



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

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Submitted in fulfilment of the requirements for the degree of Doctor  
of Philosophy from:

School of Life Sciences,  
University of Technology Sydney

**2018**

## **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.

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Date: 28<sup>th</sup> October 2018

## **Acknowledgements**

I would like to thank all the people who contributed to my PhD. Firstly, I would like to thank my supervisors, Dr Eileen McGowan and Dr Najah Nassif, for their patience, ongoing support and supervision. Without you, I would have never been in this position and our project would have not been successful.

Dr Eileen McGowan, I cannot thank you enough for helping me in everything experimental, academic and personal in the last five years. You have taught me to think critically about my work and you have provided me with lots of opportunities throughout these five years. I had many difficult (personal and academic) times through my PhD where I was about to give up, but you have gone out of your way to help me get through everything.

Thanks to Nikki Alling for making the MCF-7p14ARF cell lines and to Dr Daniel Yagoub for providing the original SILAC/MS proteomic dataset. Dr Matt Padula, Dr Alireza Ahadi and Prangwan Pateetin, thank you for helping with the proteomics and analysis of SILAC.

Thanks to Dr Sarah Bajan, Nahal Haddadi, David Moulder and all my PhD colleagues for their support. Thank you Diana Rose for your kindness and your scholarships and helping me achieve.

I would like to thank the examiners for reviewing this thesis. Also, thanks to the Australian Government Postgraduate Award Scholarship that I received in the first three and a half years of my PhD candidature.

Being a sole parent of two young children and a full-time PhD student, I struggled emotionally and financially so to my two children, Mariam and Ahmad, thank you so much for tolerating my stress and my time away from home during these five

years. To my family and to my children's adopted grandparents, John and Elizabeth, thank you so much for your support.

*“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.
2. Yagoub D, Wilkins MR, Lay AJ, Kaczorowski DC, **Hatoum D**, Bajan S, Hutvagner G, Lai JH, Wu W, Martiniello-Wilks R, Xia P, McGowan EM, (2014), Sphingosine kinase 1 isoform-specific interactions in breast cancer *Molecular Endocrinology* 28 (11). DOI: 10.1210/me.2013-1423.

### Journal Reviews

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.
4. McGowan EM, Lin Y, **Hatoum D**, (2018) Good guy or bad guy? The dual roles of wild-type p53 in breast cancer origin, treatment resistance and recurrence, Special issue, "p53 and cancer", *Cancers* 10 (6). DOI: 10.3390/cancers10060172.
5. **Hatoum D**, Haddadi N, Lin Y, Nassif NT, McGowan EM (2017) Mammalian sphingosine kinase (SphK) isoenzymes and isoform expression: challenges for SphK as an oncotarget. *Oncotarget*. DOI: 10.18632/oncotarget.16370.
6. **Hatoum D**, McGowan EM, (2015), Recent advances in the use of metformin: Can treating diabetes prevent breast cancer? *Biomedical Research International*. DOI: 10.1155/2015/548436.

### **Published Abstracts**

7. **Hatoum D**, Yagoub D, Brennan S, Nassif N, McGowan EM. P14ARF-p53-p21 alters the metabolic pathway in breast cancer a novel proteomic global approach. *Annals of Oncology– 26 (Supplement 3): iii10–iii14*, 2015.
8. **Hatoum D**, and McGowan EM. Activation of the p14ARF-p53 Pathway: A Role for p14ARF-p53 in the differential regulation of the Annexin family and S100-associated proteins. *Endocrine Reviews* 35: 3 (Mon-0292), 2014.
9. **Hatoum D**, Yagoub Y, Nassif N, Brennan S, Martiniello-Wilks R, McGowan EM. The misnomer of activating p14ARF-p53 for breast cancer therapy. *Journal of Gene Medicine* 15: (8-9), 333-334, 201.

### **Manuscripts in preparation**

10. **Hatoum D**, Yagoub D, Ahadi A, Turnbull L, Whitchurch C, Nassif NT, McGowan EM., Dissecting the p14ARF-p53 pathway in breast cancer proliferation and breast function.

### **Conference presentations**

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.
12. **Hatoum D**, Bok CF, Touw A, Nassif N, McGowan EM, 2015, Sphingosine Kinase 1 (SK1-43kDa) isoform expression may contribute to cancer aggressiveness, *New Horizons Conference 2015*, UTS.
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.
14. Moulder D\*, Mitchell H\*, **Hatoum D\***, Bajan S, Hutvagner G, Johnson M, Mills K, Brennan S, Nassif N, McGowan EM, P14ARF-p53 plays a major



role in mitochondria dynamics in breast cancer cells, *New Horizons Conference 2015*, UTS. \* Authors equal contribution.

15. **Hatoum D**, Yagoub D, Brennan S, Nassif N, McGowan EM, 2015, A Role for p14ARFp53 in Altering the Metabolic Pathway in Hormone-Dependent Breast Cancer. 2015 Innovations in Cancer Treatment and Care Conference.
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17. **Hatoum D**, Yagoub D, Brennan S, Nassif N, and McGowan EM, 2015, p14ARF-p53-p21 alters the metabolic pathway in breast cancer – a novel proteomic global approach, *IMPAKT Breast cancer conference*, Brussels May 6th-9th 2015 poster 37.
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19. **Hatoum D** and McGowan EM, 2014, Activation of the p14ARF-p53 Pathway: A Role for p14ARF-p53 in the Differential Regulation of the Annexin Family and S100-Associated Proteins, *ICE/ENDO*, Chicago June 21st-24th 2014.
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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)-methyltransferase 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 (ER $\alpha$ ) 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 be 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 ER $\alpha$  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 ER $\alpha$ + 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.