JBUON 2021; 26(2): 513-520
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Prognostic and immunomodulatory effects of PIM1 in colorectal carcinoma

Yun Zhou[1], Yongning Zhou[2], Shixiong Liu[1], Jing Wang[1], Rui Ji[2], Xiang Yan[1]

[1]Department of Geriatrics (II), The First Hospital of Lanzhou University, Lanzhou 730000, P.R. China. [2]Department of Gastroenterology, The First Hospital of Lanzhou University, Lanzhou 730000, P.R.China.

Summary

Purpose: *Proviral integration of Moloney murine leukemia virus (PIM) family kinases can promote the survival and movement of carcinoma cells and the metastatic growth of various types of carcinoma. However, there are few studies on PIM1 in colorectal carcinoma (CRC), so we decided to investigate this issue.*

Methods: *Data about PIM1 expression and clinical and mutation information were downloaded from The Carcinoma Genome Atlas (TCGA). Survival analysis was performed by Kaplan-Meier method and the cumulative incidence of survival events was calculated. The correlation of PIM1 mRNA expression and immune infiltration score with the mutation index (TMB; tumor mutational burden), MSI (microsatellite instability)) was tested by Spearman's method. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of PIM1 enrichment and carcinoma-related pathways in CRC were performed.*

Results: *PIM1 was elevated in most carcinomas, especially CRC. In CRC, PIPM1 had a correlation with the overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). They indicated that PIPM1 acts in the progression of CRC. PIM1 was correlated with MSI, immune score and immune cell infiltration. Thereby it linked its expression with the evaluation of treatment response.*

Conclusions: *Bioinformatics analysis confirmed PIM1 as a good biomarker for the prognosis and treatment evaluation of CRC.*

Key words: *colorectal carcinoma, immunomodulation, PIM1, prognosis*

Introduction

Colorectal carcinoma (CRC) is the third most frequently diagnosed malignancy in the world and the fourth leading cause of carcinoma-related death [1]. About 21% of the patients are found to be in advanced stages at diagnosis [2]. Despite continuous efforts in prevention, screening and management, the incidence of CRC elevated by 38% from 2007 to 2017 [3]. In addition, even in the case of similar treatment, patients with the same clinical and pathological conditions show different clinical results [4]. The reason of different prognosis of CRC patients may be inherent genetic heterogeneity.

The pathogenesis of CRC is still unclear. However, more and more studies have shown that epithelial-mesenchymal transition (EMT) plays an important role in infiltration, metastasis and chemoresistance [5-7]. The proviral integration site of Moloney Murine Leukemia Virus (PIM) is a serine/threonine kinase, which has been found to be involved in many kinds of EMT processes of tumors. It is also involved in the cell cycle process, cell growth, cell survival and treatment resistance of tumor cells [8]. In MET-amplified non-small cell lung cancer (NSCLC) cell lines, application of MET

Corresponding author: Yongning Zhou, MD. Department of Gastroenterology, The First Hospital of Lanzhou University, No.1 Donggangxi Rd, Chengguan District, Lanzhou 730000, P.R. China.
Tel: +86-13893351869, Email: v259gx@163.com
Received: 27/01/2021; Accepted: 16/02/2021

inhibitor resulted in the up-regulation of PIM-1, which was the drug resistance mechanism. PIM inhibitor restored the sensitivity of MET inhibitor resistant cell lines to MET inhibition [9]. PIM1 promotes EMT and stemness of breast carcinoma cells induced by IL-6 through c-myc activation [10]. MicroRNA (miR)-486 inhibits the invasion and EMT of osteosarcoma cells by targeting PIM1 [11]. Therefore, determining the role of PIM1 in CRC is of great significance to the prognosis and treatment of this disease.

In this study we performed bioinformatics analysis to evaluate PIM1 in different tissues and its possible correlation with carcinoma. We found that PIM1 was highly expressed in almost all carcinoma types, especially CRC. The level of PIM1 was obviously correlated with the survival rate, immune cell function and tumor mutation status. PIM1 was identified as a new prognostic marker of CRC and an index of response to immunotherapy. In addition, it may become a potential target for carcinoma treatment.

