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RESEARCH ARTICLE

Platelets at the Crossroads of Cancer: Activating Epithelial-Mesenchymal Transition in Colorectal Carcinogenesis via Snail1

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Abstract

Introduction: Epithelial-mesenchymal transition (EMT) was an important process in colorectal cancer progression. Activated platelets and thrombocytosis had been associated with cancer progression, but the specific mechanism in triggering EMT through the transcription factor Snail1 was not fully understood. **Methods:** This study used an observational analytical design with a cross-sectional approach. The subjects were colorectal cancer patients who underwent blood tests to determine platelet and activated platelet levels (P-selectin) and tissue to determine Snail1 and EMT transcription factors (E-cadherin and vimentin). Statistical analysis was performed using SPSS, Python, and Google Colab. **Results:** This study showed a significant role of activated platelets in triggering EMT ($p = 0.005$), activated platelets in triggering Snail1 ($p = 0.042$), and Snail1 in triggering EMT ($p = 0.002$). Causality assessment by artificial intelligence analysis of direct acyclic graphs and Granger causality tests showed that changes in platelet activation levels significantly preceded increased Snail1 expression, which in turn was followed by increased EMT markers. In addition, a decision tree was built to predict EMT from P-selectin and Snail1 levels with an accuracy of 62%. **Conclusion:** There was no significant relationship between thrombocytosis and activated platelets, and no significant role of thrombocytosis in EMT was found. Thus, the results of this study indicated a significant role of activated platelets in triggering EMT through the transcription factor Snail1 in colorectal cancer.

Keywords: Thrombocytosis- activated platelets- epithelial-mesenchymal transition- Snail1- colorectal cancer

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Introduction

Colorectal cancer still had high morbidity and mortality in worldwide [1]. Significant progress in medicine had been achieved in the last few decades in understanding the progression of colorectal cancer carcinogenesis. Among all the mechanisms of carcinogenesis, the epithelial-mesenchymal transition (EMT) process had received much attention [2].

Platelets could trigger carcinogenesis after being activated, known as activated platelets [3]. Most current studies only assessed the relationship between thrombocytosis and the EMT process and did not examine activated platelets directly. In fact, thrombocytosis could not trigger EMT because it had to be activated first [4]. Furthermore, the molecular pathway of how activated platelets could trigger the EMT process in colorectal cancer was still unclear. The role of activated platelets in triggering EMT through Snail1 and its effect on E-cadherin and vimentin expression was a topic that had not been widely explored in colorectal cancer research.

In this study, researchers used blood and tissue samples

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from colorectal cancer patients to evaluate the role of activated platelets in triggering EMT. P-selectin, expressed by activated platelets, was used as an indicator to measure the level of platelet activation in patient blood samples [5]. In addition, the expression of transcription factors Snail1, E-cadherin, and vimentin in tumor tissues was analyzed to assess the relationship between activated platelets and the EMT process [6]. Analysis between variables was carried out using an artificial intelligence approach. In addition, this study also aimed to develop a decision tree-based prediction model that could predict the probability of EMT in colorectal cancer patients based on existing data.

Materials and Methods

Study design

This study was an analytical observational study with a cross-sectional design on at least 26 patients who visited the digestive surgery polyclinic of Dr. Wahidin Sudirohusodo Makassar General Hospital with histopathology results supporting colorectal cancer. The sample size of 26 was calculated based on existing literature and prior studies, ensuring it was sufficient to detect statistically significant relationships while maintaining ethical considerations and practical constraints.

Study variables

Thrombocytosis was defined as a platelet count in the blood of more than 400,000/µL. Activated platelets were measured by ELISA (MyBiosource ©, USA). P-selectin levels in serum had a cut-off point of 34 ng/mL [5]. Snail1 was measured by examining Snail1 expression with immunohistochemistry (Affinity Bioscience©, USA) in colorectal cancer tissue with a high (strong and moderate positive) or low (negative and weak positive) classification. Epithelial-mesenchymal transition was measured by ELISA examination (MyBiosource©, USA) of E-cadherin and vimentin in colorectal cancer tissue. The cut-off point for E-cadherin was 51 ng/mL [7] and the cut off point for vimentin is 3 ng/mL [8]. When the E-cadherin value was low and vimentin was high, EMT was classified as positive. If it did not meet these conditions, EMT was classified as negative.

