

# Update in liver cancer pathology: stemness and tumor microenvironment

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1885 Chejungwon

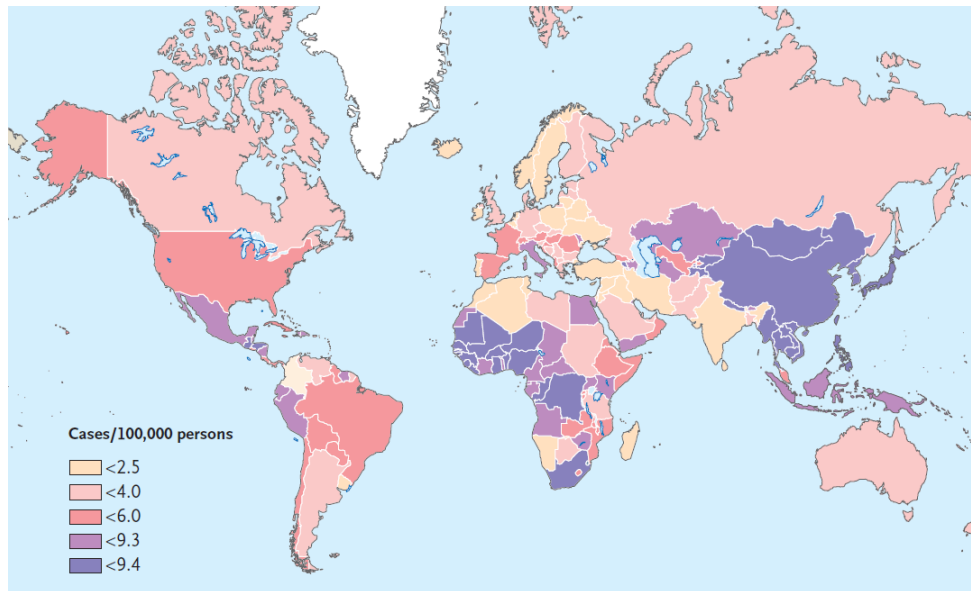
1904 Severance Memorial Hospital

1913 Severance Medical School

2005 Main building of the Severance Hospital

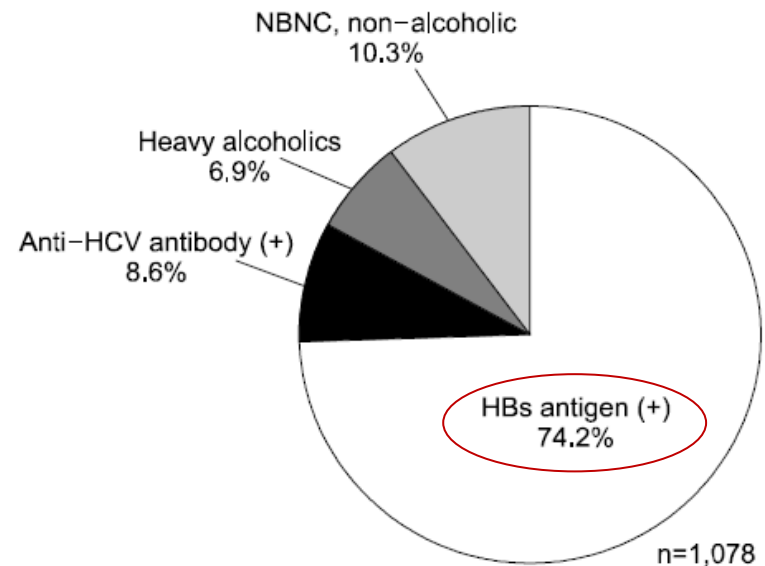
# Global burden of liver cancer

- The fifth most common neoplasm in the world
- The third mortality rate among all cancers
- ✓ One million new cases and 700,000 death worldwide annually.



