Analysis of Expression Levels and Functions of MDK and PTN Genes in Colorectal Cancer

Fengqin Jiang
Huzhou Central Hospital
Huilian Huang
Huzhou Central Hospital
Yang Liu (liuyang2017@zju.edu.cn)
Huzhou Central Hospital https://orcid.org/0000-0001-8528-1355

Research Article

Keywords: MDK, PTN, colorectal cancer, biomarker, immune infiltration

Posted Date: February 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1314782/v1

Abstract

Purpose Colorectal cancer (CRC) is the third most common and the fourth most lethal type of cancer. Current therapies for CRC mainly include chemotherapy, radiotherapy, and surgery. However, the therapeutic effects of these therapies are not satisfactory for advanced CRC patients. Therefore, there has been ongoing research looking for better diagnostic targets that may give rise to more efficient interventions. Midkine (MDK) and pleiotrophin (PTN) are two important heparin-binding cytokines. These proteins are highly expressed in many human tumor cells, but have low or no expression in normal tissues.

Methods To identify novel diagnostic targets for CRC, we herein analyzed the expression patterns of *MDK* and *PTN* genes in colorectal cancer and normal samples by using The Cancer Genome Atlas (TCGA) and tumor immune estimation resource (TIMER) databases.

Results We found that both genes were abnormal expressed in CRC samples and involved in the regulation of immune response and cell metabolism, and the expression levels of these two genes had significant prognostic value.

Conclusions In conclusion, our results provide a comprehensive understanding of the functions of *MDK* and *PTN* genes in colorectal cancer.

Introduction

Colorectal cancer (CRC) is the third most common and the fourth most lethal type of cancer [1–3]. During early stages, CRC can be effectively managed via surgical resections. In fact, patients with early-stage CRC have a long survival period and can even be completely cured. Data show that the 5-year survival rate of patients with stage I CRC is higher than 85% [4]. However, for patients with stage IV CRC, 65% may experience recurrence after surgical resections [5] and most of them exhibit disease progression due to resistance to chemotherapies and targeted drugs [6]. As a result, the 5-year survival rate of patients with stage IV CRC is less than 10% [4]. Therefore, exploring novel targets for adjuvant therapy for CRC has become more and more important.

Midkine (MDK) and pleiotrophin (PTN) are two members of the heparin-binding cytokines family. The amino acid sequences of mammalian MDKs show 50% homology to those of mammalian PTNs [7, 8]. In humans, *MDK* and *PTN* are abnormally expressed in many kinds of cancer cells [9], such as neuroblastoma [10], bladder cancer [11], lung cancer [12, 13], breast cancer [14, 15] and thyroid papillary carcinoma [16, 17]. In addition, studies have shown that both *MDK* and *PTN* are abnormally expressed in many inflammatory diseases [18]. In a previous study, we found that the *MDK* gene was highly expressed in the CRC tissue relative to the normal tissue by immunohistology analysis, and the expression of *MDK* was closely related to the clinical stage and degree of malignancy of CRC. This finding was consistent with those of some other studies, which indicated that *MDK* was abnormally expressed in CRC; in addition, according to these studies, *PTN* was also expressed abnormally in CRC [19, 20]. However,

according to existing reports, the expression patterns of *MDK* and *PTN* in CRC remain controversial. To clarify the expression patterns of these two genes and unveil their underlying regulatory mechanisms, in this study, by comprehensively analyzing the RNA-seq data on *MDK* and *PTN* genes, we explored the expression characteristics and potential biological functions of the two genes in CRC. The findings were then combined with the clinical characteristics of CRC patients to guide the establishment of new prognostic markers for CRC.

Materials And Methods

Retrieval and processing of mRNA expression data

We collected RNA-seq profiles (level 3 HTSeq-FPKM format) from the CRC cohort deposited in The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/), which contains 454 tumor samples and 41 normal samples. Then the FPKM (Fragments Per Kilobase per Million reads) data were converted into the TPM (Transcripts Per Million reads) format and log2-transformed. For data filtering, the clinical information was retained and duplicate samples were removed. R (version 3.6.3) was used for statistical analysis of data, and the “ggplot2” (version 3.3.3) package in R was used for data visualization.

Characterization of the expression levels of MDK and PTN genes

Differences in mRNA expression levels of *MDK* and *PTN* genes between the CRC and normal samples of the TCGA cohort were identified by t-test. R (version 3.6.3) was used for statistical analysis of data, and the “ggplot2” (version 3.3.3) package in R was used for data visualization.

Gene set enrichment analysis (GSEA) of MDK and PTN genes

The LinkedOmics database (http://www.linkedomics.org/) [21] was used for GSEA. Genes significantly correlated with the expression of *MDK* and *PTN* were filtered by Pearson correlation analysis using the “LinkFinder” module of LinkedOmics. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed for the selected positively or negatively correlated genes by using the “LinkInterpreter” module of LinkedOmics.

Analysis of the correlation between MDK and PTN expression levels and immune infiltration

The tumor immune estimation resource (TIMER; https://cistrome.shinyapps.io/timer/) [22] database was used to analyze the correlation between *MDK* and *PTN* expression and the levels of infiltrating immune cells, such as dendritic cells, CD4+ T cells, neutrophils, CD8+ T cells, macrophages, and B cells. The stromal score (that captures the presence of stroma in tumor tissue), immune score (that represents the infiltration of immune cells in tumor tissue), and ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) score[23] of each CRC patient were also analyzed by corresponding R packages to determine the infiltration levels of immune cells and stromal cells in CRC tissues [24]. Statistical analysis was performed by adopting Pearson's correlation coefficients.

Survival analysis of MDK and PTN expression levels

Overall survival analysis was performed by R (3.6.3). The “survival” package (version 3.2.10) in R was used for statistical analysis of survival data, while the “surviviner” package (version 0.4.9) was used for visual analysis of the survival data. For data filtering, the duplicate samples were removed. The grouping method with the minimum p value was adopted for survival analysis. The prognostic data were provided by Liu Jianfang *et al.*'s article [25]. Receiver operating characteristic (ROC) curves were established by the “pROC” package (version 1.17.0.1) and visualized by the “ggplot2” package (version 3.3.3). For data filtering, duplicate samples were removed.

Statistical analysis

All statistical analyses were conducted using R (version 3.6.3) software in this study. The significance of the expression of *MDK*/*PTN* in tumor and normal tissues was analysis using the Student’s t test (two-tailed), the Mann-Whitney test (two-tailed), or one-way analysis of variance (ANOVA). Overall survival (OS) curves were analyzed by the log-rank test. The correlation analysis was conducted using Pearson correlation test or Spearman rank correlation test. $P<0.05$ was considered statistically significant.

Results

MDK and PTN exhibited abnormal expression levels in CRC samples

We first analyzed data from the TCGA cohort and found that *MDK* was significantly upregulated (P<0.0001), while *PTN* was significantly downregulated (P<0.0001) in CRC samples versus normal tissues (Fig. 1a and 1b). Pearson correlation analysis showed no statistically significant correlation between the mRNA expression levels of *PTN* and *MDK* (r=0.050, P=0.279), suggesting that the expression of *PTN* and *MDK* may be subjected to unrelated regulatory mechanisms during the occurrence and development of CRC (Fig. 2).

