The Role of Hepatic Progenitor cells in Human Liver Regeneration

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A stem cell is an undifferentiated cell capable throughout life of renewing itself as well as of generating one or more type of differentiated cells.

- Embryonic stem cells: totipotential
- Fetus and adult: multi/pluripotential
- Closer to final differentiation: Progenitor cells, committed stem cells, transit cells
Liver progenitor cells
Liver progenitor cells:
CK7, CK19, OV6, OV1, Chrom A, NCAM
subpopul: CD34, c-kit, flt-3, sca1, CD133, EpCAM
CD133 in chronic hepatitis C pt
Control Mechanisms of Progenitor Cell Activation and Differentiation
Progenitor Cell Activation

Hepatocyte/ bile duct damage
inhibition of replication

Bile duct ep
Progenitor cell
Hepatocyte

CDAAF, galactosamine intoxication in rat
Majority of human liver diseases
Human **acute and chronic** liver diseases are characterized by replicative senescence of hepatocytes

- **Acute (sub)massive liver necrosis**
  
  (Katoonizadeh Liver Int 2007)

- **Alcoholic liver disease and viral hepatitis**
  
  (Crary Hepatol 98, Paradis Hum Pathol 01, Falkowski J Hepatol 03, Roskams Am J Pathol 03, Eleazar J hepatol 04, Marshall Gastroenterology 05)

- are associated with **inhibition of hepatocyte replication**
# Acute Severe Liver Impairment

## Histopathological correlations

<table>
<thead>
<tr>
<th>Severity of hepatocyte loss</th>
<th>No. of patients</th>
<th>No. of HPCs</th>
<th>No. of mib-1+ hep</th>
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<td>Mild (&lt; 30%)</td>
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<td>76 ± 40</td>
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<td>138 ± 53.7</td>
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Classification of patients according to the severity of hepatocyte loss and the number of HPCs or proliferating (mib-1 positive nuclei) hepatocytes for each group.

Katoonizadeh Liver International 2007
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CK7, CK19: progenitor cell activation/differentiation

< 30% necrosis

> 50% necrosis
Comparison of HPCs activation/differentiation based on the duration of disease

- <1 week: N=5
- 1-4 weeks: N=30
- > 4 weeks: N=39
Regeneration after (sub)massive necrosis
24h 1 week later

Roskams J hepatol 98
Katooni Zadeh Liver Int. 2006
Comparison of the number of proliferating (mib 1 positive nuclei) hepatocytes and HPCs with clinical parameters at the time of liver biopsy.
Comparison of histopathological parameters and outcome

<table>
<thead>
<tr>
<th>Histopathological parameter</th>
<th>Group A Alive (n=24)</th>
<th>Group B Died (n=10)</th>
<th>Group C Transplanted (n=40)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>&gt;50% hepatocyte loss</td>
<td>8% (2/24)</td>
<td>80% (8/10)</td>
<td>95% (38/40)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Number of proliferating hepatocytes/HPF</td>
<td>14.3 ± 9.3</td>
<td>2.5 ± 2.5</td>
<td>5.7 ± 7.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Number of HPCs/HPF</td>
<td>74 ± 55</td>
<td>138 ± 52</td>
<td>141 ± 58</td>
<td>0.003</td>
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</tbody>
</table>

> 50% hepatocyte loss, low proliferative activity of hepatocytes, high progenitor cell activation, independent factors for bad prognosis
HPC in Chronic Liver Diseases

normal  Stage I  Stage II-III  Stage IV

HCV hepatitis

Stage I-II  (N)ASH  Stage IV  CK7 staining
Human chronic liver diseases are characterized by replicative senescence of hepatocytes

- Telomere shortening and replicative senescence of mature hepatocytes (and not of hepatic stellate cells or lymphocytes) is a general feature of the cirrhotic stage of a variety of chronic liver diseases (Wiemann FASEB J 03, Falkowski J Hepatol 03, Rudolph Science 00, Fausto Hepatology 2004)

- This inhibition of replication is associated with progenitor cell activation in human liver diseases (De Vos Am J Pathol 92, Roskams J Hepatol 98, Lowes Am J Pathol 99, Libbrecht J Pathol 00, Roskams Am J Pathol 03, Katoonizadeh Liver Int in press...)
Survival of progenitor cells in disadvantageous environments
Stem cells: ability to exclude the Hoechst dye 33342

- Reflects possession of one of the ABC-binding cassette transporter pumps BCRP (Zhou 2001)

- On fluorescent-activated cell sorting: cells separate as ‘side-sorted’ population
BCRP in progenitor cells, giving these cells a multidrug-resistant phenotype putative side population in man

Vander Borght et al. J Pathol 2006
Upregulation of ABC transporters MDR1 and MRP3 in progenitor cells, giving these cells a multidrug-resistant phenotype, putative side population in man.

