Anti-CXCR2 directed therapy unmasks the potential for immunotherapy in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy. Therapies targeted against stromal targets in the tumour microenvironment are now in pre-clinical and clinical trial. Here, we focus on our recent findings in autochthonous models of PDAC that suggest CXCR2 expressed on neutrophils is important in establishing immunosuppression in the primary tumour and the metastatic niche at distant sites. We discuss CXCR2 as a potential therapeutic target in the context of other potential stromal targets in PDAC.

Keywords: Pancreatic cancer; CXCR2; immunotherapy

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PDAC is a virtually incurable systemic disease. Surgery for disease that appears radiologically resectable still only offers marginal survival benefit with high rates of involved margins following resection [1]. Advances in recent years mean that chemotherapy regimens have improved patient survival, with FOLFIRINOX or Gemcitabine combined with nab-paclitaxel considered the current standards of care for metastatic disease [2, 3]. Neoadjuvant use of FOLFIRINOX prior to surgery in borderline resectable or locally advanced PDAC has shown promise in some studies however, the 5-year survival rate is still only 8% [4]. The survival rates for patients with PDAC are low, in part, because at the time of diagnosis the majority of tumours are already locally invasive or metastatic, and because they are also highly resistant to most chemotherapy.

It is now becoming more apparent that microenvironmental factors both in the pancreas and at sites of metastatic disease can profoundly affect tumour biology, and thus may represent targets for novel therapy. Interactions between tumour cells, associated fibroblasts, and the extracellular matrix have been a focus of research for several years. Indeed, the role of stromal cells in establishing the metastatic niche for tumour cells at sites of distant metastases has been appreciated for many years [5]. More recently, a spotlight has fallen on the role of the immune system in tumour development and progression. There is substantial evidence that inflammation can promote tumourigenesis, but we are only now beginning to understand the mechanisms responsible for this. Likewise, the failure of anti-tumour immunity was well known, but the understanding of how to re-engage the anti-tumour response was lacking. The recent development of immune checkpoint inhibitors that activate T cells to mount an anti-tumour response have provided new
hope, and transformed treatment of melanoma [6].

We became interested in the role of neutrophils in pancreatic cancer having recently showed that neutrophil homing, regulated by CXCR2 chemokine signalling, plays a significant role in pancreatic inflammation and pancreatitis [7]. In addition, high numbers of infiltrating neutrophils in human pancreatic cancer are predictive of poor prognosis [8]. Neutrophils have also been shown to play a key role in establishing the metastatic niche for cancer cells [9]. Joan Massagué’s group first noted that immature granulocytic immune cells were present in high number at the site of breast cancer metastases [10]. Subsequent work by this group established the importance of CXCR2 signalling in sustaining this metastatic niche for tumour cells and providing resistance to chemotherapy [11]. Pancreatic tumour cells have also been shown to release factors that stimulate the chemotaxis of immature granulocytes from the circulation to the tumour microenvironment [12]. These cells, analogous to neutrophil or neutrophilprecursors, have been shown to promote primary tumour progression via immunosuppressive actions [13], hence they have been referred to as myeloid derived suppressor cells (MDSCs). Direct inhibition of these cells has shown promise in sensitising primary PDAC to immunotherapy in murine models [13].

CXCR2 is a G-protein coupled receptor expressed upon the cell surface of neutrophils that permits their chemotaxis to sites of inflammation. CXCR2 has been shown to be both tumour suppressive and promoting dependent on context. Acosta et al demonstrated that CXCR2 ligands are critical to the reinforcement of the senescence associated secretory phenotype (SASP) following Kras mediated oncogenic transformation of cells [14]. Inflammatory signalling in this context drives a senescence phenotype that requires further mutations in tumour suppressor gene function to permit tumour formation. On the other hand, we have shown that CXCR2 plays an important role in both inflammation-associated and spontaneous tumourigenesis via influx of pro-tumourigenic granulocytes [15]. Furthermore, CXCR2 expression on PDAC cells ex vivo can enhance proliferative, invasive and migratory capacity [16-18].

CXCR2 represents a potentially exciting therapeutic target in established PDAC, with potential effects on primary and metastatic tumour progression. We have recently demonstrated, in a complex autochthonous model of PDAC, that loss or inhibition of CXCR2 can profoundly suppress metastasis, and enhance survival in combination with chemotherapy [19]. Pdx1-Cre; KrasG12D/+; Trp53R172H/+ (KPC) mice [20], are considered the ‘gold-standard’ in terms of models for pancreatic cancer research [21]. These mice develop PDAC over 2-10 months with high penetrance and frequent metastasis to liver, lung and diaphragm. We found that when the Cxcr2 gene was deleted systemically in these mice, there was no effect on primary tumour burden, however metastasis was almost completely abrogated. Importantly, the effect was not recapitulated when we deleted Cxcr2 specifically from tumour epithelial cells, suggesting that non-tumour immune cells mediate the anti-metastatic effect. We were also able to inhibit metastasis using a neutrophil blocking antibody in vivo [15], confirming this hypothesis. When we examined the microenvironment of tumours, we observed a striking increase in infiltration of CD3+ T cells.

