Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

High expression of GRK3 is associated with favorable prognosis in pancreatic ductal adenocarcinoma

Wen-Jing Liu[a,1], Li Zhou[a,1], Zhi-Yong Liang[b], Wei-Xun Zhou[b], Lei You[a], Tai-Ping Zhang[a,*], Yu-Pei Zhao[a,*]

[a] Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing, China
[b] Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing, China

ARTICLE INFO

Keywords:
G-protein-coupled receptor kinase 3
Pancreatic ductal adenocarcinoma
Prognosis
Immunohistochemistry
Tissue microarray

ABSTRACT

Background: It was found that G-protein-coupled receptor kinase 3 (GRK3) played key biological roles in some cancers. However, its associations with clinicopathologic features and prognosis in pancreatic ductal adenocarcinoma (PDAC) remain unknown.

Methods and methods: Expression of GRK3 was detected, using tissue microarray-based immunohistochemistry, in paired formalin-fixed paraffin-embedded tumor and non-tumor samples from 165 patients with PDAC after curative resection, and was further correlated with clinicopathologic parameters and cancer-specific survival (CSS).

Results: It was shown that GRK3 expression was much lower in tumor than in non-tumor tissues. Moreover, expression of GRK3 in tumor tissues was significantly associated with gender and T stage. Univariately, high GRK3 expression was predictive for favorable CSS, along with some conventional clinicopathologic variables. In multivariate Cox regression test, GRK3 expression remained to be a significant prognostic marker for PDAC. Finally, combination of GRK3 with some clinicopathologic variables, especially N stage, obtained more precise prediction for CSS.

Conclusions: Our data suggested that expression of GRK3 was down-regulated in PDAC and was an independent prognostic factor.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC), accounting for 85% of pancreatic cancer (PC), is one of the most life-threatening common cancers [1]. In China, the incidence and mortality rate of PC have also been to be remarkably raised, based on recent data from a population-based registry system [2]. Thus far, the overall long-term prognosis of PC has not been significantly improved, although surgical resection and adjuvant therapy have achieved some favorable results in selected patients [3–5]. Therefore, identification of the prognostic determinants in PDAC caught much attention. Among them, some are conventional clinicopathologic variables, including lymph node involvement status, neural or perineural invasion and resection margin [6–10]. Within recent years, many molecular markers were shown to be of strong potential to predict long-term prognosis of PC [11,12]. However, further supplement and validation remain to be needed.

As one of GRK isoforms that play a key role in phosphorylatation and desensitization of G protein-coupled receptors (GPCRs) [13,14], G protein-coupled receptor kinase 3 (GRK3) was previously found to have strong biological effects in the nerve system [15–17]. Since the first article published in 2012 [18], this molecule has gradually been shown to be involved in multiple phenotypes of cancer cells, such as growth, invasion and metastasis [18–21]. What calls for special attention is that the roles of GRK3 in different malignancies might also be inconsistent, even opposite [18–21], indicating that this gene might function in a tissue-type specific manner. Previously, GRK3 was revealed to be down-regulated in hepatocellular carcinoma (HCC) and predicted good post-resectional survival [22]. Up to now, GRK3 has not been investigated in PDAC.

The current study was designed to explore GRK3 expression and its associations with clinicopathologic features and prognosis in PDAC.

* Corresponding authors.
E-mail addresses: tpingzhang@yahoo.com (T.-P. Zhang), zhao8028@263.net (Y.-P. Zhao).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.prp.2017.11.013
Received 10 October 2017; Received in revised form 6 November 2017; Accepted 16 November 2017

Table 1
Correlations between GRK3 expression and clinicopathologic features of PDAC.

Variables	n	GRK3 expression		*P*
		High	Low	
Age				0.132
> 60 years	82	65	17	
≤60 years	83	73	10	
Sex				0.008[#]
Male	100	77	23	
Female	65	61	4	
Tumor location				0.876
Head	100	84	16	
Non-head	65	54	11	
Tumor size				0.092
> 4 cm	66	51	15	
≤ 4 cm	95	83	12	
Histological grade				0.816
G1-2	115	98	17	
G3-4	43	36	7	
Peri-neural invasion				0.856
Present	90	74	16	
Absent	66	55	11	
T stage				0.018
T1-2	88	79	9	
T3	75	57	18	
N stage				0.962
N0	89	74	15	
N1	70	58	12	

GRK3, G-protein-coupled receptor kinase 3; PDAC, pancreatic ductal adenocarcinona; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; G4, undifferentiated; T, tumor; N, lymph node. Note: Partial data are not available, and statistics were based on available data. *P* values were derived from the Pearson Chi-square test (two-tailed). [#]: Chi-square test with continuity correction.

