Is the 5'UTR of HIF1α mRNA important for HIF1α regulation?

Hannah Mearns*, BSc Biomedical Sciences with Industrial Placement Year, 160284232, H.Mearns@newcastle.ac.uk. Dr Niall Kenneth. Newcastle University.

Introduction

- HIF1α is a vital cellular protein and transcription factor found on chromosome 14 in humans
- Cells including many cancer cells can respond and adapt to hypoxia, a lack of oxygen, by increasing their HIF1α levels(1)
- HIFα increases the levels of many target genes encoding specific proteins and these help to increase oxygen transport and aid the response to hypoxia

- mRNA is the code used to make proteins (Figure 1) and the process of turning mRNA into a protein is well regulated (Figure 2)
- In mRNA the 5'UTR (untranslated region) comes before the protein encoding section and this region often impacts protein levels

Figure 1: mRNA with a 5' and 3' UTRs at the ends. The middle section encodes the protein.

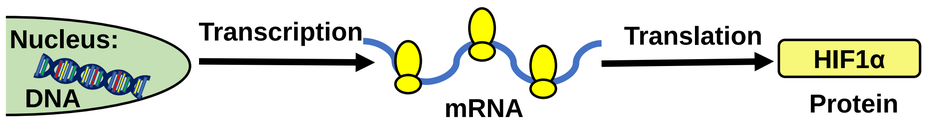

Figure 2: DNA to protein following transcription and translation.

- The aim of this project was to investigate the importance of the HIF1α 5'UTR by using CRISPR Cas9 to delete sections of the 5'UTR and then to look into the impact of this on cell responses to hypoxia

Methods

- Methods (Figure 3) using the cancer cell line PC3:
  - Used CRISPR Cas9 to modify the HIF1α 5'UTR of cells
  - Used PCR to amplify DNA
  - Investigated cellular protein levels after treatment with DMOG; a drug used to mimic hypoxia

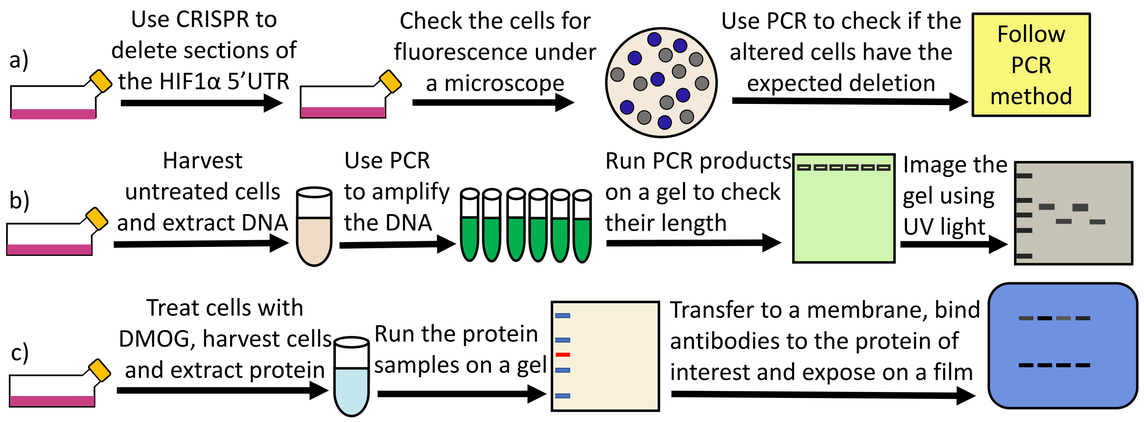

Figure 3: Methods used: a) using CRISPR Cas9 to delete sections of the 5'UTR, b) using PCR to amplify DNA and c) using Western blots to investigate protein levels

References

1. Zhou F, Guan LB, Yu P, Wang XD, Hu YY. Regulation of hypoxia-inducible factor-1alpha, regulated in development and DNA damage response-1 and mammalian target of rapamycin in human placental BeWo cells under hypoxia. Placenta. 2016;45:24-31.
2. Bos R, van der Groep P, Greijer AE, Shvarts A, Meijer S, Pinedo HM, Semenza GL, van Diest PJ, van der Wall E. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. Cancer. 2003;97:1573-1581.

