Using Specific Inhibitor Drugs for t(8:21) Acute Myeloid Leukaemia

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Introduction

Acute myeloid leukaemia (AML) is an aggressive form of cancer characterised by the overproduction of immature myeloid cells, which prevent the bone marrow from producing normal white blood cells. The most common cytogenetic abnormality in AML is translocation t(8;21), found in up to 15% of all AML cases [1].

t(8;21) results in the RUNX1/ETO fusion protein which mainly acts as a transcriptional repressor to prevent differentiation into normal white blood cells and enhances self-renewal of malignant cancer cells [2]. Despite improved treatments for AML, relapse occurs in many patients and toxic therapies often cause long term side effects. Therefore, there is a need to develop novel therapies or redeploy existing drugs to improve long term outcome for patients.

A possible drug of utility that has been identified is Palbociclib; a selective CDK4/6 inhibitor in the cell cycle. Palbociclib inhibits CDK4/6 resulting in reduction in RB phosphorylation which prevents cells from entering the S phase of the cell cycle and induces cell cycle arrest. The overall aim of this project was to evaluate whether Palbociclib is a viable treatment option for t(8;21) AML.

Aims

- To validate promising drug target genes in t(8;21) AML (as previously identified by an RNAi screen) by western blotting.
- To examine the effects of the targeted inhibitor; palbociclib on these target genes using cell lines.

Methods

Kasumi-1 cell line (Tissue culture of leukaemic cell line with an 8;21 chromosome translocation)

Drug dosing (Cells are counted manually using a haemocytometer and equally divided into flasks, at a concentration of $0.5x10^6$ cells/mL. Flasks are dosed with palbociclib and/or imatinib and incubated at 37°C for 3 days.

Isolating cells (equal numbers of numbers of cells are taken from each treated or control sample, pelleted to remove media, washed with PBS and frozen at -80°C)

Protein extraction (proteins are extracted using RIPA buffer and quantified using the BCA protein quantification technique)

Western blot (Protein samples are loaded onto a gel for electrophoresis, gel is transferred onto PVDF membrane in trans-blot cell, membranes are incubated with primary and secondary antibodies for identification of specific proteins of interest, films are developed to obtain results)

Results

1) Cells treated with palbociclib

After dosing with palbociclib, cells were counted in order to assess cell viability. The graph in figure 1 shows that cells are entering cell cycle arrest as the number of living cells per ml decreases as the concentration of palbociclib increases.

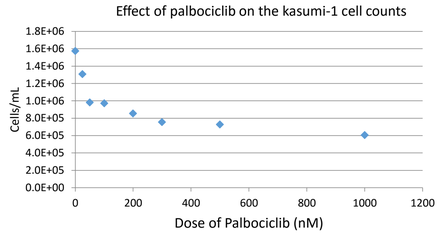

Figure 1: Graph showing that increased doses of palbociclib leads to increased cell cycle arrest in cells.

Kasumi-1 cells were treated with palbociclib and assayed for phosphorylated RB using the primary antibody phospho T821. Bands were detected at the predicted molecular weight of 110kDa (Figure 2). There was very little difference in band intensity between the doses of 0nM palbociclib and 200nM palbociclib, meaning RB phosphorylation had not been inhibited. However, at 300nM, 500nM and 1000nM palbociclib, the band intensity and therefore amount of phospho RB is decreased, showing more effective inhibition of RB phosphorylation.

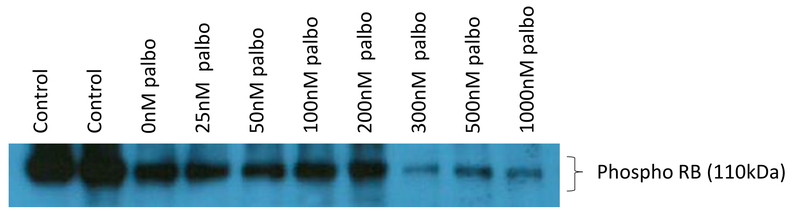

Figure 2: Western blot of phospho Rb in cells treated with increasing concentrations of palbociclib.

2) Cells treated with imatinib and palbociclib

Initially, the results suggested a pattern in RB phosphorylation between the different doses and combinations of drugs. However, once the control antibody was working, it appeared that there was an issue with the protein loading as the band intensity for the GAPDH for all the cells treated with the drugs should have been equal (see figure 3).

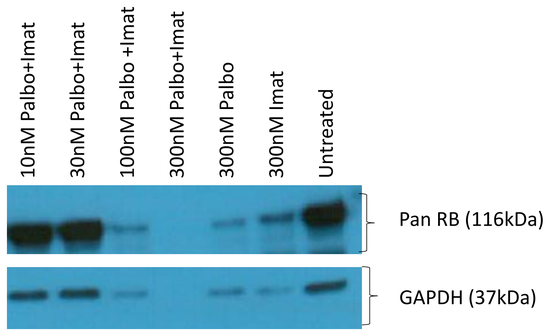

Figure 3: Western blot of Pan RB and control antibody GAPDH in cells treated with palbociclib and imatinib.

By comparing the three doses that had equal protein loading, it appeared that the 300nM imatinib had the greatest intensity phospho RB band and therefore was least effective. The intensity of the phospho RB bands for 300nM palbociclib and 100nM palbociclib and imatinib appeared equally lower, therefore making the doses more effective.

Conclusion

- Through treating cells with palbociclib, it was found that doses up to 200nM have little effect on phospho RB. However, the results suggested that increased doses of the drug lead to decreased phospho RB. This would support the theory that the drug is effectively inhibiting CDK4/6 and subsequently preventing RB phosphorylation.
- In most of the studies, there were issues with the presence of the control antibody. Therefore, further work will need to be carried out in order to conclusively compare the intensity of the bands in the results.
- Through treating cells with both palbociclib and imatinib, the results suggested a phosphorylation pattern of RB between the different doses of the drugs. However, the presence of the control antibody suggested issues with protein loading so the results cannot be conclusively compared.
- By comparing the doses which exhibited equal protein loading in the controls, it appeared that the 100nM palbociclib and imatinib combination dose was more effective than the dose of 300nM imatinib given alone. This will be investigated further to conclude whether smaller doses of each drug in combination can carry out the desired effect, as if successful, could reduce side effects in patients compared to a large dose of one drug.

References

1. Trombly et al. (2015) Genome-wide co-occupancy of AML1-ETO and N-CoR defines the t(8;21) AML signature in leukemic cells. *BMC Genomics*. 16(1):309
2. Ptasinska et al. (2012) Depletion of RUNX1/ETO in t(8;21) AML cells leads to genome-wide changes in chromatin structure and transcription factor binding. *Leukemia*. 26(8): 1829-41

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