N. Belder / Hacettepe J. Biol. & Chem., 2024, 52 (2), 117-128

Research Article

Hacettepe Journal of Biology and Chemistry

journal homepage: www.hjbc.hacettepe.edu.tr

Prognostic Implications of *MXRA8* Expression in Colorectal Cancer and Its role in Tumor Progression

MXRA8 İfadesinin Kolorektal Kanserdeki Prognostik Etkileri ve Tümör İlerlemesindeki Rolü

Nevin Belder

Ankara University Biotechnology Institute, Ankara, Turkey.

ABSTRACT

Matrix Remodeling Associated 8 (*MXRA8*) is a type I transmembrane protein capable of modulating integrin-related signaling and regulating cellular interactions, and also functions as a receptor for multiple arthritogenic alphaviruses. Although limited numbers of studies have provided evidence indicating a potential role of *MXRA8* in different types of cancer, the potential contrubition of *MXRA8* in colorectal cancer (CRC) has not yet been fully elucidated. Therefore, our aim was to conduct a comprehensive analysis elucidating the prognostic value of *MXRA8* in CRC. The results revealed that *MXRA8* was highly expressed in CRC compared to normal tissue. Notably, there was a substantial correlation with the TNM stage, and elevated *MXRA8* expression was indicative of a poorer prognosis in CRC cases. Furthermore, co-expression analysis indicated that *MXRA8* is predominantly involved in hypoxia and epithelial-mesenchymal transition pathway. In conclusion, this study demonstrates the potential roles of *MXRA8* in predicting CRC prognosis and contributes to the elucidation of how *MXRA8* might be involved in the mechanisms underlying CRC carcinogenesis.

Key Words

Colorectal cancer, *MXRA8*, prognosis, biomarker, epithelial-mesenchymal transition.

ÖZ

Matrix Remodeling Associated 8 (*MXRA8*), tip I transmembran bir proteindir ve integrin sinyalleşmesini düzenleme ve hücrelerarası etkileşimleri yönlendirme yeteneğine sahiptir; ayrıca artritojenik alfa virüsler için bir reseptör olarak işlev görür. Sınırlı sayıda çalışma, *MXRA8*'in çeşitli kanser türlerinde potansiyel bir rolünü gösteren kanıtlar sunmuştur. Bununla birlikte, *MXRA8*'in kolorektal kanser (KRK) üzerindeki rolü henüz tam olarak aydınlatılmamıştır. Bu doğrultuda, *MXRA8*'in KRK'deki prognostik değerini araştıran kapsamlı bir analiz yapılması amaçlanmıştır. Çalışmamızda, *MXRA8* ekspresyonunun, normal dokulara kıyasla KRK'de önemli ölçüde yüksek olduğu gösterilmiştir. Ayrıca, yüksek ekspresyonunun, KRK'da kötü prognozla ve TNM evresiyle anlamlı bir ilişkisi olduğ tespit edilmiştir. Koekspresyon analizi ise *MXRA8*'in çoğunlukla hipoksi ve epitelyal-mezenkimal geçiş yolunda yer aldığını göstermiştir. Sonuç olarak bulgularımız; *MXRA8*'in KRK prognozundaki potansiyel rollerini ortaya koymuş ve *MXRA8*'in KRK karsinojenez mekanizmasındaki rolünün aydınlatılmasına katkı sağlamıştır.

Anahtar Kelimeler

Kolorektal kanser, *MXRA8*, prognoz, biyobelirteç, epitelyal-mezenkimal geçiş.

Article History: Oct 20, 2023; Revised: Dec 15, 2023; Accepted: Dec 25, 2023; Available Online: Feb 9, 2024.

DOI: https://doi.org/10.15671/hjbc.1378967

Correspondence to: N.Belder, Ankara University Biotechnology Institute, 06135, Ankara, Turkey

E-Mail: nevinbelder@gmail.com

INTRODUCTION

Colorectal cancer is the most common type of gastrointestinal cancer worldwide, ranking third in terms of both incidence and mortality among all cancer types [1]. Globally, in 2020, more than 1.9 million new cases of CRC were diagnosed, and 935,000 deaths were reported [2]. Although oncogene activation and tumor-suppressor inactivation as well as methylation and other epigenetic changes are known to be involved in CRC development, progression and also drug resistance [3,4], the exact molecular mechanism of carcinogenesis is poorly understood. Furthermore, despite a great progress in CRC diagnosis and therapy strategies, the unsatisfactory prognostic outcomes, poor therapy response and high mortality rates still exist due to the complex pathogenesis and heterogeneity of the CRC [5,6]. Hence, there is still a need for identifying prognostic and diagnostic biomarkers, as well as exploring new therapeutic molecules as alternatives for colon cancer.

MXRA8, Matrix Remodeling Associated 8, also known as limitrin, is transmembrane protein that can modulate the various signaling activites through binding integrin β3, thereby regulating interactions between cells. Additionally, *MXRA8* is also known as the receptor for arthritogenic alpha viruses providing entry into the cells [7]. High *MXRA8* expression has been reported in multiple malignancies when compared to normal tissues, and this elevated expression is associated with poorer survival. Xu et al. showed that the high expression of *MXRA8* was associated with unfavorable survivals and promotes progression of glioma. They suggested that the *MXRA8* is a potential candidate as a novel prognostic biomarker and therapeutic target for glioma [8]. *MXRA8* was one of the hub genes which is upregulated in thyroid cancer with lymphatic metastasis and significantly associated with tumor immune cell infiltration [9]. Cancer-associated fibroblasts (CAFs) are one of the most prevalent and prominent component of the tumor microenvironment (TME), playing a crucial role in mediating an immunosuppressive TME and promoting the cancer progression [10]. *MXRA8* has been reported as a marker for cancer-associated fibroblasts in pancreatic ductal adenocarcinoma (PDAC) recently [11]. Furthermore, *MXRA8* has also been shown to be involved in esophageal and gingivobuccal cancer [12,13] however, the current available information on *MXRA8*'s involvement in colorectal cancer is limited and insufficient. Further investigation is necessary to elucidate the specific role of *MXRA8* in colorectal cancer and to determine its potential applications as a diagnostic biomarker, prognostic indicator, or therapeutic target in this disease.

In the current study, we conducted a comprehensive bioinformatic analysis to characterize the potential role of *MXRA8* expression in colorectal cancer (CRC). Elevated levels of *MXRA8* gene expression were identified in tumor tissues compared to normal tissues, utilizing data from The Cancer Genome Atlas (TCGA), NCBI's Gene Expression Omnibus Database (GEO), CRC datasets, and several online databases. Importantly, overexpression of *MXRA8* was linked to a poorer prognosis in colorectal cancer. Co-expression and enrichment analyses were also performed to shed light on the potential underlying mechanisms of the *MXRA8* gene in colorectal cancer. Our findings reveal a positive correlation between high *MXRA8* expression and the process of epithelial-mesenchymal transition (EMT) in CRC. Taken together, these results indicate the *MXRA8*'s involvement in CRC carcinogenesis and its potential for the therapeutic applications in patients with CRC.

