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ORIGINAL RESEARCH ARTICLE

Enhanced expression of metastasis-associated genes in colorectal cancer

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Abstract

Introduction: Globally, colorectal cancer (CRC) continues to be a major cause of cancer-related morbidity and death, with metastasis—particularly to the liver—significantly worsening patient outcomes.

Objective: The aim of this study was to investigate the expression of key epithelial-mesenchymal transition (EMT) transcription factors (Snail family transcriptional repressor 1 [SNAI1], zinc finger e-box binding homeobox 1 [ZEB1], Slug, Twist, and metastasis-associated protein 3 [MTA3]) and the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) in CRC cases with and without metastasis to the liver.

Methods: A total of 41 CRC patients (20 non-metastatic, 21 with liver metastasis) from Dr. Sardjito General Hospital, Yogyakarta, were examined utilizing reverse transcription quantitative polymerase chain reaction of the adjacent normal tissues and the tumors.

Results: SNAI1, ZEB1, Slug, Twist, and TNF-α were significantly upregulated in metastatic CRC, while MTA3 was downregulated. Expression of these markers correlated with body mass index, liver enzymes (aspartate aminotransferase), and cancer stage.

Conclusion: These findings highlight the central role of EMT-related transcription factors and inflammatory signaling in CRC metastasis and suggest that targeting these pathways could offer novel therapeutic strategies for metastatic CRC.

Keywords: Colorectal cancer; Liver metastasis; Body weight loss; Metastasis marker

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1. Introduction

Colorectal cancer (CRC), the third most common malignancy and the second leading cause of cancer-related deaths worldwide, is one of the most common cancers.[1] Incidence rates vary widely across different regions, with a higher prevalence observed in developed countries compared to developing countries. The etiology of CRC is multifactorial, involving a combination of genetic predispositions, lifestyle factors such as diet and physical inactivity, and environmental influences. Notably, polymorphisms in several genes have been studied for their potential association with CRC risk, although findings regarding their significance have been mixed.[2-5] Pathologically, CRC typically

arises from adenomatous polyps through the adenoma-carcinoma sequence (ACS), characterized by genetic mutations that lead to uncontrolled cell growth.[6,7] This development shows the importance of early detection and screening strategies in reducing CRC-related mortality.[7-9]

In addition to genetic and epigenetic alterations, metabolic reprogramming has emerged as a hallmark of CRC progression. Cancer cells frequently undergo metabolic shifts to meet the increased energy demands and biosynthetic needs associated with rapid proliferation and metastasis.[10] For instance, enhanced glycolysis, known as the Warburg effect, supports tumor growth even in the presence of oxygen.[10] Moreover, alterations in lipid metabolism and amino acid utilization have been implicated in CRC aggressiveness. These metabolic adaptations not only sustain tumor cell survival but also modulate the immune microenvironment, influencing the recruitment and function of immune cells such as tumor-associated macrophages and myeloid-derived suppressor cells.[11] Therapeutic strategies targeting metabolic vulnerabilities of CRC cells hold promise for disrupting tumor progression and overcoming resistance to existing treatments.

Chronic conditions and external stressors can significantly influence the metastasis of CRC, often leading to considerable morbidity and mortality. The metastatic process is influenced by various factors, including chronic inflammation, which can be exacerbated by conditions such as chronic hepatitis B (CHB) and psychological stress.[12,13] Paradoxically, CHB may also protect against colorectal liver metastasis due to its sclerosing effect on the liver and certain immune-mediated mechanisms.[14] Psychological stress and a perturbed gut microbiome can promote CRC growth and metastasis.[15] Single-cell RNA sequencing has revealed that chronic stress can induce an immunosuppressive environment that encourages CRC metastasis.[16] Studies have shown that chronic inflammation can activate oncogenic pathways, promoting tumor growth and metastasis.[17] Furthermore, the tumor microenvironment plays an important role in the progression of CRC, with immune cells like tumor-associated macrophages recruited to support metastatic spread.[18] Research indicates that chronic stress can alter the gut microbiome, further enhancing the metastatic potential of CRC.[19] Understanding these mechanisms is vital for developing targeted therapies, such as inhibition of related genes in CRC, aimed at preventing or reducing metastasis in patients with chronic CRC.

