppress the development of HCC[9] and "premalignant mutations" found in the RNs were independent of carcinogenesis associated with HCC development. As RNs are surrounded by fibrotic septa, the fibrosis is postulated to act as a mechanical "fence" to constrain the transformation or spatially limit the spread of cancer cells[10]. Apparently, such a novel view on the cirrhosis as a liver-protective response to various injuries, rather than a risk factor for HCC[9], has challenged the conventional view.

Few studies have been carried out to clarify the controversies on the role of cirrhosis in HCC development. Herein, this study aimed to investigate the potential association of cirrhosis with hepatitis B virus (HBV)-related HCC in two independent cohorts.

2. Materials and methods

2.1. Study subjects

Two retrospective cohorts were used in this study. The first cohort consisted of adult patients with HCC undergoing curative resection in the Fifth Medical Center of Chinese PLA General Hospital (Beijing, China) between 2015 and 2017. The inclusion criteria were: (i) Age ≥18 years; (ii) positive for hepatitis B surface antigen for at least 6 months; (iii) having complete information on laboratory data and clinical characteristics of the tumors; and (iv) pathologically confirmed for HCC and liver cirrhosis. Meanwhile, the exclusion criteria were: (i) liver disease due to co-infection with hepatitis C virus or other hepatitis, genetic and autoimmune disorders, primary biliary cirrhosis, and sclerosing cholangitis; (ii) had prior treatment for HCC or have had anti-HBV treatment within 6 months before prior to HCC diagnosis; (iii) having other malignant tumors; and (iv) being pregnant. In the first cohort, the METAVIR scoring system was used to evaluate the hepatic fibrosis stage, and the severity of liver cirrhosis was histologically staged as 4A, 4B, and 4C using the Laennec staging system, according to the size of regenerated nodules and the width of fibrous septa.

To further validate the association of the severity of liver cirrhosis with the tumor size, we included another independent cohort, in which the study subjects were recruited from four university hospitals (The Fifth Medical Center of Chinese PLA General Hospital, Beijing; The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou; Peking University Shenzhen Hospital, Shenzhen; The Third Hospital of Hebei Medical University, Shijiazhuang) in China from 2010 to 2018. The subjects in this cohort were recruited using the above-mentioned inclusion and exclusion criteria. In this cohort, HCC were histopathologically and/or clinically diagnosed, while cirrhosis was diagnosed by a combination of clinical, laboratory, and imaging approaches. This study was approved by the ethics committee of Peking University Health Science Center (IRB00001052-19081) and conducted according to the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Statistical analysis

Statistical analysis was performed by SPSS 24.0 software (IBM SPSS Statistics, New York, USA). Continuous variables are expressed as mean ± standard deviation or median and interquartile range, and qualitative variables are expressed as number and percentage (%). The *t*-test, Mann-Whitney *U* test, one-way analysis of variance, or Chi-square test were used to evaluate the differences between groups, as appropriate. Factors that are possibly associated with the tumor size were analyzed using logistic regression analysis. A forward selection method was used in the multivariate analysis. $P < 0.05$ is considered statistically significant.

3. Results

3.1. Patient characteristics

A total number of 1431 patients diagnosed with HCC were included in this study, with 334 and 1087 patients from the

first and second cohorts, respectively. In the first cohort, the mean age of the patients was 51.40 ± 9.62 years, with 283 (84.7%) males and 51 females (15.3%), and 276 (82.6%) patients had compensated cirrhosis, while the rest of the patients did not show the presence of cirrhosis. In the second cohort, there were 946 male and 141 female patients, the mean age of the patients was 51.81 ± 11.04, and 80.5% (n = 894) patients showed the presence of cirrhosis. The baseline characteristics of the two cohorts are summarized in Table 1 and 2, respectively, and the study enrollment and analysis flow chart are shown in Figure 1.

3.2. Tumor size in patients with different severity of cirrhosis

In the first cohort, patients with cirrhosis had smaller tumor size compared to those without cirrhosis (4.5 cm vs. 5.8 cm, P = 0.015). In addition, a decline trend in tumor size was observed as the severity of cirrhosis increased from no cirrhosis to Laennec stage 4A and 4B/C (5.8 cm vs. 5.0 cm vs. 4.5 cm, P = 0.020). The proportions of patients with tumor size >5 cm among the three subgroups, that is, HCC without cirrhosis, HCC with Laennec stage 4A, and HCC with Laennec stage 4B/C were 58.6%, 46.3%, and 38.8%, respectively (P = 0.025) (Table 1).

The tumor characteristics were analyzed in the second cohorts in patients with different severity of cirrhosis. The results showed that patients with cirrhosis had smaller tumor size, compared to those without cirrhosis (5.9 cm vs. 7.9 cm, P < 0.001). Besides, they had a relatively lower proportion of large tumor, which was defined as tumor size >5 cm in diameter (493/894 [54.9%] vs. 122/193 [63.2%], P = 0.035). Patients with cirrhosis were further categorized into compensated and decompensated cirrhosis groups. Among the three subgroups (non-cirrhosis, compensated cirrhosis, and decompensated cirrhosis), patients with compensated cirrhosis had the smallest tumor size (5.4 cm), and the proportion of patients with tumor size >5 cm were 286 over 524 (54.6%, P < 0.001), while patients without cirrhosis had the largest tumor size (7.9 cm), and the proportion of patients with tumor >5 cm were 122 over 193 (63.2%, P < 0.001) (Table 3).

Table 1. Comparison of the characteristics of tumor in different pathological stages of cirrhosis

Variables	HCC without cirrhosis (n=58)	HCC with Laennec 4A (n=80)	HCC with Laennec 4B/C (n=196)	P-value
Age (year)	51.71±9.68	51.21±10.12	51.39±9.43	0.956
Male, n (%)	50 (86.2)	66 (82.5)	167 (85.2)	0.803
Alanine transaminase (U/L)	34.0 (21.0, 48.0)	33.0 (21.0, 48.0)	37.0 (25.3, 58.5)	0.067
Aspartate aminotransferase (U/L)	36.9 (26.0, 56.3)	35.1 (26.3, 44.8)	34.7 (28.0, 54.0)	0.927
Albumin (g/L)	40.0 (38.0, 43.0)	39.0 (37.3, 42.0)	39.0 (36.0, 42.0)	0.022
Globulin (g/L)	27.0 (24.8, 30.0)	28.0 (25.0, 31.0)	28.0 (25.0, 31.0)	0.391
Total Bilirubin (umol/L)	13.3 (10.7, 17.8)	12.3 (9.5, 16.7)	14.6 (11.3, 18.4)	0.038
Tumor size* (cm)	5.8 (3.5, 10.0)	5.0 (3.0, 8.0)	4.5 (3.0, 6.5)	0.020
Distribution of tumor size*, n (%)				0.025
≤5 cm	24 (41.4)	43 (53.8)	120 (61.2)	-
>5 cm	34 (58.6)	37 (46.3)	76 (38.8)	-
Number of tumors, n (%)				0.397
1	53 (91.4)	73 (91.3)	169 (86.2)	
2 – 3	5 (8.6)	4 (5.0)	19 (9.7)	
>3	0	3 (3.8)	8 (4.1)	
Vascular invasion, n (%)				0.112
Yes	3 (5.2)	1 (1.3)	15 (7.7)	-
No	55 (94.8)	79 (98.8)	181 (92.3)	-
Degree of pathological differentiation, n (%)				0.566
High	2 (3.4)	0 (0)	3 (1.5)	-
Middle	54 (93.1)	78 (97.5)	186 (94.9)	-
Poor	2 (3.4)	2 (2.5)	7 (3.6)	-

Age is expressed as mean±standard deviation, and tumor size is expressed as median and interquartile range. *Tumor size indicates the maximum diameter of the tumor. HCC: Hepatocellular carcinoma

Table 2. Comparison of the characteristics of tumor between HCC patients with and without cirrhosis

Variables	HCC without cirrhosis (n=193)	HCC with cirrhosis (n=894)	*P*-value
Age (year)	50.35±12.39	52.12±10.70	0.066
Male, *n* (%)	170 (88.1)	776 (86.8)	0.631
Alanine transaminase (U/L)	39.0 (26.0, 65.5)	44.0 (30.0, 72.0)	0.003
Aspartate aminotransferase (U/L)	47.0 (29.0, 81.7)	54.0 (35.0, 95.0)	0.015
Albumin (g/L)	39.0 (35.0, 42.1)	37.0 (32.2, 40.0)	<0.001
Globulin (g/L)	28.7 (26.0, 32.7)	30.0 (26.0, 34.0)	0.159
Total Bilirubin (umol/L)	15.9 (11.7, 20.4)	18.4 (12.9, 28.9)	<0.001
Tumor size* (cm)	7.9 (4.0, 11.9)	5.9 (3.0, 10.0)	<0.001
Distribution of tumor size*, *n* (%)			0.035
≤5 cm	71 (36.8)	403 (45.1)	-
>5 cm	122 (63.2)	491 (54.9)	-
Number of tumors, *n* (%)			0.195
1	143 (74.1)	646 (72.3)	-
2 – 3	12 (6.2)	92 (10.3)	-
>3	38 (19.7)	156 (17.4)	-
Vascular invasion, *n* (%)			0.004
Yes	45 (23.3)	305 (34.1)	-
No	148 (76.7)	589 (65.9)	-
Vascular invasion or extrahepatic metastases, *n* (%)			0.012
Yes	52 (26.9)	326 (36.5)	-
No	141 (73.1)	568 (63.5)	-

Age is expressed as mean±standard deviation, and tumor size is expressed as median and interquartile range. *Tumor size indicates the maximum diameter of the tumor. HCC: Hepatocellular carcinoma

3.3. Vascular invasion and extrahepatic metastases in patients with different severity of cirrhosis

We also analyzed the rate of vascular invasion and extrahepatic metastases in these patients. As a result, in the first cohort, there were no differences in the rate of tumor vascular invasion among HCC patients without cirrhosis, with cirrhosis + Laennec stage 4A, and with cirrhosis + Laennec stage 4B/C (5.2% vs. 1.3% vs. 7.7%, $P = 0.112$). In the second cohort, a significantly higher rate of vascular invasion of HCC (34.1% vs. 23.3%, $P = 0.004$), and vascular invasion and/or extrahepatic metastases (36.5% vs. 26.9%, $P = 0.012$) were observed in HCC patients with cirrhosis compared to those without cirrhosis (Table 2). Specifically, patients with decompensated cirrhosis had the highest rate of vascular invasion and/or extrahepatic metastases (53.0%, 196/370), followed by patients with non-cirrhosis patients (26.9%, 52/193) and compensated cirrhosis (24.8%, 130/524) ($P < 0.001$) (Table 3).

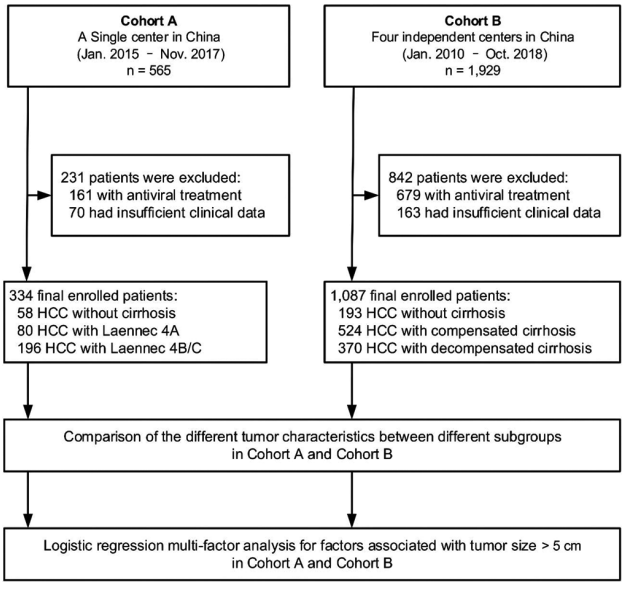

Figure 1. Study subjects enrollment and analysis flow chart.

Table 3. Comparison of the characteristics of tumor among the three subgroups

Variables	HCC without cirrhosis (n=193)	HCC with compensated cirrhosis (n=524)	HCC with decompensated cirrhosis (n=370)	P-value
Age (year)	50.35±12.39	51.01±10.57	53.71±10.70	<0.001
Male, *n* (%)	170 (88.1)	454 (86.6)	322 (87.0)	0.878
Alanine transaminase (U/L)	39.0 (26.0, 65.5)	40.5 (27.0, 65.0)	53.0 (34.0, 84.3)	<0.001
Aspartate aminotransferase (U/L)	47.0 (29.0, 81.7)	42.6 (30.0, 72.0)	80.0 (48.8, 138.0)	<0.001
Albumin (g/L)	39.0 (35.0, 42.1)	38.0 (35.0, 41.0)	34.0 (30.2, 38.0)	<0.001
Globulin (g/L)	28.7 (26.0, 32.7)	29.0 (25.6, 32.0)	32.0 (27.2, 36.4)	<0.001
Total Bilirubin (umol/L)	15.9 (11.7, 20.4)	15.7 (11.6, 21.4)	25.6 (17.7, 42.9)	<0.001
Tumor size*(cm)	7.9 (4.0, 11.9)	5.4 (3.0, 9.2)	6.5 (3.1, 11.0)	<0.001
Distribution of tumor size*, *n* (%)				0.003
≤5 cm	71 (36.8)	256 (48.9)	147 (39.7)	-
>5 cm	122 (63.2)	268 (51.1)	223 (60.3)	-
Number of tumors, *n* (%)				0.130
1	143 (74.1)	379 (72.3)	267 (72.2)	
2–3	12 (6.2)	61 (11.6)	31 (8.4)	
>3	38 (19.7)	84 (16.0)	72 (19.5)	
Vascular invasion, *n* (%)				<0.001
Yes	45 (23.3)	115 (21.9)	190 (51.4)	
No	148 (76.7)	409 (78.1)	180 (48.6)	
Vascular invasion or extrahepatic metastases, *n* (%)				<0.001
Yes	52 (26.9)	130 (24.8)	196 (53.0)	-
No	141 (73.1)	394 (75.2)	174 (47.0)	-

Age is expressed as mean±standard deviation, and tumor size is expressed as median and interquartile range, * Tumor size indicates the maximum diameter of the tumor. HCC: Hepatocellular carcinoma

3.4. Factors associated with HCC tumor size

Univariate and multivariate logistic regression analyses were conducted to analyze the possible factors which are associated with tumor size (Table 4). In the first cohort, results of univariate analysis showed that globulin (P = 0.010), vascular invasion (P = 0.012), and Laennec 4B/C (P = 0.008) were significantly associated with HCC tumor size >5 cm. On the other hand, multivariate analysis revealed that globulin (odds ratio [OR]: 1.096; 95% CI: 1.037 – 1.158; P = 0.001) and vascular invasion (OR: 4.013; 95% CI: 1.342 – 11.996; P = 0.013) were independent risk predictors of HCC tumor size >5 cm; however, cirrhosis stage Laennec 4B/C was a protective factor (OR: 0.372; 95% CI: 0.200 – 0.693; P = 0.002). As for the second cohort, univariate analysis results revealed that age (P = 0.004), alanine transaminase (P = 0.047), aspartate aminotransferase (P < 0.001), albumin (P = 0.002), globulin (P < 0.001), number of tumor (P < 0.001), vascular invasion (P < 0.001), and compensated cirrhosis (P = 0.004) were significantly related to HCC tumor size >5 cm. While using multivariate analysis, number of tumor (OR: 1.731; 95% CI: 1.364 – 2.196; P < 0.001) and vascular invasion (OR: 7.065; 95% CI: 4.238 – 11.775; P < 0.001) were identified as independent risk predictors of HCC tumor size >5 cm, while age (OR: 0.977; 95% CI: 0.962 – 0.992; P = 0.003), albumin (OR: 0.949; 95% CI: 0.916 – 0.98; P = 0.003), and compensated cirrhosis (OR: 0.551; 95% CI: 0.379 – 0.801; P = 0.002) were found to be protective factors. As vascular invasion had the largest OR values associated with tumor size >5 cm, the occurrence of vascular invasion in patients with different tumor size was compared as shown in Table 5. In short, patients with larger tumor size tend to have higher rates of vascular invasion, confirmed by the multivariate logistic regression analysis.

4. Discussion

Tumor size has been considered one of the prognostic factors for HCC[11,12]. This study demonstrated that the presence of liver cirrhosis, especially compensated cirrhosis, could be an important factor that is negatively associated with the tumor size in HBV-related HCC. Conversely, the vascular invasion showed a positive

Table 4. Univariate and multivariate logistic analysis of tumor size>5 cm and associated factors in hepatocellular carcinoma patients

First cohort	Univariate	*P*-value	Multivariate	*P*-value
	OR (95%CI)		OR (95%CI)	
Age (year)	0.994 (0.972, 1.017)	0.618		
Gender (Male)	1.528 (0.840, 2.780)	0.165		
Alanine transaminase (U/L)	1.000 (0.996, 1.003)	0.768		
Aspartate aminotransferase (U/L)	1.001 (0.998, 1.004)	0.543		
Albumin (g/L)	0.955 (0.906, 1.008)	0.094		
Globulin (g/L)	1.062 (1.015, 1.111)	0.010	1.096 (1.037, 1.158)	0.001
Total Bilirubin (umol/L)	0.992 (0.975, 1.009)	0.354		
Number of tumor (1/2–3/>3)	1.283 (0.786, 2.095)	0.319		
Vascular invasion	3.832 (1.347, 10.90)	0.012	4.013 (1.342, 11.996)	0.013
Degree of pathological differentiation (Poor/Middle/High)	1.096 (0.407, 2.949)	0.856		
Cirrhosis				
No (reference)	-	-	-	-
Laennec 4A	0.607 (0.307, 1.202)	0.152	-	-
Laennec 4B/C	0.447 (0.246, 0.812)	0.008	0.372 (0.200, 0.693)	0.002
Second cohort	**Univariate**	***P*-value**	**Multivariate**	***P*-value**
	OR (95%CI)		**OR (95%CI)**	
Age (year)	0.980 (0.967, 0.994)	0.004	0.977 (0.962, 0.992)	0.003
Gender (Male)	0.758 (0.490, 1.173)	0.214		
Alanine transaminase (U/L)	1.001 (1.000, 1.002)	0.047		
Aspartate aminotransferase (U/L)	1.003 (1.002, 1.005)	<0.001		
Albumin (g/L)	0.967 (0.946, 0.988)	0.002	0.949 (0.916, 0.983)	0.003
Globulin (g/L)	1.038 (1.017, 1.060)	<0.001		
Total Bilirubin (umol/L)	1.001 (0.999, 1.003)	0.192		
Number of tumor (1/2–3/>3)	1.751 (1.475, 2.078)	<0.001	1.731 (1.364, 2.196)	<0.001
Vascular invasion	10.056 (7.07, 14.287)	<0.001	7.065 (4.238, 11.775)	<0.001
Cirrhosis				
No (reference)	-	-	-	-
Compensated cirrhosis	0.609 (0.434, 0.855)	0.004	0.551 (0.379, 0.801)	0.002
Decompensated cirrhosis	0.883 (0.616, 1.264)	0.496		

association with the tumor size. Furthermore, it is also suggested that decompensated cirrhosis favors vascular invasion and metastasis of HCC relative to non-cirrhosis and compensated cirrhosis. Nevertheless, it should be emphasized that the association of cirrhosis with HCC is only restricted to the size of tumor but not the vascular invasion or metastasis, and to the HCC patients with compensated cirrhosis, since this stage favors other tumor biological behaviors, such as tumorigenesis, vascular invasion, metastasis, and differentiation of tumor[13]. All these results imply that compared to those without cirrhosis, HCC patients with cirrhosis tend to harbor smaller tumor, and this is consistent with the physical and mechanical constraint of tumor transformation in patients with cirrhosis[10].

The main finding of this study was that compensated cirrhosis might be a protective factor against tumor growth, and this may be counterintuitive to the clinician's perspective. However, this idea is supported by the fact that liver responds to acute injury through tissue repair, which leads to synthesis, deposition, and accumulation of extracellular matrix[14]. In addition, other previously published experimental evidence also showed that liver

Table 5. Occurrence of vascular invasion in patients with different tumor size

First cohort	Tumor size				*P*-value
	≤2cm (*n*=34)	>2 and≤5cm (*n*=153)	>5 and≤10cm (*n*=106)	>10 cm (*n*=41)	
Vascular invasion					<0.001
Yes	1 (2.9)	4 (2.6)	6 (5.7)	8 (19.5)	
No	33 (97.1)	149 (97.4)	100 (94.3)	33 (80.5)	
Second cohort	**Tumor size**				***P*-value**
	≤2cm (*n*=128)	>2 and≤5cm (*n*=346)	>5 and≤10cm (*n*=339)	>10 cm (*n*=274)	
Vascular invasion					<0.001
Yes	8 (6.2)	35 (10.1)	131 (38.6)	176 (64.2)	
No	120 (93.8)	311 (89.9)	208 (61.4)	98 (35.8)	

fibrosis may play a protective role[15-17]. Further, a recent study showed that the value of liver stiffness measurement inside the tumor and in the peri-tumoral tissue was negatively correlated with serum alpha-fetoprotein ($P < 0.05$)[18], which to some extent supports the above idea that cirrhosis is an important factor which is negatively associated with the tumor size, since the value of liver stiffness measurement would reflect the severity of cirrhosis and the alpha-fetoprotein level is strongly correlated with the tumor size[19]. Besides, the importance of fibrosis as an activator of the immune system against cancer has been previously shown in pancreatic cancers[20,21], where fibrosis development and cirrhosis-induced inflammation might prime the immune system and ensure a better response against malignant cells present in the liver[9]. However, it should be emphasized that this process is harmful in the long term, as indicated by our results that patients with decompensated cirrhosis had larger tumor size (a higher proportion of patients had tumor >5 cm) and a significantly higher rate of vascular invasion and extrahepatic metastasis than those with compensated cirrhosis and non-cirrhosis. When cirrhosis progresses to decompensated cirrhosis, immune dysfunction in HCC patients with severely decompensated cirrhosis would result in significant tumor growth and metastasis[13]. Decreased efficiency of cell durotaxis and increased level of stiffness from soft matrix to rigid matrix has been reported previously[22], which may explain the limitation on HCC tumor margins as seen in the patients with cirrhosis. However, many studies have shown that tumor cells would spread better and migrate faster in the rigid matrix than in the soft matrix[23-25]. These studies may also explain why vascular invasion occurred more frequently in HCC patients with cirrhosis, especially in patients with decompensated cirrhosis. In addition, changes of vascular permeability in these patients may also lead to a higher probability of vascular invasion and extrahepatic metastases.

The present study also showed that globulin is a risk factor for HCC tumor growth, while albumin is a protective factor. This result is justifiable since the albumin levels reflect the nutrition status of patients, and numerous studies have shown that malnourished patients with HCC with low serum albumin levels have poor overall survival and high recurrence rate[26]. In contrast, high levels of globulin indicate a systematic inflammatory response, which plays an important role in proliferation, progression, development, and metastasis of tumor cells[26-28].

The current study is restrained by several limitations. Although we had excluded the patients having anti-HBV treatment within 6 months before prior to HCC diagnosis, to reduce the potential influence of different screening frequency between patients with and without cirrhosis, some bias may still exist because of the retrospective and cross-sectional nature of this study. In addition, the disease background of both cohorts was not exactly the same; therefore, similar results observed from the two cohorts might verify its reliability only to some extent but could not be compared directly with each other. Further, only HBV-infected patients were investigated in this study; therefore, future studies including patients with other etiological factors of HCC are warranted.

5. Conclusion

In summary, this retrospective multicenter study demonstrated that HCC patients with compensated cirrhosis tend to harbor smaller tumor but severe cirrhosis favors tumor vascular invasion and metastasis, which affect tumor recurrence and survival of the patients. This further reminds us that for patients with cirrhosis, especially those with decompensated cirrhosis, risk of

vascular invasion and/or extrahepatic metastases should be given more attention. These observations may reconcile current controversies regarding the role of liver cirrhosis in HCC and may raise more heated discussions on this topic. Prospective and mechanism-related studies are needed to further clarify the effects of fibrosis and cirrhosis on HCC development and progression as these aspects would provide valuable insights into the development of anti-fibrotic therapy as HCC treatment.

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Conflict of interest

The authors declared no conflict of interest related to this article.

Author contributions

Conceptualization: Xiangmei Chen, Fengmin Lu
Data curation: Xiangjun Qian, Jingmin Zhao
Formal analysis: Yanna Liu, Xiangjun Qian
Writing – original draft: Yanna Liu, Xiangjun Qian
Writing – review and editing: Congying Wu, Weidong Pan, Jingmin Zhao, Xiangmei Chen, Fengmin Lu
All authors of this research have approved the final version of the article.

References

1. Sung H, Ferlay J, Siegel RL, *et al*., 2021, Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 71: 209–249.

   https://doi.org/10.3322/caac.21660

2. Seitz HK, Stickel F, 2006, Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol Chem*, 387: 349–360.

   https://doi.org/10.1515/BC.2006.047

3. Yuen MF, Tanaka Y, Fong DY, *et al*., 2009, Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. *J Hepatol*, 50: 80–88.

   https://doi.org/10.1016/j.jhep.2008.07.023

4. Lok AS, Seeff LB, Morgan TR, *et al*., 2009, Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology*, 136: 138–148.

   https://doi.org/10.1053/j.gastro.2008.09.014

5. Zhang DY, Friedman SL, 2012, Fibrosis-dependent mechanisms of hepatocarcinogenesis. *Hepatology*, 56: 769–775.

   https://doi.org/10.1002/hep.25670

6. Massarweh NN, El-Serag HB, 2017, Epidemiology of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *Cancer Control*, 24: 1073274817729245.

   https://doi.org/10.1177/1073274817729245

7. Idilman R, De Maria N, Colantoni A, *et al*., 1998, Pathogenesis of hepatitis B and C-induced hepatocellular carcinoma. *J Viral Hepatitis*, 5: 285–299.

   https://doi.org/10.1046/j.1365-2893.1998.00116.x

8. Chayanupatkul M, Omino R, Mittal S, *et al*., 2017, Hepatocellular carcinoma in the absence of cirrhosis in patients with chronic hepatitis B virus infection. *J Hepatol*, 66: 355–362.

   https://doi.org/10.1016/j.jhep.2016.09.013

9. Garrido A, Djouder N, 2021, Cirrhosis: A questioned risk factor for hepatocellular carcinoma. *Trends Cancer*, 7: 29–36.

   https://doi.org/10.1016/j.trecan.2020.08.005

10. Zhu M, Lu T, Jia Y, *et al*., 2019, Somatic mutations increase hepatic clonal fitness and regeneration in chronic liver disease. *Cell*, 177: 608–621.e612.

    https://doi.org/10.1016/j.cell.2019.03.026

11. Chen YL, Ko CJ, Chien SY, *et al*., 2011, Tumor size as a prognostic factor in resected small hepatocellular carcinoma: A controversy revisited. *J Gastroenterol Hepatol*, 26: 851–857.

    https://doi.org/10.1111/j.1440-1746.2010.06595.x

12. Zhang W, Wang X, Jiang R, *et al*., 2015, Effect of tumor size on cancer-specific survival in small hepatocellular carcinoma. *Mayo Clin Proc*, 90: 1187–1195.

    https://doi.org/10.1016/j.mayocp.2015.06.018

13. Albillos A, Lario M, Álvarez-Mon M, 2014, Cirrhosis-associated immune dysfunction: Distinctive features and clinical relevance. *J Hepatol*, 61: 1385–1396.

    https://doi.org/10.1016/j.jhep.2014.08.010

14. Mehendale HM, 2005, Tissue repair: An important determinant of final outcome of toxicant-induced injury. *Toxicol Pathol*, 33: 41–51.

https://doi.org/10.1080/01926230590881808

15. Bourbonnais E, Raymond VA, Ethier C, *et al*., 2012, Liver fibrosis protects mice from acute hepatocellular injury. *Gastroenterology*, 142: 130–139.e4.

https://doi.org/10.1053/j.gastro.2011.09.033

16. Nishio T, Iimuro Y, Nitta T, *et al*., 2003, Increased expression of collagenase in the liver induces hepatocyte proliferation with cytoplasmic accumulation of beta-catenin in the rat. *J Hepatol*, 38: 468–475.

https://doi.org/10.1016/s0168-8278(03)00013-8

17. Zhou XY, Jamil A, Nash A, *et al*., 2006, Impaired proteolysis of collagen I inhibits proliferation of hepatic stellate cells implications for regulation of liver fibrosis. *J Biol Chem*, 281: 39757–39765.

https://doi.org/10.1074/jbc.M605621200

18. Lashen SA, Elshafei MM, Hablass FH, *et al*., 2020, Liver stiffness as a predictor of hepatocellular carcinoma behavior in patients with hepatitis C related liver cirrhosis. *Hepatobiliary Pancreat Dis Int*, 19: 22–28.

https://doi.org/10.1016/j.hbpd.2019.11.004

19. Giannini EG, Sammito G, Farinati F, *et al*., 2014, Determinants of alpha-fetoprotein levels in patients with hepatocellular carcinoma: Implications for its clinical use. *Cancer*, 120: 2150–2157.

https://doi.org/10.1002/cncr.28706

20. Rhim AD, Oberstein PE, Thomas DH, *et al*., 2014, Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell*, 25: 735–747.

https://doi.org/10.1016/j.ccr.2014.04.021

21. Özdemir BC, Pentcheva-Hoang T, Carstens JL, *et al*., 2014, Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell*, 25: 719–734.

https://doi.org/10.1016/j.ccr.2014.04.005

22. DuChez BJ, Doyle AD, Dimitriadis EK, *et al*., 2019, Durotaxis by human cancer cells. *Biophys J*, 116: 670–683.

https://doi.org/10.1016/j.bpj.2019.01.009

23. Plotnikov SV, Pasapera AM, Sabass B, *et al*., 2012, Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. *Cell*, 151: 1513–1527.

https://doi.org/10.1016/j.cell.2012.11.034

24. Gao J, Rong Y, Huang Y, *et al*., 2019, Cirrhotic stiffness affects the migration of hepatocellular carcinoma cells and induces sorafenib resistance through YAP. *J Cell Physiol*, 234: 2639–2648.

https://doi.org/10.1002/jcp.27078

25. Prager-Khoutorsky M, Lichtenstein A, Krishnan R, *et al*., 2011, Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing. *Nat Cell Biol*, 13: 1457–1465.

https://doi.org/10.1038/ncb2370

26. Deng Y, Pang Q, Miao RC, *et al*., 2016, Prognostic significance of pretreatment albumin/globulin ratio in patients with hepatocellular carcinoma. *Onco Targets Ther*, 9: 5317–5328.

https://doi.org/10.2147/OTT.S109736

27. Vawda S, Mansour R, Takeda A, *et al*., 2014, Associations between inflammatory and immune response genes and adverse respiratory outcomes following exposure to outdoor air pollution: A HuGE systematic review. *Am J Epidemiol*, 179: 432–442.

https://doi.org/10.1093/aje/kwt269

28. Du XJ, Tang LL, Mao YP, *et al*., 2014, The pretreatment albumin to globulin ratio has predictive value for long-term mortality in nasopharyngeal carcinoma. *PLoS One*, 9(4): e94473.

https://doi.org/10.1371/journal.pone.0094473

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REVIEW ARTICLE

State-of-the-art: A taxonomy of artificial intelligence-assisted robotics for medical therapies and applications

Jinyang Wang[1], Lei Zhu[2], Po Yang[3], Ping Li[4,5], Jihong Wang[1]*, Huating Li[6]*, and Bin Sheng[7]*

[1]Shanghai University of Sport, Shanghai, China

[2]ROAS Thrust, The Hong Kong University of Science and Technology (Guangzhou), Guangzhou, China

[3]Department of Computer Science, University of Sheffield, Sheffield, U.K.

