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Tumor Discovery

REVIEW ARTICLE

Current concepts and new insights into early tumor initiation

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Abstract

Despite substantial research and clinical efforts, the mechanisms underlying tumor initiation and progression remain incompletely understood and, in some areas, controversial. In tumorigenesis, genetic mutations and abnormal gene expression can confer survival advantages on selected cells and contribute to reciprocal interactions with the tumor microenvironment (TME), thereby promoting malignant transformation. This process includes the progression from a precancerous lesion to primary cancer. To maintain their survival advantage, cancerous cells must progressively optimize and exploit the existing functions and regulatory systems of all tissue cells and maximize the body's available resources. Exploiting existing physiological systems and resources supports the clonal expansion and high dominance of tumor cells after their formation, allowing tumor cells to proliferate and infiltrate adjacent tissue. Temporally, tumor cells exhibit the growth and change characteristics of 'persistent dynamic biology' and dynamically update themselves. The patient-specific TME is the basis of cancer recurrence and metastasis. In addition, we propose the "Dysregulated Adaptive Cascade Model" (DACM) to elucidate the early processes of tumorigenesis. Incorporating these different perspectives will enormously refresh our understanding of tumorigenesis. Conventional cancer-control strategies emphasize early detection, early diagnosis, and early treatment, but they do not fully address prevention at the precancerous stage. Therefore, we propose new cancer prevention principles: early detection of precancerous lesions, early determination of patient-specific TME, and early intervention to halt tumorigenesis. These new cancer prevention principles are of great significance to the prevention and control of the occurrence and development of tumors. In conclusion, the highlights of this article include: (i) proposing the novel "persistent dynamic biology" concept and the early tumorigenesis model DACM; (ii) establishing an integrated framework of the above concept and organoid culture for individualized cancer detection, diagnosis, and treatment; and (iii) proposing three innovative cancer prevention principles targeting precancerous lesions, patient-specific TME, and early intervention.

Keywords: Tumorigenesis; Intratumoral heterogeneity; Tumor microenvironment; Persistent dynamic biology; Organoid

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1. Introduction

It is well established in traditional tumor biology that tumor development is caused by many factors, including genetic elements, living environment, physical and chemical elements, and viral infection. Within a single cell, these factors can lead to somatic gene mutations, causing the loss of normal gene function and abnormal gene expression, thereby affecting critical biological processes such as cell proliferation, metabolism, and apoptosis. Eventually, this process leads to the development of tumor cells that are significantly different from normal cells with respect to their morphology, metabolism, and function. Multiple genes participate in the process of tumor development, which occurs through a series of steps leading to continuous cellular changes. Tumor cells have an abnormal morphology, which is used clinically for tumor pathological diagnosis. Decades of in-depth study led to the identification of the following hallmarks that define tumorigenesis: sustaining proliferative and growth signaling, evading immune surveillance and growth suppressors, angiogenesis, invasion and metastasis, resisting cell death, energy metabolism reprogramming, genome instability, epigenetic reprogramming, and senescence.[1–12] Although the above hallmarks are supported by many experimental results *in vitro*, they still fail to solve the fundamental problem of tumorigenesis, which includes when, how, and what happens in the very beginning of the tumor occurrence. Additional molecular biology studies revealed that genetic mutations in specific tumor driver genes can be classified into 12 signaling pathways that regulate three core cellular processes: cell failure, cell survival, and gene maintenance.[8] Ultimately, uncontrolled and sustained high-level gene expression and proto-oncogene activation[9,11,13–15] are crucial factors in tumorigenesis. In addition, the aberrant and continuous activation of these key signal transduction systems also plays a pivotal role in driving tumor development.[10,16] Furthermore, the phenomenon of spatiotemporal heterogeneity in tumor cells, which is referred to as intratumoral heterogeneity (ITH)[17], has led researchers to question whether "current cancer research may be on the wrong track; this is because it considers cancer more as a 'monogenic' disease, tries to extract common information from thousands of patients, and fails to properly consider its complexity and individual diversity".[18(p1)] ITH reminds us to earnestly re-examine the tumorigenesis process and mechanisms underlying normal cell carcinogenesis. Doing so will allow cancer researchers and clinicians to formulate more effective prevention and treatment strategies.

Therefore, the main challenges facing current tumor research include: firstly, the origin and maintenance mechanisms of tumor heterogeneity remain controversial; secondly, the traditional mutation-centered paradigm of tumorigenesis is facing challenges; thirdly, our understanding of the precise molecular mechanisms underlying the transformation of precancerous lesions into malignant tumors is still incomplete, especially regarding the dynamic processes at the cellular and tissue levels.

To explore the fundamental problem of tumorigenesis, it is necessary to first understand the biological mechanisms by which normal cells undergo malignant transformation. We believe that cells or tissue stem cells achieve their best survival advantage by dynamically adapting to both favorable and unfavorable alterations of the cell's microenvironment. This enhanced adaptability functions as a critical driving force of malignant transformation. To achieve this phenotype, which ultimately leads to malignant transformation, cells must experience genetic mutations and/or abnormal gene expression that alters all of a cell's biological behaviors, such as metabolism, cell growth and proliferation, and interaction with many factors in the microenvironment. This process involves two stages, from precancerous lesions to primary cancer. Although the leading tumorigenesis factors of each patient will not change, the subsequent cancerous tissue and cells will change with respect to their intracellular omics due to the influence of ITH. In order to maintain their survival advantage, cancerous cells must utilize and maximize the beneficial functions and signal transduction pathways of all cells, as well as maximize the body's available resources. Through these processes, altered cells may progress from precancerous lesions to primary tumors. Subsequently, clonal expansion and tumor cell dominance lead to primary tumor formation, as well as continuous tumor cell proliferation and infiltration of adjacent tissue. Temporally, tumor cells exhibited "persistent dynamic biology" in that they can adapt according to changes in their microenvironment. Meanwhile, the patient-specific tumor microenvironment (TME) is the pathological and biological basis for cancer recurrence and metastasis.

Although there remains much to be discovered regarding the progression from a normal cell to a precancerous lesion, to primary cancer, additional comprehensive experimental data encompassing various histology, histopathology, and molecular regulation mechanisms will provide the basis for this novel tumorigenesis theory.[19–23]

In this review, we focus on the initial mechanisms of cell carcinogenesis and discuss the necessary signals and resource optimization modes that support continuous cell carcinogenesis, including gene expression changes, activation of cell signal transduction pathways, dysregulation of regulatory pathways and energy

metabolism, enhanced cell survival and resource optimization, changes in microenvironment components, and structural status of cancerous cells. Additionally, we explore the basic characteristics of the "persistent dynamic biology" of cancerous cells, while paying attention to the significance of the patient-specific TME on metastasis and recurrence, and trying to establish a model that simulates the multidimensional and dynamic process of cellular carcinogenesis, involving the participation of the TME under multi-factorial induction. With these concepts in mind, we propose potential clinical applications and future effective prevention strategies (Figure 1).

2. Basic process and mechanisms of carcinogenesis

The classic multistage theory of carcinogenesis divides the carcinogenic process into at least three stages: initiation, promotion, and progression. The initiation stage involves irreversible genetic alterations, the promotion stage is reversible and does not involve changes in DNA structure but rather alterations in genomic expression mediated by promoter-receptor interactions, and the progression stage is the final irreversible stage, characterized by karyotype instability and malignant growth.[24]

Recent research has proposed a new perspective based on the Waddington epigenetic landscape, viewing tumorigenesis primarily as a disruption of cellular development. Within this theoretical framework, cell types are defined by their specific gene expression profiles, which are determined by gene regulatory networks. These networks are regarded as attractors of network dynamics, representing specific self-stabilizing patterns of gene activity in the genome. Large-scale mutational events reshape the landscape topology, creating abnormal "non-physiological" attractors, and such changes are considered key points in the initiation process.[25]

Under the theoretical framework we proposed, normal cells survive and function under the constraints of biological evolution, organizational structure, and long-term coordination with their micro-environment. When normal cells are challenged with either favorable or unfavorable factors, some cells may lose their normal physiological features as they seek the optimal environment and cell state to ensure their continued survival. To achieve this goal, one of the following modes will appear: the incentive model or the depressive model. According to the incentive model, continuous changes in the molecular mechanisms that regulate a robust and durable induction of factors, which promote growth, survival, proliferation, and differentiation in normal cells, alter cell biological behaviors (such as alterations in the induction of growth hormones or other differentiation inductions). For the depressive model, the molecular mechanisms that regulate

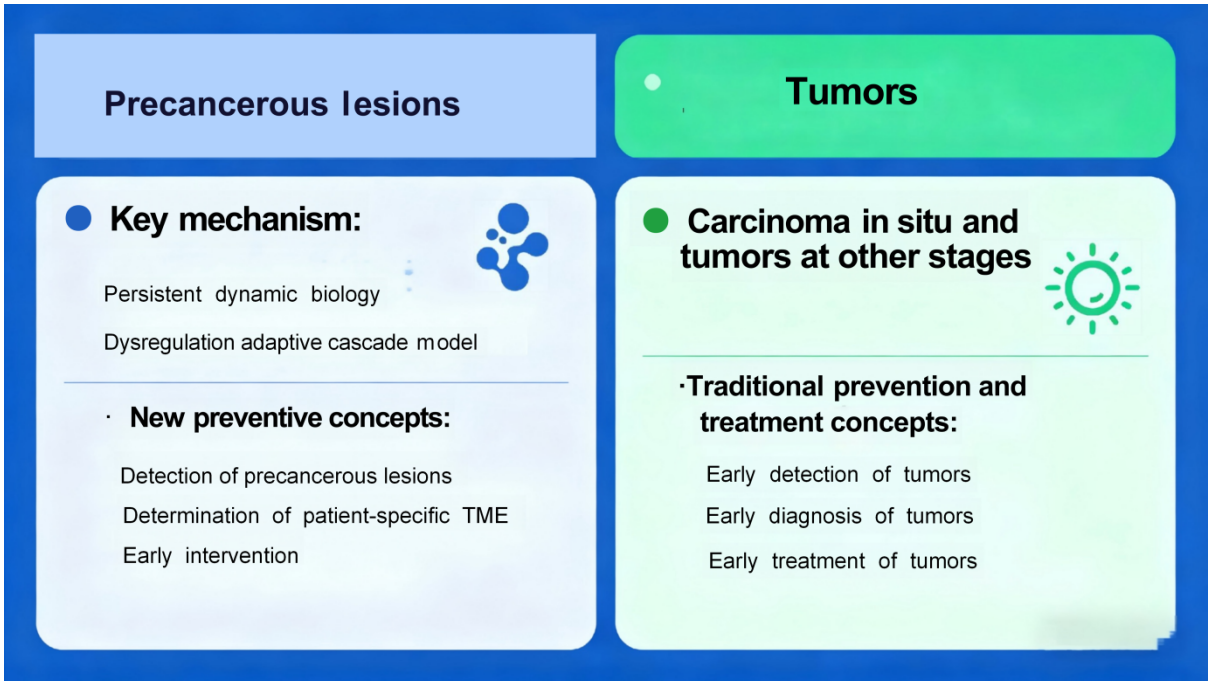

Figure 1. Key molecular mechanisms associated with precancerous lesions and their corresponding preventive strategies, compared with conventional tumor-focused prevention and treatment approaches