CRC progression and metastasis are governed by a complex interplay of genetic, epigenetic, and microenvironmental factors, each contributing to the disease's heterogeneity and clinical outcomes.[7] Beyond the classical ACS and the role of epithelial-mesenchymal transition (EMT) regulators, recent research has illuminated several additional mechanisms that underpin CRC advancement and metastatic spread.[7] One critical aspect is the dysregulation of multiple cellular signaling pathways, which orchestrate the malignant transformation and dissemination of CRC cells. Notably, the Wnt/β-catenin pathway is frequently activated in CRC, driving uncontrolled proliferation and survival of tumor cells.[20] Mutations in key genes can disrupt cellular homeostasis, thereby facilitating the accumulation of genetic changes that drive tumorigenesis. Snail family transcriptional repressor 1 (SNAI1), zinc finger e-box binding homeobox 1 (ZEB1), Slug, Twist, metastasis-associated protein 3 (MTA3), as transcription factors, and tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine signaling are also shown to be involved in CRC progression and metastasis.[21-23] In particular, SNAI1, Slug, and MTA3 are pivotal regulators of EMT, a process that enables cancer cells to acquire migratory properties. They are also members of the metastasis-associated protein family.[24,25] TNF-α has been shown to induce EMT in CRC cells by stabilizing SNAI1 through the AKT/GSK-3β signaling pathway.[26] This pathway enhances SNAI1's nuclear localization and promotes the switch from E-cadherin to N-cadherin expression, a hallmark of EMT.[21] Meanwhile, ZEB1 and Twist also contribute to this process by responding to various microenvironmental signals and facilitating the transition of epithelial cells into mesenchymal-like cells.[27] The interplay between these factors not only drives tumor invasion but also correlates with unfavorable clinical outcomes in CRC patients, underscoring their potential as targets for therapy in managing cancer metastasis.

Clinically, advancements in molecular profiling have enabled the stratification of CRC patients based on genetic and epigenetic signatures, allowing for more personalized therapeutic approaches. Biomarkers such as SNAI1, ZEB1, Slug, Twist, MTA3, and TNF-α are increasingly used to guide treatment decisions, particularly regarding the selection of targeted agents and immunotherapies. Immune checkpoint inhibitors have shown remarkable efficacy in a subset of CRC patients with high expression, highlighting the potential of immunotherapeutic approaches in against this disease.[28,29] However, resistance mechanisms and the limited response in tumor microenvironment remain challenges that necessitate further investigation. The interplay of these pathways not only promotes tumor growth but also provides potential therapeutic targets for intervention.

In summary, the progression and metastasis of CRC are driven by a complex interplay of genetic mutations,

epigenetic alterations, and dynamic interactions within the tumor microenvironment. Understanding these mechanisms is essential for developing effective diagnostic, prognostic, and therapeutic strategies. Ongoing research into the molecular and cellular mechanisms underlying CRC will continue to inform the development of targeted interventions, aiming to improve both survival and quality of life for those affected by this formidable disease.

2. Materials and methods

2.1. Respondents

This descriptive clinical study used 41 patients as respondents during the period 2021–2022 at Dr. Sardjito General Hospital, Yogyakarta. This study obtained ethical approval through the Institutional Review Board (IRB), Faculty of Medicine, Universitas Gadjah Mada, Indonesia (ethics number: KE/FK/0938/EC/2021). The respondents consisted of 20 CRC patients without metastasis and 21 CRC patients with liver metastasis. Then, each group was also compared with adjacent normal tissue. The study's inclusion criteria encompassed patients diagnosed with cancer, whether metastatic or non-metastatic, who had completed the diagnostic phase, had been declared as surgery candidates, and possessed comprehensive medical records. Patients with incomplete data, lung metastasis, or refused to participate in the study were excluded from the study. Confirmation and classification of samples were based on histopathology data and computed tomography scans assessed by medical personnel at the hospital. Basic patient information was extracted from the patient's medical records. Primary tumor samples were stored in the Biobank of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, and sent to the Molecular Biology Laboratory, Universitas Negeri Malang, for further analysis.