Statistical analysis

Data analysis was done in SPSS, Python, and Google Colab. To analyze the relationship between two variables, chi-square, Fisher's exact test, and likelihood ratio were used. Furthermore, the author conducted propensity score matching (PSM) to control bias in this study. Furthermore, a multivariate test with logistic regression was carried out to determine the most influential variables in triggering EMT. Causality analysis between variables was carried out using artificial intelligence with direct acyclic graphs (DAG) and Granger causality tests. The correlation values between variables were presented in the form of a heatmap. A decision tree was then developed to assess the percentage probability of predicting EMT from P-selectin and Snail1 values.

Ethical Approval and Consent

This study was conducted in accordance with the ethical guidelines set forth by the Institutional Review Board (IRB) at Hasanuddin University. The ethical approval number is UH24060459. Informed consent was obtained from all participants involved in the study, ensuring that their participation was voluntary and confidential. All patient data were anonymized to protect privacy and ensure compliance with ethical standards.

Results

Characterstics of the subjects

The characteristics of the study subjects were shown in Table 1. A total of 17 of 26 patients (65.4%) had a positive EMT status. Of all the characteristics of the study subjects, only the variables of lymph node status, metastasis status, and stage were significantly associated with EMT status ($p = 0.042$; $p = 0.030$; $p = 0.037$, respectively). There were no subjects with positive metastasis and stage 4 who had negative EMT status.

This study showed that there was no significant relationship between thrombocytosis and activated platelets. There was also no significant role of thrombocytosis in triggering EMT and Snail1. However, there wass a significant role of activated platelets in triggering both EMT (p=0005; PR 2.92; 95% 1.11-7.65) and Snail1 expression (p=0.042; PR 2.03; 95%CI 1.02-4.50). This study also showed that there is a significant role of Snail1 in triggering EMT (p=0.002; PR 3.97; 95%CI 1.16-13.65) (Table 2).

This study used a computational approach by utilizing artificial intelligence techniques to analyze the causal relationship between variables. The DAG graph visualized the potential causal pathway where P-selectin affected Snail1, leading to EMT, which ultimately affected lymph node involvement and metastasis (Figure 1). The results of the Granger causality test showed that changes in platelet activation significantly preceded changes in Snail1 expression, and changes in Snail1 expression preceded the occurrence of EMT. These findings also indicated that fluctuations in P-selectin, Snail1, and EMT ran in harmony (Figure 2). The heatmap showed the correlation

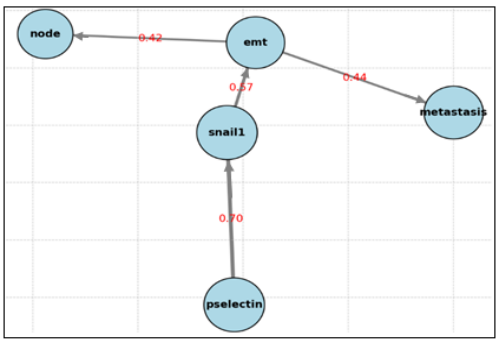

Figure 1. Direct Acyclic Graph (DAG) Illustrating the Causal Relationships between Platelet Activation (P-selectin), Snail1 expression, and epithelial-mesenchymal transition (EMT) in colorectal cancer. The graph shows how platelet activation influences Snail1 expression, which subsequently triggers EMT in cancer cells.

Table 1. Characteristics of the Subjects

Variables	EMT				
	Positive		Negative		p
	n	%	n	%	
Age					
>45 years old	14	53.9	7	26.9	0.58
≤45 years old	3	11.5	2	7.7	
Gender					
Male	6	23.1	5	19.2	0.281
Female	11	42.3	4	15.4	
Location					
Right colon	6	23.1	3	11.5	0.979
Left colon	6	23.1	3	11.5	
Rectum	5	19.3	3	11.5	
Tumor					
T2	1	3.9	1	3.9	0.521
T3	11	42.3	7	26.9	
T4	5	19.2	1	3.8	
Node					
Negative	4	15.4	6	23.1	0.042*
Positive	13	50	3	11.5	
Metastasis					
Negative	10	38.5	9	34.6	0.030*
Positive	7	26.9	0	0	
Stage					
2	4	15.4	6	23.1	0.037*
3	9	34.6	3	11.5	
4	4	15.4	0	0	

*Signifikan

values between P-selectin, Snail1, E-cadherin, Vimentin, EMT, lymph node status, and metastasis. P-selectin and Snail1 were strongly correlated with EMT, then lymph node status and metastasis, indicating that both might have been important predictors for modeling (Figure 3).

