Liver Cancer Stem Cell Biology
Contents

- What is Cancer Stem Cell (CSC) ?
- Biological Characteristics of Liver CSC ?
- Role of CSCs on Hepatocarcinogenesis ?
- Clinical Implication of Liver CSC ?
Stem cells

- Self–renewal
- Differentiation
- Multi-proliferation potential
Cancer stem cells

Def: "a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor"

by consensus of the American Association of Cancer Research

◆ Self–renewal
◆ Differentiation
◆ Multi-proliferation potential

Aberrant regulation
History of CSC

- **1855, Rudolf Virchow**: “Cancers arise from the activation of dormant, embryonic-like cells present in mature tissue” (1821~1902)
Van R. Potter and Barry Pierce “maturation arrest of tissue-determined stem cells” hypothesis

Ernest McCulloch and James Till defined the hallmark properties of stem cells: the ability to self-renew and differentiate [49]

Irving Weissman and colleagues were first to use SCID mice as an assay model for human hematopoietic cells

Michael Clarke and colleagues identified the first CSCs in a solid tumor – breast cancer stem cells (CD44+CD24−low); In the same year, P. Dirks and colleagues also identified a CSC population in brain tumors (CD133+)

CSC population identified in the skin by Meenhard Herlyn and colleagues (CD20+)

CSC population identified in the prostate by Anne Collins, Norman Maitland and colleagues (CD44+α2β1+CD133+)

John Dick, Tsvee Lapidot and colleagues identified the first CSCs – leukemic stem cells – in patients with AML (CD34+CD38−)

CSC population identified in the colon (CD133+,EpCAM+CD44+CD166+), pancreas (CD44+CD24+EpCAM+), liver (CD133+) and head/neck (CD44+)

Milestones in a concept of cancer as a stem cell disorder
Two General Models for Cancer Heterogeneity

Self renewal and differentiation are random. All cells have equal but low probability of extensive proliferation. Only cells with self renewal capacity can sustain tumor growth.

Distinct classes of cells exist within a tumor. Only a small definable subset, the cancer stem cells can initiate tumor growth.
How Do Cancer Stem Cells Arise?

1. Stem cell
   - Stem Cell
   - Normal stem cell
   - Mutated stem cell, or self-renewal genes turned on

2. Progenitor cell
   - Normal progenitor cell
   - Mutated progenitor, or self-renewal genes turned on
   - Loss of regulated cell division
   - Self-renewal genes turned on

3. Differentiated cell
   - De-differentiated cell

Cancer stem cell

Stem cell information from NIH resources
Role of Cancer Stem Cell

A Carcinogenesis
- Dysregulation of microenvironmental factors
- Stromal cells
- Immune cells
- Secreted factors
- Mutations
- Somatic stem cell
- Somatic progenitor cell
- Somatic differentiated cell

B Tumorigenesis
- Self-renewal
- Unlimited proliferative potential
- Angiogenesis
- Vasalogenic mimicry
- Immune evasion

C Tumor resistance
- Metastasis
- Chemoresistance
- Radioresistance
- Immune evasion
- Tumor progression
- Tumor recurrence
- Apoptosis
- Immune rejection
- Chemosensitivity
- Radiosensitivity
- Susceptibility to hypoxia

Frank NY et al. J Clin Invest 2010
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• What is Cancer Stem Cell (CSC)?
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Markers for identification of CSCs

- essential for early detection of cancers
- development of targeted therapy
### CSC markers in human cancers