a robust and durable induction of factors that inhibit or interfere with the growth, survival, proliferation, and differentiation in normal cells are dysregulated (for instance, in the infection of foreign substances or microorganisms and slow inflammatory processes). In response to a strong induction, the enhanced activity of the multi-signal transduction systems in the cells leads to abnormal structure or expression of downstream target genes, thereby affecting the expression of proto-oncogenes and tumor suppressor genes. Disruption of normal expression levels of these important proto-oncogenes and tumor suppressor genes promotes cell cycle progression, inhibits cell apoptosis, and alters the precise regulatory mechanisms of other critical genes and intracellular signal transduction pathways. The repeated and long-term induction of these changes promotes carcinogenesis. In the face of strong and long-term inducements, cells adapt to improve their survival status, and this represents the most primitive driving force that promotes carcinogenesis. Research in 2024 demonstrated that transient perturbations of Polycomb protein-mediated transcriptional silencing are sufficient to induce an irreversible transition to the fate of cancer cells in Drosophila. This is associated with the irreversible derepression of genes driving tumorigenesis, including members of the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling pathway and *zfh1* (the Drosophila homolog of the *ZEB1* oncogene), whose aberrant activation is essential for tumorigenesis induced by disruption of Polycomb-mediated gene repression. This latest research result supports our understanding of early tumorigenesis and development.[26]

2.1. Precancerous lesions as early-stage pathological changes in tumor development

Precancerous lesions are lesions that occur before invasive cancer and are closely associated with an increased risk of cancer. They are characterized by the fact that cancer is more likely to develop from these lesions than from seemingly normal corresponding tissues. Precancerous lesions are characterized by abnormal histological and immunohistochemical features and may manifest as adenomas, dysplasia, or benign tumors. The American Association for Cancer Research (AACR) defines precancerous lesions as non-invasive lesions that exhibit genetic abnormalities, loss of cell control functions, and certain phenotypic characteristics of invasive cancer, and indicate a substantial likelihood of developing into invasive cancer.[27]

The development of precancerous lesions follows specific molecular pathways. Taking colorectal cancer as an example, there are two main pathways for the development of precancerous lesions: the first involves the transition from adenoma to carcinoma, while the second involves the serrated pathway, which represents another major route to colorectal carcinogenesis. Research has shown that these precancerous lesions originate from crypt stem cells that have undergone a series of genetic and epigenetic alterations. The transition from adenoma to carcinoma involves the sequential activation of oncogenes such as *RAS* and the inactivation of tumor suppressor genes such as *APC* and *TP53*; the transition from serrated adenoma to carcinoma involves *BRAF* mutations and extensive methylation of CpG islands.

Cervical intraepithelial neoplasia (CIN) is a multifactorial and multistep pathological process, with persistent infection of high-risk human papillomavirus (HPV) as the core driving factor. It is also synergistically influenced by host immune function, genetic factors, and behavioral factors, which ultimately induce abnormal proliferation and disordered differentiation of cervical squamous epithelial cells, leading to the gradual progression to precancerous lesions.

Human papillomavirus is a small, non-enveloped virus with a circular double-stranded DNA genome that infects basal cells of the cervical squamous epithelium. Persistent infection with high-risk HPV genotypes, particularly HPV16 and 18 (accounting for over 70% of all CIN cases and cervical squamous cell carcinoma), is a major etiological factor for CIN. The main pathogenic mechanisms are as follows:

(i) Aberrant expression of the E6 and E7 oncoproteins encoded by high-risk HPV interferes with the *TP53* and retinoblastoma (*RB*) genes, respectively. This inhibits *TP53*-mediated apoptosis and disrupts RB protein-mediated cell-cycle control, resulting in uncontrolled cell proliferation and abnormal differentiation, which gradually progresses to epithelial dysplasia.

(ii) If the host fails to clear the infection, persistent HPV infection may be accompanied by integration of the viral genome into the host genome. This integration may disrupt viral regulatory control and increase E6/E7 expression, further promoting cell-cycle dysregulation and the transformation of abnormally proliferating cells toward a malignant phenotype. Consequently, lesions may progress from low-grade CIN to high-grade CIN and, in some cases, to invasive cervical squamous cell carcinoma.

The characteristics of CIN are mainly reflected in pathological morphology, clinical manifestations, and grading features. Histopathologic examination of cervical biopsy specimens is the diagnostic standard, while grading reflects lesion severity and the risk of progression. CIN is

characterized by dysplasia of cervical squamous epithelial cells, with the following pathological features:

(i) Cellular morphological abnormalities: Cells exhibit pleomorphism, including variation in size and shape, enlarged nuclei, increased and unevenly distributed chromatin, prominent nucleoli, increased mitotic activity, and an abnormal nuclear–cytoplasmic ratio.
(ii) Disordered cellular arrangement: The hierarchical structure of the squamous epithelium is disrupted, with active proliferation of basal-layer cells. Abnormal cells may extend upward, leading to loose intercellular connections and loss of cellular polarity.
(iii) Extent of involvement: The lesion is mainly confined to the cervical squamous epithelium and does not breach the basement membrane; breach of the basement membrane indicates progression to invasive carcinoma. CIN is classified into grades according to the degree of dysplasia and the depth of epithelial involvement by abnormal cells.

According to the degree of cellular dysplasia and the extent of epithelial involvement, CIN is divided into three grades:

(i) CIN grade 1 (low-grade squamous intraepithelial lesion): Abnormal cells are mainly confined to the lower one-third of the cervical squamous epithelium, with mild cellular dysplasia and rare mitotic figures. CIN1 is usually associated with productive HPV infection and often regresses spontaneously (approximately 60–80%), with a low risk of progression (<1%).
(ii) CIN grade 2 (intermediate-grade squamous intraepithelial lesion): Abnormal cells involve the lower two-thirds of the cervical squamous epithelium, with more pronounced dysplasia and increased mitotic figures. CIN2 is commonly associated with persistent high-risk HPV infection and has a lower spontaneous regression rate and higher progression risk than CIN1, requiring close clinical follow-up or intervention.
(iii) CIN grade 3 (high-grade squamous intraepithelial lesion): Abnormal cells involve more than two-thirds or the full thickness of the cervical squamous epithelium, with marked cellular dysplasia and frequent mitotic figures (including atypical mitotic figures). CIN3 is strongly associated with persistent high-risk HPV infection and carries a substantial risk of progression (approximately 5–10%) and a 20% probability of progressing to invasive carcinoma within 10 years. It is therefore regarded as a high-risk precancerous lesion requiring active clinical management.[28,29]

Esophageal squamous epithelial dysplasia is the only welldefined precancerous lesion of esophageal squamous cell carcinoma (ESCC). It is characterized by cytologic atypia, impaired differentiation and maturation, and dysregulated proliferation of esophageal squamous epithelial cells. The lesion is strictly confined to the epithelial layer without invasion through the basement membrane, representing a noninvasive proliferative abnormality. The development of this lesion is a multifactorial, multistep, and multigenedriven process. Its core pathogenesis involves genomic instability and the acquisition of malignant transformation potential induced by longterm mucosal injury and carcinogen exposure, with modulation by host genetic susceptibility and microenvironmental alterations.

The core driving mechanisms include the following:

(i) Esophageal squamous epithelial cells are exposed to chronic mucosal injury and a persistent inflammatory microenvironment. Longterm repeated cycles of injury and repair form the pathological basis of dysplasia. Sustained injury activates inflammatory pathways, leading to abundant release of proinflammatory factors such as nuclear factor-κB (NF-κB), tumor necrosis factor-alpha, and interleukin6. These factors induce the production of reactive oxygen species and reactive nitrogen species, causing oxidative DNA damage and genomic instability. Meanwhile, the inflammatory microenvironment promotes abnormal epithelial proliferation and inhibits apoptosis, allowing the accumulation of gene mutations during repair and gradual progression to dysplasia.
(ii) Esophageal squamous epithelial cells undergo exogenous carcinogen exposure and driver gene alterations. Longterm exposure to exogenous carcinogens is the major risk factor for dysplasia in highincidence regions of ESCC. Important carcinogens include nitrosamines, which directly induce DNA strand breaks, base modifications, and gene mutations in esophageal epithelial cells. Key molecular events include tumor suppressor gene inactivation, such as alterations in *TP53*, *CDKN2A* (inactivated by promoter hypermethylation or mutation, leading to cell cycle dysregulation), and *MGMT* (a DNA repair gene whose silencing aggravates genomic instability); activation and amplification of oncogenes, including *CCND1*, *EGFR*, and *MYC*, promoting abnormal proliferation and apoptosis resistance; and dysregulation of signaling pathways such as NOTCH1 and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) signaling, leading to disturbed cell differentiation and proliferation.
(iii) Host genetic susceptibility affects carcinogen metabolism, DNA repair capacity, and disease

risk. ESCC shows obvious familial aggregation, and individuals with a positive family history in highrisk areas have significantly elevated cancer risk. Epigenetic dysregulation may also occur early and includes promoter hypermethylation of tumor suppressor genes, leading to transcriptional silencing; abnormal histone modifications that alter chromatin structure and regulate oncogene and tumor suppressor expression; and aberrant expression of noncoding RNAs (ncRNAs), including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), involved in proliferation, apoptosis, and differentiation.

The core characteristics of esophageal squamous epithelial dysplasia are reflected in histopathology, grading risk, and clinical and endoscopic manifestations. The essence of esophageal squamous epithelial dysplasia is cellular atypia and impaired differentiation. Lesions are strictly limited to the squamous epithelium with an intact basement membrane and no invasive growth, which is the key distinction from invasive ESCC. The main histopathological findings include the following: cellular atypia, including variation in cell size and shape, an increased nuclear–cytoplasmic ratio, enlarged and hyperchromatic nuclei, prominent nucleoli, increased mitotic figures, and occasional atypical mitoses; architectural disturbance, including loss of epithelial stratification and polarity and disruption of the maturation gradient from the basal to superficial layers; and noninvasive nature, with lesions confined to the epithelium and no stromal invasion, lymphovascular invasion, or metastatic potential.

