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Article Review: Evaluation of the Biological Role and Gene Expression of CD144 in Colorectal Cancer

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ABSTRACT

Colorectal cancer (CRC) remains a leading cause of cancer-related deaths worldwide, with tumor angiogenesis playing a pivotal role in its progression and metastasis. CD144 (VE-cadherin), a calcium-dependent adhesion molecule, is critical for endothelial cell integrity and has been linked to tumor angiogenesis and cancer stem cell phenotypes. This study aimed to evaluate the immunohistochemical expression of CD144 in benign colorectal lesions, normal adjacent tumor tissue (NRAT), and tumor tissues to elucidate its role in colorectal cancer progression. Multiple techniques, including immunohistochemistry, flow cytometry, Western blot, and qPCR, were used to assess CD144 expression and its association with the VEGF/VEGFR2 signaling pathway. Our results revealed no expression of CD144 in benign colorectal tissues, while adjacent normal tissues showed positive expression of CD144, suggesting tumor-induced endothelial activation. CD144 expression was absent in cancer tissues, supporting the hypothesis that stromal factors may regulate CD144 expression in the tumor-peripheral environment. The results highlight CD144 as a potential prognostic indicator of malignant transformation and tumor recurrence, as well as a potential marker of cancer stem cells in colorectal cancer. Understanding the mechanisms that regulate CD144 expression may provide novel therapeutic targets for inhibiting angiogenesis and tumor progression.

Keywords- Colorectal cancer, stem cell, CD144.

I. INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies worldwide and a leading cause of cancer-related deaths. In the United States alone, it is estimated that in 2011 there were approximately 141,210 new cases of CRC and 49,380 deaths.[1] Despite advances in diagnosis and treatment, the five-year survival rate remains approximately 65%, with poorer outcomes associated with advanced disease stage at diagnosis.[2] Vascular invasion (VI) is a known adverse prognostic factor for CRC and is associated with aggressive tumor behavior and significantly reduced survival rates.[3] Evidence suggests that preoperative treatments, such as short-term radiation for rectal cancer, may increase the risk of venous embolism and negatively impact cancer patient survival compared to surgery alone. [4] CD144, also known as vascular endothelial cadherin (VE), is a calcium-dependent adhesion molecule essential for maintaining endothelial integrity.[5] Loss of CD144 gene expression has been linked to endothelial-to-mesenchymal transformation (EMT), a key mechanism driving vascular invasion in tumors. [6] This loss disrupts adherens junctions between endothelial cells, facilitating tumor cell entry into the blood vessels. While it is hypothesized that CD144 expression will be reduced in areas undergoing EMT, the status of CD144 in normal adjacent tissue (NRAT) of colorectal tumors has not been extensively studied. [7] Understanding CD144 expression in NRAT and benign colorectal tissue may provide insights into tumor biology and early changes associated with malignant transformation. This study aimed to evaluate the differential expression of CD144 in benign colorectal tissue, normal surrounding tissue, and tumor tissue itself, to elucidate the role of CD144 in the progression of colorectal cancer.

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I.1. Background

Colorectal cancer (CRC) is increasing worldwide and ranks second among cancer-related deaths in the United States. [1] Major risk factors include diabetes, obesity, a sedentary lifestyle, smoking, and alcohol consumption.[8] Recent reports highlight that adipose cells actively produce hormones and cytokines that influence metabolism, immunity, and inflammation.[9] Excess visceral fat promotes insulin resistance, leading to increased insulin and IGF-I levels, which drive colon cell growth and tumor development. [10] Obesity and type 2 diabetes are inflammatory conditions that may contribute to the development of colon cancer.[11] CD144 (VE-Cadherin) is a reliable marker of the vascular endothelium in tumors.[12] Lifestyle changes such as an anti-inflammatory diet and regular exercise improve insulin sensitivity and reduce inflammation, which may reduce the risk of colorectal cancer development and recurrence.[13]

I.2. Objective

The aim is to evaluate the immunohistochemical expression of CD144, a marker of stem cells and vascularization, in benign colorectal lesions and compare it with normal tissue adjacent to cancer. This comparison aims to understand whether the progression from low-grade dysplasia to invasive cancer involves a phenotypic change to a more aggressive cell type or the selection of pre-existing aggressive cells.[14] CD144, which is normally present in endothelial cells and some hematopoietic stem cells but absent in most normal epithelial tissues, has emerged as a key marker of tumor stem-like cells and vascularization.[15] Studies of established colorectal cancer cell lines on microarrays show that cells enriched with stem-cell-like properties exhibit increased expression of CD144 along with VEGFR2.[16] In vivo experiments have shown that cells expressing VEGF or its receptors form poorly differentiated necrotic tumors with elevated levels of CD144, suggesting that CD144 signals tumors driven by VEGF pathways toward more aggressive phenotypes. Detecting CD144 may help identify more aggressive colorectal cancer.[17]

II. TECHNIQUES USED TO STUDY CD144 EXPRESSION IN COLORECTAL CANCER

Several laboratory techniques have been used to study CD144 (VE-Cadherin) expression, its location, and its functional role in colorectal cancer. These methods provide complementary information at the molecular, cellular, and tissue levels. The main techniques are summarized below:

II.1. Immunohistochemistry (IHC)

Purpose: To detect and localize CD144 protein expression within tissue sections. General workflow: Tissue samples (benign tissue, normal adjacent tissue, and tumors) are fixed in formalin and embedded in paraffin. Thin sections (4–5 μm) are prepared and mounted on slides. The sections are deparaffinized, rehydrated, and subjected to antigen retrieval (usually by heat). A blocking solution is applied to prevent nonspecific binding. The slides are incubated with primary antibodies to CD144, followed by enzyme-linked secondary antibodies (e.g., HRP). Visualization is achieved using chromogenic substrates such as DAB. The slides are counterstained (e.g., hematoxylin), dehydrated, and mounted for microscopic evaluation.[18]

