Regulation of the cell cycle and cell death by protein phosphatase 4 in breast cancer cell lines

Hiba Mohammed, Mark Pickard, Gwyn Williams, Mirna Mourtada-Maarabouni

Keele University, Keele, Staffs, UK

Background

At the molecular level, cell death is often regulated by the level of phosphorylation of particular proteins, i.e. by the balance of between opposing kinase and phosphatase activities on those proteins. Protein phosphatase 4 (PP4) is a PP2A-related serine/threonine phosphatase. PP2A has already been implicated in the control of cell proliferation, cell cycle and tumorigenesis. Using a functional expression cloning strategy, we have previously identified the catalytic subunit of PP4 (PP4c) as an important gene influencing the regulation of both apoptosis and cell proliferation in human leukaemic cell lines and in normal lymphocytes. The aims of this study were to examine the effects of PP4c overexpression and silencing on the cell death and survival of breast cancer cell lines.

Method

MCF7 and MDA-MB-231 cells were transfected with pcDNA3.1 encoding PP4c (pcDNA3-PP4c) or siRNAs to different PP4c sequences. Cells transfected with scrambled siRNA or empty vector were considered as controls. Culture viability, apoptosis and cell cycle were assessed post transfection.

Results

In MCF7 and metastatic MDA-MB-231 cells, PP4c over-expression exerted an inhibitory effect on cell proliferation, enhanced spontaneous apoptosis and decreased their colony forming ability. Conversely, siRNA mediated silencing of PP4 enhanced the proliferation and survival of MCF7 and MDA-MB-231 cells, affected cell cycle kinetics by enhancing the proportion of cells in S and G2/M phases, increased the colony forming ability and stimulated the anchorage independent growth.

Conclusion

PP4c promotes cell death and inhibits proliferation in breast cells, suggestive of a role of PP4c as tumour suppressor gene. Down regulation of PP4c expression increases cell survival, proliferation and anchorage independent growth of breast cancer cells, indicating a potential link between the PP4c expression levels, tumorigenesis and metastasis.