Could CD45 have a role in the development of myeloid malignancies?

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Background

CD45, also known as Protein tyrosine phosphatase receptor type C (PTPRC), is a transmembrane glycoprotein tyrosine phosphatase, expressed on almost all hematopoietic cells except for mature erythrocytes, and is essential regulator of T and B cell antigen receptor-mediated activation[1].

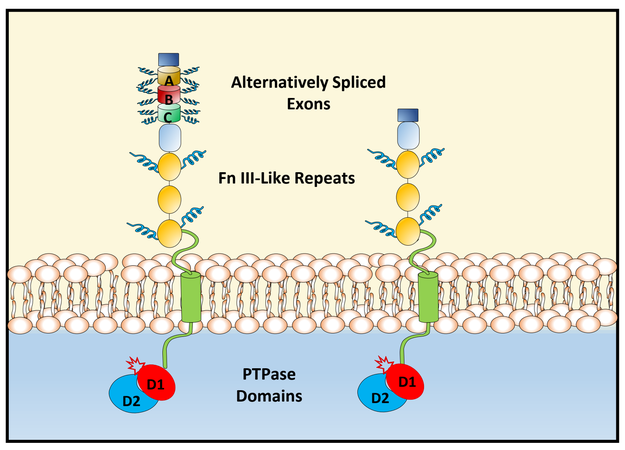


Figure 1. structure of CD45

Disruption of the equilibrium between protein tyrosine kinase and phosphatase activity (from CD45 and others) can result in immunodeficiency, autoimmunity or Malignancy[2].

Therefore, CD45 is vital for the normal blood functioning and alteration in this molecule results in serious consequences[3] and may have potential as a drug target.

Objectives

To investigate the effects caused by knockdown of CD45 in myeloid cell lines and to gain insight into pathways induced by CD45, which are disrupted in myeloid malignancies (AML, MDS and MPN) in an attempt to develop novel therapeutic strategies.

Methods

Flow cytometry, Western blot, and PCR, was used to study CD45 expression in a panel of leukaemia cell lines

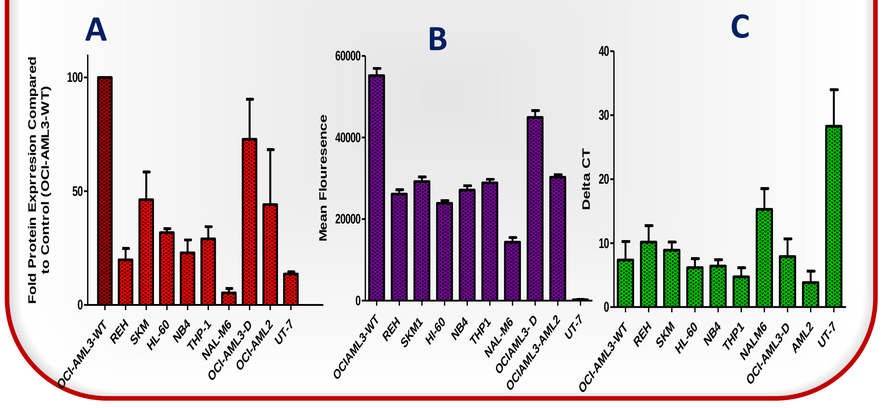
CD45 was knocked down, by siRNA, in the MD/AML cell line SKM and in OCI-AML3 to study the effect of reducing CD45 expression on cellular survival and proliferation.

To further explore the effects of lowered CD45 levels, cells were incubated with increasing concentrations of Cytarabine for 48 h and 72 h, followed by cytotoxicity study by Cell Titre Glo (CTG) assays.

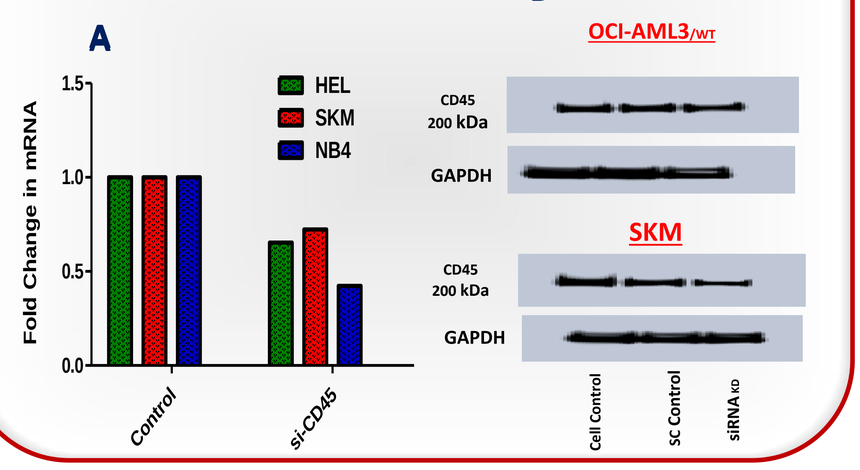
Several genes whose expression positively or negatively correlated with that of CD45 including Jak2, ACTR2, THAP-3, PBX-1 and Serglycin were investigated. The top 20 positive and negative genes were used imported as a signature into Quadratic, a powerful scalable gene expression connectivity mapping software.