2.2. Marker expression analysis

The mRNA expression levels of SNAI1, ZEB1, Slug, Twist, MTA3, and TNF-α were measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from fresh frozen tissue samples (100 mg) using the TRISURE™/Qiazol Total RNA Isolation Kit (Bio line, UK) and stored at −80°C for subsequent analysis. RNA purity was evaluated by determining the A260/280 ratio (acceptable range: 1.8–2.0) using a Nanodrop spectrophotometer, and residual genomic DNA (gDNA) was removed by treatment with RNAse-free DNase enzyme (Invitrogen, US).

Complementary DNA (cDNA) synthesis was performed using the High-Capacity ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo, Japan) following the manufacturer's protocol, using 2 μg of total RNA. The reaction mixture was processed in a PCR thermocycler (Applied Biosystems, US) with the following steps: denaturation at 65°C for 5 min, gDNA elimination at 37°C for 5 min, followed by cDNA synthesis at 37°C for 15 min, 50°C for 5 min, and 98°C for 5 min, then held at 4°C. The resulting cDNA was diluted to 0.5 μg/μL to be used as a template for subsequent RT-qPCR analysis.

PCR samples were prepared using the SensiFAST SYBR Green No-ROX kit (Bioline, UK) and analyszed on an Analytic Jena qTower instrument. Relative quantification of PCR products was performed using the $2^{-\Delta\Delta CT}$ method. Primers from Integrated DNA Technologies through Genetika Science Indonesia, Singapore, were used as follows: TNF-α, forward 5'- CCTGCCCCAATCCCTTTATT -3' and reverse 5'- CCCTAAGCCCCCAATTCTCT -3'; SNAI1, forward 5'- ACTGCAACAAGGAATACCTCAG -3' and reverse 5'- GCACTGGTACTTCTTGACATCTG -3'; ZEB1, forward 5'- AGCAGTGAAAGAGAAGGGAATGC -3' and reverse 5'- GGTCCTCTTCAGGTGCCTCAG -3'; Slug, forward 5'- ATCTGCGGCAAGGCGTTTTCCA -3' and reverse 5'- GAGCCCTCAGATTTGACCTGTC -3'; Twist, forward 5'- GCAGGACGTGTCCAGCTC -3' and reverse 5'- CTGGCTCTTCCTCGCTGTT -3'; MTA3, forward 5'- TATCAGGGGAAAGTGCAGTGTTG -3' and reverse 5'- AACAGCATTTCTGGAATGTCTGC -3'; and GAPDH, forward 5'- TGCACCACCAACTGCTTAGC -3' and reverse 5'- GGCATGGACTGTGGTCATGA -3'. The mRNA expression levels of target genes were normalized to GAPDH expression.

2.3. Statistical analysis

Data distribution was evaluated using the Kolmogorov-Smirnov normality test. Demographic features were analyzed using ANOVA test. Comparisons between groups were conducted using the independent samples t-test. Associations among variables such as body mass index (BMI), carcinoembryonic antigen (CEA), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were assessed using Pearson correlation and univariate linear regression analysis, while the staging parameters (T, N, and M) were analyzed using ordinal regression. A significance level of 5% was applied, and data are presented as mean ± standard error of the mean.

3. Results

The interplay among SNAI1, ZEB1, Slug, Twist, MTA3, and TNF-α in colon cancer involves a complex regulatory network driving EMT, metastasis, and therapeutic resistance. The results of this study provide compelling evidence for the intricate molecular interplay underlying EMT and metastatic progression in colon cancer. The

significant upregulation of SNAI1, Slug, ZEB1, Twist, and TNF-α in the liver metastasis group, as compared to both non-liver metastasis patients and adjacent normal tissue, underscores the central roles of these transcription factors and inflammatory mediators in facilitating metastatic dissemination. This pattern of expression aligns with the well-established understanding that EMT is a pivotal driver in cancer metastasis, endowing tumor cells with enhanced migratory and invasive properties. The concurrent downregulation of MTA3 in the liver metastasis group is particularly noteworthy, as it suggests a loss of EMT suppression, further promoting a pro-metastatic phenotype. The following presents a structured framework based on mechanistic insights supported by research evidence:

This framework (Table 1) highlights the central roles of SNAI1, Slug, ZEB1, and Twist in EMT, supported by stromal interactions and pro-inflammatory mediators such as TNF-α, whereas MTA3 acts as a suppressive regulator. Targeting these molecular nodes may disrupt metastatic progression in colon cancer.

