Liver Regeneration And Drug Induced Liver Injury

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Centrilobular Necrosis from Drug Induced Liver Injury
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Two-Thirds Partial Hepatectomy
Higgins and Anderson

FIG. 3. Liver regeneration after partial hepatectomy. Liver of a normal rat at operation (the excised lobes are outlined) and at 1 to 4 weeks after partial hepatectomy.

Lobes not regenerated $\rightarrow$ remaining parenchyma undergoes “compensatory hyperplasia”
Following resection, the entire regenerative response is complete in 1-2 weeks in rodents (1-2 months in humans)

- highly coordinated process
- without substantial changes in liver synthetic or metabolic function
DNA synthesis cycles after partial hepatectomy.

One-hour incorporation of $^3$H-thymidine into hepatic DNA in 200 g male Sprague Dawley rats at intervals after partial hepatectomy. Vertical lines indicate the standard error of the mean, numbers the number of rats per point. From Bucher, Patel and Cohen, with kind permission of Pergamon Press.
DNA synthesis of different hepatic cell types after partial hepatectomy.

Modified from original work by Dr. Joe Grisham
Cellular Interactions During Liver Regeneration

Mitogenic Growth Factors
- HGF
- EGF

Hepatocytes in G0

Activation of
- STAT3, NFκB
- AP1, Cyclin D1

Hepatocytes in G1

Beta catenin Notch 1

Stellate Cells
- PDGF, FGF1
- HGF, TGFβ

Kupffer Cells
- GMCSF
- TNF, IL6

Auxiliary mitogens
- TNF, IL6, Norepinephrine, Insulin, Bile acids, Leptin

Endothelial cells
- VEGF, ANG1, ANG2, TGFβ, FGF1
- HGF
Exchange of hepatotrophic factors through parabiotic circulation.

Current understanding:
Following partial hepatectomy, substances contributing to liver regeneration and known to be rising in the blood are as follows:

1. Hepatocyte Growth Factor (HGF)
2. Norepinephrine
3. Tumor Necrosis Factor
4. Interleukin 6
5. Bile acids
6. Serotonin

Of the above, HGF is the only direct mitogen and as a consequence the only one isolated by a hepatocyte proliferation bioassay.
Hepatocyte Growth Factor (HGF)

Synthesized in inactive form as a single continuous polypeptide.

Activated by urokinase plasminogen activator (uPA), Hepsin and by a Factor XII homologous protein known HGFA, by cleavage at an RVV site to the mature two-chain heterodimeric form.
HGF Receptor (MET)

UNIQUE (?) FUNCTIONS OF ACTIVATED HGF RECEPTOR (MET) IN LIVER (AND OTHER TISSUES?)

Transduction of signals related to mitogenesis and motogenesis (Ras/MAPK, etc.)
Mobilization of beta catenin to hepatocyte nuclei (in culture; same phenomenon occurs in vivo after PHx. Is Met responsible?)
Dimerization with Fas via YLGA peptide (antiapoptotic effects; SiMet caused activation of caspase 3).
Lung cancer: Dimerization between Met and Erb3 allows tumors to overcome inhibition of Erb1 (EGFR) by specific inhibitory agents.
Extracellular Signals implicated in liver regeneration

COMPLETE MITOGENS:

1. Mitogenic in hepatocyte cultures in chemically defined (serum-free) media.
2. Cause liver enlargement and hepatocyte DNA synthesis when injected into whole animals:
   - Hepatocyte Growth Factor (HGF) and receptor MET
   - Ligands of the EGF R (EGF, TGFα, HB-EGF, Amphiregulin)

AUXILIARY MITOGENS.

