

Identifying inhibitors of S100P - RAGE binding as a novel therapy for pancreatic cancer

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SUMMARY

Pancreatic ductal adenocarcinomas (PDAC), the fourth leading cause of cancer-related deaths in the western world. High prevalence of a calcium-binding protein S100P has been shown to promote PDAC cancer, and several other types of aggressive cancer progression through its interaction with the receptor for advanced glycation end products (RAGE). This project aims to identify lead compounds from a bank of 93 newly designed compounds that attach to and prevent S100P from binding to and activating RAGE. Using an in-house produced human S100P, an improved enzyme linked immunosorbent assay (ELISA) was developed based on a previously published study. This assay was used to confirm the binding of S100P with RAGE, and to assess the efficacy of the lead compounds on migration of pancreatic cancer cells. In addition, MTS and LDH release assays, and Transwell cell migration and invasion studies were performed using pancreatic cancer cells to assess the viability, and migratory and invasive properties of these cells following treatment with the lead compounds.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC), is a devastating disease resulting in approximately 227,000 deaths annually worldwide Raimondi *et al.*, (2009). There is currently no effective treatment for this disease which due mostly to the difficulty of detection at an early stage, and the inherent chemoresistance nature of cancer cells. Recent studies have shown that high levels of the calcium-binding protein S100P found in PDAC enhances cell survival, proliferation and invasion through interactions with intracellular targets and *via* extracellular interaction with the receptor for advanced glycation end products (RAGE). Previous *in vivo* studies have also shown that inhibition or silencing of S100P reduces tumour growth and metastasis, and enhances response to gemcitabine

therapy. As such, S100P has emerged as a promising biological target for novel anticancer drug design. This study aims to identify S100P-RAGE inhibiting compounds from a library of newly designed compounds to aid development of a novel therapy for PDAC.

MATERIALS AND METHODS

Computational modelling of a small-molecule binding site in S100P was used in a virtual screen to identify lead compounds, predicted to bind S100P and inhibit its tumour-promoting effects. An enzyme linked immunosorbent assay (ELISA), to detect S100P-RAGE binding, was developed based on a published protocol by Padilla *et al.*, (2014); 18 of all 93 lead compounds (purchased or synthesised in-house) which were screened for inhibition of S100P-RAGE

interaction using this assay significantly inhibited S100P/RAGE interaction. These lead compounds were assessed for their effects on two human pancreatic cancer cell lines; S100P-overexpressing cells (BxPC-3) and cells expressing reduced amounts of S100P (Panc-1) using the MTS assay (CellTiter AQ, Promega, for metabolic activity) and LDH release assay (CytoTox, Promega, for cell toxicity). Transwell invasion assays were also performed on the cell lines to determine the effect of treatment with the lead compounds.

RESULTS AND DISCUSSION

BxPC-3 cells treated with lead compounds (figure 1) Ogbeni *et al.*, (2015), for 48 hours, at 10 μ M, demonstrated a significant reduction (*P<0.0001) in cell invasion; whereas no effect was observed for the Panc-1 cells suggesting an S100P-specific mechanism (Figure 2). MTS and LDH release assays revealed that the compounds did not exhibit general cytotoxicity. Identified lead compounds will be further investigated for their effects on angiogenesis, and on the expression of key cell signalling proteins in pancreatic cancer cell.

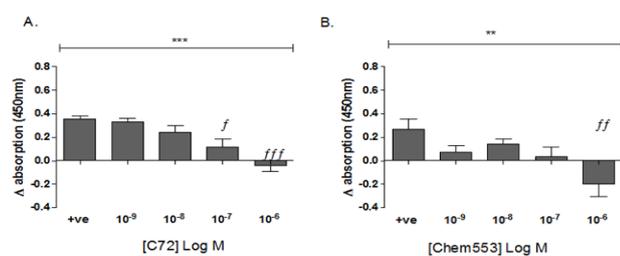


Figure 1. ELISA results from two novel compounds A. C54 and B. C56. Data are represented as the mean \pm

CONCLUSIONS

Results from this study has identified lead compounds with functional effects in S100P-expressing pancreatic cancer cells and may enable further development of a potential therapy for pancreatic cancer.

S.E.M, n=9 from 3 separate plates; significance by one-way ANOVA is denoted by *= p<0.05, and Dunnet's posthoc test f= p<0.05 compared to (+ve) control.

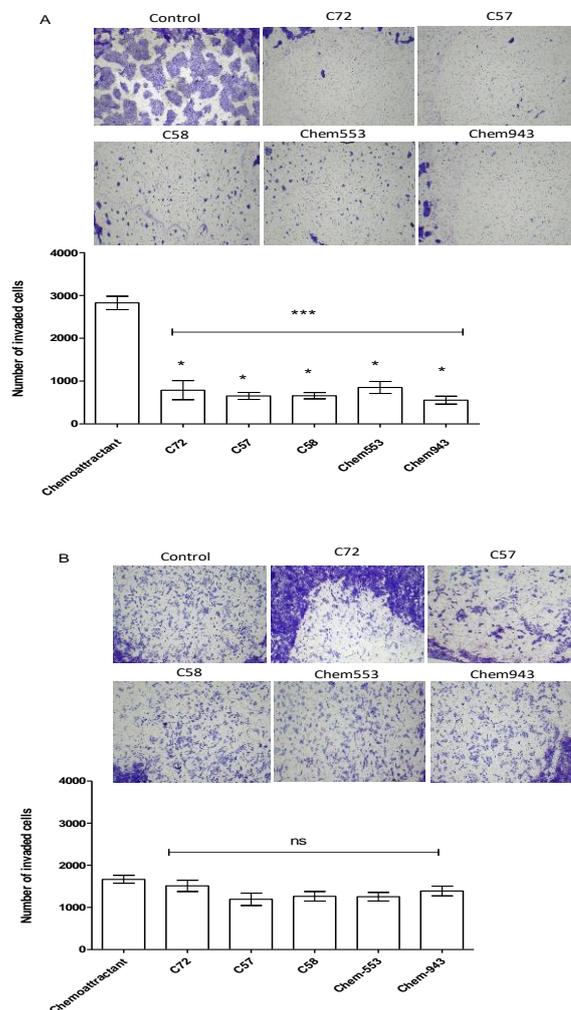


Figure 2. Transwell invasion assay from five novel lead compounds. A) Invasion of BxPC-3 treated with lead compounds B) Invasion of Panc-1 cells exposed to lead compounds. Data are the means \pm S.E.M of two independent experiments. Statistical significance was assessed by one-way ANOVA, *P<0.0001 vs. control.

REFERENCES

- Ogbeni, et al., (2015). pA2 Online, Vol. 13, No. 3, 256P.
- Padilla, L., Dakhel, S., & Hernández, J. (2014). *Biochemical And Biophysical Research Communications*, 446(1), 404-409.
- Raimondi S, Maisonneuve P, Lowenfels AB. (2009). *Nat Rev Gastroenterol Hepatol* 2009;6:699-708.