[4]Department of Computing, The Hong Kong Polytechnic University, Hong Kong, China

[5]School of Design, The Hong Kong Polytechnic University, Hong Kong, China

[6]Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

[7]Department of Computer Science and Engineering, Shanghai Jiao Tong University, Shanghai, China

Abstract

This paper presents a review on the development and major advances in artificial intelligence-assisted robotics for medical therapeutic tasks by focusing on the current challenges emerging from the clinical application process and the research efforts mitigating the problems. In this review, we searched Nature, Science, and Cell using specific keywords (*i.e.*, medical artificial intelligent robots), categorized research works over the past three decades based on therapeutic applications, and discuss the latest development and bottleneck problems of each subtopic. We first present a chronology of the artificial intelligence-assisted techniques developed for medical therapeutic tasks over the past three decades and classify them according to the principles of the algorithm and its corresponding type of medical therapeutic tasks. Artificial intelligence technologies have evolved from classic machine learning methods in the early nineties to data-driven deep learning methods. We subsequently derive a taxonomy of artificial intelligence-assisted therapeutic tasks in the past three decades based on the types of therapeutic tasks and the trending topics in relation to the problems. Using certain search criteria with Nature and Cell databases, one prosperous trend has been abstracted from highly cited research papers and the interpretation of our taxonomy. This unprecedented trend embodies the revolutionary development of artificial intelligence, a closer integration with therapeutic tasks, and a more comprehensive human-robot interaction, all of which benefit sophisticated telesurgery and microsurgery by providing surgeons with higher imaging accuracy and human-like tactile sensation. Our survey discusses the current challenges and future trends of artificial intelligence-assisted therapeutic tasks for the convenience of clinical research and applications, hoping that they would help bridge the gap between entrepreneurial translation and research.

Keywords: Artificial intelligence; Chronic disease management; Laparoscopic robots; Medical robotics; Medical therapies; Wearable medical robots

***Corresponding authors:**
Jihong Wang
(cylwsy@163.com)
Huating Li
(huarting99@sjtu.edu.cn)
Bin Sheng
(shengbin@sjtu.edu.cn)

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1. Introduction

The past decade has witnessed the advancements achieved by artificial intelligence-assisted robotics for therapeutic tasks (AI-ART). In consideration of commercial translational requirement and better artificial intelligence application to therapeutic tasks, AI-ART can be categorized into three groups based on their applications and underlying principles. The nascent application of artificial intelligence-assisted robotics for therapeutic tasks, which includes the exploration application of robotic manipulators to fulfill standard surgical procedures and the incorporation of early artificial neural network or statistics-based algorithms, has had its ups and downs. In this review, we regard medical artificial intelligent robots, such as laparoscopic robots, medical wearable equipment that has benefited from artificial intelligent algorithms, and intelligent soft medical robots, as AI-ART. The chronological development of AI-ART based on methodology and milestones is shown in Figure 1.

2. Taxonomy of artificial intelligence-assisted robotics for therapeutic tasks

By setting the search criteria and selecting influential journals, such as Web of Nature, Cell, and other journals, involving AI-ART in various aspects over a long time span, we identified the taxonomy range. The topics of the taxonomy are related to specific clinical therapeutic tasks (*e.g.*, laparoscopy surgical robots in Section 2.1) or enabling technologies that help to remove urea for dialysate regeneration for wearable artificial kidney (*e.g.*, medical wearable robots in Section 2.3). A detailed exposition of each clinical therapeutic task itself warrants a survey, but in this work, we focus on the problems during the integration process and the latest trending methods proposed in AI-ART for each clinical therapeutic task. We also survey the clinical artificial intelligence improvement in robots, which empower the effective monitoring and update of the applied AI-ART in specific clinical therapeutic task. In this section, we focus on summarizing the development of each subtopic of laparoscopy surgical robots and discuss the current challenges to lay a foundation for the three major trending research in Section 3, as shown in Figure 2.

2.1. Surgery automation

2.1.1. Gripper contact force sensing

Due to the limitations in the development of physical sensors, the contact force sensing techniques that are used in current commercial or academical research tend to "isolate" the hands of surgeons from the tissues or skin of the patients[45,47]. The tactile sensation isolation from manual instruments or artificial intelligence-assisted medical robots could be disastrous, especially in surgical tasks like tissue retraction surgery, during which deformable connective tissues would be manipulated recurrently[48]. To sense and control the interaction force while using artificial intelligence-assisted robotic techniques for therapeutic tasks, researchers are exploring the recreation of tissue palpation, temperature, and even corrosive sensing with improved gripper design[49].

To address the technical sensing problem, advancements have been made. Luca *et al.*[50] presented a simulation of Ruffini receptors with deep neural networks and optical gratings, which could be applied to manufacture tactile-sensitive skin. This bio-inspired polymetric matrix skin might be a novel research direction to implementing tactile sensation while owning a different principle with that of human. The researchers employed the convolutional neural network (CNN) to decode the fiber Bragg grating sensor signals, achieving median errors of 35 mN and 3.2 mm, and demonstrating the advantages of CNN algorithm. Tae *et al.*[51] proposed the use of leech-inspired dry electrodes for auxiliary blood pressure sensing through surgical robots. Although their work was not directly meant to sense the interaction force between the contact point of the robotic gripper and the patient's tissue or skin, it provides an optional method to monitor the fluctuations in blood pressure during the whole surgical procedure, as shown in Figure 3.

2.1.2. Automated surgery

Prolonged operation is often indicated in research or review works as the major risk for complications after surgery.

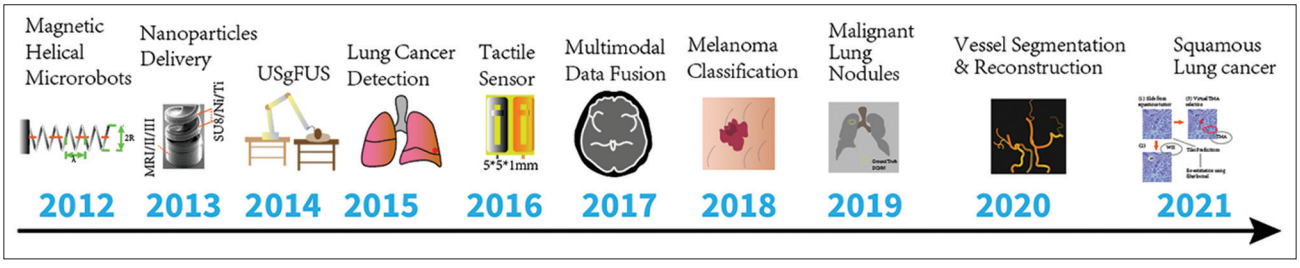

Figure 1. Chronological development of AI-ART in the past decade from the early 2010s' magnetic helical microrobots to squamous lung cancer detection and therapeutic tasks. The illustrations in each year are recreated and referenced as follows: 2012[1], 2013[2,3], 2014[4,5], 2015[6-9], 2016[10-13], 2017[14-17], 2018[18-21], 2019[22-27], 2020[28-32], and 2021[33-38].

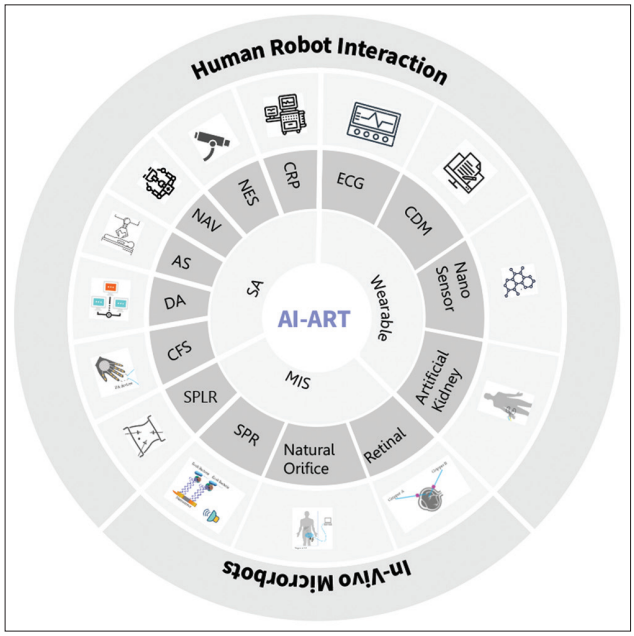

Figure 2. Taxonomy of AI-ART in three major scenarios for medical therapies. The innermost circle embodies the three major scenarios. The outermost circle states the two most trending topics through AI-ART. The middle tiles are the subbranches (from the innermost circle to the adjacent tiles surrounding it): MIS refers to minimally invasive surgery; SA refers to surgery automation; Wearable refers to medical wearable robots; CFS refers to contact force sensing, with an illustration shown[38]; DA refers to distributed architecture, with an illustration taken from the Robotic Surgery Center at Szpital na Klinach; SPLR refers to single port laparoscopy robot, with an illustration taken from intuitive surgical; AS refers to automated surgery; NAV refers to navigation techniques, with an illustration shown[39]; NES refers to naked-eye scopy, with a reprinted illustration shown[40]; CRP refers to collaborative research platform, with an illustration taken from Applied Dexterity; ECG refers to electrocardiogram, with an illustration shown[41]; CDM refers to chronic disease management, with an illustration taken from the American Society of Hematology; Nano Sensor refers to nanotechnology-based medical sensors, with an illustration shown[42]; artificial kidney refers to the artificial intelligence-assisted kidney devices, with illustration shown in[43]; Retinal refers to robots used in retinal surgeries, with an illustration shown[44]; Natural orifice refers to surgical robots that can gain access through natural orifices, with an illustration shown[45]; SPR refers to self-propelling robots, with an illustration shown[46].

Prolonged surgery mainly occurs in reconstructive surgical tasks, such as in cancer reconstruction, birth defects, full or partial mastectomy, limb salvage, and so on. Therefore, automated surgical robots are required to perform a wide range of surgical tasks, including standard and non-standard procedures. Surgeries with standard procedures are viewed as super-states, which could be decomposed into finer-grained sub-states, such as dynamic imaging of different views, pull, loop, cutting through, and so on[56]. However, a precise and robust detection of incision remains one of the biggest challenges, as point cloud generated by stereo vision is significantly affected by various light conditions.

These standard procedures could be learned through AI-ART[56]. Wang *et al.*[56] developed a medical robot system equipped with stereo vision, incision detection, and staple positioning algorithms for surgical suturing and staples removals. Lu *et al.*[54] presented an in-house vision system with efficient trajectory planning algorithms. They successfully addressed the automatic suturing problem with two collaborative grippers through visual detection algorithms, which also eliminated the risk of collisions between the two gripers. In fact, their work does not include the full automation of medical robots, but rather only as a sub-state semi-automatic assistant for surgeons. Moreover, the tactile sensation between the operated tissue or organ and the robotic gripper does not provide any feedback to the surgeons or have a monitoring module of the computer unit.

Even with artificial intelligence assistance for laparoscopic surgery, full-automation medical robots have not been approved by the Food and Drug Administration (FDA), due to the trust attitudes of patients and surgeons toward these technologies. In addition, career dilemmas and anxieties associated with full-automation smart medical robots among skilled surgeons still exist. Another issue to be considered is personal information security protection, which is either exposed to the surgeons in charge or big data, and sometimes even a virtual AI doctor outputting the treatment. Hence, governmental departments and researchers have begun to establish laws, standards, and instructions during the AI-ART process.

2.1.3. Navigation

Navigation plays a crucial role for AI-ART, providing fundamental functions such as real-time self-localization and dynamic map building. The relative positional coordinates of the abdominal cavity vary with time and breathes. The classic navigation methods used for other non-medical robots in most cases are not suitable for AI-ART, as the workspace and volume of medical robots are limited, namely, the aforementioned miniaturization challenge. Based on specific operative scenarios, corresponding navigation algorithms are leveraged with homologous hardware and sensors by AI-ART.

The commonly used sensors for AI-ART include visual charge coupled device (CCD) camera, three-dimensional (3D) laser, and time-of-flight (ToF) camera. Ebihara *et al.*[39] performed real-time vessel navigation through indocyanine fluorescence during artificial intelligence-assisted gastric tube reconstruction. Each patient was followed-up, with no reported post-operative complications, such as ischemia or adhesion of gastric tube. Although the navigation method adopted by Ebihara *et al.* was a classic approach, they

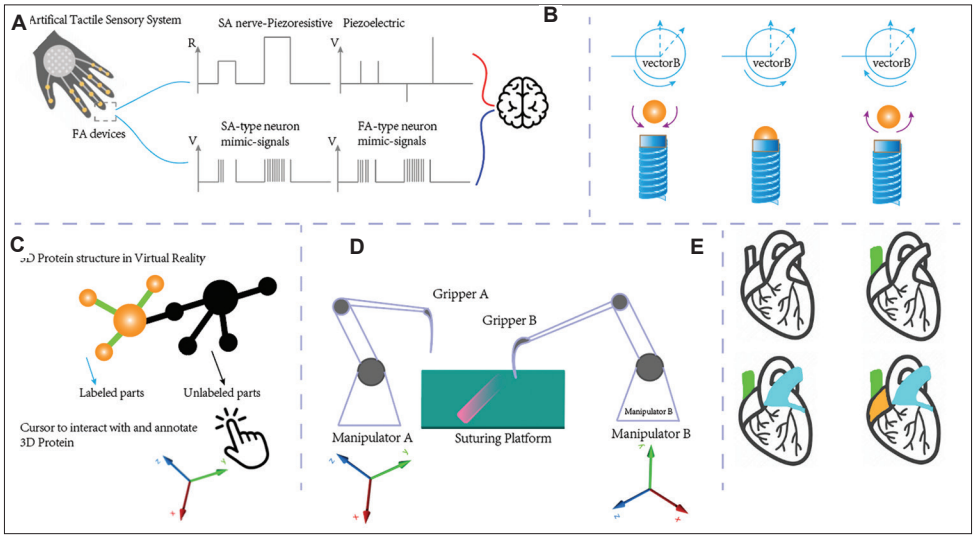

Figure 3. Representative surgery automation applications with AI-ART. (A) Mimicking human tactile sensing for laparoscopy gripper[50]. (B) A capsule robot that is capable of picking, dropping, and assembling particles and drugs[52]. (C) Augmented reality (AR)-assisted biological annotation[53]. (D) Vision-assisted suturing robots[54]. (E) Ground truth atrium (first, at top left) and predicted results (the other three) of CNN[55].

recognized the elementary function of navigation, which is to provide localization signal. Affected by the fluorescence dosage, imaging accuracy, and the positioning precision of visual algorithm, the actual relocalization and robustness of the navigation have room for further improvement. Taking the dynamics and deformability of the abdominal cavity into account, Zhang *et al.*[57] attempted to address the problems of invasive external tags and the difficulties of deformable tissue mapping and segmentation through modified 3D-3D iterative closest point (ICP), Mask R-CNN, and semi-global block matching (SGBM) algorithms. The method presented by Zhang *et al.* is suitable for the distributed form of AI-ART, as the deep learning segmentation algorithm would cost expensive computation during real-time inference. SGBM algorithm relies on the complex texture of the surgical region, which may be polluted by disinfectants or residual bloodstains. Therefore, surgery automation is expected to improve when the navigation algorithm is invariant to the slight texture variations.

The current state-of-the-art navigation technologies prefer to fuse multi-modality sensor data together to achieve accurate and multiple aspects imaging of the patients, including ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and the two-dimensional (2D) visual images of inner tissue and organs. We will discuss sensor data fusion in Section 3.2.

2.1.4. Collaborative research platform

To produce comparable and reproducible results of AI-ART, researchers of different organizations seek to construct a collaborative research platform of laparoscopic robots[57]. A collaborative laparoscopy platform would eliminate the enormous amount of duplicate work for a small or new medical research team. The bootstrapping team or experienced peers can gain access to existing and open works as well as use their own laparoscopic robots for specific therapeutic tasks[58]. On identifying this requirement and the benefits for subsequent product development, two organizations have developed their own respective collaborative laparoscopy platform for researchers. The first one is Raven II, an open-architecture laparoscopic robot, from Applied Dexterity, which has seven degrees of freedom (six DoF plus one grasp) through two cables containing monitoring, power supply, and control signals[58]. The second open platform for laparoscopy surgery is from the collaboration of intuitive surgery with practicing surgeons to perform non-clinical trials with animals for verification or proof of certain therapeutic approaches[59]. After in-depth investigation, the weakness of Raven II applied for automatic surgery lies in state estimation, as it lacks accurate encoders to indicate each coarse state. The lack of relevant evaluation standards and metrics may be a serious problem for collaborative research platforms. As a consequence, the experimental results and data produced by these collaborative research platforms lack comparability with equipment, granted by the FDA. The two collaborative platforms are verified only for research use, in which human clinical trials are not permitted.

2.2. Minimally invasive surgical (MIS) robots

MIS has evolved as a popular alternative to open-ended surgeries, due to reduced trauma and a much faster

recovery. Another reason for its popularity is an increasing proportion of the elderly. The elderly has a weaker immunity and wound healing ability than the young. Therefore, it is crucial to invest energy and money in MIS now and in the future. We have witnessed the progress made by researchers in MIS in recent years; the seminal ones are illustrated in Figure 4.

2.2.1. Self-propelling robots for endoluminal surgery

Traditional endoscopic equipment used in clinical therapeutic tasks tends to cause pain and agitate the stomach as a soft cable, containing imaging or small scalpels, would be pushed through the stomach or intestine. Therefore, several types of self-propelling endoluminal robots with different performances have been proposed by researchers[60].

Researchers have designed a new propelling method inspired by wasp ovipositor, which squeezes its tail needle into the rind of tree through multiple sliding body parts[61]. The propelling endoluminal robot is able to drive itself forward in the intestine with a rotatable camera mounted on one of its body parts. Further research is needed even after testing the novel self-propelling endoluminal robots for the risk of insufficient energy, which would cause these robots to remain inside the body, and the image sticking algorithm of the rotating camera in the dynamic human intestine is susceptible to peristalsis and breathing. To overcome the possible adverse effects produced by the friction between the sliding body part and the intestinal mucosa, researchers have turned to a new direction: Extracorporeal magnetic actuator[46,62]. Endoluminal robots that are propelled by body parts or extracorporeal magnetic actuator depend on the natural opening of the intestine or stomach, which is dynamic and continuously changing along with the heartbeat and respiration. Hence, a reliable support structure is needed for endoluminal robots to obtain better imaging of its surroundings deep inside the human body. At present, self-propelling robots are mainly developed for endoluminal examinations. They have yet to meet the requirements of drug delivery, which has higher demands for accurate localization and resistance to gastrointestinal peristalsis.

2.2.2. Surgical robots through the natural orifice

As a minimally invasive surgical approach, natural orifice transluminal endoscopic surgery (NOTES) robots are gaining widespread attention for bare skin incision, faster recovery time, and fewer complications after surgery[62,65]. The main challenge of NOTES is the localization and navigation ability when deep inside the human body. A number of surgical robots are now able to carry out natural orifice tasks. With regard to NOTES, the focus is on development of tools for multitasks and compact-sized soft continuum robots with multiple articulations and more power.

Shen *et al.* devised a multitasking robot with two hands, like humans, but equipped with multifunctional manipulators, aiming at on-site tool exchange based on the type of surgery[65]. Another important feature is the motor-like actuator with two spatial independent articulated curvature sections. They attempted to address the power deficit problem, which constrains the degree-of-freedom motion inside the lumen of the stomach, intestine, *etc.* The research team also rearranged the operation sequence

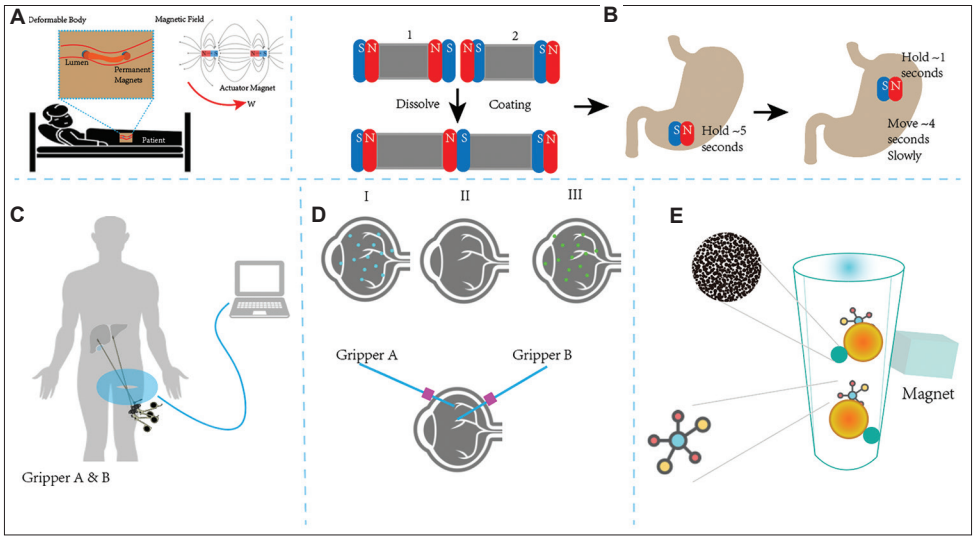

Figure 4. Three interpretations of minimally invasive surgical robots with AI-ART. (A) Soft endoluminal *in vivo* robot propelled by rotating magnetic field[60]. (B) Deglutible capsule robot propelled by rotating magnetic dipole fields[61]. (C) Operative illustration through natural orifice[62]. (D) Retinal robot with AI-ART[63]. (E) Illustration of self-propelling magneto-fluorescent nanorobot capturing tumor cells[64].

configuration to reduce the trade-off between size and power, reflecting their comprehensive efforts toward the exploration of NOTES. The navigation of NOTES robots within the lumen would be a topic of interest considering the synthesis of multiple AI technologies, such as visual semantic segmentation and recognition, inertial information fusing, and so on. The integration of NOTES and soft-bodied robots would be another technical direction, as conventional NOTES tends to cause injuries and discomfort to the natural orifices of the body. The soft-bodies design adjusts the integral positions and speed based on the pressure to the orifice. Hence, the tactile sensing technique plays a crucial role for the further development of NOTES.

2.2.3. Robots for retinal surgery

The innovation of AI-ART robots for retinal surgery predominantly aims at reducing the risk of injury to the crystalline lens, corneal injury, and retinal tearing, all of which may lead to serious consequences and pose a challenge for surgeons[63,66]. The additional benefit of AI-ART robots for retinal surgery lies in the increased dexterity and lower incidence of ocular hypotony. In the treatment of retinal vein occlusion, severe visual loss may occur; hence, retinal cannulation of retinal veins using a smaller-diameter cannula is the best approach thus far. Within the past few years, there has been an increasing interest in ultra-high precision positioning mechanism and image segmentation algorithms. In 2021, Jinno[66] proposed the use of snake-like robots to address the problem of restrained motion within a confined workspace from an appropriate direction, in which multiple surgical tasks were performed on the delicate cornea and retina. In 2020, Suzuki[44] designed a miniature remote center of motion manipulator (RCM) with a positional precision of 26.4 mm for teleoperated microsurgery. Retinal surgery robots continue to be the best assistance for surgeons. Considering the complexity of retinal surgery, automated robots can contribute from three perspectives: (1) Understanding the current semantic processing; (2) learning the control patterns or rules based on the semantic process; (3) conducting semantic processing when the surgeon requires.

2.2.4. Single-port laparoscopy

A standard laparoscopy surgical robot usually consists of 3–4 ports to supply accesses for the robotic arms, including several manipulators and one planar or stereo imaging endoscopy. Compared with manual open procedures of standard specifications, the standard laparoscopy surgical robot can effectively reduce the invasiveness of surgery. The incision is small as single-port laparoscopy robot integrates the robotic manipulation arms and the endoscopy into one trocar through the abdominal wall.

The difficulties in using single port-laparoscopic robots lie in the solvability and theoretical formula derivation of multi-arm single-port surgical coordinates trajectory mapping. As we know, the singularities of artificial intelligence-assisted robotic arms with a confined workspace are major issues. Notable innovative single-port laparoscopic robot prototypes have been proposed over the past 5 years[67,68]. Bai *et al.* proposed an optimized anthropomorphic coordinated control approach through dual-step optimization based on the dexterous arms in human boxing maneuvers[67]. Bai's method has shown to achieve higher efficiency and less invasiveness apart from addressing the issue of control. Wang *et al.* designed a comprehensive system, made up of three 6-DoF robotic arms and a camera-mounted endoscopy, which could be inserted into the abdominal cavity through one trocar with diameter <20 mm. Bai *et al.* attempted to address the miniaturization of laparoscopic robots and the sophisticated control of robotic arms[67].

2.3. Medical wearable robots

Medical wearable robots are primarily for the improvement of body functionality, including the normal functioning of internal organs and limb movement. In medical therapeutic scenarios, exoskeleton robots generally refer to rehabilitation robots. The focus now is on human-robot interaction to improve the cognition ability and physical assistance provided by robots to humans. Another aspect of medical wearable robots is wearable artificial organs, which include microfluidic lung, bioartificial heart, artificial hemodialysis (artificial kidney), artificial liver, and so on[69,70]. The representative medical wearable robots are illustrated in Figure 5.

