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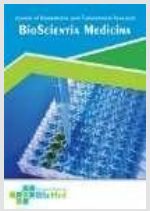

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The Significance of TGF-β Expression in Predicting Lymphovascular Invasion and Lymph Node Metastasis in Colorectal Cancer

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A B S T R A C T

Background: Colorectal cancer (CRC) is a major health burden globally. The prognosis of CRC is strongly influenced by the presence of lymphovascular invasion (LVI) and lymph node (LN) metastasis. Transforming growth factor-beta (TGF-β) is a cytokine with a complex role in CRC progression. This study aimed to evaluate the significance of TGF-β expression in predicting LVI and LN metastasis in CRC. **Methods:** This cross-sectional study involved 50 patients diagnosed with CRC. The expression of TGF-β was assessed using immunohistochemical staining and the Allred scoring system. The relationship between TGF-β expression and the presence of LVI and LN metastasis was analyzed using the Chi-square test. **Results:** High TGF-β expression was significantly associated with both LVI (p = 0.011) and LN metastasis (p = 0.012) in CRC. Patients with high TGF-β expression had a higher risk of LVI and LN metastasis compared to those with low TGF-β expression. **Conclusion:** TGF-β expression is a significant predictor of LVI and LN metastasis in CRC. This finding has potential implications for risk stratification and treatment decisions in CRC patients.

1. Introduction

Colorectal cancer (CRC) is a significant global health concern, ranking as the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide. The prognosis of CRC patients is intricately linked to the stage at which the disease is diagnosed. Early detection and treatment can dramatically improve survival rates, with 5-year survival rates reaching 90% for localized tumors. However, these rates plummet to a dismal 14% for those diagnosed with advanced metastatic disease. The progression of CRC is a multi-step process, often developing from precancerous polyps to invasive tumors. The ability of CRC cells to invade lymphatic or blood vessels, termed lymphovascular invasion (LVI), is a critical step in the metastatic cascade. LVI significantly increases the risk of lymph node (LN) involvement and distant metastasis, both of which are associated with a poorer prognosis.[1-3]

Transforming growth factor-beta (TGF-β) is a pleiotropic cytokine that plays a complex and multifaceted role in CRC development and progression. TGF-β signaling pathways are involved in regulating various cellular processes, including cell

growth, differentiation, apoptosis, and extracellular matrix remodeling. In the context of CRC, TGF-β exhibits a dual nature, acting as both a tumor suppressor and a tumor promoter. In the early stages of CRC, TGF-β primarily exerts tumor-suppressive effects. It inhibits cell proliferation, induces apoptosis, and maintains the integrity of the intestinal epithelium. However, as CRC progresses, cancer cells often acquire the ability to evade the growth-inhibitory effects of TGF-β and exploit its pro-tumorigenic properties. TGF-β can promote tumor progression through several mechanisms. It can induce epithelial-to-mesenchymal transition (EMT), a process that endows cancer cells with increased motility and invasiveness. TGF-β also stimulates angiogenesis, the formation of new blood vessels, which provides tumors with the necessary nutrients and oxygen to sustain their growth and facilitates the spread of cancer cells to distant organs. Additionally, TGF-β can suppress the anti-tumor immune response, allowing cancer cells to evade immune surveillance and destruction.[4-7]

The relationship between TGF-β expression and LVI and LN metastasis in CRC has been the subject of numerous studies. However, the findings have been inconsistent, with some studies reporting a positive association and others showing no significant correlation. This variability may be attributed to differences in study design, sample size, patient populations, and the methods used to assess TGF-β expression.[8-10] This study aimed to evaluate the significance of TGF-β expression in predicting LVI and LN metastasis in CRC.

2. Methods

This cross-sectional study was conducted at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital Padang, a tertiary referral hospital in Padang, Indonesia. The study was approved by the Research Ethics Commission of Dr. M. Djamil General Hospital Padang (ethical review number: DP.04.03/D.XVI.XI/4482024). The study population consisted of all patients diagnosed with colorectal carcinoma at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital Padang between January and December 2023. A total of 140 cases were identified during this period. From this population, a sample of 50 cases was selected using simple random sampling. This sampling method ensures that each case in the population has an equal chance of being included in the study sample, minimizing selection bias and increasing the generalizability of the findings.

To be eligible for inclusion in the study, cases had to meet the following criteria; Complete medical records: Cases were required to have complete medical record data, including information on age, gender, and tumor location. This ensured that the study sample was well-characterized and that potential confounding factors could be accounted for in the analysis; Surgical resection: Only cases that underwent surgical resection of the tumor were included. This excluded cases that only had biopsies, as biopsies may not provide sufficient tissue for a comprehensive pathological evaluation; Available slides and paraffin blocks: Cases were required to have slides and paraffin blocks available for reevaluation. This allowed for the assessment of TGF-β expression and other histopathological features. Cases were excluded from the study if they only underwent biopsy. This exclusion criterion was necessary to ensure that the study sample consisted of cases with adequate tissue for a thorough pathological examination.