Three patients in the non-liver metastasis group had a history of diabetes mellitus (DM), four had hypertension, 19 followed a high-protein diet, and two had cardiovascular disease (CVD), according to the patients' medical records. In the liver metastasis group, two individuals had a history of DM, one had hyperlipidemia, two had hypertension, six had a history of smoking, fifteen followed a high-protein diet, and two had CVD. None of the participants had a history of hepatitis or alcohol consumption. There were no significant differences ($p \geq 0.05$) between the two groups in terms of hemoglobin, hematocrit, white blood cells, red blood cells, platelets, fasting blood glucose, albumin, blood urea nitrogen, creatinine, systolic blood pressure, diastolic blood pressure, and age. However, individuals with liver metastases from CRC exhibited higher levels of circulating fasting blood glucose, albumin, platelets, and blood urea nitrogen. Notably, CEA, ALT, AST, and BMI showed significant differences between the groups ($p<0.05$) as detailed in Table 2.

It was observed that SNAI1, Slug, ZEB1, Twist, and TNF-α were most highly expressed in the liver metastasis group, whereas MTA3 was markedly decreased in this group (Figure 1). The expression levels were significantly different when compared to both the non-liver metastasis group and adjacent normal tissue. Further analysis using univariate linear regression revealed that SNAI1, ZEB1, Slug, Twist, and TNF-α markers were significantly correlated with BMI ($p \leq 0.05$), while AST was correlated with SNAI1, Slug, Twist, and TNF-α only. Notably, MTA3 showed no correlation with any of the four basic clinical characteristics (Table 3). Furthermore, each marker's association with cancer staging (T, N, and M) was specifically assessed. As presented in Table 4, all markers were found to significantly affect cancer stage. It is worth noting that MTA3 exhibited a negative Wald value, which indicates an inverse relationship with cancer staging.

The correlation between the expression of EMT markers (SNAI1, Slug, ZEB1, Twist, and TNF-α) and BMI highlights a potential link between metabolic status and the molecular mechanisms underlying metastasis (Table 3). Obesity and metabolic dysregulation have been increasingly recognized as risk factors for cancer progression, and our findings provide molecular evidence supporting this association. Elevated BMI may create a microenvironment conducive to EMT and metastasis, possibly through mechanisms such

Table 1. Framework summary of marker correlations

Marker	Role in colon cancer	Interaction partners	Clinical relevance
SNAI1	Drives EMT, stemness, and stromal remodeling; upregulated by tumor ECM	TWIST1, PDGF-BB, and TGF-β1[30,31]	Poor prognosis, metastasis[31,32]
Twist	Enhances EMT and lymph node metastasis; synergizes with SNAI1	SNAI1, ALDH1[33]	Reduced survival[33]
Slug	Supports EMT and stemness; context-dependent expression	SNAI1[34]	Clinical aggressiveness of tumors and poor patient survival[34,35]
ZEB1	Sustains mesenchymal state; potential downstream effector of SNAI1/TWIST1	SNAI1, TWIST1[36]	Promotes the proliferation and invasion of cancer[37]
MTA3	Represses SNAI1; loss of MTA3 promotes	SNAI1[31]	Metastasis suppression[31]
TNF-α	Pro-inflammatory cytokine inducing EMT via NF-κB; modulates stromal-tumor crosstalk	SNAI1, TWIST1[38,39]	Linked to advanced disease[40]

Abbreviations: ALDH1: Aldehyde Dehydrogenase 1, ECM: Extracellular matrix; EMT: Epithelial-mesenchymal transition; MTA3: Metastasis-associated protein 3; NF- κB: Nuclear factor kappa B; PDGF-BB; Platelet-derived growth factor subunit B; SNAI1: Snail family transcriptional repressor 1; TGF-β1: Transforming growth factor-beta 1; TNF-α: Tumor necrosis factor-alpha; ZEB1: Zinc finger e-box binding homeobox 1.