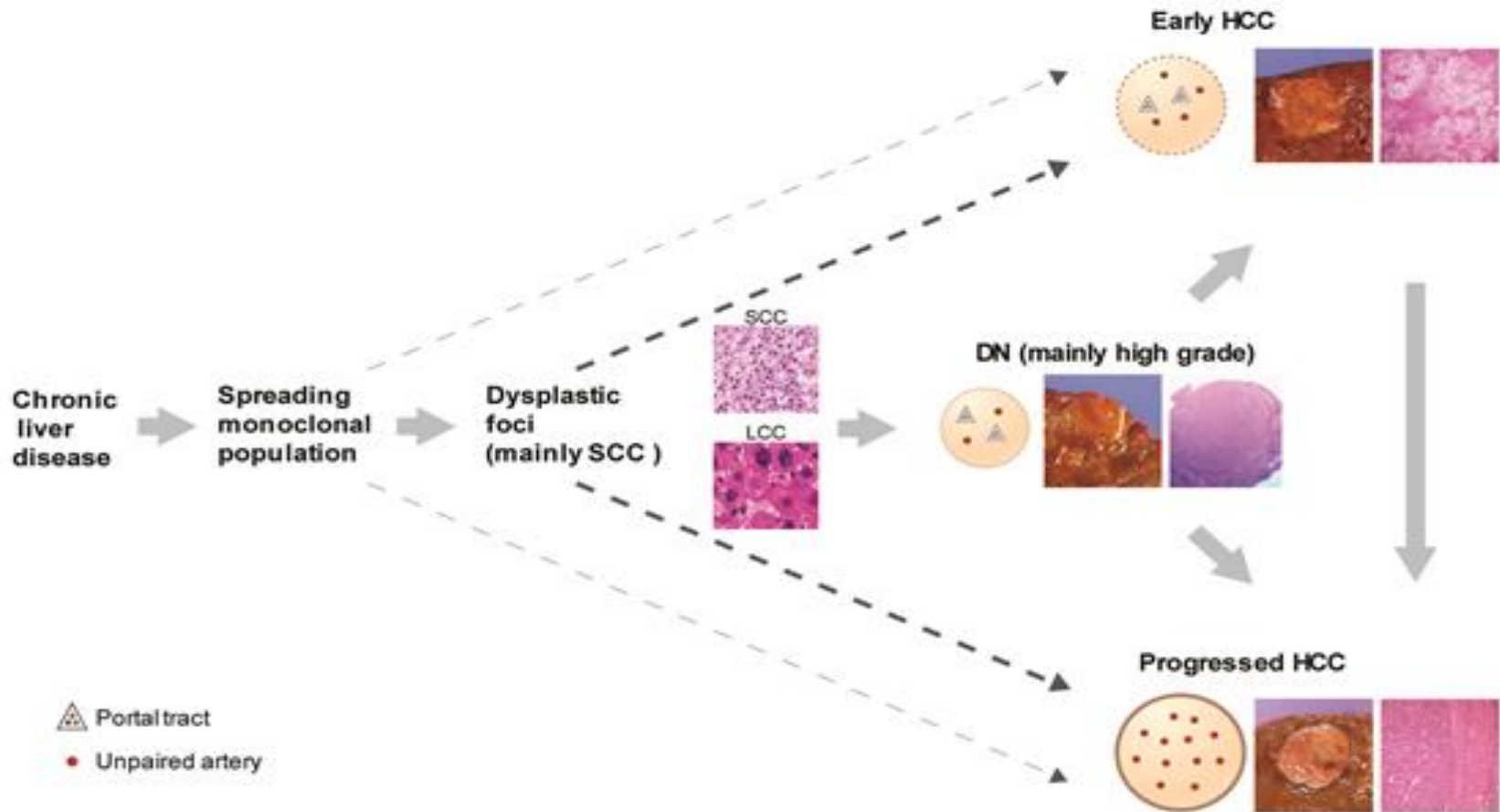
**Figure 1.** Regional Variation in the Estimated Age-Standardized Incidence Rates of Liver Cancer. The incidence rates shown (numbers of cases per 100,000 persons) pertain to both sexes and all ages. Adapted from the World Health Organization.<sup>3</sup>

