LIVER FIBROSIS REVISITED

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Fibrosis in Pathology

• A ubiquitous and deleterious mechanism
• Deposit of Extracellular Matrix (ECM)
• Transient if remodeling occurs (scaring process), permanent if no or partial remodeling
• If protracted, fibrosis impair the structure and function of any organ
• A mechanism driven by:
  – Uncontrolled chronic inflammatory process
  – Ischemia / anoxia
  – Toxic / Metabolic ...
LIVER FIBROSIS AS A PARADIGM

- Development of fibrosis is the rule for any untreated chronic liver disease
- The endpoint of fibrosis in the liver is cirrhosis
- A major clinical concern: cirrhosis is the major cause of mortality in hepatology
Histological stage of fibrosis predict survival in chronic liver diseases

Overall survival according to stage of fibrosis in index liver biopsy

Non Alcoholic liver diseases

Hepatitis C

Younossi ZM, Stepanova M, Rafiq N, et al.. Hepatology 2011

MH. Khan et al. Hepatology 2000; 31:513-520
Etiology of chronic liver disease with high risk of developing fibrosis

- Alcoholic liver disease
- NAFLD, Diabetes
- Chronic hepatitis C
- Chronic hepatitis B
- Autoimmune hepatitis
- Cholangiopathies (PBC, PSC, AIC), biliary obstruction
- Genetic diseases (Wilson, haemochromatosis, $\alpha_1$-antitrypsin deficiency, telomerase gene mutation....)
- Portal venopathy
- Bud Chiari syndrom, chronic cardiac failure
- ........
Micronodular cirrhosis
Alcoholic, NAFLD, Hepatitis C

Macronodular cirrhosis
Hepatitis B, Autoimmune

«Meganodular» cirrhosis
Vascular liver disease

irregular cirrhosis
Biliary disease
Agenda

• Liver Fibrosis: a complex and highly organized 3-D structure
• Cellular mechanisms driving liver fibrosis
• Detection and quantification of liver fibrosis
• Liver fibrosis: a dynamic process
Liver Fibrosis: a complex and highly organized 3-D structure

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Extracellular Matrix: a multimolecular network of proteins and glycoproteins

More than 100 different molecules
Composition of ECM may vary according to organs
Collagens: 25% of the proteins in the human body

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<tr>
<th>Proteins</th>
<th>Glycoconjugates</th>
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<tr>
<td>Collagens</td>
<td>Structural Glycoproteins</td>
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<td>Fibrillar: Type I</td>
<td>Fibronectin</td>
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<td>Hyaluronic acid</td>
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ECM: Highly organized 3-D structure

Light microscopy: Fibrillar collagens and Basement membranes

From: http://jpkc.scu.edu.cn/
FIBRILLAR COLLAGEN
Highly organized 3D structure

COLLAGEN MOLECULE →→→ FIBRILS →→→ FIBERS

CROSSLINKS
BASEMENT MEMBRANE
Highly organized 3-D structures:

Type IV Collagen

Laminin
Agenda

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➢ Cellular mechanisms driving liver fibrosis

• Detection and quantification of liver fibrosis

• Liver fibrosis: a dynamic process
HEPATIC STELLATE CELLS
The cellular origin of liver fibrosis
HEPATIC STELLATE CELLS
The cellular origin of liver fibrosis

HEPATIC STELLATE CELL ACTIVATION

Quiescent HSC (Lipocyte)  Activated HSC (myofibroblast)
HEPATIC STELLATE CELL ACTIVATION

Alpha-SMA immunostaining
HEPATIC STELLATE CELL ACTIVATION

Rodent hepatic stellate cells in culture

Day 0

Day 5
Block HSC activation:
- Alpha / gamma interferon
- PPARγ ligands
- PDGF-R antagonist (Glivec)
- RTK antagonist
- ET-1 and ET1-R antagonists
- NO donors
- TGF et TGF-R inhibitors
- Col inhibitors
- TIMP inhibitors
- MMPs
- Apoptotic ligands e.g. TRAIL
SOURCES OF FIBROGENIC CELL TYPES IN THE LIVER

