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Abstract

Exploring the effect of PAK inhibition in a 3D Pancreatic Cancer invasion model †

Marianne Best [1], Dr Debashis Sarker [2] and Professor Claire M. Wells [2,*]

[1] School of Cancer and Pharmaceutical Sciences, King's College London; marianne.best@kcl.ac.uk
[2] School of Cancer and Pharmaceutical Sciences, King's College London ; claire.wells@kcl.ac.uk
* Correspondence: marianne.best@kcl.ac.uk
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Abstract: Pancreatic Ductal Adenocarcinoma (PDAC) is an aggressive cancer, with over half of patients presenting with metastatic PDAC at diagnosis. Most patients receive conventional chemotherapy which invariably faces resistance, and a key facilitator in this is the PDAC stroma which acts as a functional mediator of disease progression through bilateral crosstalk between stromal cells and cancer cells. 'Migrastatics' are a new drug class which target cell migration pathway effector proteins to attenuate cancer cell invasion. Improvement in PDAC treatment strategy is well-overdue and migrastatics as adjuvant therapy is one avenue gaining traction. The p21-activated kinase (PAK) family is frequently overexpressed and/or amplified in PDAC where it regulates cytoskeletal actin contractility as well as transcription. Pre-clinical PAK inhibitors have shown reduced 3D PDAC cell invasion in vitro, yet it is unknown how the PDAC stroma would respond to a PAK inhibitor and how this could affect PDAC invasion. My PhD project investigates the stellate cells response to PAK inhibition.

Keywords: Pancreatic cancer, cell migration, cell invasion, p21-activated kinases (PAKs), kinase inhibitors, actomyosin contractility, cytoskeletal remodelling, transcription, migrastatics, 3D models

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1. Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is an aggressive and rapidly invasive cancer, with only 10% of patients surviving 5 years post-diagnosis[1]. Chemotherapy treatment invariably faces resistance, and a central facilitator of this is the PDAC stroma which acts as a functional mediator of disease progression through bilateral crosstalk between PDAC cells and stromal cells[1]. The p21-activated kinases (PAK1-6) regulate cytoskeletal actin dynamics as well as cellular transcription, and are frequently overexpressed and/or amplified in PDAC to promote cancer cell migration[2].

Cancer Research UK is developing PAK inhibitors as anti-migration cancer therapeutics called 'migrastatics'. Thus far, pre-clinical PAK inhibitors have shown promising results by attenuating 3D invasion of PDAC cells *in vitro*[3], yet the stromal response to PAK inhibition remains unknown. Pancreatic stellate cells (PSCs) are a key stromal player in PDAC and it has been shown that drug administration can alter PSC behaviour to ultimately drive the overall therapeutic outcome[4,5]. Therefore, my PhD project investigates the PSC response to PAK inhibition, with regards to 3D PDAC invasion.

2. Methods

A 3D spheroid assay is used to co-culture PDAC cells and PSCs together to model stellate promotion of PDAC invasion, and subsequently investigate the effect of a PAK inhibitor. Immunofluorescence, western blotting, and gel contraction assays are used to characterise PSC behaviour and explore PAK expression. Further, multi-photon imaging investigates PSC influence on alignment and/or degradation of the extracellular matrix (ECM) within the 3D spheroid system. Finally, a novel pipeline was developed to isolate PDAC cells and PSCs from 3D co-culture spheroids for downstream RNA-sequencing. All sequencing analysis is performed using R.

3. Results

Characterisation studies compared our in-house immortalised stellate cell model, PS-1 against the commercially available HPaSteC, validating that the latter was the more representative model to bring forward. Exploration of PSC PAK expression revealed that HPaSteC express PAKs in both mRNA and protein, with Group I PAKs (PAK1-3) appearing more highly expressed over Group II PAKs (PAK4-6) (Fig. 1).

Both HPaSteC conditioned media, as well as co-culturing of HPaSteC with PDAC cells significantly increase 3D PDAC cell invasion. However, the physical presence of co-cultured HPaSteCs is required for maximal PDAC invasion efficiency in the spheroid (Fig. 2). Treatment with the pan-PAK inhibitor was tested in the 3D PDAC:Stellate co-culture setting against group specific PAK inhibitors to show that both pan-PAK and Group I PAK reduce PDAC invasion more so than Group II (Fig. 3).

To investigate the effect of direct PAK inhibition on the stellate cell cytoskeleton, morphological characterisation was performed to show that PAK inhibition induces HPaSteC cell rounding. Currently multi-photon imaging is exploring PAK inhibition influence on HPaSteC-driven fibre realignment compared to MMP secretion – both published mechanisms by which PSCs promote PDAC invasion.

In addition to cytoskeletal dynamics, PAKs have strong links to transcriptional regulation. We developed a pipeline to isolate both PDAC and PSCs from embedded 3D invaded spheroids for downstream ultra-low bulk RNA-sequencing in order to evaluate the transcriptomic landscape of both PDAC and PSC compartments under PAK inhibition. So far, quality control shows good quality RNA was obtained and differential gene expression will be explored next.

