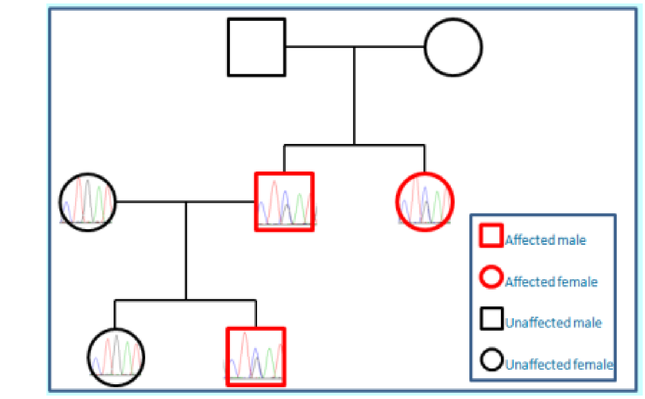
Testing a novel CD45 mutation in patient autoimmunity

Susana Keane*, Professor Sophie Hambleton, David Swan, Karin Engelhardt, Angela Grainger

INTRODUCTION

A young boy was brought to the RVI presenting with symptoms suggesting he has a primary immunodeficiency (PID) involving autoimmunity. His father also has shown symptoms throughout his life that suggest the possibility of the same PID. In addition, the father's sister suffers from IBS and ulcerative colitis. Both of these diseases are associated with autoimmune reactions.

Using whole exome sequencing (WES), a heterozygous mutation, D648H, was found in the PTPRC gene of the boy, his father, and his father's sister. The mutation was not present in the boy's mother or his healthy sister.



The PTPRC gene codes for the CD45 protein, a transmembrane phosphatase that is expressed on many cell types of the immune system[1]. CD45 is involved in the signal transduction of B cell and T cell receptors. In T cell receptor (TCR) signalling, CD45 removes an inhibitory phosphate group from Src-family kinases to allow them to initiate signal transduction from the activated TCR[2].

The lab in which I undertook my project had already gained results from one transfection experiment which suggested that this mutation resulted in a loss of CD45 phospshatase activity. These results needed to be verified.

OBJECTIVE

To determine how this mutation affects CD45 phosphatase activity in order to confirm or disprove previous experiments.

METHODS

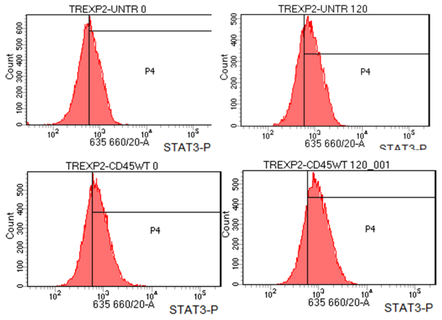
1) HEK293 cells (a human embryonic kidney cell line that does not normally express CD45[3][4]) were transfected with plasmid containing a wild type (WT), mutated (SDM), or inactive copy of the PRPRC gene, using the fugene method.

METHODS

2) Phos-flow flow cytometry was used to measure levels of the phosphorylated form of the transcription factor STAT3 (p-STAT3) in cells overexpressing CD45 in one of the three forms (WT, SDM, or inactive), and in cells which had not been transfected. The difference between the p-STAT3 expression before and after stimulation of the cells with interferon was calculated as an indication of CD45 phosphatase activity.

RESULTS

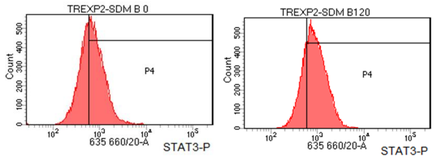
The results of the previous transfection indicated that the increase in p-STAT3 expression in response to interferon (IFN) was decreased in cells overexpressing the WT form of CD45 compared to the untransfected cells containing on CD45. This is expected with full CD45 phosphatase activity.



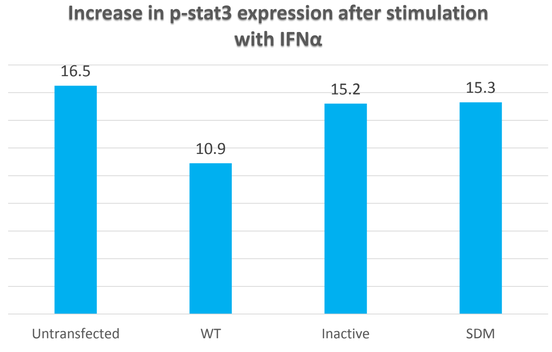
Untransfected cells:- 16.5% increase in p-STAT3 expression following IFN stimulation

WT:- 10.9% increase in p-STAT3 expression following IFN stimulation

The cells overexpressing the experimental CD45 mutant (SDM) showed higher p-STAT3 expression compared to the WT CD45, indicating reduced phosphatase activity.

