Investigating the transcriptional response to hypoxia

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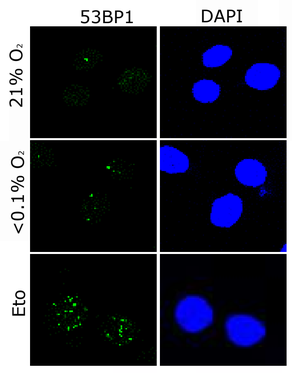
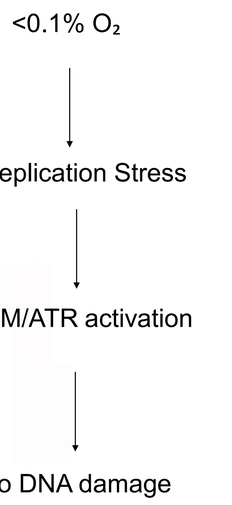
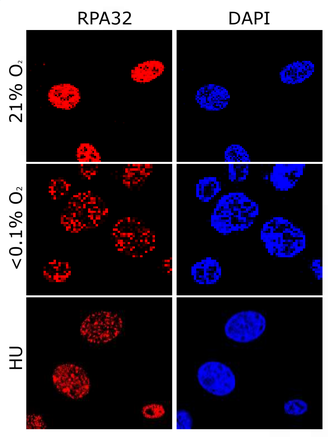
Introduction

Areas of low oxygen (hypoxia) are a common characteristic of solid tumours and are associated with therapy resistance and poor patient prognosis. The transcriptional response to hypoxia is mediated primarily through stabilisation of hypoxia inducible factor (HIF) and the induction of target genes. However, gene repression is also relevant to the hypoxic response and includes the reduction of numerous essential components of the DNA repair pathways. Repression of specific genes in hypoxia occurs through both HIF dependent and independent mechanisms, including changes in histone methylation and heterochromatin or upregulation of micro RNAs. Hypoxia leads to the rapid accumulation of replication stress and upregulation of the DNA damage response. Currently, we are investigating the hypoxia-induced signal, which initiates global transcription repression. We envisage that gaining a better understanding of transcriptional control and gene expression in hypoxia will reveal clues as to why resistance to therapies arise and help us identify factors that could be targeted in combination with radiotherapy.







Hypoxia leads to nucleolar reorganisation

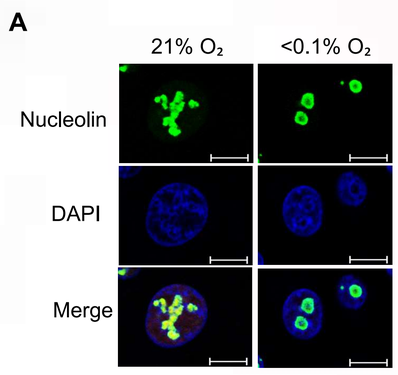
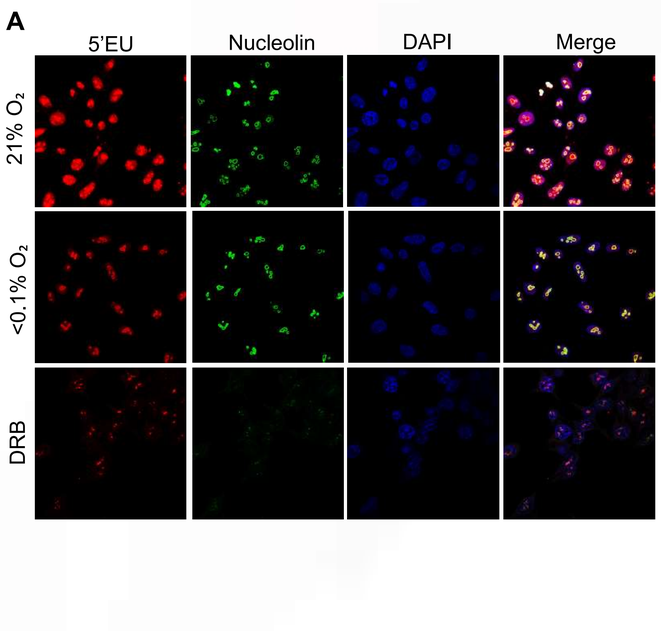


Figure 1. Immunofluorescence microscopy of RKO cells stained with nucleolin (green) demonstrate a change in nucleolar shape in long periods of hypoxia (<0.1% O_2) which is indicative of transcriptional repression. The scale bars represent 10 μm.

