ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Increased angiopoietin-like protein 8 expression in colorectal carcinoma patients with liver metastasis

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Citation: Susanto H, Handaya AY, Linangkung A, *et al*. Increased angiopoietin-like protein 8 expression in colorectal carcinoma patients with liver metastasis. *Eurasian J Med Oncol*. 2025;9(2):261-269. doi: 10.36922/EJMO025080034

Received: February 18, 2025

1st revised: March 24, 2025

2nd revised: April 2, 2025

3rd revised: April 22, 2025

4th revised: April 23, 2025

Accepted: April 24, 2025

Published online: May 9, 2025

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Abstract

Colorectal cancer (CRC) is the third most common cancer in men and the second most common in women, with a 5-year survival rate. The disease burden of CRC can be exacerbated by a sedentary lifestyle, lack of physical exercise, smoking, and obesity. Angiopoietin-like protein 8 (ANGPTL8), a liver hormone, plays a crucial role in lipid and glucose metabolism and is also implicated in the development of certain cancers. However, the basic profile and activity of this hormone in CRC with liver metastasis remains unknown. This study aims to establish the baseline expression of ANGPTL8 in tumors of CRC patients with and without liver metastasis. After histopathological screening and computed tomography scans, 41 patients were identified and included in this study, consisting of 20 CRC patients without liver metastasis and 21 with liver metastasis. Tumor samples from each group were compared to their respective adjacent normal tissues. Quantitative real-time polymerase chain reaction and enzyme-linked immunosorbent assay were employed to assess the ANGPTL8 expression at both mRNA and protein levels. The results show that ANGPTL8 expression is higher in tumors of CRC patients with liver metastasis compared to those without liver metastasis. ANGPTL8 expression is significantly correlated with body mass index (BMI) (Pearson's correlation coefficient = −0.367, $p \leq 0.05$) and could serve as a significant predictor of BMI ($p \leq 0.05$). These findings suggest that ANGPTL8 may enhance tumor development in CRC patients and is associated with body weight changes, contributing to weight loss symptoms during metastasis.

Keywords: ANGPTL8; Body weight loss; Colorectal cancer; Liver metastasis

1. Introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer-related mortality, with a low survival rate in patients with metastasis.[1] It is estimated that the global CRC incidence will significantly increase by 3.2 million cases by 2040.[2] CRC is the third most common cancer in men and the second most common in women, with a 5-year survival rate.[3] The disease burden of CRC is exacerbated by a sedentary lifestyle, lack of physical exercise, smoking, and obesity.[4] Recent studies have shown that nutrition can contribute to CRC development,[5] and Western diets, coupled with unhealthy lifestyle choices, are known to accelerate CRC progression.[6] In addition to these external factors, genetic instability plays a vital role in the development of CRC.[7] Notably, recent studies have found that the overall incidence of CRC has increased in individuals under 50 compared to older individuals.[3,8,9]

The increased mortality rate in CRC patients has been shown to correlate with the metastasis of malignant cells to specific sites within the body. The liver is the most common site of CRC metastasis, particularly due to its association with portal circulation.[10] Approximately 25% of CRC patients will develop liver metastasis, which significantly impacts the survival rate of the patients.[11] Liver metastasis in CRC patients presents a major clinical problem, with curative resection and chemotherapy being the gold standard of treatment.[12] The implementation of a preventive management strategy for CRC–liver metastasis would be beneficial for patients, potentially involving the development of accurate screening procedures, followed by the introduction of prevention programs aimed at improving the patient's quality of life.[13,14]

Recent literature has suggested that angiopoietin-like protein 8 (ANGPTL8) may contribute to dyslipidemia, particularly in relation to triglyceride (TG) metabolism. ANGPTL8 is a potential regulator for lipoprotein lipase activity in the vascular system,[15-18] as demonstrated in *in vitro* models.[19-21] Conversely, the inhibition of ANGPTL8 upregulation has been shown to reduce TG levels.[22] ANGPTL8 is also significantly associated with obesity and related complications.[15,23-31] In addition, significant alterations in ANGPTL8 expression have been linked to cancer development[32] and kidney dysfunction, such as diabetic nephropathy.[33] Previous studies have reported elevated serum levels of ANGPTL8 in patients with hepatocellular carcinoma (HCC) and non-alcoholic fatty liver disease,[34,35] where ANGPTL8 acts through the Akt-GSK3β pathway to improve insulin sensitivity. In addition, Akt plays a pivotal role in cancer-related survival and proliferation.[36] Despite these findings, the prognostic role of ANGPTL8 in cancer progression and liver metastasis remains insufficiently explored.