This decision tree model had an accuracy of 62%. This model could predict positive EMT with 67% precision, 80% recall, and 73% F1 score. On the other hand, this model could predict negative EMT with 50% precision, 33% recall, and 40% F1 score. The parameter at the top of the algorithm was Snail1 as the most influential independent variable in predicting EMT. When Snail1 level was high, the probability of positive EMT was 92.3%. When Snail1 level was low, the check was done on P-selectin. When Snail1 level was low and P-selectin level was low, the probability of negative EMT was 63.5%. When Snail1 level was low but P-selectin level was high, the probability of negative EMT dropped to 50% (Figure 4).

Discussion

Epithelial-mesenchymal transition (EMT) was a biological process that allowed epithelial cells to assume a mesenchymal cell phenotype, which included increased migratory capacity, invasive properties, increased resistance to apoptosis, and significantly increased production of extracellular matrix components [9]. In this study, it was shown that EMT had a significant relationship with lymph node status and metastasis in colorectal cancer, but had no significant relationship with tumor size. One reason why there was no significant effect between tumor size and EMT lay in the complexity of the mechanisms that regulate the metastasis process. Tumor size did reflect the existing tumor burden, but other factors such as the invasiveness of cancer cells, the ability to migrate, and the interaction of tumor cells with the microenvironment (including stromal and immune factors) played a much greater role in the process of cancer spread [10]. EMT, as a process that allowed cancer cells to transform into a more invasive and mobile form, had a key role in metastasis even though the tumor size was not too large [11].

In this study, no significant relationship was found between thrombocytosis and activated platelets. Blood sampling by Steller et al. (2013) showed that only 5-10% of tumor cells were positive for P-selectin, which is a marker of activated platelets [12]. So, while thrombocytosis was characterized by an increased number of platelets in the blood, this did not necessarily reflect how many platelets were activated. Platelet activation was the process by which platelets changed shape and function in response to vascular injury or certain stimuli, which did not always correlate directly with the number of circulating platelets [4]. In other words, someone with a high platelet count did not necessarily have a high level of platelet activation. Assessment of platelet function required specific tests that assessed platelet aggregation and activation capabilities, not just counting the number [9].

Research on the role of activated platelets in triggering EMT often showed that activated platelets were associated with a high probability of positive EMT. However, quantitatively, how many times the probability of activated platelets increasing EMT had never been studied. In the results of this study, it was shown that there was

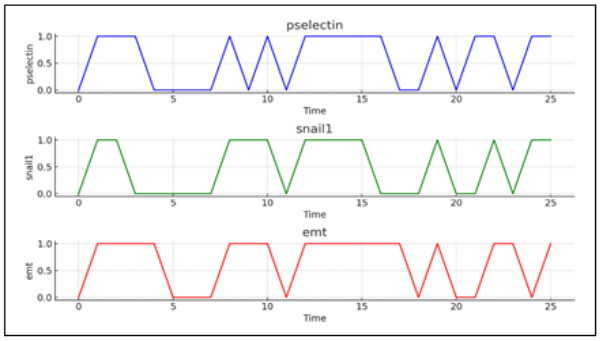

Figure 2. Granger Causality Test Results Showing the Temporal Relationships between Platelet Activation, Snail1 Expression, and the Occurrence of EMT. The graph indicates that changes in platelet activation significantly precede changes in Snail1 expression, which in turn is followed by increased EMT markers. The fluctuations in P-selectin, Snail1, and EMT markers run in harmony, suggesting a causal chain.