MATERIALS and METHODS

MXRA8 expression analysis

GEPIA2 (Gene Expression Profiling Interactive Analysis, version 2) online tool [14] was first utilized to compare the expression of *MXRA8* in colorectal cancer tissues based on TCGA COAD-READ datasets with their corresponding normal tissues. The parameters we employed for this analysis were as follows: p value cutoff of 0.05, log2FC (fold change) cutoff of 1, and the option to "Match TCGA normal data". To confirm the observed expression change of *MXRA8* in CRC, we assessed three different CRC datasets (GSE9348 (12 normal tissue and 70 CRC samples; Platform: GPL570), GSE44076 (148 normal tissue and 98 CRC samples; Platform: GPL13667) and GSE106582 (117 normal tissue and 77 CRC samples; Platform: GPL10558)) obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). In addition, the expression level of the *MXRA8* gene in various types of human cancers (TCGA project) was assessed and visualized using the Tumor Immune Estimation Resource, version 2 (TIMER2.0) (http://timer.cistrome.org/) [15].

Survival Analysis and prognostic significance of *MXRA8* gene

The association between high gene expression of

MXRA8 and advanced tumor progression (TCGA COAD-READ, GSE25701, GSE17536, GSE40967) as well as recurrence (GSE17536, GSE5206) was assessed using the GEO CRC datasets. $p<0.05$ was considered as statistically significant.

The GEPIA2 tool was utilized to analyze the prognostic significance of *MXRA8* in TCGA CRC samples by assessing overall survival (OS) and disease-free survival (DFS). Median expression of the *MXRA8* gene was used as the cutoff value for stratifying the patients. Independent CRC cohorts (GSE17536 and GSE40967) were obtained from the GEO database to further confirm the potential prognostic role of *MXRA8*. P values were obtained using the Log-rank test in GraphPad Prism 6.6, and a log-rank p-value less than 0.05 was considered statistically significant.

Co-expressed Genes, Gene Set Enrichment Analysis (GSEA) and Functional insights of *MXRA8*

Gene Set Enrichment Analysis (GSEA) from the Broad Institute (http://software.broadinstitute.org/gsea/downloads.jsp) was employed to investigate the impact of gene expression on tumors. To achieve this, samples in the GSE17536 CRC dataset were classified into two groups based on their *MXRA8* expression levels (high and low), and the enrichment of HALLMARK pathways was assessed in these two groups. For the GSEA analysis, we employed the Molecular Signatures Database (MsigDB) containing the Hallmark gene set (http://software.broadinstitute.org/gsea/msigdb). Significant Hallmark gene sets enrichments were identified based on the following criteria: Normalized Enrichment Score |NES| > 1, and False Discovery Rate (FDR) - q-value < 0.05, adhering to the GSEA threshold for significance. The analysis results revealed that patients with high-*MXRA8* colorectal cancer (CRC) showed significant enrichment of biological processes related to **epithelial-mesenchymal transition** and hypoxia. To assess co-expression between *MXRA8*-HIF1 and *MXRA8*-EMT markers in the GSE17536 CRC dataset, Pearson correlation coefficient evaluation was employed.

To further investigate the molecular mechanisms associated with *MXRA8*, the LinkedOmics analysis (http://linkedomics.org/login.php) [12] was employed to determine positively and negatively co-expressed genes with *MXRA8* in RNA-seq data for CRC patients in the TCGA cohort. by Pearson correlation analysis. The LinkedOmics database is a web-based platform that contains 32 different multi-omics datasets associated with TCGA cancer studies. LinkFinder module was performed to determine gene co-expressions related with *MXRA8*, and the results were displayed by volcano plot and heat map. Subsequently, we subjected these genes to GSEA enrichment analysis for KEGG pathway, and Hallmark gene sets analyses using the LinkInterpreter module of LinkedOmics tool.

Establishment of PPI network

GeneMANIA and STRING servers were utilized for the interaction analysis. GeneMANIA, accessible at https://genemania.org/, is an online tool that offers comprehensive insights into co-expression, protein interaction pathways, localization, and homology for protein domains of the genes provided as input. In this study, we employed GeneMANIA to construct a gene network comprising the genes interacting with *MXRA8*, and to investigate genes with functions similar to *MXRA8*. The gene interactions were visually depicted as a network, with nodes representing genes and links denoting their associations. Furthermore, we leveraged the STRING database, which encompasses predictions of both direct (physical) and indirect (functional) protein-protein interactions. This database was used to identify the functional protein partners of *MXRA8*. The selected interactions were restricted to the species "Homo sapiens," and only those with a combined score greater than 0.4 were considered for analysis.

Genetic alterations analysis

The interactive open-source platform cBioPortal, accessible at https://www.cbioportal.org/, offers extensive cancer genomics datasets for the exploration, visualization, and analysis of multidimensional cancer genomics data on a large scale. Here, cBioPortal database was utilized to conduct a comprehensive investigation of the genetic alterations (mutation, deep deletion, amplification etc.) of *MXRA8* in the TCGA CRC datasets containing 594 samples. The "cancer types summary" tab was also utilized for detection of the mutation frequency and mutation types associated with the *MXRA8* gene.

Statistical analysis

The nonparametric Mann–Whitney test was applied for the statistical analysis of the differences between two independent groups, such as tumor and normal. To assess overall survival and generate survival curves, we employed Kaplan-Meier survival analysis along with a log-rank test. Additionally, Pearson's correlation analysis was carried out to evaluate the correlation and in-

teraction, and logarithmic regression was employed to calculate R2 and derive the slope equation. Correlation strength was categorized based on the following criterias: r: 0.00-0.19=very weak, r: 0.20–0.39=weak, r: 0.40–0.59= moderate, r: 0.60–0.79=strong, and r: 0.80–1.0=very strong. Graphs were drawn with GraphPad Prism v6.01 (GraphPad Software Inc., La Jolla, CA). $p<0.05$ was considered to indicate statistical significance.

RESULTS

MXRA8 is up-regulated in colorectal cancer

To explore the potential involvement of *MXRA8* in colorectal cancer pathology, we first conducted a comparative assessment of *MXRA8* gene expression changes between CRC tissues and normal tissues. This analysis was performed in the TCGA CRC dataset through the GEPIA2 platform, revealing higher mRNA expression levels of *MXRA8* in TCGA-COAD tissues compared to normal tissues (Fig 1A). Upregulation of *MXRA8* in colorectal cancer was also confirmed in three independent GEO CRC data sets (GSE9348, GSE44076, GSE106582). As shown in Figure 1 (B, C and D), *MXRA8* expression in colorectal cancer was markedly elevated compared to normal tissues. In conclusion, these findings serve as evidence for the potential upregulation of *MXRA8* in CRC.

