

# An Integrated Analysis of NF- $\kappa$ B, MMP-9 Expression, and Tumor-Infiltrating Lymphocytes Density in Correlation with TNM Stage of Colorectal Cancer

Heru Fajar Trianto<sup>1,2</sup>, Gondo Mastutik<sup>3\*</sup>, Desak Gede Agung Suprabawati<sup>4</sup>, Mitra Handini<sup>5</sup>, Mahyarudin Mahyarudin<sup>6</sup>

<sup>1</sup>Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

<sup>2</sup>Department of Anatomical Pathology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia

<sup>3</sup>Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

<sup>4</sup>Oncology Division, Surgery Department, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

<sup>5</sup>Department of Physiology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia

<sup>6</sup>Department of Microbiology and Immunology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia

## Abstract

**Background:** Colorectal cancer progression is influenced by interactions between tumor cells and the stromal area. Nuclear factor kappa B (NF- $\kappa$ B) and matrix metalloproteinase-9 (MMP-9) contribute to cancer expansion, while tumor-infiltrating lymphocyte (TIL) density influences the host immune response. However, their complexity remains unclear. Therefore, this study aimed to integrate an analysis of the correlation between NF- $\kappa$ B, MMP-9, and TIL density with TNM stage in colorectal cancer.

**Methods and Results:** Fifty colorectal cancer paraffin blocks were analyzed. NF- $\kappa$ B and MMP-9 expression were evaluated by immunohistochemistry, while TIL density was assessed using hematoxylin-eosin (HE) staining. Correlations with TNM stage and its components were analyzed using Spearman's correlation test. NF- $\kappa$ B expression correlated positively with N stage ( $p = 0.013$ ). MMP-9 expression had a positive correlation with T stage ( $p = 0.000$ ), M stage ( $p = 0.036$ ), and TNM stage ( $p = 0.001$ ). In contrast, TIL density showed an inverse correlation with T stage ( $p = 0.010$ ), N stage ( $p = 0.003$ ), and TNM stage ( $p = 0.001$ ). NF- $\kappa$ B expression correlated positively with MMP-9 ( $p = 0.021$ ), but not with TIL density.

**Conclusion:** NF- $\kappa$ B and MMP-9 expressions contribute to progression, while TIL density contributes to the antitumor response in colorectal cancer. An integrated analysis of the biomarkers showed a dynamic interaction between tumor molecular changes and the host immune response. (*International Journal of Biomedicine*. 2026;16(2):163-168.)

**Keywords:** transcription factor • matrix metalloproteinase • immune response • cancer

**For citation:** Trianto HF, Mastutik G, Suprabawati DGA, Handini M, Mahyarudin M. An Integrated Analysis of NF- $\kappa$ B, MMP-9 Expression, and Tumor-Infiltrating Lymphocytes Density in Correlation with TNM Stage of Colorectal Cancer. *International Journal of Biomedicine*. 2026;16(2):163-168. doi:10.21103/Article16(2)\_OA2

## Abbreviations

NF- $\kappa$ B, nuclear factor kappa B; MMP-9, matrix metalloproteinase-9; TILs, tumor-infiltrating lymphocytes.

## Introduction

Colorectal cancer is ranked second with the highest mortality rate worldwide.<sup>1</sup> Several examination variables can be used to predict outcomes and therapy options, including

the TNM (tumor, lymph nodes, metastasis) stage established by the American Joint Committee on Cancer (AJCC).<sup>2,3</sup> The TNM stage reflects the biological and molecular conditions of the tumor associated with the proliferation and progression of cancer cells.<sup>4</sup>

Nuclear factor kappa B (NF- $\kappa$ B) is a transcription factor associated with the immune response, apoptosis regulation, and progression of various cancers, including colorectal cancer.<sup>5,6</sup> Its activation can trigger various molecules that enhance the ability of cancer cells to migrate, invade, and metastasize.<sup>6,7</sup> Among the kinds of molecules is matrix metalloproteinase-9 (MMP-9), which has matrix degradation capabilities and facilitates tumor invasion.<sup>8</sup> Increased MMP-9 expression is associated with advanced stage and high-grade tumors.<sup>9</sup>

In addition to influencing cancer progression, NF- $\kappa$ B plays a crucial role in inflammation and the immune response.<sup>7,10</sup> A component of the immune response in cancer is tumor-infiltrating lymphocytes (TIL), which are discovered in the tumor stroma.<sup>11</sup> Several studies have shown a relationship between TIL density and clinicopathological characteristics with survival rates in colorectal cancer patients.<sup>12,13</sup>