## Etiology of HCC in Korea



**HBsAg (+) rate in Korean  
: 3% in adult, 0.2% in children**

# Human hepatocarcinogenesis is multistep process

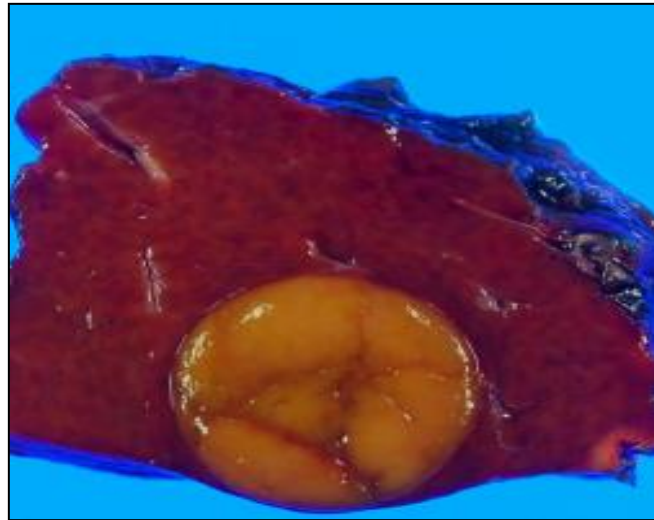


# Small HCC (< 2cm)

## Early HCC vs. Small progressed HCC

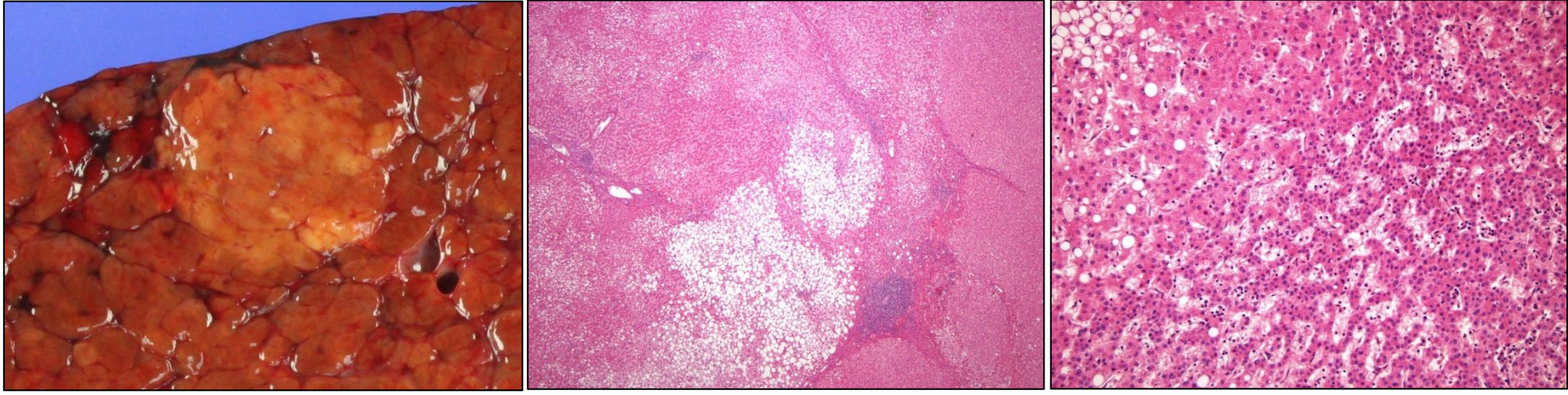


Indistinct margin



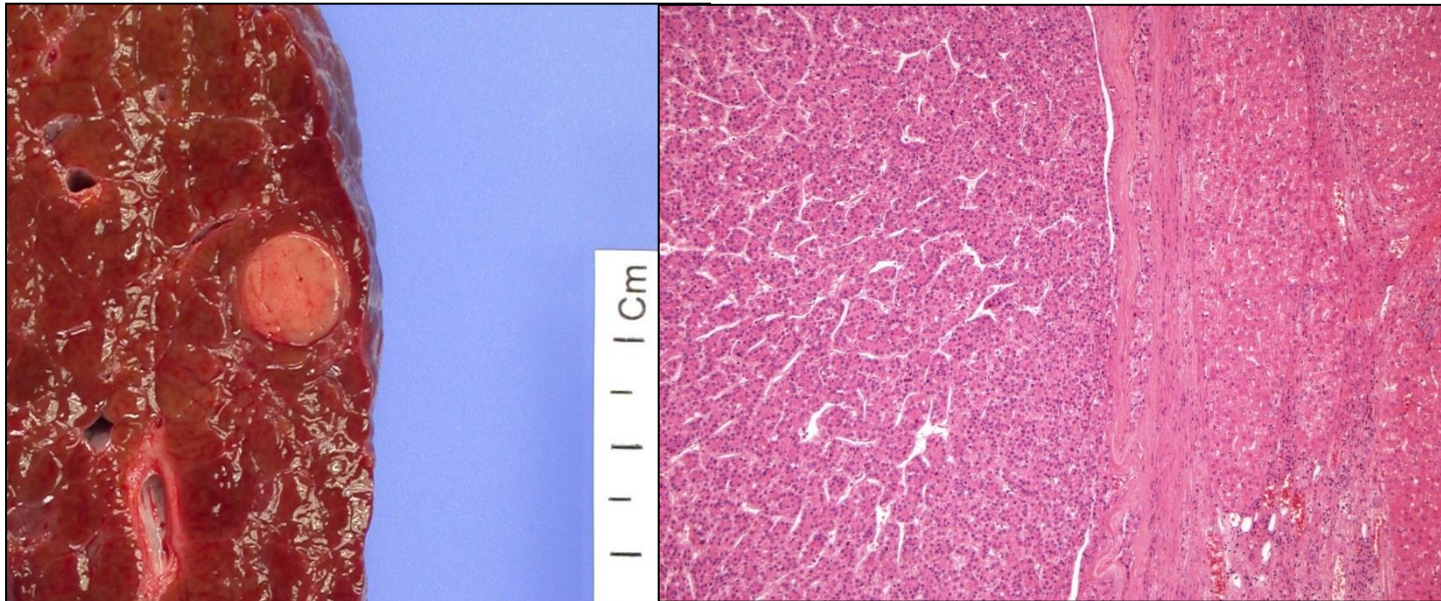
Distinct margin

# Early HCC



- Small HCC ( $11.9 \pm 3.3\text{mm}$ ) with indistinct margin
- Very well to well differentiated HCC