In addition, *MXRA8* expression was analyzed in the TIMER2.0 platform and found that *MXRA8* was expressed differently in different tumors. *MXRA8* was highly expressed, except COAD (colon adenocarcinoma) in CHOL (Cholangiocarcinoma), ESCA (esophageal carcinoma), HNSC (head and neck squamous cell carcinoma), KIRC (Kidney renal clear cell carcinoma), LUAD (lung adenocarcinoma), THCA (thyroid carcinoma), STAD (stomach adenocarcinoma) and in contrast, *MXRA8* expression in KICH (Kidney chromophobe), BRCA (Breast invasive carcinoma), BLCA (bladder urothelial carcinoma), LUSC (lung squamous cell carcinoma), and UCEC (uterine corpus endometrial carcinoma) was significantly lower

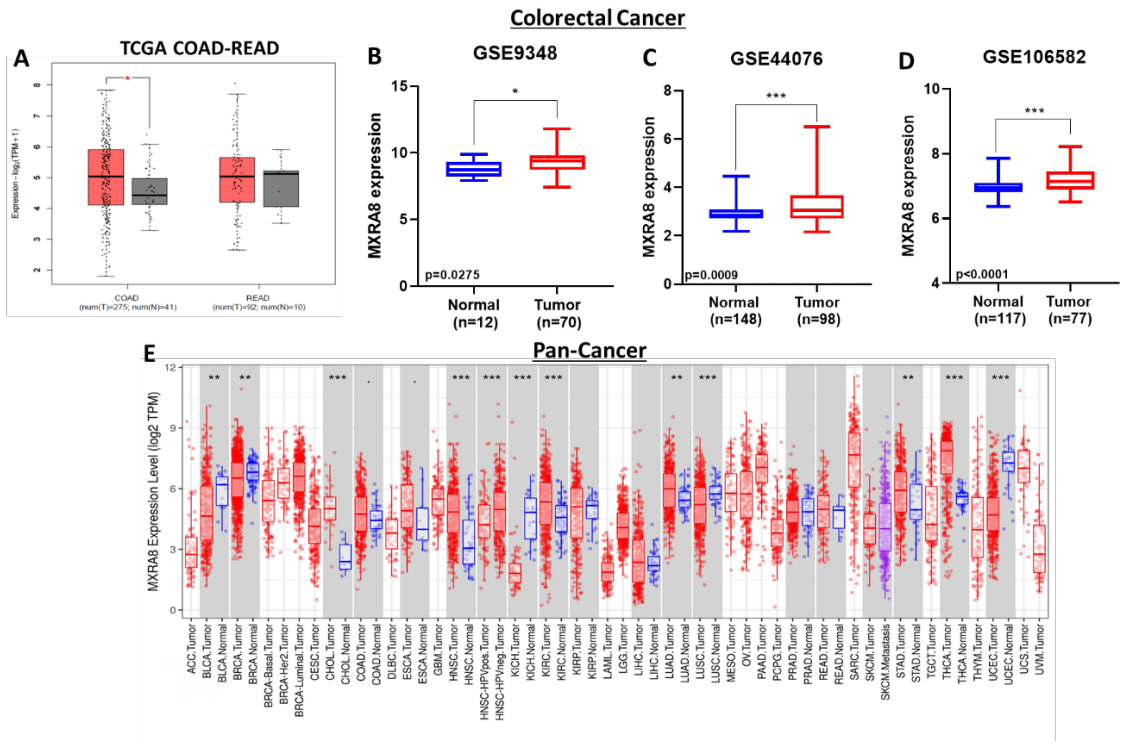

Figure 1. *MXRA8* expression levels in colorectal cancer and human malignancies. *MXRA8* mRNA expression profile in different human malignancies with normal control samples was analyzed (A) TCGA COAD-READ, (B) GSE9348, (C) GSE44076 (D) GSE106582. (E) Expression changes of *MXRA8* in different cancer types (Pan-Cancer). *p<0.05, ** p<0.01, ***p<0.001. Abbreviations: BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; FPKM, fragments per kilobase per million; KIRP, kidney renal papillary cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; ns, not significant; READ, rectum adenocarcinoma; TCGA, The Cancer Genome Atlas; THCA, thyroid carcinoma; PRAD, prostate adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

than that of normal tissues (Figure 1E). However, in the other TCGA cancer types such as adrenocortical carcinoma (ACC), pheochromocytoma and paraganglioma (PCPG), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), sarcoma (SARC), uterine carcinosarcoma (UCS), glioblastoma multiforme (GBM), and pancreatic adenocarcinoma (PAAD), *MXRA8* expression remained relatively unchanged or did not exhibit statistically significant differences.

Colorectal Cancer patients with overexpressed *MXRA8* correlates with poor prognosis

To examine the prognostic value of *MXRA8*, the correlations between the expression of *MXRA8* and the pathological stage of colorectal cancer patients were assessed. The expression of *MXRA8* showed an upward trend with the clinical stage in TCGA COAD+READ patients (Figure 2A). The correlation between tumor stage and *MXRA8* expression was further confirmed in three independent CRC datasets, GSE17536, GSE25701 and GSE40967, respectively (Figure 2B-D). Of note, the expression of *MXRA8* was significantly positively correlated with the advanced stage of CRC ($p < 0.05$). In addition, colorectal cancer patients with recurrence showed higher *MXRA8* expression, compared to patients with no recurrence (Figure 2D-E). Collectively, these data suggested that *MXRA8* might have a pivotal role in the progression of CRC.

The prognostic significance of elevated *MXRA8* expression was further investigated in TCGA CRC dataset through GEPIA 2.0 platform. Kaplan-Meier survival analysis demonstrated that high *MXRA8* expression was linked with the worst OS in TCGA COAD-READ (p=0.0017) (Figure 3A). Furthermore, TCGA COAD-READ patients with high transcriptional levels of *MXRA8* were significantly associated with a short disease-free survival (p = 0.032) (Figure 3B). GSE17536 and GSE40967 CRC datasets, as validation cohorts, were obtained from the GEO platform to demonstrate the reproducibility of the prognostic importance of *MXRA8*. The results demonstrated a significant correlation between elevated *MXRA8* mRNA expression and adverse outcomes in the patient group, including poor overall survival (OS) (p=0.0041), disease-specific survival (DSS) (p=0.0008), disease-free survival (DFS) (p=0.0055) in GSE17536, and DFS (p=0.019) in GSE40967, in contrast to patients having low *MXRA8* expression (Figure 3C-F). Taken together, high expres-

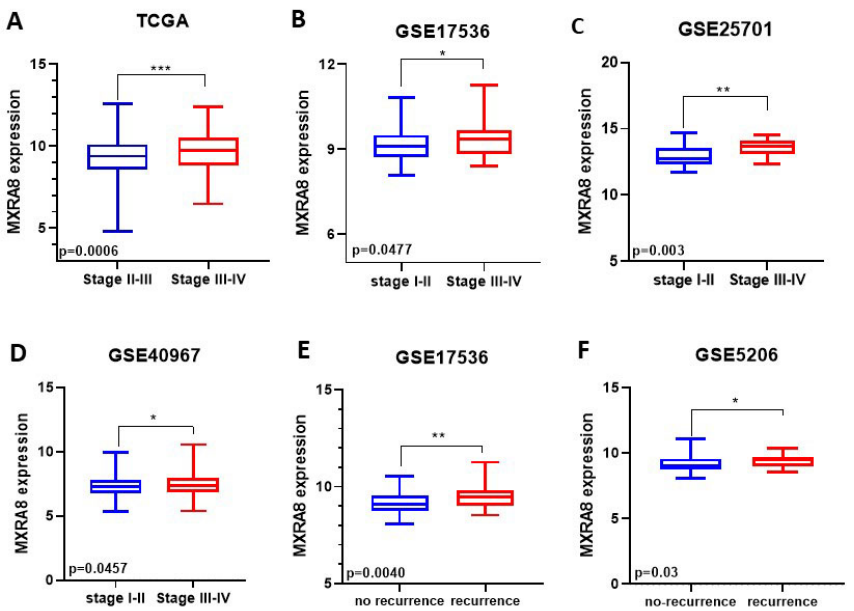

Figure 2. The prognostic value of *MXRA8* in CRC patients. Correlations between the expression of MXRA8 and tumor stage (A–D) and recurrence (E-F) in colorectal cancer patients. *p<0.05, ** p<0.01, ***p<0.001.

