Investigation into the role of protein phosphatase 4 regulatory subunit 1 (PP4R1) on the survival of leukemic Human T cells

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Introduction

The serine/threonine protein phosphatase 4 holoenzyme (PP4) has many functional roles such as DNA damage response, DNA repair, cell cycle regulation, and apoptosis, cell migration, immune response, stem cell development, glucose metabolism, and diabetes.

PP4 consists of a catalytic subunit (PP4c) which interacts with four regulatory subunits (PP4R1, PP4R2, PP4R3, PP4R4). Our previous studies showed that PP4c has an important tumour suppressor function and plays an important role in the control of cell death and survival of both leukemic T-cells and untransformed human peripheral blood T-cells. Subsequently, many studies have confirmed a role for PP4c in the regulation of cell fate. However, the role of the PP4 regulatory subunits in the regulation of cell survival remains unclear.

Aims of the study

The aim of the present work is to investigate the role of PP4R1 in the regulation of leukemic cell fate. The work examines the effects of modulation of PP4 regulatory subunits expression levels on the survival human leukemic T-cells. The work also investigates the effects of modulating PP4R1 expression levels on PP4c.

Method

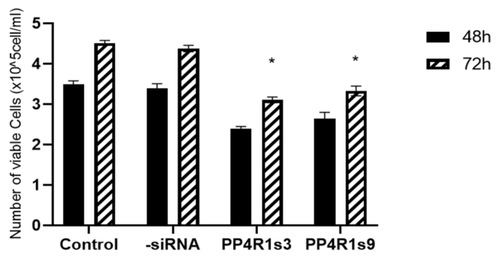
The human leukemic Jurkat T cells were transfected with pcDNA3.1-PP4R1 for overexpression studies and PP4R1 specific siRNAs for silencing studies. Controls received empty vector or scrambled siRNA.

Western blotting was used to confirm the endogenous expression level of PP4R1 and PP4c.

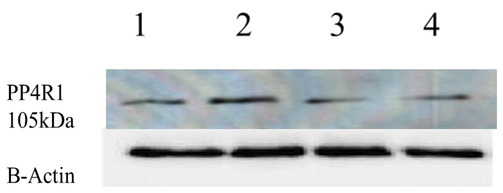
Cell viability and apoptosis of transfected cells were investigated using flow cytometry.

Results

PP4R1 silencing inhibits cell growth and increases basal apoptosis

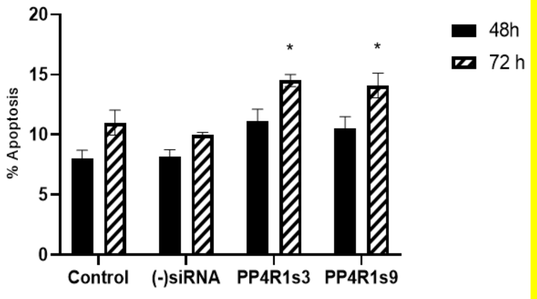


PP4R1 silencing decreases the viable cell number of leukemic T cells as determined by flow cytometry . *P<0.005



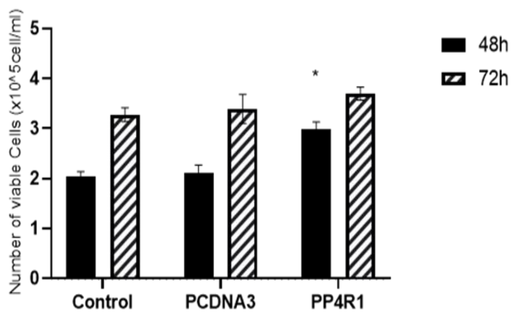
PP4R1 protein expression levels as determined by western blotting

control=lane 1, -siRNA=lane 2, S3siRNA=lane 3, and S9siRNA=lane 4)

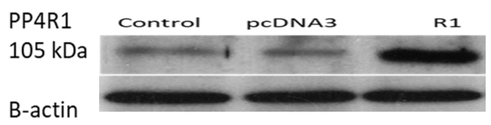


PP4R1 silencing increases basal apoptosis level of leukemic T cells as determined by Annexin V staining . *P<0.005

PP4R1 overexpression increases the viable cell number

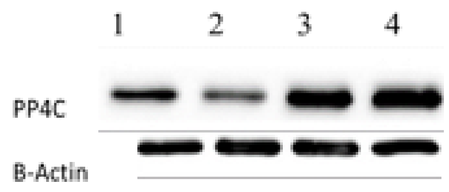


PP4R1 overexpression increases the viable cell number of leukemic T cells as determined by flow cytometry . *P<0.005



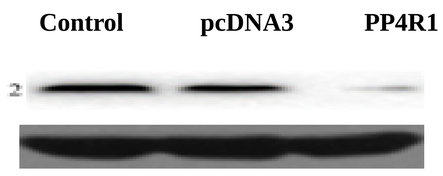
PP4R1 protein expression level as determined by western blotting

Modulation of PP4R1 expression levels affects the endogenous protein levels of PP4c



Silencing of PP4R1 leads to an increase in PP4c protein expression level as determined by western blotting .

control=lane 1, -siRNA=lane 2, S3siRNA=lane 3, and S9siRNA=lane 4)



Overexpression of PP4R1 leads to a decrease in PP4c protein expression level as determined by western blotting .

Conclusion

PP4R1 regulates the cell survival and cell proliferation in leukemic T-cells. The results suggest that PP4R1 is an important protein in the development and progression of leukaemia.

The study also shows that PP4R1 might regulate PP4c activity by affecting its stability.

In conclusion, this study provides evidence that PP4R1 plays an important role in the regulation of cell survival of Jurkat T cells and could potentially act as an important player in cell fate decision of leukemic T cells. The study also suggests that PP4R1 might regulate PP4c stability.

References

Mourtada-Maarabouni, M. and Williams, G. (2008). Protein phosphatase 4 regulates apoptosis, proliferation and mutation rate of human cells. Biochemical et Biophysica Acta (BBA) - Molecular Cell Research, 1783(8), pp.1490-1502.

Mourtada-Maarabouni, M. and Williams, G. (2009). Protein phosphatase 4 regulates apoptosis in leukemic and primary human T-cells. Leukaemia Research, 33(11), pp.1539-1551.