Ribosomal RNA transcription by RNA polymerase I is repressed in the nucleolus in hypoxia



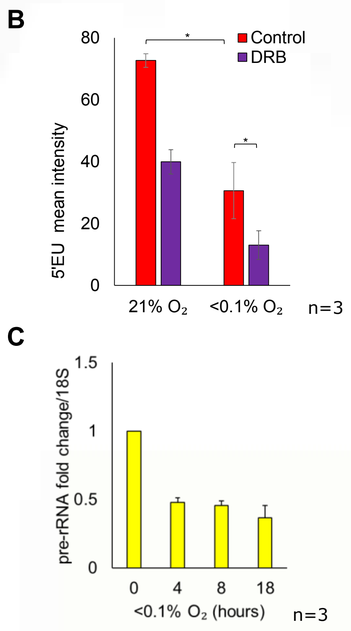


Figure 2: Co-immunofluorescence microscopy with nucleolin (green) demonstrates that 5'EU incorporation (red) is reduced in response in hypoxia (<0.1% O_2) in RKO cells at the site of rRNA transcription, the nucleolus (A). The mean intensity of 5'EU incorporation was quantified in hypoxia and treatment with DRB, a transcriptional inhibitor (B). The levels of pre-ribosomal RNA (pre-rRNA) in RKO cells were measured by RT-qPCR relative to *18S* in 21% O_2 and in hypoxia (<0.1% O_2) (C).

Conclusions

We demonstrate that nucleolus undergoes reorganisation in longer periods of hypoxia (<0.1% O_2) and that this corresponds with a swift decrease in rRNA transcription. Ribonucleotide levels are reduced in hypoxia, which suggests that insufficient ribonucleotides are a contributing factor to transcriptional repression in hypoxia. Transcriptional repression in hypoxia was found to occur independently of ATM or p53, suggesting that hypoxic conditions repress rRNA transcription through an alternative pathway than that reported to be induced by double strand breaks. Interestingly, cleavage and degradation did not occur consistently at known sites on the 5'ETS region when exposed to hypoxic conditions suggesting that there are problems with pre-rRNA processing in hypoxia.

Ribonucleotide levels are reduced in hypoxia

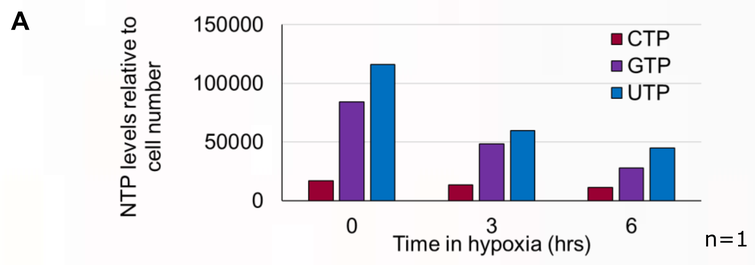


Figure 3: Ribonucleotide levels (CTP, GTP and UTP) in HCT116 cells were measured in hypoxia (<0.1% O_2). UTP levels are reduced by half in 3 hours of hypoxia.

