Liver Cancer and the Non-Canonical NFκB Pathway

Examining the potential treatment of hepatocellular carcinoma by investigating the non-canonical NFκB signalling pathway and its role in tumorigenesis.

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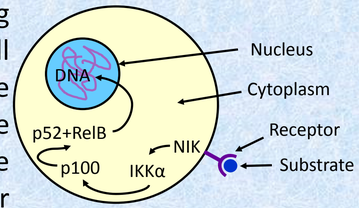
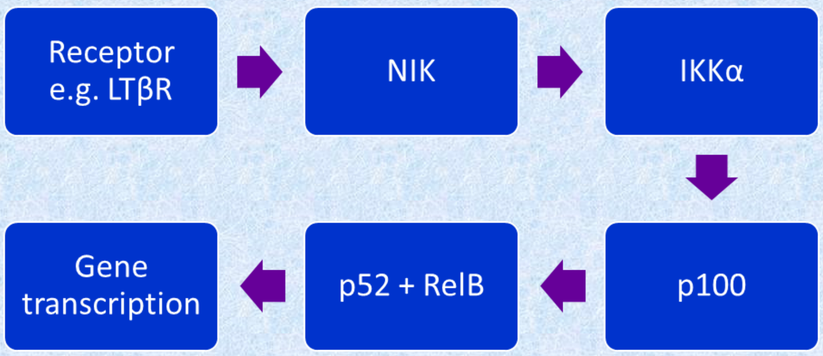
Background

NFκB is a protein complex involved in the regulation of genes that code for proteins involved in the proliferation and survival of cancer cells. It is a member of a cellular signalling pathway of which there are two halves: the classical (canonical) pathway and the alternative (non-canonical) pathway. The classical NFκB pathway has already been shown to have a significant role in the development of hepatocellular carcinoma (HCC) in the liver. However, very few studies have examined the non-canonical pathway in liver cancer, despite recent findings that loss of non-canonical pathway regulation can lead to a poor outcome for patients.

The Non-Canonical NFκB Pathway

The non-canonical NFκB signalling pathway is first stimulated by a small number of receptors, such as the lymphotoxin β-receptor (LTβR). These receptors are molecules on the surface of the cell that bind to other very specific molecules, known as substrates (e.g. lymphotoxin-β). Substrate binding leads to the activation of the enzyme NFκB inducing kinase (NIK) in the cytoplasm. NIK then activates a downstream kinase (IKKα), which adds a phosphate group to a precursor p100 and results in conversion to the DNA-binding p52 subunit. This then binds to the protein RelB and translocates to the nucleus to induce gene transcription.





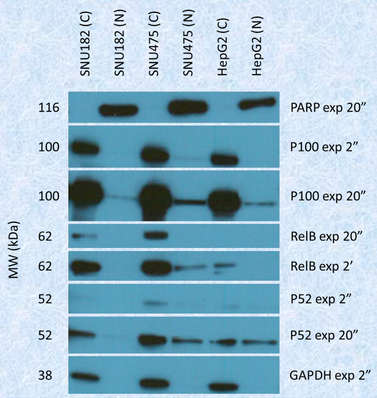
Aims

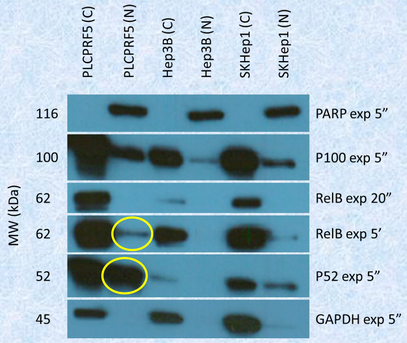
- To examine the extent of non-canonical NFκB pathway activation in human liver cell lines.
- To examine how gene silencing of key upstream mediators (e.g. IKKα) affects target gene expression in tumour cells with significant nuclear RelB/p52.
- To examine whether gene silencing also has an effect on tumour cell growth and viability.

Methods

- Nuclear-cytoplasmic extraction of 6 liver tumour cell lines, followed by Western Blot analysis to accurately determine levels of RelB and p52 in the nucleus.
- Selection of one cell line, PLCPRF5, with the highest level of p52 in the nucleus.
- siRNA transfection to deplete IKKα in the cell line selected, followed by Western Blot analysis.
- Further siRNA transfection to determine whether IKKα knockdown affects tumour cell proliferation.

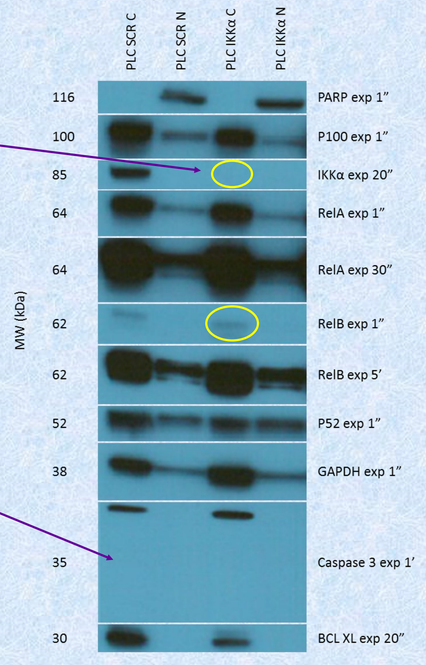
Results



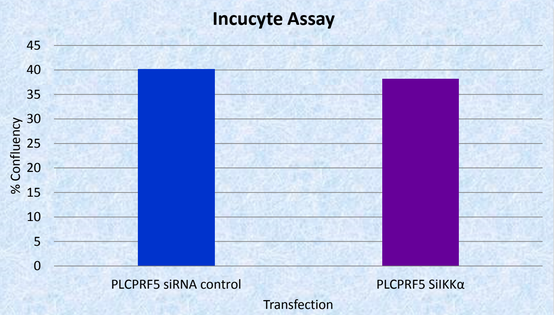


The PLCPRF5 cells had the most RelB and p52 in the nucleus (shown by a thicker band), and therefore the highest non-canonical NFκB expression.

The siRNA was shown to be very effective at knocking down IKKα as no IKKα was present in the nucleus following transfection. After confirmation of IKKα knockdown, the cell line was transfected once again. A slight reduction in RelB was seen in the cytoplasm of cells without IKKα, but no apoptosis occurred. This is shown by a lack of bands beneath the caspase 3 bands at 35kDa. Apoptosis causes caspase 3 to be cleaved into a fragment with a lower molecular weight (MW).



The growth rate of the transfected cells was determined by measuring the level of confluence (i.e. % of plate covered by cells) over 120h. Using a 2 sample t-test, the p-value was calculated to be 0.137. This is greater than 0.05, therefore there was no significant difference between the control and IKKα.



Discussion

The non-canonical NFκB signalling pathway was not found to be active in the majority of liver cell lines examined. In the PLCPRF5 cells, where non-canonical NFκB signalling is high, IKKα knockdown using siRNA did not affect downstream pathway signalling. Consistent with this, IKKα knockdown did not affect the growth rate of PLCPRF5 cells or induce apoptosis (cell death). Collectively these studies suggest inhibition of IKKα would not be beneficial in the treatment of liver cancer.