ADAMTS1 IS A PROMOTER OF METASTATIC CELL BEHAVIOUR IN MAMMARY CANCER CELLS

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“The ones crazy enough to think they can change the world are the ones that do”

- Steve Jobs

“It always seem impossible until it’s done”

-Nelson Mandela

“Everything is okay in the end. If its not okay, its not the end”

- John Lennon
Abstract

Metastatic disease is the primary cause of mortality in breast cancer. It is characterised by the dissemination of cancer cells from the primary site, infiltration into vessel networks and the establishment of new tumour growth in secondary tissues. Several events are required for metastasis to occur, including enhancement of cell-matrix adherence, augmented motility and invasiveness. The extracellular matrix (ECM) environment plays a vital role in the processes involved in metastatic progression and undergoes aberrant remodelling to permit and support the metastatic cascade.

Metalloproteinases are a group of enzymes that play a major role in ECM remodelling. The ADAMTS metalloproteinase family has been implicated in the re-organisation of the tumour microenvironment associated with cancer development and metastatic disease progression. Of the 19 ADAMTS proteases, considerable attention has been devoted to the role of its first member ADAMTS1 in cancer metastasis.

Both exogenous overexpression and upregulation of the endogenous ADAMTS1 gene have been strongly associated with metastatic disease in breast cancer. The MMTV-PyMT transgenic breast cancer model recapitulates in vivo metastasis and ablation of Adamts1 impeded the aggressive advancement and growth of pulmonary metastases. The signalling pathways and mechanistic events through which ADAMTS1 mediates its pro-metastatic effects are currently unknown. The aim of this present study is to therefore identify the causal events imposed by ADAMTS1 to promote breast cancer metastasis, with much focus on its role in matrix adhesion, cell migration and invasion.

Using isolated primary mammary carcinoma cells PyMT/Adamts1\(^{+/+}\), PyMT/Adamts1\(^{+-}\) and PyMT/Adamts1\(^{--}\) mice, I performed real-time assessment of cell-matrix adhesion, motility and invasion and found diminished capacity of PyMT/Adamts1\(^{+-}\) cells to adhere to matrigel and migrate towards a chemoattractive environment. Consistent with the reciprocal approach, introduction of Adamts1 into the MCF10A breast cell line induced the inverse effect, promoting cell adhesion and motility in cells overexpressing Adamts1. Cell-matrix adhesion is a major cue for the determination of front-rear polarity necessary in cell migration and hence, the influence of ADAMTS1 on cell-matrix adhesion underpinned its effects on breast cancer cell migration. Breast cancer cell invasion was unaffected by loss or gain of Adamts1, suggesting a redundant role for ADAMTS1 in this process.
To unravel the transcriptional differences and mechanistic pathways induced by ADAMTS1, microarray analysis was undertaken with PyMT/Adamts1+/+ and PyMT/Adamts1−/− mammary tumours. Remarkably, only 2 differentially regulated genes were identified from our analysis. Further investigation of the most dysregulated gene, BC018473, revealed a non-homologous inheritance of this strain specific gene, which unfortunately prevented conclusions being drawn on the underlying genetic effects attributable to Adamts1 ablation.

This study was the first to present a novel role for ADAMTS1 in the promotion of breast cancer cell adhesion to the ECM. This capacity to dynamically modulate adhesion through ADAMTS1 is important in cell migration and highlights a potential mechanism by which ADAMTS1 promotes breast cancer metastasis.
Declaration

This thesis contains no material, which has been accepted for the award of any other degree or diploma in other university or tertiary institution. The content of this thesis is an original body of work and does not contain any published material written by another person, except where due reference has been made.

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Publications arising from thesis


2. de Aaro Tan I, Frewin K, Ricciardelli C & Russell DL. “ADAMTS1 promotes the adhesion of mammary cancer cells to structural proteins that make up the extracellular matrix and basement membrane that in turn promotes cancer cell migration”. In preparation
Abstracts arising from thesis


5. de Arao Tan I, Frewin K, Ricciardelli C & Russell DL. “The metalloproteinase Adamts1 increases the capacity of mammary cancer cells to adhere to extracellular components”. Faculty of Health Sciences Postgraduate Conference, Adelaide, SA, August 2011

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### Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>1^oMCC</td>
<td>primary mouse mammary cancer cell</td>
</tr>
<tr>
<td>ADAMTS1</td>
<td>a disintegrin and metalloproteinase with thrombospondin motifs 1</td>
</tr>
<tr>
<td>*Adams1^+/−</td>
<td><em>Adams1</em> knockout</td>
</tr>
<tr>
<td>*Adams1^+/-</td>
<td><em>Adams1</em> heterozygous</td>
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<tr>
<td>*Adams1^+/+</td>
<td><em>Adams1</em> wild type</td>
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<tr>
<td>ADPC</td>
<td>androgen dependent prostate cancer</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>AR</td>
<td>androgen receptor</td>
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<td>bp</td>
<td>base pair</td>
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<tr>
<td>C-terminal</td>
<td>carboxyl terminal</td>
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<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
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<td>chinese hamster ovary</td>
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<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
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<td>colorectal carcinoma</td>
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<td>castrate resistant prostate cancer</td>
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<tr>
<td>DMEM</td>
<td>dulbecco’s minimum essential medium</td>
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<td>dimethyl sulfoxide</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxyribonucleic triphosphate</td>
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<tr>
<td>ECD</td>
<td>glutamic acid-cysteine-aspartic acid</td>
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<tr>
<td>ECM</td>
<td>extracellular matrix</td>
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<td>EDTA</td>
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<td>EGF</td>
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<td>ER</td>
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<td>FBS</td>
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<td>GSE</td>
<td>genomic spatial event</td>
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<td>h</td>
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<td>Abbreviation</td>
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<tr>
<td>RANKL</td>
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<tr>
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