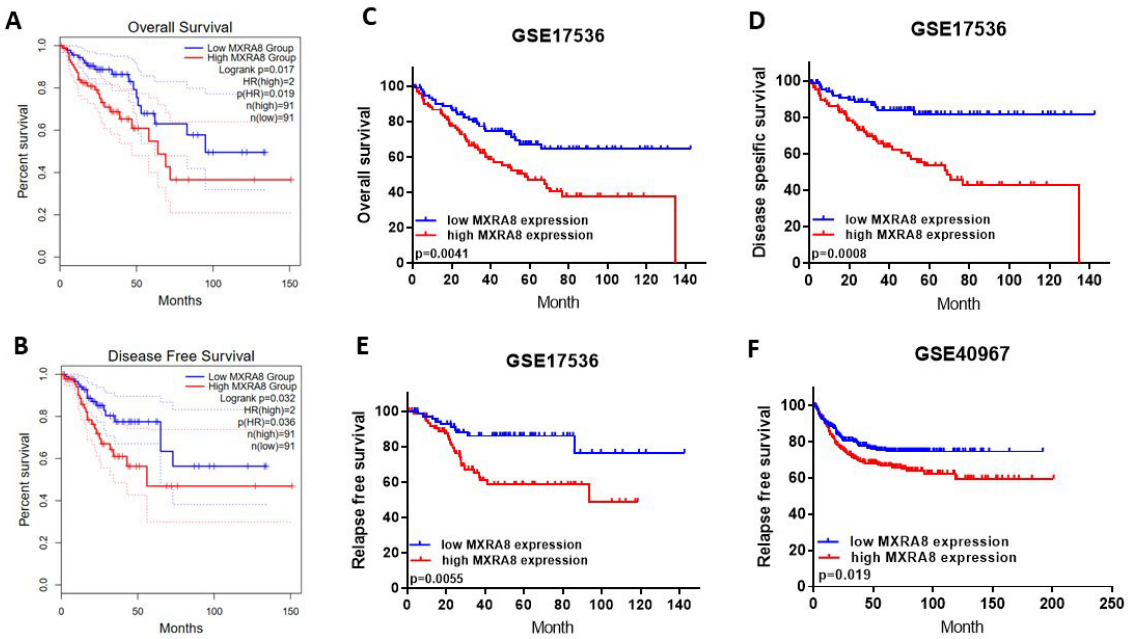

Figure 3. Survival analysis of the *MXRA8* gene in patients with colorectal cancer. The overall survival (OS) (A) Disease free survival (DFS) (B) curve of *MXRA8* in COAD+READ. GSE17536 (F). disease spesific survival (DSS) in GSE17536 (G) and relapse free survival (RFS) (H-J) in colorectal cancer patients based on the expression of MXRA8 in COAD+READ, GSE17536 and GSE40967, respectively. *P<0.05, ** P<0.01, ***P<0.001.

sion of *MXRA8* is prognostically relevant in CRC, and might be promising biomarkers for predicting survival.

MXRA8 Enrichment Analyses and Co-expression Networks

To identify the potential function of *MXRA8* in CRC, GSEA analysis was carried out in the high- and low-*MXRA8* groups from the GSE17536 CRC cohort, divided according to the median expression value. A total of 10 cancer hallmark gene sets were identified in the high-risk group (Figure 4A-J, supplementary table 1). The most enriched pathways in patients with high *MXRA8* expression are "HYPOXIA", "EPITHELIAL_MESENCHIMAL_TRANSMISION" and "ANGIOGENESIS" which are closely associated with the progression of CRC.

It has been known that epithelial-mesenchymal transition involved in driving human malignancies and sustaining tumor growth. Malignant tumors with aggressive subtypes, such as triple-negative breast cancer, which are associated with increased metastasis rates, frequently display elevated levels of EMT. Furthermore, during EMT, cancer cells evade apoptosis, leading to the promotion of cancer stem cells and stimulating angiogenesis while enhancing immunosuppression within the tumor microenvironment [16, 17]. Hypoxia can trigger the induction of EMT, leading to tumor invasion and metastasis and most studies indicate that the hypoxia-inducible factor 1 subunit alpha (HIF1A) is primarily responsible for initiating the EMT process in this context [18]. Based on our GSEA analysis results in CRC patients with high *MXRA8* expression, EMT and hypoxia were the most enriched gene sets, we evaluated the correlation of *MXRA8* expression with HIF1a and EMT-related genes. As represented by Figure 5A-F, the expression of *MXRA8* was positively correlated with HIF-1a ($r=0.372$, $p<0.0001$) and EMT-related genes such as ZEB1 ($r=0.84$, $p<0.0001$), ZEB2 ($r=0.75$, $p<0.0001$) vimentin ($r=0.81$, $p<0.0001$), MMP9 ($r=0.41$, $p<0.0001$) and SNAI1 ($r=0.35$, $p<0.0001$), suggesting that *MXRA8* plays a prominent role in progression of colorectal cancer by inducing EMT.

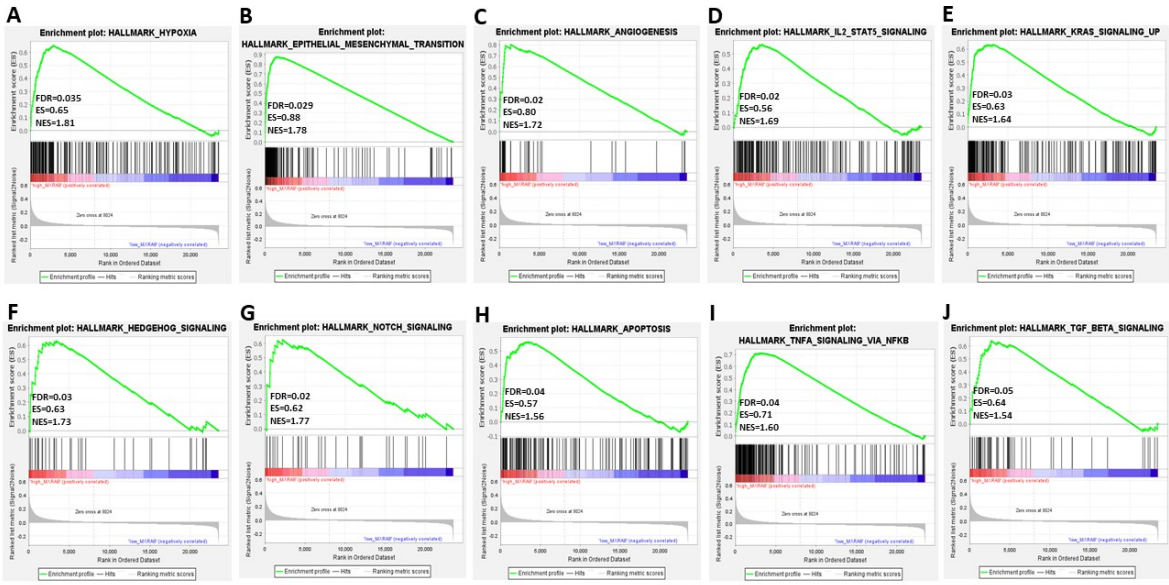

Figure 4. Gene set enrichment analysis (GSEA) in patient groups with high- and low-*MXRA8* expression for the GSE17536 CRC dataset. Ten cancer hallmark pathways exhibit significant enrichment in patients with CRC having elevated MXRA8 levels (FDR < 0.05, |NES| > 1). (A) Hypoxia (B) Epithelial-mesenchymal transition (C) Angiogenesis (D) IL2-STAT5 signaling (E) KRAS signaling up (F) Hedgehog signaling (G)Notch signaling (H) Apoptosis (I) TNFa signaling via NFkB (J) TGFb signaling.