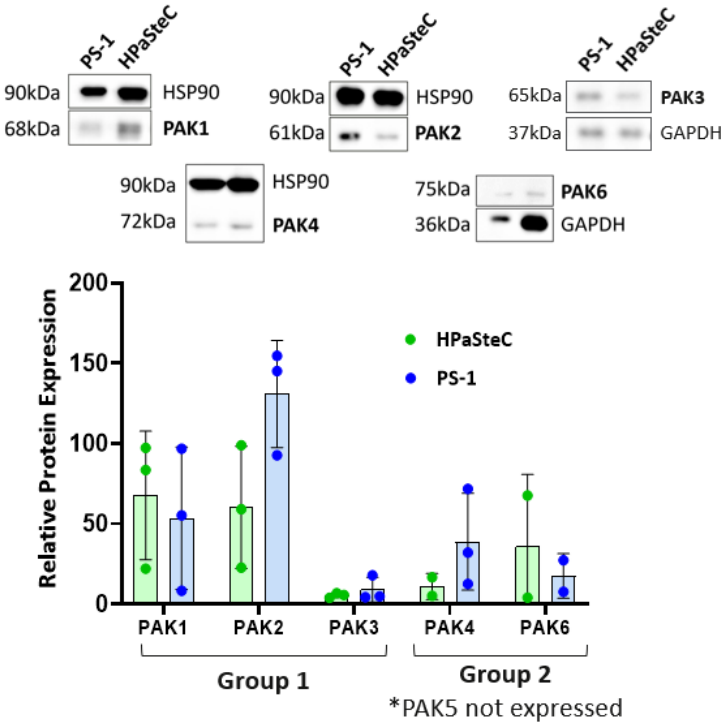

Figure 1. p21-activated kinase (PAK) expression in Pancreatic Stellate cells (PSCs). Western blots of PAK protein expression (top) and quantification (bottom) of HPaSteC and PS-1.

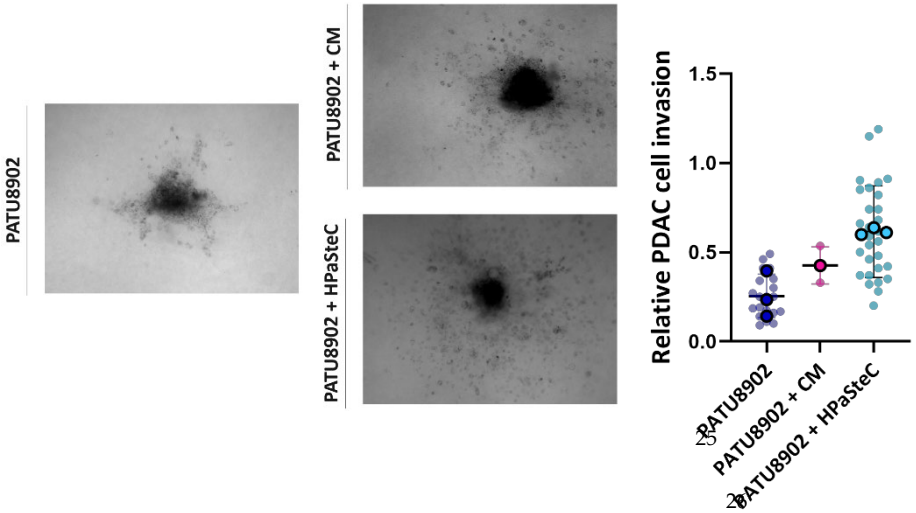

Figure 2. The presence of Pancreatic Stellate cells (PSCs) are required for maximal invasion efficiency in the 3D spheroid compared to conditioned PSC media. PDAC cell line, PATU8902 is shown cultured alone (left), compared to with HPaSteC conditioned media (middle top) and with HPaSteC co-cultured (middle bottom). Quantification of PDAC cell specific invasion relative to number of PDAC cells seeded is shown (right).

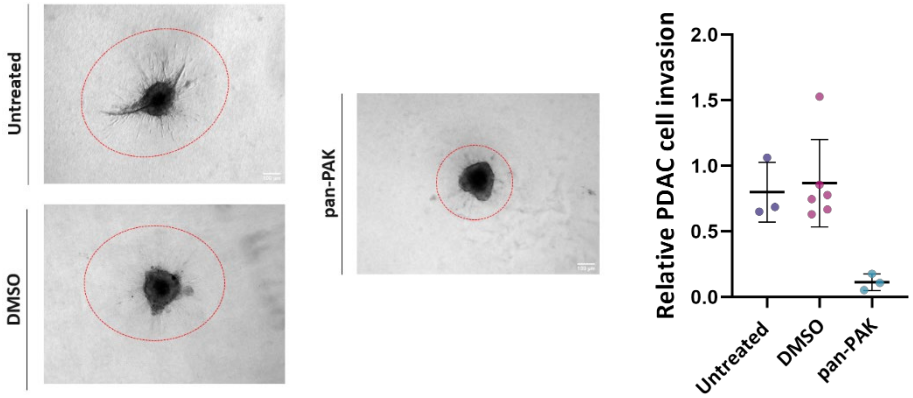

Figure 3. Treatment with a pan-PAK inhibitor which targets all 6 PAK isoforms shows reduced invasion in the 3D PDAC: Stellate co-culture spheroid assay compared to control groups Untreated and DMSO (vehicle control).

4. Conclusions

These data suggest that PAKs functionally contribute to PSC promotion of PDAC invasion, strengthening the argument for PAK inhibitors as PDAC migrastatics. Current work is further investigating the potential divergence between the two PAK groups, as well as how PAK inhibition could affect PSC interaction with ECM to facilitate PDAC invasion. RNA-sequencing analysis is underway to explore the differentially expressed genes in PAK-inhibited PDAC and stellate cells, to understand how PAK inhibition may influence the bilateral crosstalk between these two cell types.

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