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Prognostic and immunomodulatory effects of PIM1 in colorectal carcinoma
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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 Cancer 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 PIM1 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...
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, CD8+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](image-url)

**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.

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Conflict of interests

The authors declare no conflict of interests.

References

5. Fischer KR, Durrans A, Lee S et al. Epithelial-to-