

The regulation, function and expression of $\Delta 40p53$ in breast cancer

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Declarations

Statement of Originality:

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Contributions by others to the thesis:

There were a number of collaborators who have made important contributions to the unpublished work presented in this thesis as follows-

- Dr Hamish Campbell and Professor Antony Braithwaite generated the MCF-7-LeGO and MCF-7- $\Delta 40p53$ cell lines used in Chapter 3 and Chapter 4.
- Dr Trisha Al-Mazi provided technical support for the MRM-MS studies in Chapter 4, and aided in the analysis of the data generated.

List of additional publications:

Publications:

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3. Mathe, A, Wong-Brown, M., **Morten, B.**, Forbes, J.F., Braye, S.G., Avery-Kiejda, K.A., and Scott, R.J., 'Novel genes associated with lymph node metastasis in triple negative breast cancer.' *Scientific Reports*, **5**, 15832 (2015).

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Abstract:

Breast cancer is the most common malignancy in women and it is the second highest cause of cancer-related death. The tumour suppressor gene *p53* is crucial for the prevention of cancer through its role in the maintenance of cellular growth and differentiation. Inactivation of *p53* through mutations is the most common event in cancer. However, in breast cancer, *p53* is mutated in only ~24% of cases and this suggests that other mechanisms are responsible for the loss of *p53* function. Ascertaining the mechanisms responsible for its inactivation may lead to the identification of novel treatment targets or prognostic tools. The *p53* isoforms were discovered over a decade ago and they may represent one mechanism that regulates *p53* functionality. Currently, over 14 *p53* isoforms have been discovered and their expression is deregulated in many human cancers. One such isoform, $\Delta 40p53$, has been found to inhibit *p53*, or support its tumour suppressive functions, depending on its ratio to full-length *p53* (FL*p53*). Previous studies from our laboratory have shown that $\Delta 40p53$ is the most highly expressed *p53* isoform in breast cancer, but its function and the clinical implications of its expression are yet to be elucidated. Furthermore, current methods of detecting $\Delta 40p53$ at the mRNA and protein level lack the sensitivity and specificity to analyse the endogenous expression of this isoform in clinical specimens. The aims described in this thesis were to elucidate the regulation and function of this isoform in breast cancer. Further studies aimed to design and evaluate novel methods for the detection of $\Delta 40p53$ mRNA and protein expression to aid in the clinical implementation of $\Delta 40p53$ as a breast cancer biomarker.

$\Delta 40p53$ can be produced by alternative translation or alternative splicing, but our previous studies have indicated that alternative splicing is likely to be a major route of $\Delta 40p53$ production in breast cancer. G-quadruplex structures within intron 3 of *p53* are important for the production of FL*p53* mRNA and disruption of these structures by a polymorphism in intron 3 of *p53* (PIN3) has been shown to lead to increased levels of $\Delta 40p53$ mRNA *in vitro*. The first part of this thesis reports on whether PIN3 was associated with the expression level of $\Delta 40p53$ in breast tumour tissues. PIN3 was shown to be associated with a low $\Delta 40p53$:FL*p53* ratio, and that this was correlated with improved disease-free survival. This suggests that the $\Delta 40p53$:FL*p53* ratio is modified by PIN3 in breast cancer and that the $\Delta 40p53$:FL*p53* ratio and PIN3 may be potential prognostic indicators for breast cancer outcome.

Following this, the function of $\Delta 40p53$ was investigated in estrogen receptor (ER)-positive breast cancer. *p53* can interact with and regulate the expression of another important transcription factor in breast cancer, ER, and this interaction may be important in breast

cancer development and progression. However, the role that $\Delta 40p53$ may play in this interaction is unclear. In this thesis, knockdown of $\Delta 40p53$ caused a reduction in the expression of ER and its target genes PR and pS2 in the presence of estrogen, and this was supported by overexpression studies examining $\Delta 40p53$. Furthermore, a high $\Delta 40p53$:FLp53 ratio was shown to be an indicator of worse disease-free survival in ER-positive breast cancers, suggesting that the level of $\Delta 40p53$ expression is related to treatment outcomes.

