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High expression of diffuse panbronchiolitis critical region 1 gene promotes cell proliferation, migration and invasion in pancreatic ductal adenocarcinoma

Jiayi Yan [a, 1], Guanghui Chen [b, 1], Xuesong Zhao [a], Fangying Chen [a], Ting Wang [b, **], Fei Miao [a, *]

[a] Department of Radiology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Ruijin Er Road, Shanghai 200025, China
[b] Department of Bone Tumor Surgery, Changzheng Hospital, Second Military Medical University, 415 Fengyang Road, Shanghai 200003, China

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ABSTRACT

Diffuse panbronchiolitis critical region 1 (DPCR1) is located in the major histocompatibility complex (MHC) class I. It was reported to be downregulated in invasive pituitary adenoma compared with that in non-invasive tumors, but upregulated in the precursor of gastric carcinogenesis. However, the direct effect of DPCR1 on cancer cells has rarely been reported, and the role DPCR1 in pancreatic ductal adenocarcinoma (PDAC) remains unclear. The clinical sample validation and public data analysis of the present study demonstrated that DPCR1 was upregulated markedly in PDAC and this high expression was negatively correlated with the patient prognosis. Functionally, knocking down DPCR1 in PDAC cell lines inhibited cell proliferation, migration and invasion in *vitro*. Tumor xenograft experiments further showed that suppression of DPCR1 inhibited tumor growth in *vivo*. In addition, the results of RNA deep sequencing and qRT-PCR assay showed that DPCR1 participated in PADC progression by regulating nuclear factor-kappa B signaling pathway, suggesting that it might be a novel oncogene in tumor progression and a potential therapeutic target in PDAC as well.

1. Introduction

Pancreatic cancer (PCa) is the eleventh most common cancer and ranks the third leading cause of cancer-related death in the United States [1]. Despite the development of new therapeutic techniques and approaches including chemotherapy and immunotherapy in recent years, the prognosis of PCa patient remains poor, with a 5-year survival rate of 7% at present [2,3]. Pancreatic ductal adenocarcinoma (PDAC) constitutes about 90% pancreatic cancers [4]. Although a number of studies have uncovered various gene signatures and signaling pathways underlying tumor pathogenesis [5,6], the molecular mechanisms that trigger PDAC growth and progression remain largely unknown. Given the fast-growing morbidity and high mortality, it is an urgent task to identify novel diagnostic biomarkers and therapeutic targets for the treatment of PDAC patients for both basic scientists and clinicians.

Diffuse panbronchiolitis critical region 1 (DPCR1) was firstly identified in 2002 as one of the major histocompatibility complex (MHC) class I molecules located between HLA-B and HLA-A genes on chromosome 6p21.33. It was initially considered a marker for the diagnosis of diffuse pan-bronchiolitis [7]. MHC class I protein expressed on cancer cells usually functions as the activator of specific antigen recognition by the immune system and protection of the host. But studies found that some MHC molecules were associated with unfavorable outcomes in several cancers [8–11]. Recent reports further showed that part of the MHC molecules could promote cell proliferation, migration and invasion, thus accelerating tumor progression [12–14]. Lan et al. [15] reported that DPCR1 was significantly downregulated in invasive pituitary adenomas than that in non-invasive ones, and they considered that reducing DPCR1 might cause immune-escape in invasive pituitary adenomas. Shen et al. [16] showed that single-nucleotide polymorphisms (SNPs) located at DPCR1 gene were associated with

* Corresponding author. Department of Radiology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Ruijin 2nd Road, Shanghai 200025, China.
** Corresponding author.
E-mail addresses: spine_oncology_wt@163.com (T. Wang), MF11066@rjh.com.cn (F. Miao).
[1] These authors contributed equally to this work.

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esophageal squamous cell carcinoma, and preferentially existed in younger cases. However, to the best of our knowledge, the expression and role of DPCR1 in PDAC have not yet been investigated.

In the present study, we demonstrated that DPCR1 was highly expressed in PDAC and significantly correlated with poor prognosis. DPCR1 enhanced cell proliferation in *vitro* and in *vivo*, and further promoted migration and invasion in PDAC cells. Through RNA sequencing, we found that DPCR1 might act as an oncogene by regulating the nuclear factor-kappa B (NF-κB) signaling pathway.

2. Materials and methods

2.1. Tissue specimens

Forty PDAC and paired para-tumor tissue specimens were obtained from patients who underwent surgical resection at Ruijing Hospital (Shanghai, China) between January 2015 and December 2016. None of the patients received neoadjuvant chemotherapy or radiotherapy before operation. The histological grade was assessed by the experienced pancreatic pathologists. Clinical data of the patients including age, sex, tumor size and AJCC stages are summarized in Supplementary Table 1. The study was approved by the Ethnic Committee of Shanghai Jiao Tong University School of Medicine (China), and written informed consent was obtained from all participants.