Table 2. Basic demographic and clinical features of the study 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*
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*
Stage (average from T, N, and M)	4	2
Tumor	3	5
Node	1	2
Metastasis	0	2
DM		
Yes	3	2
No	17	19
Hyperlipidemia		
Yes	0	1
No	20	20
Hypertension		
Yes	4	2
No	16	19
Smoking		
Yes	0	6
No	20	15
Alcoholism		
Yes	0	0
No	20	21
High protein diet		
Yes	19	15
No	1	6
Hepatitis		
Yes	0	0
No	20	21
CVD		
Yes	2	2
No	18	19

Notes: Independent sample t-test was used to compare differences among groups. Data are presented as mean ± standard error of the mean. *Significant value of each parameter compared to the non-metastasis group by ANOVA Test ($p \leq 0.05$). The value or grading of the TNM stage is divided into three components of colon cancer, namely, T (Tumor), N (Node), and M (Metastasis). T consists of levels 0–5, N: levels 0–6, and M: levels 0–4. This grading is then used to determine the stages of cancer, including low, well, moderate, and poor differentiation.[41]
Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; CEA: Carcinoembryonic antigen; CVD: Cardiovascular disease; DM: Diabetes mellitus.

Table 3. Univariate linear regression analysis

Response	Predictor	Estimate (B)	*p*-value
BMI	SNAI1	−3.156	0.012*
CEA	SNAI1	81.303	0.501
ALT	SNAI1	10.025	0.065
AST	SNAI1	11.982	0.046*
BMI	ZEB1	−5.501	0.029*
CEA	ZEB1	70.112	0.524
ALT	ZEB1	11.383	0.070
AST	ZEB1	9.117	0.055
BMI	Slug	−6.813	0.042*
CEA	Slug	56.118	0.174
ALT	Slug	5.293	0.091
AST	Slug	7.231	0.042*
BMI	Twist	−4.532	0.021*
CEA	Twist	61.002	0.407
ALT	Twist	11.015	0.060
AST	Twist	12.740	0.048*
BMI	MTA3	1.241	0.104
CEA	MTA3	10.203	0.075
ALT	MTA3	5.746	0.081
AST	MTA3	15.801	0.159
BMI	TNF-α	−9.725	0.033*
CEA	TNF-α	51.118	0.235
ALT	TNF-α	18.964	0.136
AST	TNF-α	11.507	0.046*

Note: *Significant with $p \leq 0.05$.
Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; CEA: Carcinoembryonic antigen; MTA3: Metastasis-associated protein 3; SNAI1: Snail family transcriptional repressor 1; TNF-α: Tumor necrosis factor-alpha; ZEB1: Zinc finger e-box binding homeobox 1.

as chronic low-grade inflammation or altered adipokine signaling, which could upregulate the expression of EMT-promoting genes. The specific association of AST with SNAI1, Slug, Twist, and TNF-α further suggest the role of liver function and systemic metabolic changes are intertwined with the molecular events that drive metastatic colonization of the liver.

In addition, the observed differences in clinical and biochemical parameters between the non-liver metastasis and liver metastasis groups further contextualize the molecular findings. The significant elevations in CEA, ALT, AST, and BMI among patients with liver metastasis reflect both increased tumor burden and the systemic impact of metastatic disease. Taken together with the gene