The expression of TGF-β was assessed using IHC staining. IHC staining is a widely used technique in pathology to detect specific antigens in tissue sections. In this study, IHC staining was performed to visualize the presence and localization of TGF-β protein in colorectal carcinoma tissue. The IHC staining technique used was the Streptavidin Biotin Complex (SBC) method. This method is a highly sensitive and specific technique for detecting antigens in tissue sections. The SBC method involves a series of steps, including; Deparaffinization and rehydration: Paraffin-embedded tissue sections are deparaffinized using xylene and rehydrated through a series of

graded alcohols; Antigen retrieval: Heat-induced antigen retrieval is performed to unmask the antigens and enhance their binding to the antibodies; Blocking: Endogenous peroxidase activity is blocked using hydrogen peroxide to prevent non-specific staining; Primary antibody incubation: The tissue sections are incubated with a primary antibody specific for TGF-β. The primary antibody binds to the TGF-β protein in the tissue; Secondary antibody incubation: The sections are then incubated with a secondary antibody that is conjugated to biotin. The secondary antibody binds to the primary antibody; Streptavidin-HRP incubation: The sections are incubated with streptavidin-horseradish peroxidase (HRP) conjugate. Streptavidin binds to biotin with high affinity, forming a complex with the secondary antibody; Chromogen detection: The sections are incubated with a chromogen substrate, such as 3,3'-diaminobenzidine (DAB). HRP catalyzes the oxidation of DAB, resulting in a brown precipitate at the site of the antigen-antibody reaction; Counterstaining: The sections are counterstained with hematoxylin to provide contrast and visualize the tissue morphology; Dehydration and mounting: The sections are dehydrated through a series of graded alcohols, cleared in xylene, and mounted with a coverslip.

The level of TGF-β expression in tumor cells was evaluated using the Allred scoring system. The Allred score is a semi-quantitative method that takes into account both the proportion of positively stained cells and the intensity of staining. The proportion score is assigned as follows; 0: No staining; 1: <1% of cells stained; 2: 1-10% of cells stained; 3: 11-33% of cells stained; 4: 34-66% of cells stained; 5: >66% of cells stained. The intensity score is assigned as follows; 0: No staining; 1: Weak staining; 2: Intermediate staining; 3: Strong staining. The total Allred score is calculated by adding the proportion score and the intensity score. The total score ranges from 0 to 8. An Allred score of 2 or greater is considered positive for TGF-β expression.

LVI was assessed by examining the hematoxylin and eosin (H&E)-stained slides of the colorectal carcinoma tissue. LVI was defined as the presence of tumor cells within lymphatic or blood vessels. The presence or absence of LVI was recorded for each case. LN metastasis was assessed by examining the H&E-stained slides of the surgically resected lymph nodes. LN metastasis was defined as the presence of tumor cells within the lymph node tissue. The number of lymph nodes involved was also recorded for each case.

The relationship between TGF-β expression and the presence of LVI and LN metastasis was analyzed using the Chi-square test. The Chi-square test is a statistical test used to determine if there is a significant association between two categorical variables. In this study, the categorical variables were TGF-β expression (high vs. low) and the presence of LVI (yes vs. no) or LN metastasis (yes vs. no). A p-value of less than 0.05 was considered statistically significant. A statistically significant result indicates that there is a strong association between the two variables, and that the observed relationship is unlikely to be due to chance alone.

The data were presented in the form of tables and narratives. The tables summarized the clinicopathological characteristics of the study subjects, the relationship between TGF-β expression and LVI, and the relationship between TGF-β expression and LN metastasis. The narratives provided a detailed description of the methods used in the study and the results of the statistical analysis.

3. Results

Table 1 presents the clinicopathological characteristics of the 50 colorectal cancer (CRC) patients included in the study. The average age of the patients was 54.52 years. The majority of patients (72%) were over 50 years old, which aligns with the general trend of CRC being more common in older adults. There was a slightly higher proportion of female patients (54%) compared to male patients (46%). This difference is relatively small and may not indicate a significant gender bias in the study sample. Most tumors (94%) were classified as low-grade, meaning the cancer cells appeared relatively similar to

normal colon cells under a microscope. This suggests that the majority of tumors were detected at a relatively early stage. The T stage describes how far the cancer has grown into the wall of the colon or rectum. Most tumors (82%) were classified as T3, indicating that the cancer had grown through the muscularis propria (the main muscle layer of the colon) into the subserosa (the outermost layer). This suggests a moderate level of invasion. LVI was observed in 60% of the cases. This is a significant finding, as LVI is associated with a higher risk of cancer spreading to the lymph nodes and other parts of the body. LN involvement was found in 34% of the cases. This indicates that the cancer had already spread to nearby lymph nodes in over a third of the patients. High TGF-β expression was observed in 68% of the cases. This suggests that TGF-β may play a role in the development and progression of CRC.

Table 1. Clinicopathological characteristics.

Characteristic	Frequency (n=50)	Percentage (%)
Age (years)		
Mean (SD)	54.52 (10.21)	
≤ 50	14	28
> 50	36	72
Gender		
Male	23	46
Female	27	54
Differentiation		
Low-grade	47	94
High-grade	3	6
Depth of invasion (T stage)		
T1	0	0
T2	4	8
T3	41	82
T4	5	10
Lymphovascular invasion (LVI)		
Positive	30	60
Negative	20	40
Lymph node (LN) involvement		
Positive	17	34
Negative	33	66
TGF-β expression		
High-grade	34	68
Low-grade	16	32

Table 2 illustrates the relationship between TGF-β expression and the presence of lymphovascular invasion (LVI) in the 50 colorectal cancer cases studied. In cases where TGF-β expression was low, 11 out of 16 cases (68.7%) did *not* show LVI. Conversely, 5 out of 16 cases (31.25%) with low TGF-β expression *did* exhibit LVI. In cases with high TGF-β expression, LVI was observed more frequently. 25 out of 34 cases

(73.52%) with high TGF-β expression showed LVI, while only 9 out of 34 cases (26.47%) did not. The p-value of 0.011 is less than the significance level of 0.05. This indicates that there is a statistically significant association between TGF-β expression and LVI. In other words, it's highly unlikely that the observed relationship between high TGF-β expression and increased LVI is due to chance alone.