According to the World Health Organization 2019 Classification of Tumors of the Digestive System, esophageal squamous epithelial dysplasia is classified using a twotier grading system, comprising lowgrade and highgrade dysplasia, rather than the former threetier system of mild, moderate, and severe dysplasia. The two categories are defined as follows:

(i) Lowgrade squamous epithelial dysplasia: Atypical cells are limited to the lower half of the squamous epithelium; cytologic atypia is mild, mitoses are rare and confined to the basal and parabasal regions, and the upper half of the epithelium shows preserved maturation. Most cases have a low risk of progression (annual malignant progression rate < 1%) and may regress spontaneously (>50%); therefore, management is mainly based on endoscopic surveillance.
(ii) Highgrade squamous epithelial dysplasia: Atypical cells involve more than the lower half or the full thickness of the epithelium, including lesions previously termed squamous cell carcinoma *in situ*. It is characterized by marked cytologic atypia, frequent mitoses and atypical mitoses throughout the epithelium, and loss of polarity and surface maturation. High-grade dysplasia has a substantially higher risk of progression to invasive carcinoma if left untreated (annual progression rate to invasive cancer: 10–20%; very low spontaneous regression rate) and therefore requires active intervention, including endoscopic or surgical treatment.[30]

2.2. The mechanism of action of driver gene mutations in the early stages of tumors

2.2.1. Main molecular mechanisms of proto-oncogene activation

While precancerous lesions exhibit aberrant cellular proliferation and histopathological dysplasia that set them apart from normal somatic tissues, they lack the defining malignant traits of stromal invasion and metastatic competence. The stepwise progression from these premalignant states to frankly invasive carcinoma is a multi-stage process driven by the sequential accumulation of specific genetic and epigenetic alterations in the clonally expanding cell population. The core molecular events that underpin this malignant transformation are primarily the gain-of-function activation of proto-oncogenes and the loss-of-function inactivation of tumor suppressor genes. The mechanistic details, functional impacts, and pathological significance of these pivotal oncogenic alterations are systematically elaborated in the subsequent subsections.

The activation of proto-oncogenes is a crucial driving event in tumorigenesis, primarily achieved through four molecular mechanisms: point mutations, gene fusions, juxtaposition with enhancer elements, and gene amplification. These mechanisms lead to structural alterations or increased/dysregulated expression of proto-oncogenes, conferring growth advantages or enhanced survival to cells carrying these alterations. Point mutation is one of the most common mechanisms for the activation of proto-oncogenes. When a proto-oncogene is activated through mutation, the structure of the encoded protein undergoes changes, enhancing its transforming activity. Many types of mutations occur in proto-oncogenes, such as the *RAS* proto-oncogenes (*KRAS*, *HRAS*, and *NRAS*). When mutations occur at codons 12, 13, or 61, the encoded RAS protein remains constitutively active and continuously transduces signals for inducing sustained cell growth.[31] Gene fusion is another important activation mechanism. In prostate cancer, gene fusions occur between genes carrying promoters that are very active in target tissues.[32] Gene amplification typically occurs during tumor progression and represents the third significant mechanism of proto-

oncogene activation. For instance, the amplification of the dihydrofolate reductase gene (*DHFR*) is observed in methotrexate-resistant acute lymphoblastic leukemia. The amplification of *DHFR* is accompanied by cytogenetic alterations reflective of proto-oncogene amplification. Members of four distinct proto-oncogene families are frequently amplified: *MYC*, *CCND1*, *EGFR*, and *RAS*. Chromosomal rearrangement is the fourth mechanism of proto-oncogene activation, encompassing chromosomal inversions and translocations. For instance, in Burkitt lymphoma, translocations frequently relocate the *MYC* gene to a new position adjacent to the immunoglobulin heavy chain locus, thereby activating *MYC* transcription.[33]

2.2.2. Specific patterns of driver gene mutations in different tumor types

Different tumor types exhibit unique patterns of driver gene mutations, which reflect the differences in cellular biology characteristics and carcinogenic mechanisms of various tissues. Glioblastomas are cytogenetically heterogeneous tumors that frequently display alterations of chromosomes 7, 9p, and 10q. Gains of chromosome 7 (97%) and losses of chromosomes 9p (83%) and 10 (91%) were the most frequent alterations, and these gains consistently occur early in the clonal mutation process.[34,35]

In colorectal cancer, the classical *APC–KRAS–TP53* progression sequence has been recognized as a more complex mutation pattern. In pancreatic cancer, *K-RAS* activation is an early event in the carcinogenesis of the Syrian golden hamster pancreas induced by N-nitroso-bis(2-oxopropyl)amine.[36] In breast cancer, different molecular subtypes exhibit distinct patterns of driver gene mutations.[37]

2.2.3. Types of molecular mechanisms underlying the inactivation of tumor suppressor genes

The inactivation of tumor suppressor genes is another key mechanism underlying tumorigenesis, with its danger lying in the loss of tumor suppressor gene function. Tumor suppressor gene inactivation can occur through various means, with different unfortunate combinations converging to eliminate or weaken the function of two genes. One copy may be lost through small chromosomal deletions or inactivated through point mutations. The other copy is usually eliminated through less specific but more probable mechanisms: the chromosome carrying the remaining normal copy may be lost from the cell through chromosomal segregation errors, or the normal gene may be replaced by a mutated version through mitotic recombination or gene conversion events.[38] Biallelic inactivation follows the classic Knudson's "two-hit" hypothesis, which posits that most tumor suppressor genes require inactivation of both alleles (via mutation or epigenetic silencing) to cause phenotypic changes. Knudson formulated the two-hit hypothesis to explain the epidemiology of familial and sporadic cancers, proposing that the same genes are involved in both scenarios, which has been molecularly confirmed in studies of familial cancer genes such as *TP53*.[39] Epigenetic inactivation is a crucial mechanism for the inactivation of tumor suppressor genes. Epigenetic changes offer another significant way to inactivate tumor suppressor genes. Most commonly, genes may be packaged into heterochromatin, and the C nucleotides in their CpG sequences in the promoter may undergo methylation in a heritable manner. These mechanisms can irreversibly silence genes in cells and all their progeny.[38,40,41]

2.2.4. The role of important tumor suppressor genes in the development of early-stage tumors

During the early development of tumors, the inactivation of several key tumor suppressor genes plays a decisive role. The loss of function of these genes leads to cell cycle deregulation, DNA repair defects, and apoptosis resistance, thereby promoting the initiation and progression of tumors. The *TP53* gene, as the most important tumor suppressor gene, functions to prevent cell division in cells with mutated or damaged DNA, thereby helping to prevent the development of tumors. Since *TP53* is crucial for regulating DNA repair and cell division, when *TP53* is normal and unmutated, it helps maintain cellular health and prevents the development of abnormal cells.[42] The *RB1* gene plays a central role in cell cycle regulation. Studies have shown that the loss of RB immunoreactivity in ductal carcinoma *in situ* (DCIS) is closely associated with the risk of ipsilateral breast event and invasive breast cancer. The prognostic ability of RB loss remained statistically significant in multivariate analysis.[43] The *PTEN* gene is a key negative regulator of the PI3K/AKT signaling pathway. Loss of *PTEN* expression occurs frequently in DCIS, but PTEN loss alone is not associated with recurrence or progression. However, patients with DCIS lesions lacking both RB and PTEN have an increased risk of ipsilateral breast events, including progression to ipsilateral invasive breast cancer.[43] In studies of lung cancer, it has been found that PTEN loss drives more rapid tumor progression, characterized by a more diverse histopathology of tumor tissues ranging from adenocarcinoma to small cell lung cancer. PTEN loss also drives transcriptional heterogeneity, as significant lineage plasticity is observed across histopathological subtypes. Spatial analysis reveals that transcriptional heterogeneity exists within and between tumor foci, with transcriptional patterns correlated with spatial location, indicating that the growth environment affects gene expression.[44]

In recent years, through the integration of multi-omics data and novel algorithms based on network models, researchers have discovered a plethora of new cancer driver genes, which are redefining our understanding of the mechanisms underlying tumorigenesis. The groundbreaking discoveries of the MONet algorithm demonstrate the immense potential of computational biology in driver gene identification. Among the cancer driver genes predicted by MONet (a cancer driver gene identification algorithm based on comprehensive analysis of multi-omics data and network models), 49% are already known cancer driver genes. For the newly predicted cancer driver genes, most have multiple sources of evidence supporting their potential as driver genes, including candidate cancer genes from the Network of Cancer Genes, manually curated cancer genes from the OncoKB Precision Oncology Knowledge Base, and literature-curated cancer genes from the ONGene database.[45] Specifically, 3 genes are supported by all 3 datasets, 33 genes are supported by 2 datasets, 76 genes are supported by 1 dataset, and 80 genes are newly identified by MONet.[45] The emergence of novel gene categories driven by splicing mechanisms marks a significant breakthrough in cancer research. Researchers have utilized algorithms to identify 813 genes that facilitate cancer cell proliferation through a frequently overlooked molecular mechanism known as splicing.[46] When considering non-mutational mechanisms such as splicing, researchers believe there may be twice as many potential gene targets to control cancer. These are not classical oncogenes, but represent a novel class of potential cancer drivers that can be targeted individually or in conjunction with existing strategies, playing a role in the early stages of tumor development and progression.