After determining a role for $\Delta 40p53$ in mediating the expression and function of ER in breast cancer, the next chapter evaluated two new methods of detecting $\Delta 40p53$ expression in tumour tissues. Firstly, a method using branched DNA probes (QuantiGene 2.0 assay) was examined to determine if it was superior to real-time PCR for the detection of $\Delta 40p53$ mRNA in formalin-fixed paraffin-embedded (FFPE) tissues. The results were also compared in matched fresh frozen (FF) tissues. $\Delta 40p53$ was unable to be detected by either real-time PCR or the QuantiGene 2.0 assay in FFPE tissues, but FLp53 was detected. However, the expression of FLp53 in FFPE tissues was not correlated between the two different methods. This study confirmed the difficulties in quantitating mRNA from these archival specimens. Finally, the use of targeted mass spectrometry in the quantification of $\Delta 40p53$ protein expression was investigated in a small pilot study. While only preliminary, the results suggested that $\Delta 40p53$ endogenous expression was distinguishable from FLp53 and was accurately quantitated in breast cancer cells. This method provided a novel technique for determining the abundance of $\Delta 40p53$ protein compared to FLp53 protein.

Taken together, the work portrayed in this thesis has demonstrated that $\Delta 40p53$ expression is associated with PIN3 in breast cancer. A novel functional role for $\Delta 40p53$ has also been identified, the regulation of ER expression in breast cancer which is associated with clinical outcomes, and a novel method for the quantification of $\Delta 40p53$ protein expression has been developed that may be useful for the analysis of clinical samples. These findings highlight the importance of high $\Delta 40p53$ expression in breast cancer, which has been previously reported on by our laboratory, and suggests that $\Delta 40p53$ may be an important prognostic marker in breast cancer.

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List of abbreviations:

Abbreviation	Expanded term
AML	acute myeloid leukemia
ANOVA	analysis of variance
ATM	ataxia telangiectasia mutated
ATR	ATR serine/threonine kinase, a protein coding gene
BMI	body mass index
BRCA1/2	breast cancer 1/2
Chk2	checkpoint kinase 2, a serine/threonine kinase
COSMIC	catalogue of somatic mutations in cancer
CV	coefficient of variation
DBD	DNA binding domain
DCIS	ductal carcinoma <i>in situ</i>
DMEM	dulbecco's modified eagle medium
DNA	deoxyribonucleic acid
dPCR	digital PCR
ER	estrogen receptor
ERE	estrogen response element
ESR1	estrogen receptor 1
FCS	fetal calf serum
FF	fresh frozen
FFPE	formalin-fixed paraffin-embedded
FLp53	full-length p53
G4	g-quadruplex structure
GATA3	GATA binding protein 3, a transcription factor
HCD	higher-energy collisional dissociation
HER2	human epidermal growth factor receptor 2
IDC	invasive ductal carcinoma
IGF-1	insulin-like growth factor 1
IRES	internal ribosome entry site
kDa	kilodalton
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MAPK	mitogen-activated protein kinase
MDM2	mouse double minute 2
mp53	mutant p53
MRM-MS	multiple reaction monitoring-mass spectrometry
mRNA	messenger RNA
mTOR	mechanistic target of rapamycin, a serine/threonine kinase
NANOG	Nanog homeobox, a protein coding gene
NLS	nuclear localisation signal
NTC	non-targeting control siRNA

p53RE	p53 response element
PCR	polymerase chain reaction
PIK3CA1	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha, a protein coding gene
PIN3	polymorphism in intron 3 of p53
PR	progesterone receptor
pS2	protein PS2, trefoil factor 1
PTEN	phosphatase and tensin homolog, a protein coding gene
PUMA	p53 upregulated modulator of apoptosis
RNA	ribonucleic acid
SFRS1	serine/arginine-rich splicing factor-1
siRNA	small interfering RNA
SNP	single nucleotide polymorphism
SRM	selected reaction monitoring
SRSF3	serine and arginine rich splicing factor-3
TAD	transactivation domain
TNBC	triple negative breast cancer
wtp53	wild-type p53

