The interplay between extracellular matrix remodelling and kinase signalling in cancer progression and metastasis

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The behaviour and phenotype of cancer cells, and subsequently the progression of cancer, are dictated by the interplay between the intrinsic genotypic features of the cancer cells; stromal cells such as fibroblasts; resident and recruited immune cells; and environmental cues from the extracellular matrix (ECM). The ECM provides not only a structural scaffold to support cell growth, but is also a master regulator of cell activity and behaviour via ‘outside-in’ signalling mechanisms. The activation of intracellular signalling pathways by extracellular cues can promote, or suppress, proliferative and survival programs in both normal and cancerous tissues.

Both normal and tumour ECM are continually deposited, remodelled and degraded. However tumour ECM in particular tends to undergo aberrant remodelling through, among other events, disproportionate post-translational collagen crosslinking, leading to a stiffer fibrotic microenvironment. For years, many believed that remodelled tumour ECM merely accompanied tumour growth; however a growing body of evidence now strongly implicates ECM remodelling in driving cancer progression through activation of intracellular kinase signalling pathways, subsequently alter cancer cell behaviour.

In recent years, the existence of feed-forward regulatory loops between dynamic ECM remodelling and cancer cell survival has been uncovered. Developing therapies to target components of these networks – either intrinsic kinase signalling within tumour cells, or the remodelling of the tumour ECM – can potentially disrupt growth-permissive microenvironments to slow cancer progression and reduce metastasis.

It has been hypothesised that during the early stages of cancer growth, remodelling of the ECM serves a largely protective role by attempting to restrict the expansion of the primary tumour. This is supported by recent work focussed on engineering significant reductions in the tumour desmoplastic response in pancreatic ductal adenocarcinoma (PDAC). Through targeting either aSMA+ pancreatic fibroblasts directly, or indirectly via blocking sonic hedgehog signalling to reduce stromal density, the decrease in ECM led to a rapid, unrestricted primary tumour growth and increased metastasis. Whilst these findings support the concept of an initial protective role of the ECM, there is also evidence to suggest that the excessive accumulation of ECM and extensive remodelling subsequently activates intracellular signalling cues, as well as providing additional biomechanical cues that survival, acceleration growth, dissemination of tumour cells.

Remodelling of the ECM in many solid tumours is driven by the activity of lysyl oxidases (LOX), a family of amine oxidases that catalyse the post-translational crosslinking of collagen molecules and which are pivotal to collagen biogenesis and maturation. Aberrant LOX expression in tumours leads to elevated collagen deposition within the tumour stroma. The sustained,

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accumulative activity of LOXs are primarily responsible for increasing primary tumour stiffness. As such, high LOX expression has been correlated with poor prognosis in several solid tumours including colon, breast and pancreatic cancer\textsuperscript{9-15} (for a detailed review, see\textsuperscript{15}). LOX-mediated activation of kinase signalling, either biomechanically via ECM remodelling (Fig. 1A) or indirectly through other mechanisms such as the release of reactive oxygen species (ROS) – a by-product of amine oxidase activity\textsuperscript{16} – can promote both the growth of tumour cells within the primary tumour, and also facilitate the migration of cells out of the primary tumour to secondary sites.

Integrins are one of the major families of cell adhesion receptors\textsuperscript{17} and one of the most extensively studied in cancer, owing to their involvement in many cell-matrix interactions and strong association with cancer progression\textsuperscript{18} and chemoresistance.\textsuperscript{19} Changes in integrin conformation, localisation and avidity, via both LOX-mediated and LOX-independent ECM remodelling, are critical mediators in transducing extracellular signals that regulate intracellular kinase signalling pathways involved in determining cancer cell fate. LOX-mediated integrin activation has been shown to activate focal adhesion kinase (FAK) and its associated tyrosine kinase SRC