2.2.5. The role of epigenetic silencing in the inactivation of tumor suppressor genes

Epigenetic silencing is a crucial mechanism for the inactivation of tumor suppressor genes, primarily achieved through DNA methylation and histone modification. In human cancers, aberrant DNA methylation in promoter regions is a key mechanism for the inactivation of tumor suppressor genes. Genes involved in each step of tumor formation can be silenced through this mechanism.[38,40,41] In medulloblastoma, the *KLF4* gene is silenced through promoter methylation and may function as a tumor suppressor gene.[47] Research has shown that genes that are abnormally silenced in cancer cells exhibit an aberrant chromatin structure, characterized by hypermethylation of histone H3 lysine 9 (H3-K9) and hypomethylation of histone H3 lysine 4. This abnormal heterochromatin is incompatible with transcription initiation. H3-K9 methylation may play a role in the silencing of tumor suppressor genes in cancer.[48]

2.2.6. Gene-altered interaction network and synergistic effect

The interaction between driver genes and tumor suppressor genes: The activation of driver genes and the inactivation of tumor suppressor genes are not independent events in the process of tumorigenesis, but rather form a complex network of interactions that jointly drive the occurrence and development of tumors. Within the human cell cycle regulatory network, key tumor suppressor genes, including *TP53*, *RB1*, *CDKN2A*, and *PTEN*, exhibit elevated expression levels and abundant post-translational modifications across diverse human cell lineages.[49] There is a complex inter-regulatory relationship between the *RB1* and *CDKN2A* genes. Research has shown that, in addition to *RB1* and *CDKN2A*/*CDKN2B* themselves, cell cycle genes exhibit a positive correlation across some cancer types. However, *CDKN2A* or *CDKN2B* methylation can cause increased levels of *CDK4/6*, which in turn drives hyperphosphorylation of *RB1*, suggesting that the *CDK4/6* inhibitors may be a useful targeted therapy for *CDKN2A*/*CDKN2B*-methylated cancers, indicating that the inactivation of *RB1* or *CDKN2A* has similar downstream effects on the cell cycle pathway.[50]

2.2.7. The regulatory effect of tumor microenvironment on gene expression in early tumor development

The TME plays a crucial regulatory role in gene expression during the early stages of tumor development, involving multiple aspects such as cell–cell interactions, secretion of signaling molecules, and alterations in stromal components. Research on the impact of the immune microenvironment reveals differences in the immune microenvironment among different subtypes of DCIS: LumA DCIS is characterized by lower immune cell infiltration compared to *TN*- and *HER2*-enriched types, but there is variability both between and within cases. B cells are more abundant in *HER2*-enriched tumors, while T cells are more common in *TN* and LumA. Regulatory T cells are found in all subtypes.[51] The role of stromal cells cannot be overlooked in tumorigenesis. Studies have shown that fibroblasts promote, while normal myoepithelial cells inhibit, the progression of DCIS to invasive breast cancer. The tumor growth and progression-promoting effect of fibroblasts is at least partially due to the increased expression of cyclooxygenase-2 (*COX2*), an inducible enzyme belonging to the cyclooxygenase family, primarily involved in inflammatory reactions and prostaglandin synthesis, in tumor epithelial cells caused by their interaction with fibroblasts. The upregulation of *COX2* in DCIS xenografts

leads to increased expression of *VEGF* and *MMP14*, which may contribute to tumors expressing *COX2* having greater weight and invasive histological capacity.[52] Studies on the regulatory effects of inflammatory factors have shown that JQ-1, a bromodomain and extraterminal domain protein inhibitor, attenuates the activation of inflammatory NF-κB signaling triggered by 12-O-tetradecanoylphorbol-13-acetate. Luciferase reporter gene assays using plasmids carrying different elements of the *COX2* or *IL6* promoter indicate that JQ-1 does not directly inhibit the interaction between NF-κB and its binding sequences; instead, it affects transcription enhancement associated with cAMP response elements. Through small interfering RNA gene silencing, it was found that JQ-1 inhibits p300-dependent *COX2* transcriptional activation.[53] Research on the impact of spatial location reveals that spatial analysis indicates the presence of transcriptional heterogeneity within and between tumor foci. The transcription patterns are correlated with spatial location, suggesting that the growth environment influences gene expression. These findings confirm that *PTEN* loss is a clinically relevant genetic alteration driving the molecular and histopathological heterogeneity of neuroendocrine lung tumors initiated by *RB1*/Trp53 mutations.[44]

2.2.8. Regulatory role of non-coding RNA in early tumorigenesis

Non-coding RNA is a class of functional RNA molecules that lack the ability to encode proteins and play an important regulatory role in the early development of tumors. ncRNA has become a key regulator in various biological processes and is associated with the occurrence and progression of many diseases, including cancer.[54]

The microRNA regulatory mechanism plays a significant role in the early development of tumors. In the early stages of oral cancer development, downregulation of miR-34a-5p, miR-124-3p, and miR-125b-5p is observed in all stages, while miR-1-3p is underexpressed in dysplasia and early invasion. Regarding the interaction between miRNA and lncRNA, studies have shown that miR-21 exhibits an oncogenic role in various human cancers, including prostate cancer, breast cancer, lung cancer, and colorectal cancer. Overexpression of miR-21 is closely associated with tumor proliferation, growth, invasion, angiogenesis, and chemotherapy resistance, while inactivation of miR-21 is associated with regression of most tumor-related processes. Furthermore, the interaction between lncRNA and miRNA further complicates the regulatory mechanisms of tumor development and progression.[54]

Exosomal ncRNA, such as miRNA, lncRNA, and circRNA, are highly stable in body fluids because they are protected within exosome vesicles. Exosomal ncRNA demonstrates specific expression patterns closely related to the development stages of various cancers, which can be used for continuous monitoring of early cancer progression in patients.[55]

3. Important signal transduction pathways that regulate carcinogenesis and tumor progression

When pro-tumorigenic or tissue-stress factors promote carcinogenesis in normal tissues and cells, effective mutations of important related genes, abnormal expression of proto-oncogenes, immune evasion, and ITH become the cell's main driving force to acquire new survival and proliferative advantages, promoting tumor progression.

The mechanisms underlying tumorigenesis have been shown to include gene mutations that cause changes in gene structure and protein function. We need to emphasize that although the mutation rate of tumor-related genes is low, there may be a high effective occurrence rate (possibly due to more nonsense mutations). Secondly, the probability of structural changes of tumor-related proteins caused by gene mutations accounts for 15–70% in all tumor patients, according to the different types of tumor tissue.[8] The majority of human cancers are sporadic tumors, which arise from somatic, genetic, and epigenetic alterations leading to changes in gene sequence, structure, copy number, and expression.[8,56]

The most frequently mutated gene in the Pan-Cancer cohort is *TP53* (42% of samples), and *PIK3CA* is the second most commonly mutated gene, occurring frequently (>10%) in most cancer types except ovarian serous cystadenocarcinoma, kidney renal clear cell carcinoma, lung adenocarcinoma, and acute myeloid leukemia.[57] This raises the question of what the causes and processes are underlying these rare and faint mutation-related or non-mutational tumors. According to the clonal theory of oncogenesis, tumors start from a single cell. But the clonal origin of the tumors does not mean that a malignant tumor develops because of a single mutation. Oncogenes often act synergistically to cause malignant transformation. In such instances, the proposed mechanism suggests that tumorigenesis arises from the sequential abnormal expression of multiple genes. This occurs as cells attempt to enhance their survival capabilities under sustained and repeated stress. These adaptive responses include the activation and mutual reinforcement of key proto-oncogenes (e.g., *HER2*/*NEU* and *SVV*), the inactivation of tumor suppressor genes (e.g., *TP53*)[58,59], and the dysregulation of critical signal transduction pathways governing cell proliferation and

survival (including PI3K/AKT, MYC, JAK2/STAT3, and the HIPPO pathway).[6,60] Consequently, cells undergo malignant transformation during this chronic adaptation process. Concurrently, emerging cancer cells enhance their ability to evade immune surveillance, thereby optimizing their survival and facilitating population expansion.[61] A fundamental cellular basis for tumor development is the clonal expansion of these malignant cells coupled with the emergence of ITH. ITH enables the formation of genetically and phenotypically diverse cell populations, facilitating continuous cellular optimization and selection through evolutionary pressure.[62,63]

3.1. Abnormal activation and interaction network of key signaling pathways

The abnormal activation of key signaling pathways is the core mechanism underlying the occurrence and development of tumors. These pathways form a complex interaction network, collectively driving the malignant transformation and progression of tumors. The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinases (ERK) signaling pathway is one of the most extensively studied carcinogenic pathways. Under physiological conditions, the MAPK/ERK pathway (also known as the RAS–RAF–MEK–ERK pathway) maintains homeostasis through multilevel feedback regulation. However, *RAS* mutations (such as *KRAS* and *NRAS*) are detected in approximately one-third of solid tumors, and about 8% of tumors carry *BRAF* mutations (such as *BRAF* V600E), leading to sustained activation of the pathway and driving malignant transformation of tumors (accounting for more than 40% of human tumors).[64] The PI3K/AKT/mTOR signaling pathway controls normal cell growth and metabolism through AKT activation and downstream targets such as *mTORC1/2*, *p70S6K1*, and *4E-BP1*.[65] This pathway is frequently mutated in various cancers and serves as a key regulator of important functions such as cell proliferation, cell differentiation, cell survival/apoptosis, and metabolism.[66] Mutations in *KRAS*, present in approximately 95% of pancreatic ductal adenocarcinomas, lead to constitutive activation of the KRAS protein and downstream pathways, including the ERK and PI3K.[67] The Wingless-related integration site (Wnt)/β-catenin signaling pathway plays a central role in colorectal cancer. Over 70% of colorectal cancer patients carry biallelic *APC* mutations.[68] The abnormal activation of the Wnt/β-catenin signaling pathway leads to uncontrolled cell proliferation and abnormal differentiation, which is a key mechanism in the early occurrence and development of colorectal cancer. The network effect of inflammation-related signaling pathways is increasingly gaining attention. Research has emphasized the pivotal role of chronic inflammation in cancer development and elucidated the key molecular mechanisms underlying inflammation-related cancers. External pathways driven by inflammatory conditions and internal pathways propelled by genetic events have emerged as crucial links between inflammation and carcinogenesis. Persistent inflammation exacerbates genomic instability, providing a potential early mechanistic link between inflammation and carcinogenesis (such as NF-κB, JAK–STAT, MAPK/ERK, PI3K/AKT, Wnt, and transforming growth factor-beta [TGF-β]).[69,70] The interaction and compensatory mechanisms of signaling pathways increase the complexity of the tumor formation process. Studies on the impact of carcinogenic drivers on the immune microenvironment reveal that the amplification of growth factors (such as fibroblast-like growth factor [FGF] family members) or mutations in receptor tyrosine kinases trigger the activation of downstream signaling pathways, including the RAS/RAF/MAPK pathway and the PI3K pathway.[71–75] PTEN, a tumor suppressor gene, dephosphorylates the lipid signaling intermediate PIP3 with inhibition of AKT activity, one of the main effectors of the PI3K signaling axis. As a consequence of genetic or epigenetic aberrations, *PTEN* expression is often altered, with increased activation of the PI3K axis. The activation of these pathways induces the expression of immunosuppressive genes in tumor cells (such as *CCL2* and *VEGF*), inhibiting the recruitment and activation of $CD8^+$ T cells. Furthermore, the RAS/RAF/MAPK and PI3K pathways regulate the expression of checkpoint proteins in tumor cells (such as programmed death ligand 1 [PD-L1] and CD47).[71-75]

3.2. Network of cross-talk among key signaling pathways

The deregulation of the signal transduction and regulation system in cancer cells involves a complex interplay network among multiple key signaling pathways. The abnormal activation and interaction of these pathways ultimately lead to the complete collapse of the normal regulatory mechanisms in cells. In the crosstalk between the PI3K/AKT/mTOR pathway and the JAK2/STAT3 pathway, studies have found multiple interaction mechanisms between these two pathways. Firstly, activated AKT can phosphorylate the serine 727 site of *STAT3*, enhancing the transcriptional activity of STAT3. Secondly, mTORC1 can affect the expression levels of JAK2 and STAT3 proteins by regulating protein synthesis. Thirdly, the activation of the PI3K/AKT pathway can inhibit the activity of PTEN, and the absence of PTEN can lead to the continuous activation of *AKT*, thereby enhancing the signaling of STAT3.[64–76] This cross-talk has been observed in various tumors, particularly evident in prostate cancer and breast cancer.

