Orchestrating HCC Development by Diverse Liver Cancer Stem Cells

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The Origins of Liver Progenitor Cells

I. Progenitor Cells at Canals of Hering

II. Cholangiocytes

III. Hepatocytes

IV. Axin2+ pericentral hepatocytes

Dan YY, Hepatology 64:297-300, 2016
Liver cancer can originate from different cells of origin.

The presence of cancer stem cells is a common feature.

Histological types are linked to cell of origin.
Features of Liver Cancer Stem Cells

- **Stem cell marker**
- **Self-renewal**
- **Differentiation**
- **Oncosphere**
- **Invasive**
- **Chemoresistance**
- **Tumorigenicity**
- **Heterogeneity**

_Hematopoietic stem cell_ → _CSC_

_Hepatic stem cell_ → _CSC_

_Hepatocyte_ → _CSC_

_Carcinogenesis_ → _Primary HCC_ → _Invasion (Local/Distant Organ)_ → _Metastasis_

_Carcinogenesis_ → _Mutations_

- _Hepatocyte_

- _Hematopoietic stem cell_

**Key Features**

- Stem cell marker
- Self-renewal
- Differentiation
- Oncosphere
- Invasive
- Chemoresistance
- Tumorigenicity
- Heterogeneity

*Modified from Ji & Wang. Semin Oncol 39: 461-72, 2012*
Are EpCAM\(^{+}\) HCC Cells Tumor Initiating Cells with Normal Stem Cell Traits?

Yamashita T et al, Gastroenterology 2009

**CSC marker expression**

- EpCAM
- AFP
- DAPI
- Merge

**Stemness Genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>HCC cells</th>
<th>HpSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>[graph]</td>
<td>[graph]</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>[graph]</td>
<td>[graph]</td>
</tr>
<tr>
<td>MYC</td>
<td>[graph]</td>
<td>[graph]</td>
</tr>
<tr>
<td>TACSTD1</td>
<td>[graph]</td>
<td>[graph]</td>
</tr>
</tbody>
</table>

**Self-renewal**

- Number of colony: [graph]

**Differentiation**

- [graph]

**Oncosphere**

- Number of spheres per 1000 cells: [graph]

**Tumorigenicity**

- 200 cells
- Tumor incidence (%): [graph]
3D-Organotypic HCC Culture Models to Study Candidate Driver Genes

In Vitro Features of the Glandular Epithelium

Epithelial acinar morphogenesis
- Cyst-like spheroid
- Apicobasal polarization
- Tight control of cell growth

Ref: Debnath & Brugge, Nat Rev Cancer 2005

Acinar Morphogenesis of EpCAM⁺ AFP⁺ HCC Cells

3D Matrices (AlgiMatrix)

Ref: Nature 451, 856, 2008
TGF-β Promotes HCC Progression in HCC Organoids

Takai et al, Scientific Reports 6: 21174, 2016
Cell of Origin and Cancer Stem Cell Heterogeneity in Liver Cancer


**Mutations**
- Hematopoietic stem cell
- Hepatic stem cell
- Hepatocyte

**Carcinogenesis**

**Primary HCC**
- EpCAM$^+$
- CD133$^+$
- CD90$^+$
- CD44$^+$
- CD24$^+$
- CD13$^+$

**Invasion (Local/Distant Organ)**
- CSC
- Non-CSC

**Metastasis**
- HCC nodule
- Limited lifespan

**CSC**
Discrete Nature of EpCAM\(^+\) and CD90\(^+\) Cancer Stem Cells in Human HCC

Yamashita et al, Hepatology 2013;57:1484-1497

**Principal Component Analysis**
(1,561 genes; 172 HCC cases)

- **CD90\(^+\)** (n=49)
- **EpCAM\(^+\)** (n=34)
- **CD133\(^+\)** (n=10)
- **Triple negative** (n=79)

**EpCAM\(^+\) HCC**
- Epithelial-like cells; AFP\(^+\), \(\beta\)-catenin\(^+\)
- Sensitive to \(\beta\)-catenin inhibitor

**CD90\(^+\) HCC**
- Vascular endothelial-like cells; c-kit\(^+\)
- Sensitive to imatinib
The Etiology and Features of Liver Cancer Heterogeneity

Demographic
- Age
- Gender
- Ethnicity

Environmental
- HBV, HCV
- Chemical Carcinogens: AFB1, etc.
- Microbiota, parasites, etc

Clinical
- Tumor size
- Tumor stage
- Metastasis status
- Chronic liver diseases

Lifestyle
- Smoking
- Alcohol intake
- Dietary factors

Tumor Biology

Tumor genomics
- Somatic mutations
- Epigenetic alterations
- Aberrant transcriptome

