The role of NF-κB2 Serine 222 phosphorylation in cancer

Author: Adam Duxfield, 160381151, School of Biomedical Science, A.Duxfield@Newcastle.ac.uk
Supervisor: Prof. Neil Perkins, Institute for Cell and Molecular Bioscience

Newcastle University

Definitions

- **Serine 222 (S222)** - An amino acid residue which is found on p100 and p52 of NF-κB2.
- **Phosphorylation-** the addition of a phosphate group to a molecule or amino acid such as S222.
- **Kinase-** an enzyme which adds a phosphate group to S222 on p52/p100).
- **Transcription Factor-** a molecule which increases the production of a target gene.
- **U2-OS cells-** A Osteosarcoma (Bone cancer) cell line.

Introduction

- NF-κB is a family of transcription factors in human cells that consists of 5 different subunits. These are c-Rel, RelA, RelB, NF-κB1 and NF-κB2[1], which regulate the processes of inflammation and immunity by acting on their target genes[2]. However, these subunits also play a key role in cancer development[2]. My project focused on NF-κB2, which is synthesised as the p100 precursor protein prior to processing to its shorter form p52[2]. As shown in figure 1 below. I investigated the role that serine 222 phosphorylation of p52 and p100 plays in cancer.

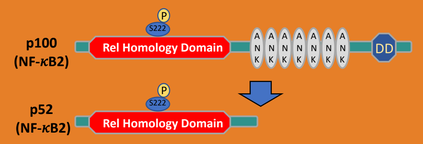

Figure 1: A Diagram showing the structure of both p100 and p52 of NF-κB2. Along with a phosphorylated Serine 222 site.

Aims

- To Identify the kinase responsible for the phosphorylation of NF-κB2 at Serine 222.
- To characterise NF-κB2 Serine 222 phosphorylation in cells from patients with chronic lymphocytic leukaemia (CLL).

Results

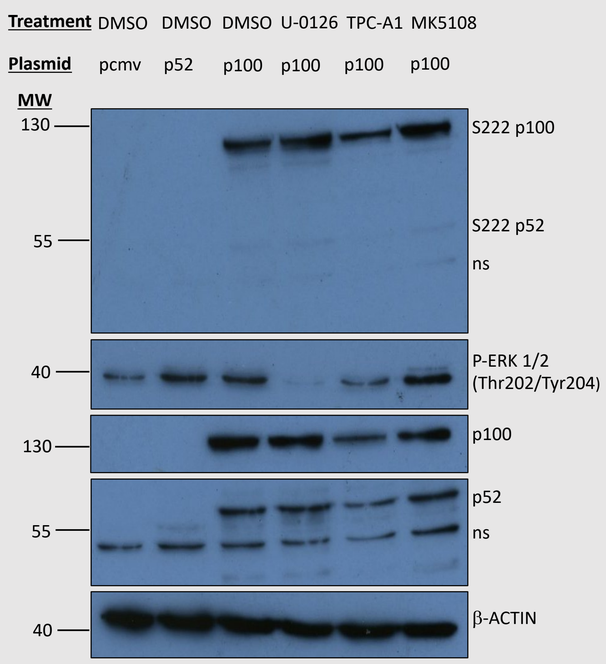

Figure 2: Inhibition of the MAP Kinase, IκB Kinase 2 and Aurora-A Kinase pathways in U2-OS cells had no effect on S222 phosphorylation. The first 3 lanes expressing the pcmv (empty control) , p52 overexpressed and p100 overexpressed plasmids were treated with DMSO a control treatment to show the basal level of p100 s2222 phosphorylation. U-0126 a MAPK inhibitor shows no change in p100 S222 levels compared to lane 3 and therefore had no effect on S222 levels. Furthermore, the fact that the P-ERK band decreases for U-0126 shows that the drug is in fact working just not on S222. TPC-A1 a IκB Kinase 2 inhibitor shows a decrease in both S222 and total p100 levels by the same amount compared to lane 3 and thus only influences the total levels of p100 rather than S222 phosphorylation. MK5108 an Aurora-A Kinase inhibitor shows no change in levels like U-0126 compared to lane 3 and has no effect effect on S222. Actin levels are equivalent for all lanes showing protein loading is equal so any difference in bands is due to the treatment added. In summary the 3 kinases MAP, IκB 2 and Aurora-A are not the S222 kinases.