- Portal vein invasion:  $< 5\%$ , Intrahepatic metastasis:  $0\%$
- "Carcinoma in situ"
- Best prognosis (5yr survival rate  $> 90\%$ )
- Recurrence rate:  $80\%$  at 4yrs after surgery
  - ✓ Metachronous multicentric occurrence

## Small and progressed HCC (< 2cm)



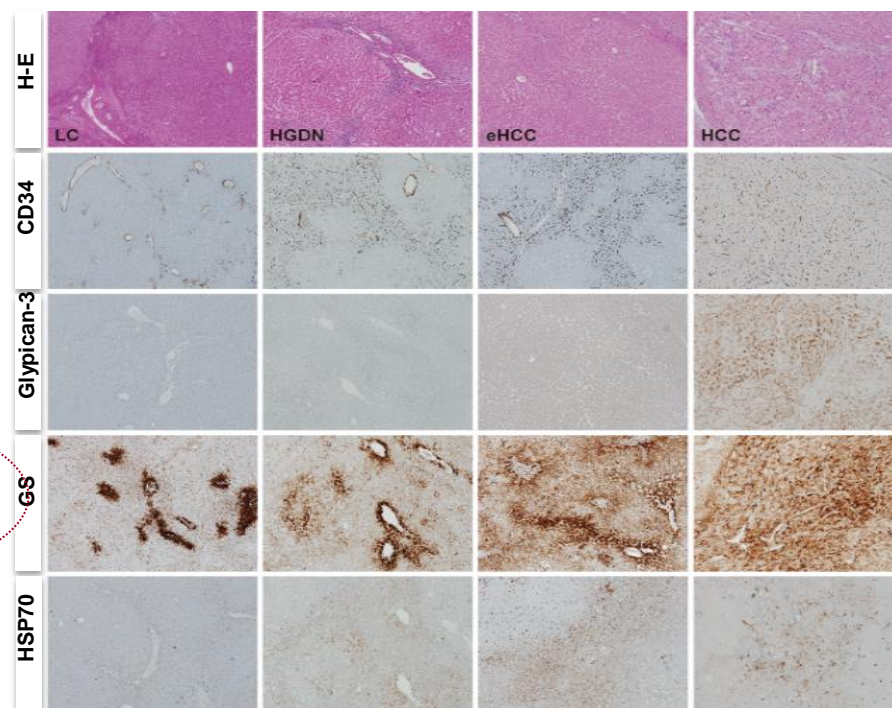
- Well defined mass with distinct margin
- Moderately differentiated HCC; 60%
- Tumor invasion in portal vein; 27%
- Intrahepatic metastasis; 10%