Several studies have indicated that ANGPTL8 may act as a trigger for cancer cell death. In an *in vitro* study on pancreatic cancer, ANGPTL8 was reported to exhibit an antiproliferative effect on Mia-PaCa and PANC-1 cell lines.[37] Exogenous ANGPTL8 treatment in cancer cell lines has been shown to induce apoptosis. Moreover, ANGPTL8 was found to reduce cell proliferation in the HepG2 cell line through the Wnt signaling pathway.[38] Although previous *in vitro* studies suggest that ANGPTL8 may induce cancer cell death, there is a lack of data regarding whether ANGPTL8 expression contributes to cancer cell proliferation and metastasis. Therefore, this study was conducted to establish the baseline data of ANGPTL8 expression in tumors of CRC patients with and without liver metastasis.

2. Materials and methods

2.1. Samples

This unicentric, observational clinical study was conducted in Indonesia, involving 41 study participants. Clinical data were extracted from the hospital database for the period between 2021 and 2022. The study was approved by the Institutional Review Board of the Faculty of Medicine, Universitas Gadjah Mada, Indonesia (ethics approval number: KE/FK/0938/EC/2021), which granted legal permission for this clinical study. All participants diagnosed with CRC who underwent surgery provided written informed consent, in accordance with the Declaration of Helsinki, 1975. This observational clinical study involved 20 CRC patients without liver metastasis and 21 patients with liver metastasis. Tumor samples from each group were compared to their respective adjacent normal tissues. The inclusion criteria for this study include: CRC patients with and without metastasis who had undergone the diagnostic stage at Dr. Sardjito General Hospital, Yogyakarta; patients who had been deemed suitable candidates for surgery; and those with complete medical records. Patients with incomplete data, pulmonary metastasis, or those who refused to participate were excluded from this study. The samples were obtained from Dr. Sardjito General Hospital in Yogyakarta, Indonesia. Confirmation and classification of the samples were determined based on histopathological and computed tomography scan data. Baseline information of the patients was extracted from their medical records at the hospital. The primary tumor samples were stored in the Biobank of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, and were subsequently transferred to the Molecular Biology Laboratory, Universitas Negeri Malang, for further analysis.

2.2. ANGPTL8 expression analysis

The protein level of ANGPTL8 expression was assessed using a human enzyme-linked immunosorbent assay kit specific for ANGPTL8 (No. E11644h, EIAab, China). The mRNA level of *ANGPTL8* expression was measured using real-time quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from fresh-frozen tissue samples (100 mg) using the TRISURE™/Qiazol synthesis kit (Bioline, United Kingdom) and stored at −20°C. The reverse transcription process was performed using the ReverTra Ace® RT-qPCR Master Mix with gDNA Remover (TOYOBO, Japan). The RT-qPCR was conducted on a qTower machine (Analytic Jena, Germany) using a mix solution from the SensiFAST SYBR Green No-ROX kit (Bioline, United Kingdom). The forward and reverse primers for *ANGPTL8* were 5'-GAGACTCAGATGGAGGAGGA-3' and 5'-ATGCTGCTGTGCCACCATCT-3', respectively. *β-actin* was used as the housekeeping gene, with primer sequences of 5'- CATGTACGTTGCTATCCAGG-3' (forward) and 5'-CTCCTT AATGTCACGCACGAT-3' (reverse). *ANGPTL8* expression was determined by quantifying the comparative cycle threshold (Ct) values. The relative gene expression levels were calculated using the $2^{\Delta Ct}$ method and analyzed with JMP.6 software (SAS Institute, United States of America).

