S100A2 and the ΔNp63 Network, an overlooked pancreatic cancer drug target

Christopher J. Scarlett1*, Jufeng Sun, Cecelia Russell, Jennifer Baker, Joey Ambrus, Peter Cossar, Jennette Sakoff, Melanie Pirinen, Hong Ngoc Thuy Pham and Adam McCluskey

1School of Environmental & Life Sciences, College of Engineering, Science & Environment, The University of Newcastle, Australia

*For correspondence: c.scarlett@newcastle.edu.au

Abstract Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with an abysmal ~10% survival rate at 5-years. With 48,000 projected deaths in 2021 it is now the third most common cause of cancer death in the USA, and will be number two by 2030. In Australia (2020) there were 3,900 new cases and a staggering 3,300 deaths. Current therapies are palliative at best highlighting the urgent need for novel therapeutic agents targeting molecular phenotypes not responsive to current treatments. The highly heterogeneous and metastatic nature of PDAC presents a major challenge in therapeutic development. Recent integratomic studies have demonstrated that PDAC is composed of two major transcriptomic subtypes - classical (pancreatic) and squamous (or quasi-mesenchymal/basal-like), which are characterised by distinct mutational landscapes. The squamous subtype is associated with gene silencing of endoderm specification genes; metabolic reprogramming; and an extremely poor clinical outcome. The squamous subtype shows significant S100A2 upregulation, and its presence is associated with poor patient prognosis. This has been overlooked in the pancreatic cancer drug development pipeline and offers an unparalleled opportunity to specifically target biomarkers known to be upregulated in PDAC. Importantly, S100A2 is considered a validated PDAC biomarker. We have identified molecular mechanisms that contribute to these aggressive, metastatic subtypes, and are targetable with novel drugs. Keywords: pancreatic cancer, S100A2, drug target, drug development

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Introduction Pancreatic ductal adenocarcinoma cancer (PDAC) is a devastating disease with an abysmal <10% survival rate at 5-years. With over 48,000 projected deaths in the USA for 2021, it is now the third most common cause of cancer death [1, 2], and is poised to number two by 2030 [3]. Current therapies, with only ~6-month life extension should be considered palliative at best, highlight the need for novel therapeutic agents targeting molecular subtypes not responsive to current treatments. The highly heterogeneous and metastatic nature of PDAC presents a major challenge in therapeutic development. There has been no real enhancement in PDAC patient outcomes in the past 5-decades, simply because we have failed to address the underlying biological pathways that promulgate this disease.

Recent integratomic studies have demonstrated that PDAC is composed of two major transcriptomic subtypes - classical (pancreatic) and squamous (or quasi-mesenchymal/basal-like), which are characterised by distinct mutational landscapes [4-6]. The squamous subtype is associated with gene silencing of endoderm specification genes; metabolic reprogramming; and an extremely poor clinical outcome. The squamous subtype shows significant S100A2 upregulation, and its presence is associated with poor patient prognosis. This has been overlooked in the pancreatic cancer drug development pipeline and offers an unparalleled opportunity to specifically target biomarkers known to be upregulated in PDAC. S100A2 is considered a validated PC biomarker. We have identified molecular mechanisms that contribute to these aggressive, metastatic subtypes, and are targetable with novel drugs. As such, we hypothesise that targeting S100A2 and the DeltaNp63 (ΔNp63) network by small molecules will address the significant unmet need in the treatment of PDAC. This will validate the S100A2/DNp63 nexus as a pancreatic cancer drug target. This has the potential to dramatically shift the PC treatment pipeline with significant, enhanced patient outcomes.
Results and discussion

Our recent landmark studies provided understanding of the genomic and molecular landscape of PDAC [4, 7, 8]. Via a leading edge molecular -omics approaches we identified subtypes and significant molecular mechanisms [5]. PDAC presents as two major transcriptomic subtypes - classical and squamous. We have demonstrated significant alignment with the Quasi-mesenchymal (QM-PDA) subtype [5], the Basal-like subtype [6] and the Squamous subtype [4] across all three classification systems, and all are associated with significantly poorer prognosis [9]. Our finding is that S100A2 overexpression is consistently observed across all three poor prognostic signatures – the squamous subtype (Figure 1).

Figure 1. Overexpression of S100A2 is indicative of the Squamous, QM-PDA and Basal-like poor prognostic signatures of PDAC [4,5,6].

