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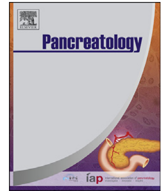

Expression of polymeric immunoglobulin receptor and stromal activity in pancreatic ductal adenocarcinoma

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A R T I C L E I N F O

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A B S T R A C T

Background/objectives: Polymeric immunoglobulin receptor (pIgR) traffics Immunoglobulins (IgA and IgM) through epithelial cells in normal mucosae but neither are expressed in the normal pancreas. Recent work from our laboratory suggested pIgR may be upregulated in pancreatic ductal adenocarcinoma (PDAC). Our aim was to assess the role of pIgR in human PDAC.
Methods: pIgR expression was manipulated (siRNA and shRNA) in cell lines to evaluate its subsequent effect on cell behaviour in 2D assays as well as 3D organotypics models. Tissue Microarrays of 88 patients with PDAC were analysed after pIgR, αSMA, E-Cadherin and Picrosirius Red staining to assess their role as a combined bio-marker panel.
Results: Cytokines such as interleukin 4 (IL4) and Tumour Necrosis Factor (TNFα) could not modulate pIgR expression in PDAC cell lines despite this effect being seen in other studies. Down-regulation in pIgR expression in Capan1 cancer cell line resulted in reduction of cellular proliferation, adhesion and migration in 2D assays. In 3D physiomimetic organotypic models, pIgR downregulation resulted in reduced cancer cell invasion, alteration of apico-basal polarity and diminished stromal activity. In human PDAC, decreased E-cadherin expression correlates with increased pIgR expression through pancreatic intra-epithelial neoplasia (PanIN) progression. In combination with enhanced stromal indices (α-smooth muscle action (SMA) and Picrosirius red), low pIgR scores had a trend towards better survival.
Conclusion: pIgR may be involved in PDAC progression and may be linked stromal activity. Further work on its precise role is mandated in *in vivo* models, to understand its influence on cancer progression.
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1. Introduction

Prognosis of patient with PDAC is dire and remains unchanged over the last 40 years [1]. Unfortunately, pancreatic resection, which improve survival significantly, is possible only in a minority of patients (5–10%) [2]. Early diagnosis using novel biomarkers, facilitating surgical resection, is considered the most important research goal for pancreatic cancer researchers. Currently, carbohydrate antigen 19–9 (CA 19–9) is used for prognostic and monitoring purposes only, as its sensitivity (70–90%) and specificity (90%) is considered too low to be of use for early detection and diagnosis [3].

The formation of a desmoplastic reaction is peculiar to PDAC [4] [5]. Pancreatic stellate cells (PSC) activated by TGFβ1, platelet-derived growth factor (PDGF) and fibroblast growth factors play a critical role in the formation and turnover of the stroma, through their ability to secrete collagen and other components of the extracellular matrix, such as matrix metalloproteinases [6]. PSC are also responsible for the poor vascularity: a characteristic of PDAC [7]. Previous work in our laboratory demonstrated PSC could modulate cancer cell behaviour by regulating gene expression affecting a range of key cellular pathways [8]. Polymeric Immunoglobulin Receptor (pIgR) was identified as a gene significantly

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upregulated in cancer cells upon exposure to PSCs.

Polymeric immunoglobulin receptor's (pIgR) main role is to transport polymeric immunoglobulins (IgA and to a lesser extent IgM) across mucosal epithelial cells [9]. Immunoglobulin A destined for the mucosal secretions is produced locally by organized mucosal-associated lymphoid tissues, as the first line of specific immune defense against gastrointestinal pathogens is secretory IgA (SIgA) [10]. High levels of pIgR on gut epithelial cells ensure selective transport of polymeric IgA (pIgA) across into the lumen [11]. However, normal pancreas, as well as pancreatic cancer have never been reported to necessitate pIgA transport. Hence, we investigated the role of pIgR in PDAC.

2. Materials and methods

2.1. Culture conditions

Pancreatic cells were cultured as adherent monolayers in sterile tissue culture flasks in a humidified atmosphere at 37 °C, 8% CO2 in either RPMI (PAA Laboratories, E15-842; AsPC1, Capan1) or Dulbecco's Modified Eagle's Medium (DMEM, PAA Laboratories, E15-843; Capan2, CFPAC1, Suit2, HS766T, Panc1, Colo357, Mia, BxPc3, 818, PaTuT/ PaTuS, and normal cells DEChTERT) medium. PS1 stellate cells were grown DMEM:F12 (Invitrogen, 11320–074) medium with 1 μg/ml Puromycin.

2.2. Stromal cells

Using the outgrowth method, pancreatic stellate cells were isolated [12] and verified as being of stellate cell origin based on the expression of characteristic stellate cell markers, such as presence of lipid droplets in the cytoplasm and expression of cytoskeletal proteins GFAP, Desmin, Vimentin and αSMA [13]. These stellate cells were immortalised, using ectopic human telomerase reverse transcriptase (hTERT) expression [14] and have been under continuous passage without loss of phenotypic characteristics over the last nine years. The cells remain in an activated state when cultured on plastic in absence of All trans retinoic acid.