MDK and PTN were associated with multiple biological pathways in CRC

To further elucidate the biological functions of *MDK* and *PTN* in CRC, we performed GO and KEGG signaling pathway enrichment analyses based on the LinkedOmics database. The results showed that *MDK* was mainly enriched with immune response and inflammatory response pathways in CRC and was also involved in RNA metabolic process and cell cycle regulation (Fig. 1c and 1d). Meanwhile, *PTN* was mainly enriched with metabolic process and DNA replication pathways (GO pathways) in CRC (Fig. 1e and 1f).

MDK and PTN were correlated with immune functions in CRC

Based on the LinkedOmics database, we performed GSEA on *MDK* and *PTN* in CRC. The results showed that the expression of *MDK* was significantly positively correlated with that of genes involved in humoral immune response, adaptive immune response, T cell activation, lymphocyte-mediated immunity, cell

killing, regulation of inflammatory response, natural killer (NK) cell-mediated cytotoxicity, differentiation of Th1 and Th2 cells, intestinal immune network for IgA production, Th17 cell differentiation, inflammatory bowel disease, and autoimmune thyroid disease, and so on, in CRC ($p<0.05$, false discovery rate [FDR]<0.05; Fig. 3). Furthermore, the expression of *PTN* was significantly negatively correlated with that of genes implicated in NADH dehydrogenase complex assembly, tricarboxylic acid metabolic process, mitochondrial RNA metabolic process, protein localization to chromosome, cytoplasmic translation, DNA damage response, detection of DNA damage, pentose phosphate pathway, glyoxylate and dicarboxylate metabolisms, pyruvate metabolism, DNA replication, fructose and mannose metabolisms, citrate cycle (TCA cycle), and so on, in CRC ($p<0.05$, FDR<0.05; Fig. 4).

Associations of MDK and PTN with immune infiltration

Since our GSEA found that *MDK* and *PTN* played important immunological roles in CRC samples, we next sought to determine the relationships between *MDK* and *PTN* expression and the levels of infiltrating immune cells. Based on the TIMER database, we discovered that the expression of *MDK* was negatively correlated with tumor purity, and positively correlated with B cell, neutrophil and dendritic cell (DC) infiltration in CRC tissues (Fig. 5a). Meanwhile, *PTN* expression was also negatively correlated with tumor purity, but positively correlated with B cell, CD8+ T cell, CD4+ T cell, macrophage, neutrophil and dendritic cell infiltration in CRC tissues (Fig. 5a). Based on the “GSVA” package (1.34.0 version) of R (3.6.3 version), the correlations of *MDK* and *PTN* expression with the infiltration of various subtypes of aDCs (activated DCs), B cells, CD8+ T cells, cytotoxic cells, DCs, eosinophils, iDCs (immature DCs), macrophages, mast cells, neutrophils, NK cells, $CD56^{bright}$ cells [26], NK $CD56^{dim}$ cells [26], NK cells, pDCs (plasmacytoid DCs), T cells, T helper cells, Tcm (T central memory) cells, Tem (T effector memory) cells, Tfh (T follicular helper) cells, Tgd (T gamma delta) cells, Th1 cells, Th17 cells, Th2 cells, and Tregs (regulatory T cells) were comprehensively analyzed. The results (Fig. 5b, Table 1) were consistent with those obtained based on the TIMER database (Fig. 5a). We also calculated the immune score, stromal score, and ESTIMATE score for each CRC patient by using the “estimate” package (version 1.0.13) in R (version 3.6.3). Mann-Whitney U analysis revealed that the expression levels of *MDK* and *PTN* were both positively correlated with immune score, stromal score, and ESTIMATE score of the CRC patients (Fig. 5c).

Table 1
Correlation analysis between *MDK* and *PTN* expression and the infiltration of immune cells in CRC

	PTN		***MDK***	
Cell type	**Correlation coefficient (Pearson)**	p **value (Pearson)**	**Correlation coefficient (Pearson)**	p **value (Pearson)**
aDCs	0.192	<0.001	0.275	<0.001
B cells	0.298	<0.001	0.157	<0.001
CD8 T cells	0.238	<0.001	0.119	0.011
Cytotoxic cells	0.170	<0.001	0.335	<0.001
DCs	0.332	<0.001	0.248	<0.001
Eosinophils	0.355	<0.001	0.190	<0.001
iDCs	0.459	<0.001	0.328	<0.001
Macrophages	0.455	<0.001	0.178	<0.001
Mast cells	0.562	<0.001	0.199	<0.001
Neutrophils	0.262	<0.001	0.168	<0.001
NK CD56bright cells	-0.109	0.021	0.301	<0.001
NK CD56dim cells	0.096	0.041	0.242	<0.001
NK cells	0.454	<0.001	0.238	<0.001
pDCs	0.197	<0.001	0.084	0.074
T cells	0.205	<0.001	0.280	<0.001
T helper cells	0.222	<0.001	-0.139	0.003
Tcm cells	0.153	0.001	-0.127	0.007
Tem cells	0.268	<0.001	0.244	<0.001
Tfh cells	0.405	<0.001	0.214	<0.001
Tgd cells	0.378	<0.001	0.037	0.432
Th1 cells	0.292	<0.001	0.252	<0.001
Th17 cells	-0.180	<0.001	-0.101	0.031
Th2 cells	0.084	0.073	-0.097	0.038
Tregs	0.290	<0.001	0.300	<0.001

Prognostic analysis of MDK and PTN in CRC samples

We analyzed the overall survival (OS), disease-free survival (DSS) and progression-free interval (PFI) of CRC patients and found that low *MDK* expression was associated with a better prognosis in terms of OS (hazard ratio [HR]=1.58, 95% confidence interval (CI): 1.00-2.49; p=0.052) (Fig. 6a) and DSS (HR=2.26, 95% CI: 1.18-4.33; p=0.016) (Fig. 6b), but was not significantly associated with the PFI of CRC patients (HR=1.34, 95% CI: 0.93-1.94; p=0.116) (Fig. 6c). We also analyzed the associations between *MDK* expression and OS of CRC patients in the T1/T2 and T3/T4 stages. The results indicated that *MDK* expression correlated negatively with the OS of T1/T2-stage CRC patients (HR=2.99, 95% CI: 0.57-15.55; p = 0.194) (Fig. 6d) but positively with the OS of T3/T4-stage CRC patients (HR=1.62, 95% CI: 1.01-2.61; p=0.048) (Fig. 6e). Meanwhile, high *PTN* expression was associated with a better prognosis in terms of OS (HR=0.65, 95% CI: 0.44-0.96; p=0.031) (Fig.e 6f), but was not significantly associated with the DSS (HR=0.66, 95% CI: 0.40-1.09; p=0.103) (Fig. 6g) and PFI (HR=0.85, 95% CI: 0.58-1.23; p=0.378) (Fig. 6h) of CRC patients. We also analyzed the correlations between *PTN* expression and OS of CRC patients in the T1/T2 and T3/T4 stages. The findings revealed that *PTN* expression had no significant correlation with the OS of patients with T1/T2-stage CRC (HR=0.74, 95% CI: 0.14-3.80; p=0.715) (Fig. 6i) but had a negative correlation with the OS of patients with T3/T4-stage CRC (HR=0.67, 95% CI: 0.44-1.00; p=0.052) (Fig. 6j). In addition, the area under the ROC curve (AUC) values for the expression of *MDK* and *PTN* were 0.682 and 0.970, respectively, in CRC patients (Fig. 6k and 6l), indicating that the expression levels of *MDK* and *PTN* were closed related with the prognosis of CRC. Therefore, these two genes are potential biomarkers for CRC.