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\textbf{Figure 1.} A summary of interactions between ECM remodelling and kinase activity. (A) LOX activity catalyses the crosslinking of collagen fibres, essential for both fibrillar collagen deposition and increases in ECM stiffness. (B) LOX-mediated ECM remodelling triggers FAK/SRC signalling pathways via integrin activation, influencing focal adhesion assembly/disassembly and cell-to-cell contact, as well as regulating cell proliferation and cell survival. (C) DDR2 is activated by fibrillar collagen and can activate the PI3K/Akt and SRC kinase pathways. (D) LOX expression can also activate the Akt signalling pathway via PDGFRβ activation, inducing both changes in cell survival and increased expression of VEGF, leading to enhanced angiogenesis. (E) LOX has also been implicated in the breakdown of TGFβ signalling by catalysing the assembly of HTR1A, resulting in disruptions to TGFβ activation. This inhibition of TGFβ signalling causes an upregulation of MATN2, which traps EGF receptors at the cell surface and leads to the activation of signalling pathways involved in proliferation and cell survival. (F) ECM-meditated FAK/SRC activation can also regulate RHO-ROCK activity to modulate cell migration and ECM remodelling via cytoskeletal changes. (G) ECM components are degraded by a range of matrix degrading enzymes such as MMPs, cathepsins, hyaluronidases and ADAMs/ADAMTSs, which can then serve as ligands for receptors. (H) Extracellular kinases can phosphorylate ECM components and potentially alter downstream kinase signalling pathways. (I) Mutations in TGFβ signalling pathway in cancer can lead to an upregulation of STAT3 kinase signalling, which can in turn alter ECM deposition and dictate tumour ECM stiffness. (J) Elastin activates elastin binding protein receptors (EBPR), which can subsequently activate PI3K/Akt and ERK1/2 signalling pathways to enhance cancer cell proliferation and migration.
in tumour cells, which promotes cell survival and proliferation in primary breast and colorectal tumours (Fig. 1B).\textsuperscript{9,13,20} Phosphorylation of FAK is also necessary for cell migration and anchorage independent growth in fibroblasts,\textsuperscript{21} and has been linked with altered epithelial cell-to-cell contact and subsequent impairment of vascular integrity, leading to enhanced migration.\textsuperscript{22} In addition to outside-in signalling activation through integrins, altered ECM deposition and remodelling has also been linked with activation of discoidin domain receptor 2 (DDR2)\textsuperscript{23} on mesenchymal stem cells (MSCs) in breast cancer. This also leads to the activation of both SRC and PI3K/Akt downstream signalling pathways, which acts to increase further collagen deposition, cell migration and support metastatic growth (Fig. 1C).\textsuperscript{24}

In addition to the activation of FAK/SRC signalling, LOX-mediated ECM remodelling has also been shown to activate the Akt pathway through activation of platelet derived growth factor β (PDGFβ) signalling, leading to increased expression of vascular endothelial growth factor (VEGF) and enhanced angiogenesis in colorectal cancer models (Fig. 1D).\textsuperscript{25} This in turn facilitates the migration of tumour cells away from the primary site. LOX has also recently been implicated in the assembly of the serine protease HTRA1, which degrades transforming growth factor β (TGFβ) and subsequently reduces TGFβ signalling. Decreased TGFβ signalling in turn leads to increased secretion of the ECM component Matrilin2 (MATN2) and its localisation at the cell surface (Fig. 1E). MATN2 presence at the extracellular cell surface anchors epidermal growth factor receptor (EGFR) and sustains the activation of downstream kinase signalling pathways involved in maintaining cell survival.\textsuperscript{26}

A range of other kinase signalling pathways such as the GTPase RHO and its associated kinase (ROCK), both of which are involved in cytoskeleton regulation and aiding cell migration, are also activated by ECM remodelling. They have recently been shown to subsequently promote further ECM remodelling and increase tumour invasiveness in squamous cell carcinoma\textsuperscript{27} and PDAC\textsuperscript{28} (Fig. 1F). A stiffer tumour microenvironment was also shown to activate ROCK-mediated contractility via increased integrin clustering in fibroblasts and mammary epithelial cells, leading to higher focal adhesions and enhanced cell growth.\textsuperscript{29}

