Hepatogenesis II: Liver progenitor cells

HB 308
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Two pathways to regenerate liver

Acute liver damage (Drug toxicity, Physical trauma)
- Hepatocyte
- Liver Progenitor Cell
- Proliferation without Differentiation

Chronic liver damage (Alcohol, Hemochromatosis, Viral hepatitis)
- Hepatocyte
- Liver Progenitor Cell
- Proliferation with Differentiation
Animal models of liver regeneration

Acute liver damage (Partial hepatectomy, CCl4)

- Hepatocyte
- Liver Progenitor Cell

Proliferation without Differentiation

Chronic liver damage (Alcohol, Viral hepatitis, Diets e.g. CDE)

- Hepatocyte
- Liver Progenitor Cell

Proliferation with Differentiation
Liver Progenitor Cells: friend

- Source of hepatocytes and cholangiocytes
- Robust - easy to isolate and establish primary cultures and cell lines
- Can be stored and thawed
- Easily transfected for gene delivery
- Potential application in gene/cell therapy
Objectives of our laboratory

- To identify factors which regulate LPCs and to understand their mechanism of action by developing in vivo and in vitro mouse models to study LPC biology

- Apply knowledge to human LPCs to:
  - i) enhance LPC mediated regeneration in vivo
  - ii) establish human LPC lines and demonstrate their utility for gene/cell therapy
CDE diet induces LPCs in mouse liver
In vivo mouse studies

- Choline deficient, ethionine supplemented (CDE) diet induces LPCs in the mouse
  - Cytokine screen shows TNF, IL-6, LTβ and IFNγ increase - suggests they may be important mitogenic/differentiation factors
- Cytokine and cytokine Rec-KO mice show impaired LPC response
  - Ablating TNF, IL-6, LTβ and IFNγ signaling impairs generation of LPCs (40-70% reduction but never 100%)
IL-6 enhances LPC response induced by a CDE diet
In vitro mouse studies

- Why in vitro?
  - Evidence for a direct effect
  - Many replicates possible
  - Longitudinal study
  - Cell lines offer homogeneous cell source
  - Elucidation of signaling pathways
  - Bank LPCs and differentiated progeny
In vitro mouse studies

- Establish primary cultures
  - Isolate LPCs from CDE treated mice
  - Characterise growth and differentiation in different culture conditions
- Establish cell lines from embryonic and adult liver
  - Test putative cytokines
  - Evaluate ability to colonise the liver and correct disease condition
Transgenic mice utilised for in vitro studies

- p53 null mouse
  - Immortalised cells
  - Potential for transformation
- TAT GRE lacZ mouse
  - Carries the reporter gene regulated by a promoter which is only active in mature hepatocytes which express the tyrosine aminotransferase gene.
The p53 null mouse liver over-produces LPCs from which cell lines can be derived. PIL (p53 immortalised liver) cell

Wild type

p53 null

Cell lines are readily established from primary cultures of small cells isolated by Percoll gradient centrifugation.
Primary cultures of LPCs from the TAT GRE lacZ mouse express beta-galactosidase on becoming hepatocytes
LPC lines can be established from mouse fetal liver by the “Plate & Wait” Method

Perth x Paris

2-3 weeks

Collagen, WEM, serum, IGF II, EGF, Insulin

4-12 weeks

Bipotential Murine Embryonic Liver Cells
Characteristics in different culture conditions

- Growth medium
  - EGF, IGF2,

- Differentiation medium
  - EGF, ITS, dexamethasone, nicotinamide

- Matrigel (BD-F)
  - Matrix - collagen IV, laminin, entactin, heparan sulphate proteoglycan
  - Cytokines - EGF, bFGF, NGF, PDGF, IGF2, TGFβ
“Plate & Wait” works with TAT-GRE lacZ fetal mouse

LPCs from TAT-GRE mouse are beta-gal negative until they are differentiated
Markers assessed

- Hepatocyte
  - TAT
  - ALB
  - G6Pase

- Cholangiocyte
  - CD34
  - GGT IV
  - c-kit
# RT-PCR analysis of LPC lines

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LPCs readily differentiate

- BMEL - PIL
- Growth Medium
- Matrigel

Differentiation

- Medium
- Hepatocytes

- BMEL - PIL
- Cholangiocytes

BMEL - PIL
Cell line is established from the TAT-GRE lacZ mouse

Growth medium

Differentiation medium
Collection of LPC lines

- PIL2 - p53 null tumorigenic
- PIL4 - p53 null non-tumorigenic
- BMEL - Bipotential mouse embryonic liver
- BMEL (TG) - BMEL from TAT-GRE lacZ
- BMOL - Bipotential mouse oval (adult) liver
- BMOL (TG) - BMOL from TAT-GRE lacZ
- BMEL (MIP-GFP)
BMEL (MIP-GFP) cells can differentiate into pancreatic islet cells

Fig. 3.2 Transdifferentiation of liver stem cells. Oval cells from MIP-GFP mice were cultured to induce transdifferentiation. The development of cells with the potential to produce insulin was monitored by the emergence of GFP+ cells. Left, bright field image. Right, visualization of GFP+ cells. Control cultured oval cells are nonfluorescent (not shown).
Cytokine Studies - IL-6

LPC and LPC lines express IL-6R and should respond to IL-6.
IL-6 enhances growth of PIL cells

Hyper IL-6 = IL-6 + sIL-6R
IL-6 increases migration of LPC line

Migration assessed in Costar transwell plates
Effects of IL-6 (summary)

- LPC and LPC lines express IL-6R
- IL-6 induces proliferation of LPC line
- IL-6 increases migration of LPC line
- IL-6 enhances the LPC response elicited by a CDE diet
Summary

- LPC cell lines have been established from fetal and adult mouse liver
- Cell lines from wild type, p53 null and TAT GRE lacZ mice are available
- Suitability for assessing the effects of cytokines has been shown with IL-6
- Future studies:
  - Test other cytokines
  - Evaluate effects on differentiation (lacZ lines)
  - Consequence of transplant
  - Human LPCs
Human liver progenitor cell cultures

Colonies of human oval cells established from cirrhotic (ALD) liver
Liver progenitor cells: foe

- In rodent models of hepatocarcinogenesis, e.g. CDE diet, AAF/PH, azo dye administration, at early stages LPCs are observed.
- The p53 null mouse liver contains large numbers of LPCs and develops HCC.
- Rat LPCs produce hepatomas in vivo, following transfection with oncogenes in vitro.
- In chronic liver disease e.g. HBV, HCV, ALD and GH, LPCs are observed; these conditions predispose patients to developing HCC.
LPCs in the liver of p53 null mice

Wildtype

p53 -/-
Liver tumour incidence and LPC numbers induced by CDE diet are reduced in TNF R1 KO mice

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<th>Early Neoplastic</th>
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[Images: αFP, M2-PK]
Some LPC lines derived from the p53 null mouse are tumorigenic

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<th>Number of tumors formed</th>
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Future studies: gene profiling to compare non-tumorigenic vs tumorigenic cell lines
Liver progenitor cells: friend or foe?

Liver Progenitor Cells

Hepatocytes

Hepatoma
Acknowledgements

“Plate & Wait” generation of BMEL cell lines
Helene Strick-Marchand & Mary Weiss
Pasteur Institute, Paris

IL-6 studies
Vance Matthews

BMOL cells
Janina Tirnitz-Parker

p53 null mice studies
Melissa Dumble

BMEL (MIP-GFP)
Emma Jamieson