2.2. Immunohistochemistry

The sample was fixed with 4% paraformaldehyde, dehydrated through a graded series of ethanol, paraffin embedded, and sliced into 5-μm sections. Immunohistochemical (IHC) staining for DPCR1 (HPA014036, Sigma, USA) was carried out using standard histological procedures described in the manual for Histostain-Plus (DAB) kit (Mingrui Biotech, China). Staining was blindly scored in accordance by two investigators with previous protocols as negative, focally positive and positive.

2.3. qRT-PCR assay

Total RNA was isolated by using TRIZOL (Invitrogen, USA) and reverse transcribed into cDNA by using Prime Script™ RT Master Mix (Takara, Japan). Gene transcripts were quantified on 7900HT Fast Real-Time PCR System (Life Technologies Corporation, USA)

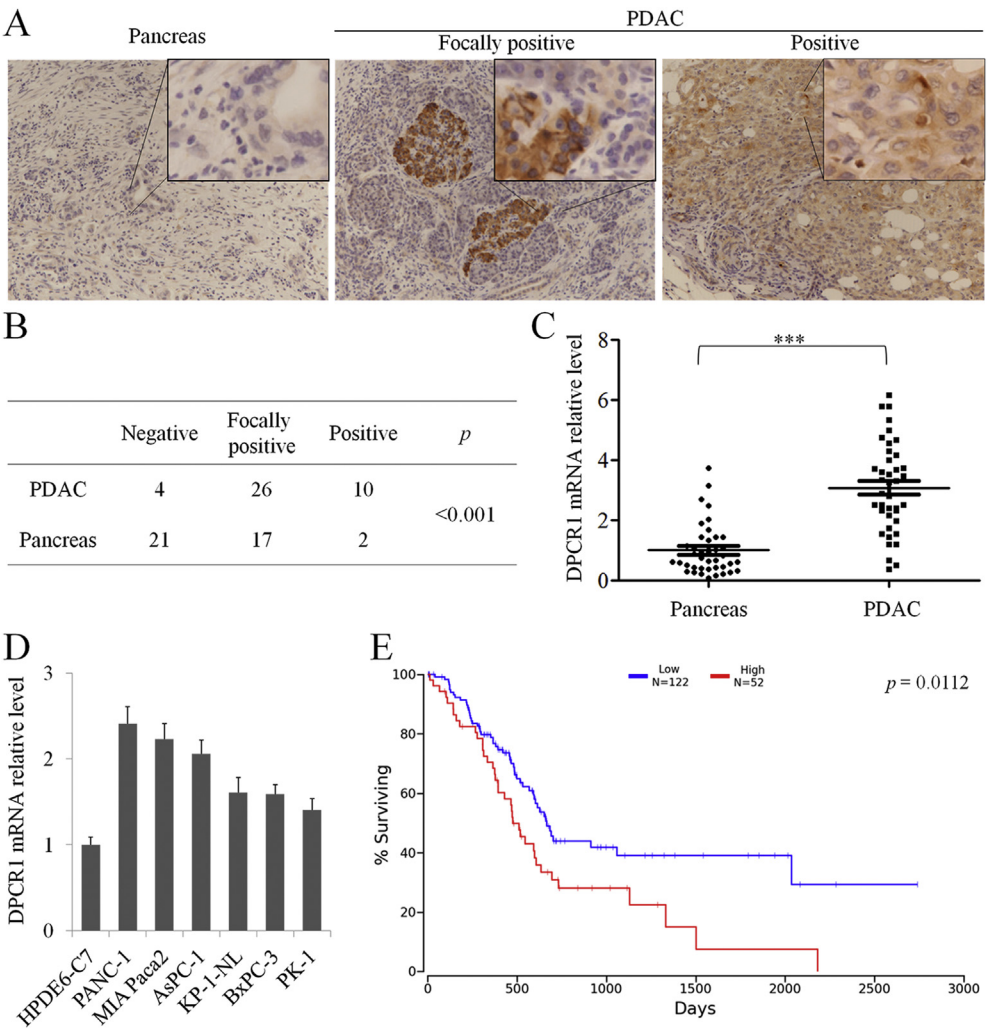

Fig. 1. DPCR1 was overexpressed in PDAC and negatively correlated with the outcome of PDAC patients. (A) IHC staining of DPCR1 in PDAC tissues and matched normal pancreatic tissues. (B) The statistical analysis of IHC staining. (C) qRT-PCR assay of mRNA level of DPCR1 in clinical samples. (D) qRT-PCR assay of mRNA level of DPCR1 in various pancreatic cancer cell lines and normal pancreatic duct epithelial cell line. (E) Kaplan-Meier analysis of DPCR1 mRNA level related to overall survival in pancreatic adenocarcinoma patients based on TCGA survival data. *** means $p < .001$.

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