Deregulation of a range of other ECM components in the primary tumour microenvironment have also been implicated with altered kinase signalling and cancer progression. Aberrant expression of decorin in oral cancer has been linked with angiogenesis via altered MMP9 and VEGF expression,\textsuperscript{30} whilst increased decorin, in combination with the downregulation of periostin, can lead to breast cancer motility and invasion.\textsuperscript{31} SPARC (secreted protein acidic and rich in cysteine) is aberrantly expressed in multiple types of solid cancer, in particular melanoma, and is associated with cancer extravasation through a p38-MAPK signalling activation and the subsequent re-organisation of the actin cytoskeleton.\textsuperscript{32} Collectively, these findings support an important role for ECM remodelling in influencing cell behaviour via the activation of a range of kinase signalling pathways, which together promote tumour cell survival and increase the propensity for tumour cells to escape the primary tumour and migrate to secondary sites.

In addition to ECM deposition and post-translational modification, another important element contributing to the dynamic ECM in tumour progression and metastasis is the proteolytic degradation of the ECM. The extracellular microenvironment is continually turned over by a range of matrix-degrading enzymes.\textsuperscript{3} These enzymes include matrix metalloproteinases (MMPs) such as gelatinases, matrilysin and collagenases; ADAMs (a disintegrin and metalloproteinases); ADAMTSs (ADAMs with thrombospondin motifs)\textsuperscript{33,34}, cathepsins; hyaluronidases and serine and threonine proteases\textsuperscript{34} (Fig. 1G). Generally speaking, most tumour driven fibrosis is a result of lower ECM turnover compared to the excessive deposition.\textsuperscript{35} However in some cases deregulation of ECM breakdown can, via the activation and suppression of kinase signalling pathways, also influence cancer cell survival. In particular, aberrant cathepsin protease activity and localisation in the tumour microenvironment has been shown to influence cancer progression, invasion and metastasis.\textsuperscript{36} A range of MMPs have also long been implicated in both the promotion and inhibition of cancer progression, in particular through their effect on ECM remodelling and subsequent changes in integrin-mediated signalling.\textsuperscript{34,37}

In addition to the interaction between ECM remodelling and intracellular kinase signalling, the existence of extracellular kinases such as VLK and FAM20C that can phosphorylate a range of ECM components has recently been discovered (Fig. 1H).\textsuperscript{38-40} There is evidence of widespread ECM phosphorylation in both normal and cancerous tissues, and phosphorylated ECM components have been found to modulate downstream signalling activity and exert changes in cell adhesion and migration.\textsuperscript{38,40-44} Although the exact role of extracellular kinases in tumour ECM remodelling, cancer progression and metastasis remains poorly understood to date, the discovery of these kinases is an exciting development and provides further evidence for the impact of subtle ECM cues on directing cell behaviour.

The effect of ECM remodelling on cancer cell survival does not always occur in isolation. Rather, it is often the
interplay between microenvironmental cues and the oncogenic genotype of tumour cells that together influence stromal properties and subsequently modulates tumour growth. This interaction between intrinsic and extrinsic factors in driving cancer progression was recently shown by Laklai et al. The authors showed that genetic alterations in TGFβ signalling in pancreatic cancer cells alters STAT3 kinase activity and leads to an increased deposition and remodelling of the tumour ECM, elevating tumour stiffness (Fig. 1I), and driving a malignant transition leading to enhanced tumour growth.45 This interplay between the inherent genotypic features and the external ECM landscape can establish a feed-forward network that promotes tumour growth through the activation of signalling pathways that facilitate further ECM remodelling. The resulting altered microenvironment can in turn activate signalling programs to drive enhanced cell survival, tumour progression and additional ECM remodelling. This reciprocal feedback between ECM cues and tumour cell signalling does not only regulate tumour progression and metastasis, but can also dictate tumour response to therapeutic agents, as well as the recruitment and activation of non-malignant stromal cells. This therefore underpins the need to uncouple or target the link between ECM remodelling and cell survival in order to improve the treatment of cancer.