2.3.1. Assisted chronic disease management

Compared with wearable nanosensors for medical signal collection, AI-ART for chronic disease management mainly focuses on stage distinction and leveraging more powerful artificial intelligent algorithms to support the decision-making process. For instance, patients with chronic lymphocytic leukemia (CLL), which is a type of cancer that originates from the lymphocytes in the bone marrow and spreads to the blood, causing infections and lowering the immunity, may endure the transformation into the next stage: Aggressive malignant lymphoma. However, determining whether the patient is in the first stage or second stage is challenging due to limited guidelines and insufficient morphologic experience of biopsy specimens. Therefore, researchers from University of Rochester Medical Center, Rochester and University of

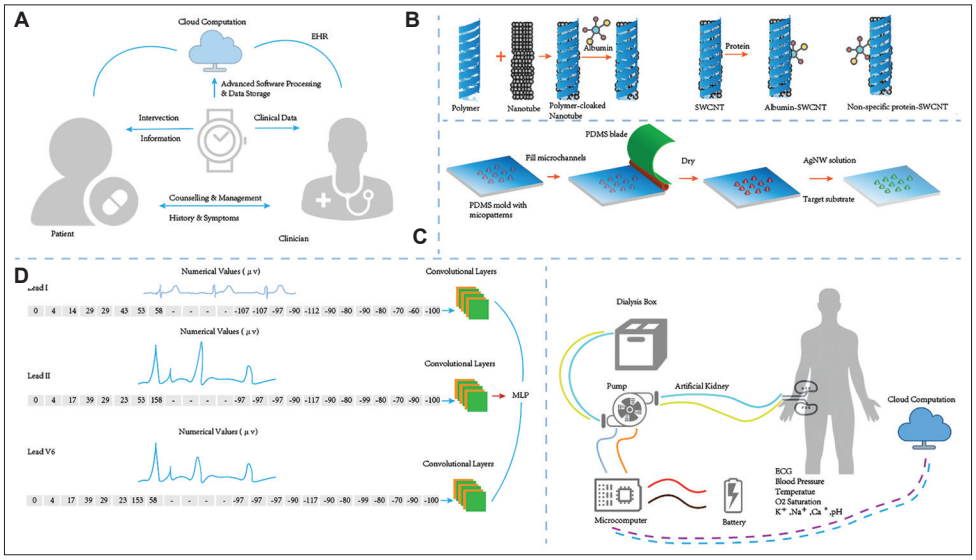

Figure 5. Data collection and flow of medical wearable robots. (A) Intelligent wearable robot with AI-ART[71]. (B) Formation process, and a proposed interaction model[72]. (C) Fabrication process of epidermal electronics with nanocomplex[71]. (D) Cardiovascular disease management method and architecture with AI-ART[73]. (E) Smart artificial kidney incorporated with proteomics, hematology, and engineering techniques of AI-ART[70].

Texas MD Anderson Cancer Center seek to establish an approach to improve the diagnostic accuracy and probe the stage or degree of CLL with artificial intelligence algorithms[73]. Besides the mainstream application for stage distinction, there are two supplementary branches of AI-ART for chronic disease management. The first is to optimize chronic disease management, involving fusion of multi-modality medical big data, patient-oriented risk evaluation, and decision support assistance[71]. Compared with the former, the second branch involves imposing the prediction function of artificial intelligence algorithms onto post-operative recovery. In spite of that, the third branch is in its infancy, involving a comprehensive framework with enormous amount of multi-modality medical data of specific diseases from wearable medical robots, multilevel progressive data mining algorithms, official guidelines, and relevant laws. One of the most important goals of chronic disease management is to recapitulate the corresponding short-term and long-term therapies for the patient in the future. The chronic disease management system collects comprehensive data from daily examination and wearable sensors, such as ECG and so on. Valuable data mining from the massive information are the key for subsequent treatment formulation.

2.3.2. Wearable electrocardiogram monitoring

Predicting when a patient would suffer from an unexpected heart attack is difficult. Wearable ECG is a revolution toward 24 h or longer ECG monitoring, which assists the doctors in detecting cardiovascular diseases before they become serious and uncontrollable. At present, wearable ECG monitor is growing as a supplementary tool of conventional heavy and costly ECG monitor[71]. AI-ART human-like interpretation, which is massively unrecognizable to human interpreters, reveals the associations between electrical signals and various phenotypes, such as patents' ages, sexual distinction, atrial fibrillation, ventricular dysfunction, and so on. Wearable ECG monitor is expected to become a potential, ubiquitous, and non-invasive biomarker[74]. Long-lasting power supply for wearable ECG monitoring can be mitigated by the rapid development of new-energy chargeable battery. Perhaps, integrating important first-aid strategies, such as defibrillation by electric shock and so on, into the ECG monitor would be beneficial.

2.3.3. Artificial kidney

The innovation of artificial kidney (portable hemodialysis) to some extent represents a center translation substantially from in-center to patient-center[75]. The kidneys remove toxic and excessive water from the blood. Hence, wearable artificial kidney plays an extremely important role for kidney failure patients. Although artificial kidney has been envisioned in 1960s, until now, artificial kidney has not been approved by the FDA for clinical use.

Researchers are gaining interest in the barriers hindering research development and commercial productization. The main challenge is how to effectively remove toxic salutes from the blood, while decreasing the dosage of dialysate, which is also an impediment for hemodialysis. The reason behind the necessity to solve the substantial usage of dialysate is that even if the patient is able to afford

a wearable artificial kidney, the patient may not have access to dialysis storage or caregiver at any time[70].

The main direction is the study of sorbent adsorption, in which highly effective activated charcoals can inherently absorb uremic toxins. Historically, the exclusive sorbents system has been proven ineffective in binding and eliminating urea. Hence, researchers have developed five types of sorbents, to the best of our knowledge, for urea adsorption. One of the five types includes TPA-COF (covalent organic framework) nanosheets and TPA-COF nanoparticle modified with -OH, which have been verified to have better urea adsorption based on molecular study. Other directions of wearable artificial kidney include enzymatic removal of urea, electro-oxidation, photo-oxidation, blood compatibility, and human factors[69]. Artificial intelligence-assisted analysis would improve the search for more efficient materials relating to the development of sorbents in three aspects: (1) Effective combination of candidate structures; (2) more reliable dynamics simulation for liquid circuit system; and (3) accurate prediction of the recharge of consumable materials.

3. Human-robot interaction for automation or telesurgery

We searched Nature, Cell, and Science website pages using keywords "medical artificial intelligent robots" to index all the related papers with a time range from 1990s to the latest issues. We then conclude that the main research trend is human-robot interaction for automation or telesurgery, as shown in Figure 6.

3.1. Feedback of tactile sensation

The application of either classic laparoscopy or AI-ART for medical robots isolates the surgeon's tactile sensation from the patient's deformable tissue or skin. However, either the surgical robot with on-site surgeons or telesurgery through 5G communication technologies is required to hold or pull the tissue with proper force without secondary classic laparoscopic injury. Researchers have explored new sensation technologies for the interaction between medical robots and soft tissue[76]. The fundamental work of AI-ART for medical robots is undoubtedly the calibration of force sensor or torque sensor, which transforms raw electrical signal into force or torque values with real physical meaning. With regard to the progress of calibration, the main focus is on end-to-end neural networks as the multi-axis data and their mutual coupling. Deep learning algorithms have been leveraged for the calibration of force and torque sensors. On the basis of calibration works, the skin-like sensor that is able to attach to arbitrary surface has drawn interests, as it can be manufactured into non-array arrangement as well as sense and transmit signals even with partial damages. Four artificial neural networks have been adopted to determine their slippage and accuracy data output as well as compared for cross-verification, with their weaknesses illustrated[50]. Generative adversarial network (GAN) learning algorithms and its variants as GAN exploits generator and discriminator can be used for searching an effective tactile sensing material to produce better generator in an adversarial manner. The produced generator by GAN

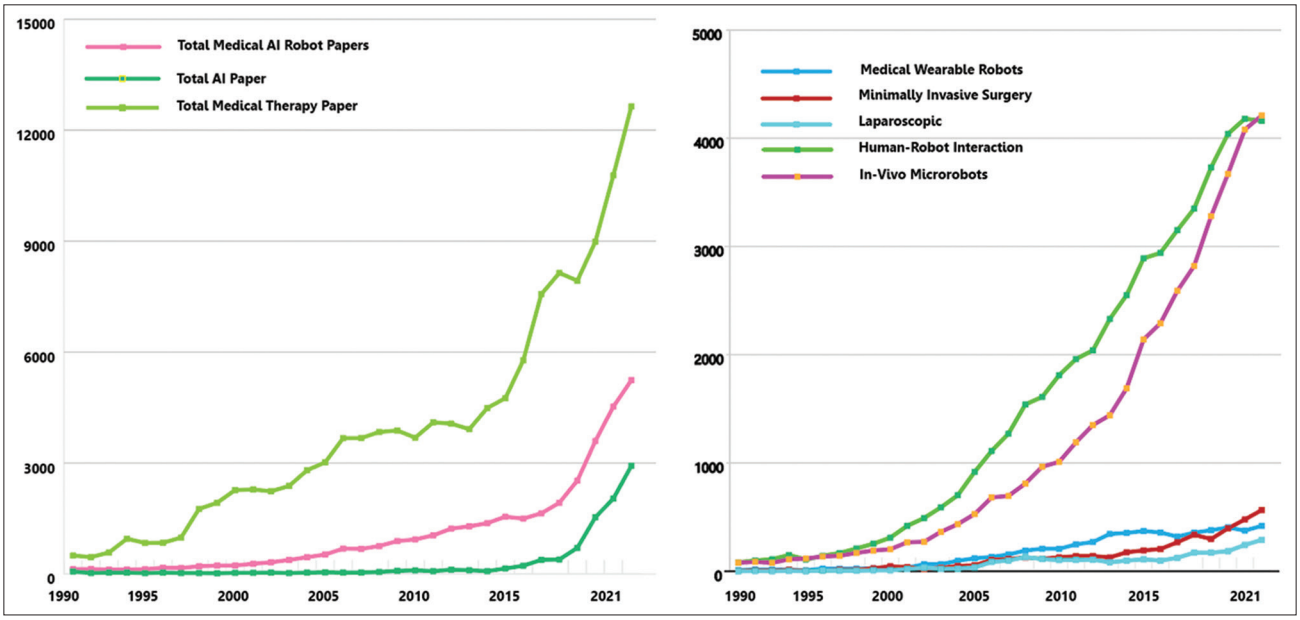

Figure 6. The left curves describe the total artificial intelligence, total medical therapy, and total medical applications combined with AI-ART papers published each year. The right curves are the trending topics associated with AI-ART medical therapies and applications. From the highest two trends of the right chart, we extract the two most trending topics: Human-robot interaction for automation or telesurgery and *in vivo* microrobots.

possesses the ability to generate candidate structures to sense different surfaces and materials. The signals collected from the sensing system can be sent to processing neural networks to extract the valid tactile sensing patterns.

3.2. Multi-sensor fusion

Recognizing and understanding the various behaviors of surgeons under different scenarios may improve the cognition and assistive ability of medical robots. The traditional data fed to assistive medical robots are often single modal, such as robotic imaging information from CT or MRI[16]. One of the focuses of current research on human-robot interaction is sensor fusion based on multi-modality information collected during or after the surgery process. For instance, in a surgery with the assistance of a laparoscopic robot, the robot must be able to comprehend the term "hemostasis" spoken by the surgeon, segment the images collected by the camera sensors mounted on the robot and obtain semantic understanding of organs or tissues of the patient, recognize the bleeding vessels, and use the correct size of hemostatic forceps to implement the action within a short period of time. The construction of a multimodal information framework is necessary for flexible interfaces of various sensor types, accuracy improvement, haptic sensation, diverse vital signs, surgeons' spoken words and gestures, and decoupling of internal modules and external software interfaces. Concrete gesture recognition incorporates the kinematics of grippers, grasping different tissues, with proper fine-grained tissue or organ segmentation under different surgical types[77,78]. Multi-sensor fusion has greatly contributed to the commercial translation from AI-ART, such as the combination of CT and MRI to obtain more precise spatial scanning results in relation to the location of lesions. However, CT and MRI images require diverse configuration parameters under different conditions to ensure a better fusion. Therefore, more emphasis should be placed on adaptive fusion by AI-ART.

3.3. Augmented reality for telemanipulated medical robot

In the past 3 years, COVID-19 has posed unprecedented challenges to both, patients who have underlying diseases and surgeons with long-distance or safety isolation concerns. As a result of the considerable advancements in both, AI-ART and hardware computing power, medical applications integrated with augmented reality are growing exponentially[57]. In cardiovascular surgery, complicated anatomical structures make the surgery more challenging. For instance, in obstructive hypertrophic cardiomyopathy, the surgeon needs to open a small slot without damaging the upper cardiac aorta, which is only a few millimeters away from it. Therefore, in cases such as this, the interactive augmented reality technique supported by robotic endoscope camera provides useful functions, such as magnified stereo imaging of the ventricle internal structure, dynamic segmentation of ventricle parts, and automatic annotation on the screen for surgical decision-making. Another challenge to accurate segmentation and annotation is the deformable tissue and organs at the operation area. The 2D shape silhouettes of the tissue or organs are extracted from images from a monocular camera to assist the 3D deformable registration models through several projective constraints of multiview geometry[53,57,76]. The allocation of communication channels by AI-ART, such as 5G, ensures real-time video transfer of the surgery process. This lays a solid foundation for telemanipulated medical robots. Simulated sensing is obtained through the tactile sensing technique equipped on these robots. AI-ART also enables multi-views of a patient through cameras mounted at different positions. The fusion of these multi-views, in which the images are stitched together, forms a panoramic view by convolutional neural network (CNN)[50,78].

4. Challenges and directions

4.1. Future challenges

The past decades have witnessed the tremendous progress of AI-ART in the fabrication of various medical robots, such as laparoscopy surgical robots, single-port laparoscopy robots, naked-eye imaging laparoscopy, capsule robots, wearable medical robots, and so on, as shown in Figure 7. Due to the breakthrough of parallel computation chips, such as graphics processing unit (GPU) and field programmable gate array (FPGA), as well as artificial intelligence algorithms like multi-layer perceptron (MLP), convolutional neural network (CNN), deep learning (DL), and the latest knowledge distillation techniques, various medical therapies and applications have been uncovered.

New surgical tool manipulation modeling and navigation methods are made feasible with more powerful artificial intelligence algorithms and computation hardware. However, the main obstacle to surgical robot automation is the interaction between surgeons and robots. Another challenge lies in *in vivo* microrobots, which have shown potential for target drug and cell delivery, bacteria killing, vascular cleanup, and other therapeutic applications. The 6-DoF motion control and navigation of the microrobots *in vivo* are pushed forward by the rotating magnetic field technique. The challenges are listed below.

i. Vision segmentation accuracy and robustness: Is the medical robot's vision algorithm capable of precisely and robustly segmenting dynamic tissue or organs from complex backgrounds with the given required metrics? This is crucial in automatic surgery and human-robot interaction.

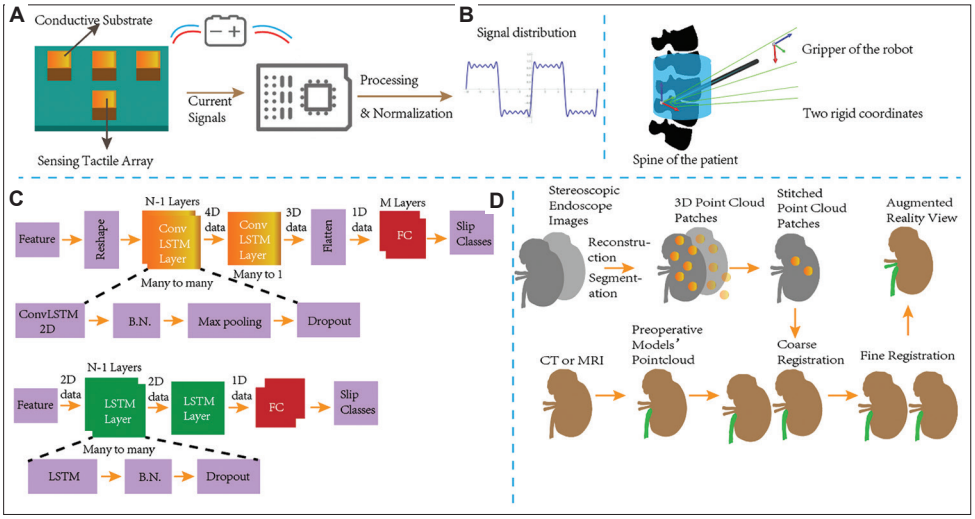

Figure 7. Trending topics and advancements over the past decades. (A) 3-DoF force feedback apparatus[76]. (B) Spinal operation robot with AI-ART constrained by a safe light cylinder workspace[78]. (C) Tactile sensing feature processing neural networks from[50]. (D) Non-marker virtual reality (VR) navigation approach[57].

ii. 6-DoF motion control: How to guarantee the robustness of real-time 6-DoF motion control, especially when the microrobot is approaching the target position?
iii. Smarter tactile sensation of surgical robot: How to apply appropriate pinching force onto different tissues or organs during surgery to prevent post-operative complications?
iv. Multi-modal data fusion: How to better fuse data generated from different sensors, such as vision sensor, robotic arms control, MRI, and ultrasound, to obtain a better integration for navigation *in vivo* or imaging?
v. Binding mechanism of nanoparticles and microrobots: In target drug or cell delivery scenarios, how to bind nanoparticles efficiently with the organic membrane of the transporter microrobot without mismatching?
vi. Formal self-verification of AI-ART: How to verify the output through AI-ART used by surgical robots, especially the recognition or segmentation results?

4.2. Technical directions

It is challenging to predict the long-term technical direction for surgical therapies and applications using AI-ART, but it is possible to predict the mid-term technical direction based on the established trends, as shown in Figure 8.

1) Gripper contact force sensing would not directly rely on the progress of AI-ART but rather the development of the structural design of the force sensor and on novel material producing electrical signals. Researchers are now seeking for arbitrary shape coverage and human-like simultaneous pressure and temperature sensation. Hence, the novel calibration methods and concrete application scenarios using AI-ART would be fairly different from the previous routines.
2) Since the navigation, control, computation, and surgical tool functional modules are suitable for spatially independent mounting, the distributed architecture of laparoscopy surgical robots would be the mainstream design for *in vitro* surgical robots. The development of 5G in the coming years would bring about improvements to the complex arrangement of cables inside surgical robots and linking modules, eventually replacing them with wireless communication.
3) In the next decade, single-port laparoscopy would progressively erode the market of natural orifice surgical robots due to its reaching limitations and the significant physical (orifice) and psychological discomforts associated with the procedure. Single-port laparoscopy also requires peritoneal membrane penetration with an extra incision.
4) The navigation of AI-ART would continue to progress toward robust segmentation and reliable depth value estimation. This fundamental technique also serves as the basis of self-propelling *in vivo* robots for target cell or drug delivery and the advancement human-robot interaction.
5) The technical directions of artificial wearable kidney could be divided into the advancements of new dialysis material and the methods of using less dialysate. Wearable chronic disease management would embody remote databases, cloud computing platforms, and the monitoring devices worn by patients, such as ECG monitor and medical nanosensor network.

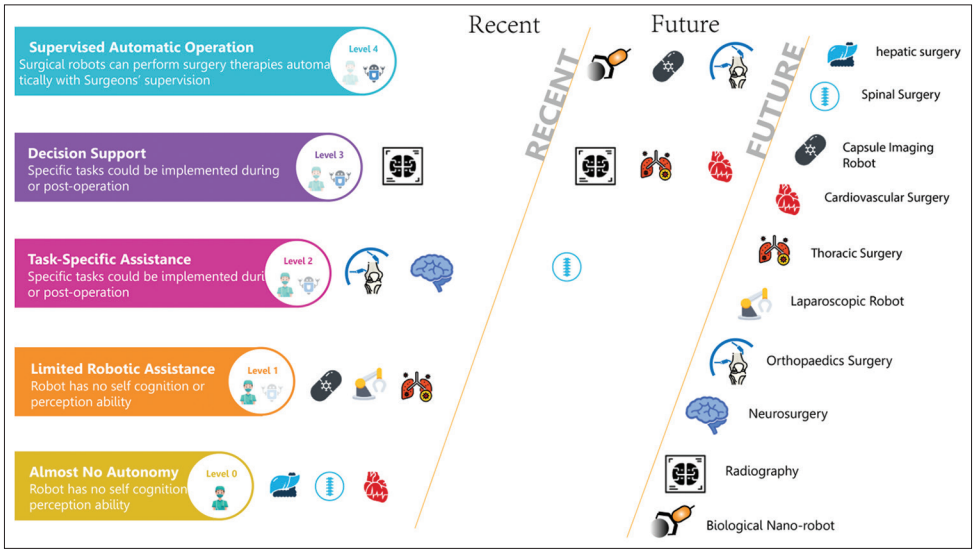

Figure 8. Recent advancements and future technical directions of medical robots with higher autonomy. The left column represents the recent stages and applications of surgical therapies with AI-ART. The middle column represents the future surgical applications with more automated assistance. The right column shows the annotations of the graphic icons adopted in left and middle columns.

6) Target drug and cell delivery microrobots weave another promising area for precision medicine, instead of the traditional one-size-fits-all approach. To ensure the goal of using the right treatment (targeted treatment) for the right patients at the right time, the patients' genetic information and the genetic profile of specific tumors would be taken into account by microrobots carrying suitable doses of drugs or functional cells.

5. Conclusion

To bridge the gap between AI-ART and commercial applications, a taxonomy of major scenarios of AI-ART was derived with three divisions: Surgery automation, minimally invasive surgery, and medical wearable robots. Surgery automation mainly focuses on the progress and challenges of artificial intelligence-assisted techniques used in automated surgeries, such as gripper contact force sensing, navigation, and collaborative research platform. Considering that minimally invasive surgery results in rapid recovery and less physical injury, we discuss its development from four aspects: Self-propelling robots, surgical robots through natural orifice, high-precision retinal surgical robots, and single-port laparoscopy. In light of the pressing demand for chronic disease management and wearable life support equipment, we also explore the applications of AI-ART in artificial kidneys and ECG monitoring, as well as the use of AI as a powerful analysis for chronic diseases. By ranking the research results, we identify the latest trend of AI-ART as human-robot interaction. Although we have witnessed the great achievements in AI-ART, there are still many challenges ahead. Accurate vision segmentation and localization would affect the quality of surgery automation. Meanwhile, the efficiency and reliability of drug or cell delivery at certain time and location within the body are determined by the spatial six-DoF control of self-propelling robots. Based on the problems encountered both, in research and commercial applications, we also discuss the promising technical directions for AI-ART.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Huating Li, Bin Sheng
Formal analysis: Jinyang Wang, Lei Zhu
Investigation: Jinyang Wang, Lei Zhu, Ping Li
Methodology: Po Yang, Bin Sheng
Writing – original draft: Jinyang Wang, Lei Zhu

Writing – review & editing: Jinyang Wang, Ping Li, Huating Li, Bin Sheng

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this work are available from the corresponding author on reasonable request.

References

1. Tottori S, Zhang L, Qiu F, *et al*., 2012, Magnetic helical micromachines: Fabrication, controlled swimming, and cargo transport. *Adv Mater*, 24: 811–816.

   https://doi.org/10.1002/adma.201103818

2. Huang TY, Qiu F, Tung HW, *et al*. 2014, Generating mobile fluidic traps for selective three-dimensional transport of microobjects. *Appl Phys Lett*, 105: 114102.

   https://doi.org/10.1063/1.4895937

3. Swangnetr M, Kaber DB. 2013, Emotional state classification in patient–robot interaction using wavelet analysis and statistics-based feature selection. *IEEE Trans Hum Mach Syst*, 43: 63–75.

   https://doi.org/10.1109/tsmca.2012.2210408

4. Menciassi A, Melzer A, Dumont E, *et al*., 2014, Robotics and Machine Learning Approaches to Improve robustness of USgFUS: Futura. *J Ther Ultrasound*, 2: A25–A25.

   https://doi.org/10.1186/2050-5736-2-s1-a25

5. Svensson CM, Krusekopf S, Lücke J, *et al*., 2014, Automated Detection of Circulating Tumor Cells with Naive Bayesian Classifiers. *Cytometry A*, 85: 501–511.

   https://doi.org/10.1002/cyto.a.22471

6. Lyndon D, Kumar A, Kim J, *et al*., 2015, Convolutional Neural Networks for Medical Clustering. In: CLEF.

7. Romero J, Diago LA, Shinoda J, *et al*., 2015, Evaluation of Brain Models to Control a Robotic Origami arm Using Holographic Neural Networks.

   https://doi.org/10.1115/detc2015-48074

8. Kondo T, Ueno J, Takao S. 2015, Medical Image Diagnosis of Liver Cancer by Hybrid Feedback GMDH-type Neural Network Using Principal Component-regression Analysis. *Artif Life Robot*, 20: 145–151.

   https://doi.org/10.1007/s10015-015-0213-1

9. Kondo T, Ueno J, Takao S. 2015, Logistic GMDH-type Neural Network Using Principal Component-regression Analysis and its Application to Medical Image Diagnosis of Lung Cancer. *Artif Life Robot*, 20: 137–144.

   https://doi.org/10.1007/s10015-015-0200-6

10. Garza-Burgos M, Sanchez-Orozco E, Bayro-Corrochano E. 2016, Medical Robot Vision Using the Conformai Geometric Algebra Framework. In: 2016 IEEE-RAS 16th International Conference on Humanoid Robots (Humanoids). p1087–1093.

    https://doi.org/10.1109/humanoids.2016.7803406

11. Nokata N, Kato S, Feng LK, *et al*., 2016, Measurement of Mechanical Characteristics for Soft Materials by Using Medical Robot with Piezoelectric Tactile Sensors. In: 2016 International Symposium on Micro-NanoMechatronics and Human Science (MHS). Piscataway: IEEE. p1–4.

    https://doi.org/10.1109/mhs.2016.7824164

12. Nguyen PB, Park JO, Park S, *et al*., 2016, Medical Micro-robot Navigation Using Image Processing-blood Vessel Extraction and X-ray Calibration. In: 2016 6th IEEE International Conference on Biomedical Robotics and Biomechatronics (BioRob). Piscataway: IEEE. p365–370.

    https://doi.org/10.1109/biorob.2016.7523653

13. Miyashita S, Guitron S, Yoshida K, *et al*. Ingestible, Controllable, and Degradable Origami Robot for Patching Stomach Wounds. In: 2016 IEEE International Conference on Robotics and Automation (ICRA). Piscataway: IEEE. p909–916.

    https://doi.org/10.1109/icra.2016.7487222

14. Jiang G, Luo M, Bai K, *et al*., 2017, A Precise Positioning Method for a Puncture Robot Based on a Pso-optimized bp Neural Network Algorithm. *Appl Sci*, 7: 969.

    https://doi.org/10.3390/app7100969

15. Sarikaya D, Corso JJ, Guru KA. 2017, Detection and Localization of Robotic Tools in Robot-assisted Surgery Videos Using Deep Neural Networks for Region Proposal and deTection. *IEEE Trans Med Imaging*, 36: 1542–1549.

    https://doi.org/10.1109/tmi.2017.2665671

16. Tang L, Qian J, Li L, *et al*., 2017, Multimodal Medical Image Fusion Based on Discrete Tchebichef Moments and Pulse Coupled Neural Network. *Int J Imaging Syst Technol*, 27: 57–65.

    https://doi.org/10.1002/ima.22210

17. Jayanthi PR, Bommannaraja K, Manju R. 2017, Early Detection of Macular Edema in Fundus Image Datasets Using Neural Network.