Methods

Data collection and processing

Whole carcinoma sequencing data and corresponding clinical information were collected from The Carcinoma Genome Atlas (TCGA), including age, sex, tumor stage and clinical stage of patients. Robust multi-array average (RMA) function in R package (version 3.6.3) was applied to filter data. The lost and duplicate results were deleted. The data were conversed through log2 (TPM+1). tumor mutational burden (TMB) and microsatellite instability (MSI) data were from TCGA database. TMB is defined as the total mutation rate per million base pairs. MSI is defined as counting the number of insertion or deletion events occurring in the repeated sequences of genes.

Cox regression and survival analysis

Cox regression analysis was used to test the correlation of PIM1 levels with patients' overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI) and progression-free interval (PFI). The best separation method was applied to group patients into high and low PIM1 expression groups. Kaplan-Meier method was used to construct the survival curves of patients, and log-rank test was used to check the difference among the curves. P<0.05 indicates the differences were significant.

Immune cell infiltration and enrichment

Tumor immune estimation resource (TIMER) was used to calculate the infiltration of immune cells. It provides infiltration scores of six common types of immune cells, including B cells, CD4+Tc cells, CD 8+Tc cells, macrophages, neutrophils and dendritic cells [12,13]. Immune cell infiltration score of whole carcinoma data from TCGA was calculated by TIMER and archived online. Here, infiltration data were downloaded and used to test the correlation with PIM1 expression.

Functional enrichment analysis

Gene set enrichment analysis (GSEA, http://www.broadinstitute.org/gsea/index.jsp) was used to explain the functional enrichment of gene expression data. The functional enrichment of PIM1 with prognostic value was explored, and the first five gene ontologies (GO) related to autophagy and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were visualized [14].

Statistics

Spearman's correlation was applied to evaluate the correlation of YIF1B expression with the target, including immune cell infiltration score, TMB and MSI. According to whether the samples were paired, the expression level of PIM1 between groups or between tumor and normal tissue was compared by paired t-test or t-test. P values below 0.05 were considered significant. All graphs were generated by R packages of ggplot2 and forestplot.

Results

Analysis of the correlation of PIM1 expression level with the survival of CRC

The mRNA expression level of PIM1 in all tissues can be determined using data from TCGA, including different tissues of healthy people (Figure 1A). Similar to other carcinomas, PIM1 was highly expressed in CRC. Kaplan-Meier analysis of the relationship between PIM1 expression level and CRC survival showed that the OS of PIM1 high expression group was shorter than that of low expression group (Figure 1B). Cox regression analysis further examined the correlation between PIM1 and carcinoma survival, and PIM1 was significantly correlated with CRC survival (Figure 1C, p<0.001).

Correlation of PIM 1 expression level with prognosis of CRC

PIM1 expression data and clinical data in TCGA database were applied to evaluate the relationship between PIM1 and CRC prognosis. During follow-up, OS may be affected by non-carcinoma related deaths. Therefore, we re-analyzed the correlation between DSS and PIM1 expression in CRC, and the results of DSS analysis were similar to those of OS analysis, with lower DSS in PIM1 high expression group (Figure 2A). We also analyzed the correlation of PIM1 expression with DFI and PFI, and the results showed that there was a significant differ-

ence in survival between high expression and low expression group (Figure 2B,C). In addition, we also analyzed the expression of PIM1 in different stages of CRC, and the results showed that PIM1 expression level was positively correlated with the stage of CRC (Figure 2D).

Regulatory effect of PIM1 on the immune micro-environment of tumor by affecting immune infiltration

TMB and MSI are effective prognostic biomarkers and immunotherapy response indicators in many kinds of tumors. In order to study the relationship between PIM1 activity and mutation of specific carcinoma types, we explored the relationship between PIM1 expression and PIM1 activity in various carcinoma types. The results showed that there was no significant correlation between PIM1 expression and TMB (Figure 3A), but PIM1 level was positively correlated with MSI (Figure 3B). In addition, in order to evaluate whether the immune micro-environment of tumor may be affected by PIM1, we tested the correlation of PIM1 expression with immune score and immune cell infiltration level in CRC. The results showed that