2.3. Statistical analyses

The data distribution was assessed using the Kolmogorov–Smirnov normality test. Comparisons between groups were performed using a parametric independent sample *t*-test. Associations between parameters or variables were evaluated using Pearson's product-moment correlation test and univariate linear regression analysis. A significance level of 5% ($p \leq 0.05$) was applied, and the results are presented as mean ± SEM.

3. Results

Based on the patients' medical records (Figure 1), in the non-liver metastasis group, three patients had a history of

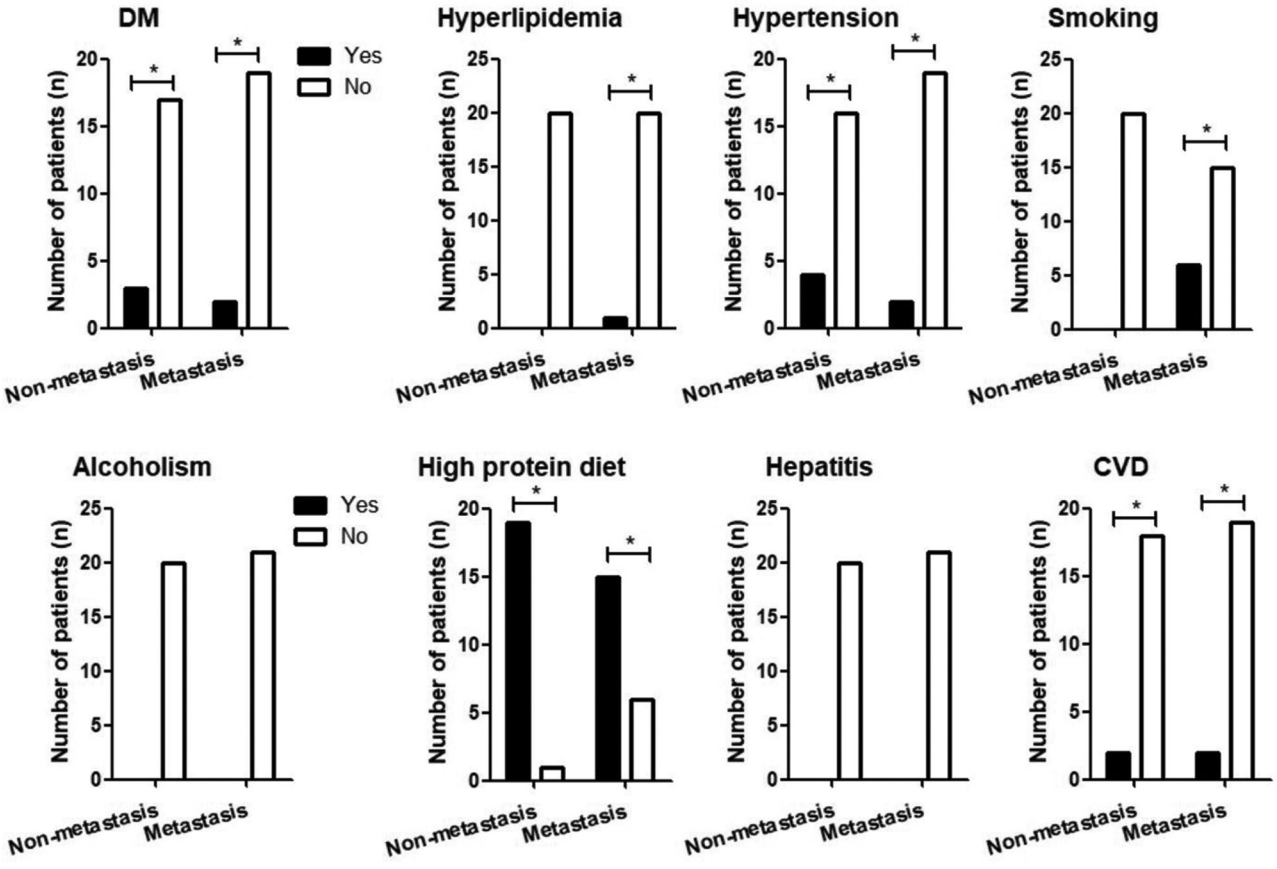


Figure 1. Characteristics of disease history in the study population across metastasis and non-metastasis groups. The graphs represent the distribution of individuals with (Yes) and without (No) history of each condition.
Note: Asterisk (*) indicates a significant difference between "Yes" and "No" within the same group (metastasis or non-metastasis), as determined by independent sample *t*-test analysis ($p \leq 0.05$).
Abbreviations: CVD: Cardiovascular disease; DM: Diabetes mellitus.

