Fibrotic Diseases

New Targets and Model Development

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In Vivo Pharmacology
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Kenneth Zhang, Ph.D.

- Associate Director, *In vivo* pharmacology at GenScript

- Tumor immunologist with expertise in tumor microenvironment
  - Published over 19 peer-reviewed publications

- Leads the development of:
  - Hepatic, renal and pulmonary fibrosis models
  - SC xenograft, orthotopic and syngeneic tumor models
Outline

1. Fibrotic diseases
2. Anti-fibrotic therapeutic targets
3. GenScript fibrotic disease models
Fibrotic Diseases

- Organ structure – parenchyma and interstitium
- Fibrotic diseases are the replacement of organ parenchyma by interstitium - excessive extracellular matrix (ECM) deposit during chronic injury, which progressively leads to impair or loss of organ function.

PubMed Indexed Publications on Fibrosis

![Graph showing the number of PubMed indexed publications per year for different keywords: Fibrosis, Lung fibrosis, Liver fibrosis, and Kidney fibrosis. The graph indicates an increase in publications over time, with a significant rise in publications related to Fibrosis.](image-url)
Hepatic Fibrosis

◆ Nature
  • A reversible wound-healing response characterized by the accumulation of ECM following liver injury. Chronic injury and inflammation leads to progressive substitution of liver parenchyma by scar tissue (fibrosis) and finally end stage liver disease (cirrhosis).

◆ Etiology
  • The diseases can be induced by HBV/HCV infection, alcohol abuse, nonalcoholic steatohepatitis (NASH), chronic cholestatic disorders (including primary biliary cirrhosis) and autoimmune hepatitis etc.

◆ Therapy
  • Currently no drug approved
  • Obeticholic acid designated Breakthrough Therapy by US FDA in 2015 (Farnesoid X receptor agonist).
Development of Liver Fibrosis and Cirrhosis

- Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) infections
- Parasites
- Alcohol (ASH)
- Cryptogenic
- Drugs and toxins
- Obesity (NAFLD)
- Metabolic diseases
- Venous obstruction

Liver cell injury leads to inflammation, which activates hepatic stellate cells (HSC) and expansion of myofibroblasts (MFB) to form fibrosis. Mediators such as IGF-1, PDGF, TGF-β, ET-1, and ROS contribute to the process. Products like collagen, elastin, glycopolymers, proteoglycans, and hyaluronan are secreted.

Cirrhosis results from the progression of fibrosis, leading to primary liver cell carcinoma.

Gressner, Comparative Hepatology, 2007
Hepatocyte injury and release of reactive oxygen species (ROS) and apoptotic bodies

Activation of immune-subset cells and hepatic stellate cells (HSC), and their cross-talk
  • Immune infiltration and Kupffer cell activation
  • Hepatic stellate cell activation, proliferation and trans-differentiation into myofibroblasts (α-SMA+)

Excessive ECM protein production by myofibroblasts in the space of Disse, blocking substance exchange between hepatocytes and the blood

Bataller, J Clin Invest, 2005
Key Soluble Factors Involved

- **TGF-β**
  - Secreted by Kupffer cells and activated HSCs
  - Enhances the transition of HSCs into collagen-producing myofibroblasts, stimulates the synthesis of ECM proteins and over-expression of TIMP.

- **PDGF**
  - Secreted mainly by Kupffer cells
  - Predominant mitogen of activated HSCs.

- **MCP-1 (CCL2)**
  - Recruits monocytes

Schuppan, *JCI*, 2013
Sources of Myofibroblasts in Liver Fibrosis

Pulmonary Fibrosis

- The development of excessive connective tissue in the lung – most cases are idiopathic pulmonary fibrosis (IPF)
- Irreversible impairment of respiratory function
- Etiology:
  - Idiopathic; Secondary effect of Inhalation of pollutants or pathogens, Cigarette smoking, Connective tissue disease, Infections, etc
- Limited therapeutic:
  - Pirfenidone and Nintedanib approved (Oct 2014) by US FDA

Wynn, *Integrating mechanisms of pulmonary fibrosis*, 2011
Renal fibrosis is the common end point of virtually all chronic (progressive) kidney diseases.

Renal fibrosis includes
- Glomerulosclerosis
- Tubulointerstitial fibrosis -> Renal failure
  - Excessive production of ECM between microtubules and peritubular vasculature
Renal Fibrosis in Response to Epithelial Injury

Common Features of Fibrotic Diseases

- Epithelial injury and dysfunction
- Accumulation of myofibroblasts
- Deposition of fibrotic ECM
- Inflammation and recruitment of immune infiltrate
- Pro-fibrotic macrophages
## Anti-fibrotic Therapeutic Targets