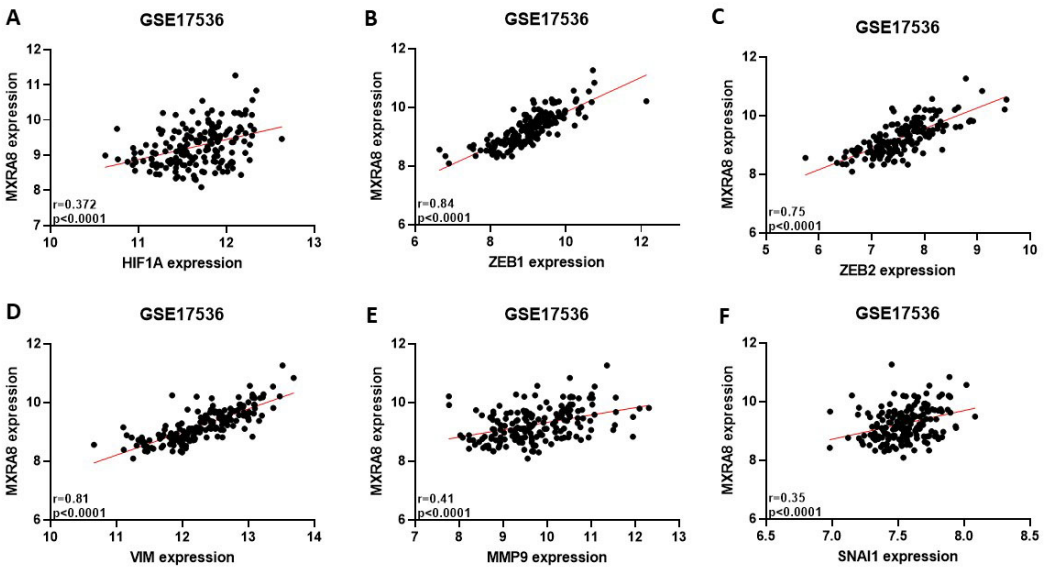

Figure 5. Correlation analysis of *MXRA8* mRNA expression and EMT-related genes. Pearson's correlations for *MXRA8* expression and HIF1 (A) ZEB1 (B) ZEB2 (C), VIM (D) MMP9 (E) SNAI1 (F) in GSE17536 CRC dataset. Pearson's r correlation coefficient was obtained for each data set.

For a more comprehensive understanding of the functional relevance of *MXRA8* in CRC, we conducted a co-expression analysis of *MXRA8* in the TCGA-CRC cohort by the LinkedOmics database. In the volcano plot representation, it's evident that out of 2804 genes linked to *MXRA8* expression, 2672 genes (indicated by dark red dots in the right panel) exhibit a positive correlation with *MXRA8*, while 132 genes (represented by dark green dots in the left panel) display a negative correlation with *MXRA8* (r>4, FDR <0.01; Figure 6A). The heatmaps in Figure 6 B&C depict the top 50 statistically significant genes exhibiting either positive or negative correlations with *MXRA8* (Figure 6B-C). Within the genes showing a positive correlation with *MXRA8* expression, the top three genes are EFEMP2 (Pearson's correlation = 0.96, adjusted p-value < 0.0001), SCARF2 (r = 0.96, adjusted p-value < 0.0001), and CCDC8 (Pearson's correlation = 0.95, adjusted p-value < 0.0001). (Supplementary table 2). GSEA Enrichment analysis revealed a primary association of these genes with hallmarks including epithelial-mesenchymal transition, myogenesis, TNFa via NFkB, and Apical junction (Figure 6D & Supplementary table 2). Additionally, Figure 6E shows KEGG results of the positively co-expressed genes with *MXRA8*. Notably, ECM-receptor interaction, Focal adhesion, and the cell adhesion molecules pathways were the most enriched pathways in TCGA COAD-READ datasets.

Prediction of Interaction networks for *MXRA8* gene

For subsequent investigations, we conducted an exploration of the gene-gene and protein-protein interactions (PPI) involving *MXRA8* using GeneMANIA and STRING online analytical tools. These platforms compile comprehensive data on various aspects, including co-expression, genetic interactions, co-localization, predictions of physical interactions, pathways involved, and shared protein domains.

The construction of gene-gene interaction networks of *MXRA8* and related neighboring genes was performed using GeneMANIA. The findings showed that the 20 genes most frequently linked to *MXRA8* alterations comprised TIMP3, ISLR, AEBP1, VIM, COL1A2, FBLN1, BGN, COL6A2, and etc. (Figure 7A). Most of the interacted genes were extracellular matrix components or associated with ECM-receptor interaction pathway. Furthermore, STRING analysis was performed to identify predicted protein partners of *MXRA8* at the protein level. The network of *MXRA8* and its interacting proteins consisted of 21 nodes and 36 edges. The PPI enrichment p-value was 0.00647, and the average local clustering coefficient was 0.845 (Figure 7B). Among the key nodes identified within this network were FAM20C, COL6A2, and ADRB2, which represent potential interacting protein partners of *MXRA8*. Consequently, these predicted interactions are likely to play a crucial role in regulating *MXRA8*-mediated cancer progression and prognosis.

Genomic alterations of *MXRA8* in CRC

Genomic alterations of the *MXRA8* were analyzed in TCGA colorectal cancer patients using the cBioPortal online data analysis tool. The results indicated that the *MXRA8* gene was altered in 56 (11%) of colorectal cancer patients, including high mRNA expression, missense mutations, truncating mutations, inframe mutation and deep deletion. Among the alterations, the most common type was "high mRNA expression" (Figure 8A). The mucinous colorectal cancer showed the highest mRNA expression frequency (Figure 8B). Both truncating and missense mutations were the main types of genetic alterations associated with the *MXRA8* gene. They were located in different protein domains with no apparent hotspot (Figure 8C).

Discussion

Colorectal cancer remains one of the most common cancers in both genders worldwide [19]. Based on the 2020 Global Cancer Statistics, approximately 1.9 million new CRC cases were reported, resulting in 935,000 CRC-related deaths. These figures represent 10% of global cancer incidents and 9.4% of cancer-linked deaths on a global scale [2]. Thus, there is a crucial need to explore more effective biomarkers and strategies for diagnosing, prognosing, and treating CRC [20].

