RESEARCH ARTICLE

Snail Expression is Positively Correlated with Depth of Invasion in Colorectal Carcinoma

Upik Anderiani Miskad[1,2,*], Fardah Akil[3], Muhammad Husni Cangara[1,2], Haslindah Dahlan[1,2], Gina Andyka Hutasoit[1,4], Muhammad Riza Ilyasa[5]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Hasanuddin, Jl. Perintis Kemerdekaan KM. 10, Makassar 90245, Indonesia
[2]Anatomical Pathology Laboratory, Universitas Hasanuddin Hospital, Jl. Perintis Kemerdekaan KM. 10, Makassar 90245, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Hasanuddin, Jl. Perintis Kemerdekaan KM. 10, Makassar 90245, Indonesia
[4]Department of Anatomical Pathology, Faculty of Medicine, Universitas Tadulako, Jl. Soekarno Hatta KM. 9, Palu 94118, Indonesia
[5]Undergraduate Program of Medical Study, Faculty of Medicine, Universitas Hasanuddin, Jl. Perintis Kemerdekaan KM. 10, Makassar 90245, Indonesia

*Corresponding author. Email: upik.miskad@med.unhas.ac.id

Received date: Oct 24, 2024; Revised date: Dec 2, 2024; Accepted date: Dec 3, 2024

Abstract

BACKGROUND: Colorectal carcinoma ranks as the second deadliest and third most prevalent cancer globally. The depth of tumor invasion and the presence of tumor-infiltrating lymphocytes (TILs) are linked to survival rates in this disease. Meanwhile, Snail expression is positively correlated with tumor grade, recurrence, metastasis and poor prognosis in various tumors. However, not many studies discuss the correlation of Snail expression with invasion depth and TILs in Indonesia. Therefore, this study was conducted to investigate the correlation between Snail expression and both the depth of invasion and TIL scoring in colorectal carcinoma.

METHODS: A cross-sectional study was conducted to evaluate 70 paraffin-embedded blocks of colorectal carcinoma patients. Snail expression was measured with immunohistochemistry using Snail rabbit polyclonal antibody. Stromal TILs were assessed on a single full-face hematoxylin and eosin (H&E) slide, and classified into high, intermediate, and low TILs.

RESULTS: The results showed that the most invasion was to the muscularis propria (42.9%) and the least invasion was to the submucosa (4.3%). In scoring TILs, the most samples with intermediate TILs (58.6%) and the least samples with low TILs (4.3%). The analysis employing a Spearman Rank coefficient shows significant positive correlation between the expression of Snail with depth of invasion (r=0.273; p=0.022) but there was no significant correlation with TILs scoring (p=0.892).

CONCLUSION: Even though, there is no significant correlation between Snail expression with TILs, there is, however, a significant positive correlation between Snail expression with depth of invasion in colorectal carcinoma. Therefore, Snail expression might be potentially used as a prognostic factor in colorectal carcinoma.

KEYWORDS: Colorectal, carcinoma, Snail, depth of invasion and TILs

Indones Biomed J. 2024; 16(6): 553-9

Introduction

Colorectal carcinoma is a malignant epithelial tumor originating in the large intestine. The most common colorectal carcinoma is adenocarcinoma which shows glandular or mucinous differentiation.(1) Colorectal carcinoma can occur in young adults and teenagers, but most cases occurs in people over 50 years old. For colorectal carcinoma, the average age at diagnosis for men is 66 years and for women is 69 years.(2,3) According to GLOBOCAN 2020, colorectal cancer is the second most deadly and the third most prevalent cancer globally, with nearly 2 million new cases and around 1 million deaths expected that year. (4) Factors contributing to the development of colorectal cancer include obesity, unhealthy lifestyles, consumption of

red meat, and the use of alcohol and tobacco.(5) The rising incidence of colorectal carcinoma in Indonesia is a growing concern, with both new cases and death rates increasing annually. GLOBOCAN data from 2020 revealed that there were 33,427 new colorectal cancer cases in Indonesia, representing approximately 8.4% of the total 396,914 cancer cases.(4) This cancer is the third most common in the country, driven by dietary changes among Indonesians due to rising prosperity and a shift toward a Western diet that is high in fat and low in fiber.(6)