Table 2. The relationship between TGF-β expression and LVI.

Variable	**Lymphovascular invasion**		**Total**	**p-value**
TGF-β expression	**Negative**	**Positive**		
Low expression	11 (68.7%)	5 (31.25%)	16 (100%)	11
High expression	9 (26.47%)	25 (73.52%)	34 (100%)	

Table 3 shows the relationship between TGF-β expression and lymph node (LN) involvement in the 50 colorectal cancer cases. In cases with low TGF-β expression, LN involvement was rare. Only 1 out of 16 cases (6.25%) showed positive LN involvement, while 15 out of 16 (93.7%) did not have LN involvement. In cases with high TGF-β expression, there was a much higher rate of LN involvement. 16 out of 34 cases (47.05%) showed LN involvement, compared to 18 out of 34 (52.94%) that did not. The p-value of 0.012 is less than the commonly used significance level of 0.05. This indicates a statistically significant association between high TGF-β expression and LN involvement. This means the observed link between high TGF-β expression and increased LN involvement is unlikely to be due to random chance.

Table 3. The relationship between TGF-β expression and LN involvement.

Variable	**Lymph node involvement**		**Total**	**p-value**
TGF-β expression	**Negative**	**Positive**		
Low expression	15 (93.7%)	1 (6.25%)	16 (100%)	0.012
High expression	18 (52.94%)	16 (47.05%)	34 (100%)	

Figure 1 shows microscopic images of colorectal adenocarcinoma (CRC) with different grades of differentiation, which is a way to describe how much the cancer cells resemble normal colon cells. Figure 1A (Low-grade adenocarcinoma) shows a low-grade CRC. The arrows show a predominant glandular pattern, which indicates that the cancer cells are forming gland-like structures. This is more typical of normal colon tissue. More than 50% of the tissue in this image is composed of these gland-like structures. This indicates that the cells are still relatively well-differentiated. Low-grade tumors tend to grow slower and are less likely to spread quickly. Figure 1B (High-grade adenocarcinoma) depicts a high-grade CRC. There are fewer distinct gland-like structures, and the cells are arranged more haphazardly. This indicates a loss of normal tissue architecture. Less than 50% of the tissue shows a glandular pattern. This signifies that the cells are poorly differentiated. The cells look very different from normal colon cells. High-grade tumors tend to be more aggressive and have a higher chance of spreading.

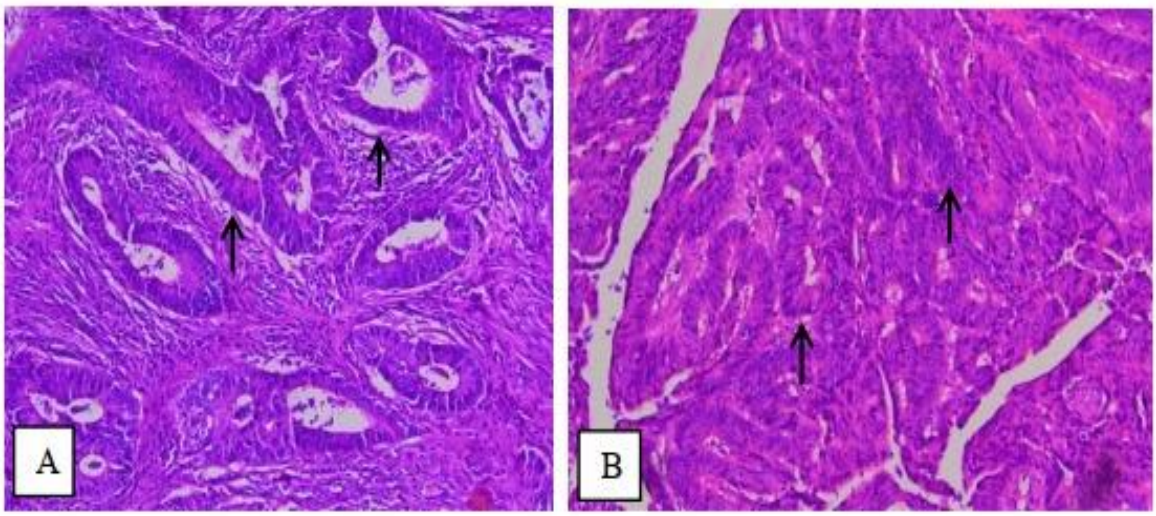

Figure 1. Histopathological features of colorectal adenocarcinoma. (A) Low grade colorectal adenocarcinoma with >50% glandular pattern (arrow). (B) High grade colorectal adenocarcinoma with <50% glandular structure (HE staining, magnification 100).

