LOXL2 in pancreatic tumourigenesis: the complexity of tumour–stromal crosstalk exemplified

Seth B Coffelt,[1,2] Jennifer P Morton[1,2]

The role of the lysyl oxidase (LOX) family of enzymes, consisting of LOX and LOX-like (LOXL) proteins 1–4, is to catalyse the crosslinking of collagen and elastin in the extracellular matrix (ECM). Members of the family have been implicated in tumour progression in a range of cancers, with multiple studies suggesting that LOX/LOXL-mediated ECM remodelling facilitates tumour cell invasion and metastasis. Increased expression, particularly of LOX and LOXL2, has been noted in many aggressive cancers and has been linked to reduced survival. However, the mechanisms of action are still unclear, and there is an ongoing debate over whether expression is tumour supporting or tumour suppressive.[1 2] This appears, to some degree at least, to be dependent on tumour site, stage and even the source of expression, highlighting the need to understand the cell and stage-specific consequences of LOX/LOXL activity.

Pancreatic ductal adenocarcinoma (PDAC) is notable for the abundance of collagen in the tumour microenvironment (TME), and as such has been the focus of several studies examining LOX or LOXL2 activity. However, the conflicting results reported have raised as many questions as answers. Indeed, more generally, there has been controversy over the stroma and ECM in PDAC, and although they clearly influence disease initiation and aggressiveness, they can exhibit tumour-suppressive activity in some contexts.[3] In this issue, Alonso-Nocelo *et al* sought to address some of these questions, investigating the role of LOXL2 in pancreatic tumourigenesis using model systems.[4]

The authors exploited genetically engineered mouse models of loss or gain of LOXL2 expression specifically within the pancreatic epithelium to demonstrate that overexpression of LOXL2 promotes tumour initiation and metastatic capacity, potentially through increased epithelial–mesenchymal transition (EMT) and stemness. Consistent with this, they found that loss of LOXL2 decreased metastasis and increased survival, primarily due to significantly less organised ECM, and reduced stiffness and mechanosignalling. Importantly, human datasets support these findings, with higher LOXL2 expression associated with decreased survival and enrichment for an EMT signature,[4] suggesting that evaluation of LOXL2 targeting in the metastatic setting may be warranted.

Given the ongoing debate around LOX and LOXL activity and the role of ECM in pancreatic tumour progression, these are important findings. Recent studies have reported conflicting data to those presented here and suggested that both collagen 1 and LOXL2 suppress PDAC progression. In one study, Jiang *et al* found that low stromal content correlated with poor prognosis in human PDAC, and using an orthotopic syngeneic transplant model, they showed that ECM ablation using an anti-LOXL2 antibody promoted PDAC progression.[5] In this setting, the tumour cells themselves had higher proliferative capacity, but there were no significant changes to cancer-associated fibroblast (CAF) or immune cell populations, or tumour vasculature. In an analogous study, Chen *et al* demonstrated that deletion of collagen 1 from alpha smooth muscle actin cells in an autochthonous model led to a reduction in stromal collagen 1 content and decreased stiffness, but a more poorly differentiated and invasive phenotype resulting in accelerated disease progression.[6] They observed myeloid cell accumulation in tumours and an ultimately immune-suppressive environment, which was important for the aggressive tumour phenotype and reduced survival since myeloid targeting therapies reversed this effect.[6]