Clinical and pathological staging is very important in determining the local and remote extent of the disease, which in turn provides a framework for determining prognosis and therapy in colorectal carcinoma.(7) The depth of tumor invasion is one of the histopathological findings that currently must be reported. Depth of invasion is associated with an increased incidence of nodal and distant metastases, as well as poorer 5-year survival.(8) Tumor differentiation is related to estrogen receptor beta (ERβ) expression. Tumor invasion is associated with the detachment of tumor cells from their host cells to move elsewhere. This is caused by the loss of E-cadherin as an intercellular adhesion molecule so that the bonds between cells disappear and the cell is separated from its parent cell.(9) The transcription factor is a repressor of E-cadherin which functions as a cell adhesion molecule and plays an important role in the formation and maintenance of integrity complex tissue is Snail.(10) Snail expression is positively correlated with tumor grade, recurrence, metastasis and poor prognosis in various tumors.(11)

Another crucial factor in tumor development is immune infiltration, which includes various lymphocytes (T cells, B cells, and natural killer cells), neutrophils, dendritic cells and macrophages, highlighting significant variability among patients. The presence of tumor-infiltrating lymphocytes (TILs) is recognized as a positive prognostic indicator in several malignancies, including colorectal carcinoma.(12) The immune response in colorectal cancer, particularly involving effector memory T cells, correlates with the cancer's histopathology and carries important prognostic implications.(13) Patients with TIL levels exceeding 5% tend to have significantly longer survival rates compared to those with lower TIL counts.(14)

We previously studied the correlation of Snail expression with colorectal adenocarcinoma, focusing on the histopathological grading, tumor budding grading, lymphovascular invasion and metastases ability of colorectal carcinoma.(15) However, not many studies discuss the correlation of Snail expression with invasion depth and TILs, especially in Indonesia. Based on the description above, this study was conducted to analyze the correlation of Snail expression with the depth of invasion and TILs in colorectal carcinomas.

Methods

Study Design and Data Collection

This research was an analytical observational study using a cross-sectional design conducted at the Anatomic Pathology Laboratory of the Faculty of Medicine, Universitas Hasanuddin, Makassar. Between January 2020 and June 2023, 70 paraffin block samples from patients diagnosed with colorectal adenocarcinoma at the Anatomical Pathology Laboratory of Dr. Wahidin Sudirohusodo Hospital and Makassar Pathology Diagnostic Center were collected. The Faculty of Medicine's Ethics Committee waived informed consent for this study (Protocol #UH23070526 – Registry No. 621/UN4.6.4.5.31/PP36/2023).

Evaluation of TILs (TILs Scoring)

Stromal TILs were assessed on a single full-face hematoxylin and eosin (H&E) slide according to the International TILs Working Group 2014 guidelines.(16) TILs were analyzed as a continuous variable and classified into three categories: high TILs (>55%), intermediate TILs (15-50%), and low TILs (≤10%).

Snail Immunohistochemistry (IHC)

IHC staining with a Snail rabbit polyclonal antibody (Cat. No. AF6032; Affinity Biosciences, Cincinnati, OH, USA) was conducted on unstained slides. The sample was then blocked and incubated with the primary antibody at 4°C overnight. An HRP conjugated anti-rabbit antibody was used as the secondary Ab. The paraffin blocks were sectioned to create slides, which were then cut to a thickness of 3 µm using a microtome. The sections were taken from a water bath onto poly-L-lysine-coated slides and subsequently deparaffinized with the Snail rabbit polyclonal antibody for staining. The expression of Snail in the nuclei or cytoplasm of tumor cells was analyzed using a 400x light microscope. Two pathologists, who were unaware of the clinical data and outcomes, performed the assessment.

Snail expression was scored in a semiquantitative manner based on the intensity and percentage of stained tumor cells, resulting in a total immunostaining score (TIS). This score ranges from 0 to 9 and is calculated by multiplying the proportion score of the positively stained

tumor area (0-3) with the intensity score of Snail staining (0-3). The proportion scores are defined as follows: 0 for none, 1 for <10% stained, 2 for 10-50% stained and 3 for >50% stained. Snail intensity is categorized as uncolored (0/negative), weak (+1), moderate (+2), or strong (+3). A TIS of 6 or higher indicates strong Snail expression, while a score below 6 indicates weak expression.