18. Namozov A, Cho YI. 2018, Convolutional Neural Network Algorithm with Parameterized Activation Function for Melanoma Classification. In: 2018 International Conference on Information and Communication Technology Convergence (ICTC). p417–419.

https://doi.org/10.1109/ictc.2018.8539451

19. Qamar S, Jin H, Zheng R, *et al*., 2018, 3d Hyper-dense Connected Convolutional Neural Network for Brain Tumor Segmentation. In: 2018 14th International Conference on Semantics, Knowledge and Grids (SKG). p123–130.

https://doi.org/10.1109/skg.2018.00024

20. Lu Y, Yu Q, Gao Y, *et al*., 2018, Identification of Metastatic Lymph Nodes in MR Imaging with Faster Region-Based Convolutional Neural Networks. *Cancer Res*, 78: 5135–5143.

https://doi.org/10.1158/0008-5472.can-18-0494

21. Alom MZ, Yakopcic C, Taha T, *et al*., 2018, Nuclei Segmentation with Recurrent Residual Convolutional Neural Networks based U-Net (R2U-Net). In: NAECON 2018- IEEE National Aerospace and Electronics Conference. p228-233.

https://doi.org/10.1109/naecon.2018.8556686

22. Sim Y, Chung MJ, Kotter E, *et al*., 2019. Deep Convolutional Neural Network-based Software Improves Radiologist Detection of Malignant Lung Nodules on Chest Radiographs. *Radiology*, 294: 182465.

https://doi.org/10.1148/radiol.2019182465

23. Pratt H, Coenen F, Harding SP, *et al*., 2019, Feature Visualisation of Classification of Diabetic Retinopathy Using a Convolutional Neural Network. In: CEUR Workshop Proceedings. p23–29.

24. Parakh A, Lee H, Lee JH, *et al*., 2019, Urinary Stone Detection on ct Images Using Deep Convolutional Neural Networks: Evaluation of Model Performance and Generalization. Radiology. *Artif Intell*, 1: e180066.

https://doi.org/10.1148/ryai.2019180066

25. Yin C, Qian B, Wei J, *et al*., 2019, Automatic Generation Of Medical Imaging Diagnostic Report with Hierarchical Recurrent Neural Network. In: 2019 IEEE International Conference on Data Mining (ICDM). p728–737.

https://doi.org/10.1109/icdm.2019.00083

26. Zhang K, Zhou X, Wu J. 2019, U-module: Better Parameters Initialization of Convolutional Neural Network for Medical Image Classification. In: 2019 IEEE International Conference on Image Processing (ICIP). p799–803.

https://doi.org/10.1109/icip.2019.8803799

27. Lee H, Jang M, Kim HC, *et al*., 2019, Association of Imaging Factors Derived from Convolutional Neural Network with Visual Outcomes in Age-related Macular degeneration and Polypoidal Choroidal Vasculopathy. *Sci Rep*, 9: 1–9.

https://doi.org/10.1038/s41598-019-56420-z

28. Weng J, Ding YJ, Hu C, *et al*. Meta-neural-network for Real-time and Passive Deep-Learning-based Object Recognition. *Nat Commun*, 11: 6309.

https://doi.org/10.1038/s41467-020-19693-x

29. Shafiei SB, Lone Z, Elsayed AS, *et al*., 2020, Identifying Mental Health Status Using Deep Neural Network Trained by Visual Metrics. *Trans Psychiatry*, 10: 430.

https://doi.org/10.1038/s41398-020-01117-5

30. Fu F, Wei J, Zhang M, *et al*., 2020, Rapid Vessel Segmentation and Reconstruction of Head and Neck Angiograms Using 3d Convolutional Neural Network. *Nat Commun*, 11: 4829.

https://doi.org/10.1038/s41467-020-18606-2

31. Ribeiro AH, Ribeiro MH, Paixão GM, *et al*., 2020, Automatic Diagnosis of the 12-lead ECG Using a Deep Neural Network. *Nat Commun*, 11: 1760.

https://doi.org/10.1038/s41467-020-15432-4

32. Noyan MA, Durdu M, Eskiocak AH. 2020, Tzancknet: A Convolutional Neural Network to Identify Cells in the Cytology of Erosive-vesiculobullous Diseases. *Sci Rep*, 10: 18314.

https://doi.org/10.1101/2020.06.22.20137570

33. Le Page AL, Ballot E, Truntzer C, *et al*., 2021, Using a Convolutional Neural Network for Classification of Squamous and Non-squamous Non-small Cell Lung Cancer Based on Diagnostic Histopathology Hes Images. *Sci Rep*, 11: 23912.

https://doi.org/10.21203/rs.3.rs-646715/v1

34. Kanakasabapathy MK, Thirumalaraju P, Kandula H, *et al*., 2021, Adaptive Adversarial Neural Networks for the Analysis of Lossy and Domain-shifted Datasets of Medical Images. *Nat Biomed Engin*, 5: 571–585.

https://doi.org/10.1038/s41551-021-00733-w

35. Elmarakeby HA, Hwang JH, Arafeh R, *et al*., 2021, Biologically Informed Deep Neural Network for Prostate Cancer Discovery. *Nature*, 598: 348–352.

https://doi.org/10.1038/s41586-021-03922-4

36. Lu H, Uddin S. 2021, A Weighted Patient Network-based Framework for Predicting Chronic Diseases Using Graph Neural Networks. *Sci Rep*, 11: 22607.

https://doi.org/10.1038/s41598-021-01964-2

37. Ahmed S, Muhammod R, Khan Z, *et al*., 2020, Acp-MHCNN: An Accurate Multi-headed Deep-convolutional Neural Network to Predict Anticancer Peptides. *Sci Rep*, 11: 313668.

https://doi.org/10.1101/2020.09.25.313668

38. Xu W, Zhang H, Yuan H, *et al*., 2021, A Compliant Adaptive Gripper and its Intrinsic Force Sensing Method. *IEEE Trans Robot*, 37: 1584–1603.

https://doi.org/10.1109/tro.2021.3060971

39. Ebihara Y, Shichinohe T, Kurashima Y, *et al*., 2021, Laparoscopic Real-time Vessel Navigation Using

Indocyanine Green Fluorescence During Laparoscopy-assisted Gastric Tube Reconstruction: First Experience. *J Minim Access Surg*, 17: 576–579.

https://doi.org/10.4103/jmas.jmas_210_20

40. Li X, Zhong J, Wang Y, *et al*., 2021, Rapid, Accurate, Multifunctional and Self-assisted Vision Assessment and Screening with Interactive Desktop Autostereoscopy. *Ann Trans Med*, 9: 23.

https://doi.org/10.21037/atm-20-3555

41. Yin L, Wang Y, Zhan J, *et al*., 2022, Chest-scale self-compensated Epidermal Electronics for Standard 6-precordial-lead ECG. *npj Flex Electron*, 6: 1–9.

https://doi.org/10.1038/s41528-022-00159-7

42. Shitiri E, Cho HS. 2021, A Tdma-based Data Gathering Protocol for Molecular Communication Via Diffusion-based Nano-sensor Networks. *IEEE Sens J*, 21: 19582–19595.

https://doi.org/10.1109/jsen.2021.3091494

43. Jahromi AM, Khedri M, Ghasemi M, *et al*., 2021, Molecular Insight into COF Monolayers for Urea Sorption in Artificial Kidneys. *Sci Rep*, 11: 12085.

https://doi.org/10.1038/s41598-021-91617-1

44. Suzuki H, Wood RJ. 2020, Origami-inspired miniature manipulator for teleoperated microsurgery. *Nat Mach Intell*, 2: 437–446.

https://doi.org/10.1038/s42256-020-0203-4

45. Matsunaga T, Ohnishi K, Wada N, *et al*., 2019, Development of Small-diameter Haptic Flexible Gripping Forceps Robot. *IEEE J Trans Ind Appl*, 2019.

https://doi.org/10.1002/eej.23269

46. Kwak B, Choi S, Maeng J, *et al*., 2021, Marangoni Effect Inspired Robotic Self-propulsion Over a Water Surface Using a Flow-imbibition-powered Microfluidic Pump. *Sci Rep*, 11: 1–13.

https://doi.org/10.1038/s41598-021-96553-8

47. Zhang Y, Yang J, Hou X, *et al*., 2022, Highly Stable Flexible Pressure Sensors with a Quasi-homogeneous Composition and Interlinked Interfaces. *Nat Commun*, 13: 1317.

https://doi.org/10.1038/s41467-022-29093-y

48. Barragan JA, Yang J, Yu D, *et al*., 2022, A Neurotechnological Aid for Semi- autonomous Suction in Robotic-assisted Surgery. *Sci Rep*, 12: 4504.

https://doi.org/10.21203/rs.3.rs-1021937/v1

49. Chun S, Kim JS, Yoo YH, *et al*., 2021, An Artificial Neural Tactile Sensing System. *Nat Electron*, 4: 429–438. https://doi.org/10.1038/s41928-021-00585-x

50. Massari L, Fransvea G, D'Abbraccio J, *et al*., 2022, Functional Mimicry of Ruffini Receptors with Fiber Bragg Gratings and Deep Neural Networks Enables a Bio-inspired Large-area Tactile Sensitive Skin. ArXiv, abs/2203.12752.

https://doi.org/10.1038/s42256-022-00487-3

51. Kim TH, Bao C, Chen Z, *et al*. 3d Printed Leech-inspired Origami Dry Electrodes for Electrophysiology Sensing Robots. *npj Flex Electron*, 6: 1–10.

https://doi.org/10.1038/s41528-022-00139-x

52. Lee S, Kim S, Kim S, *et al*., 2018, A Capsule-type Microrobot with Pick-and-drop Motion for Targeted Drug and Cell Delivery. *Adv Healthc Mater*, 7: 1700985.

https://doi.org/10.1002/adhm.201700985

53. Huaulmé A, Despinoy F, Perez SA, et al., 2019, Automatic annotation of surgical activities using virtual reality environments. *Int J Comput Assist Radiol Surg*, 14: 1663–1671.

https://doi.org/10.1007/s11548-019-02008-x.

54. Lu B, Chu HK, Huang KC, *et al*., 2019, Vision-based Surgical Suture Looping Through Trajectory Planning for Wound Suturing. *IEEE Trans Automa Sci Eng*, 16: 542–556.

https://doi.org/10.1109/tase.2018.2840532

55. Borra D, Andalò A, Paci M, *et al*., 2020, A fully Automated Left Atrium Segmentation Approach from Late Gadolinium Enhanced Magnetic Resonance Imaging Based on a Convolutional Neural Network. *Quant Imaging Med Surg*, 10: 1894–1907.

https://doi.org/10.21037/qims-20-168

56. Wang J, Yue C, Wang G, *et al*., 2022, Task Autonomous Medical Robot for Both Incision Stapling and Staples Removal. *IEEE Robot Autom Lett*, 7: 3279–3285.

https://doi.org/10.1109/lra.2022.3141452

57. Zhang X, Wang J, Wang T, *et al*., 2019, A Markerless Automatic Deformable Registration Framework for Augmented Reality Navigation of Laparoscopy Partial Nephrectomy. *Int J Comput Assist Radiol Surg*, 14: 1285–1294.

https://doi.org/10.1007/s11548-019-01974-6

58. Hannaford B, Rosen J, Friedman DW, *et al*., 2013, Raven-ii: An Open Platform for Surgical Robotics Research. *IEEE Trans Biomed Eng*, 60: 954–959.

https://doi.org/10.1109/tbme.2012.2228858

59. Kazanzides P, Chen Z, Deguet A, *et al*., 2014, An Open-source Research Kit for the da Vinci® Surgical System. In: *2014 IEEE International Conference on Robotics and Automation* (*ICRA*). p6434–6439.

https://doi.org/10.1109/icra.2014.6907809

60. Steiner JA, Pham LN, Abbott JJ, *et al*., 2021, Modeling and Analysis of a Soft Endoluminal Inchworm Robot Propelled by a Rotating Magnetic Dipole Field. *J Mech Robot*, 14: 11.

https://doi.org/10.1115/1.4053114

61. Pham LN, Steiner JA, Leang KK, *et al*., 2020, Soft Endoluminal Robots Propelled by Rotating Magnetic Dipole Fields. *IEEE Trans Med Robot Bionic*, 2: 598–607.

https://doi.org/10.1109/tmrb.2020.3027871

62. Kaouk JH, Haber GP, Goel RK, *et al*., 2010, Pure Natural Orifice Translumenal Endoscopic Surgery (Notes) Transvaginal Nephrectomy. *Eur Urol*, 57: 723–726.

https://doi.org/10.1016/j.eururo.2009.10.027

63. Rahimy E, Wilson J, Tsao TC, *et al*., 2013, Robot-assisted Intraocular Surgery: Development of the Iriss and Feasibility Studies in an Animal Model. *Eye*, 27: 972–978.

https://doi.org/10.1038/eye.2013.105

64. Wavhale R, Dhobale KD, Rahane CS, *et al*., 2021, Water-powered Self-propelled Magnetic Nanobot for Rapid and Highly Efficient Capture of Circulating Tumor Cells. *Commun Chem*, 4: 1–9.

https://doi.org/10.1038/s42004-021-00598-9

65. Shen T, Hennings DL, Nelson CA, *et al*., 2018, Performance of a Multifunctional Robot for Natural Orifice Transluminal Endoscopic Surgery. *Surg Innov*, 25: 364–373.

https://doi.org/10.1177/1553350618781225

66. Shen T, Hennings DL, Nelson CA, *et al*., 2018, Performance of a Multifunctional Robot for Natural Orifice Transluminal Endoscopic Surgery. *Surg Innov*, 25: 364–373.

https://doi.org/10.1177/1553350618781225

67. Bai W, Wang Z, Cao Q, *et al*., 20225, Anthropomorphic Dual-arm Coordinated Control for a Single-port Surgical Robot Based on Dual-step Optimization. *IEEE Trans Med Robot Bionic*, 4: 72–84.

https://doi.org/10.1109/tmrb.2022.3145673

68. Wang F, Toombs NJ, Kesavadas T, *et al*., 2019, Mechanical Design and Modeling of a Manipulator Tool for a Compact Multiple-tool Single Port Laparoscopic Robot Platform. 2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). p5836–5841.

https://doi.org/10.1109/embc.2019.8857539

69. Hueso M, Navarro E, Sandoval D, *et al*., 2018, Progress in the Development and Challenges for the use of Artificial Kidneys and Wearable Dialysis Devices. *Kidney Dis* (*Basel*), 5: 3–10.

https://doi.org/10.1159/000492932

70. Budhathoki-Uprety J, Shah J, Korsen JA, *et al*. Synthetic Molecular Recognition Nanosensor Paint for Microalbuminuria. *Nat Commun*, 10: 3605.

https://doi.org/10.1038/s41467-019-11583-1

71. Siontis KC, Noseworthy PA, Attia ZI, *et al*., 2021, Artificial Intelligence- enhanced Electrocardiography in Cardiovascular Disease Management. *Nat Rev Cardiol*, 18: 465–478.

https://doi.org/10.1038/s41569-020-00503-2

72. Siontis KC, Noseworthy PA, Attia ZI, *et al*., 2021, Artificial Intelligence- enhanced Electrocardiography in Cardiovascular Disease Management. *Nat Rev Cardiol*, 18: 465–478.

https://doi.org/10.1038/s41569-020-00503-2

73. Hussein SE, Chen P, Medeiros LJ, *et al*., 2022, Artificial Intelligence-assisted Mapping of Proliferation Centers Allows the Distinction of Accelerated Phase from Large Cell Transformation in Chronic Lymphocytic Leukemia. *Mod Pathol*, 35: 1121–1125.

https://doi.org/10.1038/s41379-022-01015-9

74. Bayoumy K, Gaber M, Elshafeey A, *et al*., 2021, Smart Wearable Devices in Cardiovascular Care: Where we are and How to Move Forward. *Nat Rev Cardiol*, 18: 581–599.

https://doi.org/10.1038/s41569-021-00522-7

75. Himmelfarb J, Ratner B. 2020, Wearable Artificial Kidney: Problems, Progress and Prospects. *Nat Rev Nephrol*, 16: 558–559.

https://doi.org/10.1038/s41581-020-0318-1

76. Mintchev S, Salerno M, Cherpillod A, *et al*., 2019, A Portable Three-degrees-of-freedom force Feedback Origami Robot for Human-robot Interactions. *Nat Mach Intell*, 1: 584–593.

https://doi.org/10.1038/s42256-019-0125-1

77. Heiligenstein X, Paul-Gilloteaux P, Belle M, *et al*., 2017, Ec-clem: Flexible Multidimensional Registration Software for Correlative Microscopies with Refined Accuracy Mapping. *Microsc Microanal*, 23: 360–361.

https://doi.org/10.1017/s1431927617002483

78. Pore A, Corsi D, Marchesini E, *et al*., 2021, Safe Reinforcement Learning Using Formal Verification for Tissue Retraction in Autonomous Robotic-assisted Surgery. In: 2021 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS). p4025–4031.

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REVIEW ARTICLE

Genetic and non-genetic risk factors of idiopathic pulmonary fibrosis: A review

Shamil R. Zulkarneev[1], Rustem H. Zulkarneev[1]*, Gulnaz Faritovna Korytina[2,3], Irshat A. Gibadullin[4], Arthur M. Avzaletdinov[4], Zhihui Niu[5], Jiayu Guo[5], Yulia Genadievna Aznabaeva[1], Guzel M. Nurtdinova[1], and Naufal Shamilevich Zagidullin[1]

[1]Department of Internal Diseases, Bashkir State Medical University, Ufa, 450008, Russian Federation

[2]Institute of Biochemistry and Genetics - Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences, IBG UFRC RAS, Laboratory of Physiological Genetics, Ufa, 450054, Russian Federation

[3]Department of Biology, Bashkir State Medical University, Ufa, 450008, Russian Federation

[4]Department of Hospital Surgery, Bashkir State Medical University, Republic of Bashkortostan, Ufa, 450008, Russian Federation

[5]Department of Pharmacology (State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education), College of Pharmacy, Harbin Medical University, Harbin, Heilongjiang 150081, China

Abstract

Idiopathic pulmonary fibrosis (IPF) is the most common form of fibrosis of internal organs. The etiology and pathogenesis of IPF are still not well understood. However, a growing line of evidence shows that both genetic and non-genetic factors contribute to IPF development. The release of pro-inflammatory cytokines activates the immune cells. The enhanced synthesis of interleukins and cytokines, especially transforming growth factor β1 leads to the proliferation of fibroblasts, increased extracellular matrix formation, and epithelial-mesenchymal transformation of the lung tissue. These pathological changes could lead to fibrosis. Polymorphisms of genes responsible for the function of mucociliary clearance (*MUC5B*), telomerases (*TERT*, *TERC*), as well as signaling pathway related-genes such as Sonic hedgehog, *Wnt,* and some other genes are also risk factors for IPF development. Epigenetic regulatory mechanisms, such as methylation and acetylation of DNA and histones, may also influence the development and progression of this disease. At present, the role of non-coding RNAs, in particular long non-coding RNAs (lncRNA) in the development of fibrotic processes, is actively studied. LncRNA is an RNA that is longer than 200 base pairs and does not code for any proteins. LncRNAs perform various functions in the cell, from nuclear compartmentation to epigenetic regulation of gene expression and post-translational modification of proteins. In this review, we present the important aspects in the pathogenesis of IPF.

Keywords: Long non-coding RNAs; Idiopathic pulmonary fibrosis, COVID-19-induced pulmonary fibrosis

***Corresponding author:**
Rustem H. Zulkarneev
(zurustem@mail.ru)

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1. Introduction

Fibrosis is a pathological process of wound healing in which connective tissue replaces the normal parenchymal tissue by increasing extracellular matrix synthesis and proliferation of fibroblasts. This leads to a considerable level of tissue remodeling and formation of permanent scar on tissues and organs, contributing to the remodeling of organ and damages to its histo- and cyto-architecture. In clinical setting, signs of functional disorders or even organ failure could be observed in patients with this condition. In short, fibrosis is the outcome of chronic inflammation, frequent tissue damage followed by proliferation processes, systemic connective tissue diseases, autoimmune diseases, tissue necrosis, and atrophy due to ischemia and metabolic disorders[1,2].

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease characterized by the destruction of the acinar structure, the growth of the extracellular matrix, and other properties of the fibrotic disease, and is histologically similar to interstitial pneumonia. Respiratory failure develops in patients with IPF, which in some cases could be fatal. IPF shares some similarities with COVID-19-induced pulmonary fibrosis. However, the mechanisms of the occurrence of both IPF and COVID-19-induced pulmonary fibrosis are poorly understood. Of particular scientific interest are the mechanisms that are somehow associated with the function of long non-coding RNA (lncRNA), the role of which in the occurrence and progression of fibrotic processes in various organs is being actively studied[3,4]. The discovery of new lncRNA-associated molecular mechanisms that are responsible for the pathogenesis of IPF and COVID-19-induced pulmonary fibrosis would facilitate the development of diagnostic systems for predicting the risk and severity of the disease and the formulation of therapeutic measures which can target key pathogenic factor with minimal side effects.

IPF is the most common form of visceral fibrosis. According to meta-analyses, the incidence of IPF in Europe and North America is 3 – 9 cases/100,000 population per year (according to other sources, up to 18 cases[5]), whereas <4 cases/100,000 population per year were reported in South America and East Asia. The prevalence of the disease in North America is 10 – 60 cases/100,000 population per year. In total, there are about 3 million patients with IPF worldwide, while there is an increase in the number of patient visits to hospitals and the frequency of deaths. In addition, IPF is more common in men, with a median age of 65 years[6,7].

The goal of the review is to summarize the genetic and non-genetic risk factors that contribute to IPF development and the role of lncRNA in this disease.

2. Non-genetic factors in the development and progression of IPF

2.1. Risk factors and pathogenesis

Approximately 1/3 of new IPF cases and the progression cases are etiologically linked to non-genetic factors. The main risk factors for IPF are older age, male sex, smoking, and living in unfavorable environmental surroundings. Aging of type 1 alveolocytes and fibroblasts, metabolic dysfunction of cellular proteins, and damage of subcellular structures are potential risk factors of lung tissue fibrosis[6]. Such alterations lead to the release of mediators from epithelium, endothelium and connective tissue cells that activate immunocompetent cells, such as polymorphic nuclear leukocytes, lymphocytes, monocytes, and macrophages (Figure 1). When the vascular wall is impaired and the blood coagulation cascade is activated, factor X is able to induce the differentiation of lung myofibroblasts, and thrombin (factor II) activates the production of the chemokine (C-C motif) ligand 2, along with low molecular weight hyaluronic acid (LMWHA) and inflammatory mediators, such as interleukin (IL)-1β, IL-6, IL-25, IL-33, which are chemoattractant for myelocytes and monocytes that could further differentiate into macrophages[8].

2.2. The role of macrophages in fibrosis development

Under the influence of LMWHA, interferon gamma (IFN-γ), ligands of toll-like receptors, macrophages are activated along the classical pathway. The secreted active forms of nitrogen, oxygen, tumor necrosis factor alpha (TNF-α) and IL-1β are strong tissue pro-inflammatory agents. IL-1β is responsible for the transition of epithelial cells to mesenchymal cells, as well as for the induction of myofibroblasts. TNF-α stimulates the expression of IL-6, an autocrine stimulator of fibroblast growth. There is also an alternative pathway for macrophage activation, which is induced by granulocyte-macrophage colony-stimulating factor, IL-4, and IL-13. As a result, the expression of the enzyme arginase-1 (Arg1) increases, as demonstrated in in vitro and murine experiments. This leads to the increase of L-proline concentration, which is necessary for the synthesis of collagen fibers[9].

2.3. The role of other immune cells in fibrosis development

Fibroblast growth factor (FGF)-2, FGF-10, FGF-9, and FGF-18 may also contribute to the development of IPF[10]. The role of eosinophils in the development of pulmonary fibrosis in allergy as well as IPF is attributed to their ability to synthesize transforming growth factor beta 1 (TGF-β1) and IL-13, which was confirmed in a study on mice[8,9]. According to a meta-analysis by Wynn and Ramalingam[1],

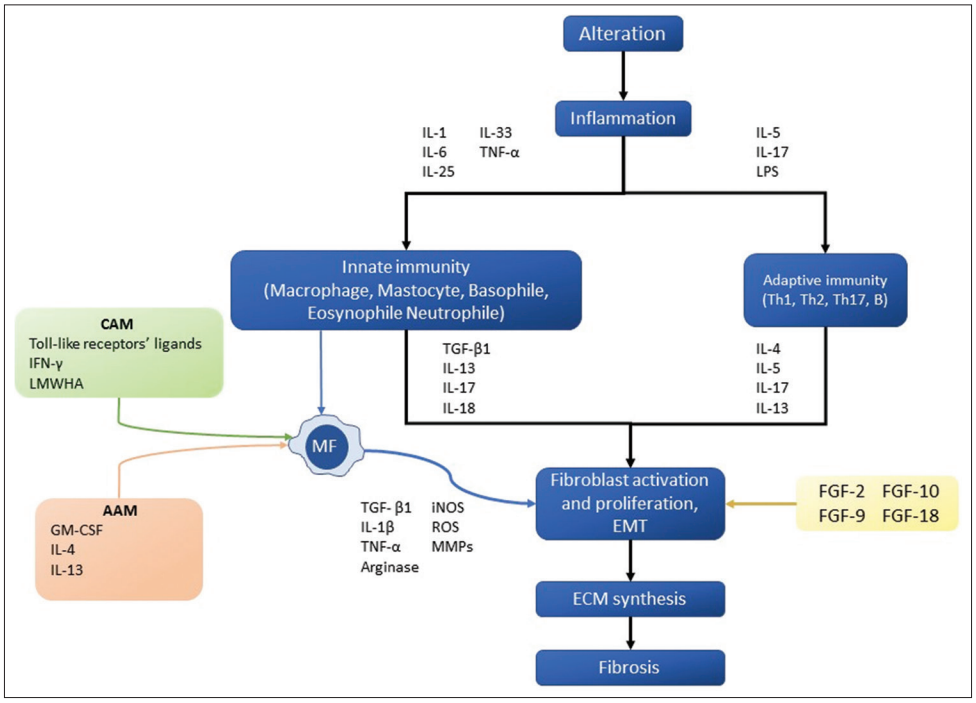

Figure 1. Immune mechanisms of fibrosis development. During inflammation, the release of interleukins, cytokines and other substances that activate both innate and acquired immunity occurs. The corresponding immune cells also synthesize interleukins and cytokines that stimulate the differentiation and proliferation of fibroblasts, ECM synthesis, and EMT of alveolocytes. An important role is played by classically or alternatively activated macrophages, since they are able to increase inflammation not only due to released cytokines, but also due to metalloproteinases, iNOS, ROS, and other enzymes that damage normal lung tissue. In summary, these factors lead to lung fibrosis. AAM: Alternatively activated macrophage; CAM: Classically activated macrophage; FGF: Fibroblast growth factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; iNOS: Inducible nitric oxide synthase; LMWHA: Low molecular weight hyaluronic acid; LPS: Lipopolysaccharide; MF: Macrophage; MMPs: Metalloproteinases; ROS: Reactive oxygen species; TGF-β1: Transforming growth factor beta 1; TNF-α: Tumor necrosis factor α.

lymphocytes have a significant impact on the development of fibrotic processes. Thus, a subpopulation of $CD4^+$ Th17 cells secretes IL-17A, which has a significant effect on the synthesis of TGF-β and causes persistent neutrophilia. Th2 produces IL-13, which activates TGF-β synthesis and fibroblast proliferation. IL-4 and IL-5 also have a profibrotic effect. In contrast, IFN-γ produced by Th1 cells inhibits TGF-β and fibroblast proliferation.

2.4. TGF-β and fibrosis development

TGF-β is a polypeptide expressed in many organs and tissues during ontogenesis. TGF-β regulates the processes of cell proliferation, differentiation and apoptosis. In this case, the signal is transmitted to the nucleus with the help of SMAD 2/3 proteins. There are three isoforms of TGF-β, with TGF-β1 isoform being the most active in the pathogenesis of IPF. Specifically, the overexpression of *TGF-β1* in type 2 alveolocytes leads to hyperplasia, while in fibroblasts, the overexpression influences cell proliferation and increases synthesis of extracellular matrix. Of note, the level of TGF-β sharply increases with age[10]. The induction of epithelial-mesenchymal transformation (EMT) in mouse type 1 alveolocytes was also proven *in vitro* when they were co-cultivated with M2 alveolar macrophages[11].

