Do WIP1 inhibitors increase the effect of p53-dependent therapies in neuroblastoma?

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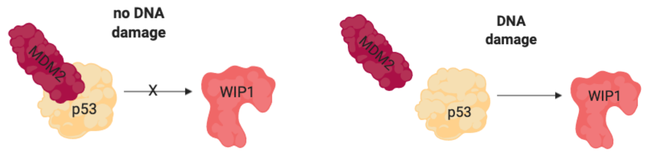
Introduction

Neuroblastoma is a rare, aggressive form of cancer that affects around 100 children in the UK each year[1]. Wild-type p53-induced phosphatase 1 (WIP1) is often present in abnormally high levels in neuroblastoma[2] .

The expression of WIP1 is increased with increasing levels of tumour suppressor protein p53. p53 is usually inhibited by the binding of a third protein MDM2 but is activated in the presence of DNA damage[2].



P53 acts to stop the cell from dividing when there is DNA damage, this gives the cells time to repair the DNA so that they don't divide with damaged DNA as this is what causes cancer. WIP1 functions to decrease levels of p53 and return cells to their normal state once the damage has been fixed[2].

Methods

Aims:

Inhibit WIP1 and observe the effect on neuroblastoma cells.

1) Cell free phosphatase assay does GSK2830371 (GSK) inhibit WIP1?

2) Drug matrix does GSK increase the affects of MDM2 inhibitor RG7388 (RG)?

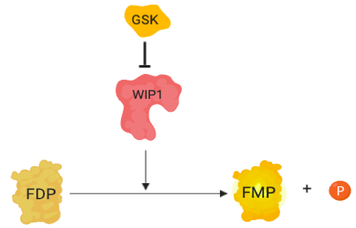
3) Western blot what affect do the drugs have on levels of p53 and genes regulated by it?

Drug Matrix

Before drug matrix was performed growth of cells was monitored over 6 days at different seeding densities to determine which seeding density would be best.

Cells are cultured and allowed to multiply → Cells are seeded → Cells are drugged with different concentrations of GSK and RG both as single treatments and in combination. → Absorbance reading is taken and matrix is analysed

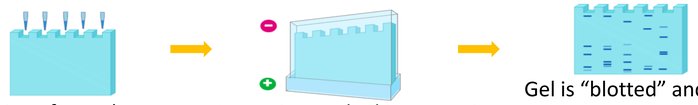
Cell Free Phosphatase Assay



- Different concentrations of GSK were introduced into wells containing WIP1 and FDP.
- WIP1 breaks down FDP to FMP and then to fluorescein which are fluorescent.
- Fluorescence is then read and lower level indicates WIP1 inhibition.

Western Blot

First cells are cultured, seeded, and drugged same as in the drug matrix, then the samples are collected and processed into 'lysates'



Lysates of samples are loaded onto gel → Current is applied to separate proteins in the sample by size → Gel is "blotted" and the filter analysed for p53 and proteins regulated by it

Results

Does GSK2830371 (GSK) inhibit WIP1?

- Figure 1 shows that **GSK acts as a WIP1 inhibitor** with there being some inhibition even at the lowest concentration of GSK used.
- 50% inhibition (under 50% activity) is achieved at a low concentration of GSK which means it can be considered an effective inhibitor.

Does GSK increase the affects of MDM2 inhibitor RG7388 (RG)?

- The first step of this experiment was to determine the seeding density of cells that should be used, this was by performing growth curves, the results for which are shown in figure 2.
- Figure 3 shows that **GSK does increase the affects of RG** as for all concentrations of RG there is more inhibition when cells are treated in combination with GSK.
- The Loewe score for this matrix came out as 34.32 indicating a synergistic relationship as anything over 10 is considered synergistic.

What affect do the drugs have on levels of p53?

- Figure 4 shows that **all drug treatments increase p53 levels** and that after 24hrs RG becomes less effective while GSK potentiate the effects of it with the highest levels of p53 after 24hrs treatment being seen with RG100 + GSK.

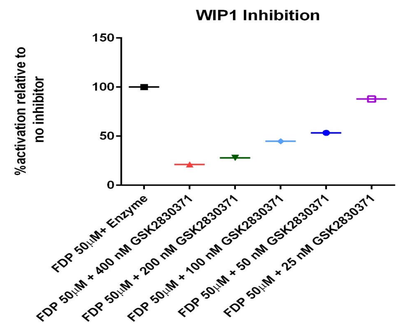


Figure 1: cell free phosphatase assay results based off percentage activation relative to no inhibitor, *graph made using PRISM.*

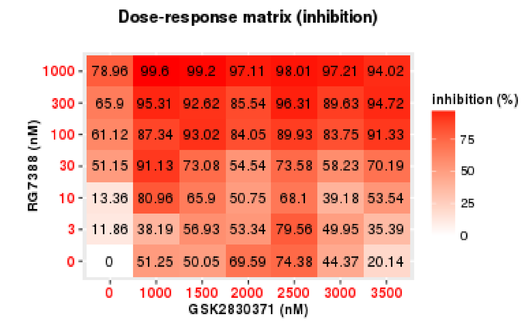


Figure 3: NGP drug matrix results based off the percentage inhibition of cell growth for each drug combination when compared to DMSO negative control, *made using synergyfinder.*

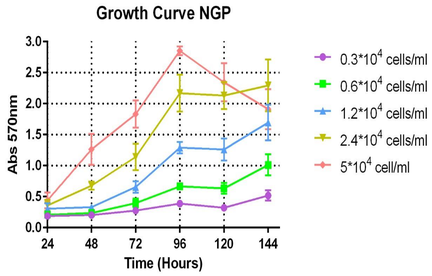


Figure 2: NGP growth curve results, *graph made using PRISM.*

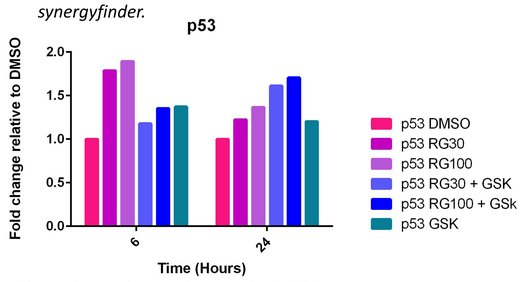


Figure 4: densitometric analysis of NGP western blot based off intensity of band on the gel relative to DMSO negative control, *made using PRISM.*

Conclusion

- Results from my research project showed that GSK is an effective WIP1 inhibitor that can be given alongside RG to increase the effects of RG.
- This allows us to decrease the dose of RG given which is beneficial as decreasing the concentration makes it less cytotoxic, meaning there is less harm to non-cancerous cells and fewer side effects.
- Additionally giving treatments in combination has been shown to decrease the chance of relapse by preventing drug resistance[4], meaning that showing GSK can potentiate the effects of existing cancer treatments is extremely important
- Further studies would need to be done to show that this kind of treatment works for multiple neuroblastoma cell lines before it can be considered for further pre-clinical testing.

References

1. NHS. Neuroblastoma. 2019 Aug 15 [cited 07/10/19]; Available from https://www.nhs.uk/conditions/neuroblastoma/
2. Gains J, Mandeville H, Cork N, Brock P, Gaze M. Ten challenges in the management of neuroblastoma. Future Oncol. 2012 Jul;8(7):839-58
3. All diagrams were made using BioRender.
4. Mokhtari RB *et al.* Combination therapy in combating cancer. Oncotarget. 2017 Jun 6; 8(23):38022-38043