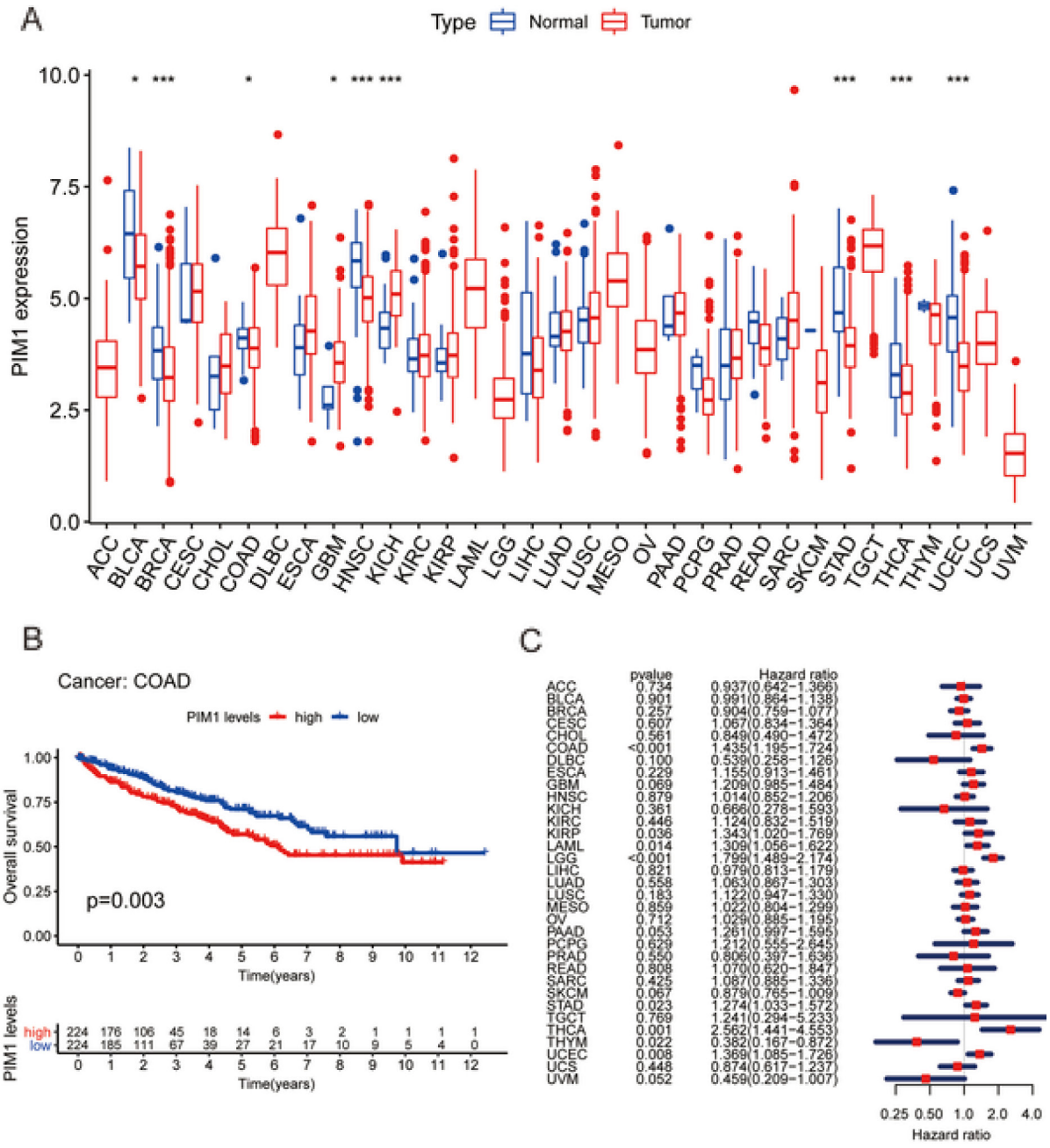

Figure 1. Correlation of PIM1 mRNA expression level with OS in multiple tumors in TCGA database. **A:** Expression of PIM1mRNA in different tumors. **B:** Correlation of PIM1 expression level with OS of CRC. **C:** Cox analysis of the survival and prognostic value of PIM1 in different tumors. P<0.05 was considered statistically significant.

PIM1 level was positively correlated with immune score and interstitial score (Figure 3C,D). The infiltration scores of six immune cell types (B cells, CD4+T cells, CD 8+T cells, neutrophils, macrophages and dendritic cells) available from the TIMER of TCGA were applied and it was found that PIM1 was obviously correlated only with CD4+T cell infiltration (Figure 3E).