Results

Characteristics of the Sample

A total of 70 block samples were used in this study. Most samples were found in the age category <55 years (52.9%) and most samples were male (51.4%). Based on location, the most tumors were found in the proximal part (44.3%), followed by the distal part (40.0%). Based on the invasion depth of the tumor, most samples were classified into tumor invades muscularis propria (42.9%) followed by tumor invades to lymph node (32.9%). Based on histopathological grade, most samples were low-grade colorectal adenocarcinoma (87.1%). Based on TILs scoring, there were samples that mostly had intermediate TILs scores (58.6%), followed by weak TILs scores (37.1%). Based on Snail expression, most samples had weak Snail expression (57,1%) (Table 1).

Snail Expression Intensity and TILs Scoring

Snail intensity could be seen in colorectal carcinoma samples (Figure 1). Snail expression was seen in the nucleus or cytoplasm of tumor cells. There were 17 samples with strong Snail intensity, 37 samples with moderate Snail intensity, 15 samples with weak Snail intensity and 1 sample was colorless (negative).

The stromal area of TILs was assessed in the peritumoral section of colorectal carcinoma subjects (Figure 2). There were 26 samples with high TILs, 41 samples with moderate TILs and 3 samples with low TILs.

Correlation of Snail Expression with Depth of Invasion and Scoring TILs

Results analysis of Spearman Rank coefficient test showed a significant correlation between Snail expression with depth of invasion (p=0.022). There was a positive correlation between Snail expression with depth of invasion (r=0.273) (Table 2). The deeper the invasion, the higher the Snail expression. Meanwhile, the results analysis showed that there was no significant correlation between Snail expression with scoring TILs (p=0.892), but there was a negative correlation between Snail expression with scoring TILs (r=-0.017) (Table 3). The higher the TILs score, the lower the Snail expression. Spearman Rank coefficient test analysis also showed that there was no significant correlation between depth of invasion with scoring TILs (p=0.075), but there was a negative correlation between depth of invasion with scoring TILs (r=-0.214) (Table 4). The deeper the invasion, the lower the TILs score.

Discussion

Classification based on tumor, node, and metastasis (TNM) is being used as a system for classifying malignancy. This classification is mainly used in solid tumors and can be used to assist in determining or predicting the prognosis of patients with colorectal cancer.(16) In addition, this system is also used to guide additional therapy after potentially curative surgery and to classify patients who will participate in clinical trials. This system has the basis for assessing tumors, regional lymph nodes, and distant metastases including colorectal carcinoma.(17)

Table 1. Characteristics of the sample.

Characteristics	n (%)
Age (years old)	
<55	37 (52.9)
≥55	33 (47.1)
Gender	
Male	36 (51.4)
Female	34 (48.6)
Tumor location	
Right	31 (44.3)
Left	28 (40.0)
Rectum	11 (15.7)
Depth of invasion	
pTis	5 (7.1)
pT1	3 (4.3)
pT2	30 (42.9)
pT3	9 (12.9)
pT4	23 (32.9)
Histopathological grade	
Low grade	61 (87.1)
High grade	9 (12.9)
Scoring TILs	
Low	3 (4.3)
Intermediate	41 (58.6)
High	26 (37.1)
Snail expression	
Weak	40 (57.1)
Strong	30 (42.9)