Results and Discussion

- The CRISPR Cas9 was successful in deleting sections of the 5'UTR (Figure 4), where populations 1, 2 and 3 have different sized deletions and 4 is the negative control for the CRISPR reaction and shows only a wild-type band
- DMOG treated cells showed lower levels of HIF1α in the mixed populations (populations 1, 2 and 3) where some cells had the deletion, compared to the control cells: population 4 (Figure 5)
- But as the population grew the percentage with the deletion diminished, impacting future experiments
- PC3 clones with HIF1α 5'UTR deletions were then used to avoid this problem and the PCR products from some of the clones (Figure 6) show some as wild-type and some with the 5'UTR deletion
- DMOG treated clones with the deletion showed lower levels of HIF1α compared to the wild-type clones (Figure 7)

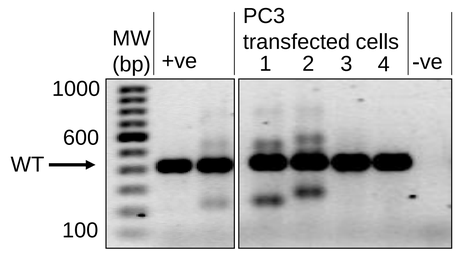

Figure 4: PCR products of PC3 transfected cells, showing successful 5'UTR deletions as bands below the wild-type (WT) bands using CRISPR Cas9.

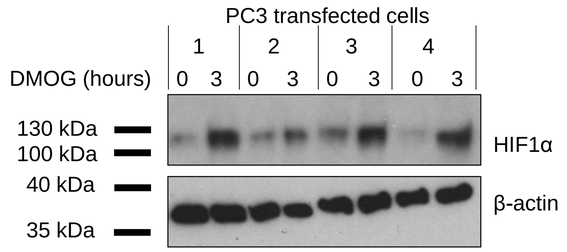

Figure 5: Western blot of PC3 transfected cells after DMOG treatment showing varying levels of HIF1α with β-actin as a loading control. Populations 1, 2 and 3 have deletions and 4 is wild-type.

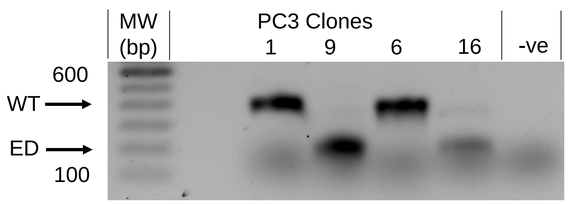

Figure 6: PCR products from the PC3 clones showing 9 and 16 with the 5'UTR deletion and 1 and 6 as wild-type. The last lane is a negative control for the PCR. The arrows point to the wild type (WT) band and where the expected deletion (ED) should be.

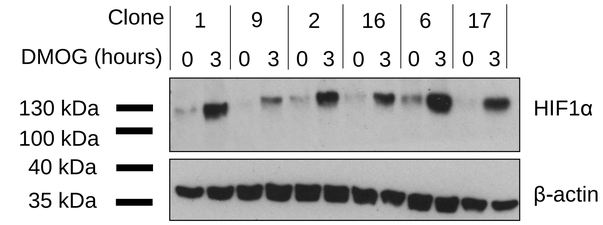

Figure 7: Western blot of PC3 clone protein extracts after DMOG treatment showing altered levels of HIF1α with β-actin as a loading control.

- Relevance:
  - Hypoxia is a component of many diseases, e.g. in cancer where cells in the centre of tumours have a lack of oxygen; by better understanding how HIF1α is regulated and how the 5'UTR is important in this we may be able to better understand how certain diseases progress and develop
  - Many cancer cell types upregulate HIFα; allowing the tumour to survive the stress of hypoxia(2)

- The future:
  - Look into which section of the 5'UTR in particular is important by completing more CRISPR Cas9 experiments
  - Repeat the experiment with more cell lines to see if the effect is conserved

Conclusions

- The 5'UTR is important in controlling and upregulating HIF1α in cells responding to hypoxia
- More research is needed to understand which region in particular of the 5'UTR is responsible

Acknowledgements

- Newcastle University for funding this project
- My supervisor Dr Niall Kenneth and the staff Professor Neil Perkin's lab