Although NF- $\kappa$ B activation, MMP-9 expression, and TIL density have been studied individually in colorectal cancer, an integrated analysis of their interrelationships and correlations with clinicopathological characteristics remains limited. The interaction between the inflammatory process and cancer progression remains poorly understood. Therefore, this study aims to evaluate the correlation between NF- $\kappa$ B, MMP-9 expression, and TIL density with TNM stage in colorectal cancer. Clarifying the associations may provide a more comprehensive understanding of the interplay between inflammation, invasion, and immune response in the progression.

## Materials and Methods

This observational analytical study used 50 colorectal cancer paraffin blocks from the Anatomical Pathology Laboratory of Dr. Soedarso General Hospital, Pontianak, West Kalimantan, Indonesia, between 2020 and 2023. Inclusion criteria included paraffin blocks from resection specimens, a diagnosis of adenocarcinoma, and data on depth of invasion (T stage), lymph node spread (N stage), distant metastasis (M stage), and TNM stage. Observations were conducted on hematoxylin-eosin (HE) slides and by immunohistochemistry (IHC) using two blinded pathologists. Ethical approval was obtained from the Health Research Ethics Committee of Dr. Soedarso General Hospital (No. 96/RSUD/KEPK/XI/2024).

### Immunohistochemistry (IHC)

Paraffin blocks were sectioned at 4  $\mu$ m, deparaffinized using xylene, and rehydrated with alcohol. Antigen retrieval was performed at 95°C in a decloaking chamber with pH 9 for 30 minutes. Slides were then incubated with NF- $\kappa$ B (Cat#BF0466, Affinity Biosciences, USA; dilution 1:200) and MMP-9 primary monoclonal antibodies (IHC109, GenomeMe, Canada; dilution 1:200) at room temperature for 60 minutes. Washing was conducted twice with phosphate-buffered saline, followed by polymer and 3,3'-diaminobenzidine for 5 minutes each. Counterstaining was performed with hematoxylin. This was followed by dehydration in graded alcohol concentrations and clearing with xylene.

### TIL Density Assessment

TIL density was assessed based on the percentage of mononuclear cells (lymphocytes and plasma cells) in the entire tumor stroma on HE slides. Observation criteria were based on a modification of the TIL working group method.<sup>14</sup> TIL was observed at 10x objective magnification and classified into three groups, namely low (0-10%), moderate (11-50%), and high (51-100%).

### NF- $\kappa$ B and MMP-9 Expression Evaluation

NF- $\kappa$ B and MMP-9 expression were examined using IHC staining. NF- $\kappa$ B expression was observed in the nucleus, while MMP-9 expression was observed in the cytoplasm of tumor cells. NF- $\kappa$ B and MMP-9 expressions were assessed using the semiquantitative H-Score method. This combined the intensity and number of stained tumor cells across the entire field of view. H-Score assessment results were categorized as negative (0-49), weak (50-99), moderate (100-199), and strong (200-300).<sup>15</sup>

### Statistical Analysis

Observation data were presented as frequencies and percentages. Furthermore, statistical analysis was performed using SPSS 25 software. The Spearman's correlation test was used to assess the strength and direction of a monotonic relationship between two variables. A *p*-value of < 0.05 was considered statistically significant.

## Results

The sample consisted of 50 colorectal cancer patients, comprising 29 men and 21 women, with the majority aged 51-60 (36%). The histopathological types were Adenocarcinoma NOS (86%) and Mucinous Adenocarcinoma (14%), with the most common grade being well-differentiated (58%). In this study, the most common T Stage was T3 (64%), while the most common N and M Stages were N0 (54%) and M0 (76%). The most common TNM stage was Stage II (26%) and III (26%), with sample characteristics presented in Table 1.

The entire sample showed NF- $\kappa$ B expression, as evidenced by nuclear staining in tumor cells (Figure 1). Most of the expressions were moderate (56%) as detailed in Table 1. There was a significant relationship between NF- $\kappa$ B expression and N stage (*p* = 0.013) with a correlation coefficient (*r*) of 0.350. No significant relationship was observed between NF- $\kappa$ B expression and T stage (*p* = 0.160), M stage (*p* = 0.468), and TNM stage (*p* = 0.086) (Table 2).