1. Ablation of their signaling pathways causes delay but does not abolish liver regeneration.
2. They are not mitogenic in hepatocyte cultures and when injected in vivo do not cause hepatocyte DNA synthesis and liver enlargement.
   - Norepinephrine and the α1 adrenergic receptor.
   - TNF and TNFR1.
   - IL6
   - Notch and Jagged (recombinant Jagged causes DNA synthesis in hepatocyte cultures)
   - VEGF and receptors I and II.
   - Bile acids
   - Serotonin
   - Complement proteins
   - Leptin
   - Insulin
   - PPAR gamma
Chronology of concurrent early (first 1 hour) signaling events after PHx

- Multiple signaling pathways involving both growth factors, cytokines, paracrine signals (Notch/Jagged) and neuroendocrine factors (Norepinephrine) occur simultaneously within the first 60 minutes after PHx. Examples:
  - Increase in urokinase activity (first 5 minutes)
  - Translocation of N(otch)ICD to the nucleus (15 minutes)
  - Translocation of beta-catenin to the nucleus (5-10 minutes to 6 hours)
  - Decrease in HGF biomatrix stores (30 minutes to 3 hours)
  - Activation of the HGF receptor (within 30-60 minutes)
  - Activation of the EGF receptor (within 30-60 minutes)
  - Increase of HGF, Norepinephrine, IL6, TNFa, TGFb1 and hyaluronic acid in the plasma.
  - Activation of AP1, NFkB and STAT3
  - Extensive gene expression reprogramming of hepatocytes within 30 minutes after PHx (Taub et al.).
Increased translocation of residual $\beta$-catenin
HGF, Hepatic Extracellular Matrix, Urokinase, Matrix Metalloproteinases

• Previous work from our laboratory has shown the following:
  – HGF protein is localized in hepatic extracellular matrix.
  – Urokinase activates single chain HGF to the active heterodimer in regenerating liver homogenates (anti-uPA antibody blocks activation)
  – Liver urokinase activity rises dramatically within minutes after PHx.
  – As expected, this leads rapidly to activation of plasminogen to plasmin.
  – Plasmin activated MMP9 is expressed by proliferating hepatocytes as a wave from periportal to centrilobular areas from 3 hours to 48 hours after PHx.
HGF was isolated from plasma of hepatectomized rats as the mitogenic substance rising in the blood after partial hepatectomy.

HGF in plasma increases 20-fold within 1 hour. HGF mRNA expression in liver and lung starts at 3 hours peaking at 20-30 hours.

Where does the plasma HGF come from?
Proteolytic cascades involved in matrix remodeling.

uPA initiates matrix remodeling and activates HGF!
uPA activity increases rapidly following partial hepatectomy.

Normal Liver

One minute after PHx

-anti uPA

+ anti uPA
Urokinase activates scHGF to tcHGF after hepatectomy.
uPA knockout mice have deficient regeneration
(borrowed from Russell et al.)
HGF infusion in normal mice causes increase in liver weight
(from Kay et al.)
proMMP2 and proMMP9 activation after PHx.
scHGF and tcHGF during liver regeneration after partial hepatectomy.

Consumption from pre-existing stores

Synthesis of new HGF

<table>
<thead>
<tr>
<th>minutes</th>
<th>hours</th>
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<tbody>
<tr>
<td>0 5 15 30 60</td>
<td>3 6 12 18 24 48 72</td>
</tr>
</tbody>
</table>

PHx

Sham

Sc
tc

Sc
tc
Tyrosine phosphorylation of Met and EGFR after PHx.
Decrease in c-met after ShMet Treatment

Real Time PCR

Phospho Met WB

mRNA & protein levels in ShMet treatment
Suppression of Cell Division after shMet treatment

Complete absence of mitoses at 24 hours.
Mitotic rate at 48 hrs surpassed the rate of the scrambled RNA treated rats.

ShRNA: 4%.
Scr RNA: 80%.
Changes in expression of cell cycle and growth regulating genes following treatment with Met Silencing RNA

