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SUPPRESSION AND ACTIVATION OF LIPID SIGNALLING IN CANCER: TUMOUR SUPPRESSOR PTEN AND ONCOGENIC SPHINGOSINE KINASE

A thesis submitted in fulfilment of the requirements of the degree of Doctor of Philosophy

Submitted by

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Nahal Haddadi declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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LIST OF PUBLICATIONS AND PRESENTATIONS

JOURNAL ARTICLES

1. Haddadi N, Lin Y, Travis G, Simpson AM, Nassif NT, McGowen E: **PTEN/PTENP1: 'Regulating the regulator of RTK-dependent PI3K/Akt signalling', new targets for cancer therapy.** *Mol Cancer* 2018, **17:**37.

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4. **Haddadi N*,** Chen H*, Li Q*, Long M, Hatoum D, Bok CF, Zhu X, Nassif NT, Lin Y, McGowan EM: **Differential expression of sphingosine kinase 1 major Isoforms in human cancer patients and cancer cell lines: implications for cancer therapy.** (Submitted to Cancers).

5. Haddadi, N, Lin Y, McGowan, EM and Nassif, NT: **Cancer-associated mutations of PTEN alter PTEN function.** (Manuscript in preparation 2019).

PUBLISHED ABSTRACTS AND CONFERENCE PRESENTATIONS

1. Nahal Haddadi, Eileen McGowan, and Najah Nassif. Alteration Cellular Function by Cancer-associated Mutations of PTEN. **Frontiers in Cancer Science (FCS) 2017, Singapore.**

2. Nahal Haddadi, Eileen McGowan, and Najah Nassif. Cancer-Associated PTEN Mutations Alter PTEN Cellular Function. **Australasian Genomic Technologies Conference (AGTA) 2017, Hobart, TAS.**

3. Nahal Haddadi, Eileen McGowan, and Najah Nassif, Determining the Frequency and Involvement of PTEN Pseudogene (PTENP1) Expression in Cancer Cell Lines. **New Horizons Conference 2015, Sydney, NSW.**

AWARDS AND RECOGNITION

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LIST OF ABBREVIATIONS

Ab	Antibody
Amp	Ampicillin
AKT	AKT/protein kinase B
ATP	Adenosine triphosphate
BAD	Bcl-2 associated death promoter
Bp	Base pairs
BSA	Bovine serum albumin
Caspase	Cystein aspartate specific proteases
CBF1	Also known as recombination signal binding protein for immunoglobulin kappa J region (RBBJ)
cGMP	Cyclic guanosine monophosphate
CK	Creatine kinase
CK2	Casein kinase II
CMV	Cytomegalovirus
$C0_2$	Carbon dioxide
cDNA	Complementary DNA
CRC	Colorectal Cancer
CDK	Cyclin dependent kinase
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
dNTPs	deoxynucleotide triphosphates
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamminetetraacetic acid
FCS	Foetal calf serum
Kb	Kilobase
MW	Molecular weight
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI	Propidium iodide

PIP2	Phosphoinositide 3,4,5-triphosphate
PIP3	Phosphoinositide 4,5-bisphosphate
PTEN	Phosphatase and tensin homologue deleted on chromosome 10
RNA	Ribonucleic acid
Tris	2-amino-2(hydroxymethyl-1,3-propaneldiol)

ABSTRACT

The *PTEN* tumour suppressor is the second most frequently mutated tumour suppressor gene in cancer. It is a lipid and protein phosphatase that negatively regulates the well-known pro-proliferative and anti-apoptotic phosphatidylinositol 3-kinase (PI3K)/AKT signalling pathway to modulate cell proliferation, cell cycle progression and cell survival. Sphingosine kinase 1 (SphK1), a known tumour promoter/oncogene, has been shown to activate the PI3K/Akt pathway to enhance resistance to apoptosis. The loss of function of PTEN and/or the overexpression of SphK1 contribute to tumorigenesis. This thesis describes the analysis of novel, cancer associated mutations of PTEN, to determine their effect(s) on wild type (WT) PTEN function, and also explores differential SphK1 isoform expression in cancers.

Previous work in our laboratory described 10 novel somatic mutations of PTEN in primary colorectal tumours. To determine the functional consequences of these novel cancer-associated PTEN mutations, the WT *PTEN* and the series of PTEN mutants (K62R, Y65C, K125E, K125X, E150Q, D153N, D153Y, N323K and the C124S and G129E controls) were transiently transfected into: (A) PTEN null U87MG glioblastoma cells, and WT PTEN expressing (A) HCT116 colon cancer and (C) MCF7 breast cancer cells. Transfected cells were then assayed for cell proliferation, cell cycle phase distribution and AKT phosphorylation. In U87MG cells, 50% of the PTEN mutants (Y65C, K125E, E150Q and D153Y) exhibited statistically significant reductions in cell proliferation, but not to the level of that of WT PTEN. In both the HCT116 and MCF7 cell lines, 80% of the PTEN mutants (K62R, Y65C, K125E, K125X, E150Q, D153Y and N323K) displayed reduced cell proliferation rates but none produced reductions comparable with WT PTEN. Further, relative to WT PTEN, 75% of PTEN mutants (K62R, K125X, E150Q, D153N and N323K) possessed functional deficiency in cell cycle inhibitory capacity in the G2 phase in U87MG cells. In contrast, 90% of PTEN mutants (K62R, K125E, K125X, E150Q, D153N, D153Y and N323K) possessed functional deficiency in the cell cycle inhibitory capacity in either the G1 or G2 phase in HCT116 cells. In MCF7 cells, 100% and 60% (K62R, Y65C, D153N, D153Y and N323K) of the PTEN mutants had functional deficiency in cell cycle inhibitory capacity in either the G1 or G2 phase, respectively. The analyses of endogenous suppression of phosphorylation of AKT revealed that 40% of PTEN mutants (K125E, K125X and D153N) show deficiency in pAKT suppression in the U87MG cell line while 60% of the mutants showed such deficiency in the HCT116 cell line (K125X, E150Q, D153N, D153Y and N323K). All but one (K62R) of the PTEN mutants showed a deficiency in the ability to suppress the level of endogenous phosphorylated AKT in the MCF7 cells. Overall, the results of the functional assays showed that the somatic mutations of the *PTEN* gene alter PTEN tumour suppressor function.

Expression of SphK1, a positive upstream regulator of the Akt pathway, is expressed as 2 major isoforms, SphK1-43kDa (SphK1a) and SphK1-51kDa (SphK1b), with similar SphK1 activity. However, to date, there is no literature on the expression of the two SphK1 isoforms in cell lines or human tissues. Profiling the expression of the two SphK isoforms in various cancer cell lines (n=24), primary cancer tissues (n=28) and paired adjacent tissues (n=28), demonstrated that the SphK1a isoform is expressed in all cell lines and tissues (both normal and cancer) studied, however, expression of SphK1b is cell and tissue specific, including breast, prostate and lung. Balancing signalling pathways and maintaining cellular homeostasis, as observed through the PTEN/SphK1 swinging pendulum is important and the study of these pathways is crucial in gaining further understanding the opposing regulatory mechanisms, which may be exploited for the future prevention and treatment of cancers.

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