Results

AML cells exhibit higher levels of CD45 expression, with all cell lines. REH, NALM-6 and UT-7 having the lowest expression. Assessment was done by western blotting analysis (A), flow cytometry (B), and PCR (C).

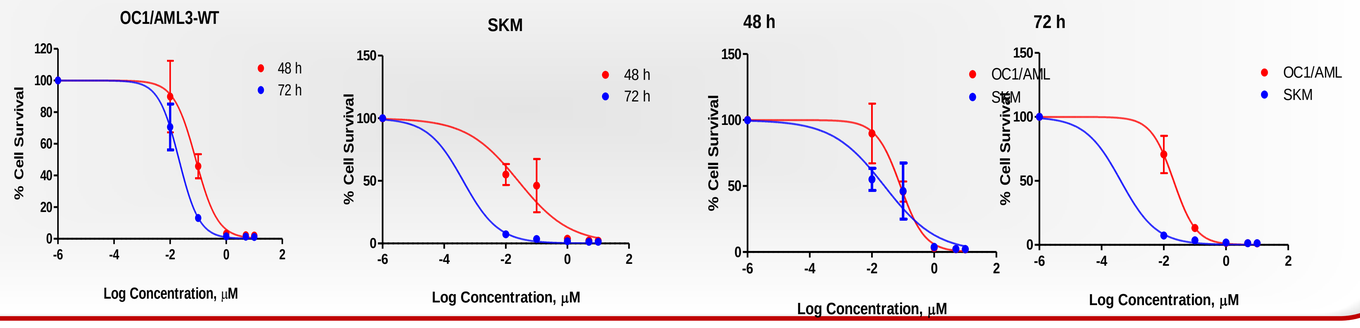


CD45 knockdown, ~8-fold, was confirmed by PCR (A) and western blotting (B) in different AML cell lines.

