The Role of hypoxia inducible factor 1 (HIF1) in Liver Fibrosis

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Aims:

- Investigate the role of HIF1 in promoting liver fibrosis in human **hepatic stellate cells**.

Hypothesis: Elevated HIF1 levels in normal oxygen concentrations promotes the fibrotic **phenotype** (activated HSC) i.e. increases the probability of liver fibrosis.

Introduction:

Fibrosis of the liver is the build-up of scar tissue involved in most chronic liver diseases that is initiated by the activation/ differentiation of **hepatic stellate cells (HSCs)**(1). Defining molecular pathways that lead to stellate cell activation is essential to direct the development of pharmaceuticals to therapeutically intervene in liver diseases.

The LX2 human hepatic stellate cell line was used during the project. They are suitable for a study of human liver fibrosis because they are extensively characterized whilst having the key features of HSCs e.g. metabolism, the development of fibrotic tissue and gene expression.

HIF1 is stabilised during HSC activation, however it is unknown whether HIF1 promotes any fibrotic phenotype.

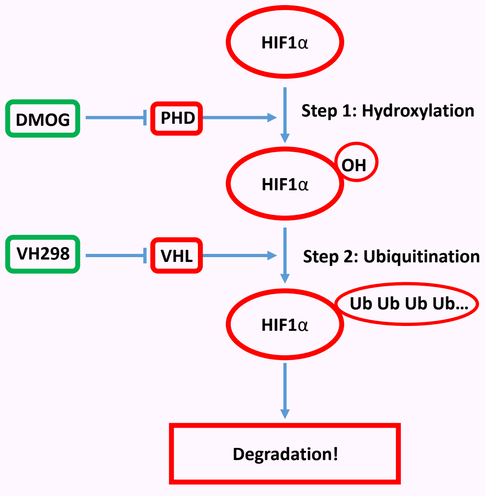

Figure 1: The HIF1α signalling pathway in **normoxia**.
In **normoxia**, cells aim to get rid of HIF1α . Therefore, to increase levels of HIF1α part of this pathway needs to be blocked. DMOG blocks the first step and VH298 blocks the second step. This stops the cell from degrading HIF1α leading to more of the protein being present in the cell.

During the project, methods to chemically increase levels of HIF1α within the HSCs in **normoxia** were investigated. This was done using 2 drugs: DMOG and VH298. These work by blocking different parts of the HIF1α signalling pathway as shown in **figure 1**. Once achieved, the effect on fibrotic markers were investigated.

References:
1. Roger Klein Moreira (*2007*) Hepatic Stellate Cells and Liver Fibrosis. Archives of Pathology & Laboratory Medicine: November 2007, Vol. 131, No. 11, pp. 1728-1734

Definitions:

Hepatic stellate cells (HSCs): Specialised liver cells that are a key player in liver fibrosis. In normal liver cells, HSCs remain inactive but are activated by damage to the liver.

Hypoxia inducible factor 1 (HIF1): a protein that regulates the transcription of target genes in response to the stress of low oxygen concentrations. HIF1α is a subunit of this protein.

Phenotype: an observable characteristic

Normoxia: normal oxygen concentrations

Fibrotic marker: a gene that can be used as an indicator of liver fibrosis in a cell

Hydroxylation: a reaction which adds the chemical group "-OH" onto an organic compound

Western blot results:

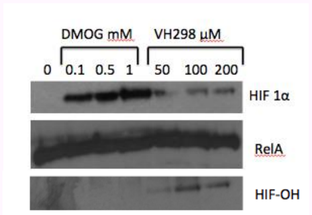

Figure 2: The effect of varying concentrations of the drugs on HIF1α levels

The LX2 cells were treated for 6 hours with a range of concentrations of DMOG and VH298 before the protein was harvested. The results in **figure 2** show that the higher the concentration of drug, the higher the level of HIF1α . The increasing intensity of the bands represent the increased stability of HIF1α .
HIF-OH is a product of the HIF1α signalling pathway due to a **hydroxylation** reaction. This is present after the addition of VH298 but not DMOG. This is consistent with the known signalling pathway shown in **figure 1**.

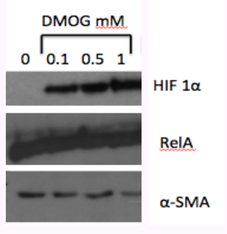

Figure 3: The effect of increasing DMOG concentration on a common marker of liver fibrosis

The LX2 cells were treated for 6 hours and the relationship between the increasing HIF1α levels and the effect on the protein α-SMA, a known fibrotic marker, was investigated. Looking at **figure 3** the level of α-SMA is unexpectedly high in the untreated samples. No increase of α-SMA is seen when treated with DMOG.

Acknowledgments:

Firstly, I would like to thank my supervisor Niall Kenneth for all his help over the summer together with the Perkins lab for the resources necessary for me to undertake the project. Also, a big thank you to Catherine Park for her guidance and lastly to the Wellcome Trust for funding the studentship.

Quantitative PCR results:

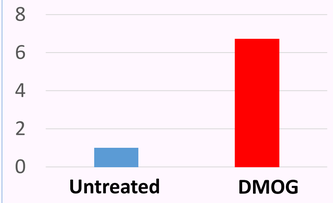

Figure 3: The effect on the expression of the Glut1 gene after addition of DMOG

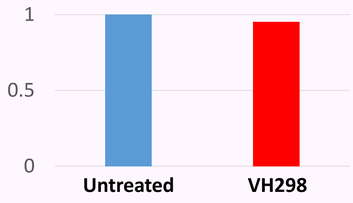

Figure 4: The effect on the expression of the Glut1 gene after addition of VH298

Glut1 is a well known target gene for HIF1α and is "switched on" by HIF1α activity. The results in **figure 3** show a large increase in HIF1α activity after the addition of DMOG, however **figure 4** indicates no effect on HIF1α activity with the addition of VH298.

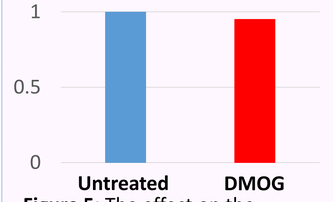

Figure 5: The effect on the expression of the TIMP1 gene after the addition of DMOG

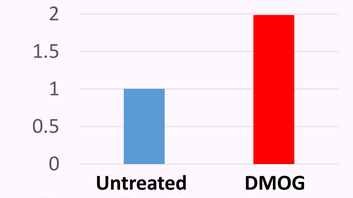

Figure 6: The effect on the expression of the COL1 gene after addition of DMOG

TIMP1 and COL1 are fibrotic markers. When these genes are switched on the products formed are known contributors to scar tissue in the liver which in turn leads to fibrosis.
Figure 5 shows no significant effect on the TIMP1 gene, whereas **figure 6** shows an increase on the expression of COL1.

Conclusions:

- HIF1α levels successfully elevated in the cell by DMOG and VH298. Both stabilised HIF1α but DMOG showed greater stability.
- DMOG is shown to increase the activity of HIF1α whereas VH298 was not.
- Hydroxylation not only targets HIF1α for degradation but appears to also inactivate it's activity.
- The results indicate that the activation of HIF1α by DMOG is sufficient to drive the expression of the fibrotic marker COL1, which codes for the protein collagen 1.
  - HIF1α activation did not have an effect on TIMP1 or α-SMA
  - Further investigation is required into more fibrotic markers.