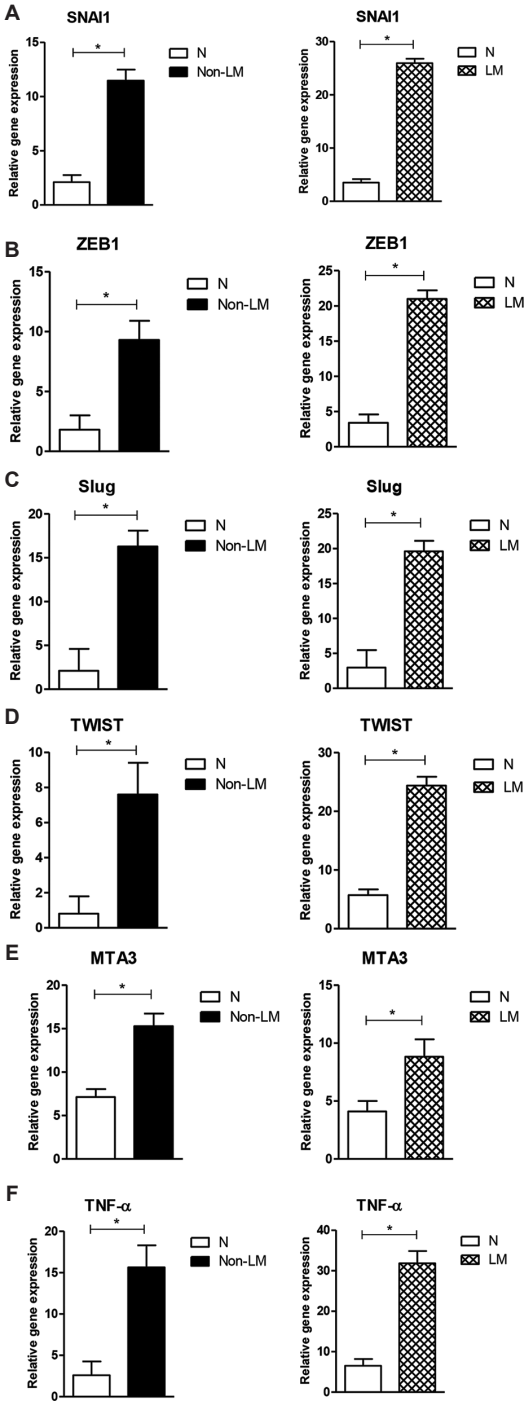

Figure 1. (A-F) Relative gene expression of markers in CRC patients with non-liver and liver metastasis, measured by RT-qPCR and compared with adjacent normal tissue

Note: *Significant difference between groups by independent sample t-test ($p \leq 0.05$).

Abbreviations: CRC: Colorectal cancer; LM: Liver metastasis; MTA3: Metastasis-associated protein 3; N: Adjacent normal tissue; Non-LM:Non-livermetastasis;SNAI1:Snailfamilytranscriptionalrepressor1; TNF-α: Tumor necrosis factor-alpha; ZEB1: Zinc finger e-box binding homeobox 1.

Table 4. Ordinal regression analysis

Response	Predictor	Wald value	*p*-value
Stage (TNM)	SNAI1	3.006	0.022*
Stage (TNM)	ZEB1	1.725	0.031*
Stage (TNM)	Slug	2.018	0.016*
Stage (TNM)	Twist	1.922	0.028*
Stage (TNM)	MTA3	−3.443	0.035*
Stage (TNM)	TNF-α	5.813	0.006*

Note: * Significant with $p \leq 0.05$.

Abbreviations: M: Metastasis; MTA3: Metastasis-associated protein 3; N: Node; SNAI1: Snail family transcriptional repressor 1; T: Tumor; TNF-α: Tumor necrosis factor-alpha; ZEB1: Zinc finger e-box binding homeobox 1.

expression profiles, these clinical markers may serve as a potential composite biomarker panel for risk stratification and early detection of metastatic spread.

The clinical significance of these molecular findings is reinforced by the ordinal regression analysis, which demonstrated that all examined markers significantly influence cancer stage (T, N, M). The negative Wald value for MTA3 is particularly intriguing, indicating that higher MTA3 expression is associated with a lower cancer stage and, conversely, that its downregulation is linked to more advanced disease. This inverse relationship supports the hypothesis that MTA3 acts as a tumor suppressor by antagonizing EMT and maintaining epithelial integrity. Therapeutically, restoring MTA3 function or mimicking its activity could represent a novel strategy to impede metastatic progression in colon cancer.

4. Discussion

Beyond the molecular insights, the clinical translation of these findings holds significant promise for improving patient outcomes in CRC. The identification of SNAI1 as a central regulator of EMT and chemoresistance suggests that SNAI1-targeted therapies could be developed to overcome treatment resistance, a major challenge in advanced CRC. Small molecule inhibitors or RNA interference strategies aimed at reducing SNAI1 expression, or disrupting its stabilization pathways such as the AKT/GSK-3β axis, may sensitize tumors to conventional chemotherapeutics.[7] Furthermore, the interplay between SNAI1 and inflammatory cytokines such as TNF-α highlights the potential for combinatorial therapies that simultaneously target both EMT and inflammation. For instance, anti-TNF-α agents, already in clinical use for inflammatory bowel disease, could be repurposed or combined with EMT inhibitors to suppress tumor progression and metastasis in CRC patients with elevated TNF-α signaling.

