Experimental platforms to address mesangial repair: What has been accomplished

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Focused on mesangial repair and restoration of the mesangium after homeostasis has been altered, various experiment models including in vitro, ex vivo and in vivo have been used in our laboratory in the last 30 years.

The most recent animal models have recreated the various mesangiopathies that are characteristic of glomerulonephritis.
Glomeruli composed of endothelial cells, visceral / parietal epithelial cells and mesangial cells,

Normally, light chains pass through the capillary walls into urinary space and catabolized in proximal tubules.

G-LCs interact with mesangial cells and alter mesangial homeostasis.
Mesangial cells and glomerulopathic light chains
Live cell micromanipulator system
6-D Live cell imaging system
Ex-Vivo kidney perfusion system
In vivo Mouse Penile injection model
Isolation technique of mouse glomeruli

1. Perfusion with Dynabeads diluted in PBS through the heart

2. Removing Kidneys and Mincing into 1mm³ pieces

3. Digestion of tissue with Collagenase

4. Filtration of the tissue with 100μm cell strainer

5. Isolation of glomeruli by a magnet

**Experiment Design**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
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</thead>
<tbody>
<tr>
<td>4d</td>
<td>Quiescent G-LC (10ug/ml)</td>
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<tr>
<td>1W</td>
<td>Apoptosis Inducer / + Stem Cell</td>
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<tr>
<td>1</td>
<td>Cell level - 2D: MCs incubated as monolayer</td>
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<tr>
<td>2</td>
<td>Cell level - 3D: MCs growing on Matrigel</td>
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<tr>
<td>3</td>
<td>Cell level - 6D: Live cell imaging system</td>
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<tr>
<td>4</td>
<td>Glomeruli level - In vitro single glomerular system</td>
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<tr>
<td>5</td>
<td>Animal level - In-Vivo penile injection</td>
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<tr>
<td>6</td>
<td>Kidney level - Ex-vivo kidney regeneration system</td>
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</tbody>
</table>
Co-localization of AL-LC-Texas red and LCDD-LC-fluorescein on HMC
Crosslinking of G-LCs to HMCs membrane

- DTSSP: 3,3′-Dithiobis (sulfosuccinimidylpropionate), is a water-soluble and membrane impermeable crosslinker.
- DTSSP is well used for cell-surface crosslinking.
Curcumin, EGCG and PDTC prevented \textbf{NFkB} translocation in \textit{G-LCs} treated \textit{HMC}
AL-LCs induced expansion of lysosomal compartment

AL-LCs but not LCDD-LCs co-localized with mature lysosomes
Numerous lysosome formed and amyloid fiber precursor processed in mouse glomerular lysosome after penile injection of ALLC for 2 weeks.
LCDD-LC induced ER expansion in HMCs
LCDD-LCs increased ribosomes and RER in HMCs
Ex-vivo Rat Kidney Perfusion Model

Control

ALLC

LCDDLC
Penile injection control group
Penile injection ALLC group

ALLC-λ 7day

ALLC-λ 14day
Mouse penile injection model SEM

Control  ALLC 1W  ALLC 2W
Amyloid fiber formed in glomeruli of WT Mouse after penile injection of ALLC for 2 weeks
Ex vivo Kidney perfusion

Control 24h

ALLC 24h
Penile injection LCDD-LC group

LCDD-k 7day

LCDD-k 14day
Penile injection LCDD-LC group

LCDD-k 7day

LCDD-k 14day

IH staining for tenascin
Mechanism of G-LC induced Glomerula Damage

1. Cell membrane interaction
   - G-LCs compete for the same receptor on HMCs surface.

2. Signal transduction
   - G-LCs induce NF-κB and c-fos into HMCs nuclei
   - C-fos modulates gap junctions of HMCs
   - There are different pathways for LCDD-LC and AL-LC in MCs

3. Secretion pathway
   - Blocking ER secretion pathway inhibits the LCDD-LC-induced accumulation of Tenascin but decrease of Collagen IV in ECM which could be modified by MMP7 and stem cells
   - Mesangial amyloid formation in the perfused kidney

4. Extracellular Matrix
   - Amyloid Fibrils
   - TENASCIN
   - Collagen IV
   - Fibronectin
   - Laminin
Stem cell differentiation, restoration to functionality and cellular regeneration
CD 68 expression enhanced in HMCs and RMCs but not in RMSCs after ALLC treatment for 96h
ALLC treated RMCs change from cytoplasmic smoothelin\(^+\) to CD68\(^+\) and reversed staining for RMSCs.
CD 29 not expressed in RMCs and faded away in RMSCs treated with G-LCs
CD 54 expressed in RMSCs but not in RMCs, and decreased in the G-LC treated RMSCs
Excellular matrix homeostasis restoration
LCDD-LCs induce mesangial ECM over-production and nodule formation

IN-VITRO:
Hman, rat, mice glomerular cells and/or MSCs remodeling or reconstruction in Matrigel model

EX-VIVO:
Rat/mice perfusion model

IN-VIVO:
Mice penile injection model
Collagen IV and tenascin-C expressions in the glomeruli obtained from renal biopsies of patients with glomerulopathies.

(a) Significantly higher expressions of tenascin-C expressions in the glomeruli from LCDD patients as compared with AL and controls,
(b) Analysis of the staining patterns showed significant differences in the expressions of both collagen IV and tenascin-C among the study groups.
MMP-7 and tenasin-C expressions in HMCs treated with different light chains for 96 h.

LCDD-LC significantly increased extracellular Tenasin-C and intracellular MMP-7

a) The addition of exogenous tenasin-C with light chains enhanced expression of MMP-7 in HMC.

b) LCDD-LC increased intracellular MMP-7 and the extracellularly tenasin-C, in which appear as accentuated globules within the cytoplasm (red circles).
MMP-1, -3, and -7 expressions in the groups of G-LC treated HMCs

(a) LCDD and ALLC induced intracellular expressions of MMP-7 but not that of MMP-1 and MMP-3
(b) MMP-1 and MMP-3 expressed stronger band in G-LC treated HMC, but MMP-7 did not.
Collagen IV decreased and Tenascin increased in LCDD-LC treated Rat MCs while MMP7 recovered the changes

Immunofluorescent double-staining of Collagen IV and Tenascin
LCDD-LC induced apoptotic HMC and Tenascin Rich ECM
HMSCs restore the LCDD-LC damaged HMCs and restore the Tenascin Rich ECM back to Collagen IV dominated ECM.
These experimental platforms have allowed us to correlate in-vitro data with animal results and have shown that the sequence of events participating in the pathological processes are identical.

Each platform offers specific advantages and disadvantages to answer different questions.
Combining the data that has been obtained has resulted in a full appreciation of what is involved in the genesis of the mesangial damage.

The use of stem cells to repair and restore the damaged mesangium has provided insights into how mesenchymal stem cells do so offering a promising new therapeutic avenue to treat conditions such as diabetic nephropathy and thrombotic microangiopathies among others.
Our long-term objective

- Development of therapeutic modalities to prevent, ameliorate or slow down irreversible renal damage
- To make the present apparently irreversible renal damage reversible in the future.
Thank you!