MXRA8 is alternatively known as limitrin, encodes for a type I transmembrane protein and *MXRA8* serves as the receptor for arthritogenic alphaviruses [7]. Additionally, the expression of *MXRA8* is detected in adhesion molecules found in epithelial cells, bone marrow cells, and mesenchymal cells [21] and has been known modulating integrin signaling and cell–cell interactions [22]. Although the role of *MXRA8* in cancer development, diagnosis, progression, and treatment has not been fully explored, a limited number of studies have shown that *MXRA8* expression is elevated in certain cancer types, such as thyroid cancer [9] and kidney renal clear cell carcinoma [23]. However, the expression profile, potential functions, prognostic significance, and the role of *MXRA8* in the mechanisms governing colorectal can-

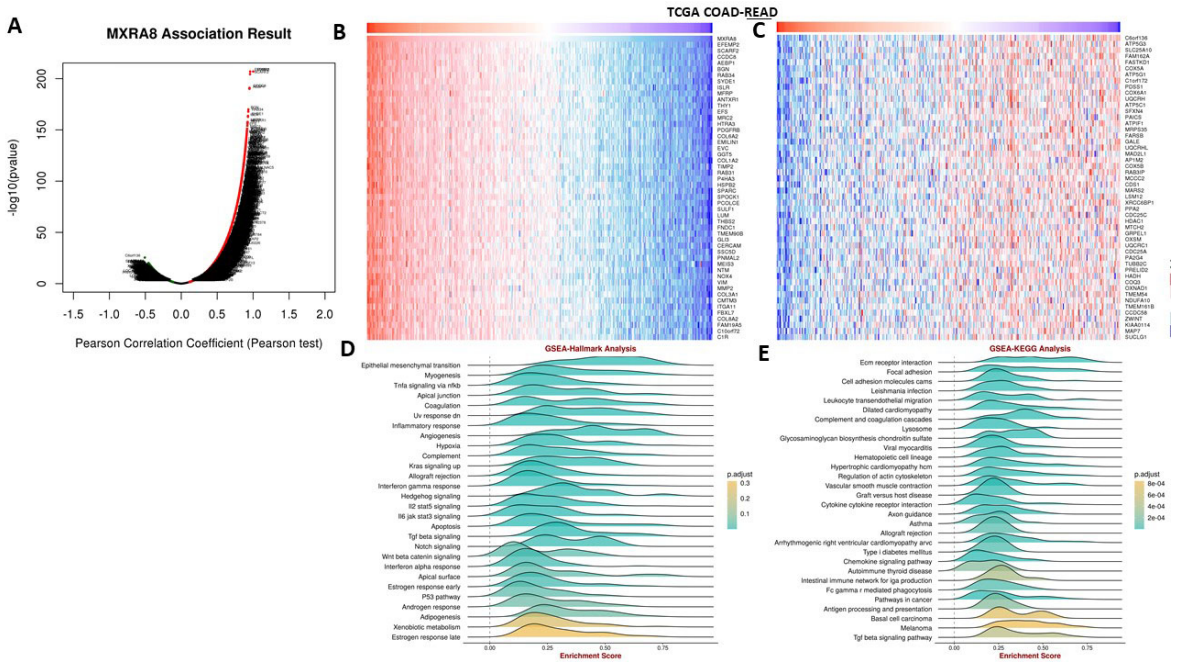

Figure 6. Enrichment analysis of *MXRA8* functional networks in TCGA COAD-READ dataset (LinkedOmics). (A) The genes strongly correlated with *MXRA8* as identified by Pearson test in colorectal cancer. (B & C) Heatmaps illustrating the top 50 genes that exhibit positive and negative correlations with MXRA8 in TCGA colorectal cancer dataset. (D–E) Significantly enriched GSEA hallmarks and KEGG pathways of genes associated with MXRA8 in TCGA CRC cohort. .

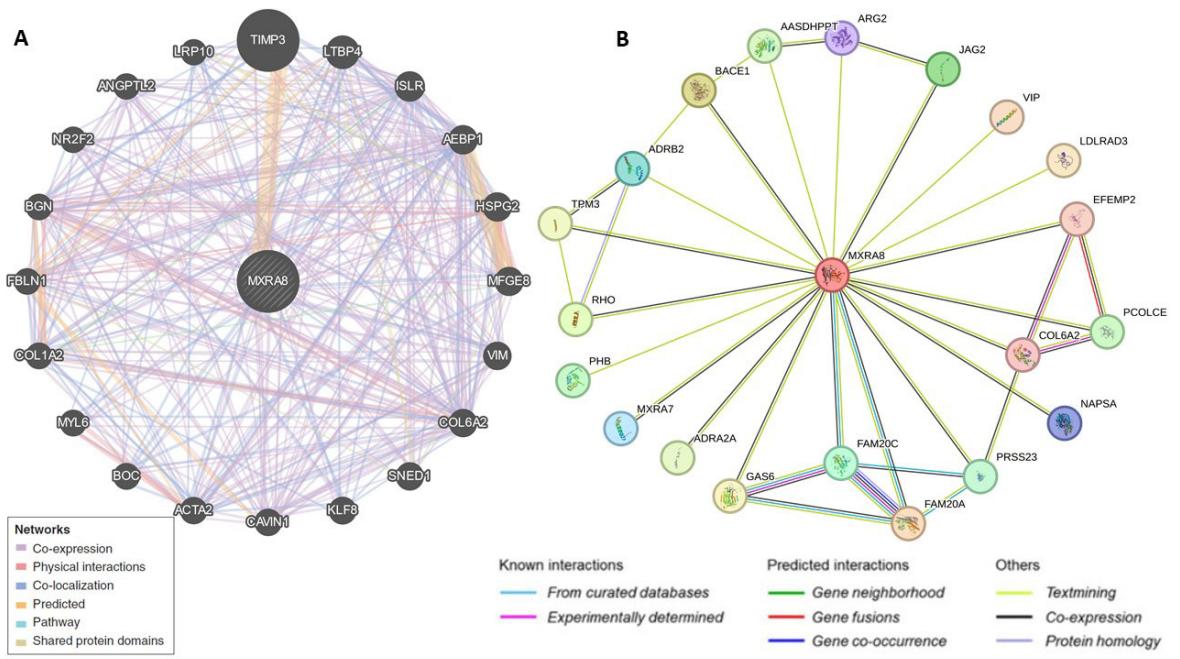

Figure 7. Gene-gene interaction network, as determined by GeneMANIA, and protein-protein interaction network, as derived from STRING. PPI network in the GeneMANIA (A) PPI network in the STRING tools with network clustering (B).