## Sequential transcriptome analysis of human liver cancer indicates late stage acquisition of malignant traits

Jens U. Marquardt<sup>1,2,†</sup>, Daekwan Seo<sup>1,†</sup>, Jesper B. Andersen<sup>1,†</sup>, Matthew C. Gillen<sup>1</sup>, Myoung Soo Kim<sup>4</sup>, Elizabeth A. Conner<sup>1</sup>, Peter R. Galle<sup>2</sup>, Valentina M. Factor<sup>1</sup>, Young Nyun Park<sup>3</sup>, Snorri S. Thorgeirsson<sup>1,\*</sup>

**Table 1. Clinicopathological information and HCC marker expression.**

Patient No.	Clinicopathological information					
	Sex	Age (yr)	Intrahepatic metastasis	Vascular invasion	Lesion	Size (cm)
1	M	68	No	No	LGDN	0.8 x 0.8
					HGDN	1.5 x 1.4
2	M	54	No	No	HGDN	0.8 x 0.8
					HGDN	0.8 x 0.7
					eHCC	1.2 x 1.0
					pHCC	3.0 x 2.0
3	F	42	No	No	HGDN	1.0 x 0.9
4	M	64	No	No	HGDN	1.5 x 1.3
5	M	61	No	No	LGDN	1 x 0.8
					LGDN	0.8 x 0.8
					LGDN	1.2 x 1.1
					HGDN	1.0 x 1.0
					HGDN	1.3 x 0.9
					HGDN	1.0 x 1.1
					eHCC	1.6 x 1.4
pHCC	3.8 x 3.2					
6	M	61	No	No	eHCC	2 x 1.9
7	M	50	No	No	eHCC	1 x 1.3
8	F	60	No	No	HGDN	1.0 x 1.0
					eHCC	1.1 x 1.0
					pHCC	1.2 x 1.0





### Sequential transcriptome analysis of human liver cancer indicates late stage acquisition of malignant traits

**LGDN**

- Oxidative Stress response
- Glutathione metabolism
- NRF2 signaling
- Apoptosis

**HGDN**

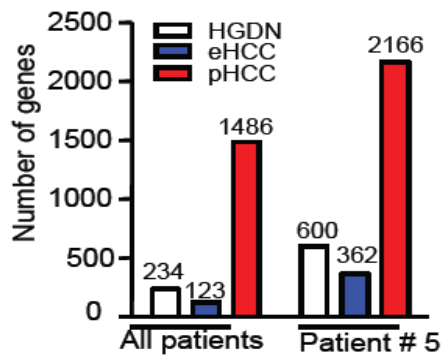
- Proliferation
- Cell cycle control
- Immune reponse
- ECM production

**eHCC**

- Cell adhesion
- Cytoskeleton remodeling
- EMT/ Invasion/ Metastasis
- Protein degradation
- TGFb signaling
- WNT / NOTCH signaling
- PI3K signaling
- MYC activation

