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Proceeding Paper

Exploring the Effect of PAK Inhibition in a 3D Pancreatic Cancer Invasion Model †

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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 PDAC cell invasion in vitro, yet it is unknown how the PDAC stromal cells would respond to a PAK inhibitor and how this could consequently affect PDAC invasion. My PhD project investigates the Pancreatic stellate cell response to PAK inhibition.

Keywords: pancreatic cancer; cell migration; cell invasion; p21-activated kinases (PAKs); kinase inhibitors; actomyosin contractility; cytoskeletal remodeling; 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. 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 to be antimigration cancer therapeutics called 'migrastatics'. Thus far, preclinical PAK inhibitors have shown promising results by attenuating the 3D invasion of PDAC cells in vitro [3], yet the stromal response to PAK inhibition remains unknown. Pancreatic stellate cells (PSCs) are key stromal players in PDAC, and it has been shown that drug administration can alter PSCs' behavior to ultimately drive overall therapeutic outcomes [4,5]. Therefore, my PhD project investigates the PSCs' responses to PAK inhibition with regard to 3D PDAC invasion.

2. Methods

A 3D spheroid assay is used to co-culture PDAC cells and PSCs together to model PDAC invasion, and subsequently investigate the effect of a PAK inhibitor. Immunofluorescence, western blotting, and gel contraction assays are used to characterize PSC behavior and explore PAK expression. 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

Characterization studies compared immortalized 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 was completed in both Pancreatic Stellate cell models to observe how group specific expression may differ.

Optimization of the 3D co-culture spheroid was completed with a panel of PDAC cell lines, choosing PATU8902 PDAC cells to take forward in the invasion model. We next investigated the effect of pan-PAK inhibitor treatment in the 3D PDAC: Stellate co-culture setting to show invasion is reduced, and current work is investigating this further.

In addition to cytoskeletal dynamics, PAKs have strong links to transcriptional regulation. We developed a pipeline to isolate PDAC and PSCs from embedded 3D invaded spheroids for downstream 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 we were able to isolate out the two cell types, and differential gene expression will be explored next.

4. Conclusions

Our findings suggest that PSCs are an important cell type in promoting PDAC invasion and should be considered in therapeutic development. Current work is investigating how PAK inhibition could affect PSC-driven PDAC invasion. RNA-sequencing analysis is underway to explore the differentially expressed genes in PAK-inhibited PDAC and stellate cells, and to understand how PAK inhibition may influence the crosstalk between these two cell types.

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