Ros et al. J Pathol 03, Gut 03
Side-population analysis in the adult liver

Bart Spee
Collaboration with Utrecht University
Experimental Setup

3-step liberase

isodensity centrifugation

pellet

supernatant

Parenchymal fraction

Non-parenchymal fraction
Side-population, cell sorting

Incubation with Hoechst33342

a.o. ABCG2/BCRP receptor

Fluorescent Activated Cell Sorting (FACS)
## Side-population, cell sorting

### RNA isolation (RNAlater)

1. Bulk population: 200,000 cells, 33.3 ng/ul, RIN 6.3
2. CD45 positive cells: 6,500 cells, 5.7 ng/ul, RIN N/A
3. CD45 negative cells: 3,500 cells, 1.9 ng/ul, RIN N/A

### Gene-expression analysis

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>CD45 positive</th>
<th>CD45 negative</th>
<th>Bulk cells</th>
</tr>
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<tbody>
<tr>
<td>c-Kit (hemato/prog)</td>
<td>+</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>CD34 (mes/hema/prog)</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>CD133/Prominin (hemato)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Thy-1/CD90 (mes/hema/prog)</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>CK7 (prog/bile)</td>
<td>+/-</td>
<td>++</td>
<td>+/-</td>
</tr>
<tr>
<td>CK19 (prog/bile)</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>CK18 (hepatocyte)</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>CSA (hepatocyte)</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>AFP (prog/hepato)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
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<tr>
<td>HNF4A (hepatocyte)</td>
<td>-</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>ABCG2/BCRP (hepato/prog)</td>
<td>-</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>FN14/TWEAKR (prog)</td>
<td>-</td>
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microscopic “niche” of stromal cells

- Nurse stem cell
- Give proper instructions
- Hair follicle of mouse: niche for melanocytic stem cells (Nature 02)
- Trabecular bone (haematopoietic stem cell niche: Nature 03)

Liver: PCs surrounded by HSCs
EXPERIMENTAL SETUP

Microdissection with Laser Capture Microscopy (LCM) of ck7+HPC

Example: Primary Billiary Cirrhosis (PBC)

Before

After
# EXPERIMENTAL SETUP

<table>
<thead>
<tr>
<th>Microdissected CK7+ HPC from</th>
<th>RNA isolation</th>
<th>RNA amplification</th>
<th>Gene-expression analysis</th>
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<tbody>
<tr>
<td>Acute hepatitis (AH)</td>
<td></td>
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<td>- Superarray (real-time PCR 84 genes)</td>
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<tr>
<td>Hepatocytic diseases (HCV-C)</td>
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<td>Billiary diseases (PBC)</td>
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IHC confirmation
Superarray (84 genes of interest)

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<td>A</td>
<td>CD133</td>
<td>KRT7</td>
<td>ABCG2</td>
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<td>AFP</td>
<td>ASMA</td>
<td>NCAM1</td>
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<td>DLL1</td>
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<td>JAG2</td>
<td>NOTCH 1</td>
<td>NOTCH 2</td>
<td>NOTCH 3</td>
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<td>DTX2</td>
<td>EP300</td>
<td>GCN5L2</td>
<td>HDAC2</td>
<td>NUMB</td>
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<td>Sox2</td>
<td>LIF</td>
<td>LEF1</td>
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<td>RB1</td>
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CD133 and NCAM are up-regulated (67.46 folds and 30.29 folds) in Acute hepatitis (AH) and PBC (19.47 folds) compared with chronic disease (HCV-C).
PRELIMINARY RESULTS: NOTCH pathway

Jag-1 and Notch-4 are up-regulated (2.99 and 7.92 folds) in AH compared with HCV-C
Jag-1 and Notch-4 are up-regulated (18.87 and 10.47 folds) in PBC compared with HCV-C.
LEF-1 is up-regulated (10,25) in AH and PBC compared with HCV-C

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PRELIMINARY RESULTS: WNT pathway
CONCLUSIONS

✓ Progenitor cells play role in human liver regeneration

✓ Control mechanisms are only partly understood

✓ Progenitor cells are in the ‘side population’: finally able to isolate progenitor cells

✓ Similar to the progenitor cell niche in other organs (gut, nervous system), wnt and notch pathways are implicated with HPC niche expansion and probably with the differentiation towards hepatocytes or cholangiocytes.
Since progenitor cells are activated in chronic liver diseases, they also form a target cell population for carcinogenesis.
Hepatocyte
Intermediate or mixed phenotype
Cholangiocyte
progenitor cell
HCC
CC
Cancer stem cells and their progeny
16-28% of HCCs have progenitor cell features

CK19 protein expression associated with:
- high serum AFP, AFP expression in tumour
- less advanced fibrosis stage
- higher prevalence of anti-Hbcore in serum
- lower incidence of nuclear beta-catenin staining
- higher recurrence rate after transplantation

Durnez A et al Histopathology 2006
**Hepatoblast signature**: significantly higher KRT7, KRT19, VIM

very bad prognosis (survival 11.9mo versus 64.4mo)

higher expression of genes involved in invasive phenotype

MMP1 (matrix metalloproteinase type 1)
PLAUR (urokinase type plasminogen activator receptor)
TIMP1 (tissue inhibitor of metalloproteinase type 1)
CD44 (cell surface glycoprotein: hyaluronan receptor)
VIL2 (gene of ezrin: actin filament - plasma membrane linker)
New Prognostically Significant Classification

Hepatocyte $\rightarrow$ HCC

Mixed hepatobiliary carcinoma

Cholangiocyte $\rightarrow$ CC

progenitor cell

CD10+ (can patt) hepar1
CK19-

CK19+ 5%+

In future additional markers will be added e.g. microvascular invasion marker, markers for targeted therapy, …

Conclusion

- PCs are activated in severe acute liver impairment and in chronic liver diseases.
- They are potential target cells for carcinogenesis.
- Their activation and differentiation depends on the microenvironment: definitions of stem cell niche!!
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- Dr. D. Bielen
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- Prof. Dr. C. Trautwein
- Dr. T. Luedde
- Dr. N Beraza
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- Prof. M. Strazzabosco
- Prof. L. Fabris
- Prof. Dr. Pinzani
- Prof. Dr. Rothuizen