rRNA transcriptional repression occurs in ATM and p53-independent manner

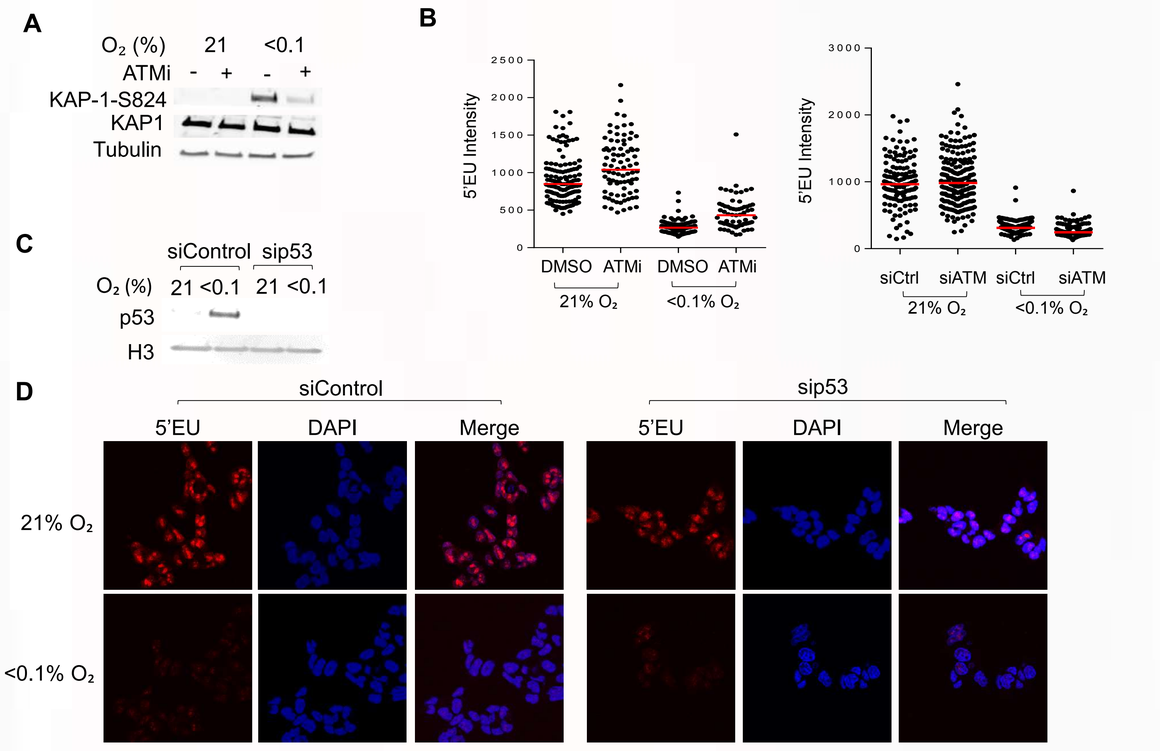


Figure 4: The levels of 5'EU in RKO cells were measured following ATM inhibition with 5 μM KU-55933 or siRNA targeting ATM in 6 hours of hypoxia (<0.1% O_2) (A/B). Each point represents one cell. siRNA knockdown of p53 in colon cancer cells (HCT116) results in similar levels of 5'EU incorporation in 6 hours of hypoxia (<0.1% O_2) (C/D) indicating that the transcriptional repression occurs through a mechanism that differs from double strand break induced rRNA transcriptional repression.

Aberrant rRNA processing in hypoxia

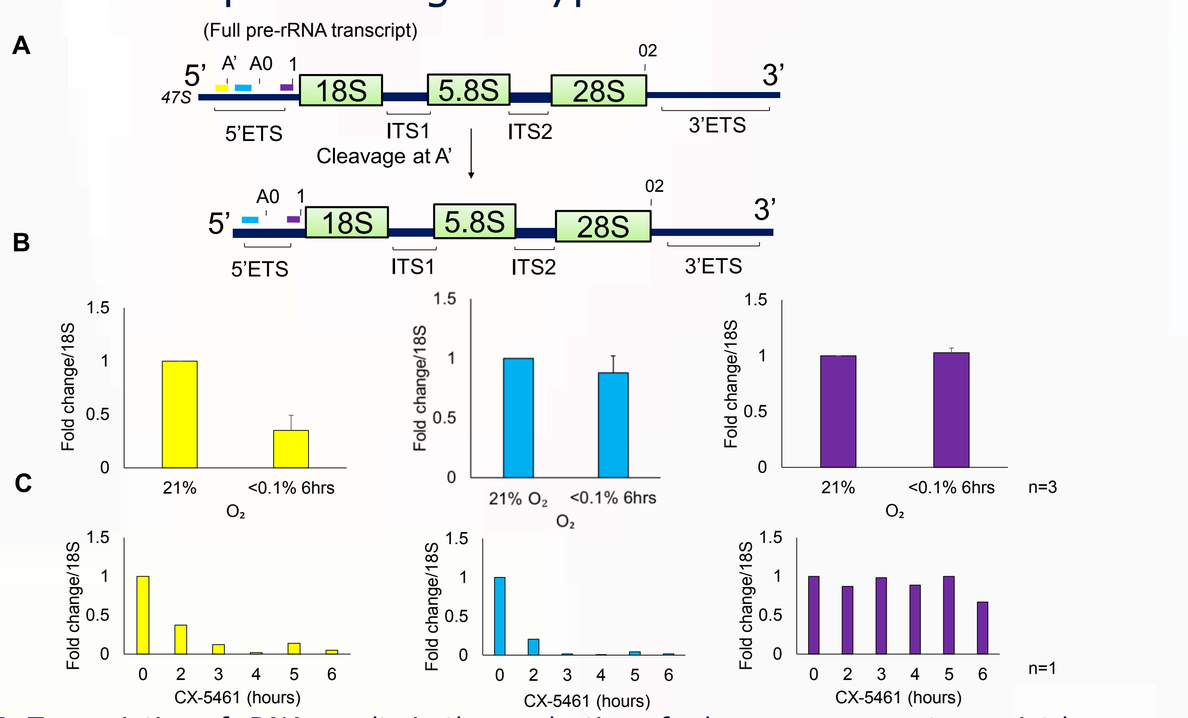


Figure 5: Transcription of rDNA results in the production of a longer precursor transcript known as the 47S. Cleavage at specific sites A', A0 and 1 occur co-transcriptionally and these products are subsequently degraded to yield the mature ribosomal RNA. Primers spanning the 5'ETS region of the 47S transcript were used in qPCR to measure nascent levels of pre-rRNA as indicated on (A). Interestingly, only one of these probes (yellow) demonstrated a decrease in 6 hours hypoxia, whereas the other products (blue and purple) remained stable over time in hypoxia (B). RKO cells were treated with 100 nM CX-5461, a RNA polymerase I inhibitor, over a time course and pre-rRNA was measured which demonstrates that cleavage occurs at sites A' and A0 following Pol I inhibition (C).

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The hypoxic conditions were created in a Don Whitley Workstation.