Figure 2 shows microscopic images illustrating lymphovascular invasion (LVI) and lymph node (LN) involvement in colorectal cancer. Figure 2A (Lymphovascular Invasion) highlights the presence of LVI. The arrow points to a cluster of tumor cells within a lymphatic or blood vessel. This is a key characteristic of LVI. The presence of tumor cells within a vessel indicates that the cancer has the potential to spread to other parts of the body through the lymphatic system or bloodstream. This increases the risk of metastasis. Figure 2B (Lymph Node Involvement) shows a lymph node with tumor infiltration. The arrows point to clusters of tumor cells that have infiltrated the lymph node. This confirms that the cancer has spread from the primary tumor site to the lymph node. The insert provides a closer look at the tumor cells within the lymph node at a higher magnification (400x). This allows for a more detailed visualization of the cancer cells and their characteristics within the lymph node.

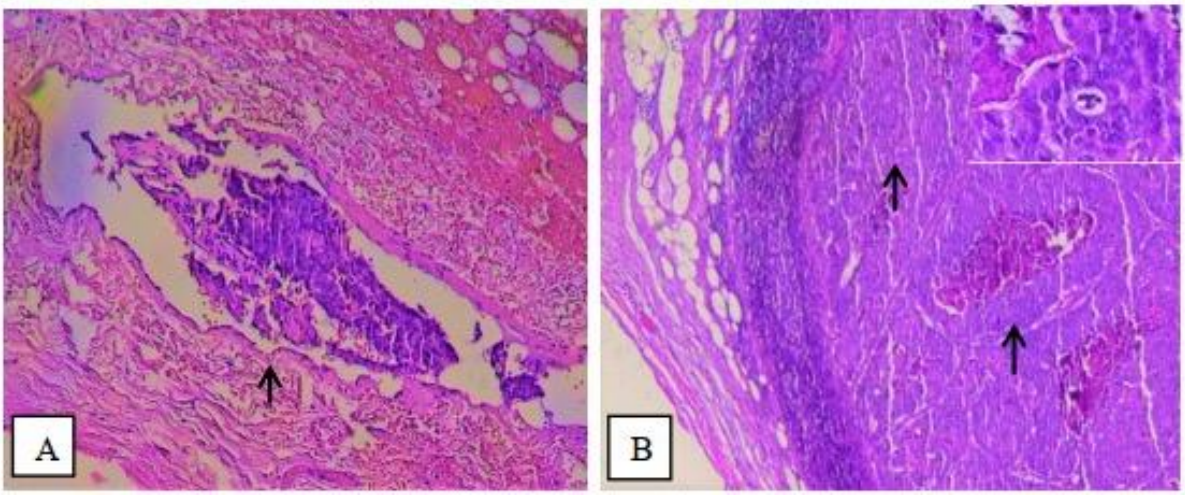

Figure 2. Histopathological features of lymphovascular invasion (A) (HE stain, magnification 200) and Infiltration of tumour cells in LN (B) (arrows) (HE stain, magnification 4x10 and insert magnification 400).

Figure 3 shows microscopic images of colorectal cancer tissue stained to reveal the expression of TGF-β. Figure 3A (Positive Control) serves as a positive control. Positive controls are used in immunohistochemical (IHC) staining to ensure that the staining procedure is working correctly. A known positive sample is used to confirm that the staining system can detect the target protein (in this case, TGF-β). Figure 3B (Moderate TGF-β Expression) shows moderate expression of TGF-β in colorectal adenocarcinoma. The brown staining (indicated by the arrows) within the cytoplasm of the glandular structures represents the presence of TGF-β. The intensity of the brown staining is moderate, suggesting a moderate level of TGF-β expression. The Allred score, which combines the proportion and intensity of staining, is less than 6 in this case. Figure 3C (Strong TGF-β Expression) shows strong expression of TGF-β in colorectal carcinoma. The brown staining (indicated by the arrows) is more intense compared to Figure 3B, indicating a higher level of TGF-β expression. The Allred score is greater than 6 in this case, reflecting the strong intensity of the staining.

