Why Inflammation and Regeneration are Key to Liver Cancer Development?

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Why Inflammation and Regeneration are Key to Liver Cancer Development?

Outline
• Introduction: liver cancer and liver regeneration following chronic liver injury
• Results
• Conclusions

Disclosure
• There is no conflict of interests
• There is no competing financial interests
Hepatocellular carcinoma (HCC) is the most frequent liver cancer

- Liver cancer is ranked as the 6th cause of cancer and the 3rd cause of cancer-related mortality worldwide, after lung and stomach cancer.

- Hepatocellular carcinoma (HCC) is the most common and one of the most aggressive and lethal liver cancer.

- 50,000 cases of HCC are diagnosed in Europe every year.

- HCC incidence is growing and with more than 700,000 people die from HCC each year in the world.

- Most patients are diagnosed at an advanced stage of the illness and they generally survive for less than 1 year.

- No treatment for advanced HCC. It is resistant to chemotherapy. Sorafenib is the best treatment to extend lifespan of 2-3 months.

- The only effective strategy is primary prevention.
**HCC risk factors**
- **Infectious:** chronic hepatitis B or C
- **Nutritional and toxic:** alcohol, obesity (nonalcoholic fatty liver disease), iron overload, aflatoxin (co-factor with HBV), tobacco
- **Genetic:** tyrosinosis, hemochromatosis (iron overload), deficiency of α1-antitrypsin (Inhibitor of neutrophil elastase and accumulation of neutrophil granulocytes), autoimmune chronic active hepatitis, primary biliary cirrhosis

**Chronic liver diseases progressing to HCC**
- Chronic hepatitis B or C virus infection
- Diabetes
- Nonalcoholic fatty liver disease (NAFLD)
- Nonalcoholic steatohepatitis (NASH)

**Cirrhosis**
- At the histological development, defined by **regenerative nodules** surrounded by **fibrous bands** in response to **chronic liver injury**, that leads to portal hypertension and end stage liver disease
- Originates from **awry and perpetual regeneration** of liver parenchyma after a chronic damage (wound healing process to restore tissue homeostasis and repair)
Liver regeneration is a complex and unique process

**Major cell types:**

1. **Hepatocytes:** liver injury, partial hepatectomy, some chemicals (CCL4)
2. **Hepatic progenitor cells (HPCs) and Hepatic stellate cells (HSCs):** severe liver failure and when hepatocyte turnover is violently affected (cirrhosis, hepatitis etc…)

**Cell types and mechanisms involved depend on:**

1. the **extent of liver damage** (mild to severe)
2. the **type of damage** (with or without necrosis, inflammation)
3. the underlying **liver disease** (acute or chronic)
4. the capacity of the **whole body to respond** (i.e. age).

**Importance of understanding mechanisms of liver regeneration:**

1. Promoting regeneration and repair is a **key challenge** of major clinical significance (fatty liver disease, fibrosis, viral infection, severe liver failure, liver cirrhosis decompensation, liver transplantation etc…).

2. **A gap** exists between the animal models used to study liver regeneration and clinically important scenarios of severe liver injury and impaired liver regeneration
URI (Unconventional prefoldin RPB5 Interactor) expression correlates with HBV/HCV infection in human HCC

Hepatic URI can be potentially a mediator of viral infection causing HCC

Tummala et al.; Cancer Cell, 2014
Generation of URI knock-in mouse model: hURI-tetOFF\textsuperscript{hep}
Ectopic URI expression in hepatocytes

A

B

Tummala et al.; Cancer Cell, 2014
Ectopic URI expression in hepatocytes induces Spontaneous and heterogeneous liver tumours

> 65 Weeks of hURI expression

Control

Mutant 1

Mutant 2

Mutant 3

Control (n=16)

Mutant (n=23)

0.5 cm

Tummala et al.; Cancer Cell, 2014
URI induces a multistep process of tumorigenesis recapitulating human HCC

