Differentiation of mesothelial cells – potential role in fibrosis

‘Scarring in the female reproductive tract’
ESHRE, Edinburgh
5-6th February 2013

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The University of Manchester, UK
• After injury…

• the ideal is regeneration…

• but usually repair with scarring occurs…

• if inadequate and weak, chronic wounds form…

• or if excessive, hypertrophic/fibrotic scarring results
Importance of the mesothelium

- **Mesothelium**
  - single layer of squamous epithelium
  - mesodermally derived
  - all three serosal cavities
- Subserosal connective tissue
- Peritoneal fluid
- Functions
  - Non-adhesive barrier-
    surfactant and microvilli
  - Solute and fluid transport
  - Immune function
- Pathology
Peritoneal repair scenarios

Injury → Close opposition of peritoneal surfaces

Normal repair → Tissue repair

Adhesions → Peritoneal sclerosis
Abdominal/pelvic surgery often leads to adhesion formation

• Form after a range of peritoneal insults
• Complications:
  – Small bowel obstruction
  – Infertility in women
  – Difficulty of repeat surgery
  – Chronic pelvic pain
• 5.5% of all abdominal repeat surgery directly due to adhesions - SCAR studies
• Reform after surgical adhesiolysis
• Limited preventative treatment – barrier devices
• Pharmaceutical interventions?
Peritoneal dialysis commonly results in peritoneal sclerosis

- Peritoneal dialysate – high glucose, low pH, high lactate
- Peritonitis
- Thickening of peritoneal membrane leads to ultrafiltration failure

- Rare condition of PD - Encapsulating Peritoneal Sclerosis (EPS)
- Cocooning of viscera by mass scar tissue leading to bowel obstruction
- Incidence linked to time on dialysis
  - 2 years – 1.9%
  - 8 years – 19.4%
- Surgical management
- Pathophysiology?

van Dellen D et al., BrJ Surg, 2011
How does tissue repair progress to fibrosis?

• Coagulation and inflammation
• Granulation tissue formation
• Re-epithelialisation
• Tissue contraction
• Remodeling and scar formation - .......fibrosis
Important role of the ‘fibroblast’ in tissue repair and fibrosis
Does the mesothelium play a role in peritoneal fibrosis?
Peritoneal repair scenarios

Injury → Close opposition of peritoneal surfaces

Normal repair → Tissue repair

Adhesions

Peritoneal sclerosis
Proposed schemes of mesothelial regeneration

1. Centripetal migration
2. Proliferation
3. Desquamation
4. Free-Floating Mesothelial cell
5. Sub-mesothelial Fibroblast-like cells
6. Circulating precursor
7. Macrophage
Rodent model of surgical injury

- Isolate and label rat cells
  - mesothelial cells
  - peritoneal lavage cells
  - peritoneal macrophages
  - lung fibroblasts
- Laparotomy and simple abrasion injury
- Intra-peritoneal injection of fluorescent Di-I labelled cells
- Assessed distribution of labelled cells post-injury

Mesothelial cells in serosal fluid increase after injury in rats

Incorporation of isolated free-floating mesothelial cells on denuded surface

Mesothelial cells
day 8

Fibroblasts
day 8

Lavage cells
day 8

Macrophages
Day 2

Macrophages
Day 5

Macrophages
Day 8
Formation of junctional components by incorporated mesothelial cells

Dil-fibroblasts  Dil-mesothelial cells

Di-I labelled mesothelial cells incorporate into multiple layers
Epithelial-Mesenchymal Transdifferentiation (EMT) of mesothelial cells *in vitro*

Epithelial – fibroblast - myofibroblast - smooth muscle-like phenotype
Pathway to epithelial-mesenchymal transdifferentiation

E-cadherin  Syndecan
Cytokeratin  MUC 1
ZO-1         Desmoplakin
Laminin      Collagen IV
Entactin     msR200

Progressive loss of epithelial markers and gain of mesenchymal markers

FSP-1  N-cadherin
Vimentin  Fibronectin
B-catenin  Syndecan 1
miR10b  Snail

Snail  Slug
Twist  α-SMA
FOXC2  LEF-1

Does mesothelial cell EMT occur \textit{in vivo}?
Mesothelium EMT contributes to blood vessels of the heart, lung, mesentery and gut during development.

Wilm B et al. Development 2005;132:5317-5328
Mesothelial cell EMT occurs with injury in adult

Peritoneal dialysis (CAPD)

Mesothelial cell damage/stress

(Tyro)fibroblast formation

Extracellular matrix deposition

Peritoneal sclerosis

Yanez-Mo et al., (2003) NEJM 348:403-413;
Transforming Growth Factor (TGF)-β

Fibrogenic cytokine:
- Wound healing
- Fibrosis
- Growth and development

3 mammalian isoforms:
- TGF-β1, TGF-β2 and TGF-β3

Secreted in latent form
- Activation
Once activated can signal through
- TGF-βRI
- TGF-βRII
- SMAD pathway

Functions
- Induction of myofibroblasts
- Increase in ECM production
- Upregulates protease inhibitors
Mouse model of peritoneal fibrosis
- Peter Margetts, McMaster University, Canada

Adenovirus delivery of TGF-β1 – 20 days – transient peritoneal fibrosis
Expression extended by helper-dependent adenovirus – 70 days – severe fibrosis/EPS

Liu L et al., Perit Dial Int 29; 508 (2009);
Peritoneal expression of mesenchymal markers after adenovirus infection

- α-SMA
- Collagen
- Snail

Is there a genetic predisposition to peritoneal fibrosis?
Mouse strain differences in the fibrotic response

<table>
<thead>
<tr>
<th>Strain</th>
<th>Susceptibility</th>
<th>Fibrosis Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>Susceptible</td>
<td>Pulmonary</td>
<td>Haston et al., 1996</td>
</tr>
<tr>
<td></td>
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<td>Hepatic</td>
<td>Hillebrandt et al., 2002</td>
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<tr>
<td></td>
<td></td>
<td>Renal</td>
<td>Kato et al., 2008</td>
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<tr>
<td>C57BL/6</td>
<td>Susceptible</td>
<td>Pulmonary, Intestinal, Hepatic, Renal</td>
<td>Schrier et al., 1983, Skwarczuk and Travis, 1998, Knight et al., 2007, Puri et al., 2010</td>
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<tr>
<td>Intermediate</td>
<td>Susceptible</td>
<td>Hepatic</td>
<td>Shi et al., 1997</td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td>Renal</td>
<td>Sugimoto et al., 2007</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Susceptible</td>
<td>Hepatic</td>
<td>Shi et al., 1997</td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td>Pulmonary, Hepatic, Renal</td>
<td>Schrier et al., 1983, Knight et al., 2007, Puri et al., 2010</td>
</tr>
<tr>
<td>BALB/cJ</td>
<td>Susceptible</td>
<td>Hepatic</td>
<td>Hillebrandt et al., 2002</td>
</tr>
<tr>
<td>A/J</td>
<td>Susceptible</td>
<td>Cardiac</td>
<td>Faulx et al., 2005</td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td>Hepatic</td>
<td>Hillebrandt et al., 2002</td>
</tr>
</tbody>
</table>
Mouse family tree – Jackson Labs

Petkov P. et al., Genome Res. 2004 14: 1806-1811
Animal model of peritoneal fibrosis - mouse strain differences

- C57BL/6J
- DBA/2J
- C3H/HeJ
- SJL/J

- Intraperitoneal injection of TGF-β1 expressing adenovirus administered to mice.
- After 4 or 10 days peritoneal tissue and omental tissue samples collected and analysed for fibrogenic differences

Louise Walkin, PhD student
Mouse stain differences in the development of peritoneal fibrosis

C57BL/6J = fibrotic
SJL/J = resistant

Mesothelial cell culture – mouse strain differences?

The Jackson Laboratory - JAX®
- Male, 8 weeks

C57BL/6J

SJL/J

Isolation & culture

+/- 1ng/ml TGF-β1

- Collagen production at 0, 24, 48 hours
Mouse strain difference in mesothelial cells – collagen production

Mesothelial cells

Fibroblasts

EMT markers?
Inhibition of EMT to prevent peritoneal fibrosis

Inhibition of Transforming Growth Factor-Activated Kinase 1 (TAK1) Blocks and Reverses Epithelial to Mesenchymal Transition of Mesothelial Cells

Fibrin-Induced Epithelial-to-Mesenchymal Transition of Peritoneal Mesothelial Cells as a Mechanism of Peritoneal Fibrosis: Effects of Pentoxifylline

BMP-7 blocks mesenchymal conversion of mesothelial cells and prevents peritoneal damage induced by dialysis fluid exposure
Human adhesions show the presence of myofibroblasts and clusters of smooth muscle

Does mesothelial cell EMT occur in the pathogenesis of adhesion formation?

Injury → Close opposition of peritoneal surfaces

Normal repair → Reduced fibrinolysis → Adhesion formation
Remodeling of Peritoneal-like Structures by Mesothelial Cells: Its Role in Peritoneal Healing

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In collagen gel

On gel with blood clot

30 mins 14 days
Inhibition of TGF-β1 and 2 reduces adhesion formation in murine model

- Neutralising antibody to TGF-β1 and 2 given topically on day of surgery and i.p 4hrs pre-surgery, post-surgery and every 4hrs for 24hrs
- Significant reduction of adhesions by blocking TGF-β1 and -β2 by 7 days

Source and plasticity of mesothelial cells?

FREE-FLOATING MESOTHELIAL CELL

Growth factors
Cytokines
ECM fragments
Plasma proteins

Mechanical forces

pH
O₂
Solutés
Inflammatory cells

EMT

RESIDENT MESOTHELIAL PROGENITOR CELL

SUBSEROUSAL CELL

Adipocyte
Chondrocyte
Myofibroblast
Smooth muscle cell
Evidence for a bone marrow source of regenerating mesothelial cells

Potential role of bone marrow-derived cells in the turnover of mesothelium

Kuo-Su Chen,¹ Chao-Hung Wang,² Tzung-Hai Yen,¹ Jim-Ray Chen,³ Ming-Jui Hung,² and Ching-Yuang Lin⁴

• GFP- bone marrow transplant into irradiated mice
• Found 2.2% of mesothelium GFP-labelled after 6 months
• Bone-marrow derived cells contribute to normal mesothelial turnover
• After injury?
Stimulated rat mesothelial cells accumulate lipid during adipogenic differentiation
- Steve Mutsaers, UWA, Perth

Stimulated rat mesothelial cells express alkaline phosphatase during chondrogenic differentiation

Bone Marrow Mesenchymal Cells (BMMC)

Mesothelial Cells
Stimulated rat mesothelial cells form mineralized bone nodules – Von Kossa staining

Bone Marrow Mesenchymal Cells (BMMC)

Unstimulated Stimulated

D18

Mesothelial Cells

Unstimulated Stimulated

D18

Conclusions

• Mesothelial cells undergo EMT during peritoneal fibrosis - adhesion formation?
• Inhibit EMT prevents peritoneal fibrosis – adhesion formation?
• Mesothelial cells show multipotential ability in culture – in people?
• Apparent bone marrow source of mesothelial cells – do these cells have stem cell-like properties?

• Need to perform cell tracking studies – which promoter?
• Use primary human mesothelial cells – source?
• Multidisciplinary approach?
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McMaster University, Canada
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