Discussion

MDK and PTN are two members of the heparin-binding growth factor family of cytokines. These two proteins are highly expressed in multiple embryonic and malignant tissues. MDK is a cysteine-rich protein with a molecular weight of 13 kDa [20, 27, 28], while PTN is a secretory protein with a molecular weight of 18 kDa [8]. Prior evidence suggests that MDK and PTN can interact with several proteins, such as anaplastic lymphoma kinase (ALK), syndecans (SDCs), receptor-type protein-tyrosine phosphatase (RPTP), low-density lipoprotein receptor related protein (LRP), integrins, neuroglycan C (NGC), and Notch [18], all of which are involved in promoting tumor growth, tumor invasion and angiogenesis [9, 29].

Many studies have demonstrated that both MDK and PTN are abnormally expressed in CRC tissues (Table 2). Aridome, K. et al. [27] analyzed the expression of MDK in various gastrointestinal carcinomas, including gastric cancer, liver cancer, pancreatic cancer, duodenal cancer, and esophageal cancer. They found that the mRNA and protein expression levels of MDK in CRC tissues were much higher than those in adjacent tissues. Barderas, R. et al. [20] found via stable isotope labeling by amino acids in cell culture (SILAC), a high-throughput proteomic analysis approach, that the protein level of MDK in KM12SM, a highly metastatic CRC cell line, was significantly higher than that in KM12C, a lowly metastatic CRC cell line. After the expression of MDK in KM12SM cells was knocked down by siRNA interference, the metastatic ability of the cells was significantly reduced. In addition, *MDK* gene had also been involved in

a six-gene prognostic signature (including *CD137L*, *CTSS*, *SOSTDC1*, *ZG16B*, *EFNA3* and *MDK*) for CRC [20]. It was found that the high expression of this signature was significantly correlated with a poor prognosis of CRC. The higher the expression of the signature genes, the shorter the survival and the worse the prognosis of CRC patients. However, some studies have also found that the expression of *MDK* gene was low in the serum of CRC patients. Kemik, O. et al. [28] found that the serum levels of albumin, MDK, adiponectin and ghrelin in esophageal, gastric, pancreatic, colon and rectal cancer patients were lower than those in the healthy control group. These findings were confirmed by another study from the same research group [30].

Table 2
Existing studies on *MDK* and *PTN* in CRC

Related studies and journals they are published in	Year of publication	Gene investigated	Main findings	Gene expression level
Mol Cell Proteomics [20]	2013	*MDK*	Knockdown of *MDK* caused a significant decrease in the migration and invasion abilities of highly metastatic cells.	High
Hum Exp Toxicol [30]	2012	*MDK*	*MDK* was lowly expressed in gastric cancer patients' serum.	Low
Int J Colorectal Dis [31]	2012	*MDK*	*MDK* was lowly expressed in colon cancer tissues.	Low
World J Surg Oncol [28]	2010	*MDK*	*MDK* was lowly expressed in colon cancer patients' serum.	Low
Jpn J Cancer Res [27]	1995	*MDK*	The increased expression of *MDK* in gastric carcinoma was more significant in well- and moderately-differentiated adenocarcinomas than in poorly-differentiated adenocarcinomas and signet ring cell carcinomas.	High
PLoS One [38]	2017	*PTN*	PTN is a secretory cytokine expressed in various cancer cell lines and human tumors such as colon cancer, lung cancer, gastric cancer and melanoma. It plays significant roles in angiogenesis, metastasis, cell differentiation and cell growth.	High
Int J Colorectal Dis [31]	2012	*PTN*	High *PTN* expression levels are accompanied by high VEGF expression and are predictive of a poor prognosis in CRC patients.	High
Recent Pat Anticancer Drug Discov [29]	2007	*PTN*	*PTN* is expressed at lower levels in CRC tissues than in adjacent normal mucosae.	Low
Cancer Lett [19]	1999	*PTN*	*PTN* and *PTPzeta* mRNA levels were decreased in CRC tissues as compared with adjacent normal mucosae.	Low
J Natl Cancer Inst [39]	1998	*PTN*	pancreatic cancer (n = 41; P<0.0001) and colon cancer (n = 65; P=0.0079) but not in patients with stomach cancer (n = 87; P=0.42)	High

PTN has also been implicated in CRC. According to Yamakawa, T. et al. [19] and Mikelis, C. et al. [29], the mRNA levels of *PTN* and protein tyrosine phosphatase zeta (*PTP zeta)* were decreased in CRC tissues compared with adjacent noncancerous tissues. However, another study found that the expression of *PTN* in CRC tissues was much higher than that in normal colorectal tissues [31]. Consistent with this study, serum levels of PTN in CRC patients were found to be significantly higher than those of healthy volunteers [31]. In the same study, *PTN* expression was also found to be associated with CRC prognosis and tumor node metastasis classification (TNM) stage [31]. Additionally, high levels of PTN were accompanied by high expression of vascular endothelial growth factor (VEGF) and were predictive of a poor prognosis in CRC patients [31].

According to the aforementioned existing studies on *MDK* and *PTN*, these two genes seem to have different expression patterns in CRC-most studies found that *MDK* was highly expressed and *PTN* was lowly expressed in CRC. This is consistent with the findings of our analysis of data from TCGA database-the expression of *MDK* in CRC tissues was significantly higher than that in adjacent normal tissues, while the expression of *PTN* in CRC tissues was significantly lower than that in adjacent noncancerous tissues. Although PTN and MDK share similar amino acid sequences [7] and have many common biological functions, such as those associated with neurodevelopment and tumor growth, but they have different expression patterns in CRC and play different roles in the regulation of CRC development. It is interesting that opposite expression patterns of *MDK* and *PTN* also exist in many other tumors, such as breast cancer, lung adenocarcinoma, esophageal carcinoma, kidney renal papillary cell carcinoma, and so on. The mechanism of this phenotype has not been confirmed, but we hypothesize that the different expression patterns may be associated with different tumor environments, and *MDK* and *PTN* may have participated in different biological processes. The GSEA results showed that *MDK* and *PTN* were associated with significantly different biological pathways in CRC. *MDK* was mainly involved in immune-related signaling pathways, while *PTN* was mainly involved in metabolic signaling pathways in CRC.