Microenvironment
- Hypoxia
- Inflammation and immune cell infiltration
- Cytokines/ Growth factors
- Extra-cellular matrix remodeling
- Vascularization
Lineage Specific Gene Expression Patterns Define Liver Tumor Subtypes

- **Hepatic lineages**: Cholangiocyte, Biliary progenitor, Liver stem/progenitor, Hepatocytic progenitor, Hepatocyte

- **Histological diagnosis**: ICC, Combined HCC-ICC, HCC

- **Molecular subtypes**: Differentiated ICC, Stem cell-like ICC, Stem cell-like HCC, Differentiated HCC

- **Survival**: Better, Poor, Better

**References**:
- Lee et al, Nat Med 2006; 12:410-6
- Oishi et al, Hepatology 2012; 56: 1792-1803
- Yamashita, Hepatology 2013; 57: 1484-97
- Yamashita and Wang, J Clin Invest 2013; 123: 1911-8
- Budhu et al, Gastroenterology 2013; 144: 1066-75
AIMS
To identify genomic and etiological factors that modify liver cancer risk, susceptibility and progression

Collaborators: NCI, Chulabhorn Research Institute and five cancer institutes in Thailand

Estimated enrollment: 6000 subjects
(1000 HCC, 2000 CCA, 2000 high risk & 1000 controls)

TIMELINE
Initiation: June 11, 2008
Protocol approval: August 2010
CRI-NCI MOU signing: December 3, 2012
Phase I study: May 2013
TIGER-LC Study Outline

Additional 180 Thai HCC/CCA patients for further validation
Mutational Landscape of Thai Cholangiocarcinoma

129 Thai ICC Patients

V
a
r
i
a
n
ts

% Freq

ICC-C1 ICC-C2 ICC-C3 ICC-C4

0.4

TP53
ARID1A
KRAS
SMAD4
APC
KMT2C
GNAS
LRP1B
CSMD3
ERBB2
RYR2
ADAMTS20
EBB3
SYNE1
ARID2
ATM
NF1
PRKDC
KAT6B
MAP2K4
PSIP1
RNF43

HBV
HCV
OV

Variants

0
10
20
30
40
50

nonsynonymous/indel mutations
no mutation

positive
negative

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Tumor Heterogeneity

- **Inter-tumor heterogeneity** (between tumors)

**Tumor cell:**
- Genomics
  - Genetic susceptibility (SNP, TSG)
  - Somatically acquired: SCNA, DNA mutations, epigenomics
- Non-genetic: transcriptomics, proteomics, metabolomics

**Microenvironment:**
- Genetic susceptibility (e.g., MHC class molecules, immune systems)

- **Intra-tumor heterogeneity** (within tumors)
  - Genomics; non-genetic
  - Tumor cell biology; evolutionary divergence
  - Collective behavior and regulation

- **Significance:** genomic/phenotypic plasticity; moving target; resistance to therapy
Genetic Intratumor Heterogeneity in A Clear-cell Carcinoma Patient

Clonal Evolution of Tumor Cell Populations

Peter C. Nowell. Science 1976
**Tumor Single Cell Genome**

**Single Cell Isolation**

- FACS
- DEPArray

**Possible Lessons**

- Molecular heterogeneity
- Tumor cell community
- Collective behavior/regulation
- Common driver signaling
- Consensus biomarkers
- Resistance to therapy

**Microfluidics**

- DNA seq
- RNA seq

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*abcam.com*

*siliconbiosystems.com*
Transcriptomic Heterogeneity of Liver Cancer Stem Cells

Hierarchical clustering of RNA-seq profiles of 64 single cells
Transcriptomic Differences Within and Between HCC Stem Cell Populations

- Differences among cell types
- Differences between marker-positive and marker-negative HCC cells
- Differences among single HCC cells

Hongping Zheng, Maria Hernandez, Yotsawat Pomyen
Glioblastoma Single Cell Genome

RNA-seq profiles of 430 single cells from 5 primary glioblastomas

- High degrees of intra-tumor and inter-tumor heterogeneity
- Continuous, rather than discrete, stemness-related gene expression
Single-cell triple omics: Liver Tumor Single Cell Genome


Single-cell triple omics sequencing (scTrio-seq)

Intra-tumor Heterogeneity

SCNA

GE
Can We Detect Cancer Stem Cells *In Vivo*?


- Improved early cancer detection
- Better therapeutic monitoring
- Better understanding of CSC, e.g., tumor persistence, dormancy, relapse, therapy failure
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