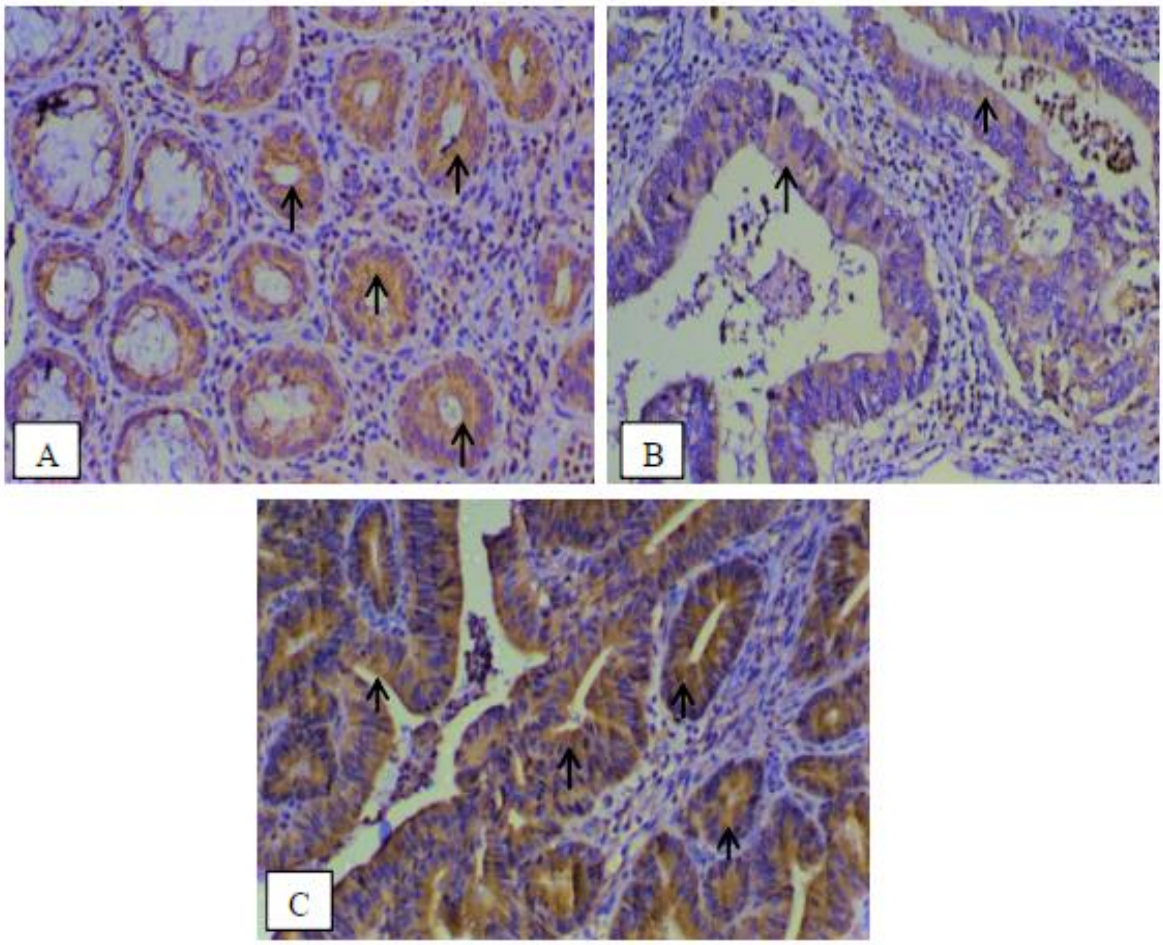

Figure 3. Expression of TGF-β. (A) TGF-β expression in the positive control. (B) Positive expression (brown colour) with moderate intensity in the cytoplasm of glandular structures (arrow) of colorectal adenocarcinoma with an Allred score <6. (C) TGF-β expression with strong intensity in colorectal carcinoma with Allred score >6 (IHK staining, magnification 200).

4. Discussion

Transforming growth factor-beta (TGF-β) is a versatile cytokine that plays a pivotal role in a myriad of cellular processes, including cell growth, differentiation, apoptosis, and the intricate modulation of the extracellular matrix—the scaffolding that provides structural and biochemical support to surrounding cells. However, in the complex landscape of cancer, TGF-β takes on a paradoxical character, acting as both a suppressor of tumor growth and a promoter of its progression. This duality is a testament to the intricate interplay between TGF-β signaling pathways, the dynamic tumor microenvironment, and the specific stage of tumor development. In the nascent stages of colorectal cancer (CRC), transforming growth factor-beta (TGF-β) emerges as a prominent guardian, diligently maintaining the delicate balance of cellular growth and death within the intestinal epithelium—the lining of the colon and rectum. It acts as a gatekeeper, inhibiting the uncontrolled proliferation of cells, a hallmark of cancer. Additionally, TGF-β induces apoptosis, a programmed cell death mechanism that eliminates damaged or abnormal cells, preventing them from becoming cancerous. Furthermore, TGF-β plays a crucial role in maintaining genomic stability, ensuring the accurate replication and preservation of the genetic material, thereby preventing the accumulation of mutations that can drive cancer development. TGF-β signaling pathways are intricately involved in regulating the cell cycle, the tightly controlled sequence of events that govern cell growth and division. By inhibiting the progression of the cell cycle, TGF-β prevents excessive cell proliferation, ensuring that cells divide only when necessary. This regulatory function is crucial in maintaining the integrity of the intestinal epithelium and preventing the uncontrolled growth that characterizes cancer. Apoptosis, or programmed cell death, is a natural process that eliminates damaged or unnecessary cells. TGF-β can trigger apoptosis in cells that have sustained DNA damage or other abnormalities, preventing them from potentially becoming cancerous. This mechanism is essential for maintaining tissue homeostasis and preventing the accumulation of potentially harmful cells. Genomic stability refers to the accurate replication and maintenance of the genetic material within cells. TGF-β signaling pathways play a crucial role in preserving genomic stability by promoting DNA repair mechanisms and ensuring the proper segregation of chromosomes during cell division. By preventing genomic instability, TGF-β helps to prevent the accumulation of mutations that can drive cancer development. The tumor-suppressive effects of TGF-β are most prominent in the early stages of CRC. However, as the tumor progresses, cancer cells often acquire the ability to evade these growth-inhibitory signals and exploit the pro-tumorigenic properties of TGF-β. This transition from tumor suppressor to tumor promoter is a critical turning point in CRC progression. Understanding the intricate mechanisms by which TGF-β switches from a tumor suppressor to a tumor promoter is crucial for developing effective therapeutic strategies that can target this versatile cytokine. By harnessing the tumor-suppressive potential of TGF-β and mitigating its pro-tumorigenic effects, we can potentially develop novel approaches to prevent and treat CRC. As colorectal cancer (CRC) progresses, the narrative takes an intriguing turn. Cancer cells, with their uncanny ability to adapt and evolve, often acquire the means to evade the growth-inhibitory signals of TGF-β. They hijack its signaling pathways, exploiting its pro-tumorigenic properties to further their agenda of uncontrolled growth and dissemination. This transition from tumor suppressor to tumor promoter is a critical turning point in CRC progression. One of the key mechanisms by which TGF-β promotes tumor progression is through the induction of EMT. EMT is a remarkable cellular transformation in which epithelial cells, the building blocks of the intestinal lining, lose their characteristic polarity and cell-to-cell adhesion, acquiring mesenchymal properties—the traits of migratory and invasive cells. This transition is akin to a chameleon changing its colors, allowing cancer cells to break free

