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INTEGRIN CELL BIOLOGY

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Although carcinoma initiation is driven by oncogenic mutations occurring in epithelia, cells bearing mutated oncogenes do not necessarily progress to form tumours. It is now clear that the microenvironment which surrounds mutated epithelial cells is key to determining the likelihood and rate at which they form malignant tumours, or whether they remain quiescent. Oncogenes are known to re-programme the cell's ribosomes, or protein synthesis machinery, and we have found that the way in which this occurs influences whether mutated cells remain quiescent or start growing. Moreover, this is because ribosome function has profound, and sometimes paradoxical, effects on the extracellular matrix components of the microenvironment. Importantly, our findings indicate that re-programming of ribosome function can engender fibrotic microenvironments and thus influence the ability of epithelial cells bearing oncogenic mutations to start growing uncontrollably. Therefore, it is important to understand how protein synthesis inhibitors affect extracellular matrix deposition before deploying these agents to target cancer in the clinic.

The translational repressor, eIF4A2 regulates protein synthesis landscapes to support fibrotic niche generation and hepatocellular carcinoma initiation - *an active collaboration with the Bushell, Sansom, and Bird laboratories*

To enable new strategies to target tumour growth, the Institute has an ongoing programme to map protein synthesis landscapes associated with cancer initiation and progression. We are, therefore, developing approaches to disrupt – genetically and pharmacologically – the protein synthesis machinery and determine how this influences outcomes in pre-clinical models of cancers, including hepatocellular carcinoma (HCC) and lung adenocarcinoma.

Translation of mRNA by ribosomes is a key event in protein synthesis, and translation initiation is mediated by the eIF4F complex. Integral to the eIF4F complex are the eIF4A helicases which are responsible for unwinding the 5'-UTR of mRNAs, thus influencing the ability of ribosomes to scan along mRNAs and find the 'translation start' codon to initiate translation and protein synthesis. Importantly, there are two eIF4A paralogues – eIF4A1 and eIF4A2 - and these are known to exert opposing effects on translation initiation. Whilst eIF4A1 is a 'classical' activator, eIF4A2 can act as a translational repressor by associating with the CCR4-NOT complex to mediate RNA degradation and shut-down of protein synthesis. Therefore, to determine how activation and repression of translation initiation might respectively influence cancer outcomes, we generated mice with conditional knockout (floxed) alleles of eIF4A1 and eIF4A2, and crossed these with a genetically-engineered mouse model of HCC which is driven by hepatocyte-specific expression of the β-catenin and cMyc oncogenes. Surprisingly, tumour cell-specific knockout of eIF4A2 (translation initiation repressor) markedly delayed the onset of β-catenin/Myc-driven HCC - leading to extended survival. By contrast, eIF4A1 (translation initiation activator) knockout affected neither tumour onset, nor survival from HCC.

To determine how mRNA translation initiation rates influence tumour onset and tumour-associated alterations to the liver microenvironment, we monitored changes in the liver shortly following activation of oncogenic β-catenin/Myc in hepatocytes. 30 days following oncogene induction, β-catenin/Myc expressing cells (identified by glutamine synthetase (GS) expression which is an indicator of activated WNT signalling) were identifiable as large non-proliferative cells which expressed markers of cell senescence, such as p21. Importantly, these enlarged senescent-like, transformed hepatocytes were associated with deposition of extracellular matrix (ECM) components, such as fibronectin (Figure 1a). Moreover, eIF4A2 knockout did not influence the number or appearance of these GS-positive/p21-expressing hepatocytes following oncogene induction. However, eIF4A2 knockout reduced deposition of fibronectin across the liver and in the vicinity of the GS-positive cells, indicating that de-repression of translation initiation opposes formation of fibrotic niches in the vicinity of recently transformed-but-not-yet-proliferative tumour initiating cells.