This study showed that MMP-9 expression was predominantly moderate (56%) (Table 1) and was observed in the cytoplasm of tumor cells. Spearman's correlation test results showed a significant, positive correlation between MMP-9 expression and T stage (*r* = 0.580, *p* = 0.000), M stage (*r* = 0.298, *p* = 0.036), and TNM stage (*r* = 0.446, *p* = 0.001). MMP-9 was not significantly correlated with N stage (*p* = 0.050) (Table 2).

Most TIL density in this study was in the moderate category (76%) as detailed in Table 1. TIL was assessed in the stromal area surrounding the tumor, as presented in

Figure 1. TIL showed significant negative correlations with T ( $r = -0.361, p = 0.010$ ), N ( $r = -0.418, p = 0.003$ ), and TNM stage ( $r = -0.454, p = 0.001$ ). TIL was not significantly correlated with M stage ( $p = 0.056$ ) (Table 2).

Spearman's test showed a positive correlation between NF- $\kappa$ B and MMP-9 ( $r = 0.325, p = 0.021$ ). These results suggest that increasing NF- $\kappa$ B expression is associated with elevating MMP-9 expression. NF- $\kappa$ B was not significantly correlated with TIL ( $r = -0.117, p = 0.418$ ) (Table 3).

Table 1.

Sample Characteristics

Parameters	n (%)
Age (Years)	
21-30	2 (4)
31-40	2 (4)
41-50	5 (10)
51-60	18 (36)
61-70	14 (28)
>70	9 (18)
Sex	
Men	29 (58)
Women	21 (42)
Histopathological type	
Adenocarcinoma, NOS	43 (86)
Mucinous adenocarcinoma	7 (14)
T Stage	
T1	3 (6)
T2	10 (20)
T3	32 (64)
T4	5 (10)
N Stage	
N0	27 (54)
N1	12 (24)
N2	11 (22)
M Stage	
M0	38 (76)
M1	12 (24)
TNM Stage	
I	12 (24)
II	13 (26)
III	13 (26)
IV	12 (24)
NF- $\kappa$ B Expression	
Negative	0 (0)
Weak	12 (24)
Moderate	28 (56)
Strong	10 (20)
MMP-9 Expression	
Negative	0 (0)
Weak	19 (38)
Moderate	28 (56)
Strong	3 (6)
TIL Density	
Low	2 (4)
Moderate	38 (76)
High	10 (20)

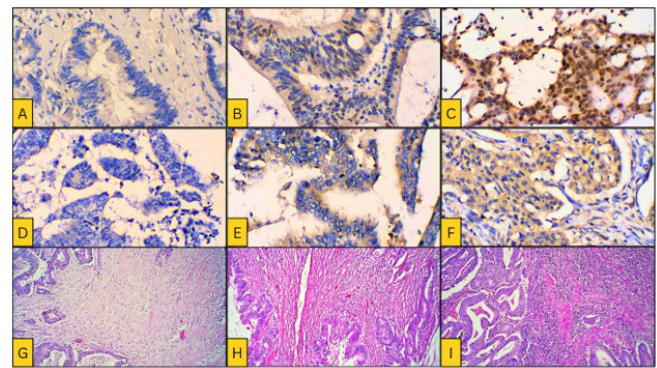


Fig 1. Immunohistochemical staining of NF- $\kappa$ B and MMP-9 (Objective, 40x) and hematoxylin-eosin staining of TILs (Objective, 10x). (A) Weak expression of NF- $\kappa$ B; (B) Moderate expression of NF- $\kappa$ B; (C) Strong expression of NF- $\kappa$ B; (D) Weak expression of MMP-9; (E) Moderate expression of MMP-9; (F) Strong expression of MMP-9; (G) Low TILs density; (H) Moderate TILs density; (I) High TILs density.

Table 2.

Correlation between NF- $\kappa$ B, MMP-9, and TIL density with clinicopathological parameters.

Parameters	NF- $\kappa$ B (n=50)		MMP-9 (n=50)		TILs (n=50)	
	p-value	r	p-value	r	p-value	r
T Stage	0.160	0.202	0.000	0.580	0.010	-0.361
N Stage	0.013	0.350	0.050	0.279	0.003	-0.418
M Stage	0.468	0.105	0.036	0.298	0.056	-0.272
TNM Stage	0.086	0.245	0.001	0.446	0.001	-0.454

Table 3.

