PhD Thesis

By

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QUINAZOLINE-BASED ALPHA1-ADRENOCEPTOR ANTAGONISTS AND PROSTATE CANCER

Submitted in the fulfilment of the requirements of the degree of Doctor of Philosophy by Research

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ABSTRACT

Early stage prostate cancer is highly manageable using definitive radical prostatectomy and/or radiotherapy techniques. Unfortunately, for some men, transition to castrate-resistant prostate cancer is both inevitable and incurable with few life-extending therapies available. Therefore, there is an urgent need for novel agents to improve the oncological and survival outcomes for these last-resort patients. One such modality may be α1-adrenoceptor antagonists. Clinically, some of these drugs reportedly increase benign and cancerous prostatic apoptosis. In vitro studies indicate that this anticancer effect occurs via α1-adrenoceptor independent mechanisms. However, the cytotoxic profile of these drugs have yet to be fully characterised, including whether these agents may be useful in improving anticancer treatment efficacy. To address the gaps in literature, the relative cytotoxic potencies and underlying cell death mechanisms (apoptosis and autophagy) were determined for six α1-adrenoceptor antagonists on castrate-sensitive and castrate-resistant prostate cancer cells. Molecular mechanisms were explored using immunoassays. The effects of these drugs were also investigated on normoxic or hypoxic irradiated prostate cancer cells to mimic outer and inner portions of a solid tumour. In an adjunct study, comparisons between the cytotoxic profile of doxazosin and the chemotherapeutic mitomycin c were made in an in vitro model of bladder cancer intravesical therapy. Overall, prazosin and doxazosin were found to be equipotent and were the most potent of all investigated drugs by inducing apoptosis and/or autophagy in a cell type-dependent manner. This cytotoxic effect was attributed to decreased mTOR/p70S6K signalling coupled with increases in p27 and p38 mitogen-activated protein kinase. Prazosin was also found to selectively radiosensitise hypoxic prostate cancer cells. This effect was characterised by increased reactive oxygen species and suppression of HIF-1α accumulation, further implicating mTOR-signalling as an underlying cytotoxic mechanism. Exploration of additional novel uses of these drugs revealed that doxazosin was 6-times more toxic than mitomycin C on bladder cancer cells in modelling of intravesical therapy. Taken together, these findings indicate that prazosin/doxazosin have potent cytotoxic actions in prostate cancer cells that are characterised by induction of apoptosis and autophagy, possibly by inhibition of the mTOR-signalling cascade. This is the first report of radiosensitising effects of these
drugs in prostate cancer cells, suggesting that these agents may have novel clinical benefits for patients undergoing radiotherapy. Likewise, the preliminarily findings of this thesis suggest that these drugs may be a novel alternative intravesical treatment option for bladder cancer and warrants further investigation.
DECLARATION

This thesis is submitted to Bond University in fulfilment of the requirements of the degree of Doctor of Philosophy. This thesis represents my own original work towards this research degree and contains no material which has been previously submitted for a degree or diploma at this University or any other institution, except where due acknowledgement is made.

_________________________________     ___________________
Amanda Lynn Forbes     Date

23 December 2015
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Lastly, I would like to dedicate this thesis to my parents Darren and Kathy Forbes, and especially to my future-husband Mick. Without their emotional, financial and physical support, this work would have not been possible. They have lead by example in teaching me resilience and the meaning of and hard work, and for that, I am forever grateful. I am eternally indebted to Mick for his unconditional support and endless patience – from countless trips driving me to and from Bond University, to pretending to understand my scientific tangents. I couldn’t imagine a better companion to support me during my challenges and celebrate my successes.
PUBLICATIONS

JOURNAL ARTICLES AS A RESULT OF THIS THESIS:


ABSTRACTS AS A RESULT OF THIS THESIS:


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ABBREVIATIONS

ADR  Adrenoreceptor
ADAMTS A disinegrin and metalloproteinase with throboosponin motifs
AR  Androgen receptor
ASTRO American Society for Radiation Oncology
AUA American Urological Association
BPH Benign prostatic hyperplasia
Cited2 Cbp/p300-interacting transactivator with Gly/Asp-rich arboxy-terminal domain 2
DCF-DA Dichloro-dihydro-fluorscein diacetate
DHT Dihydrotestosterone
DMSO Dimethyl sulfoxide
ECM Extracellular matrix
EGF Epidermal growth factor
EGFR Epidermal growth factor receptor
ERK Extracellular-related kinase
FADD Fas-associated death domain
FAK Focal adhesion kinase
FGF Fibroblast growth factor
FDA Food and drug administration (USA)
HGPIN High-grade prostatic neoplasia
HIF Hypoxia-inducible factor
LBD Ligand-binding domain
LC3 Ligand binding domain
LHRH Lutenising hormone-releasing hormone
LUTS Lower urinary tract symptoms
GAG Glycosaminoglycans
Gy Gray
MAPK Mitogen-activated protein kinase
mTOR Mammalian (mechanistic) target of rapamycin
NFAT Nuclear factor of activated T-cells
PBS Phosphate buffered saline
PI3P Phosphatidylinositol 3-phosphate
PLC Phospholipase C
PMSF Phenylmethylsulfonyl fluoride
PKC Protein kinase C
PSA Prostate specific antigen
PDGF Platelet-derived growth factor
pVHL Von Hippel-Lindau
ROS Reactive oxygen species
RP Radical prostatectomy
TIMP Tissue inhibitor of metalloproteinase
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGA</td>
<td>Therapeutic Goods Administration (Australia)</td>
</tr>
<tr>
<td>TGF</td>
<td>Tumour growth factor</td>
</tr>
<tr>
<td>TRP</td>
<td>Transient receptor potential channels</td>
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<tr>
<td>TSC</td>
<td>Tuberous sclerosis protein</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TURP</td>
<td>Transurethral resection of the prostate</td>
</tr>
<tr>
<td>TURBT</td>
<td>Transurethral resection of the bladder tumour</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Vascular endothelial growth factor receptor</td>
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