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Characterisation of PP2C β in regulating tumour suppressor pathways in cancer cell lines

A thesis presented to Massey University in partial fulfilment of the requirement for the degree of Masters in Genetics

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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.

PP2C β is a protein phosphatase which was recently identified as a negative regulator of p53 and cellular senescence. However, the function of PP2C β in cancer development is not fully understood.

The aim of this study was to characterise PP2C β 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 PP2C β 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, PP2C β 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 PP2C β gene expression. Previously, p63 was identified as a potential negative regulator of PP2C β 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 PP2C β gene expression. Mammalian expression vectors carrying either the p63 or p73 expression cassette were constructed and PP2C β 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 PP2C β 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 PP2C β 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
PP2C β	Protein Phosphatase 2C β
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

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