Regarding the interaction between the MAPK pathway and the JAK2/STAT3 pathway, the activation of the RAS–RAF–MEK–ERK pathway can affect JAK2/STAT3 signaling through multiple mechanisms. For instance, ERK can phosphorylate the serine residues of STAT3, thereby regulating its transcriptional activity; JAK2 can interact with RAF, influencing its kinase activity; and RAS can indirectly regulate JAK2/STAT3 signaling by activating various downstream effector molecules.[76] In lung cancer, the sustained activation of the MAPK pathway due to *EGFR* mutations is often accompanied by abnormal activation of the JAK2/STAT3 signaling.

In the network regulation of the TGF-β signaling pathway, TGF-β can regulate gene expression by activating SMAD2/3, and it can also exert its effects through non-SMAD pathways (such as MAPK, PI3K/AKT, JAK/STAT). During tumor progression, the TGF-β signaling pathway exhibits a dual role: it primarily exerts a tumor-suppressing effect in the early stages of the tumor, while in the later stages, it primarily exerts a tumor-promoting effect. This transition is closely related to the interaction between the TGF-β signaling pathway and other pathways.[77]

It is particularly noteworthy that the crosstalk among these signaling pathways exhibits significant heterogeneity in the context of ITH. Single-cell sequencing studies have revealed that within the same tumor, different cell subpopulations may exhibit distinct patterns of signaling pathway activation. For instance, in certain cell subpopulations, the PI3K/AKT pathway dominates; in others, the MAPK pathway is more active; and still others may simultaneously activate multiple signaling pathways. This heterogeneity not only increases the complexity of tumor development and progression but also poses challenges for the formulation of treatment strategies. In summary, the imbalance in signaling pathway regulation directly affects the determination of cell fate, leading to cancer cells acquiring abnormal proliferative capacity, survival advantage, and differentiation potential. This imbalance manifests at multiple levels, including abnormalities in cell cycle regulation, inactivation of apoptosis mechanisms, and disruption of differentiation programs, and it is of significant importance for the early generation of tumor cells.

4. Tumor cell growth and survival optimization

During the process of tumor development, tumor cells exploit related information, regulation mechanisms, signal transduction pathways, growth substances, and energy resources to optimize their growth states and achieve optimal survival status. For example, the reorganization of energy metabolism (the Warburg effect), the upregulated expression of important proto-oncogenes (*MYC*, *SVV*, *RAS*, etc.), continuous activation of important signal transduction pathways (JAK2/STAT3, PI3K/AKT, etc.), enhanced ability to exhaust and convert important metabolic substances, and inhibition of cell apoptosis act synergistically to promote uncontrolled cell proliferation.[6,60,78-80] Most importantly, the histological characteristics of cancerous cells lay the histological and molecular foundation for the enhanced survival status of tumor cells. All of these phenomena may also appear in the growth process of precancerous lesion stages, but never appear in the normal physiological processes of normal tissues.[81]

Specific cancer cells utilize tissue cell resources through several basic strategies. First, cancer cells regulate the functions of surrounding normal cells through paracrine signaling mechanisms. Studies have found that cytokines secreted by normal breast fibroblasts can establish a paracrine signaling mechanism with estrogen receptor-positive breast cancer cells, creating a microenvironment rich in interleukin-1β and promoting tumor growth.[82] In melanoma, cancer cells secrete factors to inhibit the expression of desmoglein 1 (*DSG1*) in surrounding keratinocytes, thereby enhancing melanoma metastasis through the CXCL1/CXCR2 signaling pathway.[83]

Second, cancer cells utilize exosomes as a crucial tool for intercellular communication. Tumor-derived exosomes carry a variety of bioactive molecules, including proteins, lipids, mRNA, and miRNA, which can regulate gene expression and function in recipient cells. Exosomes can induce epithelial–mesenchymal transition (EMT), promote angiogenesis, remodel the TME, and mediate the spread of therapeutic resistance.

Third, cancer cells achieve effective utilization of nutritional resources through metabolic reprogramming. The Warburg effect is a typical characteristic of cancer cell metabolic reprogramming, which manifests as preferential glycolysis over oxidative phosphorylation even under aerobic conditions. This metabolic pattern enables cancer cells to rapidly produce ATP and biosynthetic precursors, while generating large amounts of lactic acid. Lactic acid is not only a metabolic waste product, but also functions as a signaling molecule through lactate-sensing G protein-coupled receptors, promoting angiogenesis and immune escape.[84]

Fourth, cancer cells form a complex metabolic symbiosis with other cells in the TME. Studies have found that cancer-associated fibroblasts (CAFs) tend to activate glycolysis and autophagy, producing metabolites that can be utilized by cancer cells, a phenomenon known as the "reverse

Warburg effect." Cancer cells can hijack mitochondria from immune cells via physical intercellular nanotubes, a tumor immune evasion mechanism. As critical organelles, mitochondria are essential for immune cell metabolism and activation, providing energy and signaling molecules (e.g., mitochondrial DNA, reactive oxygen species) that regulate their differentiation and function. Researchers show nanotube-mediated mitochondrial transfer from immune to cancer cells has a dual effect: it boosts cancer cell metabolism by enhancing respiratory capacity and energy supply, while depleting immune cells of energy, causing impaired function or exhaustion. This one-way transfer supports cancer cell survival, proliferation, and antitumor immunity resistance.[85]

Fifth, vasculogenic mimicry refers to the phenomenon where tumor cells form vessel-like structures through their own deformation and arrangement, which can transport blood or interstitial fluid, providing nutrients and oxygen to the tumor. This concept was initially discovered in melanoma and subsequently confirmed in various solid tumors.[86] The formation of new blood vessels involves morphological changes in tumor cells, remodeling of the extracellular matrix, and interactions with endothelial cells, all of which contribute to the sustained survival of tumor cells. Ultimately, immune evasion is one of the key strategies for cancer cells to achieve clonal expansion and malignant transformation. It involves multiple levels of mechanisms, including the activation of immune checkpoints, recruitment of immunosuppressive cells, reduction of immunogenicity, and remodeling of the immune microenvironment.

5. Role of patient-specific tumor microenvironment in recurrence and metastasis

Interestingly, the TME has both systemic and local attributes. Its systemic attributes include the effects of endocrine, neural, immune, and systemic metabolic factors on the tumorigenesis process and environment. Its local attributes consist of the different cell types and structures present in tumor tissue, including microvasculature and tissue architecture, normal and tumor cell types, intercellular space, exosomes, and the interaction of all these local components.[87–89] However, once it is formed in the body of an individual patient, the TME represents the pathological basis for tumor metastasis and recurrence and includes the formation of the cell microenvironment in the metastatic niche at distant sites during the possible earliest tumorigenesis stages.[90–92] Therefore, we need to establish this novel concept and clarify the mechanisms of TME to deeply understand the mechanisms driving tumor cell recurrence and metastasis.

5.1. Historical development of the tumor microenvironment concept and the formation of patient-specific tumor microenvironment

The development of the concept of TME has undergone a historical evolution from macroscopic observation to a deep understanding of molecular mechanisms. In 1863, Rudolf Virchow first observed that leukocyte infiltration was a characteristic of solid tumors and proposed the relationship between chronic inflammation and the occurrence and development of cancer, which is considered the earliest germination of the TME concept.[80-93] Subsequently, in 1889, Stephen Paget proposed the famous "seed and soil" theory, suggesting that metastasis and colonization depend on the characteristics of the organ, which became an important milestone in the TME concept. However, before 1980, cancer research was primarily dominated by the tumor-centered theory, which believed that mutations in oncogenes and tumor suppressor genes were sufficient to determine the occurrence and progression of cancer. Most reports related to TME were descriptive and lacked mechanistic insights, thus being labeled as "epiphenomena".[93] This concept began to undergo a fundamental transformation in the 1980s, and subsequent research reports have shown that signals derived from the TME can reprogram cancer cell phenotypes, largely determining the survival and progression of tumor cells.[93] The emergence of the concept of patient-specific TME represents a significant advancement in TME research in recent years. Compared to traditional TME studies, patient-specific TME emphasizes the notable variations in TME composition and function among individuals. Research indicates that the cellular composition and functional status of TME can significantly differ depending on the organ where the tumor originates, the intrinsic characteristics of cancer cells, tumor staging, and patient characteristics. This patient-specificity is not only reflected in the cellular composition of TME but also encompasses individual differences in multiple dimensions, such as molecular expression profiles, metabolic characteristics, and immune status.

5.2. Composition and heterogeneity basis of patient-specific tumor microenvironment

Patient-specific TME is a complex ecosystem comprising tumor cells and various non-cancerous cells embedded in an altered extracellular matrix. TME includes multiple immune cell types, CAFs, endothelial cells, pericytes, and various other tissue-resident cells. These host cells were once considered bystanders in tumorigenesis, but it is now known that they play a pivotal role in the early pathogenesis of cancer.