diabetes mellitus (DM), four had hypertension, 19 reported a high-protein diet, and two had cardiovascular disease. In the liver metastasis group, two patients had a history of DM, one had hyperlipidemia, two had hypertension, six had a history of smoking, 15 patients reported a high-protein diet, and two had cardiovascular disease. No history of alcoholism or hepatitis was reported in either group.

Table 1 shows the baseline characteristics of CRC patients with and without liver metastasis in CRC patients. There are no significant differences ($p \geq 0.05$) observed between the two groups in age, systolic blood pressure, diastolic blood pressure, hemoglobin, hematocrit, white blood cells, red blood cells, platelet, fasting blood glucose levels, albumin, blood urea nitrogen, and creatinine levels. However, circulating fasting blood glucose, albumin, platelet, and blood urea nitrogen levels were observed to be higher in CRC patients with liver metastasis. Interestingly, body mass index (BMI), alanine aminotransferase, aspartate aminotransferase, and carcinoembryonic antigen levels were significantly different between both groups ($p \leq 0.05$).

Table 1. Baseline characteristics of the study population across different groups

Parameters	Groups	
	Non-metastasis (n=20)	Metastasis (n=21)
Age (years)	54.50±3.12	57.08±2.99
BMI (kg/m^2)	23.41±1.19	18.92±0.49*
SBP (mmHg)	123.15±2.43	123.33±4.32
DBP (mmHg)	78.10±1.99	79.33±2.23
Hemoglobin (g/dL)	11.38±0.47	11.63±0.47
Hematocrit (%)	34.91±1.37	35.37±1.23
WBC (10^3/µL)	8.77±0.84	10.70±1.36
RBC (10^3/µL)	4.38±0.12	4.22±0.14
Fasting glucose level (mg/dL)	120.80±10.27	142.33±10.54
Albumin (g/dL)	3.63±0.15	3.13±0.16
BUN (mg/dL)	12.69±1.49	16.77±2.32
Creatinine (mg/dL)	0.91±0.07	0.97±0.13
PLT (10^3/µL)	284.10±21.33	370.58±31.57
ALT (U/L)	11.65±1.74	28.25±5.12*
AST (U/L)	21.35±4.51	47.92±10.71*
CEA (µg/L)	14.36±5.51	307.53±1.27*

Note: Data are presented as mean±SEM. Asterisk (*) indicates a significant difference between the metastasis and the non-metastasis group ($p \leq 0.05$), as determined by an independent sample t-test. Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; BUN: Blood urea nitrogen; CEA: Carcinoembryonic antigen; DBP: Diastolic blood pressure; PLT: Platelet; RBC: Red blood cell; SBP: Systolic blood pressure; WBC: White blood cell.

The protein and mRNA expression levels of ANGPTL8 were elevated in tumors of CRC patients with liver metastasis compared to those without liver metastasis (Figures 2 and 3). Notably, parametric analysis using Pearson's product-moment correlation shows a significant negative correlation between ANGPTL8 expression and BMI (Pearson's correlation coefficient = −0.367, $p \leq 0.05$) (Table 2). In addition, univariate linear regression analysis shows that ANGPTL8 expression correlates with BMI (Table 3). These results suggest that ANGPTL8 could serve as a significant predictor for BMI ($p \leq 0.05$) and may contribute significantly to BMI changes in CRC patients.