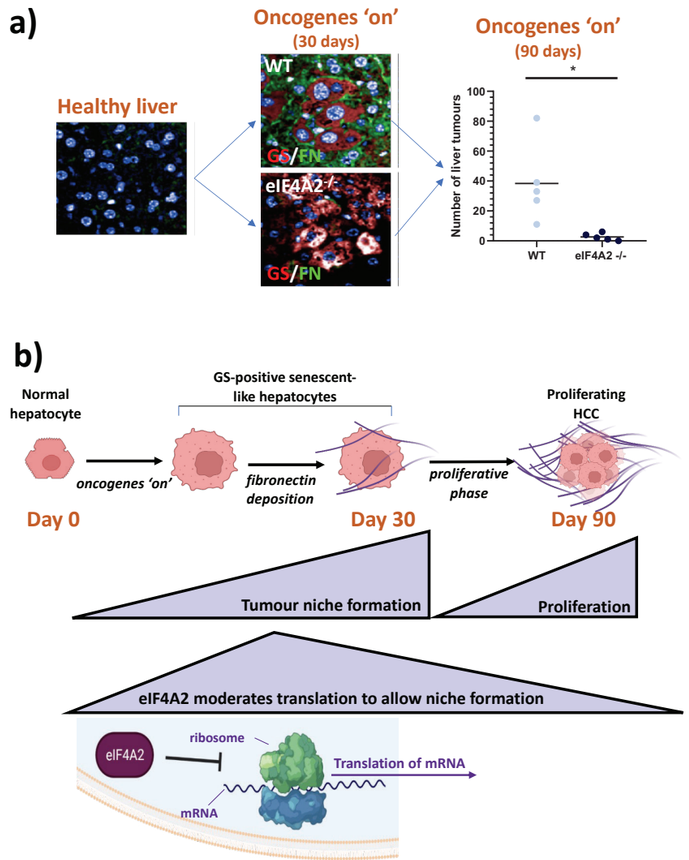

Figure 1
eIF4A2 moderates protein synthesis to allow fibronectin niche generation and HCC progression.
A Glutamine Synthetase (GS)-positive, senescent-like tumour initiating cells surrounded by fibronectin (FN)-rich niches are seen 30 days following hepatocyte-specific induction of the β-catenin and Myc oncogenes. 90 days following oncogene induction, some of these tumour initiating cells have progressed to form liver tumours. Hepatocyte-specific knockout of eIF4A2 opposes both FN-rich niche generation and establishment of liver tumours. **B** Oncogene activation drives increased translation, but this is moderated by the translational repressor, eIF4A2. This moderated level of mRNA translation fosters fibronectin-rich niche generation which allows senescent-like, growth-arrested tumour initiating cells to enter a proliferative phase to form hepatocellular carcinoma (HCC).

The assembly of soluble fibronectin into fibrils and its subsequent deposition to form insoluble/fibrotic ECM is strongly dependent on α5β1 integrin - the cell's main fibronectin receptor. We, therefore, deployed mice with floxed alleles of α5 integrin (ITGA5) to determine whether the ability of early tumour initiating cells to assemble fibronectin-rich niches might contribute to their progression to actively proliferating tumour nodules. Hepatocyte-specific ITGA5 knockout opposed fibronectin deposition following oncogene-induction, indicating that expression of α5β1 integrin by hepatocytes has a key role in generating local fibrotic microenvironments in the liver. Importantly, hepatocyte-specific knockout of ITGA5 delayed appearance of actively proliferating tumour nodules in the liver and extended survival from HCC to a similar extent as did eIF4A2 knockout. This indicated that levels of translation initiation dictated the progression of HCC by influencing α5β1 integrin function and the ensuing generation of fibronectin-rich niches in the liver.

To determine whether the increased level of translational activity resulting from eIF4A2 knockout is responsible for opposing the generation of fibrotic tumour initiation niches, we used rapamycin. Rapamycin is a drug with well-established ability to reduce mRNA translation indeed we have found it to oppose the elevated protein synthesis evoked following eIF4A2 knockout. Furthermore, because we are interested in focussing on how protein synthesis landscapes influence tumour initiation (as opposed to growth of established tumours), we administered rapamycin to mice 5 days following oncogene induction, and ceased treatment 25 days later. Administration of rapamycin in this time-window reversed the ability of eIF4A2 knockout to suppress the generation of fibronectin-rich niches associated with GS-positive, oncogene-transformed (but not-yet-proliferative) tumour initiating cells and, correspondingly, restored the ability of these cells to progress to form HCC tumour nodules.

It is now established that one of the ways in which oncogenes drive tumour growth is via their ability to promote mRNA translation and protein synthesis. Thus, agents (such as rapamycin) that oppose oncogene-driven translation, and signalling events connecting oncogenes to the protein synthesis machinery, are being evaluated as potential anti-tumour therapies. However, the present study, which involves extensive collaborative interactions between four groups at the Institute, has used sophisticated *in vivo* cancer models to demonstrate how *increased* protein synthesis can *oppose* generation of tumour initiation niches at early stages in the genesis of HCC. Therefore, we submit that it is necessary to fully evaluate the (sometimes unpredictable) consequences of protein synthesis inhibition on the microenvironment during early tumour establishment as agents targeting the mRNA translation machinery and its upstream signalling advance toward the clinic.

Publications listed on page 108