It has been reported that the *PTN* and *MDK* are aberrantly expressed under many inflammatory conditions, such as acute injury [32], hypoxia [33], atherosclerosis [34], and rheumatoid arthritis [35, 36]. However, it is not yet clear how they contribute to the inflammatory environment of cancer. In this study, the correlations between MDK and PTN expression and the infiltration of immune cells in CRC were analyzed based on the TIMER database. Interestingly, we found that the expression levels of *MDK* and *PTN* had significant correlations with the infiltration of different types of immune cells. For example, the infiltration of T helper cells and Tcm cells was negatively correlated with the expression of *MDK* but positively correlated with the expression of *PTN*. The infiltration of pDCs and CD8+ T cells was positively correlated with the expression of *PTN* but negatively correlated with the expression of *MDK*. In addition, the infiltration of CD8+ T cells, CD4+ T cells and macrophages was positively correlated with the expression of *PTN* but had no significant correlation with the expression of *MDK*. It is well known that CD8+ T cells, CD4+ T cells and macrophages can kill tumor cells. Among them, CD8+ T cells are the most lethal T cells that can kill antigen-expression tumor cells, and are important effector cells in anti-virus infection, acute allograft rejection and tumor cell elimination. Therefore, the number of CD8+ T cells

directly determines the body's ability of eliminating tumor cells [37]. This may explain why high *PTN* expression was associated with a higher survival rate of CRC patients in our study.

Limitation

Our study has several limitations. First, the exact mechanisms of action of *MDK* and *PTN* in CRC remain unclear. In addition, we are not sure if immune cells are the targets or the "providers" of abnormal expression levels of *MDK* and *PTN* in CRC. Due to these limitations, our study is still far from clarifying the detailed functions of *MDK* and *PTN* in CRC. Despite these limitations, our study lays a foundation for exploring the mechanisms of action of *MDK* and *PTN* in CRC, providing further support for the development of novel targeted drugs.

Declarations

Acknowledgments We would acknowledge the TCGA and GEO databases for providing the platforms and contributors for uploading their meaningful datasets. We also would like to thank TopEdit (www.topeditsci.com) for English language editing of this manuscript.

Author contribution All authors read and approved the final manuscript. The main contributions of each author are as follows: The authors' contributions were as follows: YL and HLH design the experiments, and drafting the article; FQJ collect and analyze the data. All of the authors reviewed and approved the final manuscript.

Funding The work was supported by the Zhejiang Provincial Natural Science Foundation of China under Grant No. LY17H160004, and the Key Project of Science and Technology Project of Huzhou City under Grant No. 2018GZ35.

Conflict of interest The authors declare no competing interests.

References

1. Atkin W, Wooldrage K, Parkin DM, et al. Long term effects of once-only flexible sigmoidoscopy screening after 17 years of follow-up: the UK Flexible Sigmoidoscopy Screening randomised controlled trial. Lancet. 2017;389(10076):1299–311. https://doi.org/10.1016/S0140-6736(17)30396-3.
2. Biller LH, Schrag D. Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. JAMA. 2021;325(7):669–85. https://doi.org/10.1001/jama.2021.0106.
3. Siegel RL, Miller KD, Goding Sauer A, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145–64. https://doi.org/10.3322/caac.21601.
4. Labianca R, Nordlinger B, Beretta GD, et al. (2010) Primary colon cancer: ESMO Clinical Practice Guidelines for diagnosis, adjuvant treatment and follow-up. Ann Oncol 21 Suppl 5:v70–77.

https://doi.org/10.1093/annonc/mdq168.

5. van der Stok EP, Spaander MCW, Grunhagen DJ, et al. Surveillance after curative treatment for colorectal cancer. Nat Rev Clin Oncol. 2017;14(5):297–315. https://doi.org/10.1038/nrclinonc.2016.199.
6. Skarkova V, Kralova V, Vitovcova B, et al. (2019) Selected Aspects of Chemoresistance Mechanisms in Colorectal Carcinoma-A Focus on Epithelial-to-Mesenchymal Transition, Autophagy, and Apoptosis. Cells 8(3). https://doi.org/10.3390/cells8030234.
7. Christman KL, Fang Q, Kim AJ, et al. Pleiotrophin induces formation of functional neovasculature in vivo. Biochem Biophys Res Commun. 2005;332(4):1146–52. https://doi.org/10.1016/j.bbrc.2005.04.174.
8. Xu C, Zhu S, Wu M, et al. Functional receptors and intracellular signal pathways of midkine (MK) and pleiotrophin (PTN). Biol Pharm Bull. 2014;37(4):511–20. https://doi.org/10.1248/bpb.b13-00845.
9. Kadomatsu K, Muramatsu T. Midkine and pleiotrophin in neural development and cancer. Cancer Lett. 2004;204(2):127–43. https://doi.org/10.1016/S0304-3835(03)00450-6.
10. Nakagawara A, Milbrandt J, Muramatsu T, et al. Differential expression of pleiotrophin and midkine in advanced neuroblastomas. Cancer Res. 1995;55(8):1792–7.
11. Lin H, Zhou Q, Wu W, et al. Midkine Is a Potential Urinary Biomarker for Non-Invasive Detection of Bladder Cancer with Microscopic Hematuria. Onco Targets Ther. 2019;12:11765–75. https://doi.org/10.2147/OTT.S235134.
12. Shin DH, Jo JY, Kim SH, et al. (2020) Midkine Is a Potential Therapeutic Target of Tumorigenesis, Angiogenesis, and Metastasis in Non-Small Cell Lung Cancer. Cancers (Basel) 12(9). https://doi.org/10.3390/cancers12092402.
13. Jager R, List B, Knabbe C, et al. Serum levels of the angiogenic factor pleiotrophin in relation to disease stage in lung cancer patients. Br J Cancer. 2002;86(6):858–63. https://doi.org/10.1038/sj.bjc.6600202.
14. Ibusuki M, Fujimori H, Yamamoto Y, et al. Midkine in plasma as a novel breast cancer marker. Cancer Sci. 2009;100(9):1735–9. https://doi.org/10.1111/j.1349-7006.2009.01233.x.
15. Chang Y, Zuka M, Perez-Pinera P, et al. Secretion of pleiotrophin stimulates breast cancer progression through remodeling of the tumor microenvironment. Proc Natl Acad Sci U S A. 2007;104(26):10888–93.
16. Jee YH, Sadowski SM, Celi FS, et al. Increased Pleiotrophin Concentrations in Papillary Thyroid Cancer. PLoS One. 2016;11(2):e0149383. https://doi.org/10.1371/journal.pone.0149383.
17. Choi YW, Kim YH, Lee J, et al. Strong immunoexpression of midkine is associated with multiple lymph node metastases in BRAFV600E papillary thyroid carcinoma. Hum Pathol. 2015;46(10):1557–65. https://doi.org/10.1016/j.humpath.2015.06.018.
18. Sorrelle N, Dominguez ATA, Brekken RA. From top to bottom: midkine and pleiotrophin as emerging players in immune regulation. J Leukoc Biol. 2017;102(2):277–86. https://doi.org/10.1189/jlb.3MR1116-475R.