4. Discussion

In this study, it was revealed that ANGPTL8 expression in tumors of CRC patients with liver metastasis is different from those without metastasis. Interestingly, ANGPTL8

Table 2. Univariate correlations with ANGPTL8 in all participants

Parameters	ANGPTL8	
	Pearson's correlation coefficient (r)	p-value
Age (years)	0.114	0.535
BMI (kg/m^2)	−0.367	0.039*
SBP (mmHg)	0.181	0.320
DBP (mmHg)	0.236	0.194
Hemoglobin (g/dL)	−0.325	0.069
Hematocrit (%)	−0.340	0.057
WBC (10^3/µL)	0.118	0.520
RBC (10^3/µL)	−0.156	0.395
Fasting glucose level (mg/dL)	0.164	0.370
Albumin (g/dL)	−0.180	0.325
BUN (mg/dL)	−0.137	0.454
Creatinine (mg/dL)	−0.160	0.383
PLT (10^3/µL)	0.004	0.981
ALT (U/L)	0.214	0.240
AST (U/L)	0.347	0.052
CEA (µg/L)	0.053	0.774
Tumor	0.037	0.840
Nodes	0.195	0.285
Metastasis	0.211	0.245

Note: Asterisk (*) indicates a statistically significant correlation ($p \leq 0.05$), as determined by Pearson's product-moment correlation test. Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; BUN: Blood urea nitrogen; CEA: Carcinoembryonic antigen; DBP: Diastolic blood pressure; PLT: Platelet; RBC: Red blood cell; SBP: Systolic blood pressure; WBC: White blood cell, ANGPTL8: Angiopoietin-like protein 8.

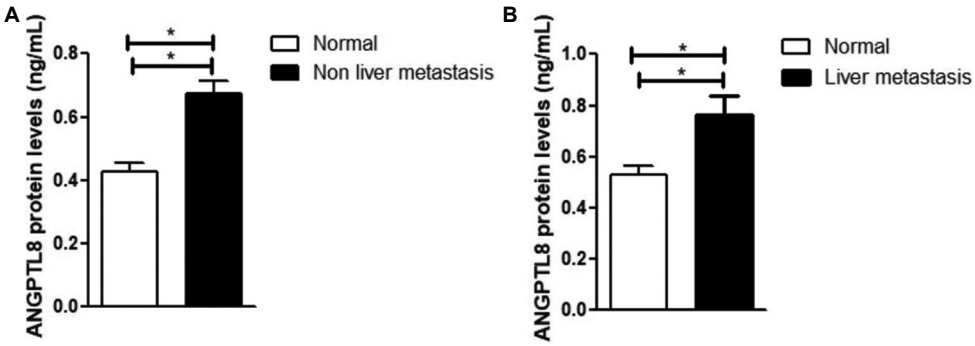


Figure 2. Angiopoietin-like protein 8 (ANGPTL8) protein levels in colorectal cancer patients with non-liver metastasis (A) and liver metastasis (B), compared to their respective adjacent normal tissues. ANGPTL8 levels were measured using an enzyme-linked immunosorbent assay.
Note: Asterisk (*) indicates a significant difference from adjacent normal tissue within each group, as determined by independent sample *t*-test analysis ($p \leq 0.05$).

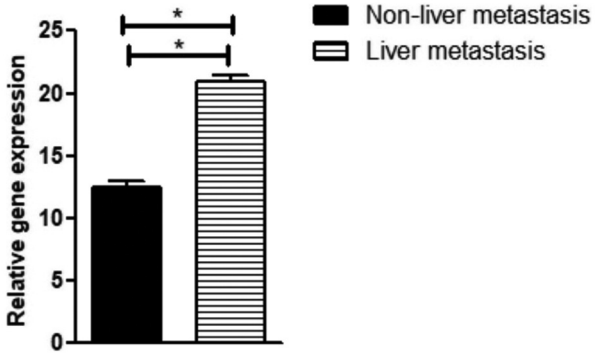


Figure 3. Relative gene expression of *Angiopoietin-like protein 8* in colorectal cancer patients with non-liver metastasis and liver metastasis were measured using real-time quantitative polymerase chain reaction.
Note: Asterisk (*) indicates a significant difference between each group, as determined by independent sample *t*-test analysis ($p \leq 0.05$).

