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INTEGRIN CELL BIOLOGY

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The microenvironment dictates how and where cancers originate and their spread throughout the body. The extracellular matrix (ECM) is an important component of the microenvironment and ECM components, such as fibronectin and collagen, are key to tumour initiation, growth and metastasis. Our laboratory is focussed on using mouse models to determine the molecular details of how the ECM influences initiation and metastasis of both liver and breast cancer and how the integrin receptors for the ECM control these processes *in vivo*. We report that integrin dependent deposition of fibronectin must occur early in tumorigenesis for cancers to propagate in the liver. Furthermore, we have found that, early in their development, breast tumours alter the metabolism of immune cells such as neutrophils to alter integrin behaviour. This leads to neutrophil recruitment to the lungs and subsequent metastasis to this organ. These studies highlight how drugs which target novel pathways controlling integrin function may be used to control both tumour initiation and metastasis.

Neutrophil pyrimidine metabolism leads to priming of the lung metastatic niche in breast cancer.

To identify circulating metabolites that may be the harbingers of metastasis, we profiled serum metabolome in the MMTV-PyMT mouse model of mammary cancer. This revealed that the circulating levels of the pyrimidine, uracil correlate closely with metastasis of mammary tumours to the lung. Further investigation indicated that the high level of circulating uracil in mice with metastatic mammary cancer emanates from neutrophils expressing the enzyme uridine phosphorylase-1 (UPP1). Indeed, the presence of a primary tumour in the mammary gland drives expression of UPP1 in neutrophils which leads to increased cleavage of the nucleoside uridine to yield ribose-1-phosphate and the pyrimidine base, uracil which is released from these neutrophils into the circulation. Moreover, we found that GM-CSF could drive increased UPP1 expression in neutrophils indicating the likelihood that this tumour-derived cytokine may be responsible for mediating increased UPP1 expression in tumour-bearing mice. We, therefore, studied the consequences of genetically deleting UPP1 on metastasis and found that MMTV-PyMT:UPP1$^{-/-}$ mice have significantly reduced lung metastases by comparison with their MMTV-PyMT:UPP1$^{+/+}$ controls. Mobilisation and recruitment of neutrophils to metastatic target organs is becoming established to prime these tissues for metastasis. We, therefore, visualised the expression of adhesion molecules and the movement of neutrophils in the lungs of mammary tumour-bearing mice and studied the influence of genetic deletion or pharmacological inhibition of UPP1 (using the specific UPP inhibitor, benzylacyclouridine (BAU)) on this. These studies indicated that the activation of UPP1 in neutrophils leads to upregulated surface expression of an integrin, CD11b which causes the neutrophils to adhere to, and become trapped in, the lungs of tumour bearing mice. These neutrophils then, in turn generate an immunosuppressed microenvironment in the lung which, we propose, favours metastasis to this organ. Conversely, inhibition or knockout of UPP1 leads to recruitment of neutrophils which are associated with increased T-cell numbers and an immunocompetent lung microenvironment consistent with decreased metastasis to this organ. These data indicate that pharmacological targeting of UPP may be an effective means to reducing lung metastasis in breast cancer (Figure 1A).

Restriction of mRNA translation is key to efficient initiation of liver cancer

The mRNA translation/protein synthesis machinery is known to drive cancer cell proliferation and tumour growth. This is thought to satisfy cancers' particular need to increase biomass. Mutated oncogenes also influence the selectivity of mRNA translation to favour synthesis of cohorts of proteins (such as cell cycle regulators) to further support cancer cell proliferation. These simple facts have fuelled the search for agents which oppose or moderate mRNA translation. Indeed, drugs which target the mRNA translation machinery – and the signalling linking mutated oncogenes to this – are now being evaluated in clinical trials as cancer therapeutics.