<table>
<thead>
<tr>
<th>Weeks in mouse</th>
<th>Mouse liver pathological state</th>
<th>Corresponding human liver pathological state</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 to 12</td>
<td>Anisokaryotic cells</td>
<td>Altered hepatic foci</td>
</tr>
<tr>
<td></td>
<td>Low grade dysplastic nodules</td>
<td></td>
</tr>
<tr>
<td>12 to 24</td>
<td>High grade dysplastic nodules</td>
<td>Large Liver Cell Dysplasia (LLCD)</td>
</tr>
<tr>
<td>24 to 32</td>
<td>High grade dysplastic nodules, Adenomas</td>
<td>Large Liver Cell Dysplasia (LLCD), Adenomas</td>
</tr>
<tr>
<td>32 to 54</td>
<td>Adenomas, Early HCCs</td>
<td>Adenomas, Early HCCs</td>
</tr>
<tr>
<td>54 to 65</td>
<td>Adenomas, Fully developed HCCs</td>
<td>Adenomas, Aggressive HCCs</td>
</tr>
<tr>
<td>&gt;65</td>
<td>Adenomas, Mixed tumors, Aggressive HCCs</td>
<td>Adenomas, Aggressive HCCs</td>
</tr>
</tbody>
</table>
URI expression in hepatocytes induces a ductular reaction/oval cell expansion

A

B

C

Tummala et al.; Cancer reports, 2017
hURI-tetOFF hep mouse crossed with Sox9-IRES-EGFP line (EpCAM, CD133, CD44, Lgr5, and DLK1)

Sox9-IRES-EGFP (Nel-Themeat et al., 2009; Yoshiya Kawaguchi)
hURI-TetOFF\textsuperscript{hep} mouse is a unique genetic tool to study cellular and molecular mechanisms of HCC

- Recapitulates many features of human HCC (anisokaryotic, oval cell expansion, LLCD, fibrosis, inflammation, NASH, hepatoma...)
- Long time interval to form full blown HCC: recapitulates human situation seen in the clinic
- Accelerate tumorigenesis by increasing URI concentration (Ki; Ki): HCC <10 weeks
- URI mouse HCC presents the human HCC signature (gene expression profiling)
- Expression level of URI in mouse hepatocytes corresponds to fold increase in human HCC
- Switch-ability
- Heterogeneous tumors
- Lung metastasis as in the human scenario
- Metabolism and changes in body physiology
hURI-tetOFF\textsuperscript{\text{hep}}: genetic model with oval cell expansion and HCC development without dramatic effects on body weight and without liver function impairment

1- **Cancer cell origin**: How liver regeneration contribute to liver tumorigenesis? What is the contribution of hepatocytes and hepatic progenitor cells (oval cells) during hepatocarcinogenesis?

2- Great model to study mechanisms of **HPC differentiation into hepatocytes**: cell therapy and liver regeneration, regenerative medicine and pharmaceutical screening
Hepatocyte labeling

Serum Albumin (SA)-CreERT2; R26-LSL-EYFP

A

<table>
<thead>
<tr>
<th>Weeks</th>
<th>0</th>
<th>5</th>
<th>Tamoxifen diet</th>
<th>7</th>
</tr>
</thead>
</table>

SA$^{\text{CreERT2};\text{R26-stop-EYFP}}$; Sacrifice

B

SA$^{\text{CreERT2}(+/+);\text{R26-stop-EYFP}}$; SA$^{\text{CreERT2}(+/-);\text{R26-stop-EYFP}}$

C

SA$^{\text{CreERT2}(+/+);\text{R26-stop-EYFP}}$; SA$^{\text{CreERT2}(+/-);\text{R26-stop-EYFP}}$

αEYFP

αHNF4α, αEYFP, DAPI

[Images showing immunostaining results]
Hepatocyte tracing in hURI-tetOFF$^{\text{hep}}$ mouse

hURI-tetOFF$^{\text{hep}}$; SA-CreERT2; R26-LSL-EYFP

**Labelling**

- **Tamoxifen** diet + Tamoxifen 2 weeks

**Tracing**

- Chow diet - Tamoxifen 65 weeks

Tumor with hepatocytic origin

Tumor with hepatocytic and non hepatocytic origin

Tumor with a non hepatocytic origin

- $\alpha$-GFP/DAPI

N= 10/59 (17%)

N= 29/59 (49%)

N= 20/59 (34%)
Summary:

1. **Hepatocytes** contribute to the tumors formation in HCC models.

