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RNA AND TRANSLATIONAL CONTROL IN CANCER

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The dysregulation of protein synthesis is an emerging hallmark of cancer, where altered translation is essential for the induction of oncogenic gene programmes. Distinct programmes of gene expression drive tumour growth and create the supportive microenvironment in which it flourishes. Our research aims to understand how components of the translation machinery are required to increase the rate of translation of key oncogenic mRNAs as well as ensuring their fidelity through the correct folding and cellular localisation.

eIF4A1 and eIF4A2 have opposing roles in tumourigenesis

The translation initiation factor eIF4A1 is a DEAD-box RNA helicase, whose role is to unwind secondary structure in the 5′ untranslated regions (5′UTRs) of mRNAs. While almost all cellular mRNAs require eIF4A1 for their translation, their requirement is not equal, with mRNAs encoding oncogenic proteins depending most upon eIF4A1 activity for their expression, which is thought to explain why eIF4A1 expression is associated with poor survival in human malignancy. Targeting eIF4A1 in cancer is currently an attractive therapeutic and there are several eIF4A inhibitors available, with EFT226 currently in clinical trials. However, all current eIF4A inhibitors target both eIF4A1 and its paralogue eIF4A2, which share roughly 90% identity at the amino acid level. Yet, eIF4A2 has a distinct role from eIF4A1, in that it acts as a translational repressor in conjunction with the CCR4-NOT complex and the role of eIF4A2 in malignancy and the effect of its targeting in cancer remain unclear.

Data from the Sansom lab showed that knock-out of either eIF4A1 or eIF4A2 in the colon of WT mice was tolerated, yet in colon cancer models, loss of eIF4A1 led to reduced proliferation and increased survival, but the loss of eIF4A2 accelerated tumourigenesis and led to decreased survival. To dissect the mechanisms that explained the divergent roles of eIF4A1 or eIF4A2 in colorectal cancer, we carried out ribosome profiling on epithelial extractions from APC$^{-/-}$/KRAS^{G12D} small intestines, following loss of either eIF4A1 or eIF4A2.

Following loss of eIF4A1, there were 283 genes that were translationally downregulated and 249 that were translationally upregulated. Gene set enrichment analysis (GSEA) showed that those mRNAs that were translationally downregulated encoded proteins that were involved in cell cycle processes (Figure 1B), which correlated with our data that loss of eIF4A1 led to reduced cell proliferation in these cells. Interestingly, those mRNAs that were translationally upregulated encoded proteins involved in the adaptive immune response and this is currently being further investigated. There were far fewer translational changes following the loss of eIF4A2. This was consistent with our data that loss of eIF4A2 did not alter the rate of proliferation in this model. Only 37 mRNAs were translationally upregulated and 1 downregulated with statistical significance. Interestingly, of the 37 mRNAs that were upregulated, 33 were TOP mRNAs, with cytoplasmic translation coming back as the main group of genes enriched in translationally upregulated mRNAs. Although only one mRNA was identified as decreasing translationally with statistical significance, when carrying out GSEA using the log2 fold-changes in translation efficiency for all mRNAs (without considering adjusted p-values), then many processes related to differentiation were identified as being enriched in translationally downregulated mRNAs (Figure 1D), which fits with eIF4A2 expression being limited to differentiated cells.

Investigating the role of stress granules in cancer

Cancer cells use translational control as a means of rapid circumvention of various environmental stresses, in particular hypoxia and oxidative stress, which they must overcome to thrive. Stress granules are liquid-liquid phase separated mRNA- and protein-rich foci that form in the cytoplasm in response to cellular stresses that have been implicated in the pathophysiology of various diseases including cancer. KRAS-mutant cell lines have been shown to have elevated levels of stress granules and overexpression of stress granule markers is associated with a poor prognosis in multiple cancers.

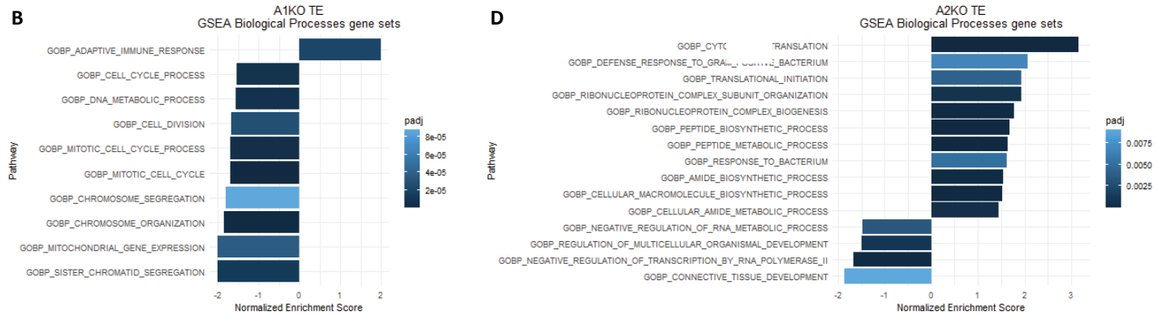

Figure 1
eIF4A1 and eIF4A2 have distinct effects on translation in APC$^{-/-}$/KRAS^{G12D} small intestines.
B GSEA shows the biological processes that are involved in translationally upregulated or downregulated mRNAs following loss of eIF4A1. **D** GSEA shows the biological processes that are involved in translationally upregulated or downregulated mRNAs following loss of eIF4A2.