<table>
<thead>
<tr>
<th>Apoptosis Related</th>
<th>Pro-survival</th>
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<tbody>
<tr>
<td>DAPK3</td>
<td>BCL-XL</td>
</tr>
<tr>
<td>MADD</td>
<td>BCL-A</td>
</tr>
<tr>
<td>fas</td>
<td>SOD2</td>
</tr>
<tr>
<td>fas</td>
<td>Galectin-3</td>
</tr>
<tr>
<td>BID apoptotic death agonist</td>
<td></td>
</tr>
<tr>
<td>Bak 1</td>
<td></td>
</tr>
<tr>
<td>bcl10</td>
<td></td>
</tr>
<tr>
<td>Apaf-1</td>
<td></td>
</tr>
<tr>
<td>PDCD4</td>
<td></td>
</tr>
<tr>
<td>DAXX</td>
<td></td>
</tr>
<tr>
<td>Death associated like kinase</td>
<td></td>
</tr>
<tr>
<td>Cell death activator-DFTA/CIDA</td>
<td></td>
</tr>
<tr>
<td>caspase 8</td>
<td></td>
</tr>
<tr>
<td>caspase 7</td>
<td></td>
</tr>
<tr>
<td>caspase 12</td>
<td></td>
</tr>
<tr>
<td>caspase 3</td>
<td></td>
</tr>
<tr>
<td>cyclophilin a</td>
<td></td>
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<tr>
<td>cyclophilin d</td>
<td></td>
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<tr>
<td>Galectin 1</td>
<td></td>
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<tr>
<td>Galectin 2</td>
<td></td>
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<tr>
<td>Galectin 9</td>
<td></td>
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<tr>
<td>stat 1</td>
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Expression levels are indicated as follows: **Increased**, **Decreased**.
Suppression of Cell Division after shEGFR treatment.

Figure 9. Mitotic index was estimated as described in Methods Section. At day 1, suppression of mitosis was seen in shEGFR treated rats, compared to controls. For comparison Data for shMet treated rats is also shown.

Pro-apoptotic genes upregulated by treatment with Sh-EGFR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>ShEGFR</th>
<th>Control</th>
<th>Fold increase after EGFR silencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>daxx</td>
<td>1550</td>
<td>366</td>
<td>4.23</td>
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<tr>
<td>apaf1</td>
<td>716</td>
<td>292</td>
<td>2.45</td>
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<tr>
<td>caspase11</td>
<td>1525</td>
<td>391</td>
<td>3.90</td>
</tr>
<tr>
<td>Caspase 8</td>
<td>261</td>
<td>53</td>
<td>4.92</td>
</tr>
<tr>
<td>Caspase 7</td>
<td>293</td>
<td>156</td>
<td>1.87</td>
</tr>
</tbody>
</table>

Suppression of Cell Division after shEGFR treatment.
Fold Regulation Treated/scr control

Proapoptotic genes

- Bak1
- Bax
- Bok
- Bid
- Bad
- Casp3
- Casp9
- Fas
- Tp53
- Apaf1
Extracellular Matrix and Hepatocyte Proliferation and Differentiation

- Hepatocytes in primary culture lose their characteristic gene expression patterns. They can be stimulated to proliferate under the influence of HGF and/or EGF.
- Addition of artificial extracellular matrix to hepatocytes in culture (e.g. Matrigel, Type I collagen gels) restores full differentiation and inhibits hepatocyte proliferation.
- Matrix breakdown and reconstitution are essential components of the processes associated with liver regeneration after partial hepatectomy.
- QUESTION: What is the role of hepatic extracellular matrix and the associated signaling through integrins in maintenance and regulation of hepatocyte proliferation and differentiation in the setting of an intact liver?
- STUDY: Eliminate matrix induced signaling from hepatocytes by liver-targeted genetic elimination of Integrin Linked Kinase. ILK^loxP/loxP^ mice were either treated with Adenovirus-Cre or mated with mice expressing Cre recombinase under hepatocyte specific promoters (AFP enhancer/Albumin promoter).
Integrin Linked Kinase Signaling Pathways

Kyle R. Legate, Eloi Montañez, Oliver Kudlacek & Reinhard Füssler
Injection of Adeno-Cre in mice with ILK $^{\text{loxP/loxP}}$ results in massive hepatocyte apoptosis and necrosis (fulminant hepatitis)

beta-gal  Cre
Crossing of the ILK-Floxed mice with the Foxa3 Cre, AFP-albumin Cre, albumin-Cre mice → conditional knock-out of ILK in the liver at different stages of development.
Hepatocyte Proliferation and Apoptosis