from the primary tumor mass and embark on a journey to distant sites. TGF-β orchestrates this cellular metamorphosis by activating transcriptional factors, the master regulators of gene expression. These factors suppress the expression of epithelial markers, such as E-cadherin, which normally hold epithelial cells together, and induce the expression of mesenchymal markers, such as vimentin, which promote cell motility and invasion. Another crucial mechanism by which TGF-β promotes tumor progression is through the stimulation of angiogenesis, the formation of new blood vessels. Angiogenesis provides tumors with the lifeline they need—a supply of nutrients and oxygen—to sustain their relentless growth. TGF-β promotes angiogenesis by inducing the expression of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), and by recruiting endothelial cells and pericytes, the building blocks of blood vessels, to construct new vascular networks. TGF-β can also suppress the anti-tumor immune response, the body's natural defense against cancer. This suppression allows cancer cells to evade the vigilant surveillance of the immune system, escaping destruction and continuing their unchecked growth. TGF-β accomplishes this by inhibiting the proliferation and activation of cytotoxic T lymphocytes (CTLs), the immune cells responsible for recognizing and eliminating cancer cells. Additionally, TGF-β can promote the differentiation of regulatory T cells (Tregs), which act as suppressors of the immune response, further contributing to the cancer cells' ability to evade immune surveillance. The pro-tumorigenic role of TGF-β is a stark contrast to its tumor-suppressive functions in early-stage CRC. This duality highlights the complexity of the tumor microenvironment and the intricate signaling networks that govern cancer progression. Understanding the mechanisms by which TGF-β switches from a tumor suppressor to a tumor promoter is crucial for developing effective therapeutic strategies that can target this versatile cytokine. TGF-β, once a guardian of the intestinal epithelium, can turn into an accomplice in cancer's insidious agenda. This Jekyll-and-Hyde behavior is driven by the intricate mechanisms through which TGF-β promotes tumor progression. TGF-β orchestrates a remarkable transformation in cancer cells, known as EMT. During this process, epithelial cells, which are normally bound together in a cohesive sheet, lose their polarity and cell-to-cell adhesion, acquiring mesenchymal characteristics—the traits of migratory and invasive cells. This transition is akin to a chameleon changing its colors, allowing cancer cells to break free from the primary tumor mass and embark on a journey to distant sites. EMT is driven by TGF-β signaling, which activates transcriptional factors—master regulators of gene expression—that suppress epithelial markers and induce mesenchymal markers. These markers are like molecular fingerprints that define the cell's identity and behavior. For example, E-cadherin, an epithelial marker, acts like a cellular glue, holding epithelial cells together. TGF-β suppresses E-cadherin expression, weakening the bonds between cells and promoting their separation. On the other hand, vimentin, a mesenchymal marker, enhances cell motility and invasion, allowing cancer cells to navigate through the extracellular matrix and spread to distant sites. Angiogenesis, the formation of new blood vessels, is essential for tumor growth and metastasis. Angiogenesis provides tumors with the lifeline they need—a supply of nutrients and oxygen—to sustain their relentless growth. TGF-β promotes angiogenesis by inducing the expression of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), and by recruiting endothelial cells and pericytes—the building blocks of blood vessels—to construct new vascular networks. VEGF is a potent signaling molecule that stimulates the growth of new blood vessels. TGF-β induces the expression of VEGF, which then binds to receptors on endothelial cells, triggering a cascade of events that lead to the formation of new blood vessels. These new vessels provide tumors with the nutrients and oxygen they need to grow and spread. TGF-β can suppress the anti-tumor immune response, the body's natural defense against cancer. This suppression allows cancer cells to evade the