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>CSC marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myeloid leukemia</td>
<td>CD34+CD38-</td>
</tr>
<tr>
<td>Breast</td>
<td>CD44+CD24-/low</td>
</tr>
<tr>
<td>Breast</td>
<td>ALDH1+</td>
</tr>
<tr>
<td>Brain</td>
<td>CD133+</td>
</tr>
<tr>
<td>Brain</td>
<td>CD133+</td>
</tr>
<tr>
<td>Head and neck</td>
<td>EpCAM\text{high} CD44+</td>
</tr>
<tr>
<td>Prostate</td>
<td>CD44+\text{high } \alpha_2\beta_1\text{high} CD133+</td>
</tr>
<tr>
<td>Melanoma</td>
<td>ABCB5+</td>
</tr>
<tr>
<td>Lung</td>
<td>CD133+</td>
</tr>
<tr>
<td>Liver</td>
<td>CD90+</td>
</tr>
<tr>
<td>Liver</td>
<td>CD133+</td>
</tr>
<tr>
<td>Liver</td>
<td>EpCAM</td>
</tr>
<tr>
<td>Liver</td>
<td>CD13</td>
</tr>
<tr>
<td>Ovary</td>
<td>CD44+ CD117+</td>
</tr>
</tbody>
</table>
CSC markers in HCC

- **Haraguchi et al. (2006):**
  - First evidence for existence of CSC in the liver
  - “side population”: subset of stem cells in hepatoma cell lines (Huh7, Hep3B)

- **Subsequent candidate markers:**
  - CD90, CD133, epithelial cell adhesion molecule (EpCAM), CD44, and CD13.
## Frequency of CSC markers in HCC

<table>
<thead>
<tr>
<th>Surface markers</th>
<th>Frequency</th>
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<tbody>
<tr>
<td><strong>Cell line</strong></td>
<td></td>
</tr>
<tr>
<td>Side population(SP)</td>
<td>0.25-0.8% (Huh7)</td>
</tr>
<tr>
<td>CD133</td>
<td>8-90% (HepG2, Huh7, PLC8024, Hep3B)</td>
</tr>
<tr>
<td>OV6</td>
<td>0.2-3.0% (Huh7, SMMC7721, Hep3B, PLC, HepG2)</td>
</tr>
<tr>
<td>EpCAM</td>
<td>58.1-99.2% (Huh1, Huh7, Hep3B)</td>
</tr>
<tr>
<td>CD90+CD44+</td>
<td>0.02-2.53% (HepG2, Hep3B, PLC, Huh7, MHCC97)</td>
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<tr>
<td><strong>Primary tumor</strong></td>
<td></td>
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<tr>
<td>CD90+CD44+</td>
<td>0.74- 4.14%</td>
</tr>
<tr>
<td>EpCAM</td>
<td>1.4-5.2%</td>
</tr>
</tbody>
</table>
Identification of CSC in HCC – CD133?

CD133+ HCC cells: marked stem cell characteristics (the ability to self-renew and to differentiate)

CD133+ HCC cells are more tumorigenic than CD133- cells in vivo.

Gastroenterology 2007
Identification of cancer stem cells in HCC – CD90?

CD90+ cells sorted from cell lines and CD45-CD90+ cells from blood samples and the tumor tissues of liver cancer patients generated tumor nodules in immunodeficient mice.

Hepatology 2008
Identification of cancer stem cells in HCC – EpCAM?

EpCAM+ HCC cells: tumor-initiating cells with stem/progenitor cell features.

Gastroentrology 2009
Characterization of hepatic stem cell marker expression in HCC cell

<table>
<thead>
<tr>
<th></th>
<th>EpCAM+ AFP+</th>
<th>EpCAM- AFP-</th>
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<tbody>
<tr>
<td><strong>HuH1</strong></td>
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<td><strong>HuH7</strong></td>
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<tr>
<td><strong>HLF</strong></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
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**Markers:**
- EpCAM
- CD133
- CD90
- CK19
- Vimentin
- Hep-Par1
- β-catenin
EpCAM+ HuH1 cells showed marked tumor-initiating capacity compared with CD133+ HuH1 cells.
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Hepatocarcinogenesis

- Aflatoxin B1
  - P53 mutations
  - P53 inactivation
- HBV or HCV viral and/or host factors
  - Oxidative stress
  - Proliferation and loss of growth control
  - Necrosis and regeneration
  - Genetic alterations
- HCV viral and/or host factors
  - Cirrhosis
  - Microenvironmental changes
- Alcohol
  - Inflammation
  - Expanded or altered stem cell compartment
- Genetic alterations