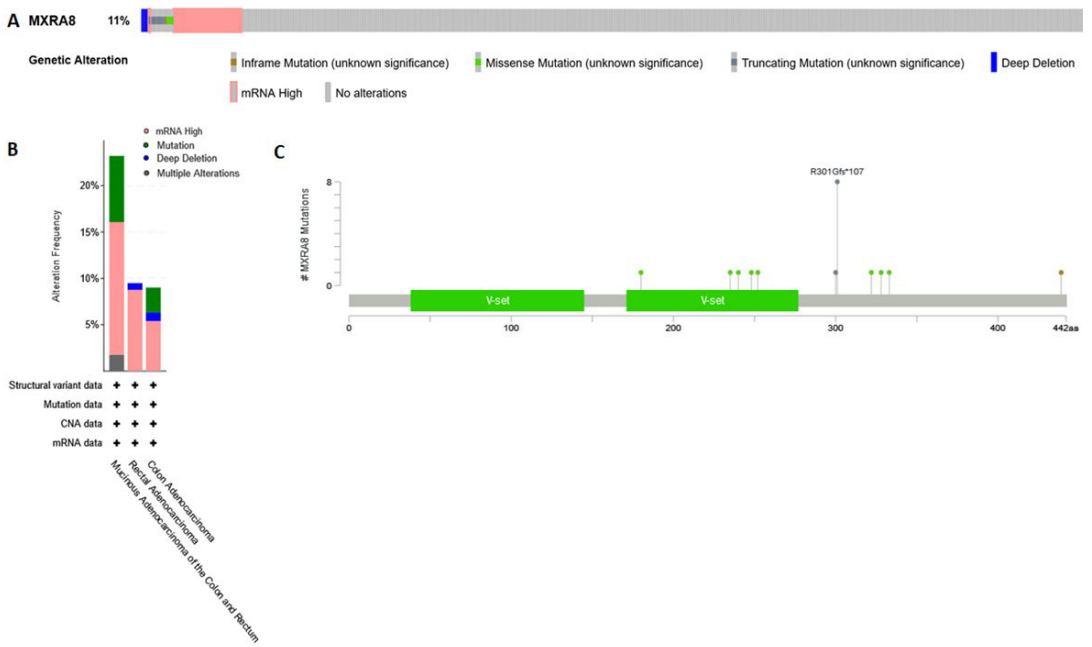

Figure 8. cBioportal analysis of TCGA colorectal cancer dataset for *MXRA8* expression. Distribution of *MXRA8* gene alterations across the CRC patient samples (%) (A) The alteration frequency based on CRC types (B) Mutation frequency of *MXRA8* gene and mutation sites (C).

cer development and progression still lack substantial clarity. Hence, in our present study, we thoroughly evaluated the expression and prognostic significance of *MXRA8* in CRC utilizing publicly accessible datasets. Furthermore, we explored the clinical implications and functional involvement of *MXRA8* in CRC pathology through bioinformatic analysis.

In our study, the mRNA level of *MXRA8* was first assessed in TCGA COAD-READ patients compared to normal tissues. Analysis of expression data for COAD-READ from the TCGA database revealed that *MXRA8* expression was significantly higher in colorectal cancer patients than in normal samples. To determine the potential of *MXRA8* as a biomarker, validation of its upregulation in different and independent tumor samples would be crucial. We observed a similarly significant upregulation of the *MXRA8* gene in CRC patients compared to healthy controls, highlighting the potential role of *MXRA8* in the growth and progression of colorectal cancer. Moreover, analysis of the TIMER 2.0 database revealed that *MXRA8* displayed significant differential expression in various cancers. We observed an upregulation of *MXRA8* expression in human COAD, CHOL, ESCA, HNSC, KIRC, LUAD, THCA, and STAD compared to their respective normal tissues. In contrast, *MXRA8* expression was downregulated in BRCA, BLCA, LUSC, UCEC, and KICH. These variations in *MXRA8* expression across different cancer types suggest potentially divergent biological functions for *MXRA8* in distinct types of cancer. This study also explored the expression of *MXRA8* in patients with various clinical characteristics. High *MXRA8* expression in CRC patients was found to be associated with a poor overall survival rate, suggesting its potential value as a diagnostic and prognostic marker. Additionally, the expression of *MXRA8* in the late stages and in patients with recurrence was significantly higher than in the early stages of colorectal cancer and in patients without recurrence. Collectively, the elevated *MXRA8* expression was associated with a poor prognosis for patients with CRC, indicating that *MXRA8* has the potential to serve as a predictive indicator for CRC prognosis. Research on *MXRA8*'s role in cancer is currently limited; however, substantial evidence points to its significant potential within the realm of cancer. Wu et al. showed that the expression of *MXRA8* was upregulated in the group with lymphatic metastasis in thyroid carcinoma (THCA) and associated with tumor immune cell infiltration [9]. Additionally, Ichihara et al. identified *MXRA8* as a new marker of cancer-associated fibroblasts (CAFs) in both human and mouse pancreatic ductal adenocarcinoma (PDAC) [11]. *MXRA8* expression was notably elevated in glioma in comparison to normal brain tissue, and it was associated with poorer survival outcomes. Furthermore, the study emphasized the significant involvement of *MXRA8* both ferroptosis and the immune

microenvironment within glioma [8]. *MXRA8* expression has also been shown to be increased in mammary tumors characterized by a high metastatic potential. In this study, Simpson et al. additionally proposed an association between *MXRA8* and breast cancer metastasis, given the pronounced elevation of *MXRA8* levels in lung metastases. Furthermore, high *MXRA8* expression correlated with unfavorable distant metastasis-free survival (DMFS) outcomes among patients diagnosed with breast cancer [24].

To gain a deeper understanding of *MXRA8*'s underlying biological role in CRC development, we conducted an investigation into its functional states using GSEA. Our results demonstrated a correlation between *MXRA8* and EMT as well as hypoxia in CRC. Additionally, we analyzed co-expression genes associated with *MXRA8* in CRC through the LinkedOmics database. GSEA and KEGG analyses revealed that these co-expression genes were implicated in EMT, hypoxia, ECM-receptor interaction, and Focal adhesion pathways. The interactions between *MXRA8* and its co-expression proteins were investigated using the GeneMania and STRING Databases. As a result, most of the interacted genes were extracellular matrix components or associated with the ECM-receptor interaction pathway. Enrichment and protein network analysis all indicated these *MXRA8*-related genes were closely connected with extracellular matrix, epithelial-mesenchymal transmission and hypoxia. Given the profound significance of *MXRA8* in colorectal cancer (CRC) development and progression through these interconnected pathways, it is essential to explore their implications. Within the tumor microenvironment (TME), a critical component, the dysregulation of the extracellular matrix (ECM), stands as a pivotal hallmark of cancer. Malignant cells contribute to the increasing of ECM stiffness, and in turn, the stiffened ECM modifies the structure of cancer cells during cancer development. Cancer is a complex and heterogeneous disease involving interactions between cancer cells, the ECM, and other cell types present in the TME. Consequently, ECM dysregulation assumes a crucial role in the cancer progression process, rendering it a promising target for cancer management [25,26]. A direct association exists between hypoxia and ECM composition and organization. Hypoxia influences the deposition, remodeling, and degradation of the extracellular matrix (ECM), potentially enhancing the metastatic potential of cancer [27]. In addition, hypoxia has been known as an important driver of EMT in cancer. Epithelial-mesenchymal transition is one of the important cellular biological process in which tumor epithelial cells undergo a loss of cell polarity and cell-cell adhesion while concurrently acquiring migratory and invasive properties, thus transforming into mesenchymal cells and undergoing metastasis [28]. Several components within the ECM, such as collagen, fibronectin, and MMPs, influence epithelial-mesenchymal transition. Likewise, hypoxia and HIF signaling have been shown to contribute to ECM remodeling through diverse mechanisms [29]. The analysis conducted in our present study revealed a comprehensive role for elevated *MXRA8* expression, dependent on its association with ECM-receptor interactions, hypoxia, and EMT pathways in CRC, enhancing our current understanding of its function during CRC development and progression.

In conclusion, the current study revealed that *MXRA8* is overexpressed and is associated with worse survival ratios in colorectal cancer patients. *MXRA8* has an important potential as both a promising prognostic indicator and therapeutic target for CRC. Furthermore, the high expression level of *MXRA8* may contribute the tumor development and progression through mechanisms such as EMT and hypoxia. However, to confirm its prognostic significance in colorectal cancer, additional in-depth experiments and further mechanistic studies are required.

Acknowledgments The author thanks Oğuzhan Tarman for his contributions to the formal arrangements of the figures.

