Characterisation of PP2Cβ in regulating tumour suppressor pathways in cancer cell lines

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A feeling of dread hangs over you.
But you stay determined
-Undertale, 2015

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Abstract

Tumour suppressor p53 is a key regulator in preventing neoplastic transformation by inducing cell cycle arrest or death in response to stress-signalling pathways. Consequently, p53 is often non-functional during the early stages of cancer development through either direct mutation or aberrant expression of negative regulators.

PP2Cβ is a protein phosphatase which was recently identified as a negative regulator of p53 and cellular senescence. However, the function of PP2Cβ in cancer development is not fully understood.
The aim of this study was to characterise PP2Cβ and its regulation of p53 pathways in human cancer cell lines. This aim was split into two objectives.

The first objective was to examine the effects of PP2Cβ on p53 pathways and cell proliferation in four cancer cell lines with various genetic backgrounds. A protein analysis using western immunoblotting procedures indicates that p53 pathways are activated in cell lines expressing wildtype p53 and Ras. Consistent with activated p53 pathways, PP2Cβ knockdown significantly reduced proliferation rates, which could be attributed to an increased expression of a p53 target gene, *p21* cell cycle inhibitor.

The second objective was to investigate the mechanisms regulating of PP2Cβ gene expression. Previously, p63 was identified as a potential negative regulator of PP2Cβ gene expression based on a modular relational database that integrated microarray results with a genome-wide search of p53 family member response elements. It was therefore hypothesized that p63 could negatively regulate PP2Cβ gene expression. Mammalian expression vectors carrying either the p63 or p73 expression cassette were constructed and PP2Cβ expression was analysed upon overexpression of p53 family members (p53, p63 and p73) in two human cancer cell lines. A reverse-transcriptase coupled quantitative PCR showed that overexpression of p63 resulted in decreased PP2Cβ expression in p53 wildtype cell line.

Taken together the results presented here suggest that restoration of tumour suppressors such as p53 and Rb activity by PP2Cβ inhibition could be used as a potential therapeutic strategy in cancer treatment

Abbreviations

BSA	Bovine Serum Albumin
CAK	CDK-Activating Kinase
CDK	Cyclin-Dependent protein Kinase
cDNA	Complimentary DNA
CIP	Calf Intestinal alkaline Phosphatase
Con	Control
Cp	Crossing Point
DBD	DNA-Binding Domain
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNase	Deoxyribonuclease
dNTP	Deoxyribosenucleotide Triphosphate
EDTA	Ethylene Diamine Tetra-acetic Acid
FBS	Foetal Bovine Serum
GUSB	β-Glucuronidase
H1299	Human Non-small cell lung carcinoma cell line
HCT116	Human Colorectal Carcinoma cell line
HEK293T	Human Embryonic Kidney 293T cell line
h	Hours
Kb	Kilo bases
kDa	Kilodaltons
KD	Knockdown
M	mol/L
mA	Milliampere
MAPK	Mitogen Activating Protein Kinase
MCF7	Human Breast adenocarcinoma cell line
Min	Minutes
mL	Millilitre
p53	Phosphoprotein-53
p63	Phosphoprotein-63

p73	Phosphoprotein-73
PCR	Polymerase chain reaction
Pen/Strep	Penicillin-Streptomycin
PP2Cβ	Protein Phosphatase 2Cβ
RCF	Relative Centrifugal Force
Rb	Retinoblastoma protein
RNase	Ribonuclease
S	Serine
s	Seconds
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
shRNA	Short-Hairpin RNA
TEMED	N,N,N',N'-Tetramethylethylenediamine
TBS/T	Tris-Buffered Saline with Tween-20
U2OS	Human Osteosarcoma cell line

Table of Contents

Results and Discussion

Chapter 3: Knockdown of PP2Cβ in Human Cancer Cell Lines

Chapter 4: Roles of the p53 Family Members in the Expression of PP2Cβ

List of Figures

List of Tables