S100A2 is hypomethylated and contributes to the aggressive squamous subtype

Our multi-omics analysis of 457 pancreatic adenocarcinomas [5] identified that the squamous subtype is enriched for mutations in TP53 and increased TP63 (∆Np63) expression, which along with S100A2, promotes invasion and metastasis, and patients with squamous tumours have significantly poorer prognosis. Significantly S100A2 was highly overexpressed in the squamous subtype due to hypomethylation (Figure 2A,B). These data are consistent with inherent differences between subtypes of PDAC and identify novel opportunities for therapeutic development, particularly the squamous subtype, which constitutes ~50% of PDAC patients in the advanced setting [10]. In 3 international patient cohorts (n>1000; Australian Pancreatic Cancer Genome Initiative (n=507); Glasgow Royal Infirmary (n=198); and University of Dresden (n=400)) we have validated high S100A2 expression as an independent poor prognostic factor for PDAC [15]. When included in a clinical variable preoperative nomogram, high S100A2 expression predicted poor patient outcome. Cancer of the pancreatic tail (poorer prognosis) is also associated with the squamous subtype, overexpresses S100A2, and is significantly associated with rapid metastatic disease recurrence post-surgery [11].
Figure 2. A. S100A2 is hypomethylated in the squamous subtype (A), leading to high S100A2 expression; B. with concomitant overexpression of DNp63; and is associated with aggressive PDAC of the pancreatic body and tail [4].

S100A2 defines a pro-metastatic PDAC subtype in vitro and in vivo

In vitro analyses identifies S100A2 as being a pro-metastatic molecule in PDAC via TGFβ, Src and CXCL5 signalling pathways. Transcriptome profiling identified de-regulation of key PDAC associated genes in S100A2 overexpressing xenografts in vivo, such as down regulation of TGFβ signalling, genes associated with regulation of the actin cytoskeleton, focal adhesions and adherens junctions. Tyrosine kinase phosphoproteomic profiling also revealed increased activation of members of the src family kinase (SFK). Src is involved in many aspects of tumour cell behaviour that impacts metastatic capacity. These changes were less evident than those observed with S100A2.

Drugs targeting S100A2

Modelling studies have identified a Drugable Pocket (DP) in the S100A2 dimer. The DP colocalises with the p53 interaction domain of S100A2 (Figure 3A). S100A2 tightly binds the p53 TET domain, as well as the nuclear localisation signal and C-terminal region. Uncommonly for S100 proteins, S100A2 also binds the p53 negative regulatory domain [12, 13]. Intriguingly, S100A proteins possess a higher affinity for the TET domains of the p53 homologs, p63/p73, than for that of p53 (S100A2 specifically to p63), suggesting that the p53 homolog p63 could be the preferred target of S100A2, and thus enable drug selectivity. Inhibition at this site is a potentially novel protein-protein inhibition approach to a drug outcome [14, 15]. As such it is likely that S100A2 plays a role in regulating the oligomerization state of the three p53 family members in an activation state dependent manner.
Figure 3. A. S100A2 homodimer with p53 co-bound; B. Docked pose of the top 10 most active analogues prepared of (follows p53 path); C & D. Chemical structures and cytotoxicity of leads PC1 and PC2, respectively. PC2 binds in the p53 path (not shown), but interacts with the ‘p53 detour’ reducing activity [16,17,18].

In silico screening of our propriety library (ca. 10,000 compounds) revealed a number of ‘hits’ against the S100A2-DP. Of these, PC1 and PC2 were the most synthetically tractable (Figure 3C & D). PC1 interacts in the DP via SO2NH oxygen H-bonds with Eb6 and Sa86 (both dimers); the aromatic amide forms a F45-π-π interaction; and the PC2 1,2,3-triazole moiety affords a Tβ14-π-π; the terminal phenyl moiety, a Ha17-π-π and the 3-OCH3 H-bonds with Da88A and N487 (McCluskey unpublished). PC1 is more potent than PC2 against MiaPaCa-2 cells, with GI50 ~ 3 μM vs. ca 50 μM (PC2). This difference is predicted by their proposed binding poses (Figure 3B), ie. the active PC1 more completely binds in the ‘p53 path’, while PC2 engages with the ‘p53 detour’. The cytotoxicity of these molecules is consistent with disruption of S100A2’s regulation of p53 and its homologues. Of the 40 PC1-based analogues screened, all returned GI50 < 30 μM. Modelling led synthesis efforts saw rapid potency enhancements with our leads with PC1 giving PC1-01 and PC2 gave PC2-1 as 1.2±0.35 and 0.48±0.08 μM inhibitors of the S100A2 expressing PC cell line, BxPC-3 (Figure 4), thus validating our S100A2 targeting approach [16,17,18].
Figure 4. A. Modelling designed 2nd generation S100A2-p53 inhibitors. GI50 values against the S100A2 overexpressing, BxPC-3 cells; B. Modelling image showing a model compound in the S100A2-p53 pocket. SA78 and YA82 are highlighted as covalent inhibitor capable DP residues; C. Specific covalent binding moieties that will be appended to leads PC1-1 and PC2-1 targeting SA78 and YA82. Boronic acids (B(OH)2) offer reversible covalent inhibition [16,17,18].

Conclusions

PDAC will be the second most common cause of cancer death by 2030. The need for novel therapeutic agents targeting molecular subtypes not responsive to current treatments is vital. We have identified S100A2 as a significant contributor to the aggressive squamous subtype of PDAC and have developed lead compounds that target S100A2. There is now an urgent need to generate further pre-clinical evidence that provide us a clear line-of-sight in translating this novel therapeutic concept into the clinic. This is an urgently required new therapeutic target for pancreatic cancer.

References