Correlation of PIM 1 with immune checkpoint expression and analysis of functional enrichment

At present, several genes are closely related to immune response and are regarded as checkpoint components. The mRNA sequence database was applied to assess the link between PIM1 expression and gene expression at such checkpoints. The analysis showed that PIM1 was highly correlated with tumor necrosis factor (TNF)-related immune genes (TNFRSF4, 8, 9, 15, 18) and CD274 in CRC ($p<0.05$), indicating that PIM1 is co-expressed with most immune checkpoint genes (Figure 4A). In addition, PIM1 was grouped according to the median value in CRC. GO and KEGG function enrichment analysis were further performed to explore the other functions of PIM1 in the progression of CRC. The results showed that highly expressed GO enrichment of PIM1 included Amide binding, Central_nervous_system_neuron_differentiation, Cytosolic calcium ion transport, and Early endosome, Glutamatergic synapse. KEGG enrichment included Allograft rejection, Cell adhesion.molecules CAMS, Cytokine cytokine receptor interaction, and Focal adhesion, T cell receptor signaling pathway (Figure 4B,C).

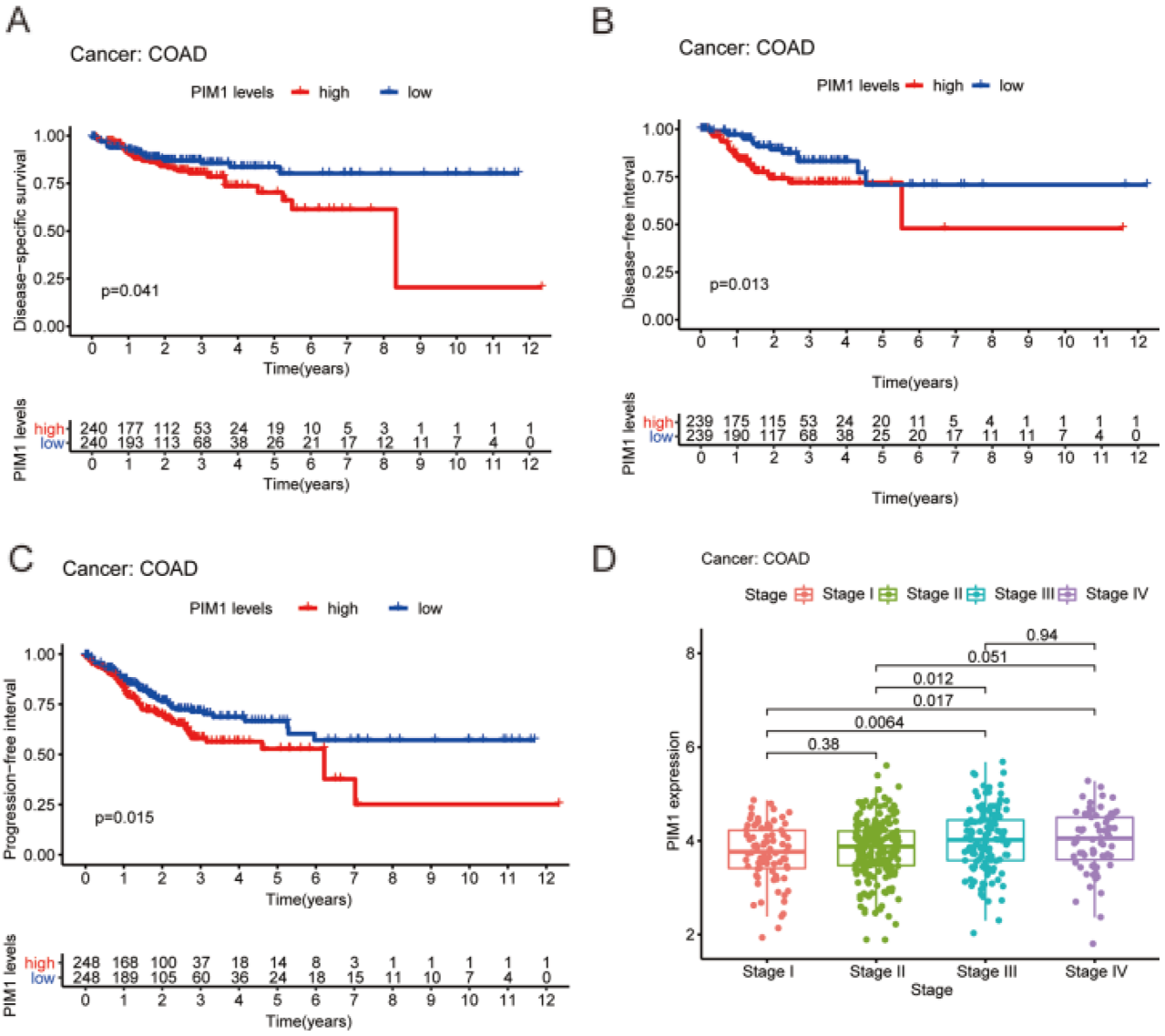

Figure 2. Correlation of PIM 1 expression level with prognosis of CRC. **A-C:** Correlation of PIM1 mRNA expression level with DSS, DFI and PFI in multiple tumors in TCGA. **D:** The expression level of PIM1 mRNA in different stages of CRC. P<0.05 was considered statistically significant.