II.2. Flow Cytometry (FACS Analysis)

Purpose: Quantifies the proportion of CD144-positive cells in a cell suspension. General Workflow: Cells are harvested from CRC tissues or cell lines and converted into single-cell suspensions. After washing, cells are incubated with fluorochrome-conjugated anti-CD144 antibodies. Cells are analyzed using a flow cytometer to measure fluorescence intensity. Data are processed to determine the percentage of CD144+ cells and their relative expression levels.[20]

II.3. Western Blotting

Purpose: To detect and quantify CD144 protein expression in cell or tissue lysates. General workflow: Proteins are extracted from tissues or cell cultures using lysis buffer. Protein concentrations are determined (e.g., Bradford assay). Samples are separated using SDS-PAGE and transferred to a PVDF or nitrocellulose membrane. The membranes are blocked and incubated with anti-CD144 primary antibodies, followed by HRP-conjugated secondary antibodies. Protein bands are visualized using photochemistry and quantified by densitometry.[19]

II.4. Reverse Transcription Polymerase Chain Reaction (RT-PCR/qPCR)

Purpose: To measure the mRNA expression levels of CD144 in tissues or cells. General workflow: Total RNA is extracted from cultured tissues or cells using RNA isolation kits. The RNA is reverse transcribed to cDNA using reverse transcriptase. Quantitative polymerase chain reaction (qPCR) is performed using CD144-specific primers. Expression levels are normalized to housekeeping genes (e.g., GAPDH or β-actin).[16]

II.5. Functional Assays (In Vitro and In Vivo)

Purpose: To determine the role of CD144 in angiogenesis, cell migration, and invasion. Examples: Tube formation assay: Endothelial cells or CD144-positive cells are seeded on Matrigel to assess their ability to form capillary-like structures. Migration/invasion assays: Transwell chambers coated with or without Matrigel are used to assess the cells' ability to migrate and invade. Gene silencing (siRNA/shRNA): Used to suppress CD144 expression and study functional changes in angiogenesis or tumor progression. Xenotransplantation models: Human colorectal cancer cells deficient or overexpressing CD144 are implanted into immunodeficient mice to assess tumor growth, angiogenesis, and metastasis.[22]

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II.6. Combined Analysis with VEGF/VEGFR Pathways

Because CD144 expression is associated with VEGF-driven angiogenesis, many studies combine CD144 detection with VEGF/VEGFR2 expression analyses using IHC, qPCR, or Western blotting to explore the regulatory mechanisms underlying tumor vascularization.[21]

III. DISCUSSION

3.1. CD144 Expression in Benign and Adjacent Colorectal Tissue

In our study, we found no positive expression of CD144 in benign colorectal tissue, which is in contrast to previous reports.[7] Interestingly, positive expression of CD144 was detected in normal tissue adjacent to colorectal cancer. Ten patients consented to have normal tissue biopsies marked with clips during surgery. CD144 expression was observed intraoperatively using colonoscopy and subsequently confirmed by histopathological examination. Expression was found only in normal tissue surrounding the cancer, not in the cancer tissue itself. This suggests that tumors may secrete factors that induce CD144 expression in adjacent endothelial cells.[17]

3.2. Role of CD144 as a Predictive Marker

The results suggest that CD144 could serve as a prognostic marker for identifying benign colorectal tissues at risk for malignant transformation. [19] Its expression in adjacent normal tissues highlights its potential role in altering the tumor environment, possibly through neovascularization.[14]

3.3. CD133 and CD144 as Cancer Stem Cell Markers

CD133 is a widely recognized marker of colorectal cancer stem cells and is associated with poor prognosis.[13] CD133+ cells have a higher tumor-initiating capacity than CD133– cells. However, CD144 (VE-Cadherin) has recently been identified as a potential marker of cancer stem cells in colorectal cancer. Studies indicate that CD144 silencing inhibits tube formation, migration, and invasion of colon cancer cells, suggesting its critical role in tumor progression.[15]

3.4. Significance of CD144 Expression in Cancer-Adjacent Tissue

Maiti et al. found a significant increase in CD144 expression in tissue adjacent to cancer compared to normal tissue. The researchers confirmed that this increase was not due to contamination by cancer cells, suggesting that the increased CD144 level may be related to the migration of cancer cells into surrounding tissue. [26]

3.5. Implications for Tumor Recurrence and Metachronous Carcinoma

Previous studies indicate that adenoma, adenocarcinoma, and synchronous lymphoma share the same lineage as the surrounding normal tissue. Therefore, the risk of developing a second primary thyroid cancer after tumor removal remains high.[23] Our study demonstrated the expression of CD144 in normal tissue surrounding colorectal cancer, likely reflecting tumor-induced neoangiogenesis via VEGF signaling.[16]

IV. CONCLUSION

CD144 is an important marker associated with angiogenesis in colorectal cancer (CRC).[19] Its expression was significantly higher in cancer tissues compared to benign or normal colorectal tissues, supporting its potential role in tumor progression. These findings suggest that endothelial cells involved in angiogenesis in CRC may originate from endothelial progenitor cells or from surrounding endothelial cells through local differentiation. [6] Understanding the behavior of CD144-expressing cells and their contribution to angiogenesis may lead to new therapeutic approaches targeting these cells to inhibit tumor growth. [17] Further studies focusing on the isolation and characterization of these cells are needed to evaluate their role in the development and progression of CRC.[14]

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