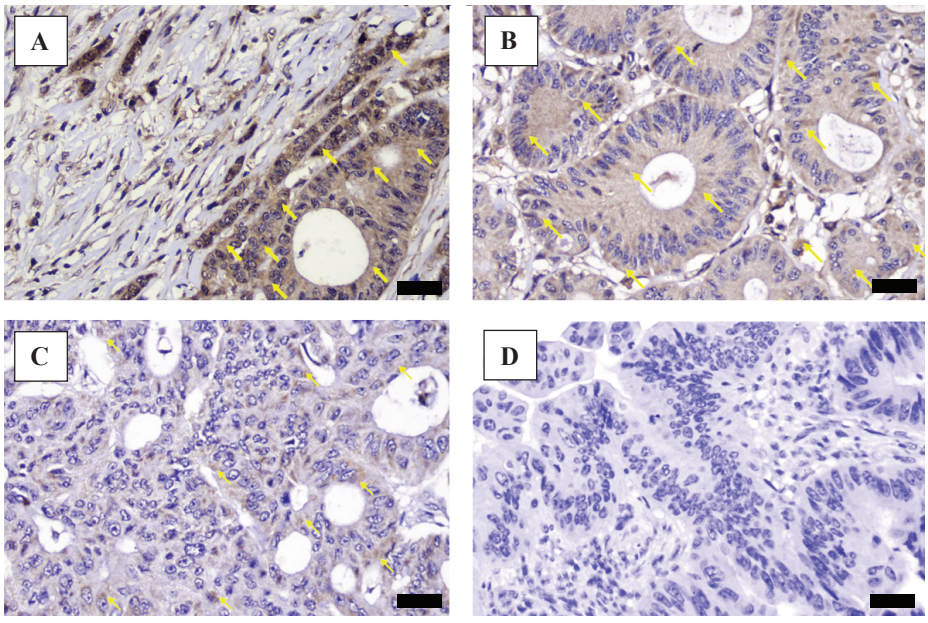

Figure 1. Snail intensity in colorectal carcinoma. A: Strong (score 3); B: Moderate (score 2); C: Weak (score 1); D: Negative (score 0). Yellow arrows: Snail expression in the cytoplasm. Black bar: 100 µm.

Tumor grading is a system used to describe the size of a primary tumor and its spread to adjacent tissues. T0 indicates no tumor, while T1-T4 categorize tumors based on their size and extent of invasion.(18) The grading varies depending on the anatomic structure affected. In colorectal cancer, for example, T1 indicates invasion into the submucosa, while T4 indicates that the tumor has extended through the entire colon and invaded the visceral peritoneum or adjacent tissues.(19)

In this study, we assessed the correlation between Snail expression and depth of invasion in colorectal carcinoma. Research data shows that there is a positive correlation coefficient between Snail expression and depth of invasion, where when snail expression is high, the depth of invasion is also high. Statistically, it shows that there is a significant correlation between Snail expression and depth of invasion. Snail is an important protein molecule in the epithelial mesenchymal transition (EMT) process and plays an important role in tumor invasion and metastasis in many types of cancer.(20)

Tumor differentiation in early-stage colorectal carcinoma is linked to the expression of ERβ.(21) The lack of reliable biomarkers for early detection of the metastatic process can hinder timely treatment for colorectal cancer. Some potential biomarkers that have been proposed include β-Catenin, E-Cadherin, and N-Cadherin.(21,22) E-cadherin is a crucial protein for cellular adhesion and plays a significant role in cell-to-cell contacts.(23) In malignant tumors undergoing EMT, E-cadherin expression is often downregulated or lost, leading to reduced adhesion among tumor cells, which enhances their invasiveness, infiltration, and metastatic potential. A lower level of E-cadherin expression is associated with a poorer prognosis for patients. Thus, the downregulation or loss of E-cadherin is

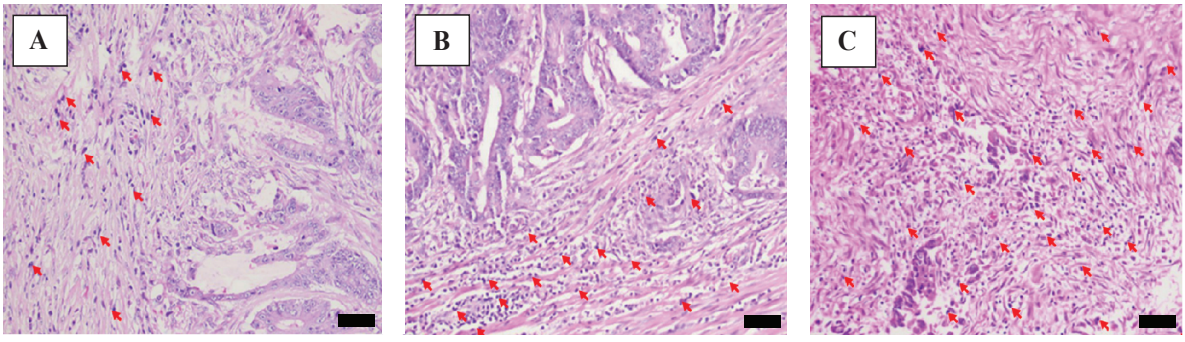

Figure 2. Scoring TILs in colorectal carcinoma. A: Low (score 1); B: Intermediate (score 2); C: High (score 3). Red arrows: Stromal area of TILs peritumoral section. Black bar: 100 µm.

Table 2. Correlation between Snail expression with depth of invasion.

	Mean±SD	Correlation Coefficient	*p*-value
Snail expression	4.87±3.106	0.273	0.022*
Depth of invasion	2.60±1.197		

Spearman correlation test, *$p<0.05$ indicates statistical significance.