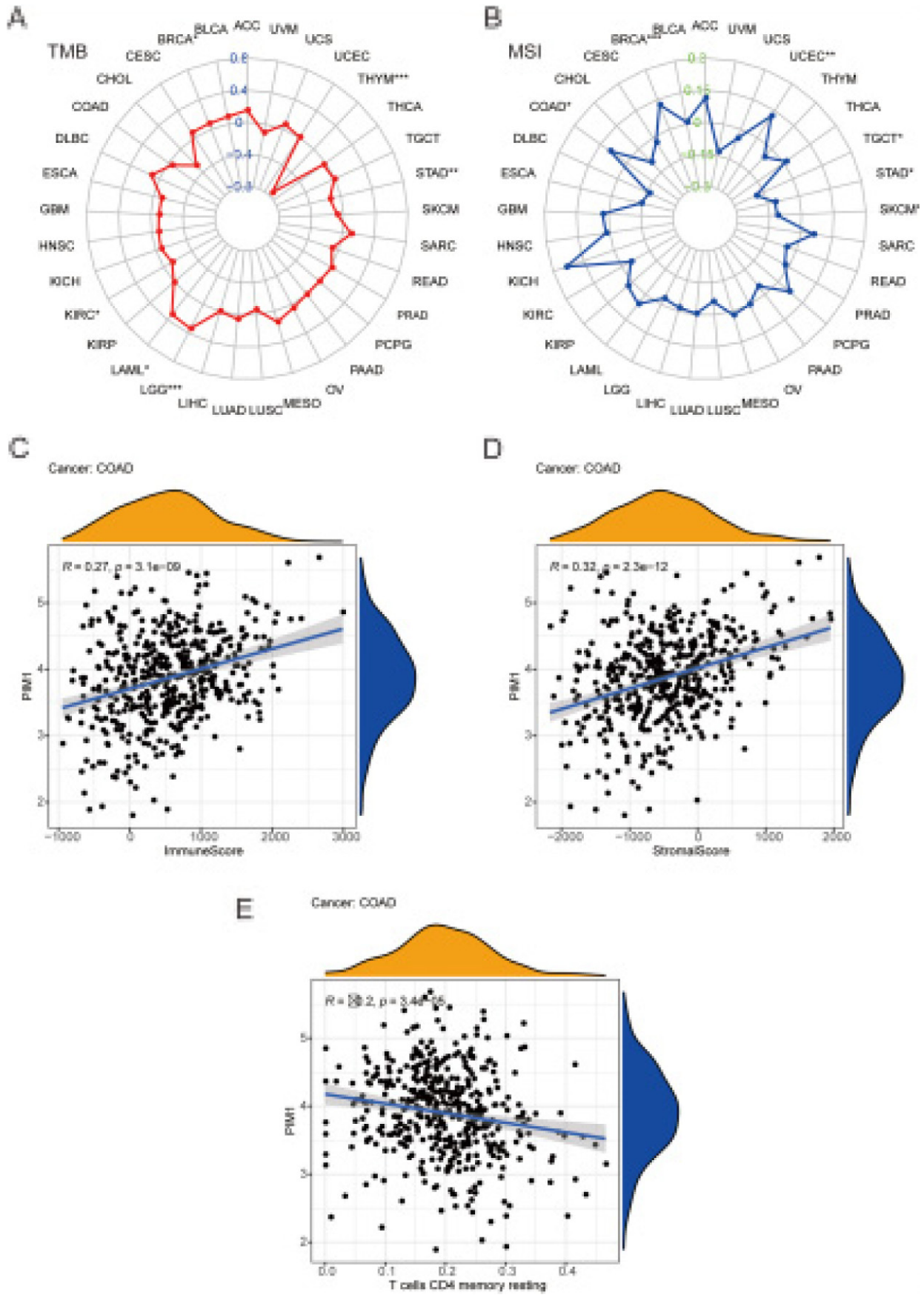

Figure 3. Regulatory effect of PIM1 on the immune microenvironment of tumor by affecting immune infiltration. **A, B:** Correlation of TMB, MSI with PIM1 mRNA expression in various tumors in TCGA. **C-E:** Correlation of PIM1 mRNA expression with immune score and interstitial score from TCGA database was investigated by TIMER, and the infiltration score of CD4+T cells was tested.