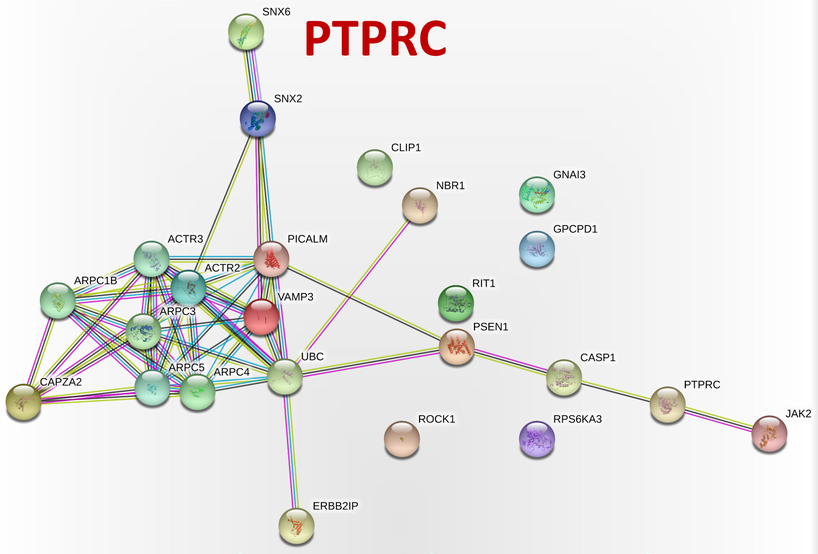


Cytrabine Alters AML Survival

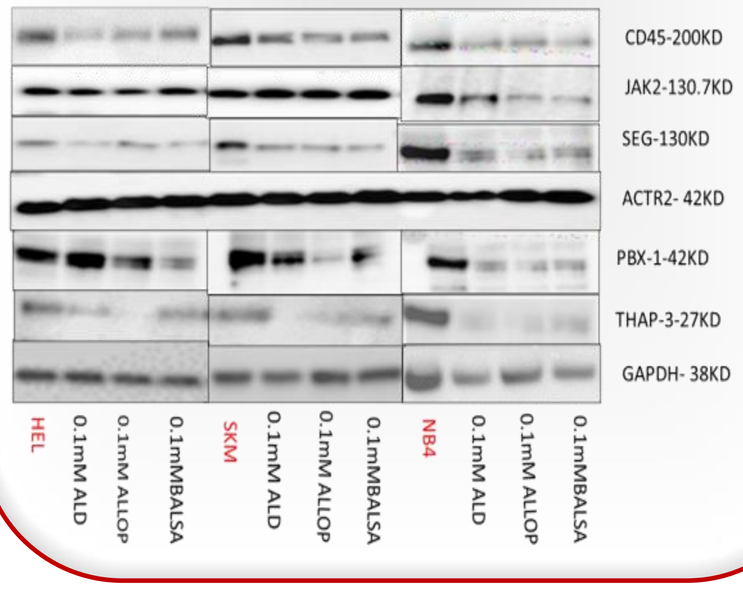
CTG assay showed a gradual decrease in cell proliferation along with progressive increases in the concentrations of cytarabine at 48 and 72 hours.



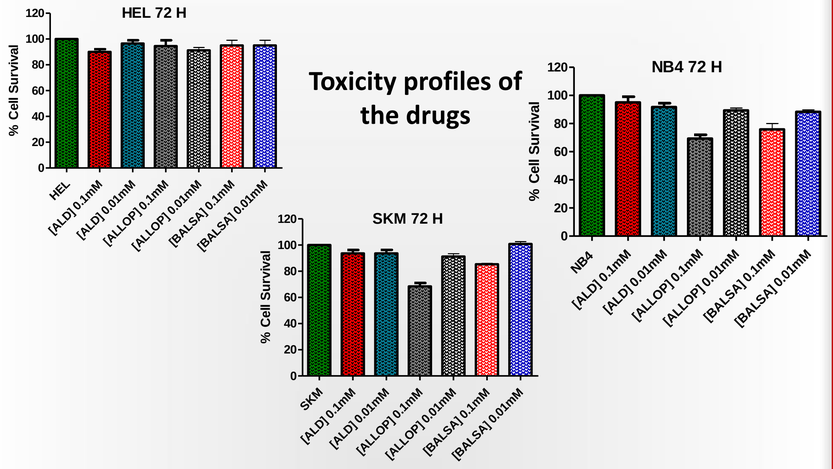
Bioinformatics analysis of patient expression databases Top 20 Correlation with PTPRC



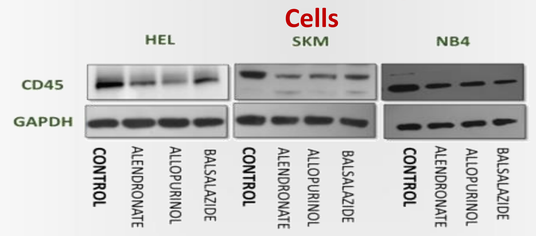
Gene Correlation with CD45 and CD45 Inhibitors at 0.1mM after 72 hours



Three drugs identified, Alendronate, Allopurinol and Balsalazide, may have the potential to act as CD45 inhibitors.

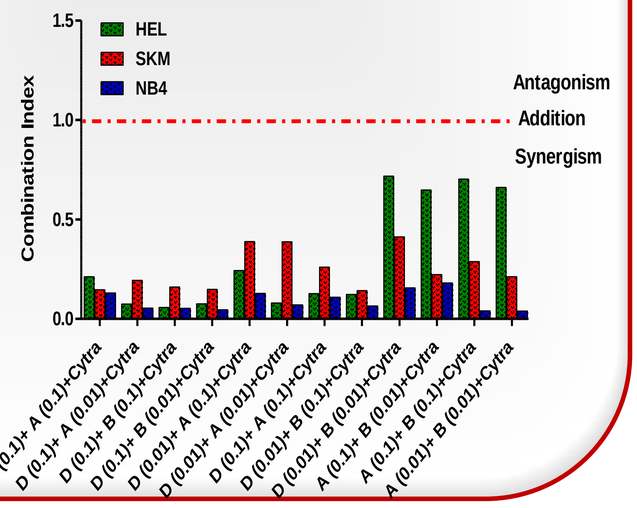


Validation of Inhibitory Effect of the Drugs on CD45 Expression on Cells



The combination of these drugs with Cytarabine has indicated a great synergy

D= Alendronate
A= Allopurinol
B= Balsalazide



Conclusion

CD45 is highly expressed in AML cell lines and the inhibition of the gene, by siRNA or novel repurposed agents targeting CD45, have given an insight into the molecular pathways and potential therapeutic options associated with this gene.

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References

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