3. Genetic factors in the development and progression of IPF

3.1. Profibrotic genes

Genetic factors account for up to a third of the risk factor for the development of IPF. The role of *TERT* and *TERC* encoding telomerase and its various components that are responsible for telomere synthesis has been well studied. The excessive shortening of telomeres in mesenchymal cells and type 2 alveolocytes accelerated their death, which in turn increases the risk of developing pulmonary fibrosis[1,5-7]. Mutations in the *TINF2*, *DKC1*, *RTEL1*, *PARN,* and *NAF1* genes that regulate telomerase function have been found in 25% of patients with IPF[7]. Polymorphism in the promoter of the mucin *(MUC5B)* gene, which is responsible for the function of mucociliary clearance, results in an increased risk of developing sporadic and hereditary pulmonary fibrosis[5,6]. Variants of the Toll-interacting protein *(TOLLIP)* gene (which encodes an inhibitor of *TGF-β*, a regulator of the toll-

like receptor-mediated signaling pathway of innate immunity) also contribute to the onset of the disease[6,7]. The role of the group of *Wnt* genes encoding a family of 19 glycoproteins has been studied. Glycoproteins bind to Frizzeled-receptors on the cytoplasmic cell membrane and activate the β-catenin-mediated signaling pathway, leading to the upregulation of the expression of T-cell factor genes, matrix metalloproteinases (MMPs), oncogenes, cell cycle regulators, and angiogenic growth factors. The second signaling pathway, which is calcium- and protein kinase C-dependent, regulates the differentiation of red bone marrow cells, cell migration and their polarity, which are modulators of embryonic development of tissues and their regeneration in response to damage. An association has been found between activation of the β-catenin-mediated signaling pathway and increased expression of IL-1β in type 2 alveolocytes, MMP-7 activation, increased profibrotic activity, and fibroblast proliferation. The latter was also found in the *Wnt5a* gene when it activated the second signaling pathway. There are scientific data on the contribution of Wnt1, Wnt7b, Wnt10b, and Frizzeled-2 and -3 receptors in the development of human IPF, which was further confirmed in murine experiments[10]. In mice, Wnt1-induced protein 1 increased proliferation of type 2 alveolocytes, EMT of lung and renal epithelial cells, and enhanced synthesis of extracellular substance[1]. The Sonic hedgehog (SHH) gene family is responsible for the morphogenesis of many organs, including the lungs. Signal transduction into the cell is carried out using SHH protein and three groups of receptors, which are cell-surface receptors Ptch1 and Ptch2, transmembrane protein Smo, and DNA-binding proteins-"zinc fingers" Gli1, Gli2, Gli3. Increased *SHH* signal transduction exacerbates the course of pulmonary fibrosis; an increase in fibroblast proliferation and resistance to apoptosis was confirmed *in vitro*[12] (Table 1).

Table 1. Genetic factors that contribute to IPF pathogenesis

Genetic factors	Examples
Genes	*TERT, TERC*[1,5-7] *TINF2, DKC1, RTEL1, PARN, NAF1*[7] *MUC5B*[5,6] *TOLLIP*[6,7] *Wnt gene family*[10] *SHH gene family*[12] *NOCH1, FBXO32*[19]
DNA methylation	Histones: H3K9[17], H3K27[25], MBD2[18] Genes and other DNA sequences: Thy-1 promoter region[20], *COX-2*[21], *14ARF*[22], promoters of *SFRP1* and *SFRP4*[23], *SMAD7, NOCH1, FBXO32*[19]
DNA acetylation	HDAC2, HDAC4[30] HDAC3[31] HDAC8[32]
LncRNAs	*PFAR*[3] *MALAT1*[4] *H19*[50-52] *MEG3*[53] *TERRA*[54] *PVT1*[55] *HOXAAS3*[56] *PFAL*[57] *DNM3OS*[58] *ZFAS1*[59] *FENDRR*[51]

HDAC: Histone deacetylase

3.2. DNA methylation

DNA methylation is the most common form of epigenetic modification, which may not only regulate gene expression, but also play a role in various life activities, such as embryonic development, aging and tumor formation. In recent years, DNA methylation has been proven to be involved in the process of multiple organ fibrosis. The in-depth studies of methylation in pulmonary fibrosis[13], renal fibrosis[14], and myocardial fibrosis[15] have confirmed that DNA methylation plays an important role in fibrosis development by regulating the expression of key genes[16].

It has been reported that changes in DNA methylation in CPG islands are related to the pathogenesis of IPF. DNA methylation can block the binding of transcription factors to cognate DNA sequences, thereby preventing the transcriptional repression of fibrotic gene expression. Coward *et al.*[17] found that histone H3 lysine 9 (H3K9) methylation inhibits the expression of the anti-fibrotic gene C-X-C motif chemokine ligand 10. Methylated DNA can also specifically bind to methyl-binding-proteins and recruit joint repressors, thereby inhibiting fibrosis-related genes. For example, Wang *et al.*[18] found that methyl-CPG-binding domain 2 (MBD2) stimulates the differentiation of fibroblasts to muscle tissue by binding to the methylated *CPG* site, the Erdr1 promoter, and inhibiting the expression and transcription of its downstream genes. Differentiation of fibroblasts leads to fibrotic processes. Clinical data have shown that fibroblast genes in patients with pulmonary fibrosis are abnormally methylated at multiple *CPG* sites in the genome of patients compared with those in healthy subjects, and the degree of abnormal DNA methylation in patients with fibrosis at different stages is different: Hypomethylation was associated with increased expression of profibrotic genes (*NOCH1*, *FBXO32*, and *TOLLIP*), whereas hypermethylation was associated with decreased expression of profibrotic genes[19].

Despite the low expression, many genes are hypermethylated in IPF patients, such as Thy-1 promoter region[20], cyclooxygenase-2 (*COX-2*)[21], *P14ARF*[22], and so on. Significantly hypermethylated promoters of *SFRP1* and

SFRP4 have been reported to result in impaired transcription and decreased expression, which in turn affects fibrosis progression[23]. The reduction of hypermethylated genes in IPF patients promotes the activation of fibroblasts and accelerates the fibrosis process. Methyltransferase also plays an important role in the process of fibrosis. Elkouris *et al.*[24] found that the methyltransferase Set9 binds to E3 ligase by promoting *SMAD7* methylation, thereby inducing ubiquitin-dependent degradation of SRSF7 and enhancing TGF-β signaling. Inhibition of DNA methyltransferases can alleviate pulmonary fibrosis by reducing abnormal DNA methylation using a classic inhibitor, 5-aza-2′-deoxycytidine. Among the regulatory mechanisms of pulmonary fibrosis, methylations of histone H3 lysine 27 (H3K27) and histone H3 lysine 9 (H3K9) are the most common histone methylations. H3K27 methylation is mainly catalyzed by the histone-lysine N-methyltransferase EZH2 and inhibited by histone demethylases KDM5, KDM6A and KDM6B[25]. Methylation of H3K9 is catalyzed by G9a or G9a analogs[26].

A variety of disease-specific biomarkers that are used for detecting diseases clinically are based methylation; for instance, *SDC2* methylation detection kits are used to detect colorectal cancer[27], but there is no mature DNA methylation biomarker for diagnosis of pulmonary fibrosis. Therefore, in-depth exploration of the mechanism of DNA methylation in pulmonary fibrosis is of great significance for the diagnosis, treatment, and prognosis of pulmonary fibrosis.

3.3. DNA acetylation

Histone acetylation is one of the most common modifications of histone tails, which regulate DNA accessibility to various transcriptional factors to control gene expression[28]. Acetyl groups are conjugated to lysine by histone acetyltransferases and removed from lysine by histone deacetylases (HDACs)[29]. It has been reported that HDAC plays an important role in setting up the imbalance of histone acetylation/deacetylation, and is the main driving force for the progression of pulmonary fibrosis. HDAC2 is mainly involved in the chronic progression of pulmonary fibrosis, while HDAC4 is mainly involved in the early stress response of pulmonary fibrosis[30]. HDAC3 promotes EMT, inflammation, and pulmonary fibrosis development by activating Notch1 and STAT1 signaling[31]. Saito *et al.* observed an increased expression level of HDAC8 in IPF lung tissue as well as in TGF-β1-treated normal human lung fibroblasts, and HDAC8 inhibitors could be employed as potential treatment of IPF as well as other fibrotic lung diseases[32]. In addition, ERK5 plays a key role in TGF-β1-induced pulmonary fibrosis by enhancing Smad3 acetylation[33]. Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, is currently approved for clinical treatment of cancer. Meanwhile, SAHA was found to be able to induce apoptosis in IPF myofibroblasts[34]. Therefore, histone acetylation has a key epigenetic regulatory role in the pathogenesis of pulmonary fibrosis, which may facilitate the development of novel therapeutic strategies against IPF (Table 1).

3.4. Non-coding RNAs (ncRNAs) and pulmonary fibrosis

3.4.1. Classification and functions of non-coding RNAs

To date, according to the NONCODE (available from: http://www.noncode.org) database of non-coding RNA (ncRNA), approximately more than 170,000 ncRNAs and about 96,000 genes encoding the ncRNAs exist in humans. Conventionally, ncRNAs are classified as "housekeeping ncRNAs," which are directly involved in the processes of protein synthesis, splicing, telomerase activity (mRNA, tRNA, rRNA, small nuclear RNA [snRNA], etc.), and as "regulatory ncRNAs," such as microRNA (miRNA), small interfering RNA (siRNA), enhancer RNA (eRNA), circular RNA (circRNA), and lncRNA, which regulate transcription and post-transcriptional processes of cells. LncRNAs are single-stranded RNA containing more than 200 base pairs. They are divided into several groups: (i) Intergenic lncRNA, which is transcribed from both DNA strands in intergenic regions; (ii) intron lncRNA, which is transcribed from introns of protein-coding genes; (iii) overlapping lncRNA, which is transcribed from the sense strand of DNA, overlapping with protein-coding genes; and (iv) antisense lncRNA, which is transcribed from the antisense strand, overlapping with exon or intron regions[35]. LncRNAs can also be divided into cis-acting lncRNAs that regulate nearby genes and trans-acting lncRNAs that regulate distant genes. In addition, lncRNAs can be spliced to form both short ncRNAs, such as miRNA, piRNA, and lncRNA isoforms[36]. LncRNAs have a variety of functions, for example, nuclear lncRNAs are involved in the enhancement and silencing of transcription, chromatin remodeling, and compartmentalization of the nucleus. In the cytoplasm, lncRNAs inhibit miRNAs (being competing endogenous RNAs for miRNAs), post-translational modification of the protein structure and formation of a "framework" for proteins of various signaling pathways, maintenance of mitochondrial homeostasis, regulation of pre-mRNA splicing (lncRNA *NEAT1*), and stabilization of intercellular contacts through interaction with membrane complexes PECAM1, p120 catenin[1,36-39].

3.4.2. LncRNAs in pathogenesis of diseases

Given such an active role of lncRNA, it is not surprising that they are involved in the pathogenesis of many diseases, especially cancers. For example, increased level of lncRNA

HOXA expression was observed in breast, stomach, pancreatic cancer, and hepatocellular carcinoma[40]; lncRNA *LUCAT1* was observed in breast, ovarian, thyroid cancer, and renal cell carcinoma[41]; and lncRNA *MALAT1* was observed in cancer of the breast, prostate, colon, liver, and uterus[37], etc. An association was found between increased expression of lncRNA *TP53TG1* and increased migration and proliferation of hepatocellular carcinoma cells[42], progression of retinoblastoma (by *miR-33b* [miRNA-33b] binding)[43], and development of pancreatic duct adenocarcinoma[44]. Activation of lncRNA *LINC00342* resulted in inhibition of *miR-545-5p*, subsequent overexpression of *CNPY2*, and progression of gastric cancer[45]. A study of Shen *et al.*[46] observed an association between increased expression of *LINC00342*, sequestration of *miR-19a-3p*, and progression of colorectal cancer. Chen *et al.*[47] found an increase in *LINC00342* expression in non-small cell lung cancer tissue; the binding of this lncRNA to *miR-203a-3p* led to increased cell proliferation, migration, and invasion. Furthermore, *LINC00342* overexpression and *miR-384* binding led to the development of thyroid cancer[48]. In turn, in gastric cancer, a decrease in lncRNA *RP11-363E7.4* expression is observed, and functional experiments performed by Chen *et al.*[49] showed the presence of antitumor activity during overexpression of this lncRNA when it affects the signaling pathways tp53, Bax/Bcl-2, and β-catenin.

3.4.3. LncRNAs in pulmonary fibrosis pathogenesis

There are studies confirming the role of lncRNA in the development of pulmonary fibrosis (Table 2). Overexpression of lncRNA *PFAR* was observed in a mouse model of bleomycin-induced pulmonary fibrosis, with *PFAR* being the competing endogenous RNA (*ceRNA*) for *miR-138*. Knockdown of *PFAR* caused attenuation of TGF-β1-induced fibrosis[3]. Yan *et al.* demonstrated the antifibrotic role of *miR-503* in pulmonary silicosis and the profibrotic role of lncRNA *MALAT1* (ceRNA for *miR-503*)[4]. The *miR-29b*-binding *H19* expression was reported to be upregulated in mice with bleomycin-induced pulmonary fibrosis, which was accompanied by an increase in the expression of *COL1A1* (responsible for the synthesis of the collagen I alpha-1 chain). Furthermore, *H19* bound *miR-196a* and *miR-140 in vitro* and *in vivo*, which led to the progression of TGF-β1- and bleomycin-induced fibrosis[50]. An analysis of the role of some lncRNAs in the development of lung diseases yielded findings as follows: *H19* sequesters hsa-*miR-140* to cause an increase in the expression of *TGF-β1* and sequester *hsa-miR-196* to enhance the proliferation and migration of fibroblasts[51,52]. *MEG3* upregulates *TP63*, *STAT3*, *KRT14*, *YAP1*, *AXL*, *TP53*, *EZH2*, and *TGF-β* genes to promote the migration of young alveolar epitheliocytes and prevent their final differentiation, which can affect tissue remodeling in IPF[53]. *Lnc TERRA* contains telomerase sequences and reduces telomerase activity. This leads to mitochondrial dysfunction in alveolar epitheliocytes, thereby contributing to fibrosis[54]. *FENDRR* can inhibit fibroblast activation and reduce pulmonary fibrosis by taking up aconitase 1, thereby reducing iron levels and sequestering profibrotic hsa-miR-214[51]. LncRNA *PVT1*, which sequesters *miR-497-5p*, has a profibrotic effect in mouse models with lung silicosis[55]. LncRNA *Hoxaas3* induced by TGF-β1/SMAD 4 signaling pathway promotes the activation of fibroblasts and EMT by inhibiting the action of *miR-450b-5p*[56]. LncRNA *PFAL* was found to have profibrotic activity, which manifested itself upon the inhibition of *miR-18a*. There was an increase in migration, proliferation of fibroblasts, EMT, and synthesis of intercellular substance, which was proven in laboratory mice with pulmonary fibrosis and activated by TGF-β1 fibroblast as shown in cell culture experiments[57]. LncRNA *DNM3OS*, whose expression is activated by both TGF-β1- and Wnt-mediated signaling pathways is the precursor

Table 2. Molecular targets of lncRNAs and their effect on lung fibrosis

LncRNA	Molecular targets	Effect
PFAR[3]	miR-138	Profibrotic
MALAT1[4]	miR-503	
H19[50-52]	miR-29b	
	miR-196a	
	miR-140	
	hsa-miR-196	
MEG3[53]	TP63, STAT3, KRT14, YAP1, AXL, TP53, EZH2, TGF-β genes	
TERRA[54]	Telomerase	
PVT1[55]	miR-497-5p	
HOXAAS3[56]	miR-450b-5p	
PFAL[57]	miR-18a	
DNM3OS[58]	Predecessor of miR199a-5p/3p, miR-214-3p	
ZFAS1[59]	miR-150-5p	Antifibrotic
FENDRR[51]	hsa-miR-214	
	Aconitase 1	

DNM3OS: DNM3 (Dynamine 3) opposite strand/antisense RNA, FENDRR: FOXF1 (Forkhead Box F1) adjacent non-coding developmental regulatory RNA, H19:H19 imprinted maternally expressed transcript, HOXAAS3:HOXA (Homeobox A Cluster) antisense RNA 3, MALAT1:metastasis-associated lung adenocarcinoma transcript 1, MEG3: Maternally expressed 3, PFAL: Pulmonary fibrosis-associated lncRNA, PFAR: Pancreatic fibrosis-associated lncRNA, PVT1: Plasmacytoma variant translocation 1, TERRA: Telomeric-repeat-containing RNA, ZFAS1: ZNFX1 (Zinc Finger NFX1-Type Containing 1) antisense RNA 1

of three microRNAs: *miR199a-5p*, *miR199a-3p,* and *miR-214-3p*, which are formed during *DNM3OS* splicing and enhance TGF-β1 signaling through TGF-β1/SMAD and TGF-β1/β-catenin pathway, leading to the development and progression of fibrosis[58]. Inhibition of lncRNA *ZFAS1* resulted in a decrease in lipid peroxidation (LPO), TGF-β1-activated migration of HFL1 fibroblasts, and a more favorable course of bleomycin-induced pulmonary fibrosis in mice due to the sequestration of *miR-150-5p*, which has inhibitory activity against *SLC38A1* (LPO controller)[59]. Li *et al.*[60] identified groups of genes whose co-expression is associated with both SARS-CoV-2 infection and the development of IPF, while their expression is largely regulated by *m6A* (N6-methyladenosine), which is one of the most common mRNA modifications in mammalian cells.

Patients with IPF are more susceptible to COVID-19, and patients who have had this infectious disease are at an increased risk of developing pulmonary fibrosis, which, in addition to gene expression features, is due to the presence of immune cell infiltrate in the lung tissue, represented by natural killer cells, mast cells, M2-macrophages, and gamma delta T (γδT) cells that secret profibrotic cytokines. The development of pulmonary fibrosis after COVID-19 is also facilitated by high levels of IL-6, IL-1, TGF-β1, TNF-α, and other pro-inflammatory cytokines, which are secreted as a result of viral infection[61,62].

4. IPF therapy and biomarkers

At present, two pharmacological drugs are used for the treatment of pulmonary fibrosis. However, they are only able to slow down the progression of the disease. Pirfenidone is a modified pyridine molecule that reduces collagen synthesis by fibroblasts, suppresses TGF-β1 and TNF-α, and has an antifibrotic and antioxidant effect. Nintedanib is an inhibitor of receptors with tyrosine kinase activity, namely receptors for endothelial growth factor 1 – 3 (EGF 1 – 3), receptors for FGF 1 – 3, and platelet-derived growth factor receptor a and b (PDGFRA, PDGFRB), which suppresses proliferation, fibroblast migration, and differentiation into myofibroblasts. Both of them have been proven to be effective during phase 3 clinical trials[63,64].

Pentraxin 2 is one of the plasma acute phase proteins. It suppresses the transformation of monocytes into macrophages and fibrocytes, as well as inhibits the synthesis of TGF-β1. The level of pentraxin 2 is reduced in patients with IPF; at present, the recombinant protein drug is at phase 3 clinical trials[63,65].

Pamrevlumab is a monoclonal antibody against connective tissue growth factor. The drug is now at phase 3 clinical trials. Based on earlier phases of the clinical trials, pamrevlumab was found to reduce mortality and improve lung function in patients taking this drug, as compared with the placebo group[64].

GLPG1690 is an inhibitor of autotoxin, an enzyme that hydrolyses lysophosphatidylcholine to lysophosphatidic acid, which has a profibrotic effect. Clinical trials of this inhibitor were terminated due to unsatisfactory safety profile[63].

TD139 is an inhibitor of galectin-3, a profibrotic protein receptor on the cell membrane of macrophages. This drug is undergoing phase 2b clinical trials[66].

There are a number of drugs that could inhibit the activity of TGF-β1, JAK 1, JAK 2, JAK 3, ROCK2, HSP47, JNK, NOX1, and NOX4 signaling pathways (drugs rhPTX-2/PRM-151, Jaktinib Dihydrochloride Monohydrate, KD025/SLx-2119, ND-L02-s0201/BMS-986263, CC-90001, GKT137831, respectively). These drugs are undergoing phase 2 clinical trials. The promising treatment is the use of small interfering RNAs, in particular TRK-250, which suppresses the expression of the TGF-β1 gene (Phase 1 clinical trials). The possibility of using monoclonal antibodies against ILs, lysyl oxidase, integrins, and leukotriene antagonists for IPF therapy is being studied[63,65].

Biomarkers to differentiate IPF patients from healthy people include Krebs von den Lungen (KL-6), a high-molecular weight glycoprotein on the surface of alveolar epithelium, chitinase-like protein (YKL40), surfactant proteins, mainly (SP)-A, -D, less -B, lysyl oxidase-like proteins, and genetic markers, such as polymorphisms of the *MUC5B*, *TERT*, and *TERC* genes. MMP1 and MMP7 are also prognostic markers; their concentration in the blood are correlated with the severity of the disease[65,67,68]. Circulating immune cells can also be a biomarker of IPF; a higher level of monocytes in blood is correlated with more severe IPF type and increased mortality risk[69].

5. Conclusion

The development of pulmonary fibrotic processes involves both genetic mechanisms (genes encoding signaling pathway proteins that activate fibroblast proliferation and extracellular matrix synthesis) and non-genetic mechanisms (immune) featuring the secretion of TGF-β, pro-inflammatory, and profibrotic cytokines. LncRNAs, being an important epigenetic regulator, also contribute to the development of pulmonary fibrosis, including the idiopathic variant, which is presented in the current review.

At present, our research group is studying the role of lncRNAs *TP53TG1*, *LINC00342*, *RP11-363E7.4* and others in the pathogenesis of IPF and COVID-19-induced

lung fibrosis. We are currently in the process of lncRNA extraction and studying their expression profile in peripheral blood leukocytes and lung tissue for further comparison with clinical parameters. The study of lncRNA-mediated mechanisms of fibrosis development is an important and urgent task, since many lncRNAs, including those we have studied, are potential disease biomarkers and targets for specific pharmacological therapy. In addition, they can serve as diagnostic and prognostic targets for early detection and prediction of the course of idiopathic and COVID-19-induced pulmonary fibrosis.

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Conflict of interest

None of the authors has conflicts of interest to report with regard to this manuscript.

Author contributions

Conceptualization: Rustem H. Zulkarneev, Gulnaz Faritovna Korytina, Naufal Shamilevich Zagidullin

Funding acquisition: Gulnaz Faritovna Korytina, Naufal Shamilevich Zagidullin

Project administration: Naufal Shamilevich Zagidullin

Writing – original draft: Shamil R. Zulkarneev, Rustem H. Zulkarneev, Gulnaz Faritovna Korytina, Irshat A. Gibadullin, Arthur M. Avzaletdinov, Zhihui Niu, Jiayu Guo, Yulia Genadievna Aznabaeva, Guzel M. Nurtdinova, Naufal Shamilevich Zagidullin

Writing – review & editing: Shamil R. Zulkarneev, Rustem H. Zulkarneev, Gulnaz Faritovna Korytina, Naufal Shamilevich Zagidullin.

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References

1. Wynn TA, Ramalingam TR, 2012, Mechanisms of fibrosis: Therapeutic translation for fibrotic disease. *Nat Med*, 18: 1028–1040.

   https://doi.org/10.1038/nm.2807

2. Rockey DC, Bell PD, Hill JA, 2015, Fibrosis a common pathway to organ injury and failure. *N Engl J Med*, 372: 1138–1149.

   https://doi.org/10.1056/NEJMra1300575

3. Zhao X, Sun J, Chen Y, *et al*., 2018, LncRNA PFAR promotes lung fibroblast activation and fibrosis by targeting miR-138 to regulate the YAP1-twist axis. *Mol Ther*, 26: 2206–2217.

   https://doi.org/10.1016/j.ymthe.2018.06.020

4. Yan W, Wu Q, Yao W, *et al*., 2017, MiR-503 modulates epithelial-mesenchymal transition *in silica*-induced pulmonary fibrosis by targeting PI3K p85 and is sponged by lncRNA MALAT1. *Sci Rep*, 7: 11313.

   https://doi.org/10.1038/s41598-017-11904-8

5. Richeldi L, Collard HR, Jones MG, 2017, Idiopathic pulmonary fibrosis. *Lancet*, 389:1941–1952.

   https://doi.org/10.1016/S0140-6736(17)30866-8

6. Lederer DJ, Martinez FJ, 2018, Idiopathic pulmonary fibrosis. *N Engl J Med*, 378: 1811–1823.

   https://doi.org/10.1056/NEJMra1705751

7. Martinez FJ, Collard HR, Pardo A, *et al*., 2017, Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers*, 3: 17074.

   https://doi.org/10.1038/nrdp.2017.74

8. Hirahara K, Aoki A, Morimoto Y, *et al*., 2019, The immunopathology of lung fibrosis: Amphiregulin-producing pathogenic memory T helper-2 cells control the airway fibrotic responses by inducing eosinophils to secrete osteopontin. *Semin Immunopathol*, 41: 339–348.

   https://doi.org/10.1007/s00281-019-00735-6

9. Henderson NC, Rieder F, Wynn TA, 2020, Fibrosis: From mechanisms to medicines. *Nature*, 587: 555–566.

   https://doi.org/10.1038/s41586-020-2938-9

10. Chanda D, Otoupalova E, Smith SR, *et al*., 2019, Developmental pathways in the pathogenesis of lung fibrosis. *Mol Aspects Med*, 65: 56–69.

    https://doi.org/10.1016/j.mam.2018.08.004

11. Zhu L, Fu X, Chen X, *et al*., 2017, M2 macrophages induce EMT through the TGF-β/Smad2 signaling pathway. *Cell Biol Int*, 41: 960–968.

    https://doi.org/10.1002/cbin.10788

12. Kugler MC, Joyner AL, Loomis CA, *et al*., 2015, Sonic hedgehog signaling in the lung. From development to

disease. *Am J Respir Cell Mol Biol*, 52: 1-13.

https://doi.org/10.1165/rcmb.2014-0132TR

13. Yang IV, Pedersen BS, Rabinovich E, *et al.*, 2014, Relationship of DNA methylation and gene expression in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*, 190: 1263–1272.

https://doi.org/10.1164/rccm.201408-1452OC

14. Bechtel W, McGoohan S, Zeisberg EM, *et al.*, 2010, Methylation determines fibroblast activation and fibrogenesis in the kidney. *Nat Med*, 16: 544–550.

https://doi.org/10.1038/nm.2135

15. Xu X, Tan X, Tampe B, *et al.*, 2015, Epigenetic balance of aberrant Rasal1 promoter methylation and hydroxymethylation regulates cardiac fibrosis. *Cardiovasc Res*, 105: 279–291.

https://doi.org/10.1093/cvr/cvv015

16. Pastore F, Bhagwat N, Pastore A, *et al.*, 2020, PRMT5 inhibition modulates E2F1 methylation and gene-regulatory networks leading to therapeutic efficacy in JAK2V617F-mutant MPN. *Cancer Discov*, 10: 1742–1757.

https://doi.org/10.1158/2159-8290.CD-20-0026

17. Coward WR, Brand OJ, Pasini A, *et al.*, 2018, Interplay between EZH2 and G9a regulates CXCL10 gene repression in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*, 58: 449–460.

https://doi.org/10.1165/rcmb.2017-0286OC

18. Wang Y, Zhang L, Huang T, *et al.*, 2022, The methyl-CpG-binding domain 2 facilitates pulmonary fibrosis by orchestrating fibroblast to myofibroblast differentiation. *Eur Respir J*, 27: 2003697.

https://doi.org/10.1183/13993003.03697-2020

19. Yang IV, Pedersen BS, Rabinovich E, *et al.*, 2014, Relationship of DNA methylation and gene expression in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*, 190: 1263–1272.

https://doi.org/10.1164/rccm.201408-1452OC

20. Sanders YY, Pardo A, Selman M, *et al.*, 2008, Thy-1 promoter hypermethylation: A novel epigenetic pathogenic mechanism in pulmonary fibrosis. *Am J Respir Cell Mol Biol*, 39: 610–618.

https://doi.org/10.1165/rcmb.2007-0322OC

21. Sun L, Xu A, Li M, *et al.*, 2021, Effect of methylation status of lncRNA-MALAT1 and MicroRNA-146a on pulmonary function and expression level of COX2 in patients with chronic obstructive pulmonary disease. *Front Cell Dev Biol*, 9: 667624.

https://doi.org/10.3389/fcell.2021.667624

22. Cisneros J, Hagood J, Checa M, *et al.*, 2012, Hypermethylation-mediated silencing of p14(ARF) in fibroblasts from idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol*, 303: L295–L303.

https://doi.org/10.1152/ajplung.00332.2011

23. Zhou J, Yi Z, Fu Q, 2019, Dynamic decreased expression and hypermethylation of secreted frizzled-related protein 1 and 4 over the course of pulmonary fibrosis in mice. *Life Sci*, 218: 241–252.