Correlation between NF- $\kappa$ B with MMP-9 expression and TIL density.

Parameters	NF- $\kappa$ B			r	p-value
	Weak	Moderate	Strong		
MMP-9				0.325	0.021
Weak	6	7	1		
Moderate	3	18	9		
Strong	1	3	2		
TIL density				-0.117	-0.418
Low	0	3	2		
Moderate	8	19	8		
High	2	6	2		

Discussion

The majority of the sample were male (58%) and aged 51-60 years (36%). The change from precancerous lesions to colorectal cancer takes approximately 10-25 years, and most incidents occur in people over the age of 50 years.<sup>1,16,17</sup> Furthermore, men have a 20.4% higher risk of colorectal

cancer than women. This is related to various factors such as tobacco exposure, mutations of several tumor suppressor genes linked to sex chromosomes, and a lower immune response around the tumor.<sup>18</sup>

The TNM stage is a classification system that assesses tumor invasion, lymph node involvement, and distant metastasis related to prognosis and therapy options in colorectal cancer.<sup>3,19</sup> The five-year survival rate decreases with increasing stage, from 0.85 in Stage I to 0.30 in Stage IV.<sup>20</sup> Based on observation, most of the samples were T3 (64%), N0 (54%), and M0 (76%). Tsabit et al.<sup>21</sup> and Yao et al.<sup>22</sup> also showed that most tumors were T3. Patients with T3 tumors had a 1.29 times greater risk of death compared to T1.<sup>22</sup> Metastatic colorectal cancer is related to several gene mutations, such as KRAS, PIK3CA, BRAF, and ERBB2, which are associated with a poor prognosis.<sup>23</sup>

The expression of NF- $\kappa$ B in this study shows a positive correlation with N stage. This signified that NF- $\kappa$ B expression increases with increasing lymph node metastasis. The result is in line with studies conducted by Farhat et al.<sup>24</sup> in patients with nasopharyngeal carcinoma and Berkovich et al.<sup>25</sup> in colonic cancer. A relationship was observed between NF- $\kappa$ B overexpression and the occurrence of lymph node metastasis. NF- $\kappa$ B plays an important role in tumor cell invasion and migration by activating transcription factors in epithelial-mesenchymal transition (EMT), such as TWIST1 and SNAIL.<sup>7,26</sup> Furthermore, it can increase the degradation of the extracellular matrix by upregulating various types of degradation enzymes, including MMP-9.<sup>7,8,26</sup> The results of this study support the above theory by obtaining a positive correlation between NF- $\kappa$ B and MMP-9 expression, featuring a biological relationship and signaling pathway between the two molecules. The NF- $\kappa$ B and MMP-9 signaling pathways, which influence tumor progression and metastasis, are related to reactive oxygen species (ROS) as well as the PI3K and AKT pathways.<sup>8,26</sup>

Matrix metalloproteinase-9 expression in this study correlated with cancer progression. Its significant positive correlation with tumor depth, distant metastasis, and TNM stage emphasized the crucial role in both local invasion and metastatic spread. The results are consistent with previous studies showing a relationship between MMP-9 and various clinicopathological factors in colorectal cancer.<sup>2,27</sup> MMP-9 degrades gelatin and collagen, facilitating tumor cell passage through the basement membrane and extracellular matrix to other sites.<sup>27,28</sup> It can influence vascular endothelial growth factor (VEGF) in angiogenesis, thereby increasing the incidence of metastasis.<sup>7,28</sup> Tumor-infiltrating lymphocytes density showed significant inverse correlations with tumor depth, lymph node status, and TNM stage, suggesting a protective role for antitumor immune responses in colorectal cancer. The results are supported by several other studies that show significant relationships between TIL and depth of invasion, lymphovascular invasion, lymph node status, and grading.<sup>12,13</sup> The TIL components are mostly CD8+ T lymphocytes, which can destroy tumor cells.<sup>11,29</sup> This destruction is mediated by the secretion of perforin and granzyme and the production of various pro-inflammatory cytokines, such as tumor necrosis

factor  $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ).<sup>11</sup> Additionally, a subset of lymphocytes, namely CD4+ T lymphocytes, enhances the immune response by activating B lymphocytes, T lymphocytes, and macrophages.<sup>11,30</sup>