Table 3. Correlation between Snail expression with scoring TILs.

	Mean±SD	Correlation Coefficient	*p*-value
Snail expression	4.87±3.106	-0.017	0.892
Scoring TILs	46.50±19.988		

Spearman correlation test, $p<0.05$ indicates statistical significance.

a characteristic feature of EMT.(24) The Snail transcription factor is instrumental in producing the Snail protein, which can inhibit E-cadherin transcription by binding to the E-box region near the E-cadherin promoter. Because the loss of E-cadherin is a hallmark of EMT, Snail is viewed as a critical regulator of this process.(25) In colorectal cancer, EMT is linked to invasive and metastatic behaviors.(26) Transforming growth factor (TGF)-β1-induced EMT facilitates invasion and metastasis by lowering E-cadherin levels and increasing Vimentin levels while activating the TGF-β1/Smads signaling pathway.(27) During EMT, epithelial cells undergo polarization and differentiation, acquiring a mesenchymal phenotype that enhances motility. Snail family proteins play a key role in regulating this EMT process. As a transcription factor, Snail governs the expression of genes that influence the EMT phenotype and contribute to neoplasm development.(10) Overexpression of Snail is associated with heightened migration, greater depth of invasion, and increased metastasis, often correlating with more advanced malignancies and poorer prognoses.(28)

Assessment of the depth of tumor invasion can help in establishing the diagnosis of colorectal lesions and inform the therapeutic strategies that can be used.(29) This research correlates with another study that states that Snail plays a key role in promoting invasion and metastasis in colorectal cancer by regulating its own expression and that of E-cadherin. High levels of Snail expression serve as a novel and effective prognostic marker for colorectal cancer. In a separate study, Western blot analysis revealed Snail expression in all layers of examined colorectal cancers, correlating with increased migratory and invasive capabilities of tumor cells. Furthermore, Snail expression was associated with a stem cell-like, spindle-shaped phenotype, typically seen with the loss of E-cadherin. As tumor stages progressed, Snail expression also increased, with a significantly higher rate of distant metastasis linked to its presence, indicating that Snail is a marker for elevated metastatic risk in colorectal carcinoma.(28) Other studies confirmed that Snail expression was markedly higher in patients with advanced tumor stages, as assessed by the depth of invasion. Additionally, Snail was identified as an independent prognostic factor for overall survival, along with lymph node metastases, which also emerged as an independent prognostic indicator for survival outcomes.(30)

Snail is a crucial transcription factor that drives EMT. Previous research in ovarian cancer using mouse models examined Snail's impact on tumor immunity.(31) Knockdown of Snail resulted in reduced tumor growth in immunocompetent mice, which correlated with an increase in tumor-infiltrating $CD8^+$ lymphocytes.(32) Additionally, tumor-infiltrating lymphocytes, particularly those expressing forkhead box P3 (FOXP3), were identified as significant contributors to tumor immunity.(33) In current study, there was no significant correlation between TILs and Snail expression or the depth of invasion. However, there was a negative correlation: higher TIL scores were associated with lower Snail expression and reduced invasion depth. Elevated levels of Twist, Slug, and Snail expression were linked to high TIL counts. Conversely, the absence of TILs or very high TIL levels correlated with shorter times to key endpoints compared to groups with intermediate TIL counts. Our findings suggest that TILs influence the prognosis of colorectal cancer and highlight the specific nature of different TIL subsets based on microsatellite instability (MSI) status. Moreover, the tumor microenvironment can create a hypoxic state, which has been shown to support tumor maintenance and function through pathways involving hypoxia-inducible factor 1-alpha (HIF-1α).(34)

Unfortunately, this study only uses one modality of protein detection through IHC. Other modalities might be needed to analyze the complexity of the relationship

Table 4. Correlation between depth of invasion and scoring TILs.

	Mean±SD	Correlation Coefficient	*p*-value
Depth of invasion	2.60±1.197	-0.214	0.075
Scoring TILs	46.50±19.988		

Spearman correlation test, $p<0.05$ indicates statistical significance.

between Snail and various other molecules involved in regulating its expression, as well as its relationship with the depth of invasion and TILs. Further cross-sectional studies with larger samples may be needed to understand how Snail or other transcription factors correlate with this mechanism.