Table 2. The relationship of Thrombocytosis and Activated Platelet with EMT and Snail1

Variables	Results		Prevalence ratio	p
Thrombocytosis	P-selectin high	P-selectin low		
Yes	8	3	-	0.277
No	8	7		
Thrombocytosis	EMT positive	EMT negative		
Yes	9	2	-	0.138
No	8	7		
Thrombocytosis	Snail1 high	Snail1 low		
Yes	9	2	-	0.138
No	8	7		
P-selectin	EMT positive	EMT negative		
High	14	2	2.92	0.005*
Low	3	7	(95%CI 1.11-7.65)	
P-selectin	Snail1 high	Snail1 low		
High	13	3	2.03	0.042*
Low	4	6	(95%CI 1.02-4.50)	
Snail1	EMT positive	EMT negative		
High	15	2	3.97	0.002*
Low	2	7	(95%CI 1.16-13.65)	

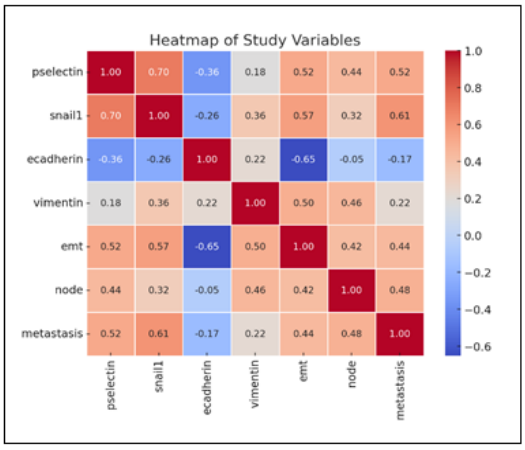

Figure 3. Heatmap Representing the Correlation Values between Platelet Activation (P-selectin), Snail1 Expression, EMT Markers (E-cadherin and vimentin), Lymph Node Status, and Metastasis. Strong correlations between P-selectin, Snail1, and EMT suggest that these variables may serve as important predictors for cancer progression.

a significant relationship between P-selectin levels and EMT status, where high P-selectin levels had a 2.9-fold risk of having a positive EMT status compared to subjects with low P-selectin levels. Contursi et al. (2023) also showed that platelet interactions with colorectal cancer cell lines increased EMT through Twist1 as indicated by decreased E-cadherin and increased Vimentin. This experiment was found to be consistent in the 4 cancer cell lines used [13].

Activated platelets activated Snail1 through the release of pro-inflammatory cytokines and growth factors, especially TGF-β. These factors bound to their receptors on epithelial cells, triggering intracellular signaling pathways such as the Smad pathway. This signaling cascade activated Snail1 transcription, which then repressed E-cadherin and promoted mesenchymal markers, which induced EMT. In addition, the interaction between P-selectin on activated platelets and PSGL-1 on epithelial cells further enhanced this process by activating pathways such as NF-κB, which also contributed to Snail1 activation [14].

This study showed that there was a significant relationship between P-selectin levels and Snail1 expression. Subjects with high P-selectin levels had 2 times more positive Snail1 expression than subjects with low P-selectin expression. This showed a very close relationship between activated platelets and Snail1, so it was an indirect hypothesis that activated platelets were likely to promote EMT through the transcription factor Snail1.

Snail1 bound to the E-box DNA sequence in the E-cadherin promoter and recruited histone deacetylases, Polycomb repressive complex 2, Lys-specific demethylase, G9a, and suppressor of variegation 3–9 homolog 1 [15]. Assembly of this complex caused various histone modifications, including methylation and acetylation of histone H3 Lys 4 (H3K4), H3K9, and H3K27, which ultimately inhibited E-cadherin promoter activity [16]. On the other hand, Snail1 increased the expression of genes involved in the mesenchymal phenotype, such as fibronectin, N-cadherin, and collagen, which supported EMT [17]. Snail1 also played a role in upregulating other EMT transcription factors, such as SLUG, TWIST, and ZEB1. In addition, Snail1 could interact with β-catenin, which then activated the WNT/β-catenin signaling pathway, an important mechanism in EMT [17]. Therefore, upregulation of Snail1 was considered a crucial step in the EMT process.