19. Yamakawa T, Kurosawa N, Kadomatsu K, et al. Levels of expression of pleiotrophin and protein tyrosine phosphatase zeta are decreased in human colorectal cancers. Cancer Lett. 1999;135(1):91–6. https://doi.org/10.1016/s0304-3835(98)00275-4.
20. Barderas R, Mendes M, Torres S, et al. In-depth characterization of the secretome of colorectal cancer metastatic cells identifies key proteins in cell adhesion, migration, and invasion. Mol Cell Proteomics. 2013;12(6):1602–20. https://doi.org/10.1074/mcp.M112.022848.
21. Vasaikar SV, Straub P, Wang J, et al. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46(D1):D956–63. https://doi.org/10.1093/nar/gkx1090.
22. Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer research. 2017;77(21):e108–10. https://doi.org/10.1158/0008-5472.CAN-17-0307.
23. Yoshihara K, Shahmoradgoli M, Martínez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nature communications. 2013;4:2612. https://doi.org/10.1038/ncomms3612.
24. Yoshihara K, Shahmoradgoli M, Martinez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun. 2013;4:2612. https://doi.org/10.1038/ncomms3612.
25. Liu J, Lichtenberg T, Hoadley KA, et al. (2018) An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics Cell 173(2):400–16 e411. https://doi.org/10.1016/j.cell.2018.02.052.
26. Michel T, Poli A, Cuapio A, et al. Human CD56bright NK Cells: An Update. J Immunol. 2016;196(7):2923–31. https://doi.org/10.4049/jimmunol.1502570.
27. Aridome K, Tsutsui J, Takao S, et al. Increased midkine gene expression in human gastrointestinal cancers. Jpn J Cancer Res. 1995;86(7):655–61. https://doi.org/10.1111/j.1349-7006.1995.tb02449.x.
28. Kemik O, Sumer A, Kemik AS, et al. The relationship among acute-phase response proteins, cytokines and hormones in cachectic patients with colon cancer. World J Surg Oncol. 2010;8:85. https://doi.org/10.1186/1477-7819-8-85.
29. Mikelis C, Koutsioumpa M, Papadimitriou E. Pleiotrophin as a possible new target for angiogenesis-related diseases and cancer. Recent Pat Anticancer Drug Discov. 2007;2(2):175–86.
30. Kemik O, Kemik AS, Begenik H, et al. The relationship among acute-phase responce proteins, cytokines, and hormones in various gastrointestinal cancer types patients with cachectic. Hum Exp Toxicol. 2012;31(2):117–25. https://doi.org/10.1177/0960327111417271.
31. Kong Y, Bai P-S, Nan K-J, et al. Pleiotrophin is a potential colorectal cancer prognostic factor that promotes VEGF expression and induces angiogenesis in colorectal cancer. Int J Colorectal Dis. 2012;27(3):287–98. https://doi.org/10.1007/s00384-011-1344-z.
32. Sato W, Kadomatsu K, Yuzawa Y, et al. Midkine is involved in neutrophil infiltration into the tubulointerstitium in ischemic renal injury. J Immunol. 2001;167(6):3463–9.

33. Weckbach LT, Groesser L, Borgolte J, et al. Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis. Am J Physiol Heart Circ Physiol. 2012;303(4):H429–38. https://doi.org/10.1152/ajpheart.00934.2011.
34. Li F, Tian F, Wang L, et al. Pleiotrophin (PTN) is expressed in vascularized human atherosclerotic plaques: IFN-{gamma}/JAK/STAT1 signaling is critical for the expression of PTN in macrophages. FASEB J. 2010;24(3):810–22. https://doi.org/10.1096/fj.09-140780.
35. Maruyama K, Muramatsu H, Ishiguro N, et al. Midkine, a heparin-binding growth factor, is fundamentally involved in the pathogenesis of rheumatoid arthritis. Arthritis Rheum. 2004;50(5):1420–9.
36. Pufe T, Bartscher M, Petersen W, et al. Expression of pleiotrophin, an embryonic growth and differentiation factor, in rheumatoid arthritis. Arthritis Rheum. 2003;48(3):660–7.
37. Jansen CS, Prokhnevska N, Master VA, et al. An intra-tumoral niche maintains and differentiates stem-like CD8 T cells. Nature. 2019;576(7787):465–70. https://doi.org/10.1038/s41586-019-1836-5.
38. Zha L, He L, Xie W, et al. Therapeutic silence of pleiotrophin by targeted delivery of siRNA and its effect on the inhibition of tumor growth and metastasis. PloS one. 2017;12(5):e0177964. https://doi.org/10.1371/journal.pone.0177964.
39. Souttou B, Juhl H, Hackenbruck J, et al. Relationship between serum concentrations of the growth factor pleiotrophin and pleiotrophin-positive tumors. J Natl Cancer Inst. 1998;90(19):1468–73.