2. **Not all tumors** derive from hepatocytes: do HPCs contribute to HCC?
Sox9 positive cell labelling

Sox9-CreERT2; R26-LSL-EYFP

Tummala et al.; Cancer reports, 2017

Sox9-CreERT2 (Furuyama et al., 2010; Haruhiko Akiyama)
HPC tracing in hURI-tetOFF\textsuperscript{hep} mouse

hURI-tetOFF\textsuperscript{hep}; Sox9-CreERT2; R26-LSL-EYFP

**Labelling**

+ Tamoxifen
2 weeks

**Tracing**

- Tamoxifen
Several weeks

[A] Control

\[\alpha\text{-GFP/}\alpha\text{-HNF4}\]

12 weeks

B

32 weeks

\[\alpha\text{-GFP}\]

Tummala et al.; Cancer reports, 2017
HPC tracing in hURI-tetOFF\textsuperscript{hep} mouse

Tumor with ductular cell origin

Tumor with ductular cell and non ductular cell origin

Tumor with a non ductular cell origin

Hepatocytes and hepatic progenitor cells contribute to the tumor heterogeneity

Tummala et al.; Cancer reports, 2017
HCC predominantly arises from hepatocytes whereas HPCs contribute to the formation of benign tumors, including RNs.

Liver regeneration involving HPCs is most likely forming a line of defense against HCC, leading to RNs.

Tummala et al.; Cancer reports, 2017
URI is upregulated by inflammatory cues to deplete NAD+ levels and induce DNA damage and p53 response.

Replenishment of NAD+ by nicotinamide riboside prevents HCC.

Tummala et al.; Cancer Cell, 2014
HPCs expansion dependents on oncogenic URI expression

Tummala et al.; Cancer reports, 2017
Hepactocytic DNA damage is essential for HPC expansion

A

Birth 0 3 weeks DNA damage 12 weeks

NR Ductular reaction

B

Control Mutant

Chow diet α-Sox9 NR

C

12 weeks

** Control Mutant Control Mutant

Chow diet NR

Sox9 (positive cells / 10x field)

D

Chow diet NR

Control Mutant Control Mutant

hURI Sox9 Vinculin

Tummala et al.; Cancer reports, 2017
Relieving the proliferative break or senescence by depleting p53 or p19ARF, respectively increases HPC expansion.

1. The type of damage is essential for HPC expansion
2. Even if hepatocytes continuously proliferate, there is still a ductular reaction

Tummala et al.; Cancer reports, 2017
Liver regeneration in chronic injury

**Chronic liver injury**
- Hepatitis B/C virus
- Alcohol
- Aflatoxin
- Iron Storage
- Obesity-Diabetes
- Genetic factors, Autoimmune disorders
- Cholestatic disorders

**Damaged hepatocytes**

**Activation of oval cells (HPCs)**

**Expansion of oval cells**

**Regenerative nodules**

**Cirrhosis**

**Fibrosis**
- Disrupted architecture
- Exert physical forces to impair liver function

**Wound healing response**

**Activated fibroblasts**
- EC Matrix deposition
- Angiogenesis
- Parenchymal cell death
MCRS1 deletion in adult hepatocytes causes mouse death at about 15 weeks of age

Garrido et al.; in preparation
Deletion in adult hepatocytes causes mouse cirrhosis at about 15 weeks of age

Garrido et al.; in preparation
Treatment of KO mice with diethylnitrosamine-induced HCC

Diethylnitrosamine (DEN)-induced carcinogenic liver injury in mice
DEN-induced HCC is prevented in KO mice

Garrido et al.; in preparation
Overexpression in hepatocytes promotes DEN-induced HCC

A

Birth

DEN

d14

†

w40

B

MCRS1\(^{+/+}\)\_hep

MCRS1\(^{+/KI}\)\_hep

C

MCRS1\(^{+/KI}\)\_hep

D

<table>
<thead>
<tr>
<th>Mice (%)</th>
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</thead>
<tbody>
<tr>
<td>No tumors</td>
</tr>
<tr>
<td>Displasia</td>
</tr>
<tr>
<td>HCA</td>
</tr>
<tr>
<td>HCC</td>
</tr>
</tbody>
</table>

n = 17, 16, 7

Garrido et al.; in preparation
MCRS1 is overexpressed in HCC patients and correlated with etiologies and poor survival.