**fHCC**

Malignant progression



**Early HCC**

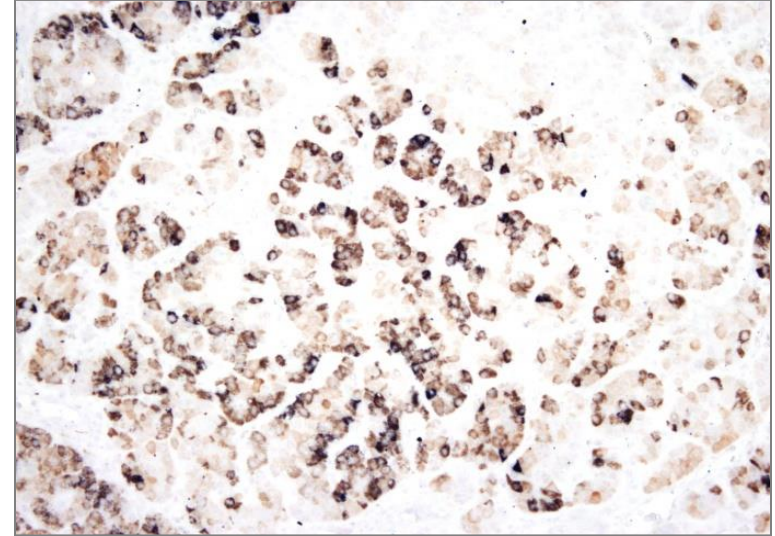
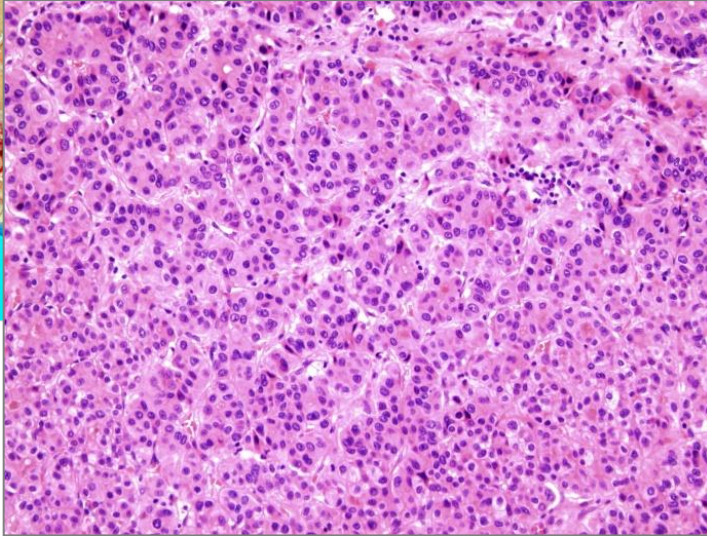
- ✓ Homogenous & modest changes in transcriptomes
- ✓ 123 genes

**Progressed HCCs**

- ✓ Extensive genetic alterations and subsequent activation of prognostic adverse signaling pathways
- ✓ 1486 genes
- ✓ Highlighting the molecular diversity of pHCC