Table 3. Univariate linear regression analysis

Response	Predictor	Estimate (*B*)	*p*-value
BMI	ANGPTL8	−8.641	0.039*
CEA	ANGPTL8	77.048	0.774
ALT	ANGPTL8	15.263	0.240
AST	ANGPTL8	50.731	0.052

Note: Asterisk (*) indicates a statistically significant association ($p \leq 0.05$), as determined by univariate linear regression analysis. Abbreviations: BMI: Body mass index; CEA: Carcinoembryonic antigen; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase, ANGPTL8: Angiopoietin-like protein 8.

expression shows a strong correlation with BMI in these patients. Previous studies have reported that ANGPTL8 expression is positively correlated with CD133, a widely recognized biological marker for cancer malignancy. Highly malignant HCC cells showed a significantly increased expression of ANGPTL8,[39,40] suggesting that ANGPTL8 expression is closely related to cancer metastasis.[39] In addition, previous studies have shown that metastasis is often associated with a decrease in muscle mass, which suggests that the reduction in body weight observed in the current study may be the result of CRC liver metastases.[41,42] To confirm whether ANGPTL8 contributes to body weight reduction, the expression of ANGPTL8 proteins and genes in the liver and metastatic cancer was analyzed. Notably, our investigation revealed that the protein levels of ANGPTL8 in CRC patients with and without liver metastasis were higher compared to the adjacent normal tissues (Figure 2). In addition, the ANGPTL8 levels in the liver metastasis group were higher than in the non-metastasis group (Figure 3). While this finding supports the correlation between ANGPTL8 levels and body weight reduction, the fundamental role of ANGPTL8 in metastasis and metabolic activities in CRC patients with liver metastasis remains to be determined.

Generally, ANGPTL8 is a liver-derived hormone with a predominant role in lipid metabolism, particularly TG metabolism.[19,20,22,29,43] Furthermore, ANGPTL8 has been proposed as a potential target for treating DM-linked obesity.[43-46] Previous studies have shown that elevated circulating ANGPTL8 levels found in HCC patients[34] and pancreatic ductal adenocarcinoma patients with diabetes[44] correlate to liver lipid content, independently of obesity and insulin resistance.[35] In addition, exogenous ANGPTL8 treatment has been shown to reduce cell viability in pancreatic cancer cell lines, particularly in PANC-1 and Mia PaCa-2 cells.[37] ANGPTL8 has also been recognized as an attractive target for development as a prognostic factor and therapeutic agent in cancer treatment.[47,48]

The present study demonstrates a significant potential correlation between ANGPTL8 expression and BMI. Several studies have reported that ANGPTL8 serum levels are significantly elevated in individuals with obesity. In addition, the increase in ANGPTL8 levels strongly correlates with TG profiles in patients with body weight disorders. Interestingly, significantly different results

were observed in the current study compared to the previous *in vitro* investigation.[49] The RNA and protein levels of ANGPTL8 in the tumors of CRC patients with liver metastasis were found to be higher compared to those without liver metastasis.[39] The higher expression of ANGPTL8 inversely correlates with the patient's body weight. Gradual changes in body weight were observed in CRC patients following liver metastasis. In line with the steady increase of ANGPTL8, the expressions of several genes contributing to tumor metastasis also exhibited an increase (data not shown). Fundamentally, there are still limited data on how ANGPTL8 is associated with body weight loss linked to TG metabolism in CRC patients with liver metastasis.

In general, loss of ANGPTL8 function or even downregulation of ANGPTL8 expression could improve TG metabolism and reduce the progression of obesity and hypertriglyceridemia.[50,51] A previous *in vivo* study demonstrated that ANGPTL8 influences adipose formation, and the knockout of ANGPTL8 resulted in significantly reduced TG levels in adipose tissue compared to wild-type mice, suggesting that ANGPTL8 may be involved in the regulation of TG.[52] Moreover, the potential mechanism by which ANGPTL8 increases TG levels involves facilitating the cleavage of ANGPTL3, thereby releasing its N-terminal domain, which suppresses lipoprotein lipase activity and promotes adipose tissue decomposition. The variation in ANGPTL8 response or expression in the tumor may be influenced by body weight loss progression. A previous study suggested that impaired ANGPTL8 response can be restored after weight loss.[53] However, in this study, it remains unclear whether the gradual weight loss observed in CRC patients with liver metastasis contributes to the elevated ANGPTL8 expression in the tumor. Another possible mechanism is that reduced ANGPTL8 expression in the liver, possibly due to liver dysfunction-linked liver metastasis, could lead to hyperglycemia and impaired insulin sensitivity. This could consequently drive increased fat metabolism in adipose tissue, leading to body weight loss.

