Tetraspanins as Biomarkers and Causative Proteins in Prostate Cancer

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B.Biotech (Hons)

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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 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.

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Abstract

Prostate cancer (PCa) is the most commonly diagnosed solid cancer and the cause of the second highest mortality rates in men in the majority of western counties. There are two major unmet needs in dealing with prostate cancer. Firstly, since the majority of deaths from prostate cancer are attributed to the largely untreatable late stage metastatic forms of the disease, understanding molecules involved in the metastatic cascade of PCa may prove beneficial in regards to therapeutic options. Secondly, PCa is a very heterogeneous disease and in many cases follows an indolent course. There are currently no reliable biomarkers to gauge which patients will progress on to advanced disease. Hence, biomarkers that can be implemented into the diagnostic process to stratify patients diagnosed with PCa in regards to their likely outcome allowing the assignment of the most effective and less invasive treatment options are urgently needed.

Tetraspanins are membrane bound proteins that associate with motility related molecules such as integrins. In vivo and in vitro experimental studies have indicated tetraspanins may be important regulators of tumour invasion and metastasis in a number of cancers. Furthermore clinical studies have shown that high expression levels of the tetraspanins CD82 and CD9 have been correlated to good prognosis, while in contrast increased expression of the tetraspanins CD151 and Tspan8 have been correlated with more aggressive cancers and poor outcomes. In this study, for the first time the effects of gene ablation of the pro- and anti-tumourigenic/metastatic tetraspanins, Cd151 and Cd9 respectively, have been evaluated in a de novo developing and spontaneously metastasising murine model of prostate cancer. In addition analysis of clinical tissue microarrays containing a cohort of various prostate tissue samples have been assessed by immunohistochemistry for CD151, Tspan8, CD82 and CD9 expression levels.
The \textit{Cd9} and \textit{Cd151} knock-out mouse models were independently crossed onto the TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP) mouse model. We report here for the first time that development of primary prostate tumours was not affected by ablation of either \textit{Cd9} or \textit{Cd151}. However ablation of \textit{Cd9} resulted in an increase of metastatic lesions (number of foci and total area) to the liver. Conversely ablation of \textit{Cd151} resulted in a decrease of metastatic lesion (number of foci and total area) to the lungs. No change in average area of individual metastases was observed in either case.

Normal and matched PCa tissue samples on tissue micro-arrays obtained from the Australian Prostate Cancer Consortium (APCC) were analysed by IHC. The expression of CD151 and Tspan8 was shown to be positively correlated to PCa progression. In contrast, CD9 and CD82 expression was shown to be negatively correlated to cancer progression. Our results showed weaker correlation with prognosis than previous reports and possible reasons are discussed. In adjunct to the classical pathological IHC manual scoring method, automated digital pathology (Aperio) systems were evaluated in an attempt to standardise IHC scoring. The automated scoring showed similar trends with manual scoring, in regards to tetraspanin expression and cancer progression, however resulted in less significant associations.

In summary, the tetraspanins \textit{Cd9} had anti-metastatic effects while conversely \textit{Cd151} had pro-metastatic effects. Both \textit{Cd9} and \textit{Cd151} had no effect on the development of primary prostate tumours in the TRAMP model. These molecules may be beneficial therapeutic options as metastatic modulators. More extensive evaluation the tetraspanins CD151, Tspan8, CD9 and CD82 as prognostic markers that can delineate PCa patients whose disease may remain indolent and those who will progress is needed.
Papers published


Conference Presentations

Oral presentations

**Ben T Copeland, Matthew J Bowman, Claude Boucheix and Leonie K Ashman.**


**Ben T Copeland, Matthew J Bowman, Claude Boucheix and Leonie K Ashman.**


**Ben T Copeland, Matthew J Bowman and Leonie K Ashman.**

_Tetraspanins Influence on Initiation and Progress of Prostate Cancer._ Hunter Medical Research Institute Cancer Research Symposium, November 2011. Newcastle, Australia. _Awarded best talk from selected abstracts._

**Ben T Copeland Matthew J Bowman and Leonie K Ashman.**

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**Ben T Copeland Matthew J Bowman, Claude Boucheix and Leonie K Ashman.**

_Tetraspanins CD151 and CD9: Metastatic Regulators in Prostate Cancer._ FASEB Summer Research Conference, Membrane Organization by Molecular Scaffolds, July 2011. Vermont, United States. _Awarded travel grant from abstract._
Ben T Copeland, Matthew J Bowman and Leonie K Ashman.  

Ben T Copeland, Ricardo Vilain, Leonie Ashman.  

**Poster presentations**

Ben T Copeland and Leonie K Ashman.  