Reticulin at 10 weeks
ILK-KO (Liver)
Control

Proliferation of HNF1 pos. biliary epithelial cells
In ILK-KO (Liver) at 16 weeks

Proliferation of stellate cells
ILK-KO (Liver) at 16 weeks
Increased Liver weight in ILK-KO mice due to higher proliferation

PCNA Positive Cells

TUNNEL Positive Hepatocytes

Mitoses

Liver to body weight ratio

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>KO</th>
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<tbody>
<tr>
<td>% Liver Weight to Body Weight</td>
<td>2.3</td>
<td>5.7</td>
</tr>
</tbody>
</table>
What terminates liver Regeneration?

Candidate signals:

1. **TGF beta 1:**
The protein inhibits hepatocyte proliferation in culture and delays regeneration in vivo. Transgenic expression of TGFb1 in hepatocytes does not block regeneration. Deletion of TGF beta receptor 1 does not alter termination of regeneration (work by S. Thorgeirsson et al.).

2. **Activin.**
Mitoinhibitory to hepatocytes in cultures

3. **Extracellular Matrix.**
In hepatocyte cultures it maintains cell differentiation and inhibits cell proliferation. ECM is degraded early in regeneration and re-synthesized at the end of regeneration.
Matrix-derived HGF and duodenum-derived EGF.

Enhanced production of TGFβ1.

Enhanced synthesis of extracellular matrix

Enhanced binding and inactivation of HGF and TGFβ1 in pericellular matrix surrounding hepatocytes.

Feedback loop between mitogens HGF and EGF, cytokine TGFβ1 and ECM leading to initiation and termination of liver regeneration.

Norepinephrine? IL6?

Synthesis of new HGF

Inhibition of expression of urokinase

ECM derived Integrin and ILK mediated signaling to hepatocytes

Hepatocytes return to G0
Norepinephrine induces DNA synthesis in hepatocyte cultures balanced between EGF and TGFβ1.

Contributing non-mitogenic cytokines:
- TNF
- IL-6
- Norepinephrine
- Bile acids
- Complement
- Serotonin
- Leptin
- Wnt
- Hedgehog

Ichikawa T., Hepatology 2001 Nov;34(5):918-25
Early Stages Of Liver Regeneration

- Local release and activation of HGF
- Increased output of EGF from duodenum
- Removal of TGFβ1

Late Stages Of Liver Regeneration

- Single chain inactive HGF bound to matrix
- TGFβ1 bound to decorin around hepatocytes
- Low EGF levels from duodenum

Normal Liver

- Growth Factors
- Mitoinhibitors

Hepatocyte Homeostasis

- Synthesis of new matrix
- Re-deposition of TGFβ1
- Binding of inactive HGF by matrix
- Decrease in EGF levels

Hepatocytic Proliferation

- Growth Factors
- Mitoinhibitors

Mitoinhibition

- Hepatocyte Mitoinhibition
Lab Collaborators:

- William Bowen      Senior Research Technician
- Shirish Paranjpe  Assist. Professor    HGF and Met silencing
- Bowen Liu         Graduate Student     Glypican 3
- Shashi Donthamsetty Postdoctoral fellow ILK Liver knockout
- Vishakha Bhave    Postdoctoral fellow Hepatocytes as stem cells
- Ann Orr           Histologist
- Amantha Michalopoulos Undergraduate Student
Independent Collaborators

- Wendy Mars: IL6 synthesis, NFkB and HGF
- Paul Monga: beta Catenin
- Reza Zarnegar: Met and Fas
- Aaron Bell: Hepatocyte transcription factors, Matrix, PB and HNF4
- Cary Wu: Integrin Linked Kinase
- Jianhua Luo: HCC Gene Expression and Genomics
- George Tseng: Biostatistics
- A. Jake Demetris, Mike Nalesnik and Erin Ochoa: Liver Pathology, liver cancer and hepatocyte <-> biliary transdifferentiation
- Steve Strom: Human Hepatocytes
- Yuhua Liu: HGF Plasmids
- Marie C. DeFrances: Liver Regeneration
Pittsburgh, Steelers Town…