(A) Typical expression levels of MCRS1 from HCC tissues. (B) Etiologic distribution and relative expression of MCRS1 by immunohistochemistry. (C) Kaplan-Meier survival analysis of MCRS1 expression levels from HCC tissues. Figure C: MCRS1 negative or low, MCRS1 high. (Garrido et al.; in preparation)
Liver regeneration in chronic injury (cirrhosis) might be an adaptive response to protect against HCC rather than predisposing to HCC.

TCGA database: OncoLnc (http://www.oncolnc.org/)

Garrido et al.; in preparation
Liver regeneration in chronic injury

Chronic liver injury
Damaged hepatocytes

Activation of oval cells (HPCs) → Expansion of oval cells → Regenerative nodules

Inflammation → NASH

Activation of hepatic stellate cells (HSCs) → Myofibroblast → Fibrosis

Disrupted architecture
Loss of function

Hepatitis B/C virus
Alcohol
Aflatoxin
Iron Storage
Obesity-Diabetes
Genetic factors, Autoimmune disorders
Cholestatic disorders

Wound healing response

Activated fibroblasts
EC Matrix deposition
Angiogenesis
Parenchymal cell death

Cirrhosis
NASH: liver injury, inflammation and fibrosis

Prevalence of NAFLD & NASH in general adult population

Worldwide prevalence (Younossi et al., 2016):
- NAFLD: **25.2%**
- NASH: **1.5 – 6.45%**

US prevalence (Kim et al., 2013, Williams et al., 2011):
- NAFLD: **34%** *(estimated to be 65% between 2020-2030)*
- NASH: **12%**

Pediatric NAFLD
- General children: **3 – 10 %**
- Obese children: **70%**

Populations at risk: prevalence in population with obesity

- NAFLD: **70% or more**
- NASH: **25-30%**

Populations at risk: prevalence in population with type 2 diabetes

- NAFLD: **65% to 70%**
- NASH: **25-30%**

- 1 billion individuals worldwide with NAFLD at the present time
- Absolute burden of NASH-related HCC is higher than that of HCV-related HCC
- In the United States, NASH is expected to become the leading cause of liver transplantation by 2020
Nutrient surpluses increase hepatic URI expression

Hepatic URI can be a mediator of nutrient surpluses and HCC progression?

Gomes et al.; Cancer Cell, 2016
Working model: does metabolic inflammation-associated IL-17A cause NASH and HCC?
Digoxin inhibits RORγT and blocks T_{H}17 Cell differentiation

T_{H}17 Cells may cause NASH

Gomes et al.; Cancer Cell, 2016

Huh et al. 2011, Nature
Gomes et al. 2016, Cancer Cell
Digoxin prevents HCC

Gomes et al.; Cancer Cell, 2016
Genetic ablation of IL-17RA in myeloid cells alleviates HCC in URI mice

**hURI-tetOFF**

**IL-17RA-lox X LysMCre**

Deletion of IL-17RA in myeloid cells

Gomes et al.; Cancer Cell, 2016
Metabolic inflammation-associated IL-17A causes non alcoholic steatohepatitis and hepatocellular carcinoma

- Digoxin
- \(\alpha\)-IL-17A blocking antibodies
- IL17RA deletion in granulocytes

Nutrients → DNA damage → Th17 cells → IL-17A → NASH → HCC

- Nicotinamide Riboside (NR) (NAD\(^+\) booster)

Fatty acid release → Insulin resistance → Neutrophil recruitment
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Transformed hepatocytes

- Decreased NAD+
- DNA Damage
- Altered TCA
- HPC Differentiation
- Galectin-3
- alpha-KG
- HPC Proliferation
- Tumorigenesis
- HCCs
- HCAs Regenerative nodules

Tummala et al.; Cancer reports, 2017