Ben T Copeland, Matthew J Bowman, Claude Boucheix and Leonie K Ashman.  

Ben T Copeland, Matthew J Bowman, Claude Boucheix and Leonie K Ashman.  


Ben T Copeland, Mark Formby, Ricardo Vilain and Leonie K Ashman.
*Evaluation of Tetraspanin Expression in Prostate Cancer Tissue Microarrays.*
Hunter Medical Research Institute Cancer Research Symposium, November 2010. Newcastle, Australia.

Ben T Copeland, Ricardo Vilain, Leonie Ashman

Ben T Copeland, Ricardo Vilain, Leonie Ashman.

Ben T Copeland, Ricardo Vilain, Leonie Ashman.
Media releases

From the Copeland et al. (2013b) paper published in the International Journal of Cancer, figure 4 was chosen to grace the cover.

Scientific photo with accompanying plaque outlining project details. The *HMRI through the lens* competition. Displayed at Wallsend District Library; May-July 2010, John Hunter Hospital; July-September 2010, Waiting hall of the chief scientific advisor office, Canberra; ongoing. University of Newcastle, ongoing; Hunter Medical Research Institute, ongoing. Electronically displayed on the ABC website [http://www.abc.net.au/local/photos/2010/05/28/2912341.htm](http://www.abc.net.au/local/photos/2010/05/28/2912341.htm).

Newspaper article in the *Newcastle Herald* outlining the research project and importance to the community. November 2008. Fairfax Media Limited, Sydney, Australia.
# Abbreviations

<p>| 1° | Primary |
| 2° | Secondary |
| A.B.R | Australian Bio Resource |
| A.P.C.C | Australian Prostate Cancer Collaboration |
| AP | Anterior prostate |
| AR | Androgen receptor |
| ARBS | Androgen receptor binding site |
| B6 | C57BL/6 |
| bp | Base pair |
| BPH | Benign prostatic hyperplasia |
| CCG | Cysteine-cysteine-glycine |
| CO₂ | Carbon dioxide |
| DAB | Diaminobenzidine |
| ddH₂O | Double distilled water/Milli Q water |
| DNA | Deoxyribonucleic acid |
| DP | Dorsal prostate |
| DRE | Digital rectal examination |
| EC1 | Extracellular loop 1 |
| EC2 | Extracellular loop 2 |
| ECM | Extracellular matrix |
| FFPE | Formalin fixed paraffin embedded |
| FVB | FVB/n |
| GEMMs | Genetically engineered mouse models |
| H&amp;E | hematoxylin and eosin |
| het | Heterozygous |
| HIER | Heat induced antigen epitope retrieval |
| HRP | Horse radish peroxidase |
| HRPC | Hormone refractory prostate cancer |
| IF | Immunofluorescence |
| IgG | Immunoglobulin G |
| IHC | Immunohistochemistry |
| KO | Knockout |
| LEL | Large extracellular loop |
| LP | Lateral prostate |
| mAb | Monoclonal antibody |
| mCRPC | Metastatic castrate resistant prostate cancer |
| MD | Moderately differentiated |
| MMPs | Matrix metalloproteinases |
| n | Number |</p>
<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>O/N</td>
<td>Overnight</td>
</tr>
<tr>
<td>OCT</td>
<td>Optimal cutting temperature</td>
</tr>
<tr>
<td>PAP</td>
<td>Prostatic acid phosphatase</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCa</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PD</td>
<td>Poorly differentiated</td>
</tr>
<tr>
<td>PIN</td>
<td>Prostatic intraepithelial neoplasia</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
</tr>
<tr>
<td>Rab</td>
<td>Rabbit</td>
</tr>
<tr>
<td>ROI</td>
<td>Regions of interest</td>
</tr>
<tr>
<td>rPB</td>
<td>Minimal rat probasin promoter</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEL</td>
<td>Small extracellular loop</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SV</td>
<td>Seminal vesicle</td>
</tr>
<tr>
<td>SV40</td>
<td>Simian virus 40</td>
</tr>
<tr>
<td>T/N/M</td>
<td>Tumour/node/metastases</td>
</tr>
<tr>
<td>Tag</td>
<td>Early tumour antigen</td>
</tr>
<tr>
<td>TEM</td>
<td>Tetraspanin enriched micro-domain</td>
</tr>
<tr>
<td>TMA</td>
<td>Tissue microarray</td>
</tr>
<tr>
<td>TRAMP</td>
<td>Transgenic adenocarcinoma of the mouse prostate</td>
</tr>
<tr>
<td>TURP</td>
<td>Transurethral resection of the prostate</td>
</tr>
<tr>
<td>UoN</td>
<td>University of Newcastle</td>
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