Although NF- $\kappa$ B plays a crucial role in the inflammatory process, this study discovered no significant association with TIL density. The NF- $\kappa$ B-mediated antitumor immune response in cancer is a complex process that involves various factors in the tumor microenvironment (TME), including TILs, dendritic cells (DCs), macrophages, natural killer (NK) cells, and cytokines such as interleukin-6 (IL-6) and TNF- $\alpha$ .<sup>7,26,31</sup> The nuclear factor can indirectly influence TIL by activating DCs, which act as antigen-presenting cells (APCs), presenting tumor antigens to CD8+ T lymphocytes.<sup>31</sup> Additionally, activation of B and T lymphocytes by NF- $\kappa$ B is influenced by activation of surface immune receptors such as the B-cell receptor (BCR) and T-cell receptor (TCR).<sup>7,31</sup>

**In conclusion**, this study showed that tumor-promoting signaling pathways were mediated by NF- $\kappa$ B and MMP-9, and protective immune responses were reflected in TIL density. The opposing correlations of MMP-9 expression and TIL density with TNM stage supported the concept that colorectal cancer progression is determined by the dynamic interaction between molecular drivers of invasion and the host immune response. This integrated analysis provided a more comprehensive understanding of tumor behavior than the assessment of individual biomarkers.

## Ethics Statement

All procedures of this study were approved by the Health Research Ethics Committee of Dr. Soedarso General Hospital (No. 96/RSUD/KEPK/XI/2024).

## Author Contributions

**Heru Fajar Trianto:** Investigation, Data curation, Formal analysis, Literature search, Writing – original draft.

**Gondo Mastutik:** Supervision, Methodology, Validation, Writing – review & editing.

**Desak Gede Agung Suprabawati:** Supervision, Methodology, Writing – review & editing.

**Mitra Handini:** Visualization, Software, Writing – original draft.

**Mahyarudin Mahyarudin:** Investigation, Data curation, Literature search.

All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

## Conflicts of Interest

The authors have declared no conflict of interest.

## Acknowledgments

The author is grateful to all staff of the Anatomical Pathology Laboratory at Dr. Soedarso General Hospital for granting permission to collect data.