The genesis of tumours is not simply a process in which mutated oncogenes drive cell proliferation. Acquisition of mutated oncogenes may not, initially, drive tumorigenesis. Indeed, under many circumstances, oncogenes promote senescence (not proliferation) and the mutated cell's ability to overcome oncogene-induced senescence dictates whether tumorigenesis occurs. Importantly, senescent cells are highly secretory, including many extracellular matrix (ECM) components which are deposited locally. We have developed and utilised transgenic mouse models to show how the genesis of liver cancer requires an ECM-driven override of oncogene-induced senescence. The hyperproduction of secretory proteins associated with oncogene-induced senescence would be expected to require reprogramming of the mRNA translation machinery in cancer cells. Indeed, we identify that a negative regulator of mRNA translation initiation (eIF4A2) *restrains* synthesis of ECM proteins, thus maintaining membrane trafficking and facilitating ECM protein deposition. Importantly, in the absence of eIF4A2 senescent hepatocytes do not progress to proliferating liver cancer because of a lack of ECM deposition rather than a lack of production. As we have seen, lack of appropriate restraint of secretory protein synthesis following oncogene activation can delay tumorigenesis through matrix suppression (Figure 1B). Consistently, we demonstrate that administration of rapamycin shortly following oncogene activation strongly promotes senescence override and allows progression to proliferating liver cancer. Thus, although inhibition of protein synthesis may be an effective way to reduce tumour biomass and the growth of established tumours, it is important to consider that high levels of mRNA translation can extend a period of senescence occurring following oncogene activation. Thus, use of drugs which reduce mRNA translation, if administered shortly following oncogene activation, may awaken senescent cells, and promote tumour progression.

Publications listed on page 125

1A

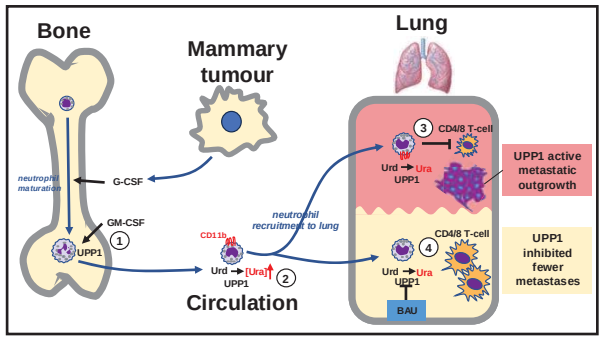

Figure 1A Neutrophil pyrimidine metabolism leads to priming of the lung metastatic niche in breast cancer. [1] Mammary tumours release factors such as G-CSF to drive neutrophil maturation in the bone marrow, and GM-CSF which leads to activation of uridine phosphorylase-1 (UPP1) in neutrophils. **[2]** Circulating neutrophils expressing UPP1 generate high levels of serum uracil (Ura). UPP1-expressing neutrophils express high levels of the surface adhesion protein CD11b (integrin αM). **[3]** CD11b leads to capture of UPP1-expressing neutrophils in the lung. These neutrophils suppress T-cells and metastasis in the lung. **[4]** When UPP1 is inhibited using benzylacyclouridine (BAU), T-cells number in the lung increase to suppress metastasis.

1B

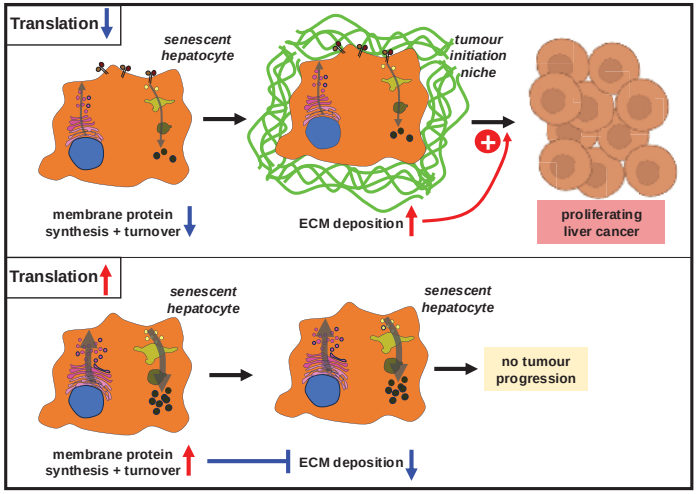

Figure 1B Restriction of mRNA translation is key to efficient initiation of liver cancer. When the translation of secretory mRNAs is restrained (upper panel), protein synthesis and membrane protein turnover is moderate. This maintains membrane trafficking and facilitates ECM protein deposition to promote progression of liver cancer from a senescent phase to form highly proliferating hepatocellular carcinoma. Conversely, when mRNA translation is rapid and unrestrained (lower panel), ECM deposition is inhibited and senescent hepatocytes harbouring oncogenic mutations remain senescent and do not progress to proliferating liver cancer.