Based on the previous study, it was suggested that ANGPTL8 could improve insulin sensitivity through the Akt-GSK3β pathway and contribute to cancer cell survival and proliferation through the Akt signaling pathway.[36] In line with this finding, our data show that glucose levels in CRC patients with liver metastasis are higher compared to those without metastasis. It is predicted that the steadily increasing glucose levels may promote cell proliferation, survival, and metastasis in the local tumor sites. In addition, disturbances in glucose levels – caused by abnormal glucose metabolism – could potentially trigger cancer cell proliferation, invasion, and migration by mediating antiapoptotic responses. In this study, it was also found that the expression of ANGPTL8 in the tumor likely correlates with cell migration. This finding contradicts the previous studies suggesting that ANGPTL8 can induce cell apoptosis and proliferation.[39,40] Similar to our findings, previous studies and several databases have reported that ANGPTL8 expression tends to increase in colorectal adenocarcinoma, kidney renal clear carcinoma, and rectum adenocarcinoma compared to breast cancer, cholangiocarcinoma, lung adenocarcinoma, and uterine corpus endometrial carcinoma.[36,54,55] In addition, the expression of ANGPTL8 in the metastasis group is higher than in the non-metastasis group.[36] Moreover, the predictive property and survival rate analysis of ANGPTL8 expression suggest that this liver hormone could significantly and potentially serve as a biomarker in those cancers. However, it remains unclear whether the higher expression of this hormone at the tumor site will trigger an increase in glucose synthesis to support malignant cell growth and facilitate detachment from the original site to the liver.

Collectively, the preliminary findings of this study propose a hypothetical framework suggesting that ANGPTL8 could be a potential contributor and part of a panel of markers for CRC with liver metastasis. However, there are several limitations in this study. For instance, our clinical investigation could not provide a comprehensive database for the baseline data of the patients. In addition, our study focused solely on the fundamental profile analysis of ANGPTL8 expression in the tumors of CRC patients, without any supporting data from serological markers or other metabolic factors related to ANGPTL8. Future investigations are warranted, involving a larger sample size, comparisons across different racial groups of clinical patients, and further laboratory analyses. A comprehensive correlational approach that includes microscopic, serological, and bioinformatics analysis will be essential to support these preliminary findings.

5. Conclusion

The findings of this study suggest that ANGPTL8 may contribute to the development of tumor-associated metastasis in CRC patients. The observed changes in ANGPTL8 expression are hypothesized to correlate with body weight loss in CRC patients with liver metastasis. This study highlights the potential of ANGPTL8 not only as a therapeutic target but also as a promising prognostic marker for CRC. Future investigations, including larger sample sizes and comprehensive analyses, are warranted to validate these findings and fully explore the clinical application of ANGPTL8 in managing CRC.

Acknowledgments

We would like to express our deepest gratitude to the PhD biotechnology team at Universitas Negeri Malang for their contributions to this research. We also extend our sincere appreciation to our collaborators from the Division of Digestive Surgery, Department of Surgery, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, and Dr. Sardjito from General Hospital Yogyakarta, Indonesia.

Funding

This study was supported by the 2024 Universitas Negeri Malang PNBP research grant (Contract number: 4.4.602/UN32.14.1/LT/2024).

Conflict of interest

There was no conflict of interest among all authors in this study.

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Writing – review & editing: Adeodatus Yuda Handaya, Zulqarnain Mohamed, Moch Sholeh

Ethics approval and consent to participate

This clinical investigation was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Institutional Review Board of the Faculty of Medicine, Universitas Gadjah Mada, Indonesia (Approval number: KE/FK/0938/EC/2021). All participants provided written informed consent before the study.

Consent for publication

All participants have provided written informed consent before the study, which includes the use of their samples for further research purposes. In addition, the identities of the participants have been deidentified to ensure confidentiality.

Availability of data

All the datasets used and/or analyzed during the current study are available from the corresponding author upon a reasonable request.

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