The role of remodelled ECM in promoting or suppressing cancer cell survival is a dynamic process that can be induced systemically or driven by a range of local microenvironmental factors. This link can be disrupted by either targeting the process of tumour stromal remodelling and normalising the stroma, or by blocking cellular response via kinase signalling networks to the remodelled tumour ECM. These approaches, either independently or in combination, have the potential to significantly reduce both cancer progression and metastatic outgrowth.

Firstly, using the example above, the level of LOX-mediated ECM remodelling itself can be targeted through the use of small molecule LOX inhibitors or activity blocking antibodies. LOX inhibition can significantly reduce growth of breast cancer cells, both in vitro46 and in mouse models, where the growth at both the primary and secondary sites is inhibited.5,12 Furthermore, LOX inhibition can decrease the development of organ fibrosis by reducing the extent of collagen biogenesis and crosslinking, subsequently preventing the colonisation of metastatic tumour cells at these sites.50 Blocking LOX activity also leads to decreases in cancer associated fibroblast activation via FAK signalling in breast cancer.47 In addition to this, inhibition of LOX in PDAC mouse models decreased tumour cell migration and invasion, completely blocking metastasis, and the combination of LOX inhibition with standard-of-care therapy gemcitabine, resulted in significantly improved survival.10 Of note is that LOX inhibition alone completely inhibited metastasis without affecting primary tumour growth, that LOX mediated crosslinking may have context dependent roles in the different stages of pancreatic tumour progression.

Secondly, a recent pioneering study has revealed that the remodelling of tumour ECM in pancreatic cancer can also be targeted by blocking the ability of fibroblasts in the stroma to respond to ECM-mediated signalling using short-term priming with Fasudil, a small molecule kinase inhibitor of ROCK.48 This approach led to tumour ECM ‘relaxation’ and sensitised tumour cells to standard of care chemotherapy (gemcitabine/Abxaxane), resulting in a significant reduction in primary tumour growth and metastasis.

Finally, the ability for downstream signalling pathways in tumour cells to respond to changes in the ECM can also be targeted through inhibitors of FAK/SRC pathways49,50 which can uncouple the effects of ECM remodelling on cell proliferation and tumour progression. A similar role has been shown in a non-cancer setting for cardiac fibroblasts following myocardial infarction.50 Collectively, this approach to targeting ECM remodelling and its effects on signalling programs within tumour and stromal cells has the potential to reduce tumour burden both at the primary tumour and also at secondary sites.

In order for successful colonisation of secondary sites to occur, tumour cells must establish a growth permissive microenvironment. Recently, it has been shown that this can proceed prior to the physical arrival of tumour cells at secondary sites via the establishment of pre-metastatic niches (for a comprehensive review, see51). ECM remodelling is a significant component in the establishment of these pre-metastatic niches, which have the potential to alter tumour cell kinase activity following their arrival and enhance their ability to colonise and survive. LOX secreted from primary tumours can mediate pre-metastatic ECM remodelling at distant sites, which subsequently supports metastatic outgrowth and colonisation. This occurs through the recruitment of CD11b+ bone marrow derived cells (BMDCs)12 and/or non-malignant VEGFR1+ haematopoietic progenitor cells at these secondary sites,52 which acts to enhance colonisation by metastatic cells. In addition, high expression of LOX in primary breast tumours has also been shown to drive the formation of pre-metastatic lesions in the bone, through altering NFATc1 expression and kinase signalling in osteoclasts. This then increases their osteolytic activity and results in the generation of bone pre-metastatic...
niches that promote the colonisation and proliferation of metastasing breast cancer cells.\textsuperscript{73}