https://doi.org/10.1016/j.lfs.2018.12.041

24. Elkouris M, Kontaki H, Stavropoulos A, *et al.*, 2016, SET9-mediated regulation of TGF-β signaling links protein methylation to pulmonary fibrosis. *Cell Rep*, 15: 2733–2744.

https://doi.org/10.1016/j.celrep.2016.05.051

25. Jiang Y, Xiang C, Zhong F, *et al.*, 2021, Histone H3K27 methyltransferase EZH2 and demethylase JMJD3 regulate hepatic stellate cells activation and liver fibrosis. *Theranostics*, 11: 361–378.

https://doi.org/10.7150/thno.46360

26. Irifuku T, Doi S, Sasaki K, *et al.*, 2016, Inhibition of H3K9 histone methyltransferase G9a attenuates renal fibrosis and retains klotho expression. *Kidney Int*, 89: 147–157.

https://doi.org/10.1038/ki.2015.291

27. Müller D, Győrffy B, 2022, DNA methylation-based diagnostic, prognostic, and predictive biomarkers in colorectal cancer. *Biochim Biophys Acta Rev Cancer*, 1877: 188722.

https://doi.org/10.1016/j.bbcan.2022.188722

28. Zhang X, Liu H, Zhou JQ, *et al.*, 2022, Modulation of H4K16Ac levels reduces pro-fibrotic gene expression and mitigates lung fibrosis in aged mice. *Theranostics*, 12: 530–541.

https://doi.org/10.7150/thno.62760

29. Schuetze KB, McKinsey TA, Long CS, 2014, Targeting cardiac fibroblasts to treat fibrosis of the heart: Focus on HDACs. *J Mol Cell Cardiol*, 70: 100–107.

https://doi.org/10.1016/j.yjmcc.2014.02.015

30. Li M, Zheng Y, Yuan H, *et al.*, 2017, Effects of dynamic changes in histone acetylation and deacetylase activity on pulmonary fibrosis. *Int Immunopharmacol*, 52: 272–280.

https://doi.org/10.1016/j.intimp.2017.09.020

31. Zheng Q, Lei Y, Hui S, *et al.*, 2022, HDAC3 promotes pulmonary fibrosis by activating NOTCH1 and STAT1 signaling and up-regulating inflammasome components AIM2 and ASC. *Cytokine*, 153: 155842.

https://doi.org/10.1016/j.cyto.2022.155842

32. Saito S, Zhuang Y, Suzuki T, *et al.*, 2019, HDAC8 inhibition ameliorates pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol*, 316: L175–L186.

https://doi.org/10.1152/ajplung.00551.2017

33. Kim S, Lim JH, Woo CH, 2013, ERK5 inhibition ameliorates pulmonary fibrosis via regulating Smad3 acetylation. *Am J Pathol*, 183: 1758–1768.

https://doi.org/10.1016/j.ajpath.2013.08.014

34. Barnes PJ, Adcock IM, Ito K, 2005, Histone acetylation and deacetylation: Importance in inflammatory lung diseases. *Eur Respir J*, 25: 552–563.

https://doi.org/10.1183/09031936.05.00117504

35. Peng WX, Koirala P, Mo YY, 2017, LncRNA-mediated regulation of cell signaling in cancer. *Oncogene*, 36: 5661–5667.

https://doi.org/10.1038/onc.2017.184

36. Zhang P, Wu W, Chen Q, *et al*., 2019, Non-coding RNAs and their integrated networks. *J Integr Bioinform*, 16: 20190027.

https://doi.org/10.1515/jib-2019-0027

37. Ferrè F, Colantoni A, Helmer-Citterich M, 2016, Revealing protein-lncRNA interaction. *Brief Bioinform*, 17: 106–116.

https://doi.org/10.1093/bib/bbv031

38. Hulshoff MS, Del Monte-Nieto G, Kovacic J, *et al*., 2019, Non-coding RNA in endothelial-to-mesenchymal transition. *Cardiovasc Res*, 115: 1716–1731.

https://doi.org/10.1093/cvr/cvz211

39. Bridges MC, Daulagala AC, Kourtidis A, 2021, LNCcation: lncRNA localization and function. *J Cell Biol*, 220: e202009045.

https://doi.org/10.1083/jcb.202009045

40. Wang J, Su Z, Lu S, *et al*., 2018, LncRNA HOXA-AS2 and its molecular mechanisms in human cancer. *Clin Chim Acta*, 485: 229–233.

https://doi.org/10.1016/j.cca.2018.07.004

41. Xing C, Sun SG, Yue ZQ, *et al*., 2021, Role of lncRNA LUCAT1 in cancer. *Biomed Pharmacother*, 134: 111158.

https://doi.org/10.1016/j.biopha.2020.111158

42. Lu Q, Guo Q, Xin M, 2021, LncRNA TP53TG1 promotes the growth and migration of hepatocellular carcinoma cells via activation of ERK signaling. *Noncoding RNA*, 7: 52.

https://doi.org/10.3390/ncrna7030052

43. Wang H, Zhang Z, Zhang Y, *et al*., 2021, Long non-coding RNA TP53TG1 upregulates SHCBP1 to promote retinoblastoma progression by sponging miR-33b. *Cell Transplant*, 30: 9636897211025223.

https://doi.org/10.1177/09636897211025223

44. Zhang Y, Yang H, Du Y, *et al*., 2019, Long noncoding RNA TP53TG1 promotes pancreatic ductal adenocarcinoma development by acting as a molecular sponge of microRNA-96. *Cancer Sci*, 110: 2760–2772.

https://doi.org/10.1111/cas.14136

45. Liu R, Yang X, 2021, LncRNA LINC00342 promotes gastric cancer progression by targeting the miR-545-5p/CNPY2 axis. *BMC Cancer*, 21: 1163.

https://doi.org/10.1186/s12885-021-08829-x

46. Shen P, Qu L, Wang J, *et al*., 2021, LncRNA LINC00342 contributes to the growth and metastasis of colorectal cancer via targeting miR-19a-3p/NPEPL1 axis. *Cancer Cell Int*, 21:105.

https://doi.org/10.1186/s12935-020-01705-x

47. Chen QF, Kong JL, Zou SC, *et al*., 2019, LncRNA LINC00342 regulated cell growth and metastasis in non-small cell lung cancer via targeting miR-203a-3p. *Eur Rev Med Pharmacol Sci*, 23: 7408–7418.

https://doi.org/10.26355/eurrev_201909_18849

48. Lv D, Tan L, Ma H, *et al*., 2020, WITHDRAWN: LINC00342 promotes thyroid carcinoma progression by targeting miR-384/CHMP5 pathway. *Pathol Res Pract*, 223: 153272.

https://doi.org/10.1016/j.prp.2020.153272

49. Chen C, Wang X, Liu T, *et al*., 2020, Overexpression of long non-coding RNA RP11-363E7.4 inhibits proliferation and invasion in gastric cancer. *Cell Biochem Funct*, 38: 921–931.

https://doi.org/10.1002/cbf.3514

50. Jiang X, Ning Q, 2020, The mechanism of lncRNA H19 in fibrosis and its potential as novel therapeutic target. *Mech Ageing Dev*, 188: 111243.

https://doi.org/10.1016/j.mad.2020.111243

51. Poulet C, Njock MS, Moermans C, *et al*., 2020, Exosomal long non-coding RNAs in lung diseases. *Int J Mol Sci*, 21: 3580.

https://doi.org/10.3390/ijms21103580

52. Xiao T, Zou Z, Xue J, *et al*., 2021, LncRNA H19-mediated M2 polarization of macrophages promotes myofibroblast differentiation in pulmonary fibrosis induced by arsenic exposure. *Environ Pollut*, 268: 115810.

https://doi.org/10.1016/j.envpol.2020.115810

53. Zhang S, Chen H, Yue D, *et al*., 2021, Long non-coding RNAs: Promising new targets in pulmonary fibrosis. *J Gene Med*, 23: e3318.

https://doi.org/10.1002/jgm.3318

54. Gao Y, Zhang J, Liu Y, *et al*., 2017, Regulation of TERRA on telomeric and mitochondrial functions in IPF pathogenesis. *BMC Pulm Med*, 17: 163.

https://doi.org/10.1186/s12890-017-0516-1

55. Li Y, Sun W, Pan H, *et al*., 2021, LncRNA-PVT1 activates lung fibroblasts via miR-497-5p and is facilitated by FOXM1. *Ecotoxicol Environ Saf*, 213: 112030.

https://doi.org/10.1016/j.ecoenv.2021.112030

56. Lin S, Zhang R, Xu L, *et al*., 2020, LncRNA Hoxaas3 promotes lung fibroblast activation and fibrosis by targeting miR-450b-5p to regulate runx1. *Cell Death Dis*, 11: 706.

https://doi.org/10.1038/s41419-020-02889-w

57. Li X, Yu T, Shan H, *et al*., 2018, LncRNA PFAL promotes lung fibrosis through CTGF by competitively binding miR-18a. *FASEB J*, 32: 5285–5297.

https://doi.org/10.1096/fj.201800055R

58. Savary G, Dewaeles E, Diazzi S, *et al*., 2019, The long noncoding RNA DNM3OS Is a reservoir of fibromirs with major functions in lung fibroblast response to TGF-β and pulmonary fibrosis. *Am J Respir Crit Care Med*, 200: 184–198.

https://doi.org/10.1164/rccm.201807-1237OC

59. Yang Y, Tai W, Lu N, *et al*., 2020, LncRNA ZFAS1 promotes lung fibroblast-to-myofibroblast transition and ferroptosis via functioning as a ceRNA through miR-150-5p/SLC38A1 axis. *Aging* (*Albany, NY*), 12: 9085–9102.

https://doi.org/10.18632/aging.103176

60. Li X, Peng C, Zhu Z, *et al*., 2021, The networks of m6A-SARS-CoV-2 related genes and immune infiltration patterns in idiopathic pulmonary fibrosis. *Aging* (*Albany, NY*), 13: 6273–6288.

https://doi.org/10.18632/aging.202725

61. Zhang C, Wu Z, Li JW, *et al*., 2020, Discharge may not be the end of treatment: Pay attention to pulmonary fibrosis caused by severe COVID-19. *J Med Virol*, 93: 1378–1386.

https://doi.org/10.1002/jmv.26634

62. George PM, Wells AU, Jenkins RG, 2020, Pulmonary fibrosis and COVID-19: The potential role for antifibrotic therapy. *Lancet Respir Med*, 8: 807–815.

https://doi.org/10.1016/S2213-2600(20)30225-3

63. Spagnolo P, Kropski JA, Jones MG, *et al*., 2021, Idiopathic pulmonary fibrosis: Disease mechanisms and drug development. *Pharmacol Ther*, 222: 107798.

https://doi.org/10.1016/j.pharmthera.2020.107798

64. Glass DS, Grossfeld D, Renna HA, *et al*., 2022, Idiopathic pulmonary fibrosis: Current and future treatment. *Clin Respir J*, 16: 84–96.

https://doi.org/10.1111/crj.13466

65. Somogyi V, Chaudhuri N, Torrisi SE, *et al*., 2019, The therapy of idiopathic pulmonary fibrosis: What is next? *Eur Respir Rev*, 28: 190021.

https://doi.org/10.1183/16000617.0021-2019

66. Hirani N, MacKinnon AC, Nicol L, *et al*., 2021, Target inhibition of galectin-3 by inhaled TD139 in patients with idiopathic pulmonary fibrosis. *Eur Respir J*, 57: 2002559.

https://doi.org/10.1183/13993003.02559-2020

67. Guiot J, Moermans C, Henket M, *et al*., 2017, Blood biomarkers in idiopathic pulmonary fibrosis. *Lung*, 195: 273–280.

https://doi.org/10.1007/s00408-017-9993-5

68. Drakopanagiotakis F, Wujak L, Wygrecka M, *et al*., 2018, Biomarkers in idiopathic pulmonary fibrosis. *Matrix Biol*, 68–69: 404–421.

https://doi.org/10.1016/j.matbio.2018.01.023

69. Kreuter M, Lee JS, Tzouvelekis A, *et al*., 2021, Monocyte count as a prognostic biomarker in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*, 204: 74–81.

https://doi.org/10.1164/rccm.202003-0669OC

ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Succinate metabolism in cardiovascular diseases

Wenxin Shan[1], Hongtu Cui[2], Yangkai Xu[1], Jing Xue[3], and Lemin Zheng[1,3]*

[1]The Institute of Cardiovascular Sciences and Institute of Systems Biomedicine, School of Basic Medical Sciences, Key Laboratory of Molecular Cardiovascular Science of Ministry of Education, NHC Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides, Beijing Key Laboratory of Cardiovascular Receptors Research, Health Science Center, Peking University, Beijing 100 191, China

[2]Department of Cardiology and Institute of Vascular Medicine, Peking University Third Hospital; NHC Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides; Key Laboratory of Molecular Cardiovascular Science, Ministry of Education; Beijing Key Laboratory of Cardiovascular Receptors Research; Research Unit of Medical Science Research Management/Basic and Clinical Research of Metabolic Cardiovascular Diseases, Chinese Academy of Medical Sciences; Haihe Laboratory of Cell Ecosystem, Beijing 100 191, China

[3]Beijing Tiantan Hospital, China National Clinical Research Center for Neurological Diseases, Advanced Innovation Center for Human Brain Protection, The Capital Medical University, Beijing 100 050, China

Abstract

Cardiovascular disease (CVD) refers to a class of diseases related to the heart or blood vessels that have high global incidence. Succinate is generally considered an important intermediate product of the tricarboxylic acid cycle. Recent studies have shown that succinate is related to the pathophysiology of CVD, such as atherosclerosis, acute aortic dissection, hypertension, myocardial ischemia-reperfusion injury, and heart failure. It may represent a potential target or biomarker for CVD. It has been demonstrated that succinate not only participates in various energy metabolic pathways but also plays an important role in various pathophysiological activities as a signaling molecule. Given the significance of metabolism in CVD, it is important to focus on the metabolic regulation mechanism of succinate in CVD. This review outlines the latest evidence pointing to the potential role of succinate in CVD, along with its mechanisms, and updates the current understanding on the role of succinate in CVD. Further studies may focus on identifying succinate, its receptor, and its downstream signaling molecules as new targets for the prevention and treatment of CVD.

Keywords: Cardiovascular diseases; Metabolism; Succinate; Succinate receptor 1

***Corresponding author:**
Lemin Zheng
(zhengl@bjmu.edu.cn)

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1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide[1]. It includes atherosclerosis, acute aortic dissection (AAD), hypertension, myocardial ischemia-reperfusion injury (MIRI), heart failure, and metabolic cardiomyopathy[2,3]. The progression of the disease cannot be well controlled with medication and surgery. Therefore, it is particularly important to look for new prevention and treatment targets.

Succinate is an important metabolic intermediate of the tricarboxylic acid (TCA) cycle and glutamine metabolism[4,5]. In addition, it acts as a signaling molecule by binding

to its receptor and regulating metabolism and immune homeostasis in various pathophysiological activities[6]. However, the exact underlying mechanism of succinate in CVD has not been elucidated. As a potential biomarker for CVD, succinate plays an important role in the early diagnosis and treatment. The role of succinate in CVD and the available evidence, including some mechanisms, recent progress, and clinical significance of succinate in CVD, is outlined in this review.

2. Source and metabolism of succinate

Succinate is an important metabolic intermediate that participates in various metabolic pathways, such as the TCA cycle and glutamine metabolism. Succinate is an intermediate metabolite of the TCA cycle, located downstream of α-ketoglutarate. The α-ketoglutarate dehydrogenase complex (OGDH) catalyzes the oxidative decarboxylation of α-ketoglutarate into succinyl-CoA. Under the catalysis of succinyl-CoA synthase, the sulfur lipid bond of succinyl-CoA is broken to form succinate, in which this reaction is reversible. Subsequently, the generated succinate is oxidized into fumaric acid under the action of the succinate dehydrogenase complex (SDH)[7]. Succinate generates a large number of reactive oxygen species (ROS) in the oxidation process. This is a crucial step in the production of ATP in the TCA cycle[8].

2.1. α-ketoglutarate dehydrogenase complex

OGDH is a rate-limiting enzyme in the TCA cycle. It consists of three enzymes (α-ketoglutarate dehydrogenase, dihydrolipoamide succinyltransferase, and dihydrolipoyl dehydrogenase)[9]. While the secretion of inflammatory factors increases in an inflammatory milieu, the increase in OGDH activity in macrophages promotes the decarboxylation of a-ketoglutarate into succinate, resulting in a decrease in the ratio of α-ketoglutarate to succinate. The addition of α-ketoglutarate increases the ratio and decreases the level of cellular inflammation[10].

2.2. SDH

The SDH, also known as mitochondrial respiratory complex II, is located within the inner mitochondrial membrane. It consists of six subunits, namely, SDHA, SDHB, SDHC, SDHD, SDHAF1, and SDHAF2. SDH is also involved in the TCA cycle and electron transport chain[11]. In the TCA cycle, SDH oxidizes succinate into fumaric acid, and subsequently, as a part of oxidative phosphorylation, SDH transfers electrons from succinate to coenzyme Q. SDH is located at the intersection of two important metabolic pathways: The TCA cycle and electron transport chain. Growing evidence reveals that the activity of SDH is regulated by post-translational modifications, such as succinylation, acetylation, deacetylation, and phosphorylation, to cope with various external stimuli[12-14].

2.3. Intestinal flora as a source

The intestinal flora is also an important source of succinate, especially in the fermentation of polysaccharides and oligosaccharides. Microbiota-derived succinate is generally considered an intermediate product of propionate synthesis, and it accumulates less in bacteria in view of its high utilization rate.

Since succinate is produced by microbiota, there are low levels of succinate in the intestinal contents of specific pathogen-free (SPF) mice and almost none in sterile mice[15]. Succinate concentrations range from 1 to 3 mM in human intestinal contents and feces, accounting for 2 – 4% of the total organic anions in feces, which are much higher than the level of succinate in plasma. The high level of succinate in feces implies that succinate is produced by microorganisms and then absorbed into the blood through the intestinal epithelium, participating in host-microbiota interactions and host cell metabolism. The main source of succinate in the intestine is *Bacteroidetes*. *Bacteroides fragilis*, *Prevotella copri*, and *Enterococcus faecalis* produce succinate through the fermentation of dietary fiber[16,17]. Dietary fiber supplements can significantly increase the concentration of succinate in the cecum of mice and participate in the process of small intestinal gluconeogenesis, thus playing an important role in maintaining glucose homeostasis[17]. Succinate levels in the cecum may also increase with dietary fiber supplements during a high-fat diet (HFD)[18]. In the intestinal flora, there are also some succinate-consuming bacteria, such as *P. faecium* and *Ruminococcus*, which convert succinate into propionate[19]. An imbalance in intestinal homeostasis may occur as a result of antibiotics and intestinal inflammation, where there is an increase in succinate-secreting bacteria, but a decrease in succinate-consuming bacteria, thus resulting in the accumulation of succinate in the intestine[20]. In a study, the concentration of succinate in the feces of patients with inflammatory bowel disease was significantly higher than that of the control group; in a dextran sulfate sodium (DSS)-induced colitis mouse model, there was also an increase in the concentration of succinate in feces[21,22].

2.4. Other pathways of succinate production

In addition to the formation of succinate from a-ketoglutarate through the decarboxylation of OGDH, many metabolic pathways are also involved in the production of succinate, such as reverse SDH activity, γ-aminobutyric acid (GABA) shunt, and glutamine metabolism[23].

When a tissue is hypoxic, there will be SDH activity reversal; that is, it mediates the reverse production of succinate from fumarate. Following myocardial ischemia and hypoxia, the SDH activity in cardiomyocytes is reversed. Fumaric acid produced by aspartic acid and adenosine monophosphate (AMP) metabolism generates a large amount of succinate under the action of SDH, resulting in the accumulation of succinate in hypoxic myocardial tissues. Following reperfusion, the accumulated succinate is rapidly oxidized by normally active SDH to produce excess ROS, resulting in further damage to myocardial tissues[24].

When macrophages undergo pro-inflammatory M1 polarization, the glutamine metabolic pathway is activated and the expression of glutamate dehydrogenase is upregulated. The latter catalyzes glutamine to produce a-ketoglutarate and provides the substrate for OGDH to produce succinate. Meanwhile, lipopolysaccharide (LPS) stimulation also leads to an increase in GABA levels and GABA transferase activity in macrophages. GABA is catalyzed by GABA transferase to produce succinic semialdehyde (SSA), which is subsequently converted into succinate by SSA dehydrogenase[25].

2.5. Transport of succinate

Intracellular succinate is involved in mitochondrial TCA cycle and is incapable of crossing the cell membrane. However, when there is an abrupt increase in energy demand and the energy supply cannot be maintained, the anaerobic pathway will be activated, resulting in excessive lactic acid production and cell acidification. The decrease in pH value will lead to the protonation of succinate, which involves the transformation of dicarboxylate trapped in the cell into monocarboxylate so that it can cross the cell membrane and escape into the extracellular matrix. A specific membrane carrier transport is required for succinate to pass through the cell membrane[26]. The solute carrier (SLC) family is composed of a large class of transmembrane solute transporters. SLC25A10 is a mitochondrial dicarboxylate carrier located on the mitochondrial membrane. It mainly transports dicarboxylic acids, such as malic acid and succinate, from the mitochondria to the cytoplasm for the exchange of phosphate, sulfate, and thiosulfate, thus providing substrates for gluconeogenesis and urea synthesis, as well as maintaining the distribution and homeostasis of intermediate products in and out of the mitochondria during the TCA cycle[27]. SLC25A10 transports succinate from the mitochondrial matrix to the cytosol, which is the first step of succinate transport to the extracellular space. Monocarboxylic acid transporter 1 (MCT1), a member of the SLC16 family, is a protein that transports monocarboxylic acids, such as lactic acid and pyruvate, to cells. Recent studies have shown that MCT1 can transfer succinate to the extracellular space through the plasma membrane of myocardium, skeletal muscle, and retina[28,29]. In addition to succinate produced by cells themselves, extracellular succinate uptake is another major source of intracellular succinate. Extracellular succinate can also be absorbed and recovered by sodium-dependent dicarboxylic acid transporters. The plasma membrane transporter of the SLC13 family is responsible for transporting succinate from the circulation into cells and regulating succinate homeostasis.

2.6. Succinate receptor 1 (SUCNR1)

SUCNR1 (also known as GPR91) is a G protein-coupled receptor responsible for succinate signaling and is widely expressed in systemic cell types[30,31]. Emerging evidence suggests that the succinate-SUCNR1 pathway plays an important role in regulating immune homeostasis. In different microenvironments, succinate activates SUCNR1, which leads to different immune cell responses. Therefore, the SUCNR1 pathway can help reduce inflammatory damage in diseased tissues. In chronic inflammation, succinate is released into the extracellular matrix as a signaling molecule to regulate the function of other cells through the interaction with receptors.

SUCNR1 is expressed in various cells of the immune system and plays an important role in regulating cellular immune homeostasis and inflammatory response. SUCNR1 is also widely expressed in the adaptive immune system, such as T-cells (including $CD4^+$ and $CD8^+$ T-cells) and B-cells. Large amounts of interleukin (IL)-10 and succinate are released as a result of the activation of T-cells in patients with systemic lupus erythematosus. When cocultured with B-cells, the activation of T-cells is inhibited by the neutralization of SUCNR1 on B-cells[32]. However, it remains unclear whether succinate acts synergistically with other cytokines to regulate adaptive immunity. The effect of SUCNR1 activation in innate immune cells is environment dependent. For example, in human immature dendritic cells, SUCNR1 controls its chemotaxis in a succinate concentration-dependent manner[33]. SUCNR1 and toll-like receptor-3 (TLR-3) or TLR-7, independent of TLR-2 or TLR-4, act in synergy, increasing the expression of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-1β, leading to the enhancement of antigen presentation ability and the activation of $CD4^+$ T-cells[33]. However, the activation of SUCNR1 pathway seems to occur only in the acute phase of stimulation, since SUCNR1 is rapidly downregulated following the activation of dendritic cells. In a mouse experimental arthritis model, SUCNR1-mediated chemotaxis of dendritic cells into lymph nodes *in vivo*

resulted in the expansion of Th17 cells, which, further, led to autoimmune diseases[34].

In addition to its effect on chemotaxis, SUCNR1 also plays an important role in macrophage inflammation. However, there are conflicting results in the current research. SUCNR1 plays a pro-inflammatory role in M2 macrophages derived from human peripheral blood monocytes. IL-4 or IL-10 stimulates macrophages, resulting in the upregulation of SUCNR1 expression in macrophages. However, the treatment of macrophages with succinate or SUCNR1 agonists decreases the secretion of IL-10 and increases TNF-α expression in macrophages, thus enhancing the inflammatory response[35]. Macrophages are activated by inflammatory signals release succinate to the extracellular environment, activate SUCNR1 through autocrine and paracrine signaling, and promote the production of IL-1β, thus further aggravating tissue inflammation[36]. Although the previous studies have shown that the knockout of SUCNR1 does not affect the secretion of IL-1β, TNF-α, and IL-6 in peritoneal macrophages stimulated by LPS (10 ng/mL, 24 h)[37], another study found that SUCNR1 knockdown resulted in a significant decrease in IL-1β expression in bone marrow-derived macrophages (BMDMs) when stimulated with a higher dose (100 ng/mL) of LPS. IL-1β stimulation results in an increased expression of SUCNR1 in BMDMs[36]. These results indicate that there may be some positive feedback between SUCNR1 and inflammatory cytokines. Other studies have shown that LPS-stimulated BMDMs of SUCNR1 knockout mice had increased IL-6, TNF-α, and nitric oxide (NO) release compared to the control group[38]. This finding reveals that SUCNR1 may play an anti-inflammatory role, thus contradicting previous studies. Hence, the mechanism needs to be further explored.

In conclusion, succinate is generated and transported through various pathways, and it plays different roles, depending on physiological and pathological conditions (Figure 1). Enzymes, intermediate metabolites involved in succinate metabolism, or SUCNR1 may become potential therapeutic targets for CVD in the future.

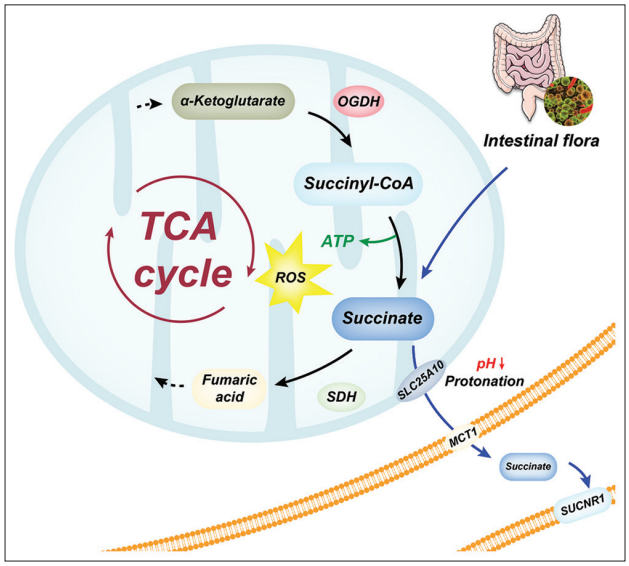

Figure 1. Succinate metabolism and transport. The main source of succinate is the oxidative decarboxylation of α-ketoglutarate by oxoglutarate dehydrogenase in the tricarboxylic acid cycle to form succinyl-coenzyme (Co)A, which is catalyzed by succinyl-CoA synthase to generate succinate. The other source of succinate is derived from the metabolism of intestinal flora through the fermentation of dietary fiber. Succinate generates excessive reactive oxygen species during oxidation. The metabolic pathway of succinate includes the oxidation to fumaric acid in the presence of SDH and then protonation due to the decrease in mitochondrial pH. Succinate is transported to the extracellular level through SLC25A10 and monocarboxylic acid transporter 1.

3. Succinate and cardiovascular disease

3.1. Succinate and atherosclerosis

The pathophysiological mechanism of atherosclerosis involves inflammatory response, endothelial cell dysfunction, and macrophage polarization, all of which eventually lead to plaque formation.

