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Neutrophils infiltrating pancreatic ductal adenocarcinoma indicate higher malignancy and worse prognosis

Yufu Wang [a], Tianyi Fang [a], Lining Huang [a], Hao Wang [a], Lei Zhang [b], Zhidong Wang [a, **], Yunfu Cui [a, *]

[a] Department of Hepatopancreatobiliary Surgery, Second Affiliated Hospital of Harbin Medical University, Harbin, 150000, Heilongjiang Province, China
[b] Department of Pathology, Harbin Medical University, Harbin, 150000, Heilongjiang Province, China

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ABSTRACT

CD177 is considered to represent neutrophils. We analyzed mRNA expression level of CD177 and clinical follow-up survey of PDAC to estimate overall survival (OS) from Gene Expression Omnibus (GEO) dataset (GSE21501, containing samples from 102 PDAC patients) by R2 platform (http://r2.amc.nl). We also analyzed correlated genes of CD177 by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to predict the potential relationship between neutrophils and prognosis of PDAC. We then performed hematoxylin and eosin (H&E) staining and immunohistochemical staining of surgical specimens to verify infiltration of neutrophils in PDAC tissues. After analyzing mRNA expression data and clinical follow-up survey provided in the GEO dataset (GSE21501, containing samples from 102 PDAC patients) and clinicopathological data of 23 PDAC patients, we demonstrated that CD177 was correlated with poor prognosis. The univariate Kaplan–Meier survival analysis revealed that OS was inversely associated with increased expression of CD177 ($P = 0.012$). Expression of phosphodiesterase (PDE)4D was positively related to CD177 in gene correlation analysis ($R = 0.413$, $P < 0.001$) by R2 platform. H&E staining and immunohistochemistry of CD177 in 23 PDAC surgical samples showed accumulation of neutrophils in the stroma and blood vessels around the cancer cells. In addition, immunohistochemical staining showed that CD177 was highly expressed in the stroma and blood vessels around tumor tissues of PDAC, which was similar to H&E staining. Expression of CD177 can be used to represent infiltration of neutrophils, which may have potential prognostic value in PDAC.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly heterogeneous alimentary tract malignancy with poor prognosis [1–4]. Since the Whipple procedure was adopted as the standard of care [5,6], histopathological examination based on surgical specimens has become the gold standard for final diagnosis [7–9]. To understand better the relationship between pancreatic cancer cells and the surrounding interstitial/cellular components, oncological pathologists have tried to describe and study PDAC at the tissue level, but current technology limits the tissue level testing, and verification remains hardly accessible. Infiltration of PDAC cancer cells by inflammatory cells such as neutrophils can sometimes be observed in resected pancreatic cancer samples [10,11]. However, it has been difficult to investigate directly the relationship between neutrophils and PDAC cells, because models based on traditional cell lines cannot accomplish this goal. Analysis of transcriptome sequencing data from real pathological specimens provides a comprehensive background for understanding the association between immune and cancer cells.

The transcriptome is the complete set of transcripts in a cell for a specific developmental stage or physiological condition [12]. The main purpose of transcriptomics in our study was to quantify the expression of each transcript during disease progress and under different tumor conditions. It is essential to understand the transcriptome to reveal the molecular constituents of tissues and

* Corresponding author. Department of Hepatopancreatobiliary Surgery, Second Affiliated Hospital of Harbin Medical University, No. 246 Xuefu Road, Nangang District, Harbin, 150001, Heilongjiang Province, China.
** Corresponding author. Department of Hepatopancreatobiliary Surgery, Second Affiliated Hospital of Harbin Medical University, No. 246 Xuefu Road, Nangang District, Harbin, 150001, Heilongjiang Province, China.
E-mail addresses: wangzhidong@hrbmu.edu.cn (Z. Wang), estel@hrbmu.edu.cn (Y. Cui).

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understand tumor development. Tumor microenvironment, due to the progress of transcriptomics, has attracted attention. Worse tumor microenvironment increases the possibility of a higher degree of malignancy [13–15]. It is thought that the microenvironment of a malignant tumor is inflammatory, where tumor and immune cells, such as macrophages, secrete a large number of inflammatory factors [16,17]. These factors can activate the inflammatory pathways of tumor cells, leading to metastasis. Another possible factor associated with inflammation and tumors is neutrophils. Neutrophils are some of the earliest inflammatory cells that respond and participate in the inflammatory response, and they can secrete a large number of cytotoxic factors and reactive oxygen species (ROS), resulting in destruction and killing of stromal cells in the tumor microenvironment [18,19]. It is believed that neutrophils have the highest proportion among the innate immune cells [20], as well as a wide range of profound effects in the development of PDAC [21]. CD177, also known as neutrophil-specific antigen, is distributed on the neutrophil surface [22,23]. Its RNA distribution levels directly reflect the recruitment of neutrophils in the tissues. However, little is known about the interaction between survival probability and neutrophils in the microenvironment of PDAC.