Importantly, we were able to replicate the anti-metastatic effect of genetic Cxcr2 deletion using a small molecule inhibitor of CXCR2. Treating KPC mice with CXCR2 inhibitor alone or in combination with standard adjuvant chemotherapy gemcitabine, from a time-point when they exhibit extensive advanced pancreatic neoplasia, resulted in a significant suppression of metastatic disease. Furthermore, CXCR2 inhibition in this context also significantly extended the lifespan in these mice. We hypothesised that CXCR2’s potential role in promoting senescence in early tumourigenesis may account for discrepancies seen between genetic and inhibitor driven studies owing to the different chronological points of CXCR2 inhibition. Once more, CXCR2 small molecule inhibition significantly increased the infiltration of CD3+ T cells into the microenvironment of PDAC.

Therefore, CXCR2 signalling modulates progression of both primary tumours and metastatic burden. As discussed, the role of microenvironmental signals in facilitating metastasis by establishing a metastatic niche is well known. When we examined metastases in untreated KPC mice we observed high numbers of neutrophils. Similarly, in the pre-metastatic livers of untreated KPC mice, we observed a substantial number of neutrophils. In the pre-metastatic liver of mice where CXCR2 signalling was abrogated, either genetically, or pharmacologically, however, the number of myeloid cells was dramatically reduced. Thus, we hypothesised that in the absence of CXCR2 signalling and neutrophil homing there was a failure to establish a metastatic niche.

Immunotherapy has elicited impressive responses in several types of tumour, most notably melanoma, however, pancreatic tumours have proved refractory to treatment thus far. Given the profound influence that CXCR2 deletion or inhibition had on intra-tumoural T cell numbers we questioned whether CXCR2 inhibition would now enhance the ability of T cell checkpoint inhibitors to promote
anti-tumour immunity in this model. When mice with late-stage tumours were treated with the combination of CXCR2 small molecule inhibitor and a blocking antibody against the T cell checkpoint inhibitor, PD1, their survival was dramatically increased, in 2 cases to over 100 days from point of therapy. This phenomenon is all too seldom observed in the robust KPC model of PDAC and represents a significant finding.

Together, these findings highlight distinct opportunities for targeting CXCR2 therapeutically in PDAC. They provide evidence that CXCR2 positive neutrophils are critical in the establishment of the metastatic niche, and suggest that targeting the neutrophil population may be worthwhile in preventing metastatic recurrence in resectable PDAC. In late-stage unresectable disease, the additional benefit of immunotherapy when combined with CXCR2 inhibition suggests that this combination should be urgently explored in patients.

Conclusions

Our work demonstrates the potential of targeting CXCR2. Many other equally interesting stromal targets within the primary tumour and at metastatic sites exist, including macrophage secreted granulin [22], CSFR1 and JAK/STAT pathways [23, 24]. A complex interplay exists between tumour cells and the microenvironment in PDAC, and it is expected that PDAC will find resistance pathways to stromally targeted therapies, as with traditional chemotherapeutic agents. Thus, it is likely that a multi-pronged approach will be required to target this complex systemic disease in future.

Over the coming years these combinatorial treatments are likely to be trialled in human PDAC patients as part of an all-encompassing precision medicine approach to therapy [25]. The approach to PDAC therapy is likely to change as a result, viewing each patient as having an individual disease. Moves towards neoadjuvant therapies are being universally adopted. We see no reason why immunotherapies, including CXCR2-directed therapies, cannot form part of neoadjuvant treatment regimens in future. Indeed introduction of early therapy prior to surgery may help select patients for surgery and allows declaration of micrometastatic disease following the re-staging process. Well-designed human PDAC trials will allow assessment of the applicability of our experimental findings to patients with this disease.

Conflicting interests

The authors have declared that no conflict of interests exist.

Author Contributions

CWS prepared the manuscript; NBJ, CRC, and OJS edited the manuscript; JPM prepared and completed the final edit of the manuscript.

Abbreviations

PDAC: Pancreatic ductal adenocarcinoma; MDSCs: myeloid derived suppressor cells; SASP: senescence associated secretory phenotype; KPC: Pdx1-Cre; KrasG12D+/-; Tp53R172H+/-.

References

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