The effect of the S42/45A mutation in the NF-κB subunit RelA

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RelA is a transcription factor that activates the expression of a wide range of target genes. RelA target genes are involved in inflammation and cell proliferation, meaning that when RelA becomes aberrantly activated it can lead to inflammatory diseases or cancer. Previously, the Eyers laboratory at Liverpool University had demonstrated that RelA becomes phosphorylated at Serine 42 and 45 following TNF stimulation and that this reduced RelA's affinity for its target gene promoters meaning they were expressed at a lower level. In my project I studied the effect of mutating RelA residues Serine 42 and 45 to Alanine, to prevent their phosphorylation. By comparing these mutants to the cell expressing wild type RelA I was then able to investigate the effect of phosphorylating these residues following DNA damage.

To do this I used a tetracycline expression (T-REx) system, created by Life Technologies. U2OS osteosarcoma cells were transfected with a plasmid containing the RelA gene but with a promoter that is only activated by the presence of tetracycline in the cell media. One cell line contained a wild-type RelA T-REx system and the other contained S42/45A RelA T-REx System. These mutations and cell lines were created by Dr George Schlossmacher. As can be seen in Figure 1, the addition of tetracycline massively induces both wild type and mutant RelA expression, showing that the system is functional.

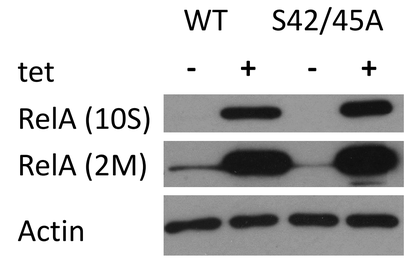


Figure 1: T-REx system enhances RelA expression in wild-type and mutants to a similar level in the presence of tetracycline. Actin levels are unaffected, showing that protein loading is equivalent.

Using these cell lines I then investigated how the S42/45A mutation affected the cellular response to DNA damage.

Aims

-To determine the effect of the S42/45A mutation on phosphorylation of Chk1 and IκBα which are key proteins in the DNA damage and NF-κB response pathways.
- To investigate how the S42/45A mutation affects expression of RelA target genes such as IL-8, BCL-XL and Clspn.

Methods

- Western blotting to measure protein expression
- Polymerase Chain Reaction (PCR) and quantitative PCR
- Agarose gel electrophoresis to overcome and understand inaccuracies regarding PCR primers
- Cell culture techniques to maintain and treat my cells and to harvest RNA or protein from them
- CRISPR gene editing technology

Conclusions

The S42/45A mutation in RelA:

- reduces phosphorylation and expression of Chk1 following DNA damage
- Increases phosphorylation of IκBα, the inhibitor of NF-κB
- Mutation of S45A alone has a stronger effect than the S42/45A double mutation

Results

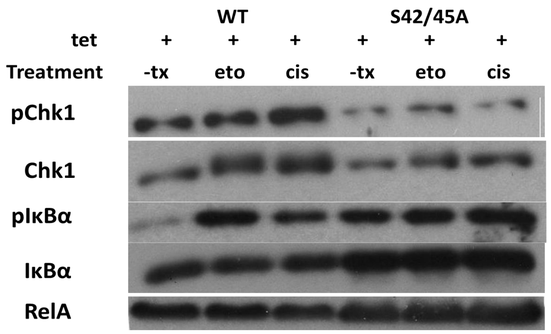
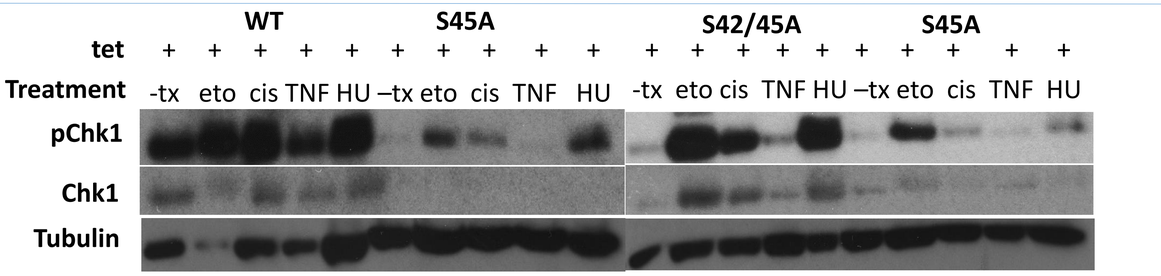


Figure 2: Phosphorylation of Chk1 and IκBα is altered by the presence of the S42/45A mutation. Upon DNA damage induced by treatment with etoposide or cisplatin, the ATM and ATR kinases are activated. ATR then phosphorylates Chk1; ATM can activate IKK complexes which then phosphorylate IκBα. Therefore, I treated my cells with etoposide (eto) and cisplatin (cis) to determine whether the S42/45A mutation affects phosphorylation and expression of Chk1 and IκBα (Figure 2). The data demonstrates that the S42/45A mutation decreases Chk1 expression and phosphorylation. The mutation also induced IκBα phosphorylation in unstimulated cells.
Tx = Treatment, eto = etoposide, cis = cisplatin, tet = tetracycline

Figure 3: The S45A mutation alone results in reduced phosphorylation and expression of Chk1. To learn whether S45 phosphorylation alone mediates these effects I examined an additional Trex U2OS cell line where RelA was mutated only at this residue. This experiment also included stimulation with TNF and an inducer of DNA replication stress called hydroxyurea. Interestingly, the S45A mutation alone inhibited Chk1 after all forms of DNA damage and appeared more potent than the S42/45A double mutation.



Other work

During my project, I used a technique called quantitative polymerase chain reaction (QPCR) to determine the mRNA levels of NF-κB target genes. However, there was not time to produce statistically significant data to show that the mutation affected BCLXL, IL8 or Claspin expression and therefore it has not been included on the poster.
I also completed the novel gene editing technique, CRISPR, to introduce RelA with a HA-tag into U2OS cells. This would act as a control to see if the process worked before undertaking more complex mutation analysis which would be to produce the S42/45A RelA mutations.