## References

1. Roshandel G, Ghasemi-Kebria F, Malekzadeh R. Colorectal Cancer: Epidemiology, Risk Factors, and Prevention. *Cancers* (Basel). 2024 Apr 17;16(8):1530. doi: 10.3390/cancers16081530. PMID: 38672612; PMCID: PMC11049480.
2. Weiser MR. AJCC 8th Edition: Colorectal Cancer. *Ann Surg Oncol*. 2018 Jun;25(6):1454-1455. doi: 10.1245/s10434-018-6462-1. Epub 2018 Apr 3. PMID: 29616422.
3. Fleming M, Ravula S, Tatischev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. *J Gastrointest Oncol*. 2012 Sep;3(3):153-73. doi: 10.3978/j.issn.2078-6891.2012.030. PMID: 22943008; PMCID: PMC3418538.
4. Coebergh van den Braak RRJ, Ten Hoorn S, Sieuwerts AM, Tuynman JB, Smid M, Wilting SM, et al. Interconnectivity between molecular subtypes and tumor stage in colorectal cancer. *BMC Cancer*. 2020 Sep 4;20(1):850. doi: 10.1186/s12885-020-07316-z. PMID: 32887573; PMCID: PMC7473811.
5. Sadati S, Khalaji A, Bonyad A, Khoshdooz S, Hosseini Kolbadi KS, Bahrami A, et al. NF- $\kappa$ B and apoptosis: colorectal cancer progression and novel strategies for treatment. *Eur J Med Res*. 2025 Jul 14;30(1):616. doi: 10.1186/s40001-025-02734-w. PMID: 40660346; PMCID: PMC12261797.
6. Lukas K, Nguyen J, Necas C, Dave K, Venkataraman V. Targeting the NF- $\kappa$ B Pathway in Cancer: Mechanisms, Resistance, and Therapeutic Potential Across Tumor Types. *Pharmaceuticals* (Basel). 2025 Nov 20;18(11):1764. doi: 10.3390/ph18111764. PMID: 41305005; PMCID: PMC12655786.
7. Mao H, Zhao X, Sun SC. NF- $\kappa$ B in inflammation and cancer. *Cell Mol Immunol*. 2025 Aug;22(8):811-839. doi: 10.1038/s41423-025-01310-w. Epub 2025 Jun 25. PMID: 40562870; PMCID: PMC12310982.
8. Veljkovic A, Stanojevic G, Brankovic B, Roumeliotis S, Leivaditis K, Djordjevic B, et al. MMP-9 Activation via ROS/NF- $\kappa$ B Signaling in Colorectal Cancer Progression: Molecular Insights and Prognostic-Therapeutic Perspectives. *Curr Issues Mol Biol*. 2025 Jul 17;47(7):557. doi: 10.3390/cimb47070557. PMID: 40729026; PMCID: PMC12293130.
9. Mudatsir, Labeda I, Uwuratuw JA, Hendarto J, Warsinggih, Lusikooy RE, et al. Relationship between metalloproteinase-9 (MMP-9) expression and clinicopathology in colorectal cancer: a cross-sectional study. *Ann Med Surg (Lond)*. 2023 Jul 25;85(9):4277-4282. doi: 10.1097/MS9.0000000000000892. PMID: 37663709; PMCID: PMC10473300.
10. Antonangeli F, Natalini A, Garassino MC, Sica A, Santoni A, Di Rosa F. Regulation of PD-L1 Expression by NF- $\kappa$ B in Cancer. *Front Immunol*. 2020 Nov 25;11:584626. doi: 10.3389/fimmu.2020.584626. PMID: 33324403; PMCID: PMC7724774.
11. Bai Z, Zhou Y, Ye Z, Xiong J, Lan H, Wang F. Tumor-Infiltrating Lymphocytes in Colorectal Cancer: The Fundamental Indication and Application on Immunotherapy. *Front Immunol*. 2022 Jan 14;12:808964. doi: 10.3389/fimmu.2021.808964. PMID: 35095898; PMCID: PMC8795622.
12. Iseki Y, Shibutani M, Maeda K, Nagahara H, Fukuoka T, Matsutani S, Kashiwagi S, Tanaka H, Hirakawa K, Ohira M. A new method for evaluating tumor-infiltrating lymphocytes (TILs) in colorectal cancer using hematoxylin and eosin (H-E)-stained tumor sections. *PLoS One*. 2018 Apr 26;13(4):e0192744. doi: 10.1371/journal.pone.0192744. PMID: 29698402; PMCID: PMC5919485.
13. Karki S, Pariyar S. Tumor-infiltrating lymphocytes in colorectal carcinoma. *J Pathol Nepal*. 2021;11(2):1859-63. doi: 10.3126/jpn.v11i2.38227.
14. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al.; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol*. 2015 Feb;26(2):259-71. doi: 10.1093/annonc/mdu450. Epub 2014 Sep 11. PMID: 25214542; PMCID: PMC6267863.
15. Soraya F, Sandhika W, Wiratama PA. 8-OHdG and Nrf2 Protein are Expressed Consistently in Various T Stages of Invasive Breast Carcinoma. *Asian Pac J Cancer Prev*. 2025 Jan 1;26(1):301-307. doi: 10.31557/APJCP.2025.26.1.301. PMID: 39874013; PMCID: PMC12082425.
16. Nguyen LH, Goel A, Chung DC. Pathways of Colorectal Carcinogenesis. *Gastroenterology*. 2020 Jan;158(2):291-302. doi: 10.1053/j.gastro.2019.08.059. Epub 2019 Oct 14. PMID: 31622622; PMCID: PMC6981255.
17. Malki A, ElRuz RA, Gupta I, Allouch A, Vranic S, Al Moustafa AE. Molecular Mechanisms of Colon Cancer Progression and Metastasis: Recent Insights and Advancements. *Int J Mol Sci*. 2020 Dec 24;22(1):130. doi: 10.3390/ijms22010130. PMID: 33374459; PMCID: PMC7794761.
18. Tsokkou S, Konstantinidis I, Papakonstantinou M, Chatzikomnitsa P, Liampou E, Toutziari E, et al. Sex Differences in Colorectal Cancer: Epidemiology, Risk Factors, and Clinical Outcomes. *J Clin Med*. 2025 Aug 6;14(15):5539. doi: 10.3390/jcm14155539. PMID: 40807160; PMCID: PMC12347225.
19. Chen K, Collins G, Wang H, Toh JWT. Pathological Features and Prognostication in Colorectal Cancer. *Curr Oncol*. 2021 Dec 13;28(6):5356-5383. doi: 10.3390/curroncol28060447. PMID: 34940086; PMCID: PMC8700531.
20. Wang R, Lian J, Wang X, Pang X, Xu B, Tang S, et al. Survival rate of colorectal cancer in China: A systematic review and meta-analysis. *Front Oncol*. 2023 Mar 3;13:1033154. doi: 10.3389/fonc.2023.1033154. PMID: 36937415; PMCID: PMC10020492.
21. Tsabit SS, Trianto HF, Pratiwi SE, Hartono H. Clinicopathological Profile of Colorectal Adenocarcinoma in the Anatomical Pathology Laboratory of Dr . Soedarso Hospital Pontianak. *Indones J Cancer*. 2023;17 (4):292-8. doi: <https://doi.org/10.33371/ijoc.v17i4.1004>.
22. Yao N, Li W, Wang J, Chu H, Duan N, Niu X, et al. Prognostic implications of T stage in different pathological types of colorectal cancer: an observational study using SEER population-based data. *BMJ Open*. 2024 Feb 29;14(2):e076579. doi: 10.1136/bmjopen-2023-076579. PMID: 38423773; PMCID: PMC10910631.
23. Testa U, Castelli G, Pelosi E. Genetic Alterations of Metastatic Colorectal Cancer. *Biomedicines*. 2020 Oct