Succinate acts as an inflammatory signal ligand, which can be transmitted through the receptor SUCNR1. SUCNR1 is inactive in normal tissues and can be activated under certain conditions, such as hypoxia and tissue injury[39]. Succinate accumulates in ischemic tissues and is involved in perfusion injury through mitochondrial ROS[24]. The binding of succinate to SUCNR1, which is expressed in human umbilical vein endothelial cells (HUVECs) and macrophages, activates transcription factor hypoxia-inducible factor (HIF)-1α, stimulates the succinate/IL-1β signaling axis, promotes the expression of IL-1β to produce excess pro-inflammatory cytokines, and exacerbates the inflammatory process of atherosclerosis[23,40].

SDHB is one of the six subunits of the succinate dehydrogenase complex. Low shear stress downregulates the expression of tet methylcytosine dioxygenase 2, inhibits the recruitment of histone deacetylase 2, and upregulates the expression of SDHB. SDHB mediates mitochondrial damage, increases the production of ROS, and subsequently induces vascular endothelial cell pyroptosis[41]. Trimethylamine N-oxide (TMAO) promotes the production of ROS in HUVECs and endothelial cell pyroptosis by upregulating the expression of SDHB[42].

Succinate in mitochondria generates a large amount of ROS through the oxidation of SDH, which promotes the conversion of macrophages into M1 pro-

inflammatory macrophages[43]. In macrophages, IFN-β antagonizes JMJD3-IRF4 pathway by controlling the ratio of α-ketoglutaric acid to succinate, thus regulating the activation and polarization of macrophages[44]. In the tumor microenvironment, macrophages are the main cells, and cancer cells secrete succinate, activate SUCNR1, induce M2 polarization of macrophages into tumor-related macrophages, and increase macrophage migration[45]. Succinate pre-treatment enhances IL-1β and pro-IL-1β levels in LPS-stimulated bone marrow-derived macrophages and increases HIF-1α levels. Moreover, the oxidation of succinate produces mitochondrial ROS, which affects the inflammatory phenotype of macrophages[43].

There is significant evidence showing that succinate increases the level of ROS, promotes vascular endothelial cell pyroptosis and macrophage polarization, and ultimately worsens atherosclerosis.

3.2. Succinate and AAD

AAD occurs when the arterial wall is unable to withstand high pressure in the blood vessel, resulting in the tearing of the middle membrane and the formation of a false lumen (arterial dissection). Once it tears, the mortality is as high as 65 – 85%[46]. Untargeted metabolomics studies showed that the level of succinate in plasma was significantly higher in patients with AAD. The direct phosphorylation of cAMP-response element-binding protein (CREB) by P38α in inflammatory macrophages leads to an increase in CREB-mediated transcription of OGDH and an elevated succinate level. The secretion of succinate outside cells leads to an increase in ROS levels in the vascular wall, which aggravates the progress of AAD[47].

3.3. Succinate and hypertension

Hypertension is an independent risk factor for cardiovascular disease. Succinate plays an important role in the regulation of blood pressure and is closely related to the renin-angiotensin system (RAS). Succinate activates RAS through SUCNR1 in the kidney to mediate hypertension[30]. High glucose levels stimulate the paracrine apparatus in the glomerulus and trigger the release of renin through the activation of succinate and its receptor SUCNR1[48]. SUCNR1, which is present in macula densa cells, is activated by succinate and regulates renin release[49]. The intravenous administration of succinate increases plasma renin activity and leads to a dose-dependent increase in blood pressure. This can be prevented with angiotensin-converting enzyme inhibitors[50]. In another study, the level of succinate in the blood of spontaneously hypertensive rats was found higher than that of normotensive rats[51]. A new succinate homeostatic pathway, which may be associated with hypertension, has also been identified, as it leads to the formation of calcium oxalate. The transport of citric and oxalic acids is regulated by the citric and succinate transporter protein Na^+-dependent dicarboxylate (NaDC)-1 and the oxalic acid transporter protein SLC family 26 member 6 (SLC26A6), both of which form a complex. This mechanism regulates the transepithelial transport of succinate. In SLC26A6 knockout mice, calcium oxalate stones, hyperoxaluria, and hypocitraturia are often seen with impaired succinate homeostasis, elevated serum succinate levels, and elevated plasma renin levels, exhibiting activity-dependent hypertension. Succinate acts on SUCNR1 to induce the translocation of the scaffold protein IRBIT and regulate transepithelial succinate transport. IRBIT interacts with SLC26A6-NADC1 complex to inhibit NADC1-mediated succinate transport[52]. In addition, oxidative stress is an important mechanism in the pathophysiology of hypertension, and succinate is known to aggravate oxidative stress *in vivo* by activating HIF-1α, thus leading to hypertension[24]. At present, the molecular mechanism by which succinate activates RAS is not well understood; however, the succinate-SUCNR1 signaling pathway and succinate transport mechanism may become potential therapeutic targets for hypertension.

3.4. Succinate and MIRI

Ischemic heart disease is the leading cause of CVD-related deaths. The main treatment strategy is to restore blood flow to the ischemic area in a timely and effective manner, but reperfusion itself may also lead to myocardial tissue injury, which is known as MIRI.

The release of succinate during reperfusion is mediated by MCT1. In ischemic cardiomyocytes, the intracellular environment acidifies, and succinate transforms into a protonated monocarboxylic form. During reperfusion, succinate monocarboxylate flows out of cells through MCT1, resulting is a reduction in intracellular succinate levels[28]. Blocking MCT1 causes succinate to reside in cells, thus exacerbating ROS production and IR injury[53]. Under hypoxic and ischemic conditions, myocardial extracellular succinate accumulation increases the translocation of dynamin-related protein 1 (Drp1) to mitochondria by SUCNR1 activation of protein kinase C-δ (PKCδ) and induces the phosphorylation of mitochondrial fission factor (MFF) by extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) activation, leading to mitochondrial fission[54]. Succinate drives ROS production during reperfusion. Preventing succinate accumulation or oxidation is a therapeutic target for cardioprotection[55]. Elevated levels of succinate inhibit the proliferation and regeneration of neonatal mouse cardiomyocytes through SDH, while malonic acid (a competitive inhibitor of SDH) inhibits the activity of SDH, preventing succinate

accumulation and inducing cardiomyocyte proliferation and heart regeneration[56]. SDH is the most crucial enzyme for succinate accumulation and oxidation to produce ROS during ischemia-reperfusion. Dimethyl malonate has a protective effect on ischemia-reperfusion injury in pre-ischemia or ischemia[24]. In a porcine ischemia-reperfusion model, coronary administration of dimethyl malonate was found to be cardioprotective[57]. In addition to its role in myocardial infarction, SDH inhibitors can also be used in predictable ischemic processes, such as ischemic stroke, kidney disease, and organ transplantation. Dimethyl malonate has been shown to reduce brain damage after cardiac arrest in rats[58]. Malonic acid may emerge as a potential treatment for reducing injuries during organ transplantation.

Isolated organs are in a state of hypoxia, which leads to the accumulation of succinate in the organs and oxidation after reperfusion, resulting in tissue injury and inflammation. The cold storage solution can slow down the metabolism and the production of succinate, thus reducing the production of mitochondrial ROS during reperfusion and in reperfusion injury[59]. In a recent study related to organ transplantation, a new storage method was designed to preserve the heart for transplantation. Hypothermia oxygenation was used to raise the level of adenosine triphosphate/adenosine diphosphate (ATP/ADP) in the perfusion tissue, which reduced the level of cardiac succinate and cell injury, thus achieving a protective effect on the heart[60]. A large amount of succinate tends to accumulate in ischemic tissue, but following reperfusion, succinate is rapidly oxidized by SDH, and excess ROS are produced through mitochondrial respiratory complex I, resulting in calcium imbalance and ATP depletion, which lead to further damage and myocardial cell death[24].

3.5. Succinate and myocardial hypertrophy and heart failure

Cardiac overload is the primary cause of heart failure. Myocardial hypertrophy is the main compensatory mechanism with an increase in cardiac afterload. The apoptosis of cardiomyocytes has a significant role in the transition from myocardial hypertrophy to heart failure[61]. Pathological myocardial hypertrophy is a major risk factor for various CVDs and sudden death, but there is no effective treatment strategy at present.

Succinate triggers ERK1/2 phosphorylation, Ca^{2+}/calmodulin-dependent protein kinase II delta (CaMKIIδ) expression, and intracellular histone deacetylase 5 (HDAC5 translocation) through SUCNR1, leading to cardiomyocyte hypertrophy[62]. Succinate-SUCNR1 is involved in right ventricular hypertrophy induced by pressure overload through the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway[63]. In a previous study, a patient who presented with congestive heart failure was found deficient in succinate dehydrogenase, which caused succinate to accumulate extracellularly[64]. Succinate activates the cardiomyocyte PKA pathway, regulates cardiomyocyte Ca^{2+} transients through SUCNR1, reduces ventricular cardiomyocyte viability, increases caspase-3 activity, and leads to cardiomyocyte apoptosis[65]. The Ca^{2+} transient is an important indicator of myocardial hypertrophy[66]. These results suggest that succinate promotes cardiomyocyte apoptosis and Ca^{2+} transients, resulting in myocardial hypertrophy and heart failure.

3.6. Succinate and metabolic cardiomyopathy

Metabolic cardiomyopathy is a type of cardiomyopathy caused by metabolic disorders, primarily glucose, and lipid metabolism disorders, some of which include heart failure with preserved ejection fraction, diabetic cardiomyopathy, and Takotsubo syndrome.[67,68] Obesity, body fat, and body mass index are significant risk factors for these cardiomyopathies, and succinate may this condition.

Obesity may lead to metabolic disorders of adipose tissue, leading to macrophage infiltration and chronic inflammation. Succinate-SUCNR1 mediates adipose tissue macrophage infiltration and glucose intolerance in obesity[37]. The knockout of SUCNR1 results in a significant reduction in macrophage infiltration in adipose tissues[37]. In addition to worsening obesity, inflammation and glucose intolerance may occur as a result of macrophage-specific deficiency of SUCNR1[69]. The elevated plasma level of succinate is related to metabolic abnormalities. In obese people, the level of succinate in the circulation increases, while the expression of SUCNR1 in adipose tissue-resident macrophages decreases[70]. The thermogenic activity of brown adipose tissue (BAT) plays a significant role in obesity. Uncoupling protein 1 (UCP1), which is a key thermogenic protein expressed in brown and beige adipocytes, regulates the removal of succinate from the circulation of brown and beige fat. High levels of succinate are rapidly absorbed by adipocyte mitochondria, producing ROS through SDH-mediated succinate oxidation, driving UCP1-dependent thermogenic respiration, and then regulating liver inflammation and glucose intolerance under obesity conditions[71,72]. In a cold exposure mouse model, succinate accumulated in brown adipocytes, reduced HFD-induced obesity, and enhanced thermogenesis in BAT through non-adrenergic signaling pathways[71]. Therefore, succinate can be regarded as an activator of BAT thermogenesis. Interestingly, the exogenous supplementation of succinate to pregnant and lactating female mice was found to promote the development of brown fat in newborn mice

and prevent obesity as well as metabolic dysfunction caused by HFD in newborn mice[73]. It is associated with enhanced succinylation and H3K4me3 modification in the PPARGC1A promoter. These results suggest that succinate can promote energy consumption and counter obesity by activating thermogenic fat, and this effect can be passed on to offspring.

Succinate can increase the differentiation of beige adipocytes. Stearoyl COA desaturase 1 (SCD1) is a rate-limiting enzyme of monounsaturated fatty acids. SCD1 induces the differentiation of preadipocytes into beige adipocytes through mitochondrial complex II activity that is driven by succinate[74]. Succinate inhibits the decomposition of triglycerides in white fat through SUCNR1[75]. The mitochondrial dicarboxylate vector SLC25A10 is mainly expressed in white adipose tissue (WAT), which enhances mitochondrial respiration, promotes succinate transport to the outside of cells, and inhibits lipolysis in adipocytes[27]. The highly expressed SUCNR1 in WAT is significantly higher than that in BAT. In the white adipose tissue of SUCNR1 knockout mice, the inhibitory effect of succinate on lipolysis is weakened. Succinate exerts its anti-lipolysis effect through SUCNR1 in WAT[76]. This is in contradiction with the role of succinate in BAT in promoting fat combustion to counter obesity. Further, exploration of the metabolism of succinate in adipose tissue and its role in obesity is needed, and the study of the role of succinate in metabolic cardiomyopathy is the future research direction (Figure 2).

4. Potential therapeutic targets of succinate metabolism in CVD

In view of the key role of the succinate-SUCNR1 signaling pathway, it is particularly important to develop a SUCNR1 agonist. Using a structural model based on crystal structure, researchers have found that the crystal had 27% homology with the purinergic receptor P2Y1 and further determined its potential ligands through simulation screening[36]. Several agonists have been screened, among which the most effective potential agonist has the structure of the succinate main chain, and its amide-linked hydrophobic structure is connected with the side chamber of the SUCNR1-binding site. Moreover, several potential agonists may be 10 – 100 times more potent than succinate itself and exert effects on SUCNR1 in both, mice and humans. *In vitro*, it may produce a similar effect to succinate on human macrophages. Further experiments have found a new nanomolar agonist with excellent stability *in vitro* but with high hydrophilicity[77]. At present, the best succinate analog found is cis-epoxy succinate, with a half maximal effective concentration

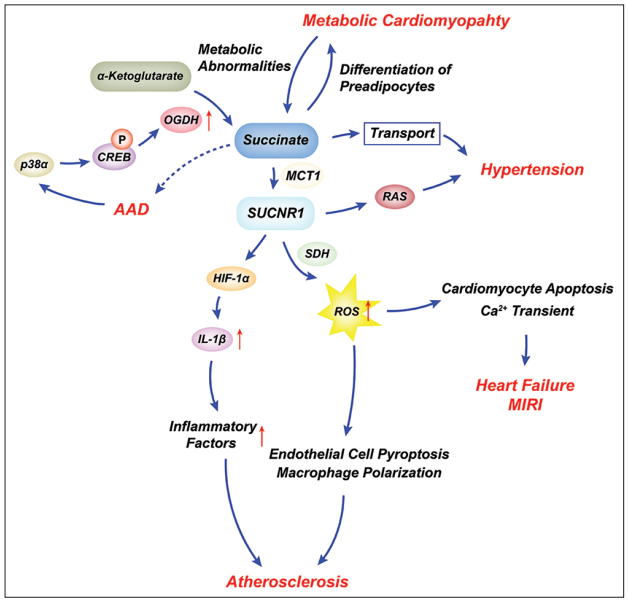

Figure 2. Succinate is associated with various cardiovascular diseases. In atherosclerosis, succinate binds to succinate receptor 1 (SUCNR1), which is expressed in human umbilical vein endothelial cells and macrophages, activates hypoxia-inducible factor (HIF)-1α, promotes IL-1β expression, produces large amounts of inflammatory factors, as well as affects endothelial cell pyroptosis and macrophage polarization; alternatively, through a non-HIF-1α-dependent pathway, succinate upregulates succinate dehydrogenase B (SDHB) expression to promote reactive oxygen species (ROS) production, exacerbating the atherogenic inflammatory process of atherosclerosis. In heart failure and MIRI, succinate accumulates in ischemic tissues. After reperfusion, succinate is rapidly oxidized by SDH, generating excess ROS, which leads to cardiomyocyte apoptosis and calcium ion (Ca^{2+}) transients. In hypertension, succinate is mediated by SUCNR1 through the activation of renin-angiotensin system or the transport mechanism of succinate to increase blood pressure. In acute aortic dissection, p38α in inflammatory macrophages is involved in its pathogenesis by phosphorylating cAMP-response element-binding protein, increasing oxoglutarate dehydrogenase expression, and increasing succinate levels. In metabolic cardiomyopathy, succinate affects the differentiation of preadipocytes. Metabolic abnormalities in obese people are associated with elevated plasma succinate levels.

(EC50) of 2.7 μM. The compound will not be oxidized by SDH but can play the role of succinate as a SUCNR1 ligand. Following intravenous administration, it can significantly increase blood pressure in rats[78]. The EC50 of SUCNR1 activated by succinate is in the range of 17 – 56 μM, and the concentration of succinate in human plasma is about 2 – 30 μM.

Since SUCNR1 plays a key role in the chemotaxis of immune cells and inflammation, SUCNR1 inhibitors may play a protective role in inflammatory diseases. Several potential small molecule inhibitors of SUCNR1 have been screened out from commercial compounds, of which the two most significant compounds are 2c and 4c, with their half maximal inhibitory concentration (IC50) values reaching 30 nM and 7 nM, respectively[79].

Although both compounds have poor bioavailability, they significantly alleviated hypertension in rats induced by succinate intervention. The study also screened several compounds with good bioavailability but poor specificity and used another compound (2d) for intervention. The 2d can inhibit the expression of type I collagen in rat hepatic stellate cells induced by high glucose or succinate[80], suggesting that it may play a certain role in alleviating non-alcoholic fatty liver. High-throughput screening identified another compound, NF-56-EJ40, which may be used as an inhibitor of SUCNR1. Its IC50 for human SUCNR1 is 25 nM, indicating good performance[31]. Through further crystal structure analysis, the structural basis of species differences in this inhibition has been clarified, thus providing direction for the design and selection of inhibitors in the future. The inhibitors studied in the previous stage have poor permeability due to their polar zwitterionic properties. Hence, to design effective drugs with good bioavailability, recent studies have systematically optimized them by adding internal salt bridges.

The therapeutic effect of the SUCNR1 inhibitor has not been reported at present. However, the designer of the SUCNR1 inhibitor based on naphthyridine has applied for a series of patents, in which it has been alluded that SUCNR1 inhibitor may be used in the treatment of non-alcoholic fatty liver disease and other related diseases, revealing a certain potential therapeutic value. Fibroblast growth factor 21 and co-recombinant peptide analogs have been found to inhibit the production of α-smooth muscle actin and reduce fibrosis in mice by inhibiting the succinate-SUCNR1 signaling pathway[81]. Metformin, a miracle drug for the treatment of type 2 diabetes, has also been shown to inhibit the hepatic succinate-SUCNR1 signaling pathway[82].

Exploring the decrease in SUCNR1 expression at the mRNA level is also an important means for researchers to explore the succinate-SUCNR1 signaling pathway. SUCNR1 is an important target of microRNA (miR)-758. Oxidized low-density lipoprotein stimulates the expression of miR-758 in endothelial cells and further downregulates SUCNR1 and its downstream signaling pathway, resulting in human vascular endothelial cell injury[83]. In a rat retinopathy model, the knockdown of rat SUCNR1 by small interfering RNA (siRNA) resulted in decreased vascular endothelial growth factor secretion, abnormal neovascularization, loss of pericytes, and areas without blood vessels[84]. The optimization of the structure and pharmacokinetics of SUCNR1 inhibitors enables researchers to identify new compounds and verify them in animal models (Table 1).

Table 1. Potential therapeutic targets of succinate metabolism

Treatment	Name of compound	Species	EC50/IC50	References
SUCNR1 agonist	cis-Epoxysuccinic acid	Rat	2.7 μM	[78]
SUCNR1 inhibitor	2c 4c	Human/Rat	30 nM 7 nM	[79]
	2d	Rat	40 nM	[80]
	NF-56-EJ40	Human	25 nM	[31]
mRNA	miR-758	Mouse		[83]

Due to the complex environment-dependent functions of SUCNR1, its current research is not thorough enough. Despite the fact that large pharmaceutical companies have submitted patents for screening SUCNR1 regulatory drugs or using SUCNR1 as an immune cell marker, it does not seem to have received enough attention. At present, several research groups and small companies are exploring superior performance regulators of SUCNR1 and their applications in diseases, but more research is needed to explain its complex functions and important role in diseases.

5. Conclusion and perspectives

Numerous clinical diagnoses of CVD have revealed changes in succinate levels. Succinate is regarded as a potential biomarker of CVD. The accumulation of succinate in ischemic tissues indicates the presence of ischemia[24]. Elevated plasma succinate levels are associated with increased cardiovascular risk factors in young adults, and its levels are positively correlated with visceral adipose tissue mass, which may serve as a biomarker for CVD risk in young adults[85]. Serum succinate was found to be significantly elevated in patients with coronary heart disease compared with healthy controls[40]. Circulating succinate levels are elevated in obese patients and are associated with poor metabolic status[86]. In patients with ST-elevation myocardial infarction, the level of succinate in the coronary sinus increases significantly[43]. Serum succinate levels also increase in patients with cardiac hypertrophy associated with acute or chronic obstructive coronary artery disease[62]. Early AAD is usually asymptomatic; hence, it is a challenge for an early diagnosis to be made. Since plasma succinate levels are significantly elevated in patients with AAD, it can be used to distinguish AAD from patients with acute myocardial infarction and pulmonary embolism[47].

This review focuses on the mechanism of succinate metabolism and its related factors in CVD. The existing

evidence reveals that succinate metabolism has a significant role in the pathophysiology of CVD. Succinate, as an important metabolic intermediate and signaling molecule, is a potential biomarker of cardiovascular disease. Further studies on the biological function, signaling pathway, and regulatory mechanism of succinate may provide new strategies and targets for the diagnosis and treatment of CVD in the coming era of precision medicine.

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Conflict of interest

The authors declare that they have no competing interest.

Author contributions

Conceptualization: Wenxin Shan, Hongtu Cui, and Lemin Zheng
Visualization: Yangkai Xu and Lemin Zheng
Writing – original draft: Wenxin Shan, Hongtu Cui, and Lemin Zheng
Writing – review & editing: Jing Xue and Lemin Zheng

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References

1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. 2017, Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet*, 390: 1211–1259.

   https://doi.org/10.1016/S0140-6736(17)32154-2

2. Ren J, Bi Y, Sowers JR, *et al*., 2021, Endoplasmic reticulum stress and unfolded protein response in cardiovascular diseases. *Nat Rev Cardiol*, 18: 499–521.

   https://doi.org/10.1038/s41569-021-00511-w

3. Nishida K, Otsu K, 2017, Inflammation and metabolic cardiomyopathy. *Cardiovasc Res*, 113: 389–398.

   https://doi.org/10.1093/cvr/cvx012

4. Lussey-Lepoutre C, Hollinshead KE, Ludwig C. *et al*., 2015, Loss of succinate dehydrogenase activity results in dependency on pyruvate carboxylation for cellular anabolism. *Nat Commun*, 6: 8784.

   https://doi.org/10.1038/ncomms9784

5. Burch JS, Marcero JR, Maschek JA, *et al*., 2018, Glutamine via alpha-ketoglutarate dehydrogenase provides succinyl-CoA for heme synthesis during erythropoiesis. *Blood*, 132: 987–998.

   https://doi.org/10.1182/blood-2018-01-829036

6. Winther S, Trauelsen M, Schwartz TW, 2021, Protective succinate-SUCNR1 metabolic stress signaling gone bad. *Cell Metab*, 33: 1276–1278.

   https://doi.org/10.1016/j.cmet.2021.06.009

7. Martinez-Reyes I, Chandel NS, 2020, Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun*, 11: 102.

   https://doi.org/10.1038/s41467-019-13668-3

8. Markevich NI, Galimova MH, Markevich LN, 2020, Hysteresis and bistability in the succinate-CoQ reductase activity and reactive oxygen species production in the mitochondrial respiratory complex II. *Redox Biol*, 37: 101630.

   https://doi.org/10.1016/j.redox.2020.101630

9. Wagner M, Bertero E, Nickel A, *et al*., 2020, Selective NADH communication from alpha-ketoglutarate dehydrogenase to mitochondrial transhydrogenase prevents reactive oxygen species formation under reducing conditions in the heart. *Basic Res Cardiol*, 115: 53.

   https://doi.org/10.1007/s00395-020-0815-1

10. Liu PS, Wang H, Li X, *et al*., 2017, Alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat Immunol*, 18: 985–994.

    https://doi.org/10.1038/ni.3796

11. Alston CL, Davison JE, Meloni F, *et al*., 2012, Recessive germline SDHA and SDHB mutations causing leukodystrophy and isolated mitochondrial complex II deficiency. *J Med Genet*, 49: 569–577.

    https://doi.org/10.1136/jmedgenet-2012-101146

12. Acin-Perez R, Carrascoso I, Baixauli F, *et al*., 2014, ROS-triggered phosphorylation of complex II by Fgr kinase regulates cellular adaptation to fuel use. *Cell Metab*, 19: 1020–1033.

    https://doi.org/10.1016/j.cmet.2014.04.015

13. Kim SC, Sprung R, Chen Y, *et al*., 2006, Substrate and functional diversity of lysine acetylation revealed by a

proteomics survey. *Mol Cell*, 23: 607–618.

https://doi.org/10.1016/j.molcel.2006.06.026

14. Du J, Zhou Y, Su X, *et al*., 2011, Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. *Science*, 334: 806–809.

https://doi.org/10.1126/science.1207861

15. Faith JJ, Ahern PP, Ridaura VK, *et al*., 2014, Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. *Sci Transl Med*, 6: 220ra211.

https://doi.org/10.1126/scitranslmed.3008051

16. Macy JM, Ljungdahl LG, Gottschalk G, 1978, Pathway of succinate and propionate formation in *Bacteroides fragilis*. *J Bacteriol*, 134: 84–91.

https://doi.org/10.1128/jb.134.1.84-91.1978

17. De Vadder F, Kovatcheva-Datchary P, Zitoun C, *et al*., 2016, Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. *Cell Metab*, 24: 151–157.

https://doi.org/10.1016/j.cmet.2016.06.013

18. De Vadder F, Kovatcheva-Datchary P, Goncalves D, *et al*., 2014, Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell*, 156: 84–96.

https://doi.org/10.1016/j.cell.2013.12.016

19. Watanabe Y, Nagai F, Morotomi M, 2012, Characterization of phascolarctobacterium succinatutens sp. nov., an asaccharolytic, succinate-utilizing bacterium isolated from human feces. *Appl Environ Microbiol*, 78: 511–518.

https://doi.org/10.1128/AEM.06035-11

20. Fernandez-Veledo S, Vendrell J, 2019, Gut microbiota-derived succinate: Friend or foe in human metabolic diseases? *Rev Endocr Metab Disord*, 20: 439–447.

https://doi.org/10.1007/s11154-019-09513-z

21. Connors J, Dawe N, Van Limbergen J, 2018, The role of succinate in the regulation of intestinal inflammation. *Nutrients*, 11: 25.

https://doi.org/10.3390/nu11010025

22. Ariake K, Ohkusa T, Sakurazawa T, *et al*., 2000, Roles of mucosal bacteria and succinic acid in colitis caused by dextran sulfate sodium in mice. *J Med Dent Sci*, 47: 233–241.