The heterogeneity of patient-specific TME is primarily manifested in the following aspects:

(i) Genetic background differences: Genetic variations in individuals directly affect the composition and function of TME. Studies have found that in the occurrence and development of pancreatic cancer, the interaction between the loss of relevant tumor suppressor genes or the activation of proto-oncogenes and the TME can lead to the formation of a specific tumor phenotype during tumor pathology.[94]

(ii) Epigenetic modification differences: Epigenetic changes play a significant role in defining the regulatory state of the tumor stroma. Studies have shown that tumor-associated myofibroblasts exhibit phenotypes distinct from those derived from healthy tissues, and these differences are associated with epigenetic rather than genetic alterations.[95] Changes in specific acetylation and methylation histone levels, such as reduced trimethylation of H3K9 and acetylation of H4K16, have been observed in tumor-associated myofibroblasts.[95]

(iii) Differences in immune status: The immune status of patients is a key factor determining the characteristics of TME. Patients with immunosuppression have a significantly increased risk of cancer recurrence. For example, among Merkel cell carcinoma patients, the 5-year recurrence rate for immunosuppressed patients is 43%, compared to only 5% for non-immunosuppressed patients.[96] In squamous cell carcinoma of the skin, the likelihood of adverse outcomes for immunosuppressed patients is approximately 11 times higher than for immunocompetent patients.[97]

(iv) Individual differences in microenvironment composition: The application of single-cell sequencing technology has revealed significant individual differences in the composition of the TME. By analyzing 233,591 single cells from 36 patients with lung cancer, colorectal cancer, ovarian cancer, and breast cancer, a pan-cancer stromal cell heterogeneity blueprint was constructed, identifying 68 stromal cell populations, of which 46 are shared across cancer types and 22 are unique.

5.3. Role of patient-specific tumor microenvironment in recurrence and metastasis

5.3.1. Individual differences and patient-specificity in immune escape mechanisms

Immune evasion is one of the hallmarks of cancer, representing a mechanism that allows tumors to survive and evade immune recognition and destruction after encountering the host immune system. The mechanisms of immune evasion in patient-specific TME exhibit significant individual differences, primarily manifested in various aspects such as the expression of immunosuppressive factors, regulatory T cell function, and myeloid-derived suppressor cell activity. Studies have shown that intrinsic hyporesponsiveness or anergy of T cells, extrinsic inhibition by regulatory cell populations, inhibitory ligands such as PD-L1, soluble factors such as TGF-β, and the activity of nutrient metabolic enzymes such as indoleamine 2,3-dioxygenase may contribute to immune evasion under different circumstances.[98] In patients with hepatocellular carcinoma undergoing immunosuppressive therapy, cyclosporine exposure exceeding 189.6 ng/mL is associated with hepatocellular carcinoma recurrence. Multivariate analysis reveals that cyclosporine exposure is the sole independent prognostic determinant.[99] This suggests that the use of immunosuppressive drugs significantly affects the immune balance within the TME, promoting tumor recurrence.

Myeloid-derived suppressor cells also show patient-specific patterns. In squamous cell carcinoma of the lung, a positive correlation was observed between the abundance of CAFs and monocyte-derived myeloid cells. Further verification revealed significant spatial interactions between CAFs and monocyte-derived myeloid cells in the TME.[100] Macrophage polarization may also vary by patient and disease context. In ascites from patients with gastric cancer accompanied by peritoneal metastasis, it was found that tumor-associated macrophages (TAMs) underwent a continuous lineage shift from cathepsin (*CTS*) high expression to complement 1q (*C1Q*) high expression. Initially, *CTS*-high TAMs attract metastatic tumor cells to the ascites, and subsequently undergo terminal differentiation into *C1Q*-high TAMs to trigger excessive proliferation and immune evasion of disseminated tumor cells in the ascites.[101]

5.3.2. Patient-specific patterns and mechanisms of metabolic reprogramming

Metabolic reprogramming is one of the hallmarks of cancer. The metabolic patterns within patient-specific TME exhibit significant individual differences, which are manifested not only in the selection of metabolic pathways but also in various aspects such as the expression of metabolic enzymes, levels of metabolites, and modes of energy production. For example, studies have used a dual-scoring system to quantify glycolysis and oxidative phosphorylation in 9,668 patients across 33 tumor types, classifying patients into four metabolic subtypes.[102] Compared to patients with low glycolysis and high oxidative phosphorylation, patients with high glycolysis and low oxidative phosphorylation consistently exhibited poorer prognoses. These individual

differences in metabolic patterns may be related to factors such as the patient's genetic background, tumor type, and disease stage.

Mitochondrial metabolism also contributes to metabolic heterogeneity. Single-cell metabolic analyses have shown that malignant cells typically exhibit higher metabolic activity and greater metabolic variability than those observed in previous bulk tumor comparative studies. Most of the metabolic variations observed in individual tumor and normal cells are inconsistent with those in bulk tumor samples. Variations in mitochondrial program expression are a major contributor to metabolic heterogeneity within tumors.

Tumor subtypes may also exhibit distinct metabolic dependencies. In prostate cancer, the two major subtypes of castration-resistant prostate cancer, androgen receptor-expressing prostate cancer (ARPC) and aggressive variant prostate cancer (AVPC), exhibit different metabolic dependencies. Transcriptomic analysis reveals that, compared to AVPC, the gene set involved in oxidative phosphorylation is enriched in ARPC tumor samples. ARPC C4-2B cells rely on aerobic respiration, while AVPC PC3 cells are more heavily dependent on glycolysis.[103] Organ-specific metabolic reprogramming may further shape these dependencies. IACS-10759 (a clinical-grade inhibitor of oxidative phosphorylation) had no effect on subcutaneous PC3 tumors but inhibited PC3 tumor growth in bone, indicating the importance of microenvironment-induced metabolic reprogramming.[103] These results suggest that castration-resistant ARPC and AVPC exhibit different metabolic dependencies and can undergo further metabolic reprogramming in bone.[103]

Metabolic enzyme expression also differs across tumor contexts. In breast cancer, marked metabolic differences were associated with hormone receptor status. Compared to estrogen receptor-positive cancers, 18% of metabolites were elevated in estrogen receptor-negative cancers, including many glycolysis and glycogenolysis intermediates, consistent with enhanced Warburg effect. Components of the glutathione pathway were also elevated in estrogen receptor-negative tumors, consistent with the increased need to handle higher levels of oxidative stress.[104]

5.3.3. Patient-specific mechanisms regulating angiogenesis

Angiogenesis is a crucial process for tumor growth and metastasis. The regulatory mechanisms of angiogenesis in patient-specific TME exhibit significant individual differences, which are mainly reflected in the expression of vascular endothelial growth factor (VEGF), variations in angiogenic factor receptors, and abnormalities in vascular structure.

(i) Individual differences in VEGF expression: VEGF is one of the most important and specific factors affecting angiogenesis in tumor development. VEGF and VEGFR2 transmit signals to the intracellular tyrosine kinase cascade. Polymorphic variations in the *VEGF* gene significantly affect the expression levels of endothelial growth factor and its receptor, leading to changes in angiogenesis activation during tumor pathology.[105]

(ii) Functional differences among VEGF isoforms: Studies have found that tumor cells expressing a single VEGF isoform exhibit distinct growth, survival, and migration characteristics. Fibrosarcoma cells expressing *VEGF*188 typically exhibit mesenchymal-like features, forming folds and exhibiting strong stromal binding activity. Cells producing *VEGF164* and *VEGF120* are less typically mesenchymal-like, lacking folds but forming abundant intercellular contacts.[106]

(iii) T-cell-regulated angiogenesis: A novel angiogenesis regulatory mechanism has been discovered in breast cancer patients, where regulatory T cells can secrete VEGF-A to promote angiogenesis. As the disease progresses, Treg cells from breast cancer patients are capable of secreting large amounts of VEGF-A. VEGF-A secreted by Treg cells induces endothelial neovascularization *in vitro*.[107]

(iv) Stromal cell-mediated angiogenesis: Studies have found that the enhancement of *FOSL2* in breast cancer CAFs is significantly correlated with angiogenesis and clinical progression in patients. The supernatant of CAFs with high *FOSL2* expression strongly promotes the tube formation of human umbilical vein endothelial cells in a VEGF-independent manner, as well as angiogenesis and tumor growth *in vivo*.[108]

(v) Hypoxia-induced angiogenesis differences: Hypoxia is one of the hallmarks of the TME, which is prevalent in the tumor core. In 1955, Thomlinson and Gray observed during the analysis of cancer tissue sections that tumor areas close to blood vessels were viable, while areas in the tumor center, relatively far from the blood supply, were necrotic. This observation was interpreted as the presence of hypoxia in the tumor core.[95] There are significant differences in the degree of hypoxia and hypoxia-induced angiogenesis responses among different patients.

5.3.4. Individual-specific network of stromal cell interactions

The interaction between stromal cells and tumor cells constitutes a crucial component of patient-specific TME.

This interaction network exhibits significant individual differences, primarily manifested in CAF function, immune cell recruitment, and extracellular matrix remodeling.

(i) Individual differences in CAFs: CAFs constitute a major component of the TME. Through pan-cancer analysis of 226 samples across 10 solid cancer types, the TME was delineated at single-cell resolution, elucidating the commonalities and plasticity of heterogeneous CAFs.[109] The activation trajectories of major CAF types were categorized into three states, exhibiting distinct interactions with other cellular components and correlating with immunotherapy prognosis.

(ii) CAF-induced phenotypic switch of tumor cells: In pancreatic cancer, CAFs are found to drive transcriptional changes from the classical phenotype to the basal phenotype in tumor cells and promote EMT. The classical phenotype of tumor cells refers to a well-differentiated epithelial state characterized by intact cell polarity, strong cell–cell adhesion, and expression of luminal/glandular epithelial markers, associated with relatively low invasiveness. In contrast, the basal phenotype represents a poorly differentiated, basal epithelial-like state marked by high expression of basal cytokeratins (e.g., *CK5*, *CK14*, *CK17*), increased stemness, invasiveness, and therapeutic resistance. This classical-to-basal phenotypic switch is often coupled with EMT and can be induced by paracrine signals from CAFs. CAFs secrete a panel of paracrine factors (e.g., TGF-β, IL-6, hepatocyte growth factor) and remodel the extracellular matrix to activate multiple oncogenic signaling pathways (TGF-β/SMAD, PI3K/AKT, MAPK, YAP/TAZ, STAT3) in tumor cells. These signals coordinately suppress the well-differentiated classical epithelial transcriptional program and induce the expression of basal phenotype markers (e.g., *CK5*, *CK14*, *EGFR*) together with epithelial-mesenchymal transition transcription factors (*SNAIL*, *ZEB1*, *TWIST*). Consequently, tumor cells undergo a phenotypic switch from a differentiated classical state to a poorly differentiated, invasive, and therapy-resistant basal-like state, which is tightly coupled with EMT progression. Bulk RNAseq of fluorescence-activated cell sorting-sorted CAF–patient-derived organoid co-cultures from 12 patients revealed that, compared to patient-derived organoid monoculture, 42% of the samples exhibited Moffitt-classified transcriptional changes, indicating a CAF-driven basal tumor phenotype.[110]

