Redefining the Role of p14ARF-p53 Wild Type Function in Breast Cancer

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Certificate of Original Authorship

I, Diana Hamze Hatoum, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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“If the facts don't fit the theory, change the facts.”

(Albert Einstein 1879 – 1955)

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Publications arising from this thesis

Journal original articles

1. **Hatoum D**, Yagoub D, Ahadi A, Nassif NT, McGowan EM (2017) Annexin/S100A Protein Family Regulation through p14ARF-p53 Activation: A Role in Cell Survival and Predicting Treatment Outcomes in Breast Cancer. *PLOS ONE* 12(1): e0169925. DOI: 10.1371/journal.pone.0169925.

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3. Moulder DE, **Hatoum D**, Tay E, Lin Y, and McGowan EM**,** (2018), The Roles of p53 in Mitochondrial Dynamics and Cancer Metabolism: The Pendulum between Survival and Death in Breast Cancer? Special issue, "p53 and cancer", *Cancers*.10 (6). DOI: 10.3390/cancers10060189.

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11. **Hatoum** D, Yagoub D, Nassif N, Ahadi A, McGowan E, Turning p53 back on in breast cancer: predicted functions for p14ARF-p53 in cell cycle braking and pre-secretory differentiation through SILAC proteomic analysis*, 2017 NCRI Cancer Conference*, 5-8 November 2017, Liverpool, UK.

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13. **Hatoum D**, Yagoub D, Brennan S, Nassif N, McGowan EM, P14ARF-p53-p21: reprogramming metabolic regulation and function in breast cancer. *New Horizons Conference 2015,* UTS.

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List of Abbreviations

A1-A9: Annexins A1-9

ACTB: Beta-actin

ALDH4A1: Aldehyde dehydrogenase 4 family member A1

ARF: Alternate reading frame

ATP: Adenosine triphosphate

β2M: β2-microglobulin

DMEM: Dulbecco's modified eagle medium

DNAJC15: DnaJ heat shock protein family (Hsp40) Member C15

DNMT1: DNA (cytosine-5)-methytransferase 1

ERα: Estrogen Receptor Alpha

FCS: Foetal calf serum

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

GFP: Green fluorescent protein

GSN: Gelsolin

IPTG: Isopropyl-β-D-thiogalactopyranoside

MCM: Minichromosome maintenance complex

MDC1: Mediator of DNA damage checkpoint protein 1

PAPSS2: 3'-phosphoadenosine-5'-phosphosulfate synthase 2

PBS: Phosphate-buffered saline

PBST: Phosphate-buffered saline with Tween20

RT-qPCR: Quantitative real time polymerase chain reaction

SILAC: Stable isotope labelling with amino acids in cell culture

Rb: Retinoblastoma

RT: Room temperature

TNBC: Triple negative breast cancers

TRIS: Tris(hydroxymethyl)aminomethane

Abstract

Background: Breast cancer is endemic, ranking the number one cancer in women worldwide. About 80% of all breast cancers are late onset, arising in post-menopausal women, and are mainly estrogen receptor alpha (ERα) positive and p53 wild type. However, the function of p53 is compromised in many cancers due to constitutive degradation of p53 by the ubiquitin ligase human double 2 (hdm2). Degradation of p53 by hdm2 can by blocked by the p14ARF tumour suppressor protein; however p14ARF is frequently deleted in breast cancer. By re-introducing p14ARF into breast cancer cells p53 function can be restored. In this study, an inducible p14ARF ERα positive breast cancer model was used to determine the effects of reactivating p53 in hormone-dependent breast cancer. While the role of p53 in the breast cancer treatment is well recognised as a tumour suppressor, the evidence supporting an opposite action of p53 in treatment resistance and recurrence in breast cancer is emerging.

Aims of this thesis: The overall aim of this thesis was to define the role of p53 in ERα+ breast cancer cells. This aim was addressed by three specific aims, 1) to determine the global proteomic changes associated with p14ARF-p53 activation in breast cancer, 2) to characterise and validate novel p53 regulated proteins and associated signalling pathways, detected by the proteomic analysis, and 3) to examine the morphological and protein expression changes occurring in the cellular metabolism focusing on mitochondria dynamics post p53 activation.

Methods: Stable isotopic labelling in cell culture and mass spectrometry (LC-MS/MS) techniques were used for proteomic profiling of simultaneous global

protein changes in breast cancer cells post activation of the p14ARF-p53 signalling pathway. High resolution immunofluorescent microscopy, conventional Western blots and RT-qPCR and bioinformatics analyses were used for p14ARF-p53 signalling validation.

Results: 1) SILAC LC-MS/MS analyses identified a unique global differential profile of protein expression changes upon activation of the p14ARF-p53 pathway over a 24 h and 72 h period. Listings of the proteome changes have been deposited in the PRoteomics IDEntifications (PRIDE) archive with identification numbers PXD009334. Significantly downregulated proteins were associated with cell cycle arrest, DNA repair, and anti-apoptosis. Many of the upregulated proteins were specifically associated with modulation of the metabolic pathways in favour of oxidation and mitochondria regulation. 2) The tumour suppressor p53 is usually associated with the modulation of the calcium regulator protein annexin A5 to promote cell death or to permanently facilitate cell cycle arrest to prevent tumour growth. Due to the sequence similarity of the annexin family of proteins, it is difficult to determine how these multi-faceted proteins are regulated. Using unbiased, quantitative proteomics we identified p53-differential regulation of the annexin/S100A family through unique peptide recognition at the N-terminal regions. This report is the first to describe how p53 acts as the central orchestrator of these calcium regulators and its role in cell survival and function in breast cancer. 3) Changes in the mitochondria occurring after p53 activation were studied using immunofluorescence, visualising on the high resolution DeltaVision OMX Blaze™ microscope and analysing using IMARIS x64 software. Activation of the p14ARF-p53 pathway resulted in unique changes in cellular metabolism. Activation of this pathway had dramatic effects on the morphological,

activity and protein expression changes in the mitochondria, observed by an increase in mitochondrial biomass, activity, cellular distribution, sphericity and volume.

Summary: Overall, the work presented in this thesis provides a unique insight into the key proteins involved in the changing cell metabolism in hormone dependent breast cancer cells upon p53 activation and elucidates the role for p53 as a master regulator of cellular processes. Specific proteins and signalling pathways that are synchronised to rapidly hit the brakes on proliferation and coordinate metabolic cellular switching in breast cancer are discussed. These p53 proteome snapshots will provide valuable information on the duplicity of p53 in cell survival and its potential role in latency and resistance to treatment in breast cancer.