References

1. L.H. Biller, D. Schrag, Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review, JAMA, 325 (2021) 669-685.
2. H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA Cancer J. Clin., 71 (2021) 209-249.
3. K. Forrester, C. Almoguera, K. Han, W.E. Grizzle, M. Perucho, Detection of high incidence of K-ras oncogenes during human colon tumorigenesis, Nature, 327 (1987) 298-303.
4. E.A. Kheirelseid, N. Miller, K.H. Chang, M. Nugent, M.J. Kerin, Clinical applications of gene expression in colorectal cancer, J. Gastrointest. Oncol., 4 (2013) 144-157.
5. Z. Fang, S. Xu, Y. Xie, W. Yan, Identification of a prognostic gene signature of colon cancer using integrated bioinformatics analysis, World J. Surg. Oncol., 19 (2021) 13.
6. J. Liu, C. Dong, G. Jiang, X. Lu, Y. Liu, H. Wu, Transcription factor expression as a predictor of colon cancer prognosis: a machine learning practice, BMC Med. Genomics, 13 (2020) 135.
7. R. Zhang, A.S. Kim, J.M. Fox, S. Nair, K. Basore, W.B. Klimstra, R. Rimkunas, R.H. Fong, H. Lin, S. Poddar, J.E. Crowe, Jr., B.J. Doranz, D.H. Fremont, M.S. Diamond, *Mxra8* is a receptor for multiple arthritogenic alphaviruses, Nature, 557 (2018) 570-574.

8. Z. Xu, X. Chen, L. Song, F. Yuan, Y. Yan, Matrix remodeling-associated protein 8 as a novel indicator contributing to glioma immune response by regulating ferroptosis, Front. Immunol., 13 (2022) 834595.
9. L. Wu, Y. Zhou, Y. Guan, R. Xiao, J. Cai, W. Chen, M. Zheng, K. Sun, C. Chen, G. Huang, X. Zhang, Z. Qian, S. Shen, Seven genes associated with lymphatic metastasis in thyroid cancer that is linked to tumor immune cell infiltration, Front. Oncol., 11 (2021) 756246.
10. R. Kalluri, The biology and function of fibroblasts in cancer, Nat. Rev. Cancer, 16 (2016) 582-598.
11. R. Ichihara, Y. Shiraki, Y. Mizutani, T. Iida, Y. Miyai, N. Esaki, A. Kato, S. Mii, R. Ando, M. Hayashi, H. Takami, T. Fujii, M. Takahashi, A. Enomoto, Matrix remodeling-associated protein 8 is a marker of a subset of cancer-associated fibroblasts in pancreatic cancer, Pathol. Int., 72 (2022) 161-175.
12. P.G. Bhosale, S. Cristea, S. Ambatipudi, R.S. Desai, R. Kumar, A. Patil, S. Kane, A.M. Borges, A.A. Schaffer, N. Beerenwinkel, M.B. Mahimkar, Chromosomal alterations and gene expression changes associated with the progression of leukoplakia to advanced gingivobuccal cancer, Transl. Oncol., 10 (2017) 396-409.
13. D. Zhang, C. Qian, H. Wei, X. Qian, Identification of the prognostic value of tumor microenvironment-related genes in esophageal squamous cell carcinoma, Front. Mol. Biosci., 7 (2020) 599475.
14. Z. Tang, C. Li, B. Kang, G. Gao, C. Li, Z. Zhang, GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res., 45 (2017) 98-102.
15. T. Li, J. Fu, Z. Zeng, D. Cohen, J. Li, Q. Chen, B. Li, X.S. Liu, TIMER2.0 for analysis of tumor-infiltrating immune cells, Nucleic Acids Res., 48 (2020) 509-514.
16. A.D. Redfern, L.J. Spalding, E.W. Thompson, The Kraken Wakes: induced EMT as a driver of tumour aggression and poor outcome, Clin. Exp. Metastasis, 35 (2018) 285-308.
17. G. Wang, D. Xu, Z. Zhang, X. Li, J. Shi, J. Sun, H.Z. Liu, X. Li, M. Zhou, T. Zheng, The pan-cancer landscape of crosstalk between epithelial-mesenchymal transition and immune evasion relevant to prognosis and immunotherapy response, NPJ Precis. Oncol., 5 (2021) 56.
18. H. Li, C. Peng, C. Zhu, S. Nie, X. Qian, Z. Shi, M. Shi, Y. Liang, X. Ding, S. Zhang, B. Zhang, X. Li, G. Xu, Y. Lv, L. Wang, H. Friess, B. Kong, X. Zou, S. Shen, Hypoxia promotes the metastasis of pancreatic cancer through regulating NOX4/KDM5A-mediated histone methylation modification changes in a HIF1A-independent manner, Clin. Epigenetics, 13 (2021) 18.
19. E.J. Kuipers, W.M. Grady, D. Lieberman, T. Seufferlein, J.J. Sung, P.G. Boelens, C.J. van de Velde, T. Watanabe, Colorectal cancer, Nat. Rev. Dis. Primers, 1 (2015) 15065.
20. X. Leng, J. Yang, T. Liu, C. Zhao, Z. Cao, C. Li, J. Sun, S. Zheng, A bioinformatics framework to identify the biomarkers and potential drugs for the treatment of colorectal cancer, Front. Genet., 13 (2022) 1017539.
21. A. Kumar, E. Rathi, S.G. Kini, Exploration of small-molecule entry disruptors for chikungunya virus by targeting matrix remodelling associated protein, Res. Pharm. Sci., 15 (2020) 300-311.
22. L. Tan, D. Fu, F. Liu, J. Liu, Y. Zhang, X. Li, J. Gao, K. Tao, G. Wang, L. Wang, Z. Wang, *MXRA8* is an immune-relative prognostic biomarker associated with metastasis and CD8(+) T cell infiltration in colorectal cancer, Front. Oncol., 12 (2022) 1094612.
23. S. Li, W. Xu, Mining TCGA database for screening and identification of hub genes in kidney renal clear cell carcinoma microenvironment, J. Cell Biochem., 121 (2020) 3952-3960.
24. K.E. Simpson, K.L. Watson, R.A. Moorehead, Elevated expression of miR-200c/141 in MDA-MB-231 cells suppresses *mxra8* levels and impairs breast cancer growth and metastasis in vivo, Genes (Basel), 13 (2022).
25. J. Huang, L. Zhang, D. Wan, L. Zhou, S. Zheng, S. Lin, Y. Qiao, Extracellular matrix and its therapeutic potential for cancer treatment, Signal Transduct. Target Ther., 6 (2021) 153.
26. J. Winkler, A. Abisoye-Ogunniyan, K.J. Metcalf, Z. Werb, Concepts of extracellular matrix remodelling in tumour progression and metastasis, Nat. Commun., 11 (2020) 5120.
27. D.M. Gilkes, G.L. Semenza, D. Wirtz, Hypoxia and the extracellular matrix: drivers of tumour metastasis, Nat. Rev. Cancer, 14 (2014) 430-439.
28. K. Saxena, M.K. Jolly, K. Balamurugan, Hypoxia, partial EMT and collective migration: Emerging culprits in metastasis, Transl. Oncol., 13 (2020) 100845.
29. R.Y. Hapke, S.M. Haake, Hypoxia-induced epithelial to mesenchymal transition in cancer, Cancer Lett., 487 (2020) 10-20.