In order to assess stress granule formation in response to oxidative and hypoxic stress, we expressed a fluorescently tagged stress granule protein GFP-G3BP1 and stressed cells using sodium arsenite or 0.1% oxygen (hypoxia), respectively. Confocal microscopy showed that granule morphology was dependent on the stress applied (Figure 2A), with arsenite-induced stress granules displaying the canonical cytosolic puncta with P-body docking on the outer surface, while hypoxia-induced stress granules were considerably larger, occupying a large proportion of the cytosol and had no P-bodies docking on their outer surface. Distinct upstream signalling events regulating granule formation were also observed between the two conditions (Figure 2B), namely phosphorylation of eIF2α in response to arsenite and hypophosphorylation of 4EBP1 in hypoxia.

We purified stress granules using multiple steps of differential centrifugation followed by an anti-GFP-G3BP1 immunoprecipitation and analysed the proteome by mass spectrometry. This highlighted a number of key differences between arsenite- and hypoxia-induced granules, including the presence of large ribosomal subunits and an enrichment of eIF4A1 in hypoxia. These data, in context with the current literature, suggest that hypoxia-induced stress granules might be even more translationally active than what has already been observed and promote pro-survival gene expression programmes in response to stress. Previous studies have implicated DEAD-box helicases as regulators of liquid-liquid phase separation, and eIF4A1 specifically as playing a pivotal role in regulating stress granule size and limiting P-body docking. Treatment with hippuristanol, an eIF4A inhibitor, reduced stress granule size and restored P-body docking (Figure 2C). G3BP1 and eIF4A1 are coexpressed in lung adenocarcinoma (LUAD) patients in the Cancer Genome Atlas (TCGA) cohort. High expression of both G3BP1 and eIF4A1 in these patients was associated with the worst survival outcomes, whilst low expression of both was associated with the best survival outcomes (Figure 2D). G3BP1 was identified as an eIF4A1-interacting protein by co-immunoprecipitation suggesting that these proteins could directly interact or be in complex together (Figure 2E). We are currently using ribosome profiling and other complementary high-throughput sequencing techniques to better understand how these two RNA-binding proteins regulate liquid-liquid phase separation, and as a result help drive pro-oncogenic gene expression programmes.

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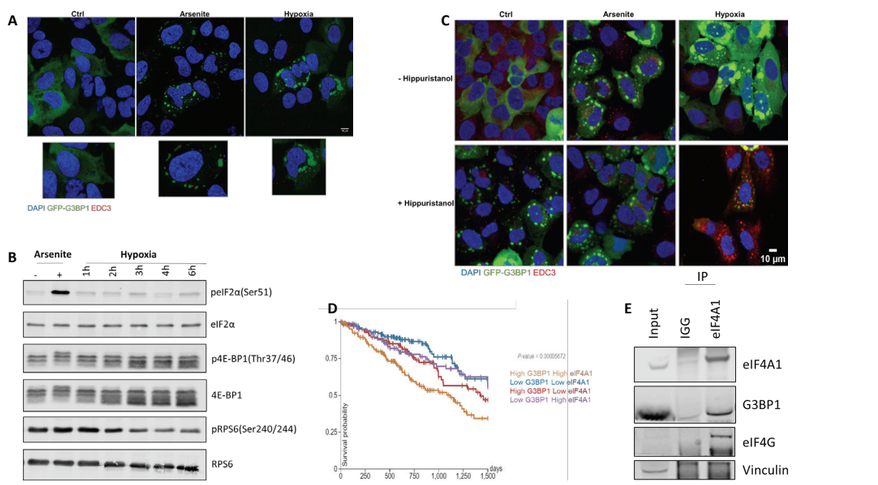

Figure 2
Arsenite- and hypoxia-induced stress granules are distinct.
A Confocal microscopy images of GFP-G3BP1-expressing U20S cells treated with either sodium arsenite or 0.1% oxygen (hypoxia) and stained with DAPI and EDC3 (p-body marker) antibodies. **B** Western blot from arsenite- or hypoxia-treated U20S cells probed with the stated antibodies. **C** Confocal microscopy images of GFP-G3BP1-expressing cells treated with either sodium arsenite or 0.1% oxygen (hypoxia) and stained with DAPI and EDC3 (p-body marker) antibodies, as in (A) plus and minus eIF4A inhibition with hippuristanol. **D** Kaplan-Meier plot of LUAD TCGA cohort stratified by *G3BP1* and *eIF4A1* expression. E Co-IP from A549 cells, demonstrating a direct interaction between eIF4A1 and G3BP1.