To clarify this phenomenon, we analyzed the CD177 expression in PDAC tissues from the Gene Expression Omnibus (GEO) database to estimate overall survival (OS) probability, by R2 analysis platform. Gene Correlation analysis, Gene Ontology (GO) analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis were performed at the level of transcriptomics. We hypothesized that neutrophils play a potential role in promoting a higher degree of malignancy and worse prognosis of PDAC.

2. Materials and methods

2.1. Specimens

The present study included 23 patients (13 male and 10 female) from the Department of Hepatopancreatobiliary Surgery, the Second Affiliated Hospital of Harbin Medical University, China between 2015 and 2017. The patients ranged in age from 52 to 76 years, with a median age of 66 years. No patients had received radiotherapy or chemotherapy prior to the present study. The PDAC specimens were histopathologically verified.

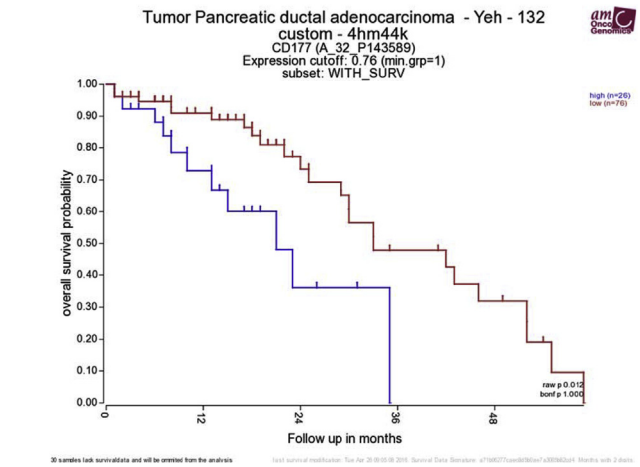

Fig. 1. OS probability of cases with high expression of CD177 was lower than that of those with low expression. 30 samples lacked survival data and were omitted from the analysis. 102 cases were separated into two groups: with high or low expression of CD177. OS was then compared between the two groups using the Kaplan–Meier method.

2.2. Tissue collection and histology

We fixed pancreatic tissue in 4% paraformaldehyde for 24 h. For paraffin sections, we dehydrated and embedded tissues in paraffin blocks. We stained paraffin sections with hematoxylin and eosin (H&E) using standard reagents and protocols [24].

2.3. Immunohistochemistry

We conducted antigen retrieval in sodium citrate solution (pH 6.0) for 30 min. We blocked sections in a buffer containing 5% bovine serum albumin and 0.1% Triton X-100 in phosphate-buffered saline, and incubated them overnight at 4 °C in primary antibodies diluted in blocking buffer. Primary antibodies used were: CD177 (1:200, Abcam, Cambridge, MA, USA). Corresponding horseradish-peroxidase-labeled secondary antibodies were used for detection [24]. The density of positive staining in the entire view was measured using a computerized imaging system [21]. We captured images of five representative fields at 100 or 200 × magnification.

2.4. Statistical analysis

The RNA sequencing (RNA-seq) data of GEO database (https://www.ncbi.nlm.nih.gov/geo) was downloaded, which contained CD177 RNA expression data for human PDAC profiles, including 132 tissue samples. Gene correlation analysis was performed with the R2 bioinformatic platform (http://r2.amc.nl), and the genes and pathways associated with CD177 were analyzed by GO and KEGG pathway analysis. The Kaplan–Meier method was used for survival data processing. Correlation statistics were calculated using the R2 platform and $P < 0.05$ was statistically significant.

3. Results

3.1. High expression of CD177 indicated poor prognosis of PDAC

The interaction between the RNA expression level of CD177 and clinical follow-up survey provided in the GEO dataset (GSE21501, containing samples from 102 PDAC patients) was analyzed. Gene correlation analysis was performed by R2 bioinformatic platform (https://r2.amc.nl). CD177-related genes and pathways were analyzed by GO and KEGG pathway analysis. Univariate Kaplan–Meier survival analysis revealed that overall survival (OS) was inversely associated with increased expression of CD177 ($P = 0.012$).

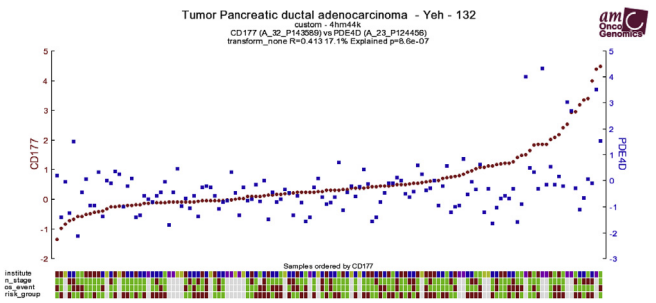

Fig. 2. By analyzing the genes with high expression of CD177, we found that expression of PDE4D was positively related to CD177. The red points show the expression level of CD177 and the blue points expression of PDE4D. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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