23. Tannahill GM, Curtis AM, Adamik J, *et al*., 2013, Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature*, 496: 238–242.

https://doi.org/10.1038/nature11986

24. Chouchani ET, Pell VR, Gaude E, *et al*., 2014, Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*, 515: 431–435.

https://doi.org/10.1038/nature13909

25. Mills E, O'Neill LA, 2014, Succinate: A metabolic signal in inflammation. *Trends Cell Biol*, 24: 313–320.

https://doi.org/10.1016/j.tcb.2013.11.008

26. Reddy A, Bozi LH, Yaghi OK, *et al*., 2020, pH-gated succinate secretion regulates muscle remodeling in response to exercise. *Cell*, 183: 62–75 e17.

https://doi.org/10.1016/j.cell.2020.08.039

27. An YA, Chen S, Deng Y, *et al*., 2021, The mitochondrial dicarboxylate carrier prevents hepatic lipotoxicity by inhibiting white adipocyte lipolysis. *J Hepatol*, 75: 387–399.

https://doi.org/10.1016/j.jhep.2021.03.006

28. Prag HA, Gruszczyk AV, Huang MM, *et al*., 2021, Mechanism of succinate efflux upon reperfusion of the ischaemic heart. *Cardiovasc Res*, 117: 1188–1201.

https://doi.org/10.1093/cvr/cvaa148

29. Bisbach CM, Hass DT, Thomas ED, *et al*., 2022, Monocarboxylate transporter 1 (MCT1) mediates succinate export in the retina. *Invest Ophthalmol Vis Sci*, 63: 1.

https://doi.org/10.1167/iovs.63.4.1

30. He W, Miao FJ, Lin DC, *et al*., 2004, Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature*, 429: 188–193.

https://doi.org/10.1038/nature02488

31. Haffke M, Fehlmann D, Rummel G, *et al*., 2019, Structural basis of species-selective antagonist binding to the succinate receptor. *Nature*, 574: 581–585.

https://doi.org/10.1038/s41586-019-1663-8

32. Macaulay IC, Tijssen MR, Thijssen-Timmer DC, *et al*., 2007, Comparative gene expression profiling of *in vitro* differentiated megakaryocytes and erythroblasts identifies novel activatory and inhibitory platelet membrane proteins. *Blood*, 109: 3260–3269.

https://doi.org/10.1182/blood-2006-07-036269

33. Rubic T, Lametschwandtner G, Jost S, *et al*., 2008, Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. *Nat Immunol*, 9: 1261–1269.

https://doi.org/10.1038/ni.1657

34. Saraiva AL, Veras FP, Peres RS, *et al*., 2018, Succinate receptor deficiency attenuates arthritis by reducing dendritic cell traffic and expansion of Th17 cells in the lymph nodes. *FASEB J*, 32: 6550–6558.

https://doi.org/10.1096/fj.201800285

35. Trauelsen M, Rexen Ulven E, Hjorth SA, *et al*., 2017, Receptor structure-based discovery of non-metabolite agonists for the succinate receptor GPR91. *Mol Metab*, 6: 1585–1596.

https://doi.org/10.1016/j.molmet.2017.09.005

36. Littlewood-Evans A, Sarret S, Apfel V, *et al*., 2016, GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. *J Exp Med*, 213: 1655–1662.

https://doi.org/10.1084/jem.20160061

37. van Diepen JA, Robben JH, Hooiveld GJ, *et al*., 2017, SUCNR1-mediated chemotaxis of macrophages aggravates obesity-induced inflammation and diabetes. *Diabetologia*, 60: 1304–1313.

https://doi.org/10.1007/s00125-017-4261-z

38. Harber KJ, de Goede KE, Verberk SG, *et al*., 2020, Succinate is an inflammation-induced immunoregulatory metabolite in macrophages. *Metabolites*, 10: 372.

https://doi.org/10.3390/metabo10090372

39. Fernandez-Veledo S, Ceperuelo-Mallafre V, Vendrell J, 2021, Rethinking succinate: An unexpected hormone-like metabolite in energy homeostasis. *Trends Endocrinol Metab*, 32: 680–692.

https://doi.org/10.1016/j.tem.2021.06.003

40. Xu J, Zheng Y, Zhao Y, *et al*., 2022, Succinate/IL-1beta signaling axis promotes the inflammatory progression of endothelial and exacerbates atherosclerosis. *Front Immunol*, 13: 817572.

https://doi.org/10.3389/fimmu.2022.817572

41. Chen J, Zhang J, Wu J, *et al*., 2021, Low shear stress induced vascular endothelial cell pyroptosis by TET2/SDHB/ROS pathway. *Free Radic Biol Med*, 162: 582–591.

https://doi.org/10.1016/j.freeradbiomed.2020.11.017

42. Wu P, Chen J, Chen J, *et al*., 2020, Trimethylamine N-oxide promotes apoE(-/-) mice atherosclerosis by inducing vascular endothelial cell pyroptosis via the SDHB/ROS pathway. *J Cell Physiol*, 235: 6582–6591.

https://doi.org/10.1002/jcp.29518

43. Mills EL, Kelly B, Logan A, *et al*., 2016, Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell*, 167: 457–470 e413.

https://doi.org/10.1016/j.cell.2016.08.064

44. Ming-Chin Lee K, Achuthan AA, De Souza DP, *et al*., 2022, Type I interferon antagonism of the JMJD3-IRF4 pathway modulates macrophage activation and polarization. *Cell Rep*, 39: 110719.

https://doi.org/10.1016/j.celrep.2022.110719

45. Wu JY, Huang TW, Hsieh YT, *et al*., 2020, Cancer-derived succinate promotes macrophage polarization and cancer metastasis via succinate receptor. *Mol Cell*, 77: 213–227 e215.

https://doi.org/10.1016/j.molcel.2019.10.023

46. Sampson UK, Norman PE, Fowkes FG, *et al*., 2014, Global and regional burden of aortic dissection and aneurysms: Mortality trends in 21 world regions, 1990 to 2010. *Glob Heart*, 9: 171–180 e110.

https://doi.org/10.1016/j.gheart.2013.12.010

47. Cui H, Chen Y, Li K, *et al*., 2021, Untargeted metabolomics identifies succinate as a biomarker and therapeutic target in aortic aneurysm and dissection. *Eur Heart J*, 42: 4373–4385.

https://doi.org/10.1093/eurheartj/ehab605

48. Toma I, Kang JJ, Sipos A, *et al*., 2008, Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. *J Clin Invest*, 118: 2526–2534.

https://doi.org/10.1172/JCI33293

49. Vargas SL, Toma I, Kang JJ, *et al*., 2009, Activation of the succinate receptor GPR91 in macula densa cells causes renin release. *J Am Soc Nephrol*, 20: 1002–1011.

https://doi.org/10.1681/ASN.2008070740

50. Robben JH, Fenton RA, Vargas SL, *et al*., 2009, Localization of the succinate receptor in the distal nephron and its signaling in polarized MDCK cells. *Kidney Int*, 76: 1258–1267.

https://doi.org/10.1038/ki.2009.360

51. Sadagopan N, Li W, Roberds SL, *et al*., 2007, Circulating succinate is elevated in rodent models of hypertension and metabolic disease. *Am J Hypertens*, 20: 1209–1215.

https://doi.org/10.1016/j.amjhyper.2007.05.010

52. Khamaysi A, Anbtawee-Jomaa S, Fremder M, *et al*., 2019, Systemic succinate homeostasis and local succinate signaling affect blood pressure and modify risks for calcium oxalate lithogenesis. *J Am Soc Nephrol*, 30: 381–392.

https://doi.org/10.1681/ASN.2018030277

53. Milliken AS, Nadtochiy SM, Brookes PS, 2022, Inhibiting succinate release worsens cardiac reperfusion injury by enhancing mitochondrial reactive oxygen species generation. *J Am Heart Assoc*, 11: e026135.

https://doi.org/10.1161/JAHA.122.026135

54. Lu YT, Li LZ, Yang YL, *et al*., 2018, Succinate induces aberrant mitochondrial fission in cardiomyocytes through GPR91 signaling. *Cell Death Dis*, 9: 672.

https://doi.org/10.1038/s41419-018-0708-5

55. Pell VR, Chouchani ET, Frezza C, *et al*., 2016, Succinate metabolism: A new therapeutic target for myocardial reperfusion injury. *Cardiovasc Res*, 111: 134–141.

https://doi.org/10.1093/cvr/cvw100

56. Bae J, Salamon RJ, Brandt EB, *et al*., 2021, Malonate promotes adult cardiomyocyte proliferation and heart regeneration. *Circulation*, 143: 1973–1986.

https://doi.org/10.1161/CIRCULATIONAHA.120.049952

57. Valls-Lacalle L, Barba I, Miro-Casas E, *et al*., 2018, Selective inhibition of succinate dehydrogenase in reperfused myocardium with intracoronary malonate reduces infarct size. *Sci Rep*, 8: 2442.

https://doi.org/10.1038/s41598-018-20866-4

58. Xu J, Pan H, Xie X, *et al*., 2018, Inhibiting succinate dehydrogenase by dimethyl malonate alleviates brain damage in a rat model of cardiac arrest. *Neuroscience*, 393: 24–32.

https://doi.org/10.1016/j.neuroscience.2018.09.041

59. Martin JL, Costa AS, Gruszczyk AV, *et al*., 2009, Succinate accumulation drives ischaemia-reperfusion injury during organ transplantation. *Nat Metab*, 1: 966–974.

https://doi.org/10.1038/s42255-019-0115-y

60. Wyss RK, Mendez Carmona N, Arnold M, *et al*., 2021, Hypothermic, oxygenated perfusion (HOPE) provides cardioprotection via succinate oxidation prior to normothermic perfusion in a rat model of donation after circulatory death (DCD). *Am J Transplant*, 21: 1003–1011.

https://doi.org/10.1111/ajt.16258

61. Kolwicz SC Jr., Purohit S, Tian R, 2013, Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes. *Circ Res*, 113: 603–616.

https://doi.org/10.1161/CIRCRESAHA.113.302095

62. Aguiar CJ, Rocha-Franco JA, Sousa PA, *et al*., 2014, Succinate causes pathological cardiomyocyte hypertrophy through GPR91 activation. *Cell Commun Signal*, 12: 78.

https://doi.org/10.1186/s12964-014-0078-2

63. Yang L, Yu D, Mo R, *et al*., 2016, The succinate receptor GPR91 is involved in pressure overload-induced ventricular hypertrophy. *PLoS One*, 11: e0147597.

https://doi.org/10.1371/journal.pone.0147597

64. Davili Z, Johar S, Hughes C, *et al*., 2007, Succinate dehydrogenase deficiency associated with dilated cardiomyopathy and ventricular noncompaction. *Eur J Pediatr*, 166: 867–870.

https://doi.org/10.1007/s00431-006-0310-1

65. Aguiar CJ, Andrade VL, Gomes ER, *et al*., 2010, Succinate modulates Ca(2+) transient and cardiomyocyte viability through PKA-dependent pathway. *Cell Calcium*, 47: 37–46.

https://doi.org/10.1016/j.ceca.2009.11.003

66. Banerjee P, Bandyopadhyay A, 2014, Cytosolic dynamics of annexin A6 trigger feedback regulation of hypertrophy via atrial natriuretic peptide in cardiomyocytes. *J Biol Chem*, 289: 5371–5385.

https://doi.org/10.1074/jbc.M113.514810

67. Palomer X, Salvado L, Barroso E, *et al*., 2013, An overview of the crosstalk between inflammatory processes and metabolic dysregulation during diabetic cardiomyopathy. *Int J Cardiol*, 168: 3160–3172.

https://doi.org/10.1016/j.ijcard.2013.07.150

68. Singh T, Khan H, Gamble DT, *et al*., 2022, Takotsubo syndrome: Pathophysiology, emerging concepts, and clinical implications. *Circulation*, 145: 1002–1019.

https://doi.org/10.1161/CIRCULATIONAHA.121.055854

69. Keiran N, Ceperuelo-Mallafre V, Calvo E, *et al*., 2019, SUCNR1 controls an anti-inflammatory program in macrophages to regulate the metabolic response to obesity. *Nat Immunol*, 20: 581–592.

https://doi.org/10.1038/s41590-019-0372-7

70. Ceperuelo-Mallafre V, Llaurado G, Keiran N, *et al*., 2019, Preoperative circulating succinate levels as a biomarker for diabetes remission after bariatric surgery. *Diabetes Care*, 42: 1956–1965.

https://doi.org/10.2337/dc19-0114

71. Mills EL, Pierce KA, Jedrychowski MP, *et al*., 2018, Accumulation of succinate controls activation of adipose tissue thermogenesis. *Nature*, 560: 102–106.

https://doi.org/10.1038/s41586-018-0353-2

72. Mills EL, Harmon C, Jedrychowski MP, *et al*., 2021, UCP1 governs liver extracellular succinate and inflammatory pathogenesis. *Nat Metab*, 3: 604–617.

https://doi.org/10.1038/s42255-021-00389-5

73. Liu X, Chen Y, Zhao L, *et al*., 2022, Dietary succinate supplementation to maternal mice improves fetal brown adipose tissue development and thermogenesis of female offspring. *J Nutr Biochem*, 100: 108908.

https://doi.org/10.1016/j.jnutbio.2021.108908

74. Liu K, Lin L, Li Q, *et al*., 2020, Scd1 controls de novo beige fat biogenesis through succinate-dependent regulation of mitochondrial complex II. *Proc Natl Acad Sci U S A*, 117: 2462–2472.

https://doi.org/10.1073/pnas.1914553117

75. Regard JB, Sato IT, Coughlin SR, 2008, Anatomical profiling of G protein-coupled receptor expression. *Cell*, 135: 561–571.

https://doi.org/10.1016/j.cell.2008.08.040

76. McCreath KJ, Espada S, Galvez BG, *et al*., 2015, Targeted disruption of the SUCNR1 metabolic receptor leads to dichotomous effects on obesity. *Diabetes*, 64: 1154–1167.

https://doi.org/10.2337/db14-0346

77. Rexen Ulven E, Trauelsen M, Brvar M, *et al*., 2018, Structure-activity investigations and optimisations of non-metabolite agonists for the succinate receptor 1. *Sci Rep*, 8: 10010.

https://doi.org/10.1038/s41598-018-28263-7

78. Geubelle P, Gilissen J, Dilly S, *et al*., 2017, Identification

and pharmacological characterization of succinate receptor agonists. *Br J Pharmacol*, 174: 796–808.

https://doi.org/10.1111/bph.13738

79. Bhuniya D, Umrani D, Dave B, *et al*., 2011, Discovery of a potent and selective small molecule hGPR91 antagonist. *Bioorg Med Chem Lett*, 21: 3596–3602.

https://doi.org/10.1016/j.bmcl.2011.04.091

80. Sakai M, Sumiyoshi T, Aoyama T, *et al*., 2020, GPR91 antagonist and TGF-beta inhibitor suppressed collagen production of high glucose and succinate induced HSC activation. *Biochem Biophys Res Commun*, 530: 362–366.

https://doi.org/10.1016/j.bbrc.2020.07.141

81. Le CT, Nguyen G, Park SY, *et al*., 2018, LY2405319, an analog of fibroblast growth factor 21 ameliorates alpha-smooth muscle actin production through inhibition of the succinate-G-protein couple receptor 91 (GPR91) pathway in mice. *PLoS One*, 13: e0192146.

https://doi.org/10.1371/journal.pone.0192146

82. Nguyen G, Park SY, Le CT, *et al.,* 2018, Metformin ameliorates activation of hepatic stellate cells and hepatic fibrosis by succinate and GPR91 inhibition. *Biochem Biophys Res Commun*, 495: 2649–2656.

https://doi.org/10.1016/j.bbrc.2017.12.143

83. Zhang H, Zheng J, Lin J, *et al*., 2018, miR-758 mediates oxLDL-dependent vascular endothelial cell damage by suppressing the succinate receptor SUCNR1. *Gene*, 663: 1–8.

https://doi.org/10.1016/j.gene.2018.04.029

84. Li T, Hu J, Gao F, *et al*., 2017, Transcription factors regulate GPR91-mediated expression of VEGF in hypoxia-induced retinopathy. *Sci Rep*, 7: 45807.

https://doi.org/10.1038/srep45807

85. Osuna-Prieto FJ, Martinez-Tellez B, Ortiz-Alvarez L, *et al*., 2021, Elevated plasma succinate levels are linked to higher cardiovascular disease risk factors in young adults. *Cardiovasc Diabetol*, 20: 151.

https://doi.org/10.1186/s12933-021-01333-3

86. Serena C, Ceperuelo-Mallafre V, Keiran N, *et al*., 2018, Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. *ISME J*, 12: 1642–1657.

https://doi.org/10.1038/s41396-018-0068-2

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Each submission should be accompanied by 3-6 keywords. Avoid using abbreviations and acronyms in keywords, unless they are established standard keywords. Separate keywords with semi-colons (i.e, term1; term2; term3).

Abbreviations and acronyms

Define abbreviations and acronyms upon their first appearance, **separately**, in the abstract, main text, table legends, and figure captions and legends.

Sections in article

(1) Section headings

Section headings should be in boldface. Examples of section headings of different levels are shown in the following:

Primary level : **1. Heart disease**

Secondary level : **1.3. Risk factors for heart disease**

Tertiary level : **1.3.2. Hypertension**

Authors are suggested **NOT** to introduce further sub-sections after the tertiary level section (e.g., **1.3.2.1. High-salt diet**).

(2) Special sectioning requirements for an original research article

- The introduction should provide a background that gives a broad readership an overall outlook of the field and the research performed. It tackles a problem and states its important regarding with the significance of the study. Introduction can conclude with a brief statement of the aim of the work and a comment about whether that aim was achieved.

- **Materials and Methods.** This section provides the general experimental design and methodologies used. The aim is to provide enough detail to for other investigators to fully replicate the results. It is also required to facilitate better understanding of the results obtained. Protocols and procedures for new methods must be included in detail for the reproducibility of the experiments. Informed consent should be obtained from patients or parents before the experiments start and should be mentioned in this section. For human and/or research, research ethics information, such as ethics approval identifiers and the name of Institutional Ethics Review Board or Institutional Review Board, should be indicated in this section.

- This section focuses on the results and findings of the experiments performed. After (statistical) analysis, all results, including tables and figures, must be neatly presented. If necessary, this section can be sub-divided into multiple topical sub-sections.

- This section should provide the significance of the results and identify the impact of the research in a broader context. It should not be redundant or similar to the content of the results section.

- Use this section for interpretation only, and not to summarize information already presented in the text or abstract.

It is acceptable to merge both Results and Discussion as a single section.

Data and image processing

Post-acquisition processing of images, photos and figures should be kept minimum to ensure that the final figures accurately reflect the original data as it was captured and/or produced. Any alterations should be applied to the entire image. Any kind of alteration, including but not limited to brightness, contrast and color balance, has to be clearly stated in the figure legend and in Materials and Methods section. For simulated or model figures, the software used for production, editing, and/or processing should be mentioned. Presenting images in the same figure must be made apparent and should be explicitly indicated in the appropriate figure legends.

Data comparisons should only be made from comparative experiments (or data from the same experiment). Same piece of data or figure should not be used in multiple instances, unless the images/data describe different aspects of the same experiment (reasons must be stated, wherever appropriate, in this regard). If inappropriate image/data manipulation is identified after publication, the editors reserve the right to ask for the original data and, if that is not satisfactory, to issue a correction or retract the paper, as appropriate.

Unit of measurements

Use SI units.

Nomenclature of genus and species

Write in italics (e.g. *Escherichia coli*). The full genus and species names must be mentioned both in the manuscript title at the first appearance of an organism in an article. The abbreviation (e.g. *E. coli*) is allowed after first mention.

Nomenclature of genes, mutations, genotypes, and alleles

Write in italics. *Global Translational Medicine* highly encourages the use the recommended names found in the gene nomenclature databases, for instance, **HUGO Gene Nomenclature Committee**.

Chemical compounds

Global Translational Medicine requires authors to fulfill the requirements below while reporting and/or describing a chemical compound in articles:

Scenario	Requirements
Naming chemical compounds	Use either IUPAC conventions or common names such as cholesterol and cephalosporins
Reporting a new chemical compound	Provide the exact structure of the compound as well as sufficient data regarding the purity and identity of the compound
Reporting the use of a known chemical compound	Provide sufficient data regarding the source, purity and identity of the compound

Figures

Include all figures, including photographs, scanned images, graphs, charts and schematic diagrams, at the back of manuscript. Avoid unnecessary decorative effects (e.g., 3D graphs) and minimize image processing (e.g., changes in brightness and contrast applied uniformly for the entire figure should be avoided or minimized). All images should be set against white background.

All figures should be numbered (e.g., **Figure 1**, **Figure 2**) in boldface. Label all figures (e.g., axis, structures), and add caption (a brief title) and legend as a description of the illustration below each figure. Explain all symbols and abbreviations used. Each figure should have a brief title (also known as caption) that describes the entire figure without citing specific panels, followed by a legend, which is either the description of each panel or further description about the single image. Identify each panel with uppercase letters in parenthesis (e.g. (A), (B), (C), etc.) Figures must be cited in chronological manner in the text.

The preferred file formats for any separately submitted figure(s) are JPEG, PNG and TIFF. All figures should be of optimal resolution. Optimal resolutions preferred are 300 dots per inch (dpi) for RBG colored, 600 dpi for grayscale and 1,200 dpi for line art. Although there is no file-size limitation imposed, authors are highly encouraged to compress their figures to an ideal size without unduly affecting the legibility and resolution of figures.

If necessary, the editors may request author(s) to supply high-resolution and/or unprocessed images after submission or paper acceptance for pre-screening/review and production purposes, respectively.

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Include all tables at the back of manuscript. Editable tables created using Microsoft Word are preferred. A table should be accompanied by a caption on top of it. Captions and legends (which are placed beneath table) should be concise. All tables should be numbered (e.g., **Table 1**, **Table 2**) in boldface. Explain all symbols and abbreviations used. Tables must be cited in chronological manner in the text.

Lists and math formulae

Lists and math formulae should be properly aligned and included within the main body of the manuscript. List them using Roman numerals in parenthesis (e.g. (I), (II), (III), (IV), etc.) Lists and math formulae must be cited in chronological manner in the text.

Lists and math formulae should be given in editable text and not as images. Use the solidus (/) for small fractional terms, e.g., X/Y. In principle, variables should be italicized.

Footnotes

Do not use footnotes.

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Reference citations in the text should be numbered consecutively in superscript square brackets. Some examples:

- Negotiation research spans many disciplines[3,4].
- This result was later contradicted by Becker and Seligman[5].
- This effect has been widely studied[1–3,7].

Do not include citations in the Abstract.

Personal communications and unpublished works can only be used in the manuscript and are not to be placed in the References section. Authors are advised to limit such usage to the minimum. These should be made identifiable by stating the authors, year of personal communications or unpublished works, and the words “personal communication” or “unpublished” in parenthesis, e.g., (Smith J, 2000, unpublished).

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This section is compulsory and should be placed at the end of all manuscripts. Do not use footnotes or endnotes as a substitute for a reference list. The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should be excluded from this section.

Authors being referenced are listed with their surname or last name followed by their initials. All references should be numbered (e.g., 1, 2, 3, and so on) and sequenced according to the order they appear as the in-text citations. References (especially journal article's) should follow the general pattern: author(s), followed by year of publication, title of publication, abbreviated journal name in italics, volume number, issue number in parenthesis and lastly, page range or article ID. If the referred article has more than 3 authors, list only the first 3 authors and abbreviate the remaining authors as italicized "*et al*." (meaning "and others"). Use of DOI is highly encouraged; include DOI, if available, after the page range or article ID. Examples of references for different types of publications are as follows:

(1) Journals

Journal article (print) with 1-3 authors:

Younger P, 2004, Using the internet to conduct a literature search. *Nurs Stand*, 19(6): 45–51.

Journal article (print) with more than 3 authors:

Gamelin FX, Baquet G, Berthoin S, *et al*., 2009, Effect of high intensity intermittent training on heart rate variability in prepubescent children. *Eur J Appl Physiol,* 105(1): 731–738.

Journal article (online) with 1-3 authors:

Jackson D, Firtko A, Edenborough M, 2007, Personal resilience as a strategy for surviving and thriving in the face of workplace adversity: A literature review. *J Adv Nurs*, 60(1): 1–9. http://doi.org/10.1111/j.1365-2648.2007.04412.x

Journal article (online) with more than 3 authors:

Hargreave M, Jensen A, Nielsen TSS, *et al*., 2015, Maternal use of fertility drugs and risk of cancer in children — A nationwide population-based cohort study in Denmark. *Int J Cancer*, 136(8): 1931–1939. http://doi.org/10.1002/ijc.29235

(2) Books

Book with 1-3 authors:

Schneider Z, Whitehead D, Elliott D, 2007, *Nursing and Midwifery Research: Methods and Appraisal for Evidence-based Practice*, 3rd edn, Elsevier Australia, Marrickville, NSW, 112–130.

Book with more than 3 authors

Davis M, Charles L, Curry MJ, *et al*., 2003, *Challenging Spatial Norms*, Routledge, London, 12–30.

Chapter or article in book

Knowles MS, (eds) 1986, Independent study, in *Using Learning Contracts*, Jossey-Bass, San Francisco, 89–96.

(3) Preprints

Preprint article with 1-3 authors:

Ulgen A, Gurkut O, Li W, 2019, Potential Predictive Factors for Breast Cancer Subtypes from a North Cyprus Cohort Analysis. *medRxiv*. https://doi.org/10.1101/19010181

Preprint article with more than 3 authors:

Wu S, Sun P, Li R, *et al*., 2020, Epidemiological Development of Novel Coronavirus Pneumonia in China and Its Forecast. *medRxiv*. https://doi.org/10.1101/2020.02.21.20026229

(4) Others

Proceedings of meetings and symposiums, conference papers:

Chang SS, Liaw L, Ruppenhofer J, (eds) 2000, *Proceedings of the twenty-fifth annual meeting of the Berkeley Linguistics Society, February 12–15, 1999: General session and parasession on loan word phenomena*. Berkeley Linguistics Society, Berkeley, 12–13.

Conference proceedings (from electronic database):

Wang T, Cook C, Derby B, 2009, Fabrication of a glucose biosensor by piezoelectric inkjet printing. *Proceedings of the Third International Conference on Sensor Technologies and Applications, 2009 (SENSORCOM-M'09)*, 82–85.

Online document with author names:

Este J, Warren C, Connor L, *et al*., 2008, Life in the clickstream: The future of journalism, Media Entertainment and Arts Alliance, viewed May 27, 2009, http://www.alliance.org.au/documents/ foj_report_final.pdf

Online document without author name:

Developing an argument, n.d., viewed March 30, 2009, http://web.princeton.edu/sites/writing/Writing_Center/WCWritingResources.htm

Thesis/Dissertation:

Gale L, 2000, The relationship between leadership and employee empowerment for successful total quality management, thesis, *Australasian Digital Thesis database*, University of Western Sydney, 110–130.

Standards:

Standards Australia Online, 2006, Glass in buildings: selection and installation, AS 1288-2006, amended January 31, 2008, *SAI Global database*, viewed May 19, 2009.

Government report:

National Commission of Audit, 1996, *Report to the Commonwealth Government*, Australian Government Publishing Service, Canberra.

Government report (online):

Department of Health and Ageing, 2008, Ageing and aged care in Australia, viewed November 10, 2008, http://www.health.gov.au/internet/main/publishing.nsf/Content/ageing

No author:

Guide to agricultural meteorological practices, 1981, 2nd ed, Secretariat of the World Meteorological Organization, Geneva, 10–20.

Note: When referencing an entry from a dictionary or an encyclopedia with no author there is no requirement to include the source in the reference list. In these cases, only cite the title and year of the source in-text. For an authored dictionary/encyclopedia, treat the source as an authored book.

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This section should be included in original research articles, review articles and case reports. In *Global Translational Medicine*, we encourage authors to use **Contributor Roles Taxonomy (CRediT)** in describing each contributor's specific contribution to the scholarly output in the Author Contributions section.

Definitions of each contributor role as per CRediT are as follows:

Contributor role	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims.
Data curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use.
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Project administration	Management and coordination responsibility for the research activity planning and execution.
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Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.
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Authors are advised to follow *Global Translational Medicine*'s preferred style of writing the Author Contributions statement. See an example below:

Conceptualization: Ali Jackson, Helen Meyer

Investigation: Ali Jackson, Tom Lewis-Hans, Han Xiang

Formal analysis: Han Xiang

Writing – original draft: Ali Jackson

Writing – review & editing: Helen Meyer, Joshua O'Brien

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This section is optional and contains all materials and figures that are excluded from the manuscript. These materials, figures or additional information are relevant to the manuscript but remain non-essential to readers' understanding of the manuscript's main content. All supplementary information should be submitted as a separate file during submission.

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If the editorial decision for a submission is major revision or minor revision, authors are advised to revise the manuscript (and possibly, the supplementary files) as per the review reports and resubmit the revision file, including the manuscript, title page and back matter, cover letter, and response/rebuttal letter, before the due date.

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Global Translational Medicine

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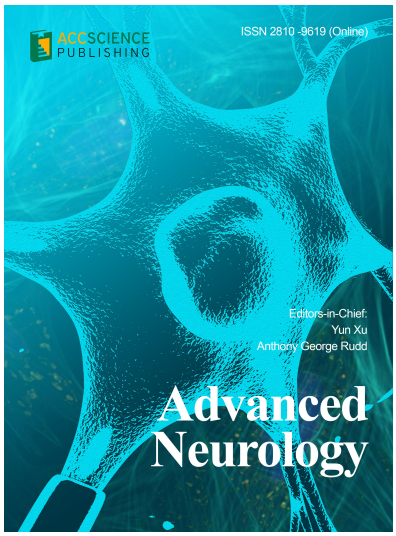

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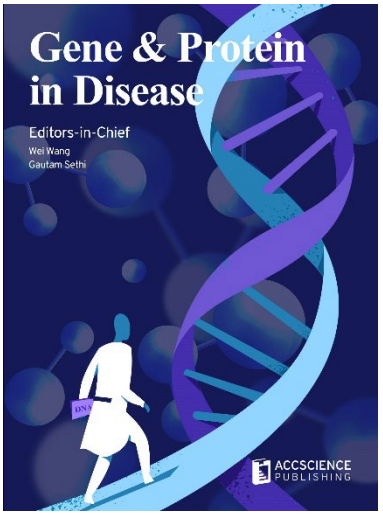

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