Influence of the Tumor Microenvironment on Prostate Cellular Behavior

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Discovery of EMT

First observed and defined by Elizabeth Hay in late 1960’s at Harvard

First associated with early stages of embryonic development.

Process is reversible

EMT ↔ MET ↔ EMT
EMT

- Loss of E-cadherin
  - Increase in translational repressors (Snail or Zeb1)
  - Methylation promoter
- Secretion of proteolytic enzymes
- Gain of mesenchymal proteins
- Fibroblastic morphology
- Increased cell motility and invasion
Factors for metastatic progression

EMT $\rightarrow$ Dissemination $\rightarrow$ Micrometastasis $\rightarrow$ M-E-T

- Reversible (epigenetic)
- Environmental factors
- Location
  - bone marrow
  - liver (hepatocytes)
Molecular Events of Metastasis

Nature Reviews Cancer, 2002
EGFR Modulates DU145 E-Cadherin Levels

Yates et al 2005
E-cadherin/EGFR in Co-culture

Figure 1

A.

<table>
<thead>
<tr>
<th></th>
<th>DU-145</th>
<th>PC-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td><img src="image1" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>E-cadherin</td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td>Cytokeratin 18</td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
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<tr>
<td>Tubulin</td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
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B.

<table>
<thead>
<tr>
<th></th>
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<tr>
<td>E-cadherin</td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
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<tr>
<td>GAPDH</td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
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</tbody>
</table>

Green = Hepatocytes
Red = DU-145 prostate cancer
Blue = Anti-E-cadherin or EGFR staining
Human PCa Mets to Liver

Top row = IHC with anti-E-cadherin, anti-β-catenin
Bottom row = anti-p120, anti-p120

E-cadherin

EGFR

p120

B-catenin

p-EGFR 1068
Reepithelialization of PCa Mets in the Liver

Cytokeratin 18

Vimentin
ARCaP: Classic EMT Model

A. ARCaPE → ARCaPM

B. RT-PCR

<table>
<thead>
<tr>
<th></th>
<th>ARCaP</th>
<th>M</th>
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<tbody>
<tr>
<td>E-cadherin</td>
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<tr>
<td>Vimentin</td>
<td><img src="image" alt="Vimentin" /></td>
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<tr>
<td>N-cadherin</td>
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<td>GAPDH</td>
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C. Western blot

<table>
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<th>M</th>
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<tr>
<td>E-cadherin</td>
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<tr>
<td>Vimentin</td>
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<tr>
<td>CK18</td>
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<tr>
<td>CK19</td>
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<td><img src="image" alt="CK19" /></td>
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<tr>
<td>β-actin</td>
<td><img src="image" alt="β-actin" /></td>
<td><img src="image" alt="β-actin" /></td>
</tr>
</tbody>
</table>

D. IHC E-cadherin

![IHC E-cadherin](image)
Prostate Cancer in Presence of Bone Stromal Cells

Josson et al 2010

cadherin and N-cadherin at Day 1 and 4
A total of 1,220 prostate TMA samples were analyzed. High (normal) E-cadherin expression was seen in 87% of 757 benign, 80% of 41 high-grade PIN, 82% of 325 prostate carcinoma and 90% of hormone-refractory prostate carcinoma TMA samples.
Clinical implications of Repithelialization during Metastatic Seeding of Tumor Microenvironment
Metastatic Seeding within Tumor Microenvironment

A. GFP- HS-27a

RFP-ARCaP_E

RFP-ARCaP_M

B. Relative Growth (Relative Fluorescent Unit)

Days

C. Clonogenic Survival

Josson et al 2010
Prostate/Bone stromal cells decrease Radiation Sensitivity

Josson et al 2010

A.

B.

C.
Blocking Cell-Cell Adhesion Increases Radiation Sensitivity
Blocking Cell-Cell Adhesion Increases Radiation Sensitivity
Establishment and Characterization of non-malignant and malignant cell lines from African American Prostate Cancer Patient.
African American Prostate Cell lines.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Age</th>
<th>Race</th>
<th>Morphology</th>
<th>Clinical Stage</th>
<th>Tumor Grade</th>
<th>Gleason Score</th>
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</thead>
<tbody>
<tr>
<td>RC77N</td>
<td>62</td>
<td>AA</td>
<td>Epithelial</td>
<td>Non-malignant</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>RC44N</td>
<td>59</td>
<td>AA</td>
<td>Epithelial</td>
<td>Non-malignant</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>RC77T</td>
<td>62</td>
<td>AA</td>
<td>Epithelial</td>
<td>Primary Adenocarcinoma</td>
<td>Poorly Differentiated</td>
<td>7</td>
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<tr>
<td>RC44T</td>
<td>59</td>
<td>AA</td>
<td>Epithelial</td>
<td>Primary Adenocarcinoma</td>
<td>Poorly Differentiated</td>
<td>7</td>
</tr>
<tr>
<td>MDA-2Pca-2b</td>
<td>63</td>
<td>AA</td>
<td>Epithelial</td>
<td>Adenocarcinoma</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PrEC</td>
<td>59</td>
<td>White</td>
<td>Epithelial</td>
<td>Non-malignant</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>RC-92a</td>
<td>57</td>
<td>White</td>
<td>Epithelial</td>
<td>Primary Adenocarcinoma</td>
<td>Well-Differentiated</td>
<td>3 + 3</td>
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<tr>
<td>PC-3</td>
<td>62</td>
<td>White</td>
<td>Epithelial</td>
<td>Metastatic Adenocarcinoma</td>
<td>Undifferentiated</td>
<td>NA&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

AA = African American
NA = not applicable
NA<sup>c</sup> = Not available

Theodore et al 2010
Properties of Newly Established Cell lines

Table 1. Properties of RC-77T/E and RC-77N/E cell lines

<table>
<thead>
<tr>
<th></th>
<th>RC-77T/E</th>
<th>RC-77N/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life Span</td>
<td>&gt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Gene Expression by RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NKX3.1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cytokeratin 8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AR</td>
<td>+</td>
<td>+</td>
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<tr>
<td>p16</td>
<td>+</td>
<td>+</td>
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<tr>
<td>PSA</td>
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<td>+</td>
</tr>
<tr>
<td>GAPDH</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3D-organoid formation</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tumorigenicity in SCID mice</td>
<td>3/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Theodore et al 2010
Figure 2

A.

B.

C.

i. 

ii. 

iii. 

Theodore et al. 2010
Acknowledgements

**Major Collaborators**

**University of Pittsburgh**
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- Sajni Josson

**Tuskegee University**
- Tim Turner

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- Ritu Aneja