Hepatocellular carcinoma

2006 Nat Rev Cancer
Signaling pathways involved in Hepatocarcinogenesis

- p53
- retinoblastoma protein (pRb)
- Wnt/β-catenin
- Janus kinases (JAK)/signal transducers, MAPK
- EGF/TGF-β pathways
- stress response signaling, and activators of transcription (STAT) pathways
SOX4 overexpression regulates the p53-mediated apoptosis in hepatocellular carcinoma

(Yoon et al. Carcinogenesis, 2010)

- SOX4 was expressed at high levels in 37 of 58 (63.8%) HCC tissues
- SOX4 interacts with p53 in HCC cells
- SOX4 suppresses p53-mediated transactivation of p53-responsive promoters
- SOX4 suppresses p53-mediated apoptosis induced by γ- irradiation in HCC cells

**Sox4**: interacts with p53 and then modulates p53-mediated transcription → inhibition of apoptosis via suppression of Bax gene exp.
How CSCs are regulated?

- Aberrant gene exp. in CSCs is linked to genetic and epigenetic deregulation of key signaling pathways controlling stem cell maintenance, self-renewal and pluripotency, such as Wnt/β-catenin, TGF-β, Notch, Hedgehog and MYC.
Key signaling pathways regulating CSCs

- **Wnt/β-catenin signaling**: activation of Wnt/β-catenin signaling enriched the EpCAM(+) HCC cell population → *EpCAM is a Wnt/β-catenin signaling target gene*

- **TGF-β signaling**: correlation of its late expression signature with *more invasive* tumor characteristics and *poor clinical outcome*, including metastasis and survival

- **Notch and Hedgehog signaling**: play an essential role in regulation of cellular processes, including *proliferation, angiogenesis, and self-renewal*
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Clinical Implications of Liver CSCs

- Role of liver cancer stem cells in *chemoresistance*
- Role of liver cancer stem cells in *radioresistance*
- Role of liver cancer stem cells in *clinical outcomes*
Why is HCC not sensitive to Chemotherapy?

- **Multidrug resistance gene 1 (MDR1)**
  - encoding glycoprotein (170 kD):
  - members of ABC transporter superfamily
  - "drug pump" function; increases the cellular outflow of cytotoxic agents

- **Hypoxia**: increase of the expression of protective stress protein
  - cytokines and growth factors, VEGF, transcription factors (AP-1, HIF)
  - anti-apoptosis & cellular proliferation

- **Anatomical alteration of Cirrhosis**:
  - not only impairs drug metabolism, but also affects the manufacture of plasma-binding proteins → influence the serum proportion of active drug.
  - the accumulation of fluid → alter drug distribution volumes.
Silencing of 14-3-3ζ over-expression in hepatocellular carcinoma inhibits tumor growth and enhances chemosensitivity to cis-diammined dichloridoplatium

Jung Eun Choi a, Wonhee Hur a, Chan Kwon Jung b, Lian Shu Piao a, KwangSoo Lyoo a, Sung Woo Hong a, Sung Woo Kim a, Hye-Yeon Yoon d, Seung Kew Yoon a,c,∗

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bDepartment of Hospital Pathology, The Catholic University of Korea, Seoul 137-701, Republic of Korea
cDepartment of Internal Medicine College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea
dDepartment of Life Science, The Ewha Womens University, Seoul 158-056, Republic of Korea
Identification and characterization of 14-3-3ζ