(iii) CAF-mediated immunosuppression network: A novel mechanism of CAF-mediated immunosuppression has been discovered in squamous cell carcinoma of the lung. The study established a positive correlation between CAFs and monocyte-macrophage abundance, with CAFs derived from patients with lung squamous cell carcinoma promoting the recruitment of *CCR2*$^+$ monocytes through *CCL2*. Using a three-dimensional culture system, it was found that CAFs polarized monocytes to adopt a myeloid-derived suppressor cell phenotype, characterized by strong inhibition of autologous CD8$^+$ T cell proliferation and interferon gamma production.[100]

(iv) Patient-specific CAF signaling network: Through research on 53 patients with endometrial cancer, it was found that endometrial CAFs express mRNA of signaling proteins and receptor ligands of multiple signaling pathways, including (i) cell-cycle pathway, (ii) TGF pathway, (iii) FGF pathway, (iv) Wnt-β-catenin pathway, (v) HER pathway, (vi) tyrosine kinase receptor ligands, and (vii) steroid receptors.[111]

6. Characteristics and significance of "persistent dynamic biology" of tumor cells

Although ITH has been demonstrated in many studies, regional heterogeneity has also led to the concept of "spatial biology".[112,113] Traditionally, researchers have been limited by only being able to observe changes in tumor cell histology, cytology, and molecular biology changes from single-cell sequencing technology detection, a unitary specimen, one tissue section, and at a single time point. Due to this limited lens, researchers have been unable to consider the dynamic state of tumor cell growth over time, severely limiting our ability to truly implement personalized and precision treatment. Being mindful of the concept of ITH, we completed an analysis of the dynamic changes of tumor cell cellular molecular biology over time (data unpublished). The results show that this process has the following characteristics:

(i) Tumor cells retain the important cellular mechanisms required for normal physiological functions. Tumor cells have the same systems as normal cells, but their functionality is enhanced.

(ii) The related genes and constituent genes in various cell signal transduction pathways might be different.

(iii) New regulatory relationships and mechanisms emerge within tumor cells to promote ITH and continuous cell renewal.

(iv) There must be spatiotemporal differences among patients with the same tumor type. These characteristics suggest that tumor cells undergo continuous adaptive changes over time and provide the driving forces for their "persistent dynamic biology."

These findings strongly suggest that we need to establish an individualized "persistent dynamic biology"

system for patients and study individualized dynamic biological markers, which is essential for the successful implementation of individualized precision therapy.

7. The Dysregulated Adaptive Cascade Model from chronic repeated induction to cancer development

7.1. Limitations of existing tumorigenesis models and the need for a new model

The traditional model of carcinogenesis induced by chronic repeated stimulation serves as an important theoretical framework. It emphasizes the central role of sustained injury and repair induced by single or multiple factors in cellular carcinogenesis, particularly highlighting the pivotal status of gene mutations and inflammatory processes. However, the main limitation of this model is that it oversimplifies the highly complex multi-step, multi-factorial, and multi-level carcinogenic process, neglecting host genetic susceptibility, epigenetic alterations, the dynamic heterogeneous evolution of the TME, as well as the synergistic inductive effects of multiple genes and various factors. Modern theories of carcinogenesis tend to favor a comprehensive and dynamically evolving model, in which the important dynamic biological changes in intracellular metabolism, proliferation, and survival caused by repeated chronic stimulation with multiple factors, as well as the dynamic evolution of cellular heterogeneity, are all regarded as crucial risk factors.

It is necessary to construct a multi-dimensional and multi-level comprehensive model integrating four key components: genetic-epigenetic crosstalk, cell fate plasticity, reactivation of developmental pathways, and selection pressure in the TME, to overcome the limitations of traditional models.

The Dysregulated Adaptive Cascade Model from chronic repeated induction to cancer development is proposed. The core cascade is as follows:

Chronic repeated induction → Epigenetic/transcriptional plasticity → Cell state transition → Clonal selection in the TME with the characteristics of ITH.

This model integrates stimulation-inhibition dynamics, cell fate plasticity, and heterogeneity, and is clearly distinct from "chronic stress-induced carcinogenesis."

7.2. Molecular mechanisms of the model

Chronic repeated induction (recurrent physical, chemical, biological, or environmental stimuli) persistently activates stress and inflammatory pathways such as MAPK, PI3K/AKT, and NF-κB, inducing the secretion of pro-inflammatory cytokines and forming a chronic inflammatory microenvironment. Meanwhile, it regulates and alters the expression status of these oncogenes and tumor suppressor genes, converting their regulatable normal expression levels into uncontrollable sustained high expression of oncogenes or sustained low expression of tumor suppressor genes. This, in turn, induces corresponding alterations in the critical cellular signaling transduction systems governing cell proliferation, metabolism, and survival, ultimately driving malignant transformation of cells toward a tumorigenic phenotype.

Epigenetic plasticity is a key node in the cascade, mediating gene expression reprogramming through DNA methylation, histone modification, and chromatin remodeling. The Adaptive Dysregulation of Epigenome Induced by Repeated Stimulation hypothesis is proposed: imbalanced epigenetic adaptation caused by repeated stimulation is a major driver of malignant transformation.

Cell fate plasticity and heterogeneity enable state transitions via EMT, acquisition of cancer stem cell properties, and other processes. Aberrant reactivation of developmental pathways is regulated by signaling gradients and transcription factors, following the developmental constraint model. Cancer cells switch among accessible states along developmental trajectories, generating tumor heterogeneity.

Heterogeneous conditions in the TME, including hypoxia and nutrient deprivation, exert immune, metabolic, and physical-chemical selection pressures. These select for invasive and drug-resistant tumor cell clones, determining tumor malignancy and progression.

7.3. Integrated mechanism of the stimulation–inhibition model and cell fate plasticity

The core is the disruption of the dynamic balance between pro-tumorigenic stimuli (stress, pro-inflammatory factors, etc.) and anti-tumor protective factors (cell cycle checkpoints, anti-inflammatory factors, etc.). Chronic repeated induction breaks this balance and drives malignant transformation through positive and negative feedback networks, bidirectional regulation of cell fate, reactivation of developmental pathways, and microenvironmental selection pressure.

7.4. Theoretical framework, validation, and prediction of the Dysregulated Adaptive Cascade Model

The model adopts a four-layer spatial architecture (molecular, cellular, tissue, organ) and a temporal dimension (acute vs. chronic induction). Its core hypotheses include: cumulative effects of chronic repeated induction, the key role of epigenetic plasticity, bidirectional

regulation of cell fate, cell heterogeneity, and the decisive role of microenvironmental selection pressure.

The model supports cancer risk prediction, therapeutic response evaluation, prognosis assessment, and drug development. It is applicable to cancers associated with chronic repeated induction, but not to cancers driven primarily by genetic mutations.

7.5. Modelbased therapeutic strategies and clinical translation

Four major intervention targets are defined corresponding to each cascade step: (i) sources of chronic repeated induction, (ii) epigenetic reprogramming, (iii) cell fate plasticity and heterogeneity, and (iv) the TME. Therapeutic strategies follow the principles of systematicity, temporality, individualization, and synergy, achieving precision therapy through multi-target combination and multi-modal coordination. Clinical translation involves four phases: basic validation, preclinical research, clinical trials, and application, with technical, economic, and ethical feasibility.

7.6. Summary and perspective

This model overcomes the limitations of traditional linear models and systematically integrates carcinogenic mechanisms related to chronic repeated induction. It provides a novel theoretical framework for cancer prevention and precision therapy. Future studies will further validate the core mechanisms, optimize clinical translation pathways, and promote its application in personalized medicine.

8. Emerging technologies unveil novel mechanisms underlying tumorigenesis

The development of emerging technologies has brought revolutionary breakthroughs to the study of tumorigenesis mechanisms, especially the application of technologies such as single-cell omics, spatial transcriptomics, and artificial intelligence, which have revealed many previously unknown mechanisms of tumorigenesis. Liquid biopsy technology has also advanced substantially. Detecting these subtle cancer traces in blood was once an insurmountable technical challenge, but the breakthroughs in liquid biopsy presented at the AACR Annual Meeting in April 2025 revealed the progress these technologies have made and their tremendous clinical potential. The interim results of the VICTORI study showed that liquid biopsy detection based on ultra-sensitive ctDNA can detect colorectal cancer recurrence, providing critical prognostic value within one month after surgery.[114] New insights from single-cell sequencing technology. Single-cell sequencing technology is revealing new mechanisms underlying tumorigenesis. For instance, in hepatocellular carcinoma research, single-cell transcriptome sequencing has uncovered tumor stem cells and their molecular characteristics.[115] In squamous cell carcinoma of the head and neck, single-cell sequencing has revealed complex interactions and heterogeneity among cancer stem cells.[116]

In 2009, H. Clevers proposed the concept of organoid culture based on the discovery that adult stem cells proliferate and self-organize into three-dimensional organoid cell structures.[117] The protocol and its associated growth factor cocktail were adapted to allow for the generation of organoid cultures derived from human tissues.[118,119] Additionally, patient-derived cancer organoids can be established from primary and metastatic cancer tissue. Additionally, normal organoids may be transformed into cancer organoids through gene editing.[120] The new concepts of tumorigenesis that we proposed above should be combined with clinical applications. Therefore, it is necessary to establish an individualized organoid culture system, regulation, and biomarker detection system for clinical cases. Doing so will achieve the most spatiotemporally precise individualized diagnosis, determine the best individualized treatment means and measure effectiveness, and represent truly individualized precision treatment.

The application of artificial intelligence in tumor research. The application of artificial intelligence and machine learning technologies in tumor research is becoming increasingly widespread, demonstrating great potential from driver gene prediction to treatment response prediction. The MONet algorithm is a typical example, which identifies cancer driver genes based on comprehensive analysis of multi-omics data and network models, and exhibits superior performance compared to traditional methods.[45]

9. New principles of tumor prevention

Based on our current understanding of tumor occurrence and development, we realize that the three principles of tumor prevention in the past decades (early detection, early diagnosis, and early treatment for patients with tumors) do not focus on tumor occurrence. These principles only affect the early detection, early diagnosis, and treatment of clinical cancer patients, but have a limited impact on preventing tumor initiation.