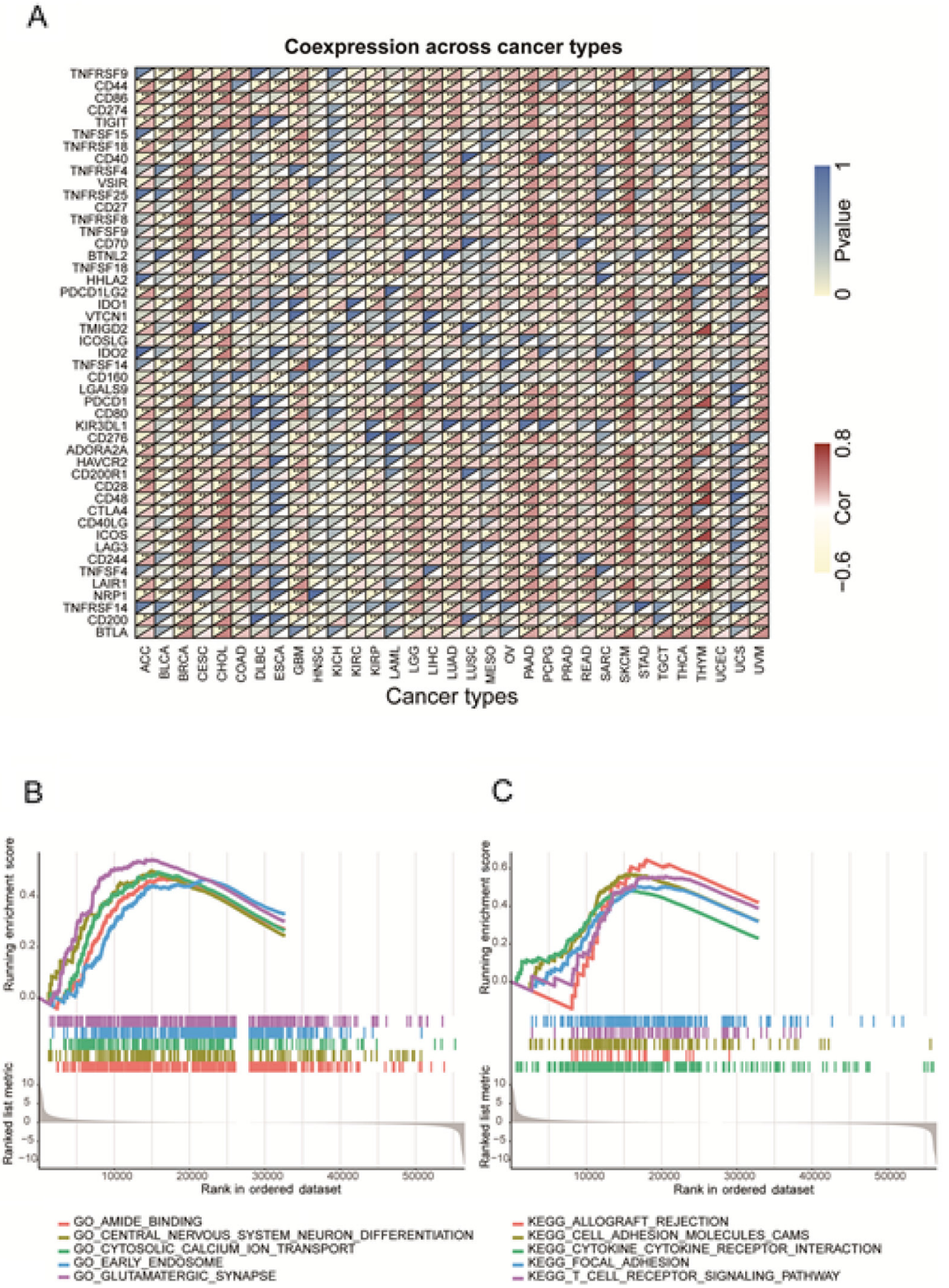

Figure 4. Correlation of PIM 1 with immune checkpoint expression and analysis of functional enrichment. **A:** Correlation of PIM1 mRNA expression level in several tumors in TCGA WITH mRNA expression in recognized immune checkpoints. **B, C:** Patients were divided into high and low expression according to the median value of PIM1, and the PIM1 high expression enrichment was tested using GO and KEGG enrichment.

Discussion

CRC is one of the major carcinomas threatening human health and life, but its pathogenesis is still unclear. However, it is of great significance to explore new diagnostic and treatment strategies of CRC patients. More and more studies have widely proved that EMT plays an important role in the occurrence and development of CRC [15-17]. In recent years, bioinformatics methods have been gradually used to screen various genes related to carcinoma phenotype, including EMT-related genes [18,19]. In this study, PIM1 was proved to be an EMT-related gene, and the prognosis and immune regulation function of PIM1 were analyzed.

Our results indicated that PIM1 was widely expressed in different tissues, especially in CRC. PIM1 was obviously correlated with the OS, DSS, DFI and PFI of CRC. It was proved that PIM1 can be used as a gene related to the prognosis of CRC. In addition, by analyzing the expression of PIM1 in different stages of CRC, PIM1 was significantly positively correlated with stages, which is consistent with the previous results. Previous studies have shown that carcinogenic PIM kinases support tumor growth and the formation of adhesion, migration, invasion and metastasis of carcinoma cells through various signaling pathways [20]. PIM family included PIM1, PIM2 and PIM3, which regulate the activity of downstream proteins by phosphorylating them. PIM1 has been proved to be the target gene of miR-33a in K562 lymphoma cell line and LS174T CRC cell line [21]. MiR-542-3p inhibits invasion and metastasis by targeting PIM1 in melanoma [22]. According to previous studies, PIM1 was confirmed to be a prognostic factor for CRC.

