Hepatic cells derived from human skin precursors as an *in vitro* model to study non-alcoholic fatty liver disease

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OUTLINE

1. The disease
   A. NAFLD?
   B. Main causes
   C. Molecular mechanisms

2. The model
   A. What are hSKP?
   B. hSKP-derived hepatic cells
   C. Application in hepatotoxicity testing

3. *In vitro* modeling of NAFLD

4. Conclusion/perspectives
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The Disease – NAFLD?

Normal Liver → Simple steatosis → NASH → Cirrhosis → Hepatocellular carcinoma

Normal Liver

Steatosis

NAFLD
non-alcoholic fatty liver disease

NASH
non-alcoholic steatohepatitis

Fibrosis/Cirrhosis

Cohen JC et al. Science 2011;332(6037):1519-1523
The Disease – NAFLD?

- High prevalence world wide:
  - NAFLD: ~20% of the adult population
  - NASH: ~2% (100m people)
  - still increasing in industrialized countries due to strong correlation to lifestyle and unhealthy diets...

steatosis $\rightarrow$ NASH $\rightarrow$ fibrosis $\rightarrow$ cirrhosis $\rightarrow$ cancer

mostly asymptomatic, reversible, benign(!)
The Disease – Main Causes

Multiple factors drive the onset of NAFLD:

A. Environmental/genetic inducers:
   - high-fatty diet
   - obesity
   - diabetes
   - insulin resistance → metabolic syndrome

B. Chemical inducers:
   - drugs (valproic acid, tamoxifen, tetracycline, amiodarone)
   - toxicant associated fatty liver disease (TAFLD): exposure to industrial chemicals and environmental pollutants
The Disease - Mechanisms

1. Increased FA uptake (obesity)
2. De novo lipogenesis
3. Reduced FA oxidation
4. Reduced VLDL secretion

Hepatic TG accumulation
The Disease - Mechanisms

E.g. insulin-induced NAFLD (hyperinsulinemia in the case of IR):

SREBP: sterol regulatory element-binding proteins; ChREBP: carbohydrate responsive element-binding protein; ATGL: adipose triglyceride lipase; HSL: hormone sensitive lipase

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The Disease - Mechanisms

How to investigate NAFLD?

A. Animal-based models
   Rodents, zebrafish,... → diet, genetic modified (eg *ob/ob* mice)
   → do not recapitulate the human pathophysiology accurately

B. *In vitro* hepatic models
   - human primary hepatocytes (hHEP):
     • gold standard
     • scarce, expensive, low quality for *in vitro* applications
   - hepatic cell lines (HepG2, HepaRG, ...)
     • surrogates to hHEP
     • limited functionality, abnormal genetic background...

→ Lack of an appropriate human-based *in vitro* model to study NAFLD!
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The Model – what are hSKP?

**hSKP** - Human **SK**in-derived **P**recursors

- Postnatal/adult stem cells isolated from human skin
- Thought to reside in hair follicle/dermal papillae
- Contribute to dermal maintenance, wound healing and hair follicle morphogenesis
- Robust protocol for isolation from foreskin, eyelid,... (mechanic/enzymatic, selective media)

**The Model – what are hSKP?**

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The Model – what are hSKP?

→ hSKP express typical markers

- SNAIL: snail homolog 1; PAX3: paired box3; MSX1: muscle segment homeobox 1

→ High self renewal capacity
→ Multipotent cells?
The Model – what are hSKP?

Comparison to ESC and other postnatal stem cells:

- adipose tissue: **hAT-MSC**
- bone marrow: **hBM-MSC**
- umbilical cord: **hWJ-MSC**

**hSKP show highest intrinsic expression of stemness genes**

- Differentiation into cell types from the three germ lineages:
  - **Mesoderm:** adipocyte-, chondrocyte-, osteocyte-, smooth muscle-like cells
  - **Ectoderm:** Schwann cells, neuron-like cells
  - **Endoderm:** hepatic cells?
The Model – hSKP-derived hepatic cells

Cells with mixed phenotype of mature and immature hepatocytes

hSKP – derived hepatocyte progenitor-like cells or hSKP-HPC
The Model – hSKP-derived hepatic cells

→ **Early hepatic** markers (EPCAM, GATA6, PROM1, NCAM, SMAD4 and THY1)

![Graph showing mRNA levels of various markers](image1)

- EPCAM: epithelial cell adhesion molecule
- GATA: GATA motif binding protein
- PROM: promin
- NCAM: neural cell adhesion molecule
- SMAD4: stem cell factor receptor
- THY1: thymocyte differentiation antigen

→ **Mature hepatic** markers (e.g. albumin, HNF1α, HNF4α and AHR)

![Bar chart showing relative normalized mRNA levels](image2)

→ **Basal expression of fetal and mature hepatic** biotransformation enzymes

![Graph showing mRNA levels of fetal and mature enzymes](image3)

- FMO: flavin containing mono-oxigenase
- MAO: monoamine oxidase
- CYP: cytochrome P450
- hHEP: primary hepatocytes

→ **Induced expression of fetal and mature** CYP enzymes

![Graph showing normalized mRNA levels](image4)
**The Model – hepatotoxicity testing**

Ultimate functionality test: can these cells show hepatotoxicity induced by reference compounds?