A range of other ECM components such as tenascins, versican, peristin, hyaluronan and elastin have also been implicated in the formation of the pre-metastatic niche and in the activation of signalling pathways that enhance metastatic colonisation.\textsuperscript{1,54,55} The upregulation of tenasin C expression has been observed in primary breast\textsuperscript{56} and colon carcinoma cells,\textsuperscript{57} as well as in stromal cells at distant sites that promote colonisation and metastatic outgrowth.\textsuperscript{58} Versican is involved in the proliferation, adhesion and motility of cells through activation of intracellular signalling pathways, and is upregulated in many types of solid cancer such as breast, lung and colon.\textsuperscript{59} Versican interacts with a large number of ECM and cell surface structural components through its central glycosaminoglycan-binding region and N-(G1) and C-(G3) terminal globular domains. Versican has also been shown also promote metastatic outgrowth through the establishment of a pro-inflammatory tumour microenvironment via Toll-like receptor 2 (TLR2) activation in myeloid cells.\textsuperscript{60}

Upregulation of peristin is associated with oral,\textsuperscript{61} pancreatic\textsuperscript{62} and breast cancer metastasis\textsuperscript{63–65}, as well as metastatic colorectal cancer via aberrant Akt/PKB signalling.\textsuperscript{66} The arrival of metastatic head and neck tumour cells at secondary sites can also induce expression of peristin in cancer-associated fibroblasts (CAFs) via TGF\beta signalling, leading to accelerated growth, migration and metastatic colonisation.\textsuperscript{67} Furthermore, peristin can promote metastatic colonisation through the recruitment of WNT ligands and activation of WNT signalling in cancer stem cells.\textsuperscript{68}

The glycosaminoglycan hyaluronan is often aberrantly deposited in cancer and has been associated with the activation of cell surface receptors such as CD44 and the subsequent downstream activation of Rho-ROCK\textsuperscript{69} and PI3K/AKT kinase signalling networks,\textsuperscript{70} as well as changes in tumour cell survival, migration and invasion (for a full review, see\textsuperscript{71}). Binding of elastin binding protein receptors (EBPRs) by elastin or elastin fragments/peptides has been shown to activate the ERK1/2 and PI3K/AKT kinase signalling pathways in dermal fibroblasts (Fig. 1).\textsuperscript{72} As such, aberrant expression of elastin and EBPRs has been linked with cancer cell proliferation and invasive capacity in a range of solid cancers including melanoma\textsuperscript{73} lung cancer\textsuperscript{74} and glioblastoma\textsuperscript{75} (for a detailed review, see\textsuperscript{76}). Collectively, the complex, reciprocal interactions between tumour cells, stromal cells and a diverse array of ECM molecules lead to the establishment and maintenance of pro-tumourigenic and pro-metastatic microenvironments at both primary and secondary sites.

Taken together, ECM remodelling, the enlistment and activation of non-malignant cells, and the activity of tumour secreted factors – all mediated in part by the activation of intracellular kinase signalling – work to establish growth permissive tumour microenvironments and are critical in supporting overt colonisation at secondary sites. The interplay between these components highlights the significant role extrinsic factors such as the dynamic ECM – at both the primary tumour and within pre-metastatic niches at secondary sites – have in driving cancer progression, and how these factors can influence the behaviour of cancer cells by enhancing proliferation and metastatic potential.