A

Normal liver  HepG 2  Huh7  Hep3B

14-3-3ζ

β-actin

B

a  b  c

d  e  f

C

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<td>2.14</td>
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<td>β-actin</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>14-3-3ζ</td>
<td>1.13</td>
<td>1.00</td>
<td>1.56</td>
<td>1.00</td>
<td>1.05</td>
<td>1.00</td>
<td>0.86</td>
<td>1.00</td>
<td>0.68</td>
<td>1.00</td>
<td>2.57</td>
<td>1.00</td>
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</table>
Control of 14-3-3ζ expression might not only control tumorigenicity but also increase the chemosensitivity of HCC cells to CDDP. By altering the activation of JNK and p38/MAPK.
Role of liver cancer stem cells in chemoresistance

- **ATP–binding cassette (ABC) transporters**: expressed on cellular membranes of CSCs include MDR1 and BCRP drug transport pumps. They play an essential role in expelling anticancer drugs from cells → *chemoresistance*

- **Huh7 “Side Populations”** cells expressing ABC transporters, such as MDR1 and BCRP1: *chemoresistance* to doxorubicin

- **ALDH1**: expressed in CSCs → facilitates metabolism → *chemoresistance*

- **CD133+** HCC cells contribute to *chemoresistance* through preferential activation of Akt/PKB and Bcl-2 cell survival response

- **CD13**: a marker for semiquiescent CSCs in liver cancer cell lines
  - CD13 predominated in the G0 phase of the cell cycle
  - decreased ROS-induced DNA damage after genotoxic chemo/radiation stress → protected cells from *apoptosis*
CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of Akt/PKB survival pathway.
To increase the efficiency and the safety of the treatment, CSCs pathways, HCC subtypes, and drugs transporter inhibitions should be considered.
Role of liver cancer stem cells in Radioresistance


  - **CD133+ glioma cells**

  - Activation of *checkpoint response and repair mechanisms in response to DNA damage*

  - Radioresistance
CD133+ glioma stem cells promote radioresistance by preferential activation of the DNA damage response.
Strategy against Radioresistance
Some hepatic stem/progenitor cell markers predict prognosis of HCC

Role of liver cancer stem cells in Clinical Outcome

Integrative genomic analysis and its implication for cancer stem cell research

Genomic and epigenomic analysis of putative CSC can be used for cross comparison and validation using independent data from different sources (cell culture, human patients, and mouse models).
Lee JS et al. (Nat Med 2006)

Genomic evidence for a progenitor cell origin in liver cancer.

The comparative functional genomic analysis of 139 human HCCs and rat hepatoblasts/hapatocytes

Worse prognosis in HCC expressing HB(fetal progenitor cell)-related gene
Expression and clinical significance of the stem cell marker CD133 in hepatocellular carcinoma (N=60)

→ Patients with increased CD133 expression had a significantly shorter DFS and OS
The role of CD133 + CSCs for radioresistance in HCC
# Indicators of cancer stem cell identification in different human cancer types

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>CSC markers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematopoietic neoplasms</strong></td>
<td></td>
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<tr>
<td>Acute myeloid leukemia</td>
<td>CD34⁺CD38⁻</td>
</tr>
<tr>
<td></td>
<td>CD34⁺CD71⁻HLA-DR⁻</td>
</tr>
<tr>
<td></td>
<td>CD44⁺</td>
</tr>
<tr>
<td></td>
<td>ALDH⁺</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>CD34⁺CD19⁻ or CD34⁺CD10⁻</td>
</tr>
<tr>
<td></td>
<td>CD34⁺CD4⁻ or CD34⁺CD7⁻</td>
</tr>
<tr>
<td><strong>Solid tumors</strong></td>
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<tr>
<td>Breast cancer</td>
<td>CD44⁺CD24⁻/low</td>
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<tr>
<td></td>
<td>ALDH⁺</td>
</tr>
<tr>
<td>Brain cancer</td>
<td>CD133⁺</td>
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<tr>
<td>Prostate cancer</td>
<td>CD44⁺α₂β₁ hiCD133⁺</td>
</tr>
<tr>
<td></td>
<td>CD44⁺, CD44⁺α₂β₁⁺</td>
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<tr>
<td></td>
<td>CD133⁺CXCR4⁺</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>CD133⁺</td>
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<td>EpCAM⁺CD44⁺CD166⁺; ALDH⁺</td>
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<td>Melanoma</td>
<td>CD20⁺</td>
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<tr>
<td>Liver Cancer</td>
<td>CD133⁺, CD133⁺ALDH⁺</td>
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<td>CD90⁺CD45⁻CD44⁺</td>
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<td>Pancreatic Cancer</td>
<td>CD44⁺CD24⁺EpCAM⁺</td>
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<tr>
<td></td>
<td>CD133⁺CXCR4⁺</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>CD44⁺</td>
</tr>
</tbody>
</table>