Figures

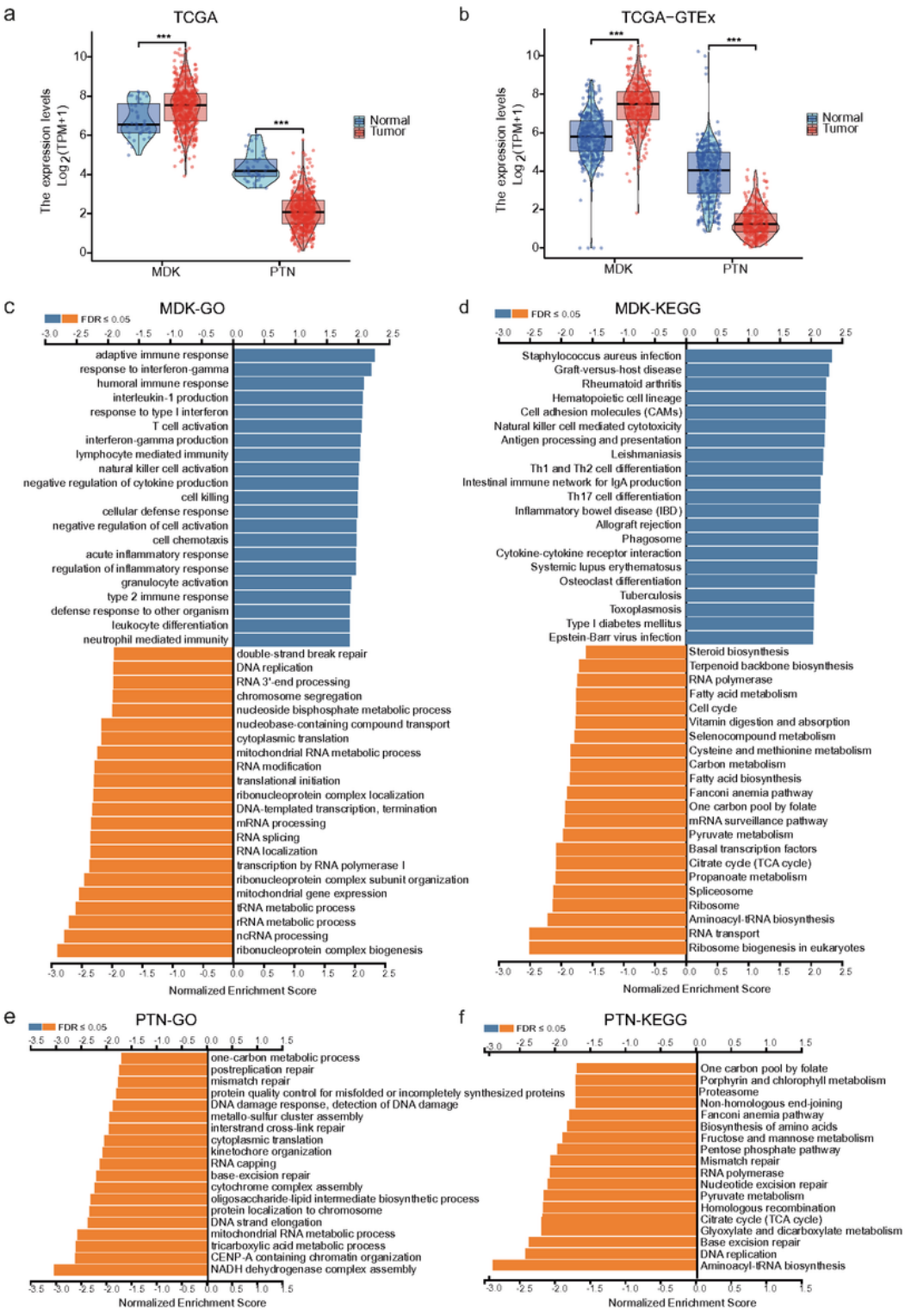

Figure 1

Expression levels and functions of *MDK* and *PTN* in CRC. Expression levels of *MDK* and *PTN* in CRC according to TCGA (a) and TCGA-GTEx (b) databases. GO analysis of *MDK* (c) and *PTN* (e) and KEGG analysis of *MDK* (d) and *PTN* (f) based on the LinkedOmics database.

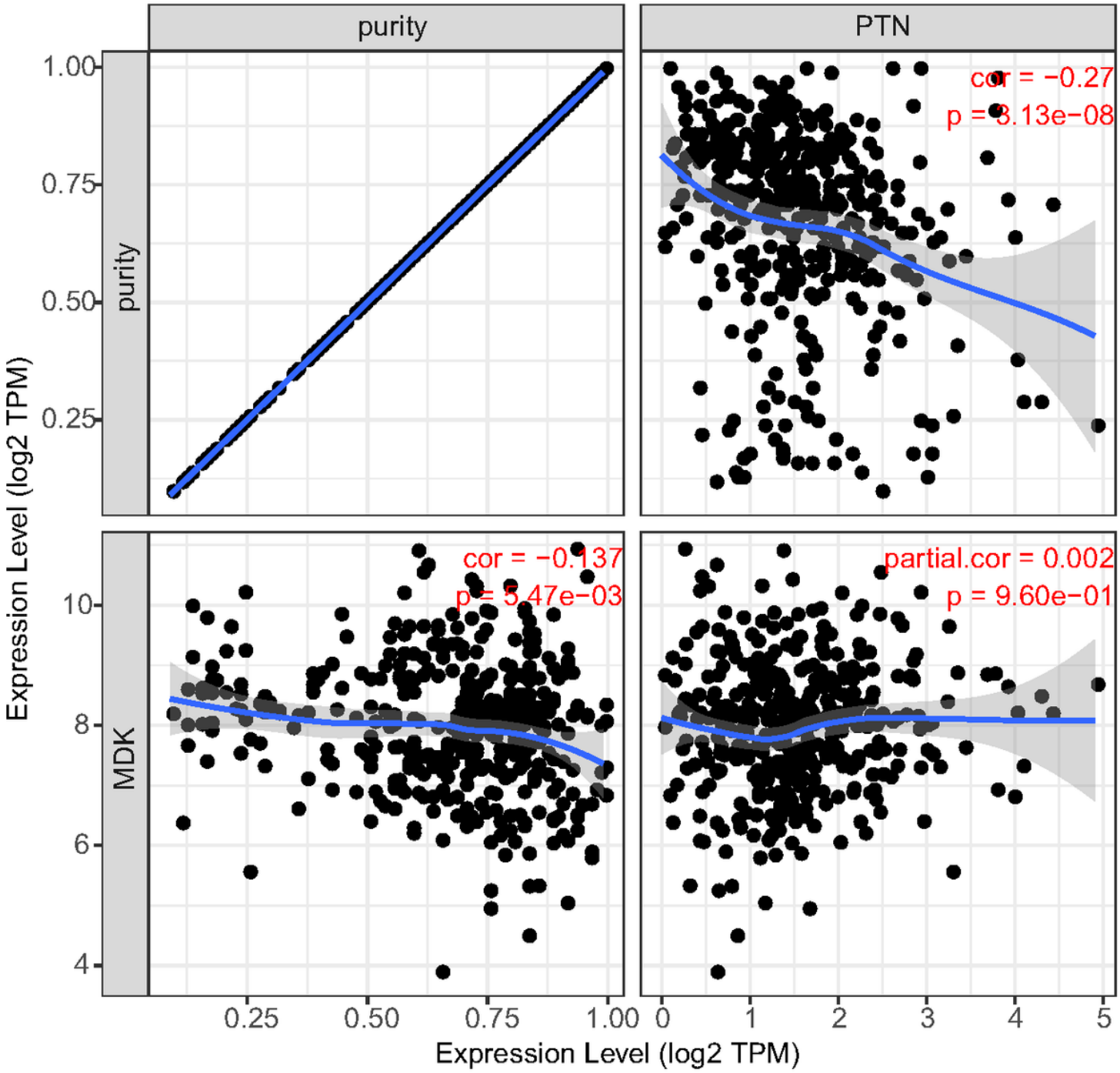

Figure 2

Correlations between expression levels of *MDK* and *PTN* and cancer purity.