The use of artificial intelligence (AI) methods, such

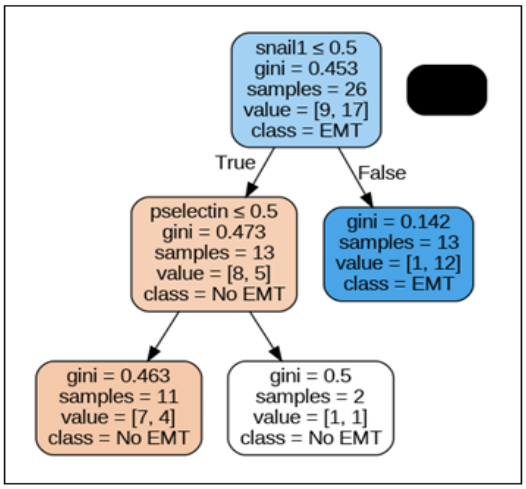

Figure 4. Decision Tree Model for Predicting EMT Status in Colorectal Cancer Patients based on P-selectin and Snail1 Levels. The model shows a prediction accuracy of 62% and provides insight into how P-selectin and Snail1 levels influence the likelihood of positive or negative EMT, with Snail1 being the most influential variable.

as Directed Acyclic Graphs (DAG) and Granger causality, offered a novel approach to understanding the causal relationships between activated platelets, Snail1, and EMT in colorectal cancer. These methods differed from more conventional statistical techniques, which typically rely on correlation and regression analysis to identify associations. DAGs allowed for a more sophisticated visualization of the causal pathways and interactions between variables, helping to infer the directionality and strength of these relationships. The Granger causality test, on the other hand, enabled the identification of temporal dependencies between platelet activation, Snail1 expression, and EMT, offering insights that are not always captured by traditional methods. These AI-based approaches provided a more dynamic and detailed understanding of the underlying mechanisms driving EMT, which could complement conventional approaches and improve the precision of predictive models in cancer research.

While this study provided important insights into the role of activated platelets in triggering EMT, there are several limitations to consider. One of the main limitations was the reliance on a single-center cohort, which might have introduced biases and limited the generalizability of the findings to other populations. The decision tree model, despite its utility, showed relatively low accuracy and had limitations in predicting negative EMT cases, which could reflect the need for further refinement of the model and inclusion of additional variables. This issue should be addressed as a limitation in the discussion. Factors such as the limited number of features, potential data imbalance, or the need for a more robust model could have contributed to the model's lower performance in predicting negative EMT cases. Further refinement of the decision tree model, along with the inclusion of additional relevant features or alternative machine learning models, may improve its predictive accuracy, particularly for EMT-negative cases.

The novelty of this study was the innovative approach in exploring the relationship between activated platelets, Snail1, and EMT in colorectal cancer, using the Directed Acyclic Graph (DAG) model and Granger Causality Test, also building the decision tree. On the other hand, most previous studies focused only on platelet counts in general without considering that platelets needed to be activated to trigger the EMT process. This suggested that this study not only filled the gap in existing knowledge but also provided a new perspective in understanding the biological mechanisms underlying colorectal cancer metastasis.

This study opened several avenues for future research. First, larger, multi-center studies with independent validation cohorts would have helped confirm the findings and improved the robustness of the results. Additionally, expanding the dataset to include a more diverse population could have provided insights into how activated platelets and Snail1 influence EMT across different demographics and cancer subtypes. The findings from this study also had the potential to drive new therapeutic interventions. Targeting platelet activation or the Snail1 signaling pathway could have offered novel strategies for preventing or treating metastatic colorectal cancer. Future studies could have explored the use of platelet inhibitors or Snail1 antagonists as part of a targeted therapy approach, potentially improving patient outcomes. Moreover, integrating AI models with more comprehensive datasets and clinical variables may have led to the development of more accurate predictive tools for personalized cancer treatment.

In conclusion, there was no significant relationship between thrombocytosis and activated platelets and no significant role of thrombocytosis in EMT was found. Thus, the results of this study indicated a significant role of activated platelets in triggering EMT through the transcription factor Snail1 in colorectal cancer.

Acknowledgments

Statement of Transparency and Principals

- Author declares no conflict of interest
- Study was approved by Research Ethic Committee of author affiliated Institute.
- Study's data is available upon a reasonable request.
- All authors have contributed to implementation of this research.

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