Stellate cell → Activated stellate cell → Myofibroblasts

Bone marrow

Fibrocytes → Portal fibroblast

Hepatocytes and cholangiocytes

EMT: ↑Hh signaling ↑BMP7

MET

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Non invasive alternatives to biopsy for assessing liver fibrosis

- Serum markers
- Imaging modalities: MRE
- Stiffness (Fibroscan)
Normal liver: soft consistency - high viscosity - slow velocity - low stiffness <5 kPa

Cirrhosis: hard consistency – high velocity → high stiffness > 7-11kPa

- Vibration transmitted toward the liver produces elastic shear wave
- Measurement of the velocity of wave propagation with ultrasound.
- Stiffness expressed in Kilopascals: 2.5 - 75 kPa
Correlation between stiffness and stage of fibrosis

- Strong accuracy for detection of advanced fibrosis / cirrhosis
- Overlap between adjacent stage
- Used as a screening tool to direct patients to biopsy in mass screening

Biopsy and semi-quantitative staging systems
Collagen Percentage Area

Automated quantitation of relative fibrosis area
(sirius red staining, digital images, appropriate algorithm)

(2500 biopsies, NAFLD, CPA vc semiQ scoring)

Morphometry of fibrosis / linear and accurate scaling
Two-photon Microscopy and Second Harmonic Generation

- Ex vivo imaging and quantification of liver fibrosis using second-harmonic generation microscopy Sun TL et al. Journal of Biomedical Optics, May/June 2010
qFibrosis: 100 Collagen Features Extracted for Analysis

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**Central Vein region**

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Two-photon microscopy and second harmonic generation

- Specificity for fibrillar collagen +++
- Assessment and quantification of multiple parameters (fibers shape, length, width, orientation, cross-linkage...)
- Label free, FFPE
- Quantitative, highly reproducible
- Clinical relevance to be demonstrated

* Dual-Photon Microscopy-Based Quantitation of Fibrosis-Related Parameters (q-FP) to Model Disease Progression in Steatohepatitis Wang Y et al, Hepatology 2017
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▶ Liver fibrosis: a dynamic process
DYNAMIC OF FIBROSIS / CIRRHOSIS
Changing concept: From progression to regression
REVERSION OF CIRRHOSIS IS REAL IN ANIMAL MODELS

Thinning of fibrous septa

- In situ production of MMPs: zymography in situ

### Synthesis

- HSC activation (Fibrogenesis - fibrosis)

### Digestion

- Matrix-degrading enzymes (Metalloproteases)
Liver cell regeneration

- Liver has a huge capacity of regeneration
- Chronic inflammation block hepatocyte regeneration: **halting inflammatory process**
Virus – Inflammatory reaction – Fibrogenesis – Antiviral drug

[Diagram showing the relationship between virus replication, immune response, inflammatory reaction, and fibrogenesis, indicating inhibition by antiviral drug and increased regeneration.]
Regeneration is extended in hepatocyte but not unlimited.

→ Multiple mitotic cycles shorten telomeres
→ Aging decreases telomere length and ability to regenerate
→ Repeated necrosis and regeneration decreases potential for regeneration (atrophic cirrhosis)
Condition for cirrhosis regression

- Thinning of fibrous septa:
  - Enzymatic digestion of fibrous tissue (metalloproteases, MMP)
  - MMP resistant: Collagen cross-links, elastic fibres
  - Fibrous tissue digestion easier in « Early » cirrhosis

- Hepatic regeneration:
  1. Halting inflammatory reaction → eradication of the noxious agent (viral eradication, immunosuppressive drugs, iron chelation....)
  2. Hepatic regeneration → Aging (physiologic and pathologic) limit regenerative capacity

- From nodular to lobular organization: Perinodular vascularization to translobular blood flow → No vessel thrombosis
Take-home Messages / consideration for treatment

- Fibrosis is a dynamic process that can progress and regress even at an advanced stage (cirrhosis)
- Regression of fibrosis in the liver is partly related to the enormous ability for the liver cells to regenerate
- Antifibrotic drugs will be useless until the background disease stimulating liver injury is not halted
- Stimulating liver cell regeneration after controlling the aetiological factor might be sufficient to induce fibrosis / cirrhosis regression
- Although cirrhosis can regress, there is a point of no return where the disease will never go back
THANK YOU FOR YOUR ATTENTION !