There are a few differences to highlight here. In the studies by Alonso-Nocelo *et al* and Chen *et al*, LOXL2 or collagen 1 was deleted from epithelial cells or myofibroblasts, respectively, in contrast to the systemic therapeutic approach taken by Jiang *et al*.[4–6] Interestingly, proteomic analyses have shown that while the majority of ECM proteins originate from stromal cells, tumour cell-derived ECM proteins are more clearly associated with poor prognosis.[7] Thus, the cell type targeted may influence outcomes in preclinical studies. Both Alonso-Nocelo *et al* and Chen *et al* also noted changes to the immune TME, which may impact on the phenotypes observed. These studies both used autochthonous models in which tumour progression and TME establishment occur spontaneously and over a longer time, while Jiang *et al* used a transplant model.[4–6] Clearly, the choice of model system may influence study outcomes, a point underscored by the lack of LOXL2 expression in many PDAC cell lines.[4] Finally, in both the Alonso-Nocelo *et al*'s and the Chen *et al*'s study, deletion of LOXL2 or collagen 1 occurred from birth, prior to tumour development, whereas, in the anti-LOXL2 therapeutic study conducted by Jiang *et al*, treatment commenced post-transplant.[4–6] Again, the timing of treatment could significantly influence outcomes, and the effects of LOXL2 inhibition in established tumours in the autochthonous setting remain to be seen. Indeed, in the clinic, a trial of the LOXL2 blocking antibody, simtuzumab, in combination with gemcitabine, failed to improve survival in patients with metastatic PDAC,[8] although it is worth noting that this may not be the most appropriate disease setting.

Over the past few years, several studies have proposed targeting the stroma as a potential therapeutic strategy in pancreatic cancer; however, despite many of these reaching the clinic, results have been disappointing. More recently, evidence has emerged to suggest that the stroma can have tumour-restricting potential, with complete ablation resulting in tumour promotion. Thus, the consensus is now that more nuanced targeting of signalling within the stroma is more likely to yield clinical benefit. The prospects for more selective approaches have been enhanced by the description of different cellular subtypes within the PDAC microenvironment, notably subpopulations of CAFs, which can exhibit both tumour-promoting versus restrictive behaviour.[9]

Interestingly, it is not only the CAFs that exhibit functional heterogeneity in the PDAC microenvironment. Many studies have highlighted a tumour-promoting role for tumour associated macrophages (TAMs) in PDAC, but these too display heterogeneity and can have distinct profiles. In PDAC, TAMs can originate from both the yolk sac and the bone marrow; however, the yolk sac-derived

[1]Cancer Research UK Beatson Institute, Glasgow, UK
[2]Institute of Cancer Sciences, University of Glasgow, Glasgow, UK

Correspondence to Professor Jennifer P Morton, Cancer Research UK Beatson Institute, Glasgow, UK; Jennifer.Morton@glasgow.ac.uk

tissue-resident macrophages are reported to exhibit a profibrotic transcriptional profile and tumour-promoting capacity, distinct from that of the infiltrating monocytes.[10] On the other hand, evidence suggests that these tissue-resident macrophages have tumour-suppressive properties during the early stages of tumorigenesis.[11] Thus, macrophage targeting is also an area where a reprogramming, rather than ablative, approach may be appropriate. Intriguingly, Alonso-Nocelo *et al* highlight a novel mechanism by which TAMs may promote tumour progression. They reveal that LOXL2 expression by PDAC cells is mediated in paracrine fashion by macrophage-secreted oncostatin M (OSM), and subsequently, show that macrophage depletion in vivo can reduce expression of OSM and LOXL2, alter collagen structure and reduce metastasis.[4] These findings are supported by work from the Jørgensen lab, which described how macrophage-secreted OSM could reprogramme fibroblasts to drive a more tumourigenic TME and facilitate metastasis in vivo.[12] Of course, the efficacy of macrophage depletion in this study, and others, is likely not due solely to LOXL2 reduction. However, these findings could at least partly explain some of the efficacy observed in macrophage targeting experiments, and interrogation of the effects of LOXL2 depletion or OSM inhibition in tissue resident macrophages compared with infiltrating monocytes could be of interest.

Ultimately, much more work is still needed in this field. The differing reports of the role played by LOXL2 and, more generally, the influence of the ECM on pancreatic tumourigenesis highlight further the complex nature of tumour–stromal crosstalk and the need to better understand these interactions in this devastating disease.

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