vigilant surveillance of the immune system, escaping destruction and continuing their unchecked growth. TGF-β accomplishes this by inhibiting the proliferation and activation of cytotoxic T lymphocytes (CTLs), the immune cells responsible for recognizing and eliminating cancer cells. Additionally, TGF-β can promote the differentiation of regulatory T cells (Tregs), which act as suppressors of the immune response, further contributing to the cancer cells' ability to evade immune surveillance. CTLs are specialized immune cells that can recognize and destroy cancer cells. They are like the body's elite soldiers, trained to identify and eliminate threats. However, TGF-β can disarm these soldiers, inhibiting their proliferation and activation, rendering them ineffective against cancer cells. Tregs are another type of immune cell that plays a role in regulating the immune response. They are like the peacekeepers of the immune system, ensuring that the immune response does not become excessive and cause damage to the body's own tissues. However, in the context of cancer, Tregs can be detrimental, as they can suppress the anti-tumor immune response, allowing cancer cells to escape destruction. TGF-β can promote the differentiation of Tregs, further contributing to the cancer cells' ability to evade immune surveillance. Transforming growth factor-beta (TGF-β) is a pleiotropic cytokine that plays a pivotal role in a myriad of cellular processes, including cell growth, differentiation, apoptosis, and the intricate modulation of the extracellular matrix—the scaffolding that provides structural and biochemical support to surrounding cells. However, in the complex landscape of cancer, TGF-β takes on a paradoxical character, acting as both a suppressor of tumor growth and a promoter of its progression. This duality is a testament to the intricate interplay between TGF-β signaling pathways, the dynamic tumor microenvironment, and the specific stage of tumor development. In the nascent stages of colorectal cancer (CRC), TGF-β primarily functions as a tumor suppressor, diligently maintaining the delicate balance of cellular growth and death within the intestinal epithelium—the lining of the colon and rectum. It acts as a gatekeeper, inhibiting the uncontrolled proliferation of cells, a hallmark of cancer. Additionally, TGF-β induces apoptosis, a programmed cell death mechanism that eliminates damaged or abnormal cells, preventing them from becoming cancerous. Furthermore, TGF-β plays a crucial role in maintaining genomic stability, ensuring the accurate replication and preservation of the genetic material, thereby preventing the accumulation of mutations that can drive cancer development. However, as CRC progresses, the narrative takes an intriguing turn. Cancer cells, with their uncanny ability to adapt and evolve, often acquire the means to evade the growth-inhibitory signals of TGF-β. They hijack its signaling pathways, exploiting its pro-tumorigenic properties to further their agenda of uncontrolled growth and dissemination. This transition from tumor suppressor to tumor promoter is a critical turning point in CRC progression. The paradoxical behavior of TGF-β is attributed to the intricate interplay between its signaling pathways, the dynamic tumor microenvironment, and the specific stage of tumor development. In early-stage CRC, the tumor microenvironment is relatively stable, and TGF-β signaling pathways are intact, allowing TGF-β to exert its tumor-suppressive effects. However, as the tumor progresses, the tumor microenvironment becomes increasingly complex and heterogeneous, and cancer cells often acquire mutations or epigenetic alterations that disrupt TGF-β signaling pathways. These alterations can lead to the evasion of TGF-β's growth-inhibitory signals and the exploitation of its pro-tumorigenic properties. The dual role of TGF-β in cancer has significant clinical implications. On the one hand, TGF-β's tumor-suppressive effects offer a potential therapeutic target for cancer prevention and treatment. On the other hand, TGF-β's pro-tumorigenic effects pose a challenge for cancer therapy, as it can promote tumor growth, invasion, and metastasis. Therefore, understanding the intricate mechanisms by which TGF-β switches from a tumor suppressor to a tumor promoter is crucial for

developing effective therapeutic strategies that can target this versatile cytokine.[11-14]

The findings of this study are consistent with the hypothesis that TGF-β plays a critical role in promoting LVI and LN metastasis in CRC. The significant association between high TGF-β expression and both LVI and LN metastasis suggests that TGF-β may be involved in driving the metastatic cascade. The mechanisms by which TGF-β promotes lymphovascular invasion (LVI) and lymph node (LN) metastasis are likely multifaceted, involving the induction of epithelial-to-mesenchymal transition (EMT), stimulation of angiogenesis, and suppression of the anti-tumor immune response. By inducing EMT, TGF-β can increase the motility and invasiveness of cancer cells, facilitating their entry into lymphatic or blood vessels, leading to LVI and subsequent LN metastasis. EMT is a process in which epithelial cells lose their polarity and cell-to-cell adhesion, acquiring mesenchymal characteristics, such as increased migratory and invasive capabilities. This transition is akin to a chameleon changing its colors, allowing cancer cells to break free from the primary tumor mass and embark on a journey to distant sites. TGF-β signaling is a key driver of EMT. It activates transcriptional factors that repress epithelial markers, such as E-cadherin, which normally hold epithelial cells together. Additionally, TGF-β signaling induces mesenchymal markers, such as vimentin, which promote cell motility and invasion. The stimulation of angiogenesis by TGF-β can further promote tumor growth and metastasis by providing tumors with the necessary nutrients and oxygen to sustain their growth and by facilitating the dissemination of cancer cells throughout the body. Angiogenesis is the formation of new blood vessels from pre-existing ones. In cancer, angiogenesis is often dysregulated, leading to the formation of abnormal blood vessels that are leaky and tortuous. These abnormal vessels provide tumors with the lifeline they need—a supply of nutrients and oxygen—to sustain their relentless growth. TGF-β promotes angiogenesis by inducing the expression of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), and by recruiting endothelial cells and pericytes—the building blocks of blood vessels—to construct new vascular networks. The immunosuppressive effects of TGF-β can also contribute to tumor progression and metastasis by allowing cancer cells to evade immune surveillance and destruction. The immune system plays a critical role in recognizing and eliminating cancer cells. However, cancer cells can evolve mechanisms to evade immune surveillance and destruction. One such mechanism is immunosuppression, which can be mediated by TGF-β. TGF-β can inhibit the proliferation and activation of cytotoxic T lymphocytes (CTLs), the immune cells responsible for recognizing and eliminating cancer cells. Additionally, TGF-β can promote the differentiation of regulatory T cells (Tregs), which act as suppressors of the immune response, further contributing to the cancer cells' ability to evade immune surveillance. The findings of this study have potential clinical implications for the management of CRC patients. TGF-β expression could be used as a prognostic marker to identify patients at high risk of LVI and LN metastasis. This could aid in risk stratification and treatment decisions. Patients with high TGF-β expression may benefit from more aggressive treatment approaches, such as adjuvant chemotherapy or targeted therapies. The significant association between high TGF-β expression and both LVI and LN metastasis suggests that TGF-β could serve as a prognostic marker in CRC. Prognostic markers are used to predict the likelihood of disease progression and patient outcomes. By identifying patients with high TGF-β expression, clinicians can better assess their risk of LVI and LN metastasis, and tailor treatment strategies accordingly. Currently, the TNM staging system is widely used to assess the risk of CRC progression. However, the TNM system has limitations in its ability to predict individual patient outcomes. The addition of TGF-β expression to the risk assessment could potentially improve the accuracy of prognostication and help identify patients who may benefit from more aggressive treatment