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β expression and activation</td>
<td>Pirfenidone; αvβ6 antibody; ATI and ATII receptor blockers; ACE inhibitors; CAT-192 (anti-TGFβ1 mAb)</td>
</tr>
<tr>
<td>TGF-β signaling pathways</td>
<td></td>
</tr>
<tr>
<td>TGFβR1 (ALK5)</td>
<td>SM305</td>
</tr>
<tr>
<td>SMAD3</td>
<td>SIS3</td>
</tr>
<tr>
<td>CTGF</td>
<td>Antibody</td>
</tr>
<tr>
<td>Tyrosine kinase inhibitors of non-canonical TGF-β and other signaling pathways</td>
<td></td>
</tr>
<tr>
<td>c-Abl, c-kit, PDGFR</td>
<td>Imatinib</td>
</tr>
<tr>
<td>PDGFR, c-Abl, src</td>
<td>Dasatinib</td>
</tr>
<tr>
<td>PDGFR, c-Abl, c-kit</td>
<td>Nilotinib</td>
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<tr>
<td>PDGFR, VEGFR, FGFR</td>
<td>Nintedanib (BIBF1120)</td>
</tr>
<tr>
<td>VEGFR, PDGFR</td>
<td>Sorafenib</td>
</tr>
<tr>
<td>Transcription factors</td>
<td></td>
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<tr>
<td>FXR</td>
<td>Agonist (Obeticholic acid)</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Agonist (Rosiglitazone)</td>
</tr>
<tr>
<td>AP-1</td>
<td>T-5524</td>
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Adapted from Rosenbloom, *Biochimica et Biophysica Acta*, 2013
## Anti-fibrotic Therapeutic Targets (cont’d)

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
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<tbody>
<tr>
<td>Recruitment of fibrocytes and monocytes/macrophages</td>
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<tr>
<td>CXCL12 (SDF-1)</td>
<td>Antibody</td>
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<tr>
<td>CXCR4</td>
<td>AMD3100</td>
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<tr>
<td>MCP-1/CCR2</td>
<td>Inhibitors (PF-04136309)</td>
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<tr>
<td>Immune response regulator</td>
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</tr>
<tr>
<td>IL-13</td>
<td>Antibody</td>
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<tr>
<td>IL-6 receptor</td>
<td>Tocilizumab</td>
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<tr>
<td>TLR</td>
<td>TLR inhibitors (E5564, TAK-242)</td>
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<td>Other intracellular pathways</td>
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<td>JAK2</td>
<td>TG101209</td>
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<tr>
<td>Wnt</td>
<td>Dkk-1</td>
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<td>RhoA</td>
<td>Statins, GGT inhibitor</td>
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<td>JNK</td>
<td>CC-930</td>
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<td>NOX4 (production of ROS)</td>
<td>GKT136901</td>
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<tr>
<td>Endothelin-1</td>
<td>Bosentan, other ET receptor blockers</td>
</tr>
<tr>
<td>Matrix stiffness, collagen crosslinking</td>
<td>D-penicillamine, clostridial collagenase</td>
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</tbody>
</table>

Adapted from Rosenbloom, *Biochimica et Biophysica Acta*, 2013
GenScript Fibrotic Disease Models

- Hepatic fibrosis
  - $\text{CCl}_4$-induced hepatic injury and fibrosis in rats
  - $\text{CCl}_4$-induced hepatic injury and fibrosis in mice
  - BDL-induced hepatic injury and fibrosis in rats
  - BDL-induced hepatic injury and fibrosis in mice
  - TAA-induced hepatic fibrosis in mice (in development)

- Renal fibrosis
  - UUO-induced renal fibrosis in rats
  - UUO-induced renal fibrosis in mice

- Pulmonary fibrosis
  - Bleomycin-induced pulmonary fibrosis in mice

Note:
BDL, Bile duct ligation
TAA, Thioacetamide
UUO, Unilateral ureteral obstruction
CCl\textsubscript{4}-induced Liver Fibrosis in Mouse
**CCl₄-induced Fibrosis (Intoxication)**

- Animal: Balb/c mice
- CCl₄-olive oil mixed solution; i.p. injection
- Induction of hepatocyte damage, necrosis, inflammation and fibrosis

### Available endpoints of measurement

<table>
<thead>
<tr>
<th>Liver and body weight</th>
<th>IHC for myofibroblasts &amp; macrophages</th>
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<tbody>
<tr>
<td>Serum biochemistry</td>
<td>Quantitative analysis of histol. images</td>
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<tr>
<td>HE staining</td>
<td>Hydroxyproline assay</td>
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<tr>
<td>Sirius Red staining</td>
<td>Profiling of markers by RT-qPCR</td>
</tr>
<tr>
<td>Masson trichrome staining</td>
<td></td>
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</tbody>
</table>
Disease Development at 6 Weeks

H&E
Olive oil only

Sirius Red

Myofibroblasts (α-SMA)

Macrophages (F4/80)

CCR₄

Olive oil only

CCl₄
Disease Development at 6 Weeks (continued)

A

**ALT**

<table>
<thead>
<tr>
<th></th>
<th>Olive oil</th>
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<td>IU/L</td>
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**AST**

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<tr>
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<tbody>
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<td>IU/L</td>
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<td>1500</td>
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B

**Hydroxyproline**

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<tr>
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<th>Olive oil</th>
<th>CCl4</th>
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<tr>
<td>µg/g</td>
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<td>500</td>
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C

**Acta2**

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<td>Fold change (mRNA/β-actin)</td>
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**Col1a1**

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**Tgfb1**

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Olive oil

CCl4
Case Study
Therapeutic Effect of Pirfenidone at 6 Weeks

A

Olive oil  CCl₄  CCl₄+PFD

B

Sirius Red

Relative Sirius Red⁺ Area

C

Tgfβ1

Fold change (mRNA/β-actin)

Col1a1

Fold change (mRNA/β-actin)
Bile-duct Ligation (BDL)-induced Liver Fibrosis in Mouse and Rat
Bile-duct Ligation-induced Fibrosis (Cholestasis)

- Animal: C57BL/6 mice; Wistar rats
- Surgery: Ligation of common bile duct (BDL)
- Study conclusion at 2-4 weeks after surgery.