Next, we further explored the role of PIM1 in immune regulation. The results showed that PIM1 was positively correlated with microsatellite instability (MSI) in CRC, indicating that PIM1 was involved in genomic instability and carcinogenesis. In addition, PIM1 was highly correlated with immune score and interstitial score, suggesting that PIM1 was involved in regulating immune microenvironment of CRC. PIM1 could regulate CD4+T cell infiltration, which also confirmed its regulatory effect on immune microenvironment. And lastly, we used Spearman's correlation test to analyze the relationship between PIM1 and common immune checkpoint genes, and found that PIM1 was highly correlated with tumor necrosis factor (TNF)-related immune genes (TNFRSF4, 8, 9, 15, 18) and CD274. Studies have shown that PIM1 mutation may participate in the regulation of immune microenvironment and promote the progression of primary central nervous system lymphoma [23]. PIM kinase family regulated the transcriptional activity of NFκB and STATs, supported the survival of Reed-Sternberg (RS) cells of classical Hodgkin's lymphoma and enhanced their immune privilege [24]. However, the immunomodulatory function of PIM1 in CRC has not been fully studied, and further verification of its biological function and mechanism is needed.

In conclusion, PIM1 is highly expressed in various tumors, and this high expression is related to poor survival and disease progression, especially for CRC. PIM1 expression is also related to immune cells, gene expression of immune checkpoint and MSI invasion of tumor. To sum up, PIM1 is a valuable new biomarker for CRC, which can be used to evaluate prognosis and immunotherapy response.