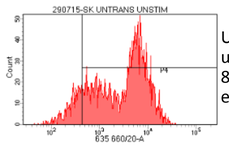


SDM:- 15.3% increase in p-STAT3 expression following IFN stimulation

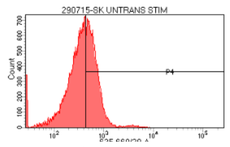


RESULTS

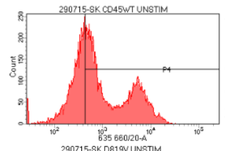
In my experiment the transfection process was unsuccessful, giving some odd flow cytometry histograms.



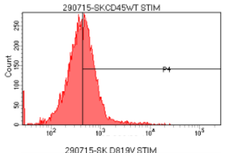
Untransfected cells, unstimulated:- 85.3% of cells expressing p-STAT3



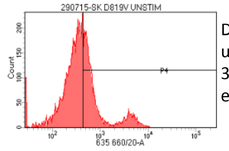
Untransfected cells, stimulated:- 40.9% of cells expressing p-STAT3



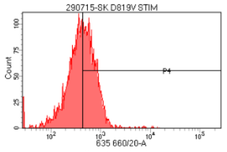
WT, unstimulated:- 63.7% of cells expressing p-STAT3



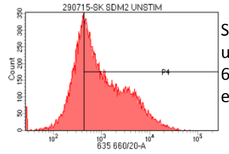
WT cells, stimulated:- 45.0% of cells expressing p-STAT3



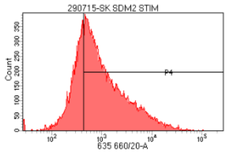
D819V, unstimulated:- 37.8% of cells expressing p-STAT3



D819V, stimulated:- 46.2% of cells expressing p-STAT3



SDM, unstimulated:- 67.8% of cells expressing p-STAT3



SDM, stimulated:- 70.9% of cells expressing p-STAT3

None of the histograms are as expected. The shapes, with extra peaks and shoulders, could be due to many factors within the multistep transfection process.

There appeared to be lots of dead and dying cells in all cell groups, possibly caused by the transfection process. These could have obscured the results if the fluorochromes used to visualize the p-STAT3 bound non-specifically to them or if they gave off their own autofluorescence.

Subsequent attempts at replicating the original results have also seen lots of cell death. This may be due to the age of the HEK cells used, which have been passaged (a technique to keep a cell line alive for extended periods of time) many times. The age of the HEK cells could also explain the large amounts of basal p-STAT3 activity and the low levels of activation on stimulation observed.

The major problem was that the flow cytometry of the untransfected cells showed expression of CD45 and GFP, signs of transfection. This means that these results are invalid and cannot be used for comparison with the other cells, therefore no useable readings can be taken from this experiment.

CONCLUSIONS

No results could be taken from this experiment, therefore the results of the previous transfection were neither confirmed or disproved.

The experiment has since been repeated but the initial results could not be replicated.

Another repeat will soon be tried with fresh HEK293 cells.

References:
1.Hermiston ML, Zikherman J, Zhu JW. CD45, CD148, and Lyp/Pep: critical phosphatases regulating Src family kinase signaling networks in immune cells. Immunological reviews. 2009;228(1):288-311.
2.Zamoyska R. Why Is There so Much CD45 on T Cells? Immunity. 2007;27(3):421-3.
3.Thomas P, Smart TG. HEK293 cell line: A vehicle for the expression of recombinant proteins. Journal of Pharmacological and Toxicological Methods. 2005;51(3):187-200.
4.Perron MD, Chowdhury S, Aubry I, Purisima E, Tremblay ML, Saragovi HU. Allosteric noncompetitive small molecule selective inhibitors of CD45 tyrosine phosphatase suppress T-cell receptor signals and inflammation in vivo. Molecular pharmacology. 2014;85(4):553-63.

*Susana Keane (120063824) School of Biomedical Sciences; s.keane2@newcastle.ac.uk