Conclusion

Even though, there is no significant correlation between Snail expression with TILs, however, there is a significant positive correlation between Snail expression with depth of invasion in colorectal carcinoma. Therefore, Snail expression might be potentially used as a prognostic factor in colorectal carcinoma.

Acknowledgments

We thank the Anatomical Pathology Laboratory staff at Universitas Hasanuddin Hospital who have contributed to the feasibility of this study in part. This research was supported by Faculty of Medicine, Universitas Hasanuddin University.

Authors Contribution

All author was involved in the conceptualization, preparation of methodology, data curation, resources collection, original draft writing, as well as reviewing and editing the manuscript. All authors approved the final manuscript.

References

1. World Health Organization. WHO Classification of Tumours Digestive System Tumours. 5th ed. Geneva: WHO; 2019.
2. American Cancer Society [Internet]. About Colorectal Cancer; What Is Colorectal Cancer? [cited 2024 Feb 20]. Available from: https://www.cancer.org/cancer/colon-rectal-cancer/about/what-is-colorectal-cancer.html.
3. Nagtegaal I, Arends MJ, Odze R, Lam A. Tumours of the colon and rectum. In: WHO Classification of Tumours Digestive System Tumours. 5th ed. Geneva: WHO; 2019. p.157–88.
4. Global Cancer Observatory [Internet]. Cancer Incidents in Indonesia [cited 2024 Feb 20]. Available from: https://gco.iarc.who.int/media/globocan/factsheets/populations/360-indonesia-fact-sheet.pdf.
5. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019; 14(2): 89–103.
6. Menteri Kesehatan Republik Indonesia. Pedoman Nasional Pelayanan Kedokteran Tata Laksana Kanker Kolorektal. Jakarta: Kemetrian Kesehatan Republik Indonesia; 2018.
7. Chen K, Collins G, Wang H, Toh JWT. Pathological features and prognostication in colorectal cancer. Curr Oncol. 2021; 28(6): 5356–83.
8. Diaconescu M, Burada F, Mirea CS, Moraru E, Ciorbagiu MC, Obleaga C V, *et al.* T4 Colon cancer - Current management. Curr Heal Sci J. 2018; 44(1): 5–13.
9. Zhou Y, Xia L, Wang H, Oyang L, Su M, Liu Q, *et al.* Cancer stem cells in progression of colorectal cancer. Oncotarget. 2018; 9(70): 33403–15.
10. Yastrebova MA, Khamidullina AI, Tatarskiy VV, Scherbakov AM. Snail-family proteins: Role in carcinogenesis and prospects for antitumor therapy. Acta Naturae. 2021; 13(1): 76–90.
11. Ganesan R, Mallets E, Gomez-Cambronero J. The transcription factors Slug (SNAI2) and Snail (SNAI1) regulate phospholipase D (PLD) promoter in opposite ways towards cancer cell invasion. Mol Oncol. 2016; 10(5): 663–76.
12. Miskad UA, Hamzah N, Cangara MH, Nelwan BJ, Masadah R, Wahid S. Programmed death-ligand 1 expression and tumor-infiltrating lymphocytes in colorectal adenocarcinoma. Minerva Med. 2020; 111(4): 337–43.
13. Zadka Ł, Chabowski M, Grybowski D, Piotrowska A, Dzięgiel P. Interplay of stromal tumor-infiltrating lymphocytes, normal colonic mucosa, cancer-associated fibroblasts, clinicopathological data and the immunoregulatory molecules of patients diagnosed with colorectal cancer. Cancer Immunol Immunother. 2021; 70(9): 2681–700.
14. Brummel K, Eerkens AL, de Bruyn M, Nijman HW. Tumour-infiltrating lymphocytes: From prognosis to treatment selection. Br J Cancer. 2023; 128(3): 451–8.
15. Hutasoit GA, Miskad UA, Akil F, Cangara MH, Dahlan H, Yamin A, *et al.* Snail expression as a prognostic factor in colorectal adenocarcinoma. Asian Pac J Cancer Prev. 2024; 25(9): 3143–9.
16. Iseki Y, Shibutani M, Maeda K, Nagahara H, Fukuoka T, Matsutani S, *et al.* A new method for evaluating tumor-infiltrating lymphocytes (TILs) in colorectal cancer using hematoxylin and eosin (H-E)-stained tumor sections. PLoS One. 2018; 13(4): e0192744. doi: 10.1371/journal.pone.0192744.
17. Li J, Yi CH, Hu YT, Li JS, Yuan Y, Zhang SZ, *et al.* TNM staging of colorectal cancer should be reconsidered according to weighting of the T stage: Verification based on a 25-year follow-up. Medicine. 2016; 95(6): e2711. doi: 10.1097/MD.0000000000002711.
18. Wang S, Guan X, Ma M, Zhuang M, Ma T, Liu Z, *et al.* Reconsidering the prognostic significance of tumour deposit count in the TNM staging system for colorectal cancer. Sci Rep. 2020; 10(1): 89. doi: 10.1038/s41598-019-57041-2.
19. Rosen RD, Sapra A. TNM Classification. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.
20. Wang W, Jin J, Zhou Z, Wang Y, Min K, Zuo X, *et al.* Snail inhibits metastasis via regulation of E-cadherin and is associated with prognosis in colorectal cancer. Oncol Lett. 2023; 25(6): 271. doi: 10.3892/ol.2023.13857.
21. Theodora I, Sudiana IK, Budipramana VS, Erwin F, Dewi S, Novita BD. Tumor differentiation is correlated with estrogen receptor beta (ERβ) expression but not with interleukin-6 (IL-6) expression in colorectal carcinoma. Indones Biomed J. 2024; 16(5): 481–6.
22. Luminturahardjo W, Soeatmadji DW, Mintaroem K, Rahajoe P, Sandra F. N-cadherin as an important marker in colorectal cancer: An investigation of β-catenin and cadherin expressions of SW-480 and HCT-116 cell lines. Indones Biomed J. 2021; 13(3): 289–94.

