The Renin-Angiotensin System in Endometrial Cancer

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Human Physiology
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DECLARATION STATEMENT

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or being examined, for the award of any other degree or diploma in any university or other tertiary institution 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 the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository, subject to the provisions of the copyright Act 1968 and any approved embargo.

Signed………………………………

7th August 2018
Date……………………………………..
ACKNOWLEDGEMENTS

“Good teachers know how to bring out the best in students”

– Charles Kuralt

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Abstract

Endometrial cancer is one of the fourth most common cancer in the developed world and its incidence is increasing rapidly. Several studies have shown that there is an upregulation of the pro-angiogenic arm of the renin angiotensin system in endometrial cancer. Endometrial cancers express both prorenin and (pro)renin receptor ((P)RR) mRNA and have significantly greater levels of these proteins than normal adjacent endometrial tissue. Prorenin acting via the (P)RR can activate both RAS dependent and independent signaling pathways.

To determine the functional role of (P)RR in endometrial cancer growth, we used siRNA transfection to knock down (P)RR expression in three endometrial cancer cell lines (Ishikawa, HEC-1A and AN3CA). All three of the endometrial cancer cell lines examined (Ishikawa, HEC-1A and AN3CA) expressed (P)RR and prorenin (REN) mRNA, however levels of (P)RR and AGTR1 were much higher in Ishikawa cells. Transfection with a (P)RR siRNA resulted in knockdown of (P)RR at both gene and protein level in three cell lines. Furthermore, there was a significant reduction in endometrial cancer cell growth (proliferation and cell viability) in Ishikawa and AN3CA cells.

Several studies show that (P)RR is released in a soluble form (s(P)RR) into blood and urine. We therefore hypothesized that s(P)RR could be released from endometrial cancer cells and that levels of s(P)RR in blood and uterine fluid would be elevated in women with endometrial cancer. The levels of s(P)RR were measured with a specific s(P)RR ELISA it was found that all three cell lines secrete s(P)RR into the cell culture supernatant. Also, we found that there was significant amount of s(P)RR levels in plasma samples.

Therefore, we postulated that endometrial cancer growth can be inhibited by drugs that block Angiotensin (Ang) II/Ang II type 1 receptor interactions and prorenin/(P)RR mediated signaling pathways.
Further we looked at the individual effects and combined effects of RAS blockers with ATP6AP2 siRNA on cell viability and cell proliferation in three endometrial cancer cell lines. There was no effect of aliskiren (a renin inhibitor) on cell viability in HEC-1A and AN3CA cell lines. Perindoprilat (an ACE inhibitor) and losartan (an AT1R receptor antagonist) had no effect on the cell viability of any cell line. Another AT1R antagonist, telmisartan, which also acts as a selective agonist of peroxisome proliferator-activated receptor gamma (PPAR-γ), did however significantly reduce the viability of the three cell lines (Ishikawa 75%, HEC-1A 50% and AN3CA 60%). The combination of telmisartan + troglitazone had a similar effect to that of telmisartan on its own. Aliskiren + perindoprilat reduced the viability of HEC-1A cells, there was no effect in Ishikawa and AN3CA cells. Conversely, the combination of (P)RR siRNA + telmisartan significantly reduced cell viability and cell proliferation in Ishikawa cells. We also looked at the effect of ovarian steroids (estrogen and progesterone) on RAS expression in two other cancer cell lines (an endometrial cancer cell line (RL-952) and a breast cancer cell line (MCF-7). Treatment with estrogen had no effect on RAS expression in RL-952 or MCF-7 cells.

This study is the first to characterize the functional role of prorenin and (P)RR in endometrial cancer, and to demonstrate that drugs that inhibit the (P)RR and the RAS pathway could reduce endometrial cancer growth. Finally, measurement of s(P)RR could be used as a biomarker for endometrial cancer detection.
Conference Abstracts

Effect of prorenin receptor ((P)RR) knock down and telmisartan on endometrial cancer growth, *Cancer Research*, 2017; (77)-13

**Riazuddin Mohammed**, Sarah J. Delforce, Yu Wang, Nicole M. Verrills, Eugenie R. Lumbers, Kirsty G. Pringle

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Riazuddin Mohammed, Sarah J. Delforce, Yu Wang, Nicole M. Verrills, Eugenie R. Lumbers, Kirsty G. Pringle
Abbreviations

A-LAP - Adipocyte-derived leucine aminopeptidase
ACE - Angiotensin converting enzyme
ACE-1 - ACE mRNA
ACEI - Angiotensin converting enzyme inhibitor
ACTB - Beta-actin mRNA
ADH - Antidiuretic hormone
AGT - Angiotensinogen
AGT - AGT mRNA
AGTR1 - AT1R mRNA
AGTR2 - AT2R mRNA
Ang - Angiotensin
APA - Aminopeptidase A
ARB - Angiotensin receptor blocker
AT1R - Angiotensin receptor blocker
AT2R - Angiotensin II type 2 receptor
ATP6AP2 - (P)RR mRNA
BCA - Bicinchoninic acid assay
cDNA - Complementary deoxyribonucleic acid
CI - Confidence interval
CT - Cycle threshold
D - Deletion
DNA - Deoxyribonucleic acid
DMSO - Dimethyl sulfoxide
DMEM - Dulbecco Modified Eagle Medium
dNTP- Deoxynucleotide triphosphate
DTT - Dithiothreitol
EC - Endometrial cancer
ECC-1 - Endometrial carcinoma cell line 1
EDTA- Ethylenediaminetetraacetic acid
EGF- Epidermal growth factor
ELISA- Enzyme linked immunosorbent assay
ER- Estrogen receptor
ER-α - Estrogen receptor alpha
ERK - Extracellular signal-regulated kinases
FGF-β Fibroblast growth factor beta
FIGO- International Federation of Gynecology and Obstetrics
Fwd - Forward
G - Grade
h - Hour
HEC-1A- Human endometrial carcinoma cells
HRP- Handle region peptide
HRT- Hormone replacement therapy
hVSMCs- Human vascular smooth muscle cells
I- Insertion
IHC - Immunohistochemistry
IRAP- Insulin regulated aminopeptidase
LRP5/6- Low density lipoprotein receptor-related protein 5/6
MAPK- Mitogen-activated protein kinase
MAS1- Mas receptor mRNA
MEM- Minimum essential media
MgCl₂- Magnesium chloride
mM- Millimolar
MRI -Magnetic resonance imaging
mRNA- messenger ribonucleic acid
MVD -Microvascular density
NaOH- Sodium hydroxide
ng/ml- Nanogram/milliliter
nt -Nucleotide
NTC -Non template control
PBS -Phosphate buffered saline
PCR- Polymerase chain reaction
pg/ml- picogram/milliliter
PPAR-γ -Peroxisome proliferator-activated receptor gamma
PVDF- Polyvinylidene difluoride
qPCR -Quantitative polymerase chain reaction
RAS- Renin angiotensin system
REN- Prorenin mRNA
Rev- Reverse
RIPA buffer- Radioimmunoprecipitation assay buffer
RNA- Ribonucleic acid
RR -Relative risk
RT- Reverse transcription
s -Seconds
siRNA- Small interfering ribonucleic acid
TBE - Tris/Borate/EDTA
Tm - Melting temperature
UV - Ultraviolet
V-ATPase - Vacuolar-type H+ ATPase
VEGF - Vascular endothelial growth factor
VEGF - VEGF mRNA
w/v - weight/volume
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