9.1. Precancerous lesions

Precancerous lesions are preneoplastic tissue abnormalities characterized by dysregulated proliferation and dysplasia, carrying an elevated but not absolute risk for malignant

progression. Representative examples include colorectal adenomas in colon carcinogenesis, CIN, and esophageal squamous epithelial dysplasia. These lesions exhibit heterogeneous natural histories, including spontaneous regression, persistent stability, or progression to invasive carcinoma, depending on dysplasia grade, etiology, host immunity, and microenvironmental factors.[121,122] To characterize the TME, it is necessary to clarify how TME features can be assessed and at which stages of tumor progression they should be evaluated. Importantly, the TME undergoes continuous remodeling and dynamic changes throughout tumor development. In the early phases of carcinogenesis, TME alterations are often mild and not pronounced, becoming progressively more prominent and complex as lesions advance from premalignant stages to invasive and metastatic cancer.[123]

9.2. Tumor microenvironment detection methods for precancerous lesions

The core of the TME detection method for precancerous lesions lies in the systematic evaluation of stromal, immune, metabolic, and cellular interaction characteristics at the tissue, cellular, molecular, spatial, and bodily fluid levels. It requires coverage of the entire dynamic cycle of changes, from early mild remodeling to complex abnormalities in advanced stages.

9.2.1. For tissue-level detection

For tissue-level detection, the main methods include multiplex immunohistochemistry (mIHC), triple immunofluorescence (IF), and imaging mass cytometry (IMC).

9.2.2. For single-cell and spatial omics

For single-cell and spatial omics (which enable in-depth analysis of TME heterogeneity), the commonly used methods are single-cell RNA sequencing (scRNA-seq), spatial transcriptomics (Visium), and single-cell spatial sequencing (Xenium/MERFISH).

9.2.3. For molecular and metabolic detection

Methylation sequencing (WGBS/EPIC) is used to detect the promoter methylation of tumor suppressor genes (such as *P16* and *MLH1*), thereby reflecting the epigenetic remodeling of the TME. Metabolomics (liquid chromatography–mass spectrometry/gas chromatography–mass spectrometry) is applied to detect metabolites in tissues or bodily fluids (such as lactate and tryptophan metabolites), so as to assess the metabolic reprogramming of the TME. Liquid biopsy (including ctDNA and exosomes) is utilized to detect tumor-derived DNA and exosomal miRNA/protein in plasma or urine, which can reflect the molecular characteristics of the TME.

9.2.4. For optical and functional imaging (for in vivo and non-invasive assessment)

High-resolution microendoscopy (HRME), combined with fiber imaging and fluorescent dyes (such as proflavin), allows real-time observation of nuclear morphology and density. Optical coherence tomography (OCT), laser-excited molecular vibrational spectroscopy, and artificial intelligence (AI)-based classification of normal, precancerous, and cancerous tissues are employed to assess metabolic and structural changes.

9.2.5. Detection strategy and application suggestions

A hierarchical detection strategy may be used to evaluate precancerous lesions and their TME. In the preliminary screening stage, non-invasive or minimally invasive methods (HRME, OCT, liquid biopsy) may be used to identify high-risk lesions. For definitive diagnosis and risk stratification, mIHC/IF can be used to assess cellular composition and spatial localization, together with methylation sequencing and metabolomics. For in-depth mechanistic exploration, scRNA-seq, IMC, and spatial omics can be applied to construct single-cell spatial atlases and analyze cell–cell interactions (such as the CAF–macrophage–epithelial cell signaling axis).

The key assessment targets may vary according to lesion stage. For early precancerous lesions (such as atypical adenomatous hyperplasia and low-grade CIN), assessment should focus on mild inflammation, extracellular matrix remodeling, and immune cell infiltration. For advanced precancerous lesions (such as high-grade CIN and high-grade colorectal adenoma), assessment should focus on immunosuppression (PD-L1$^+$ cells, M2 macrophages), CAF activation, and metabolic reprogramming.

It should be noted that some of the suggested approaches for detecting early cancer lesions and their TME are still in the experimental stage, which could represent promising future research avenues. Examples include multi-omics liquid biopsies, AI-enhanced imaging, and deep endoscopic screening.

9.3. Early intervention strategies for tumorigenesis

Precancerous lesions represent a reversible and critical intervention window during tumor development. These lesions are characterized by genomic instability and abnormal proliferative potential, yet have not undergone invasive malignant transformation. The progression from precancerous lesions to invasive cancer generally takes 5–15 years. Early intervention can significantly reduce the risk of malignant transformation and decrease the need

for invasive or morbid treatment. Core interventions focus on five areas: immunity, metabolism, microenvironment, epigenetics, and inflammation control. This review summarizes the core mechanisms and key clinical/preclinical evidence for each intervention approach.

9.3.1. Immune prevention and restoration of immune surveillance

This strategy aims to restore the ability of the immune system to recognize and eliminate precancerous cells and to prevent the early development of immune escape.

Prophylactic HPV vaccines can prevent infection with HPV types 16/18. In one study, vaccine efficacy against persistent HPV16/18 infection lasting ≥12 months was 83.7% after two doses and 92.6% after three doses.[124] Regions with high HPV vaccination coverage have experienced a profound transformative effect on public health, characterized by a significant reduction of up to 30% in cervical cancer incidence.[125]

Therapeutic vaccines have also been investigated for cancer prevention and early intervention. The MUC1 long-peptide vaccine reduces polyp recurrence by 38% in high-risk colorectal adenoma patients.[126] Dendritic cell vaccines targeting HPV E6/E7 induce antigen-specific T-cell responses in cervical precancerous lesions, achieving long-term lesion regression.[127]

Preclinical studies have further suggested that personalized neoantigen vaccines may selectively eliminate precancerous cells carrying high-risk mutations, such as *TP53* and *KRAS*, thereby preventing malignant transformation.[128] Immune checkpoint modulators have also shown preventive potential in preclinical models. TIM-3 blockade has been reported to reduce tumor burden in lung adenocarcinoma precursor models, with effects observed mainly during the precancerous stage rather than in advanced tumors. In mouse models of Lynch syndrome, combined anti-PD-L1 and anti-LAG-3 therapy has been reported to markedly reduce intestinal tumorigenesis.[129,130]

9.3.2. Targeting metabolic reprogramming

Targeting metabolic reprogramming aims to reverse abnormal metabolic features of precancerous cells, limit uncontrolled proliferation, and block metabolic support of malignant transformation. Potential strategies include AMPK activators, glycolysis inhibitors (2-deoxyglucose), and fatty acid synthase inhibitors.[131–133]

9.3.3. Normalization of the tumor microenvironment

Normalization of the TME may correct abnormal stroma, vascular, and immunosuppressive conditions surrounding precancerous lesions, thereby eliminating the "soil" for tumor development.

Vascular normalization strategies, including VEGF inhibition, may remodel abnormal vascular structures, improve vascular permeability and perfusion, alleviate tissue hypoxia, reduce mutation risk, enhance immune cell infiltration, and improve drug delivery.[134]

Stromal remodeling and immune microenvironment regulation are also important approaches. In preclinical studies, knockout of *Adam12* (a key checkpoint for CAF function) remodeled the TME, increased $CD8^+$ T-cell infiltration, and blocked malignant transformation in colorectal precancerous models.[135] Reversal of immunosuppression may also be achieved by targeting tumor-promoting immune cells and suppressive cytokine pathways. Colony-stimulating factor 1 receptor inhibitors can reduce M2-like macrophage activity, whereas TGF-β inhibitors may relieve T-cell suppression. These strategies have shown preventive effects in animal models of precancerous lesions.[136,137]

9.3.4. Epigenetic regulation intervention

Epigenetic intervention aims to reverse potentially reversible epigenetic abnormalities in precancerous lesions, reactivate silenced tumor suppressor genes, and restore normal cellular differentiation.

DNA methylation inhibitors, such as azacitidine and decitabine, are approved for the treatment of myelodysplastic syndromes (a hematological precancerous lesion), significantly reducing the risk of progression to acute leukemia.[138] Histone deacetylase (HDAC) inhibitors suppress the expression of HPV E6/E7 oncoproteins and may have potential value in cervical precancerous lesions or cervical carcinogenesis models.[139-141] HDAC6 inhibitors block PD-L1 expression, enhance sensitivity to immunotherapy, and reverse lesion progression in precancerous models of colorectal cancer and triple-negative breast cancer.[142]

9.3.5. Inflammation control and chemoprevention

Inflammation control and chemoprevention aim to reduce chronic inflammation-driven gene mutations, uncontrolled proliferation, and immunosuppression. Potential strategies include COX-2 inhibitors (celecoxib) and NF-κB pathway inhibitors.[143-145]

Early intervention in tumorigenesis is a core strategy to reduce cancer incidence and mortality. Abundant preclinical evidence supports five major intervention approaches: immune prevention, metabolic reprogramming, microenvironment normalization, epigenetic regulation, and inflammation control, with

multiple strategies already translated into clinical applications. In the future, precise intervention will become the mainstream direction of cancer prevention and early treatment. Therefore, we propose three novel principles for cancer prevention: (i) early detection of precancerous lesions, (ii) early determination of patient-specific TME, (iii) early intervention in the process of tumorigenesis. This new set of three principles can not only effectively prevent tumor occurrence and reduce the incidence but can also guide the clinical individualized precision treatment of cancer patients.

10. Conclusion

This paper incorporates the latest research to provide a new perspective on tumor occurrence and development. We further discussed the original driving force of tumorigenesis and the enhancement and reprogramming of the cellular and molecular biological mechanisms underlying this process. We clarified the multiple characteristics of tumor cell growth and proliferation of tumor cells during tumorigenesis, and we proposed the concept of "persistent dynamic biology" and a Dysregulated Adaptive Cascade Model from chronic repeated induction to cancer development for the first time. Additionally, we proposed a framework that could establish the foundation for the accurate and individualized detection, diagnosis, and treatment of cancer patients through the combination of the laboratory organoid culture system and other techniques with "persistent dynamic biology." Lastly, we introduced three new principles of cancer prevention, and their implementation may represent an effective way to reduce the incidence of cancer. Admittedly, to support the new concepts and systems proposed in this paper, we need to continue to explore more complex relationships between oncogenes, between oncogenes and important cancer-related signal transduction pathways, and between oncogenes and newly discovered molecular regulatory mechanisms. By doing this, we can deepen our understanding of the true process of tumorigenesis, continue to improve our understanding of tumor "persistent dynamic biology" to discover its internal regularity, increase detection and analysis of tumor patients' organoid culture system to verify and enrich our understanding of earlier tumorigenesis mechanisms, and provide an effective basis for prevention and individualized clinical treatment.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

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