Myoepithelial Cell Model

1089 before sorting

1089 after sorting

- Immortalised population of normal breast cells from Prof Mike O’Hare.
- Magnetic beads coated with ITGB4 & CD10 antibody isolated a pure myoepithelial cell population
- Introduced \( \alpha v \beta 6 \) to create a model of DCIS-associated myoepithelial cells
Myoepithelial Cell Model

<table>
<thead>
<tr>
<th>Primary LEC</th>
<th>Primary MEC</th>
<th>Galectin-7</th>
<th>Cytokeratin 8</th>
<th>Cytokertain 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-1089</td>
<td>b6-1089</td>
<td>Primary LEC</td>
<td>Primary MEC</td>
<td></td>
</tr>
<tr>
<td>Gal-7</td>
<td>- 14 kDa</td>
<td>MCF7</td>
<td></td>
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<tr>
<td>CK8</td>
<td>- 50 kDa</td>
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<tr>
<td>CK14</td>
<td>- 50 kDa</td>
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<tr>
<td>P-Cadherin</td>
<td>- 120 kDa</td>
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<tr>
<td>E-Cadherin</td>
<td>- 120 kDa</td>
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<tr>
<td>HSC70</td>
<td>- 70 kDa</td>
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<tr>
<td>N-1089</td>
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</table>
αvβ6+ve Myoepithelial Cells promote invasion

Transwell Invasion Assays

Organotypic Assay

αvβ6+ve Myoepithelial Cells promote tumour growth

Linear effects model; p=0.001

N=10 each group; * p<0.05
Invasion is MMP-9 dependent

Zymography

Transwells Invasion + MMP9 Inhibitor (50nM)
Angiogenesis and Breast Cancer

Markers of increased angiogenesis (e.g. MVD, VEGFmRNA) associated with reduced survival in both breast cancer and DCIS


2 vessel patterns associated with DCIS: Rim and Stromal (Guidi et al 1991)
Angiogenesis and $\alpha\nu\beta6$

- Cases stained for $\alpha\nu\beta6$ and CD31 on contiguous slides
- 112 ducts from 41 cases
**αvβ6 associated with enhanced Angiogenesis**

Significant correlation between myoepithelial staining for αvβ6 and rim pattern CD31 p<0.001

Significant correlation between myoepithelial staining for αvβ6 and MicroVascular Density p<0.001
A vb6-+ve Myoepithelial Cells promote ‘Vessel’ Formation

<table>
<thead>
<tr>
<th></th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
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</thead>
<tbody>
<tr>
<td>N-1089</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>β6-1089</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

AngioKit Assay exposed to N-1089 or β6-1089 conditioned media in varying ratio with Optimised Growth Media
Assessing Tube Complexity

A) Raw image captured of CD31 stained endothelial cell tubes
B) Blue marker identifies stained tubes to determine tubule area (in pixels)
C) Red marker defines centre line of tubules to derive total vessel length and average tubule thickness (total area divided by total vessel length (in pixels))
D) Yellow marker used to define branch points (nodal points)
\(\alpha v\beta 6^{+ve}\) Myoepithelial Cells generate more complex ‘vessels’

\[\begin{array}{c}
\text{Vessel Length} \\
\hline
\text{N-1089} & 0 \\
\text{B6-1089} & 1 \\
\end{array} \quad \begin{array}{c}
\text{Branch Points/Unit Length} \\
\hline
\text{N-1089} & 0 \\
\text{B6-1089} & 5 \\
\end{array} \]

\(\beta 6\text{-1089}\) conditioned media promotes longer tubule formation and more complex branching compared to N-1089 CM (\(p<0.001\))
Aortic Ring Assay

- 6 week Blk6/C57 mice
- Remove and chop aorta into rings
- Incubate in Optimem overnight
- Embed rings in collagen and culture in conditioned media changed every 3 days
- Count number of sprouts coming directly from ring, not branch points

Courtesy of Prof Kairbaarn Hodivala-Dilk, BCI.
αvβ6+ve Myoepithelial Cells promote Angiogenic Sprouting

\[ \beta6-1089 \text{ CM enhances aortic ring sprouting and is reversed by transfection with } \beta6 \text{ SiRNA} \]
Angiogenesis in-vivo

Co-injected myoepithelial cells and MDA-MB231 cells into mammary fat pad

Growth rate and end tumour volume

Vessel count per unit area (section)

Chalkley (tumour section)

Ten mice per group (N-1089, B6-1089)
Enhanced angiogenesis in-vivo

Tumours co-injected with $\beta$6-1089 show higher vessel counts per unit area than those with N-1089 cells ($p=0.01$)
**αvβ6 status associates with disease recurrence**

UK-DCIS Trial: 100 cases locally excised DCIS with long-term follow-up assessed for

**αvβ6 status**

αvβ6 significantly associated with disease recurrence (in-situ and invasive) independent of age, grade, extent.

- **αvβ6-neg** mean 11.4 yrs
- **αvβ6 pos** mean 2.3 yrs

Myoepithelial Cells Regulate Microenvironment

Acknowledgements

Breast Group:
Dr Mike Allen
Dr Jenny Gomm
Dr Linda Haywood
Dr Sally Dreger
Dr Adrienne Morgan
Dr Alastair Ironside
Dr Natalie Allen
Ms Mary-Kate Hayward
Dr Kathryn Hawkesford
Mr Sarantos Kaptanis
Dr Sally Smith
Ms Rachel Nelan
Mr Iain Goulding
Ms Rachel Jagger
Ms Shakira Chowdhury

Mr George Elia

Beta 6 Group:
Prof John Marshall
Dr Kate Moore
Prof Gareth Thomas
University of Southampton

Funding
Breast Cancer Now
MRC
CRUK