23. Lu J, Kornmann M, Traub B. Role of epithelial to mesenchymal transition in colorectal cancer. Int J Mol Sci. 2023; 24(19): 14815. doi: 10.3390/ijms241914815.
24. Li W, Kang Y. Probing the fifty shades of EMT in metastasis. Trends Cancer. 2017; 2(2): 65–7.
25. Ni Q, Li M, Yu S. Research progress of epithelial-mesenchymal transition treatment and drug resistance in colorectal cancer. Technol Cancer Res Treat. 2022: 21: 15330338221081219.
26. Vu T, Datta PK. Regulation of EMT in colorectal cancer: A culprit in metastasis. Cancers. 2017; 9(12): 171. doi: 10.3390/cancers9120171.
27. Ji Q, Liu X, Han Z, Zhou L, Sui H, Yan L, *et al.* Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-β1/Smads signaling pathway mediated Snail/E-cadherin expression. BMC Cancer. 2015; 15(1): 97. doi: 10.1186/s12885-015-1119-y.
28. Kaufhold S, Bonavida B. Central role of Snail1 in the regulation of EMT and resistance in cancer: A target for therapeutic intervention. J Exp Clin Cancer Res. 2014; 33(1): 62. doi: 10.1186/s13046-014-0062-0.
29. Maeyama Y, Mitsuyama K, Noda T, Nagata S, Nagata T, Yoshioka S, *et al.* Prediction of colorectal tumor grade and invasion depth through narrow-band imaging scoring. World J Gastroenterol. 2018; 24(42): 4809–20.
30. Kwon CH, Park HJ, Choi JH, Lee JR, Kim HK, Jo HJ, *et al.* Snail and serpinA1 promote tumor progression and predict prognosis in colorectal cancer. Oncotarget. 2015; 6(24): 20312–26.
32. Taki M, Abiko K, Baba T, Hamanishi J, Yamaguchi K, Murakami R, *et al.* Snail promotes ovarian cancer progression by recruiting myeloid-derived suppressor cells via CXCR2 ligand upregulation. Nat Commun. 2018; 9(1): 1685. doi: 10.1038/s41467-018-03966-7.
33. Rustamadji P, Wiyarta E, Pramono M, Maulanisa SC. Differential effects of anthracycline-based neoadjuvant chemotherapy on stromal and intratumoral FOXP3+ tumor-infiltrating lymphocytes in invasive breast cancer of no special type. Indones Biomed J. 2024; 16(2): 135–43.
34. Børretzen A, Gravdal K, Haukaas SA, Mannelqvist M, Beisland C, Akslen LA, *et al.* The epithelial–mesenchymal transition regulators Twist, Slug, and Snail are associated with aggressive tumour features and poor outcome in prostate cancer patients. J Pathol Clin Res. 2021; 7(3): 253–70.