Understanding the Apoptotic Signaling Pathways In Breast Cancer Using Microarrays, Proteomics and Bioinformatics

Ibrahim Oqla Alanazi

A thesis submitted for the degree of Doctor of Philosophy

Discipline of Genetics

School of Molecular and Biomedical Science

The University of Adelaide
# Table of Contents

Table of Contents ................................................................................................................... II
Abstract ................................................................................................................................. III
Declaration ............................................................................................................................ V
Acknowledgements ................................................................................................................ VI
List of Publications contributed to during Ph.D candidature ................................................... VII

**CHAPTER ONE** .................................................................................................................. 9
1 Introduction ......................................................................................................................... 9
1.2 Literature Review ........................................................................................................... 10
  1.2.1 Mammary Development And Function ................................................................. 10
    1.2.1.1 The Human Epidermal Growth Factor Receptors (EGFRs) And The Role Of Their
    Signaling During Mammary Development And Neoplasia ............................................. 12
    1.2.1.2 The Role Of Hedgehog, Wnt And Notch Signaling During Mammary Development
    And Neoplasia .................................................................................................................. 15
  1.2.2 Progression Of Breast Cancer .................................................................................... 15
    1.2.2.1 Causes Of Breast Cancer ...................................................................................... 15
    1.2.2.2 Molecular Causes Of Malignant Or Invasive Breast Cancer ................................. 19
    1.2.2.3 Basic Concepts Of Metastasis .............................................................................. 19
    1.2.2.4 The Environment Of The Primary Tumour ............................................................. 20
  1.2.3 Stem Cells .................................................................................................................. 22
    1.2.3.1 Role Of Stem Cells In Tumorigenesis ................................................................. 23
    1.2.3.2 Breast Cancer Stem Cells And Mammary Stem Cells ......................................... 24
    1.2.3.3 Targeting Stem Cells For Elimination ................................................................. 28
  1.2.4 Breast Cancer Implications ....................................................................................... 29
  1.2.5 Experimental Approach ........................................................................................... 32

**CHAPTER TWO** ................................................................................................................ 43
Combined Gene Expression and Proteomic Analysis of EGF Induced Apoptosis in A431 Cells
Suggests Multiple Pathways Trigger Apoptosis. .................................................................... 43

**CHAPTER THREE** .......................................................................................................... 45
MicroRNAs are part of the regulatory network that controls EGF induced apoptosis, including
elements of the JAK/STAT pathway, in A431 cells. ............................................................. 45

**CHAPTER FOUR** ........................................................................................................... 47
A comprehensive Catalogue of Differentially Expressed Genes and Proteins From
Epidermal growth factor (EGF) induced Apoptosis in A431 cells ........................................ 47

**CHAPTER FIVE** ............................................................................................................. 76
Conclusion and Future Directions ......................................................................................... 76
Supplemental Materials ........................................................................................................ 79

**Appendix 1: Additional materials for chapter two** ......................................................... 81
Abstract
Breast cancer is one of the most common causes of cancer death to women worldwide. In this study A431 cells, derived from epidermoid carcinoma, are used as a model to study breast cancer. This cell line over-expresses the Epidermal Growth Factor Receptor (EGFR/HER1) and when treated with a high dose of EGF will undergo apoptosis via the activation of EGFR/HER1 signaling. However, little work has been conducted to identify the underlying molecular mechanisms. The limited available data implicates components of the interferon response pathway as mediators of the apoptotic signal in cancer cells. The genetic network through which EGFR/HER1 can induce apoptosis is not known at the present time. With understanding of the genetic regulatory hierarchy and the molecular mechanisms linking EGFR/HER1 signaling and apoptosis, better drug targets that might regulate apoptosis in cancers that over express HERs can be identified. This thesis focuses on the hypothesis that an apoptosis specific signaling cascade can be triggered by HERs in A431 cells. Activation of HER receptors has led us to identify downstream components by global analyses of gene expression and associated regulatory miRNAs and protein levels using microarray and proteomics platforms. A high dose of EGF leads to the induction of apoptosis in A431 cells by activating a number of pathways, which are known to promote apoptosis. These include the STAT pathway and downstream components including cytokines and suppressors of cytokine signalling, cleavage of serpinb1 into L-DNAaseII and down regulation of mutant TP53, which may perturb the cytoskeleton and cell adhesion proteins. Furthermore, our data showed that gene expression and proteomic data were quite different, with very little overlap in terms of transcripts and proteins. Therefore, we considered that post-transcriptional regulation might be crucial. MicroRNAs are known to be important post-transcriptional regulators; as a result, we sought to identify potential regulatory miRNAs with respect to induction of cell death by EGF. We identified novel interaction regulatory networks based on the crosstalk between miRNAs and mRNA/protein that resulted in the induction of apoptosis in A431 cells. We also identified a number of miRNAs that may play an important role in the regulation of apoptosis in A431 cells after EGF treatment. We have used bioinformatics databases and in silico analysis tools to create a comprehensive catalogue of genes/proteins that may induce apoptosis in A431 cell after EGF treatment.
We have shown that using various complimentary platforms including gene expression, miRNA expression, proteomics, and network prediction to analyse the induction of apoptosis in treated A431 cells, we have achieved a greater understanding of the molecular mechanisms at work. This in turn may provide strategies for combined therapies and/or predict novel potential mechanisms/targets for chemotherapy aimed at inducing cell death in tumor cells.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Ibrahim Alanazi and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue, and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed………………………………………………..Date……………………………………..
Acknowledgements

I would like to express my sincere gratitude to the following people:

Dave Adelson, for supervision and guidance, and for giving me the opportunity and support to do the PhD. I am so fortunate to have an excellent supervisor. Dave, Not only the knowledge that I learned from you is precious, but also you taught me how to be easygoing guy and humble (I am going to miss you). Peter Hoffman, my co-supervisor for encouraging and supporting during my PhD journey. Joy Raison, for helping me to generate nice figures; Dan Kortschak, for giving his time to proof read my manuscript and providing valuable suggestions, and all other members of the Adelson lab, past and present, for making it such a supportive and enjoyable place to work. Zhipeng Qu (my Chinese brother), for great support and friendship. Past and present members of the Genetics discipline for their support and advice over the last few years.

My wife, for being a continuous support and always being ready to assist me. You have been there from the start to finish. Thank you very much honey. Parents and brothers, for always encouraging and supporting me throughout my PhD. I would like to also specially thank the King Abdualaziz City for Science and Technology (KACST), who provided me this scholarship to support my PhD.
List of Publications contributed to during Ph.D candidature

Combined gene expression and proteomic analysis of EGF induced apoptosis in A431 cells suggests multiple pathways trigger

Ibrahim Alanazi · Esmaeil Ebrahimie · Peter Hoffmann · David L. Adelson