Role and Effect of Serine 222 Phosphorylation of p100/p52 in U2-OS Osteosarcoma Cells

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Background

- NF-kB is a protein family involved in the regulation of cell cycle processes, and an important factor involved in the initiation and progression of cancer[1,2].
- Protein modifications, in particular phosphorylation of amino acids such as Serine, can be used to adjust NF-kB activity[1].
- My project concerns the non-canonical pathway, and the effect of phosphorylation of an amino acid reside (Serine 222) on one of the subunits – p100/p52.
- This effect is investigated in CRISPR/Cas9 genetically engineered osteosarcoma cells, one of which is wildtype and the other contains a mutation at Serine 222 to Alanine (S222A) which can not be phosphorylated .

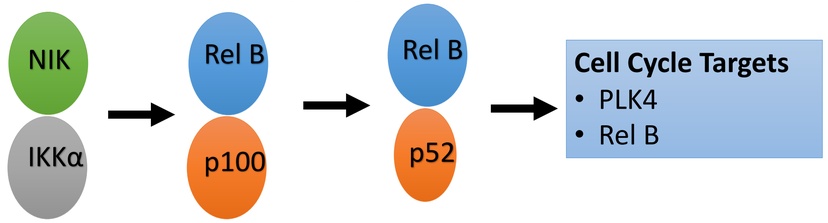


Figure 1: Non-canonical NF-kB pathway leads to the regulation of target genes associated with the cell cycle.

Aims and Objectives

- Investigate whether phosphorylation of Serine 222 (S222) contributes to the regulation of other NF-kB subunits or p100/p52 specific target genes.
- Examine the role of S222 in cell survival.

Method

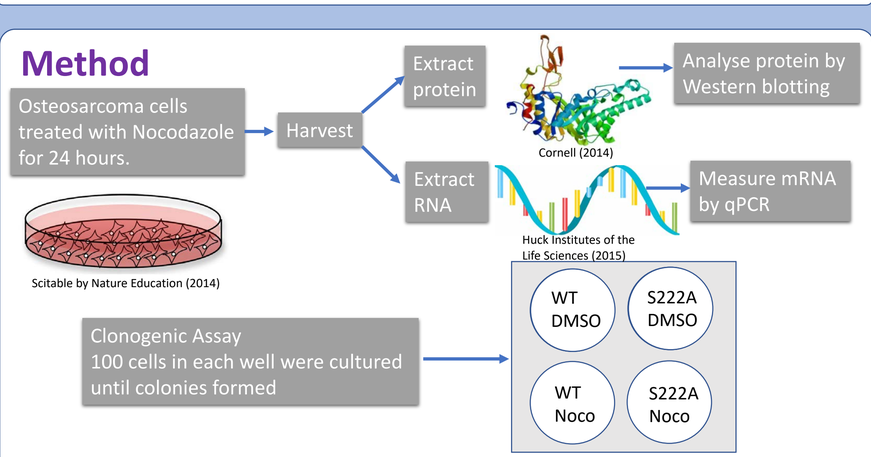
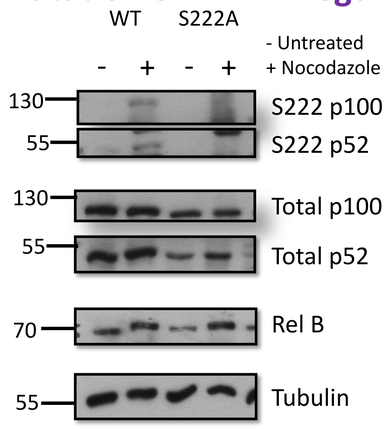
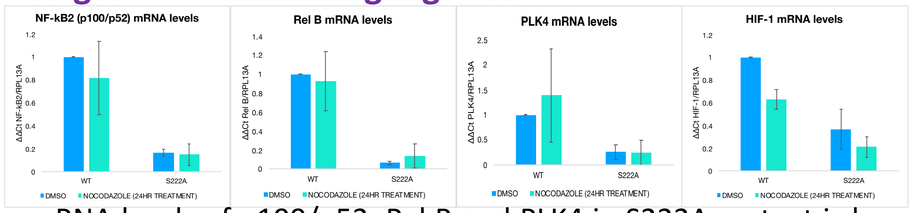


Figure 2: Western blot analysis shows effects of S222A mutation on NF-kB regulated genes.



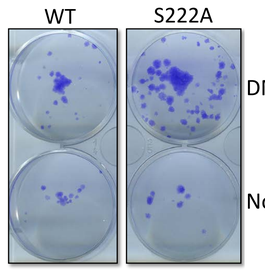
- S222 is phosphorylated in WT cells blocked in mitosis following treatment with the drug Nocodazole. Phosphorylation was absent in S222A cells.
- Total p100/p52 levels decrease in S222A compared to WT.
- Rel B level is lower in untreated S222A compared to WT, but is induced with Nocodazole.

Figure 3: S222A mutation reduced mRNA levels of target NF-kB2 and target genes.



- mRNA levels of p100/p52, Rel B and PLK4 in S222A mutant is less compared to WT.
- Rel B mRNA levels show a significant decrease in S222A compared to WT, which does not correlate with Western blot findings.
- HIF-1 mRNA levels show no significant changes between WT and S22A, as it is involved in the canonical NF-kB pathway.

Figure 4: S222A mutation does not affect cell survival, but has a possible effect on clone formation.



- Cell survival of WT and S222A cells were unaffected.
- WT and S222A form similar number of colonies.
- The effect of Nocodazole treatment appears to have a larger effect on S222A.

Discussion

- Serine 222 is phosphorylated in treated WT cells, but not in S222A cells which is expected as the Serine to Alanine mutation removes the site of phosphorylation (Figure 2).
- Low levels of Rel B in S222A mutant could possibly be due to low levels of total p100/p52, as Rel B forms a complex with p100/p52 (Figure 2).
- Confirming the Western results, mRNA levels showed that S222A cells are unable to form full length, functioning p100/p52 and so the ability to regulate target genes (e.g. PLK4) is compromised (Figure 3).
- mRNA levels of Rel B shows low levels in S222A which does not correlate with Western results, and this suggests that Rel B undergoes post-transcriptional modifications in order to increase the levels of Rel B protein (Figure 3).
- The cell survival of WT and S222A cells are not affected by phosphorylation of S222, however the ability of S222A cells to form clones appears greater (Figure 4).

Conclusion

- mRNA levels of p100/p52 are depleted following phosphorylation of S222, and this has a negative effect on p100/p52 specific regulated target genes (e.g. Rel B and PLK4).
- Genes independent from the non-canonical pathway are unaffected (e.g. HIF-1).
- Phosphorylation does not affect growth rate of cells, but may have an affect on clone formation.

References
1. Christian F, Smith EL, Carmody RJ. The Regulation of NF-κB Subunits by Phosphorylation. Cells. 2016;5(1).
2. Webster GA, Perkins ND. Transcriptional Cross Talk between NF-κB and p53. Molecular and Cellular Biology, 1999;19(5): 3485-3495.