# Cellular heterogeneity in HCC



K19: blue/ Brown: HepPar1

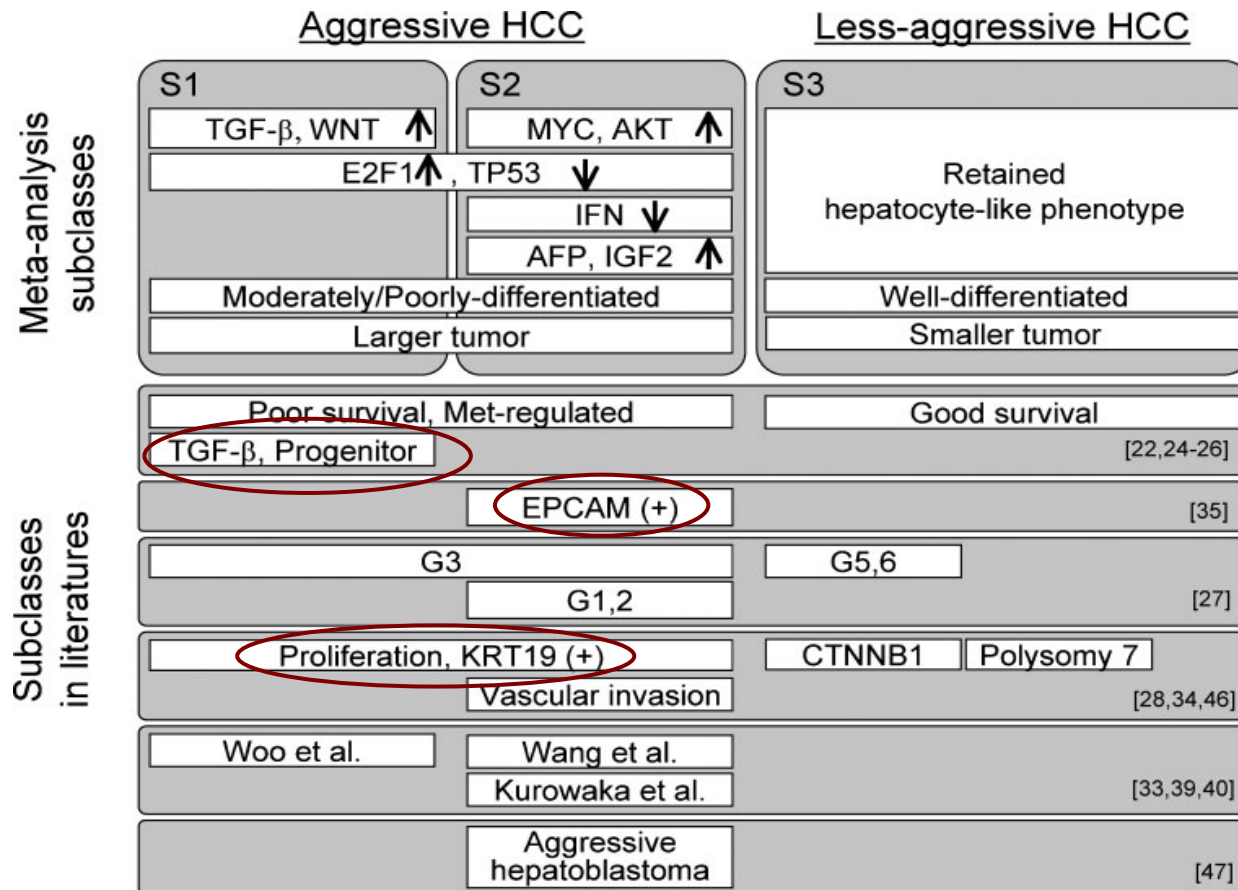
Cancer cell  
expressing stem  
cell marker

"Cancer stem  
(like) cell"

# “Cancer stem cell”

- Two ways to be used
  - ① ‘stem or progenitor cells from which some cancers derive’
  - ② ‘cancer cells, regardless of origin, which have stem cell-like functions’.
- ☞ **‘Cancer stem cell’** to those malignant cells within a cancer that perform stem cell like functioning
  - whether developing these functions de novo during malignant transformation or maintaining them as progeny of transformed stem/progenitor cells.

# ? Molecular classification might be mirrored by tumor morphology and phenotype



# HCC with stem cell markers

## 1) Pathological characteristics

### 2) Tumor microenvironment

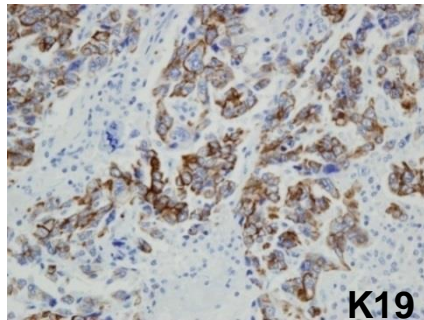
- ① Fibrous stroma
- ② K19 and its regulation mechanism
- ③ Response to locoregional treatment (TACE)

# 1-1) Pathological characteristics of HCC with stem cell markers

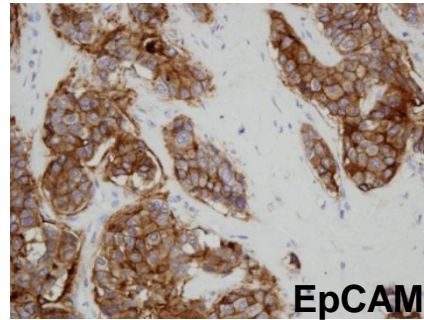
Human HCC cohort 1 (n=137, HBV+ 67.2%)