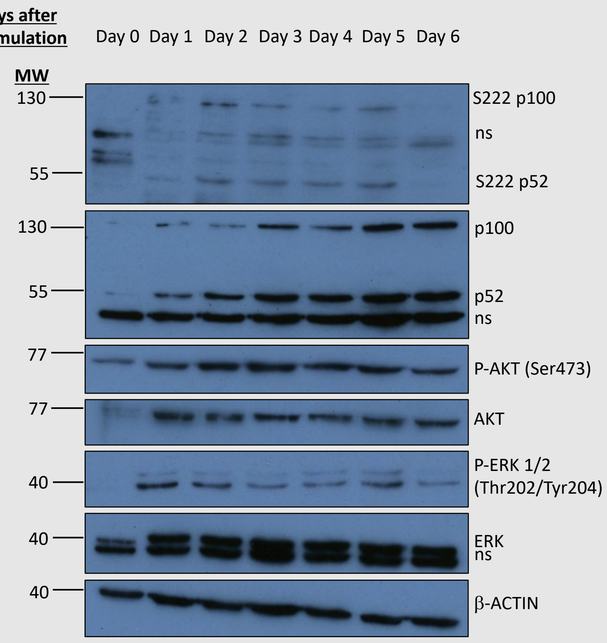

Figure 3: CLL Patient 1104 Serine 222 phosphorylation is induced from day 1 to day 5 and is lost in day 6. On day 0 the CLL cells are stimulated with CD40 Ligand a known activator of the NF-κB2 pathway. The bands for S222 phosphorylation of p100 between days 1 to 5 are not only induced compared to day 0 but also remain fairly constant in intensity over that period. On day 6 the band for S222 phosphorylation decreases. Which also happens to be the day which the CLL cells are believed to start proliferating. This suggests that loss of S222 phosphorylation in CLL cells could in fact trigger this proliferation and influence the development of this cancer.

Also shown are western blots for kinases that might regulate S222 phosphorylation. It can be seen that the CD40 ligand treatment also activates AKT and ERK1/2 activity.

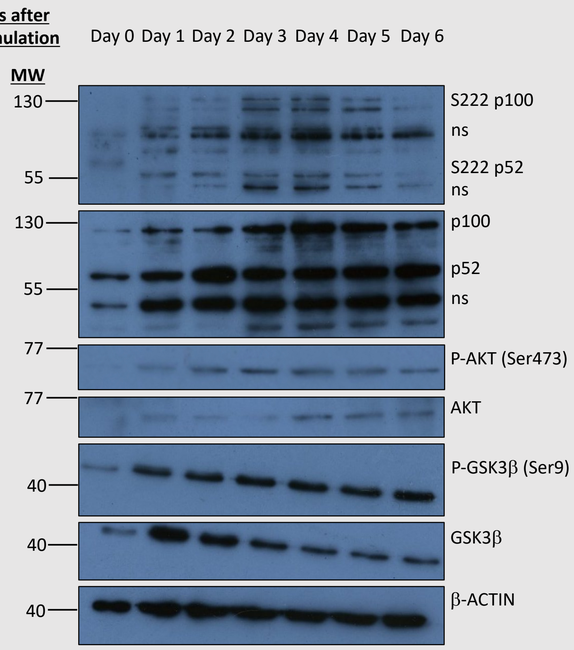

Figure 4: CLL Patient 1110 The induction of Serine 222 phosphorylation is delayed until between day 3 and day 5 and is then lost in day 6. On day 0 the CLL cells are stimulated with CD40 Ligand a known activator of the NF-κB2 pathway just like patient 1104. The bands for S222 phosphorylation of p100 at days 1 and 2 are barely visible showing that phosphorylation has not yet been induced compared to day 0. The increase in intensity of p100 S222 bands from day 3 to 5 show that this is the point in which the induction takes place. On day 6 the band for p100 S222 phosphorylation decreases. Which also happens to be the day which the CLL cells are believed to enter cells proliferation. This suggests that loss of S222 phosphorylation in CLL cells could in fact trigger this proliferation and influence the development of this cancer.

Methods

- Cell culture to grow the U2-OS cell line, transfect with pcmv (empty control), p52 and p100 plasmids, treat with various inhibitors and to harvest these cells to obtain protein extracts.
- Western Blotting to measure protein expression.
- Bacterial Culture to create new stocks of p100 and p52 expression plasmids to transfect into U2-OS cells.

Conclusion

- The inhibitors U-0126, TPC-A1 and MK5108 when added to the U2-OS cells as a treatment appear to have no direct effect on S222 phosphorylation.
- Genetic differences between CLL cancer patients result in varied S222 phosphorylation levels to be observed between patients.

Future work

- To repeat the experiment shown in Figure 2 with further kinase inhibitors to see if they influence S222 phosphorylation within U2-OS cells.
- During my project I conducted an Immunoprecipitation assay, which is not shown on the poster. This technique aims to identify which molecules bind or associate with p100 and p52 directly. The results showed an apparent interaction between p100 and the kinase GSK3β. However, when I tested the GSK3β inhibitor it had no effect on S222 phosphorylation at p100 (not included on poster). However, this should be repeated but this time using a range of concentrations of the inhibitor to see if any effect on S222 phosphorylation can be observed.

Acknowledgements

- I would like to thank Prof. Neil Perkins for helping me during my project, as well as, all members of the Perkins Lab for their help.
- I would also like to thank Newcastle University for the opportunity to undertake this research project and for funding the project.

References

1. Lawrence T, The Nuclear Factor NF-kB Pathway in Inflammation, Cold Spring Harb Perspect Biol. 2009, 1: a001651
2. Hoesel B, Schmid JA, The complexity of NF-kB signalling in inflammation and cancer, Molecular Cancer, 2013, 12:86.