Given the recent focus on the role of ECM remodelling in promoting tumour growth at both primary and secondary sites, the effectiveness of drug therapies to renormalise the tumour ECM or block aberrant remodelling and ECM-mediated signalling is under intense investigation. Indeed, many clinical trials aimed at targeting these processes are underway with varying degrees of success. Unfortunately, a range of MMP inhibitors have, in the past, proven largely unsuccessful in the clinic, likely due to their lack of specificity\textsuperscript{77} (for a full review see\textsuperscript{78}) Similarly, the Lysyl Oxidase Like-2 antibody; Simtuzumab, recently showed no significant improvement in overall survival in a phase II trial in late-stage metastatic pancreatic cancer,\textsuperscript{79} or a phase II trial in metastatic KRAS mutant colorectal adenocarcinoma.\textsuperscript{80} However given the late-stage terminal nature of disease in these patients, it may be that earlier treatment could yield more promising results in line with pre-clinical studies. Inhibitors of SRC family members (Dasatinib, saracatinib, bosutinib, KX01),\textsuperscript{81,82} and TGF\beta inhibitors\textsuperscript{83} have also yielded disparate results in clinical trials for multiple solid tumours, although there is evidence that improved biomarker identification and patient stratification may yield more promising outcomes in the future.

Of the 24 known human integrins, 3 are currently targeted therapeutically by monoclonal antibodies, peptides or small molecules.\textsuperscript{84} However, despite a number of strong in vitro and in vivo studies highlighting integrins as a potential target for disrupting cell-ECM interactions in cancer,\textsuperscript{85} and the successful inhibition of integrins in a range of other diseases including inflammatory bowel disease (IBD), none of the six integrin-targeting drugs currently on the market in 2016 have shown a significant therapeutic benefit on cancer progression.\textsuperscript{86} That said, recent trials of antibody-based integrin-targeting drugs have seen improved success rates in phase III clinical trials for melanoma, lung and renal cell carcinoma.\textsuperscript{87}
has reached phase II clinical trials and shown very promising results in increasing progression-free survival (PFS) when combined with standard of care gemcitabine/Abraxane in pancreatic cancer (Halozyme).88 Furthermore, small molecule kinase inhibitors that target signalling pathways mediated by ECM remodelling such as FAK89,90 and ROCK91 have also proven effective in targeting fibrosis in cancer and pulmonary diseases. However, what has become increasingly clear is that targeting ECM remodelling alone is unlikely to yield significant improvements in patient outcome in highly aggressive diseases such as cancer, without the addition of tumour debulking approaches such as cytotoxic agents and/or radiotherapy.

The emerging concept of tissue ‘priming’ is also showing promise, with a recent study demonstrating that disrupting ECM integrity through short-term priming with the ROCK inhibitor Fasudil, followed by gemcitabine/Abraxane treatment, showed significantly reduced PDAC tumour size and metastasis compared to chemotherapy alone.48 These pre-clinical findings are an exciting development and present a promising alternative to current treatment options, where the sequence and timing of multiple therapy administration becomes increasingly important. Their effectiveness in treating human tumours still require verification in clinical trials, and history has taught us that even the most promising preclinical studies can fail to translate to patients. Moreover, long-term monitoring of patients will also need to be conducted to investigate whether ECM targeting/disrupting treatments trigger long-term ECM alterations that can safely be maintained throughout remission and prevent recurrence or the activation of disseminated dormant tumour cells. In addition to these focus areas, there should also be further research into developing, or tailoring therapies to target aberrant kinase signalling specifically in tumour cells or tumour stroma while sparing the surrounding normal tissues.

In summary, recent research points to the existence of a reciprocal feedback mechanism between ECM remodelling and cancer cell survival, mediated by kinase signalling, that can promote cancer progression at the primary site and also enhance migration from the primary tumour. ECM remodelling at distant sites can also establish a favourable microenvironment that supports the colonisation and survival of tumour cells. It is this interplay between the intrinsic factors of cancer cells themselves, including their genotype and signalling programs, and extrinsic factors of tumour stroma, such as ECM remodelling, which together determine the fate and behaviour of cancer cells, in turn promoting tumour growth, enhancing metastasis, and worsening patient prognosis. The ability to uncouple this interaction between tumour cells and the ECM through the development of targeted therapeutics therefore presents promising potential in the treatment of cancer to reduce both tumour burden and also the incidence of metastasis.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclose

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