2.3. Small interfering RNA (siRNA)

Cells were transfected with a pool of siRNA oligos and non-targeting siRNA oligo (ON-TARGET plus SMART pool human pIgR, Dharmacon, L0017729-00-0010 and D-001810-10-05) using INTERFERin™ (Polyplus, 409–10) and OptiMEM (Invitrogen, 51985–042) and cell lysed between 48 and 144 h for confirmation of knockdown by Western blot.

2.4. shRNA

pIgR shRNA plasmids were donated by Jing Ai, Shanghai

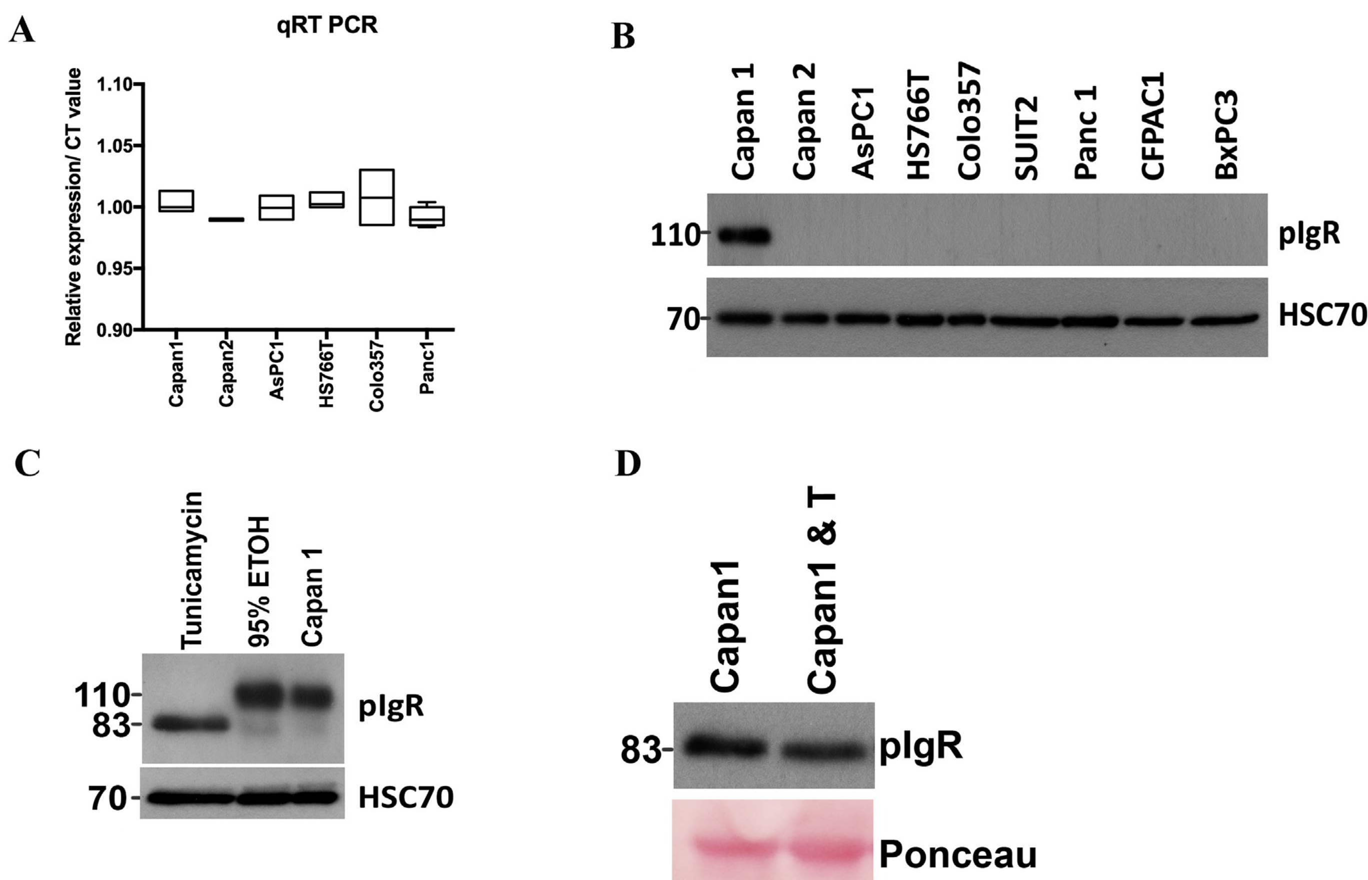

Fig. 1. pIgR is expressed in PDAC cancer cells at mRNA, but only transcribed to protein in Capan1.
(A) qRT-PCR of pancreatic cancer cells and PS1 cells. CT values were normalised to Capan1 (positive at protein level). N = 3. No significant difference in CT expression across cell types. (B) pIgR expression across all PDAC cells confirms only protein expression with Capan 1 cells. (C) Treatment with Tunicamycin confirms post-translation glycosolation of pIgR, demonstrating an effect of breaking down glycosylation bonds renders molecular weight at 83 kDa. HSC70 was used as loading control. (D) Secreted pIgR with or without Tunicamycin (T) treatment. Collected media was concentrated and Ponceau S determined equal loading.

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