→ Acute liver failure induced by APAP

→ **APAP**: acetaminophen, paracetamol
→ Analgesic and antipyretic drug
→ Safe therapeutic dose
→ **Overdose (>10g)** induces hepatic toxicity (depletion of GSH) → **ALF**

**APAP**: N-Acetyl-p-Aminophenol
**NAPQI**: N-acetyl-p-benzoquinoneimine
SULT: sulfotransferase; UGT: UDP-glucuronosyltransferase; GST: glutathione S-transferase
The Model – hepatotoxicity testing

→ Comparison to human primary hepatocytes (hHEP), cell lines (HepaRG, HepG2) and liver samples of patients suffering from APAP-induced ALF
→ Toxicity evaluation by analysis of gene expression of whole genome (microarrays; transcriptomics)

- PCA analysis:
→ Clustering by cell type
→ APAP: shift in same direction (similar modulated genes)

- PCA: principle component analysis -
The Model – hepatotoxicity testing

- **Pathway analysis**: identification of toxicity gene classes
  
  → Same ALF-specific toxicity responses in *in vitro* models
  
  → Higher percentage of significant modulated genes in **hSKP-HPC!!**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>hSKP-HPC</th>
<th>hHEP</th>
<th>HepaRG</th>
<th>HepG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Failure</td>
<td>20% (16/82)</td>
<td>11% (9/82)</td>
<td>17% (14/82)</td>
<td>-</td>
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<tr>
<td>Liver Proliferation</td>
<td>18% (61/339)</td>
<td>9% (30/339)</td>
<td>14% (47/339)</td>
<td>2% (6/339)</td>
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<tr>
<td>Liver Necrosis</td>
<td>11% (61/583)</td>
<td>4% (25/583)</td>
<td>9% (54/583)</td>
<td>1% (6/583)</td>
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<tr>
<td>Liver Damage</td>
<td>8% (55/656)</td>
<td>6% (37/656)</td>
<td>10% (68/656)</td>
<td>1% (5/656)</td>
</tr>
</tbody>
</table>

→ **hSKP-HPC exposed to APAP** identify the same toxicity gene classes as primary human hepatocytes and hepatic cell lines exposed to APAP
The Model – hepatotoxicity testing

- Comparison to liver samples of patients suffering from APAP-induced ALF:

→ a higher number of commonly modulated genes found between hSKP-HPC and clinical ALF samples
- Prediction chart «Damage of liver» based on modulated genes selected for clinical APAP-ALF samples (functional interpretation)
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→ hSKP-HPC, hHEP and HepaRG show a predicted activation of “damage of liver”

→ HepG2 show no activation based on these genes

Rodrigues et al Toxicology Letters (2016)
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In vitro modeling of NAFLD

NAFLD (first step steatosis)

Expose hSKP-HPC to:

→ Chemical inducers:
  - sodium valproate (Na-VPA): steatogenic drug
  - tetracycline: steatogenic drug
  - 2-ethylhexanol: industrial chemical

→ Environmental inducers:
  - sodium oleate: mimicking high-fat diet
  - insulin: mimicking insulin resistance
In vitro modeling of NAFLD

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In vitro modeling of NAFLD – Na-VPA

Na-VPA induces intracellular lipid accumulation in hSKP-HPC

Effect is more pronounced than in hHEPs
Transcriptomics analysis:

- Comparison to hHEP and liver samples of patients suffering from mild and severe steatosis
- Clustering of *in vitro* systems
- Higher variation in the clinical samples
- Shift in same direction (similar modulated genes)
**In vitro modeling of NAFLD – Na-VPA**

**Pathway analysis**: identification of toxicity gene classes

<table>
<thead>
<tr>
<th></th>
<th><strong>IN VITRO</strong></th>
<th><strong>IN VIVO</strong></th>
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<tbody>
<tr>
<td></td>
<td><strong>hSKP-HPC</strong> + NA-VPA</td>
<td><strong>hHEP</strong> + NA-VPA</td>
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<td>Liver Damage</td>
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<tr>
<td>Liver Necrosis</td>
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<td>Liver Steatosis</td>
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<tr>
<td>Liver Fibrosis</td>
<td>✗</td>
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</table>

→ Transcriptomics analysis of hSKP-HPC exposed to Na-VPA reveals the activation of steatotic functions
**In vitro modeling of NAFLD - tetracycline**

Tetracycline induces triglyceride accumulation in **hSKP-HPC** by three different mechanisms: (i) an increase in de novo lipogenesis, (ii) a decrease of the \( \beta \)-oxidation and (iii) a decrease in the secretion of VLDL. HepaRG show only a decrease of \( \beta \)-oxidation.

**ACADSB**: acyl-CoA dehydrogenase; **SCD1**: stearoyl-coenzyme A desaturase 1; **APOB**: apolipoprotein B; **FASN**: Fatty acid synthase; **CD36**: fatty acid synthase.
In vitro modeling of NAFLD - insulin

Insulin induces TG accumulation in hSKP-HPC by increasing de novo lipogenesis and decreasing VLDL secretion.

ACADSB: acyl-CoA dehydrogenase; ACC: acetyl-coenzyme A carboxylase; DGAT: diacylglycerol acyltransferase; CPT1: carnitine palmitoyltransferase 1; PPAR: peroxisome proliferator-activated receptor
**In vitro** modeling of NAFLD – insulin + treatment

- Investigation of potential compounds that can revert effect of insulin (anti-NAFLD NCE?)
  - PPAR-α (peroxisome proliferator-activated receptor α) = transcription factor and a regulator of lipid metabolism → possible drug target
  - Expose hSKP-HPC to insulin and PPAR-α agonist (bezafibrate) or antagonist (GW6471)
Conclusions:

→ **hSKPs** are **multipotent adult stem cells** that can be isolated from small human skin segments.

→ These cells can be efficiently **differentiated** into cells with **hepatic properties** that can be used in exploratory *in vitro* toxicology.

→ **Proof-of-principle** experiments show that differentiated cells exposed to insulin can be used as a **disease model for NAFLD**.

Perspectives:

→ Further characterization of the model...
   (incl. further evaluation of anti-NAFLD compounds)

→ Further developments towards a NASH model...
   (bring in inflammatory conditions)
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