- EpCAM, CD13,
Materials & Methods

Cell cycle (PI staining)

Cell death (Annexin V staining)

Cell proliferation (MTS assay)

Western blot

Cell Migration, invasion assay

Gelatin Zymography

cDNA Microarray

Huh-7 cell (60%)

CD133 + cells

CD133 – cells

γ-radiation
Enrichment of CD133+ cell subpopulations after irradiation of Huh-7 cells (at 72hr)

More CD133+ cells survived or proliferated after radiation treatment and conferred to radiation resistance in HCC.
The alteration of cell cycle arrest after irradiation

CD133+ cells displaying greater activation of cell cycle related protein, to control radiation-induced G2/M cell cycle arrest
CD133+ cells were resistant to radiation-induced apoptosis.

CD133+ cells could have more anti-apoptotic activities through increase Bcl-2 expression and resistance to radiation.
CD133+ cells exhibit more proliferation activity after radiation exposure compared to CD133- cells.

Radioresistance in CD133+ cells seems to be associated with the activation of the survival-promoting pathway ERK MAPK pathways.
**CD133+ cells are highly tumorigenic in nude mice than corresponding CD133- cells**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>IR</th>
<th>Tumor incidence</th>
<th>Latency (day)</th>
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<tbody>
<tr>
<td>CD133+</td>
<td>-</td>
<td>5/5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1/5</td>
<td>60</td>
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<tr>
<td>CD133-</td>
<td>-</td>
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<tr>
<td></td>
<td>+</td>
<td>0/5</td>
<td>-</td>
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</tbody>
</table>
Differential expression regulation between CD133+ and CD133− after radiation exposure: identified total 592 differentially expressed genes

GOBP enriched in DEG | P-value
--- | ---
apoptosis | 8.30E-03
(cell development | 2.60E-02
transport | 2.90E-02
sterol metabolic process | 2.30E-02
establishment of localization | 4.10E-02
cell adhesion | 4.30E-02
cell proliferation | 5.00E-02

The difference in cell proliferation and apoptosis between CD133+ and CD133− is significant.
Hypothetical network model

The network model shows the increased activity of cell proliferation and also the decreased activity of apoptosis in CD133+ cells.
Liver cancer cells expressing CD133 are more resistant to radiation treatment and have more faster proliferating activity compared to CD133− cells.

Activation of the MAPK pathway may be involved in the mechanism of radioresistance in these CD133+ liver cancer initiating cells.
Unraveling of cancer pathophysiology:
angiogenesis, metastasis, resistance to chemo/radiotherapy

Accumulating Evidences:
epigenetic regulation, microRNA, tumor environment

Candidate surface markers:
identification and characterization
Strategies for CSC sensitization

Conventional therapy → Conventional therapy → Relapse

CSC target therapy → Conventional therapy → Regression

Differentiation therapy → Conventional therapy → Regression

Immune therapy → Regression

Legend:
- CSC
- PC
- DC
- Dead cells
Collaborative Approach to Target CSCs

- Subtype of HCC
  - Signaling pathways molecules
  - Drug transporter inhibitors
  - Phenotypic marker of CSC

- Drug delivery systems
  - Combined therapeutic approach
  - Safety and efficacy

CSCs-targeted therapy
Acknowledgements
감사합니다