2. Patients and methods

2.1. Patients

One hundred and sixty-five PDAC patients who underwent surgical resection were included. The inclusion criteria were as follows: (1), with histological confirmation; (2), with radical resection; (3), without preoperative therapy; (4), with paired tumor and non-tumor tissues. There were 100 males and 65 females, with a median age of 60 years (range: 34–85 years). The patient clinicopathologic characteristics are shown in Table 1. This project was approved by the Institutional Ethics Committee.

2.2. Construction of tissue microarray (TMA)

Tissue microarray construction was performed using formalin-fixed paraffin-embedded blocks. After careful review and selection of representative tumor and non-tumor areas, two cores (1.5 mm) of corresponding tissues for each patient were punched. The tissue microarray was then constructed by a manual tissue arrayer (Beecher Instruments, 686 Progress Way, Sun Prairie, WI).

2.3. Immunohistochemical staining

A rabbit anti-human GRK3 monoclonal antibody (Abcam, Cambridge, UK) and a two-step staining kit (EnVision™ + kit, Dako, Denmark) were applied for staining. Briefly, 4 μm-thick sections were mounted, deparaffinized and rehydrated. Antigen retrieval was performed in an autoclave. Slides were then incubated with 3% hydrogen peroxide for 10 min, so as to block endogenous peroxidase. Subsequently, slides were incubated with the primary antibody (dilution: 1:100) overnight at 4 °C, and washed in PBS. Horseradish peroxidase (HRP)-labeled secondary antibody was added for a reaction of

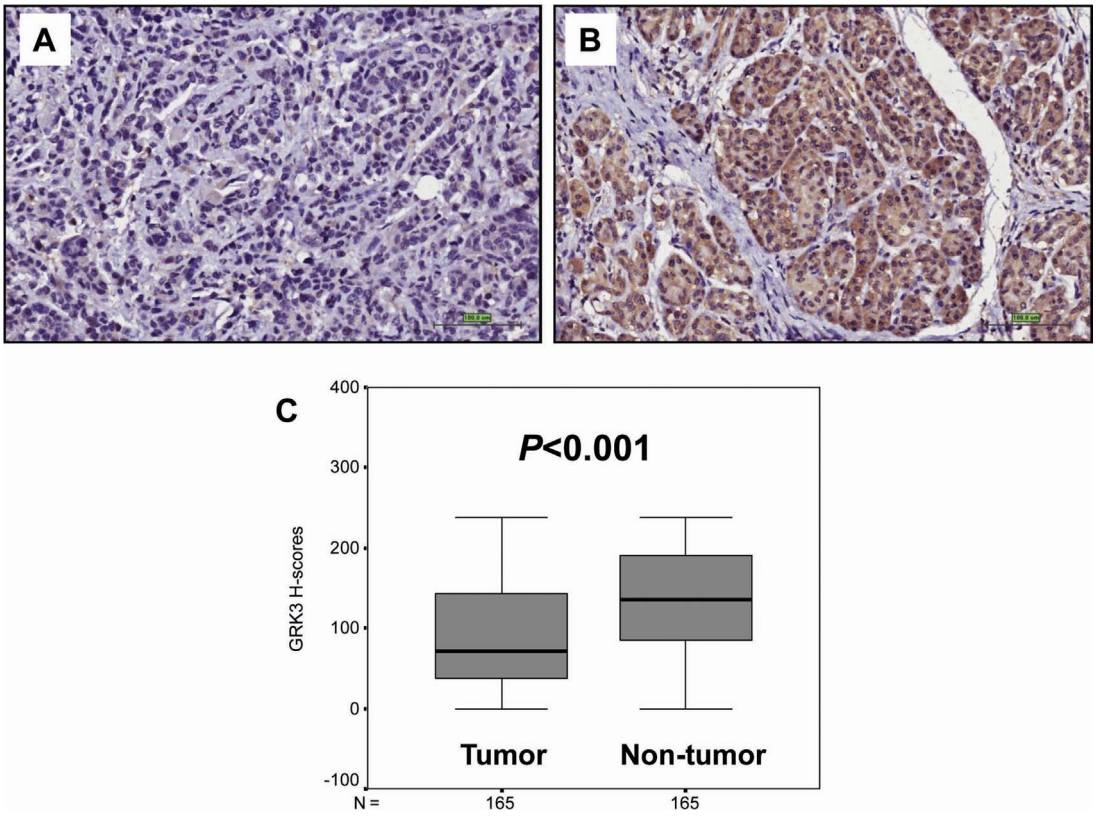

Fig. 1. Expression of GRK3 in pancreatic ductal adenocarcinoma. (A) Low expression in tumor tissue (original magnification × 200); (B) High expression in non-tumor tissue (original magnification × 200); (C) Comparison of H-scores between tumor and non-tumor tissues (Mann-Whitney *U* test; $P < 0.001$). GRK3, G-protein-coupled receptor kinase 3.