Molecular mechanisms of Fibrosis: Targets of Therapy

John P Iredale
University of Edinburgh, UK
Take home messages:

• The wound healing MFBs of the liver and the hepatic macrophages are key players in progressive and resolving fibrosis.

• The fibrotic liver retains a significant capacity for matrix degradation which is held in check.

• Resolution of fibrosis is characterised by apoptosis of the HSC/MFBs and matrix degradation associated with diminished hepatic TIMP levels.

• Strategies based on harnessing the matrix degrading capacity of the liver and using HSCs and MSCs as delivery vehicles remain attractive.
Can we demonstrate the influence of macrophages on the fibrotic response in a mechanistic experimental system?

6wks  8wks  12wks CCl₄

Macrophages populate hepatic scars during injury
Using the DTr mouse MØ can be effectively and specifically deleted in progressive CCl$_4$ induced fibrosis.
Fibrosis: Effect of experimental Macrophage depletion

12 weeks CCl₄

+ Macrophages

- Macrophages

Duffield....Iredale JCI 2005
Galectin-3

β-galactoside binding lectin

Found in all 3 cellular compartments

One of a family of 14 currently identified galectins

Galectins highly conserved through evolution
Galectin-3 regulates liver fibrosis

**WT**

**Galectin-3**

Collagen

% Collagen

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<td>Galectin-3⁻⁻</td>
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Procollagen/I/18S

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Henderson Iredale et al, PNAS 2006
Myofibroblast activation is defective in vivo.

α-SMA

WT

Galectin-3⁻/⁻

Henderson..Iredale et al, PNAS 2006
Macrophage-derived Galectin-3 drives myofibroblast activation in the kidney following UUO

Henderson et al Am J Path 2008
GELATIN SEPHAROSE CHROMATOGRAPHY OF HSC CONDITIONED MEDIA

Gelatinase activity in culture media following TIMP-1 removal

µg Gelatin degraded/18 hr

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<td>46.8</td>
<td>2.4</td>
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% inhibition of gelatinase activity

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HSC and Other NPCs/ICs express MMPs

Latent capacity for degradation of fibrillar matrix is held in check

Activated HSC/MFB

TIMP

Matrix degrading metalloproteinase (MMP)

MMP activity inhibited

No matrix degradation occurs

Collagen I-rich scar matrix

Iredale et al JCI 1992
REVERSIBILITY OF CCl₄ MODEL

4 weeks

4 weeks + 10 days recovery

Iredale JCI 1998
Collagenase activity during recovery

TIMP is reduced
Matrix degradation occurs

Iredale JCI 1998
REVERSIBILITY MODEL: NUMBERS OF ACTIVATED STELLATE CELLS

αSMA positive cells per high power field

Fibrosis
Days of recovery

A
B
C
D

21 1 2 7 42 sham
Fibrosis

PROGRESSION

QUIESCENT HSC

ACTIVATED HSC

APOPTOTIC HSC

Products of damaged cells

HEPATOCYTE KUPFFER CELL INFLAMMATORY CELL

Number HSC

↑ TIMPs

↑ Collagen

↓ Collagenase

Gal 3

TGF B1

PDGF etc

TIMP
Collagen Cross-linking may limit recovery from fibrosis

12 Week CCl₄ Days of Recovery

0Days  |  84Days  |  366Days

Elastin

+tTG

Issa et al Gastroenterol 2004
Stylised diagrammatic summary of resolution in micronodular cirrhosis

12 Weeks CCl\textsubscript{4} MICRONODULAR CIRRHOSIS

12 Weeks CCl\textsubscript{4} + 168d – 366d MACRONODULAR CIRRHOSIS

X-Linked Elastin rich

PT
Evidence for limited matrix degradation in human explant material

Wanless 2000
Assessing the role of collagen-I in mediating HSC survival

Wild Type collagen I

- MMP
- Complete Degradation

Mutant collagen I

- MMP
- Persistence

Day 0

- αSMA
- S Red

Day 28

- αSMA
- S Red

Issa et al. FASEB J 2002
Apoptosis in Cell Populations in WT and r/r mice During Recovery from Fibrosis Determined by TUNEL

Mean TUNEL positive cells in the fibrotic band /HP

WT  r  WT  r  WT  r  WT  r
PF0  PF4  PF7  PF28

Activated HSC
Fibrosis

PROGRESSION

QUIESCENT HSC
- Number HSC
- TIMPs
- Collagen

ACTIVATED HSC
- Number HSC
- TIMPs
- Collagen
- MMPase

APOPTOTIC HSC
- Number HSC
- TIMPs
- Collagen
- MMPase

Factors favouring survival:
- Cytokines/soluble factors
- Matrix stabilisation
- Cell-cell receptor stabilisation

Factors favouring apoptosis:
- Death receptor activation
- Withdrawal of survival factors:
  - Matrix degradation
  - Cell receptor degradation

HEPATOXYTE
KUPFFER CELL
INFLAMMATORY CELL
Fibrosis

PROGRESSION

QUIESCENT HSC

Number HSC
↑TIMPs
↑Collagen
↓MMPase

ACTIVATED HSC

Number HSC
↑TIMPs
↑Collagen
↓MMPase

APOPTOTIC HSC

↓Number HSC
↓TIMPs
↓Collagen
↑MMPase

What is the source of the Collagenase and other key MMPs? Is there a role for M’phages/inflamm cells in recovery?

KUPFFER CELL
MACROPHAGE
In the long term, persistent scars are hypocellular:

** relative paucity of (partic) inflammatory cells
HSC/MFBs express abundant TIMP-1 mRNA, Macrophages express MMPs 12 and 13: *in situ*
Depletion of scar associated macrophages attenuates resolution of liver fibrosis

Peak fibrosis

7d resolution: control

7d resolution: depletion

Duffield JS et al, J Clin Invest 2005
Effect of conditional macrophage depletion on MMP-13 mRNA: *in situ* hybridisation

Cells per 10 high power fields

\[ * p<0.05 \]

Fallowfield et al JI 2007
Fibrosis

**PROGRESSION**
- QUIESCENT HSC
- ACTIVATED HSC
- APOPTOTIC HSC

**RESOLUTION**

- TIMP-1
- Number HSC down
- TIMPs down
- Collagen down
- MMPase up

HEPATOECTY
KUPFFER CELL
INFLAMMATORY CELL
Summary

Progressive Fibrosis
- Proliferative response to hepatocyte damage
- Hepatocytes
- Intact Collagen-I
- Activated MFB
- TFBSurvival
- Collagen-I
- TIMP/MMP Balance
- MØ as regulator and ?Vehicle

Resolution of Fibrosis and Parenchymal Renewal
- Proliferative response to hepatocyte damage
- Degraded Collagen-I
- MFB apoptosis
- Collagen-I
- Block/vehicle
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