approaches. Adjuvant chemotherapy is the administration of chemotherapy after surgery to reduce the risk of cancer recurrence. The decision to administer adjuvant chemotherapy is often based on the risk of recurrence, which is assessed using factors such as TNM stage, LVI, and LN metastasis. The addition of TGF-β expression to the risk assessment could potentially improve the selection of patients for adjuvant chemotherapy. Targeted therapies are drugs that specifically target molecules involved in cancer cell growth and survival. Several targeted therapies have been developed for CRC, and the selection of the appropriate targeted therapy is often based on the molecular profile of the tumor. TGF-β signaling pathways could potentially serve as targets for novel therapies in CRC.[15-17]

The findings of this study have potential clinical implications for the management of CRC patients. TGF-β expression could be used as a prognostic marker to identify patients at high risk of LVI and LN metastasis. This could aid in risk stratification and treatment decisions. Patients with high TGF-β expression may benefit from more aggressive treatment approaches, such as adjuvant chemotherapy or targeted therapies. The significant association between high TGF-β expression and both LVI and LN metastasis suggests that TGF-β could serve as a prognostic marker in CRC. Prognostic markers are used to predict the likelihood of disease progression and patient outcomes. By identifying patients with high TGF-β expression, clinicians can better assess their risk of LVI and LN metastasis, and tailor treatment strategies accordingly. Currently, the TNM staging system is widely used to assess the risk of CRC progression. However, the TNM system has limitations in its ability to predict individual patient outcomes. The addition of TGF-β expression to the risk assessment could potentially improve the accuracy of prognostication and help identify patients who may benefit from more aggressive treatment approaches. Adjuvant chemotherapy is the administration of chemotherapy after surgery to reduce the risk of cancer recurrence. The decision to administer adjuvant chemotherapy is often based on the risk of recurrence, which is assessed using factors such as TNM stage, LVI, and LN metastasis. The addition of TGF-β expression to the risk assessment could potentially improve the selection of patients for adjuvant chemotherapy, ensuring that those who are most likely to benefit from this treatment receive it. Targeted therapies are drugs that specifically target molecules involved in cancer cell growth and survival. Several targeted therapies have been developed for CRC, and the selection of the appropriate targeted therapy is often based on the molecular profile of the tumor. TGF-β signaling pathways could potentially serve as targets for novel therapies in CRC. By targeting TGF-β signaling, these therapies could potentially inhibit EMT, angiogenesis, and immunosuppression, thereby hindering tumor progression and metastasis. The development of TGF-β-targeted therapies is an active area of research. Several approaches are being investigated, including the use of monoclonal antibodies to block TGF-β signaling and the development of small molecule inhibitors that target TGF-β signaling pathways. These therapies hold promise for improving the treatment of CRC, particularly in patients with high TGF-β expression who are at increased risk of LVI and LN metastasis. The ultimate goal is to use TGF-β expression and other molecular markers to personalize treatment decisions for CRC patients. This approach, known as personalized medicine, aims to tailor treatment to the individual needs of each patient, based on their unique molecular profile and risk factors. By incorporating TGF-β expression into the risk assessment, clinicians can better predict the likelihood of disease progression and select the most effective treatment strategies for each patient.[18-20]

5. Conclusion

This study investigated the relationship between TGF-β expression, lymphovascular invasion (LVI), and lymph node (LN) involvement in colorectal carcinoma. The significant findings underscore the role of TGF-β as a potential prognostic marker for identifying high-

risk CRC patients. The results show a strong association between high TGF-β expression and the presence of both LVI and LN metastasis. This suggests that TGF-β may be a crucial driver in the progression and metastatic spread of CRC. These findings have potential clinical implications for refining risk assessment and personalizing treatment decisions in CRC patients. Incorporating TGF-β expression into clinical evaluation could enhance the accuracy of prognostication and guide the selection of appropriate therapies, such as adjuvant chemotherapy or targeted therapies. Further research is needed to validate the clinical utility of TGF-β as a prognostic and predictive marker in CRC. Larger, prospective studies should be conducted to confirm these findings and assess the impact of TGF-β expression on patient outcomes. The development of TGF-β-targeted therapies holds promise for improving treatment strategies and patient outcomes in the future.

6. References

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