

Post-transcriptional Regulation of Tetraspanins CD151 and CD9 by micro-RNAs in Prostate and Breast Cancers

Danielle Bond

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Declaration

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Danielle Bond

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Abstract

Tetraspanins CD151 and CD9 play important roles in cancer progression and metastasis. CD151, referred to as a metastasis enhancer, is typically upregulated in solid malignancies including breast and prostate cancers. In contrast, CD9 is commonly considered a metastasis suppressor, with downregulation of CD9 protein levels in advanced stage cancers. Therefore, CD151 and CD9 are potential targets for new therapeutics to combat cancer progression. However, CD151 and CD9 are not typical “druggable” targets, therefore other ways to change tetraspanin expression such as manipulation of tetraspanin regulation are required. Regulation of CD151 and CD9 expression has been minimally investigated. Therefore, the aim of this thesis was to investigate post-transcriptional regulation of CD151 and CD9 by miRNAs in non-tumourigenic and tumourigenic prostate and breast cell lines.

CD151 mRNA and protein levels were found to inversely correlate in prostate cell lines, with highly tumourigenic prostate cancer cells expressing high levels of CD151 protein. Breast cancer cell lines displayed low levels of CD151 mRNA and protein compared to non-tumourigenic breast cells, however triple negative MDA-MB-231 breast cancer cells showed similar CD151 protein expression to that of non-tumourigenic breast cells. The degree by which the CD151 3'UTR regulates protein expression was determined with a dual luciferase assay, with greater repression of protein expression found in the tumourigenic cell lines. Bioinformatic analysis of miRNA predicted to bind CD151 together with miRNA expression profiling in prostate cells was used to identify miRNA

that had expression levels matching the luciferase output. miR-637, which was upregulated in prostate cancer cell lines, was shown to regulate expression at the CD151 3'UTR, with transfection of miR-637 mimic into RWPE1 and DU145 prostate cells resulting in a 10-20% decrease in CD151 protein expression. However, miR-637 had no effect on CD151 protein expression in non-tumourigenic 184A1 and MDA-MB-231 breast cancer cells, suggesting a role specifically in prostate cancer.

CD9 mRNA and total protein levels were similar across all prostate cell lines, with typically slightly lower levels of CD9 cell surface levels in tumourigenic cells. In addition breast cancer cell lines displayed lower levels of CD9 mRNA, total protein and cell surface protein expression compared to non-tumourigenic breast cells. A 3'UTR luciferase reporter assay showed that the CD9 3'UTR is differentially regulated in prostate and breast cell lines, with highly tumourigenic prostate cancer cells showing more repression of luciferase compared to other cells. In the panel of breast cells, CD9 3'UTR activity was similar across all lines, however 184A1 breast cells showed increased luciferase, which suggests that the CD9 3'UTR is partly responsible for high CD9 protein levels in normal breast cells. Using the same approach to identifying miRNA as for CD151, miR-518f* was found to bind to the CD9 3'UTR *in vitro*. Overexpression of miR-518f* in non-tumourigenic prostate RWPE1 and prostate cancer DU145 cells as well as non-tumourigenic breast 184A1 and MDA-MB-231 breast cancer cells led to a significant decrease in CD9 protein expression. Furthermore, transfection of miR-518f* increased migration of RWPE1, 184A1 and MDA-MB-231 cells and decreased migration of DU145 prostate cancer cells. Moreover, overexpression of miR-518f* significantly decreased RWPE1 adhesion to fibronectin

and basement membrane extract and increased 184A1 cell proliferation and adhesion to BME, but had no effect on adhesion in other cell lines or proliferation.

In conclusion, tetraspanins such as CD151 and CD9 are at least partially regulated by miRNAs in prostate and breast cell lines. miRNAs such as miR-518f* may be novel and effective biomarkers and/or therapeutic targets for inhibiting cancer progression in the future.

Publications and conference abstracts arising from this thesis

- D Bond, J Brzozowski, K Skelding, S Roselli & J Weidenhofer. **Use of tetraspanins CD151 & CD9 as biomarkers for breast cancer**, *Breast Cancer Management*, March 2014 Vol. 3 issue 2.
- Bond D, Cairns M, Ashman LK & Weidenhofer J. **Post-transcriptional regulation of tetraspanins CD151 & CD9 in breast & prostate cancers** (poster), AACR 2014 San Diego, USA.
- Bond D, Cairns M, Ashman LK & Weidenhofer J. **Post-transcriptional regulation of tetraspanins CD151 and CD9 by micro-RNAs in prostate cancers** (oral presentation), Hunter Translational Cancer Conference, Newcastle city hall 2013.
- Bond DR, Cairns MJ, Ashman LK & Weidenhofer J. **Post-transcriptional regulation of CD151 and CD9 in prostate and breast cancers** (oral presentation), ComBio 9 September – 3 October 2013, Perth, Australia.
- Danielle Bond, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Investigating miRNA Regulation of Tetraspanins CD151 & CD9 in Prostate and Breast Cancers** (poster presentation), ASMR NSW Scientific Meeting, 3rd June 2013, Australian Technology Park Redfern NSW, Australia.
- Danielle Bond, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Post-transcriptional Regulation of CD151 & CD9 in Prostate Cancers** (oral presentation), The 5th Annual Hunter Cancer Research Symposium, 5th November 2012, Newcastle NSW, Australia.
- Danielle Bond, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Post-transcriptional Regulation of CD151 and CD9 in Breast and Prostate Cancer** (oral & poster presentation), 5th European Conference on Tetraspanins, 26-28 September 2012, Nijmegen, the Netherlands.
- Danielle Bond, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Investigating micro-RNA Regulation of Tetraspanins CD151 & CD9 in**

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Spain.

- Danielle Bond, Matthew J Bowman, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Investigating Regulation of Tetraspanin Expression in Breast and Prostate Cancers** (poster), ASMR NSW Scientific Meeting 2012, Australian Technology Park, Redfern, NSW, Australia.
- Danielle Bond, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Investigating Regulation of Tetraspanin Expression in Prostate Cancer** (poster), HMRI Cancer Research Symposium, 4th November 2011, Newcastle, NSW, Australia.
- Danielle Bond, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Investigating Micro-RNA Regulation of Tetraspanins in Prostate Cancer** (poster), Australian Society for Medical Research (ASMR) XIX NSW Scientific Meeting, 6th June 2011, The University of Sydney, Camperdown, NSW, Australia.
- Danielle Bond, Matthew J Bowman, Murray Cairns, Leonie K Ashman & Judith Weidenhofer, **Investigating Regulation of Tetraspanin Expression in Breast and Prostate Cancers** (poster), 11th Hunter Cell Biology Meeting, 22-25 March 2011, Hunter Valley Vineyards, NSW, Australia.

Abbreviations

Abbreviation	Word
BC	Breast cancer
BME	Basement membrane extract
cDNA	Complimentary DNA
DCIS	Ductal carcinoma <i>in situ</i>
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
Exp.	Expression
FN	Fibronectin
miRNA	Micro ribonucleic acid
NTC	Non-targeting control
PC	Prostate cancer
RBP	RNA binding protein
Refs	References
RNA	Ribonucleic acid
TEM	Tetraspanin-enriched microdomain