The roles of SNAI1, ZEB1, Slug, Twist, and TNF-α in colon cancer progression reveal complex interactions between EMT-related transcription factors and inflammatory signaling, as shown in recent studies. Overexpression of SNAI1 induces partial or complete EMT in colon cancer cells, correlating with chemoresistance, reduced apoptosis, and increased tumor growth.[42,43] Mechanistically, TNF-α stabilizes SNAI1 protein through the AKT/GSK-3β signaling, bypassing transcriptional upregulation.[44] In addition, phosphorylation of sirtuin 1 at Ser27 further stabilizes SNAI1, enhancing its deacetylase activity and promoting interleukin-6 and interleukin-8-mediated tumor progression.[45] These findings position SNAI1 as a critical node in EMT regulation, with its expression levels dictating phenotypic outcomes.[46] As shown in Figure 1, SNAI1 expression is highest in the liver metastasis group. This indicates the role of SNAI1 as a central driver of EMT and chemoresistance.

The context-dependent roles of ZEB1 and Slug in colon cancer, as revealed in this study, also underscore the necessity for patient stratification in future therapeutic interventions. Since ZEB1 and Slug expression is elevated in liver metastatic lesions, their detection could serve as biomarkers of aggressive disease and guide the application of more intensive or targeted therapeutic regimens.[47] Liquid biopsy approaches, such as the measurement of circulating tumor cells or cell-free nucleic acids expressing these EMT-transcription factors (EMT-TFs), may provide non-invasive means for real-time disease monitoring and early detection of metastasis. Although ZEB1 and Slug contribute to EMT in other cancers, their involvements in colon cancer appear context-dependent.[48,49] For instance, TNF-α induces Slug in papillary thyroid cancer, while in colon cancer, SNAI1 predominates in regulating EMT. Some herbal or natural bioactive compounds have been shown to suppress SNAI1 through NF-κB inhibition, highlighting SNAI1's primacy over other EMT-TFs in therapeutic targeting.[50,51] As in Figure 1, it is clear that ZEB1 and Slug expression increase in the liver metastasis group.

Although less studied in colon cancer, Twist has also been implicated in contributing to metastasis by promoting cancer stem cell-like traits. Another study found that Manuka honey downregulates Twist, Slug, and SNAI1 in colonospheres, thereby reducing migration and angiogenesis.[52] This suggests that combinatorial targeting of EMT-TFs could enhance therapeutic efficacy. TNF-α also orchestrates a pro-metastatic niche by stabilizing SNAI1 and activating stromal fibroblasts expressing SNAI1, which, in turn, enhances cancer cell proliferation and invasion.[21,53] This stromal-epithelial crosstalk underscores the importance of microenvironmental inflammation in colon cancer progression. Therefore, TNF-α has a dual role in both inflammation and EMT, with both showing a similar expression pattern in the liver metastasis group.

In addition, the observed downregulation of MTA3 in metastatic cases suggests a tumor-suppressive function. Restoring MTA3 activity could thus represent another avenue for therapeutic intervention. Epigenetic drugs that modulate MTA3 expression or activity warrant further investigation in preclinical CRC models. While MTA3 has not been directly addressed in the recent studies, its known role in repressing SNAI1 in breast cancer suggests a potential cross-talk in colon cancer.[54] Future studies should explore whether MTA3 loss contributes to SNAI1 activation in colorectal tumors. In this study, MTA3 expression was found to be decreased in the liver metastasis group and elevated in the non-metastasis group (Table 1), supporting its proposed role as a metastasis suppressor.