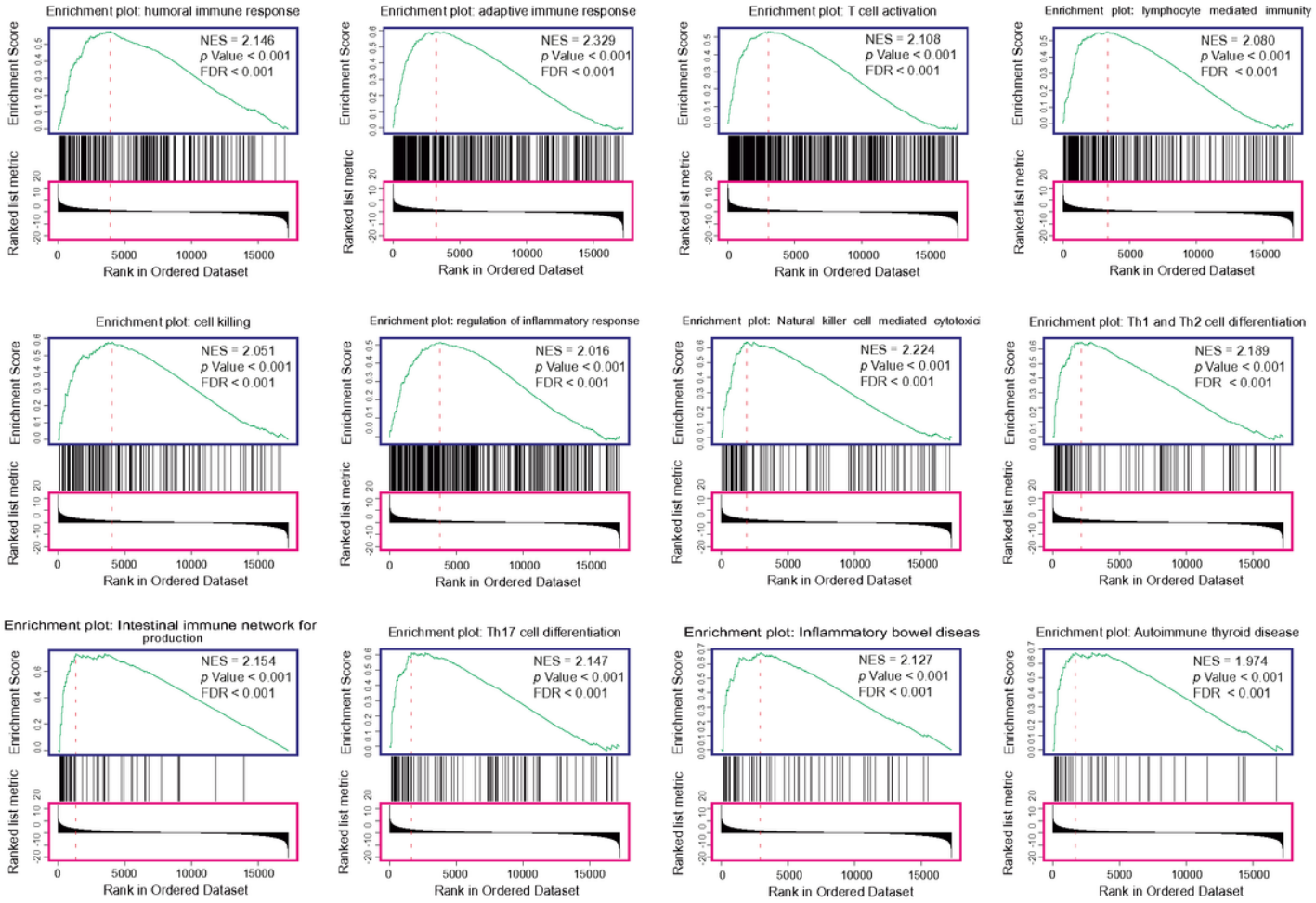

Figure 3

GSEA of *MDK* in CRC.

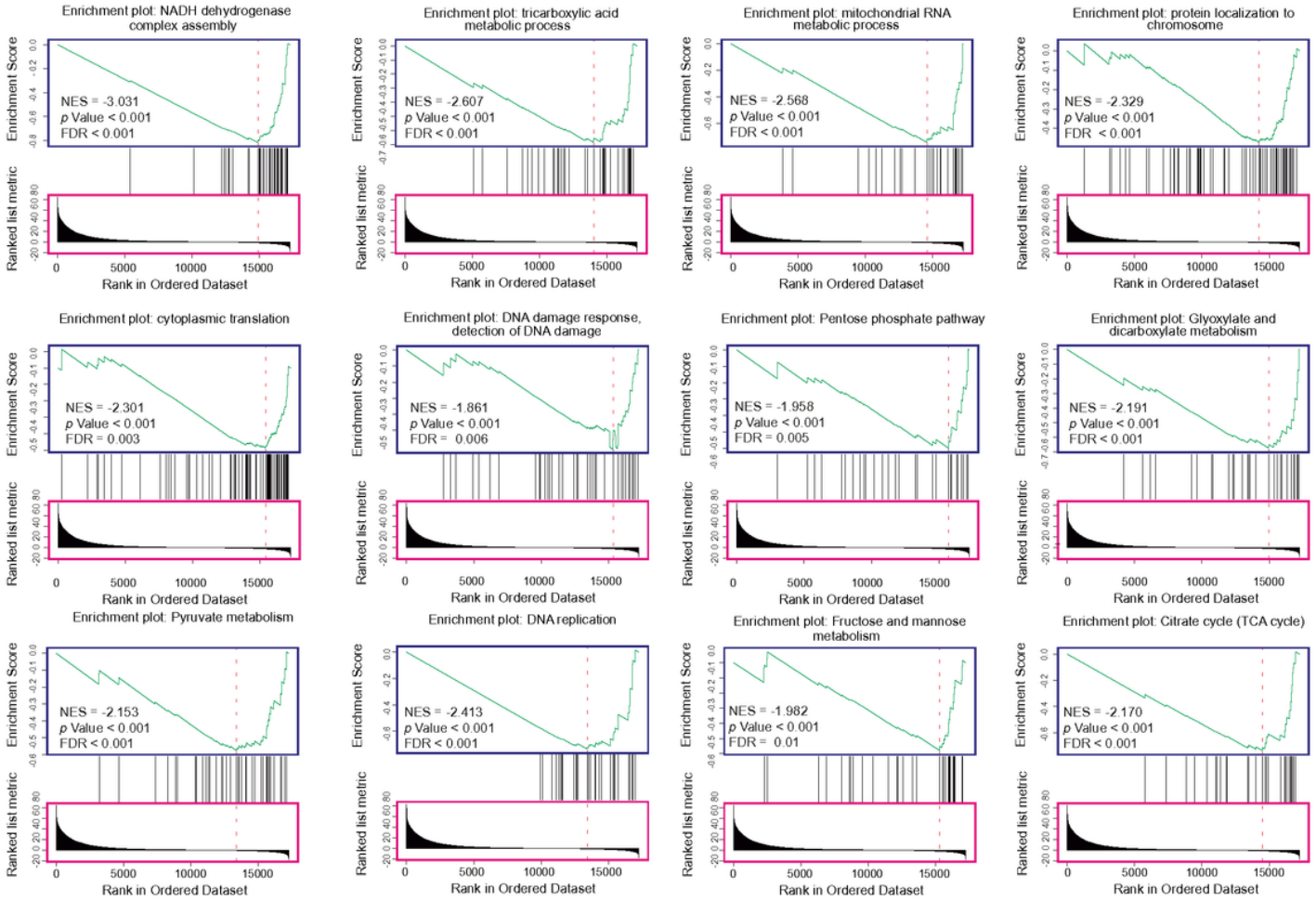

Figure 4

GSEA of *PTN* in CRC.