Funding

Intra-hospital fund of the first hospital of Lanzhou University (LDYYYN2018-70); 2020 Gansu Province Health Industry Research Project (GSWSKY2020-12).

Conflict of interests

The authors declare no conflict of interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7-34.
3. Ferlay J, Colombet M, Soerjomataram I et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019;144:1941-53.
4. Inadomi JM. Screening for Colorectal Neoplasia. N Engl J Med 2017;376:1599-1600.
5. Fischer KR, Durrans A, Lee S et al. Epithelial-to-

mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature 2015;527:472-6.

6. Gavert N, Ben-Ze'ev A. Epithelial-mesenchymal transition and the invasive potential of tumors. Trends Mol Med 2008;14:199-209.
7. Li N, Babaei-Jadidi R, Lorenzi F et al. An FBXW7-ZEB2 axis links EMT and tumour microenvironment to promote colorectal cancer stem cells and chemoresistance. Oncogenesis 2019;8:13.
8. Attili I, Bonanno L, Karachaliou N et al. SRC and PIM1 as potential co-targets to overcome resistance in MET deregulated non-small cell lung cancer. Transl Lung Cancer Res 2020;9:1810-21.
9. An N, Xiong Y, LaRue AC, Kraft AS, Cen B. Activation of Pim Kinases Is Sufficient to Promote Resistance to MET Small-Molecule Inhibitors. Cancer Res 2015;75:5318-28.
10. Gao X, Liu X, Lu Y et al. PIM1 is responsible for IL-6-induced breast cancer cell EMT and stemness via c-myc activation. Breast Cancer 2019;26:663-71.
11. Liu Y, Zhang J, Xing C, Wei S, Guo N, Wang Y. miR-486 inhibited osteosarcoma cells invasion and epithelial-mesenchymal transition by targeting PIM1. Cancer Biomark 2018;23:269-77.
12. Li B, Severson E, Pignon JC et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol 2016;17:174.
13. Li T, Fan J, Wang B et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer Res 2017;77:e108-10.
14. Zhou W, Zhang S, Li HB et al. Development of Prognostic Indicator Based on Autophagy-Related lncRNA Analysis in Colon Adenocarcinoma. Biomed Res Int 2020;2020:9807918.
15. Brabletz T, Hlubek F, Spaderna S et al. Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. Cells Tissues Organs 2005;179:56-65.
16. Cheriyamundath S, Ben-Ze'ev A. Wnt/beta-Catenin Target Genes in Colon Cancer Metastasis: The Special Case of L1CAM. Cancers (Basel) 2020;12:3444.
17. Lin S, Chen S, Chen Z, Dai Q, Ke C. X-ray-induced epithelial-mesenchymal transition in SW480 colorectal cancer cells and its potential mechanisms. JBUON 2017;22:1457-62.
18. Zhang Z, Zheng S, Lin Y et al. Genomics and prognosis analysis of epithelial-mesenchymal transition in colorectal cancer patients. BMC Cancer 2020;20:1135.
19. Liu D, Zhang J, Li L, Wang Q, Lan Y. Detection of critical genes associated with poor prognosis in breast cancer via integrated bioinformatics analyses. JBUON 2020;25:2537-45.
20. Santio NM, Koskinen PJ. PIM kinases: From survival factors to regulators of cell motility. Int J Biochem Cell Biol 2017;93:74-85.
21. Thomas M, Lange-Grünweller K, Weirauch U et al. The proto-oncogene Pim-1 is a target of miR-33a. Oncogene 2012;31:918-928.
22. Rang Z, Yang G, Wang YW, Cui F. miR-542-3p suppresses invasion and metastasis by targeting the proto-oncogene serine/threonine protein kinase, PIM1, in melanoma. Biochem Biophys Res Commun 2016;474:315-20.
23. Gandhi MK, Hoang T, Law SC et al. EBV-tissue positive primary CNS lymphoma occurring after immunosuppression is a distinct immunobiological entity. Blood 2021;137:1468-77.
24. Szydłowski M, Prochorec-Sobieszek M, Szumera-Ciećkiewicz A et al. Expression of PIM kinases in Reed-Sternberg cells fosters immune privilege and tumor cell survival in Hodgkin lymphoma. Blood 2017;130:1418-29.