The correlation of SNAI1, ZEB1, Slug, Twist, and TNF-α with BMI and AST in cancer involves their roles in EMT, cancer progression, and inflammation, though direct correlations with BMI and AST specifically are not extensively detailed in the provided sources. However, based on the results of this study, these markers were found to correlate with BMI and AST (Table 3). The decrease in BMI, as shown in Table 2, indicates the occurrence of cachexia in the subjects studied. Cachexia in colon cancer involves complex interactions between transcription factors and inflammatory mediators, with SNAI1, ZEB1, Twist1, and TNF-α playing significant roles.[55-57] Cachexia progression in colon cancer involves energy metabolism disruption (e.g., impaired amino acid metabolism) and elevated serum lysine/acetate, which may serve as diagnostic biomarkers.[58] EMT-related transcription factors (ZEB1, Twist1, and SNAI1) contribute to tumor resilience and metastasis, indirectly perpetuating cachexia by sustaining tumor-derived catabolic signals (e.g., activin A).[55-57] ZEB1, Twist1, and SNAI1 drive tumor progression and cachexia in colon cancer through EMT, anti-apoptotic signaling, and muscle degradation pathways, while TNF-α and systemic inflammation amplify these effects.

According to another study, TNF-α also showed a direct, concentration-dependent correlation with AST.[59] The relationship of AST was not directly correlated with SNAI1, ZEB1, Slug, and Twist, but these markers contribute to the development of cancer and liver-related pathologies, potentially increasing AST through metastasis, immune infiltration, or hepatocyte damage. The prognostic value of an increased AST ratio independently predicted the risk and mortality of cancer, which underlines the need to monitor liver function in cancer patients.[60] This is in line

with the results obtained in this study, where AST levels were higher in the metastasis group than in the non-metastasis group (Table 2).

Therapeutic implications for targeting SNAI1, ZEB1, Slug, Twist, MTA3, and TNF-α or their regulators show promise. Natural compounds sensitize cells to chemotherapy by disrupting EMT and inhibiting cancer development, while stromal inhibition of SNAI1, ZEB1, Slug, Twist, MTA3, and TNF-α could mitigate pro-tumorigenic signaling. However, partial EMT states may persist as reservoirs for recurrence, necessitating combination therapies. As shown in Table 4, the markers used in this study affect cancer development, as expressed in the T, N, and M (stage) grading scores.

In conclusion, SNAI1 emerges as the dominant EMT-TF in colon cancer, modulated by TNF-α and stromal interactions. ZEB1, Slug, and Twist play ancillary roles, while MTA3's involvement remains speculative. Therapeutic strategies must account for SNAI1's multifaceted regulation and the complexity of the tumor microenvironment. However, we recognize several limitations in this study, including: (a) the lack of a comprehensive database for patient baseline data, and (b) the focus on profiling the expression of several markers in CRC patient tumors without supporting or validating data from additional tests, such as somatic mutation analysis. Future studies should involve larger sample sizes, comparisons across racial groups of clinical patients, and further laboratory analysis. A comprehensive correlational approach that includes microscopic, serological, and bioinformatics analysis will be essential to support these initial findings.

5. Conclusion

Through this study, it was found that SNAI1, ZEB1, Slug, Twist, and TNF-α were associated with cancer progression toward metastasis, as their expression levels increased accordingly. In contrast, MTA3, a known metastasis suppressor, showed relatively lower expressing during metastasis progression. In addition, SNAI1, ZEB1, Slug, Twist, and TNF-α were also correlated with BMI and AST levels

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Conflict of interest

The authors declared that there were no conflict of interest in this work.

Author contributions

Conceptualization: Adeodatus Yuda Handaya, Hendra Susanto
Formal analysis: All authors
Investigation: All authors
Methodology: Hendra Susanto, Adeodatus Yuda Handaya
Writing–original draft: Adeodatus Yuda Handaya, Moch Sholeh
Writing–review & editing: Adeodatus Yuda Handaya, Moch Sholeh

Ethics approval and consent to participate

The participants consented to complete the informed consent form for the clinical observational study. With the certificate of ethics number: KE/FK/0938/EC/2021, this clinical study was authorized by the IRB, Faculty of Medicine, Universitas Gadjah Mada, Indonesia, in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable, because the respondents' identities have been completely masked, and they have also agreed in the "written consent to participate" section that the samples will be used for further research purposes.

Availability of data

All the datasets generated in the current study are available from the corresponding author based on reasonable request.

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