Available endpoints of measurement

- Liver and body weight
- Serum biochemistry
- HE staining
- Sirius Red staining
- Masson trichrome staining
- IHC for myofibroblasts & macrophages
- Quantitative analysis of histol. images
- Hydroxyproline assay
- Profiling of markers by RT-qPCR
Development of the Experimental Disease in Mouse

- Hepatocellular injury and proliferation
- Portal inflammatory response
  - Neutrophils, B/T cells, Kupffer cells
- Cholangiocellular proliferation
- Fibrosis development

Georgiev, Br J Surg., 2008
Model Development in Mouse

A

 ALT

<table>
<thead>
<tr>
<th>Time after BDL (days)</th>
<th>Sham</th>
<th>BDL</th>
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<tbody>
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<td>2</td>
<td><img src="image1.png" alt="Graph" /></td>
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AST

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<td>14</td>
<td><img src="image6.png" alt="Graph" /></td>
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B

2 wks

Sham

BDL

4 wks

8 wks

Sham

BDL
Model Development in Rat – Serum Biochemistry

ALT

AST

TBIL

Days after surgery

Days after surgery

Days after surgery

Sham
BDL

NS
NS

NS
NS

NS
NS

NS
NS
Model Development in Rat - Histology

Hydroxyproline content (μg/g)

Days after surgery

Sham
BDL

**

40x
100x

H&E

BDL vs Sham:
**

Sham
BDL

Days after surgery

14
28

Hyp content (μg/g)
Unilateral Ureteral Obstruction (UOO)-induced Renal Fibrosis in Rat
Method

- Animal: SD rats
- Surgery: Ligation of the ureter of left kidney, leaving the contralateral kidney as a control
- Induction of chronic epithelial injury, inflammation and tubulointerstitial fibrosis in left kidney

Available endpoints of measurement

- Kidney and body weight
- Serum biochemistry
- HE staining
- Sirius Red staining
- Masson trichrome staining
- IHC staining
- Quantification analysis of histological images
- Hydroxyproline assay
- RT-qPCR
Model Development

A. Renal weight/body weight

B. Hydroxyproline content

C. BUN

C. CREA
Model Development Shown by Sirius Red Staining (100x)

Sham

Day 3

Day 7

Day 14

Day 21

Day 28
Case Study
Therapeutic Effect of Pirfenidone at 4 Weeks

A

Sham
UUO
UUO+PFD

Sirius Red
100x

H&E
200x

B

Sirius Red

C

Hydroxyproline content

Sham
UUO
UUO+PFD

**
*
Bleomycin-induced Lung Fibrosis in Mouse
Model Development

- Animal: C57BL/6 mice
- Disease induction: Intratracheal instillation of bleomycin (BLM)
  - Induction of lung epithelial injury, inflammation and fibrosis

Available endpoints of measurement

- Lung and body weight
- BALF cell count
- HE staining
- Masson trichrome staining
- IHC staining
- Quantification analysis of histological images
- Ashcroft score
- Hydroxyproline assay
- RT-qPCR
Histopathological Changes 3 Days after Bleomycin Instillation (HE Staining)

Saline

Bleomycin dose gradient
Model Development

A. Animal Survival

B. Body weight

C. BAL total cells

D. Lung weight/body weight

E. Hydroxyproline content

F. Ashcroft score
## Model Development - Histology

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Bleomycin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>H&amp;E staining</td>
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</tr>
<tr>
<td>(40x)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masson’s trichrome staining</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(40x)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- H&E staining (40x)
- Masson’s trichrome staining (40x)
There is huge unmet clinical need for effective and potent therapeutics of fibrotic diseases.

Properly developed animal models that mimic diseases in human are critical for efficacy evaluation of leads.

GenScript has developed multiple fibrotic disease models and will develop more to facilitate drug discovery and development for fibrotic diseases.
Discovery Biology Services

**Antibody and Protein Engineering**
- Single domain antibody generation
- Antibody sequencing
- Affinity maturation and humanization

**In-vitro Pharmacology**
- CellPower™ custom stable cell line for assays
- GenCRISPR™ custom knock-out or knock-in cell lines
- Cell-based assays and Ion channel and GPCR assays

**In-vivo Pharmacology**
- Tumor models including SC xenograft, orthotopic and syngeneic
- Bioluminescence imaging of tumors
- Fibrosis models
Thank you for your participation
We wish you all success in your research
Email me: Maxine.Chen@GenScript.com

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