- 13;8(10):414. doi: 10.3390/biomedicines8100414. PMID: 33066148; PMCID: PMC7601984.
24. Farhat F, Daulay ER, Chrestella J, Williamson O, Syari RP. Expressions of Nuclear Factor-kappa B and Peroxisome Proliferator-activated Receptor-Gamma Proportional with Clinical Staging of Nasopharyngeal Carcinoma. *Open Access Maced J Med Sci.* 2021;9(B):1347–51. doi: 10.3889/oamjms.2021.6261.
25. Berkovich L, Gerber M, Katzav A, Kidron D, Avital S. NF-kappa B expression in resected specimen of colonic cancer is higher compared to its expression in inflammatory bowel diseases and polyps. *Sci Rep.* 2022 Oct 5;12(1):16645. doi: 10.1038/s41598-022-21078-7.
26. Bahrami A, Khalaji A, Bahri Najafi M, Sadati S, Raisi A, Abolhassani A, et al. NF- $\kappa$ B pathway and angiogenesis: insights into colorectal cancer development and therapeutic targets. *Eur J Med Res.* 2024 Dec 19;29(1):610. doi: 10.1186/s40001-024-02168-w. PMID: 39702532; PMCID: PMC11658081.
27. Ghadyani R, Mozooni Z, Sohbatzadeh Z, Gachkar L, Sepehr Kahrizi, Movafagh A. Expression patterns and clinical significance of MMP-8, MMP-9 and MMP-13 in colorectal cancer. *Cell Mol Biol (Noisy-le-grand).* 2025 Oct 7;71(9):111-116. doi: 10.14715/cmb/2025.71.9.14. PMID: 41054365.
28. Shoari A, Ashja Ardalan A, Dimesa AM, Coban MA. Targeting Invasion: The Role of MMP-2 and MMP-9 Inhibition in Colorectal Cancer Therapy. *Biomolecules.* 2024 Dec 30;15(1):35. doi: 10.3390/biom15010035. PMID: 39858430; PMCID: PMC11762759.
29. Matsutani S, Shibutani M, Maeda K, Nagahara H, Fukuoka T, Iseki Y, et al. Tumor-infiltrating Immune Cells in H&E-stained Sections of Colorectal Cancer Tissue as a Reasonable Immunological Biomarker. *Anticancer Res.* 2018 Dec;38(12):6721-6727. doi: 10.21873/anticancer.13041. PMID: 30504382.
30. Kraja FP, Jurisic VB, Hromić-Jahjefendić A, Rossopoulou N, Katsila T, Mirjadic Martinovic K, et al. Tumor-infiltrating lymphocytes in cancer immunotherapy: from chemotactic recruitment to translational modeling. *Front Immunol.* 2025 May 22;16:1601773. doi: 10.3389/fimmu.2025.1601773. PMID: 40475782; PMCID: PMC12137109.
31. Lalle G, Twardowski J, Grinberg-Bleyer Y. NF- $\kappa$ B in Cancer Immunity: Friend or Foe? *Cells.* 2021 Feb 9;10(2):355. doi: 10.3390/cells10020355. PMID: 33572260; PMCID: PMC7914614.

---

*\*Corresponding author: Prof. Gondo Mastutik, PhD. E-mail: gondomastutik@fk.unair.ac.id*