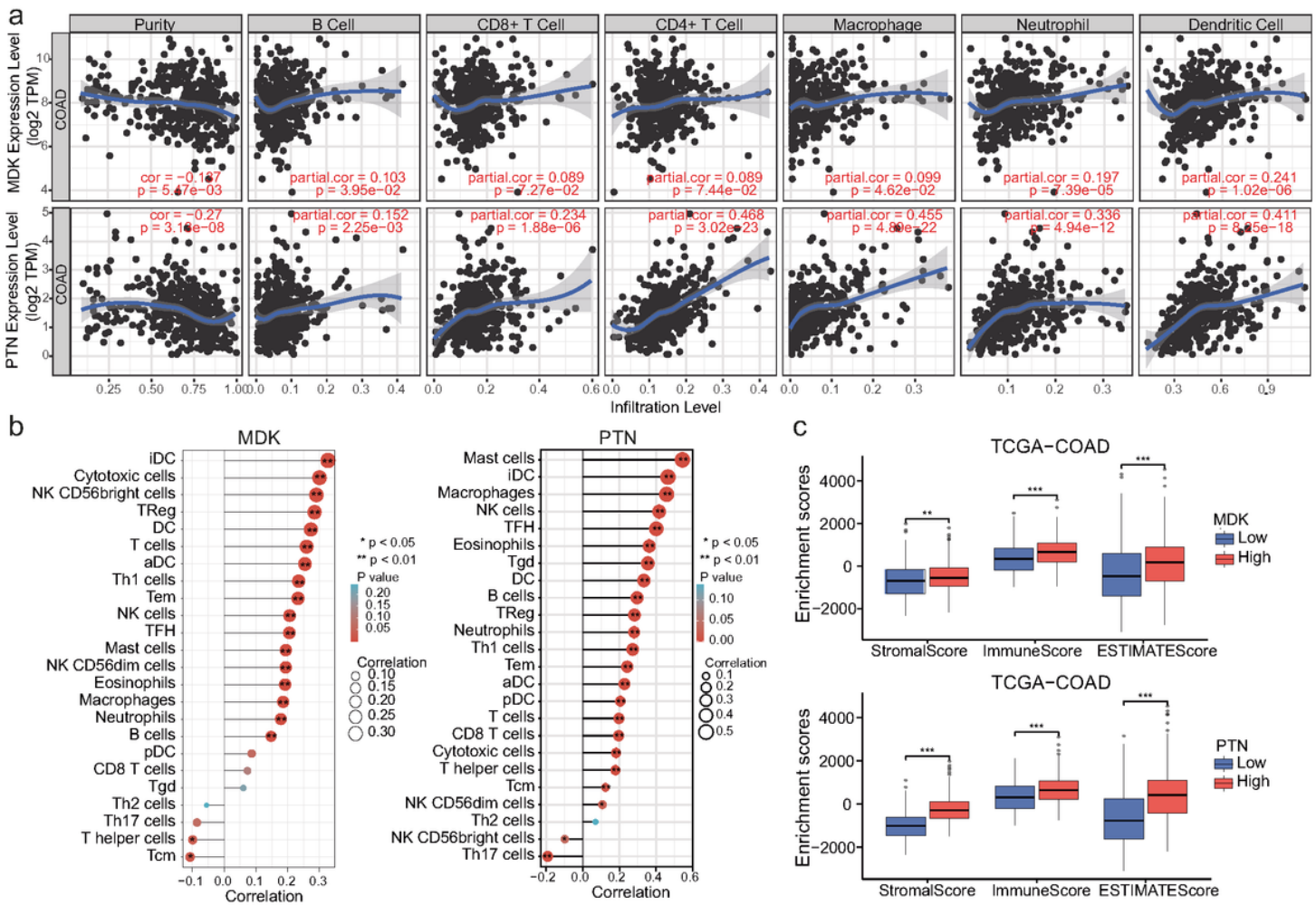

Figure 5

a, Correlations between expression levels of *MDK* and *PTN* and tumor purity, B cell infiltration, neutrophil infiltration and dendritic cell infiltration in CRC. b, Correlations between expression levels of *MDK* and *PTN* and the infiltration of various subtypes of immune cells. c, Correlations between expression levels of *MDK* and *PTN* and immune score, stromal score, and ESTIMATE score for each CRC patient.

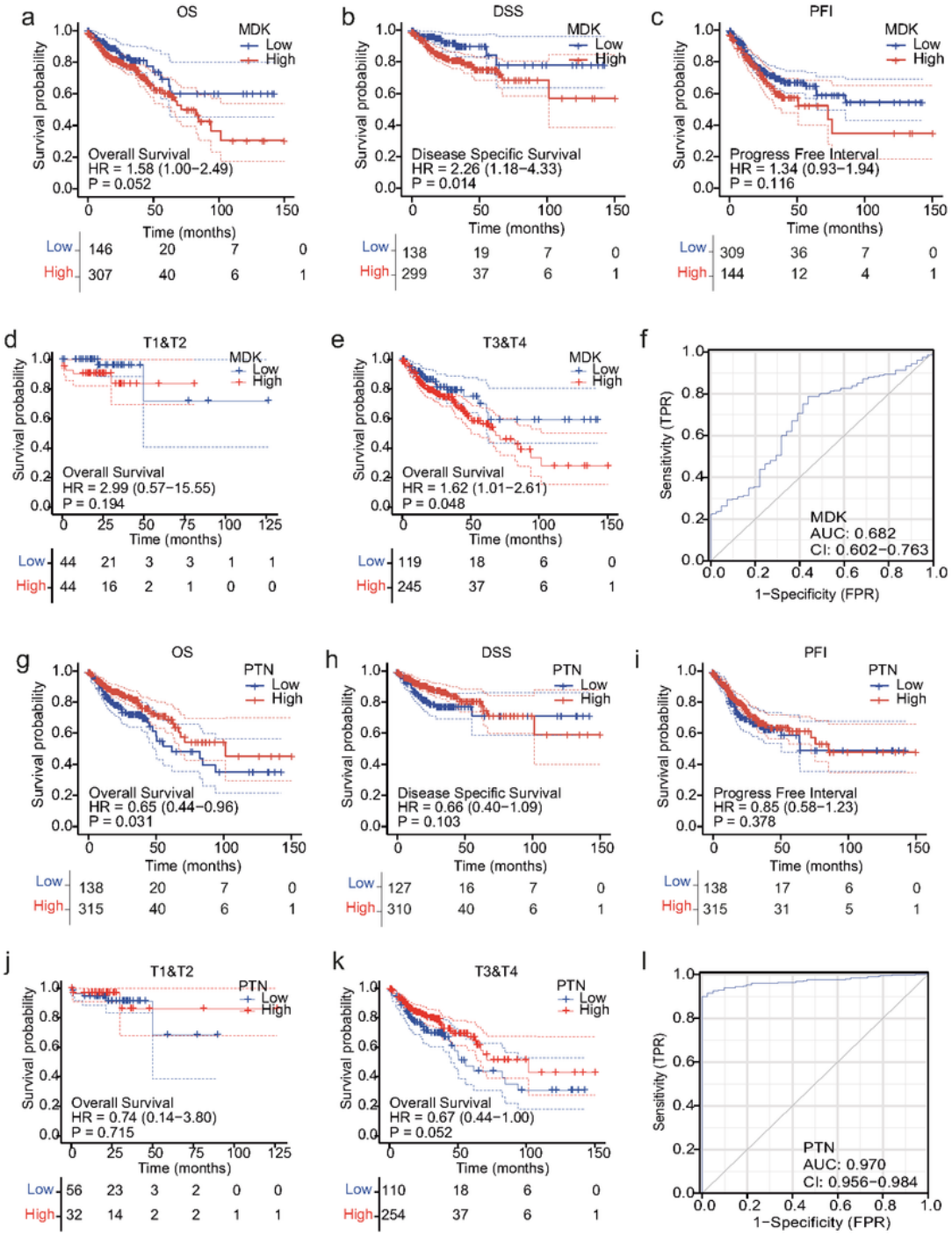

Figure 6

Prognostic value of *MDK* and *PTN* in CRC. The OS (a), DSS (b), and PFI (c) of CRC patients with different levels of *MDK* expression. The OS of T1/T2-stage CRC (d) and T3/T4-stage CRC (e) patients with different levels of *MDK* expression. The AUC of *MDK* expression in CRC patients (f). The OS (g), DSS (h), and PFI (i) of CRC patients with different levels of *PTN* expression. The OS of T1/T2-stage CRC (j) and

T3/T4-stage CRC (k) patients with different levels of *PTN* expression. The AUC of *PTN* expression in CRC patients (l).