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). 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.

Definitions:
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 conditions

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

Western blot results:

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.

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.

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 its 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.

References: