The potential of stem cells therapies for kidney disease

KRUK Alumni Meeting 6th December 2013
University of Manchester

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Institute of Translational Medicine
University of Liverpool
• Overview of kidney research at University of Liverpool

• Current research programme

• Future outlook
**Long-term aims of Liverpool stem cell group**

To determine it stem cells can:

- replace or repair damaged renal tissue and ameliorate kidney disease
- generate renal cell types *in vitro* for drug discovery, toxicology studies and disease modelling

**Main objective of KIDSTEM:**

To determine the ‘nephrogenic potential’ of different stem cell types

**Stem cell types:**

- Embryonic stem cells (ESC)
- Mesenchymal stem cells (MSC)
- Amniotic fluid stem cells (AFSC)
- Kidney stem/progenitor cells (KSC)
KIDNEY DEVELOPMENT

MESODERM

ureteric bud   metanephric mesenchyme

collecting tubules & ureters   nephrons

Pax2  Wt1

developing nephron
Can the stem cells generate cells of the developing nephrons and ureteric buds?

To compare nephrogenic potential of the different stem cell types, necessary to develop a common assay.
Development of a common assay

Disaggregate -> re-aggregate

Nat Biotech 2002
Generation of histocompatible tissues using nuclear transplantation.
Lanza et al, group of Atala

Pax2 Wt1
intact kidney rudiment

Pax2 Wt1
re-aggregated kidney rudiment

Unbekandt & Davies  Kidney Int, 2010
Evaluating the nephrogenic potential of the different stem cell types
Embryonic stem cells (ESCs) and ESC-derived mesoderm
Effect of exogenous cells on chimera development

Low magnification of kidney chimeras stained for Wt1

Higher magnification of control kidney chimeras stained for Wt1 (green)
Effect of exogenous cells on chimera development

- Number of developing nephron-like structures per mm²
  - KRC
  - ESC
  - Bra+  
  - Bra-

- Area [µm²] of developing nephron-like structures
  - KRC
  - ESC
  - Bra+ 
  - Bra-
ESC-derived mesoderm integrated into developing nephrons

Extent of integration of QD-labelled cells into developing nephrons [%]

KRC  ESC  Bra+
Expression of kidney specific markers by QD\(^+\) cells in chimeras

ESC-derived mesoderm can generate MM
ESC-derived mesoderm can generate podocytes and proximal tubule cells
Expression of kidney specific markers by QD$^+$ cells in chimeras

ESC and ESC-derived mesoderm can generate UB cells
Proximal tubule cells in intact kidney rudiments display secretory function.
ESC-derived mesodermal cells in chimeric rudiments display secretory function
Summary of mouse ESC work

• non-mesodermal precursors derived from ESCs inhibited kidney growth and nephrogenesis

• ESC-derived mesodermal cells have nephrogenic potential as they can integrate into glomeruli & transporting tubules

• ESC-derived mesoderm could potentially replace damaged cells in diseased kidneys?
Mouse kidney-derived stem cells
Cells isolated from neonatal kidneys can be expanded in vitro and express key MM-specific markers.

Question: does the population contain stem cells?
H6 clonal line can give rise to podocyte and proximal tubule-like cells in culture
Developing culture substrates to direct PTC differentiation

H6 mKSC clonal line, 3000 cells/13mm cover slip, 4d culture

Isabel Hopp, collaboration with Biomer Technology Ltd
Collagen IV expression

H6 mKSC clonal line, 1000 cells/13mm cover slip, 4d culture
Atomic Force Microscopy – Roughness

The image shows AFM images of samples labeled ESP 03, ESP 04, ESP 07, and BTLAT 15. Each sample is represented by an AFM image and a bar graph showing RMS roughness. The RMS roughness values for each sample are:
- ESP 03: 230.3 nm
- ESP 04: 451.5 nm
- ESP 07: 64 nm
- BTL AT 15: 643.8 nm

The bar graphs also show a trend in roughness across the samples.
H6 kidney stem cells display megalin-dependent uptake of albumin

Can the cells integrate into the kidney rudiment?
Rudiment assay used to investigate nephrogenic potential of H6 kidney stem cells
H6 kidney-derived stem cells (KSC) integrate into nascent nephrons

WT1/Vybrant-labelled KSC
Extent of KSC integration is similar to that of metanephric mesenchyme.
KSCs generate proximal tubule cells and podocytes within developing nephrons

Megalin  QDot

Synaptopodin  Laminin  QDot
Summary of mouse KSC work

• KSCs from neonatal mouse kidney are clonogenic

• KSCs can generate podocyte and proximal tubule-like cells (PTCs) \textit{in vitro}

• Some synthetic substrates appear to promote PTC differentiation

• KSC-derived PTCs show some functionality

• KSCs can integrate into developing nephrons to a similar extent as MM

• KSCs can generate podocytes and PTCs within kidney rudiments \textit{ex vivo}
Mouse and human mesenchymal stem cells
Mouse MSCs do not integrate into developing nephrons

**Wt1 Laminin**
Qdot-labelled MSC

**Wt1 Laminin**
Qdot-labelled KSC

Human MSCs do not integrate into developing nephrons

**Wt1** human nuclear-specific antigen
- hMSC
+ hMSC
MSCs have a detrimental effect on kidney development

**Wt1 laminin**
No stem cells

**Wt1 laminin**
QD-labelled mMSC
Normal medium
mMSC-conditioned medium
Integration of mMSCs following culture in KSC-conditioned medium
Summary of MSC work

• MSCs do not integrate into developing nephrons

• MSCs have a detrimental effect on kidney development

• Culturing mMSCs in KSC-conditioned medium can improve their integration potential and prevent the adverse effects on kidney development.
## Overall summary of KIDSTEM

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Ureteric bud</th>
<th>Nephron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney progenitors</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>mESC-derived mesoderm</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>hESC-derived mesoderm</td>
<td>-</td>
<td>+</td>
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<tr>
<td>mMSC</td>
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<tr>
<td>hMSC</td>
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<td>-</td>
</tr>
<tr>
<td>mKSC</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Amniotic fluid stem cells</td>
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AIM: To investigate the potential of human kidney-derived stem/progenitor cells for use in drug discovery and regenerative therapy programmes

1. Isolate and characterise human kidney stem cell lines
2. Direct differentiation to renal cell types using novel biomaterials
3. Investigate physiological and pharmacological properties to determine which lines have potential for drug discovery
4. Determine which lines have potential for use in cellular-based therapies to treat kidney disease
Potential of cell based therapies to treat kidney disease in animal models

Rat cisplatin and remnant kidney model
University of Heidelberg

Foetal kidney cells
*UCL*

Infant/juvenile kidney cells
*University of Liverpool*
Ilaria Santeramo

Adult kidney cells
*University of Turin*

Reprogrammed cells
*Tel Aviv*

**Efficacy** will be monitored by standard biochemical and histological analyses and by measuring the GFR using a novel electronic device.
Potential of kidney stem/progenitor cells KSPCs to generate specialised renal cells \textit{in vitro}

- Polyacrylate substrates
  - Biomer Technology and University of Liverpool
  - Isabel Hopp
- Hydrogels
  - University of Dresden
- Aligned polymeric substrates
  - University of Liverpool
  - Yonghong Yang

- PTCs and podocytes
  - University of Liverpool
  - University of Nijmegen
  - University of Leuven
  - University of Turin
Safety of therapies for regenerative medicine

Kevin Park and Patricia Murray

Safety Hub Director          Scientific Lead
Safety Issues Associated with Regenerative Medicine Therapies

• Biodistribution

• Purity and phenotype of administered cells

• Toxicology

• Tumorigenicity

• Immunogenicity
University of Liverpool Centre for Preclinical Imaging (CPI)

9.4T Bruker system. High resolution with excellent soft tissue contrast with no depth limitation and functional imaging techniques but has low sensitivity. Cell tracking methodologies

MRI

Multispectral optoacoustic imaging (MSOT)

Bioluminescence/Fluorescence

Safety Hub PhD students taking part in MSOT training course (L-R, Oihane, Lauren, Joe)

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Ivis Spectrum. Sensitive imaging of luciferase positive cells. Cell tracking and cell viability imaging methodologies are based on cells that have been genetically modified to express luciferase. Fluorescence component permits functional imaging using near infrared probes to detect apoptosis, inflammation, etc.
Future Outlook

• 4 years: establish risk:benefit ratio of cell therapies in rodent models of kidney disease.
  ....Is human translation appropriate?

• How do the administered cells elicit their beneficial effects?
Researchers and collaborators

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