• Stem/progenitor cell marker expression

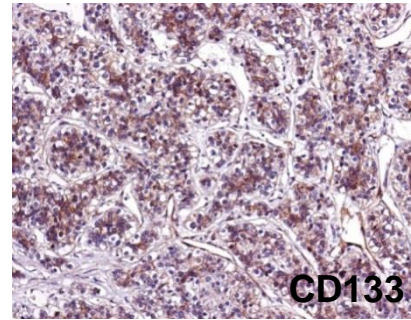
K19 (18%), EpCAM(35%), c-kit (34%), CD133(25%)



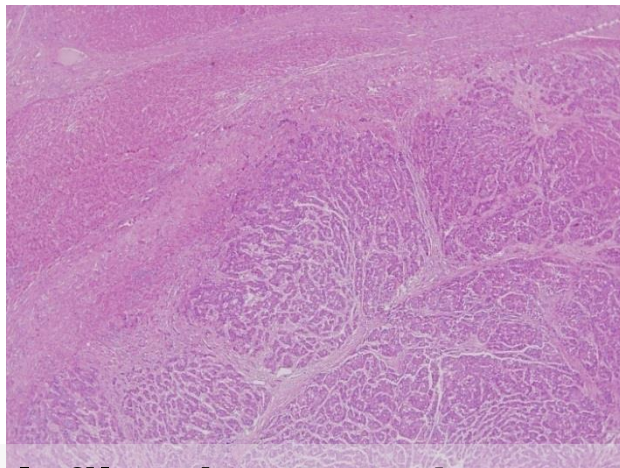
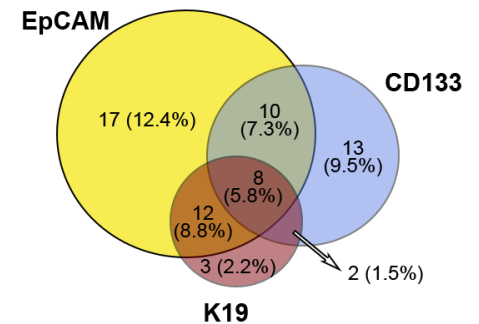
K19



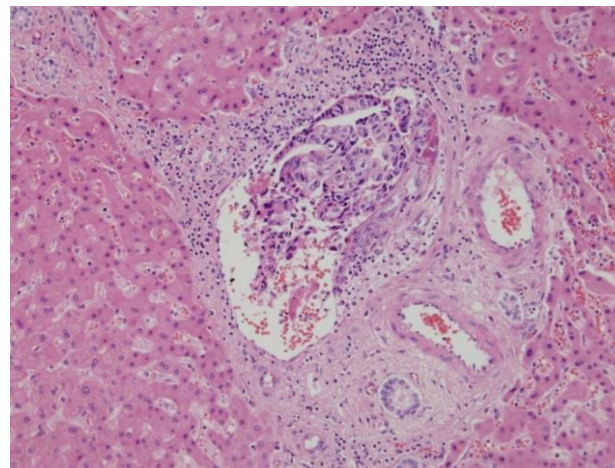
EpCAM



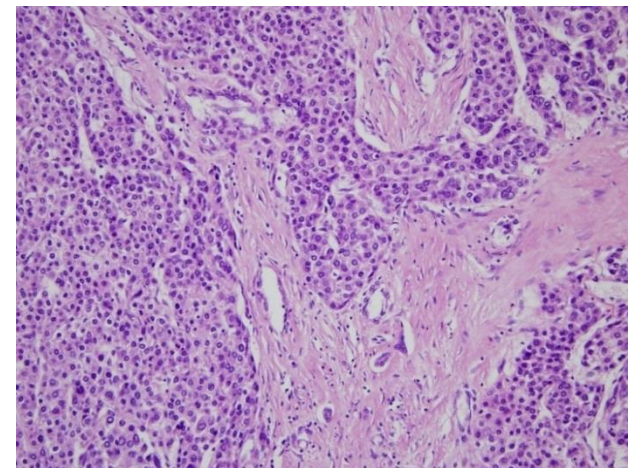
CD133



Infiltrative growth without tumor capsule

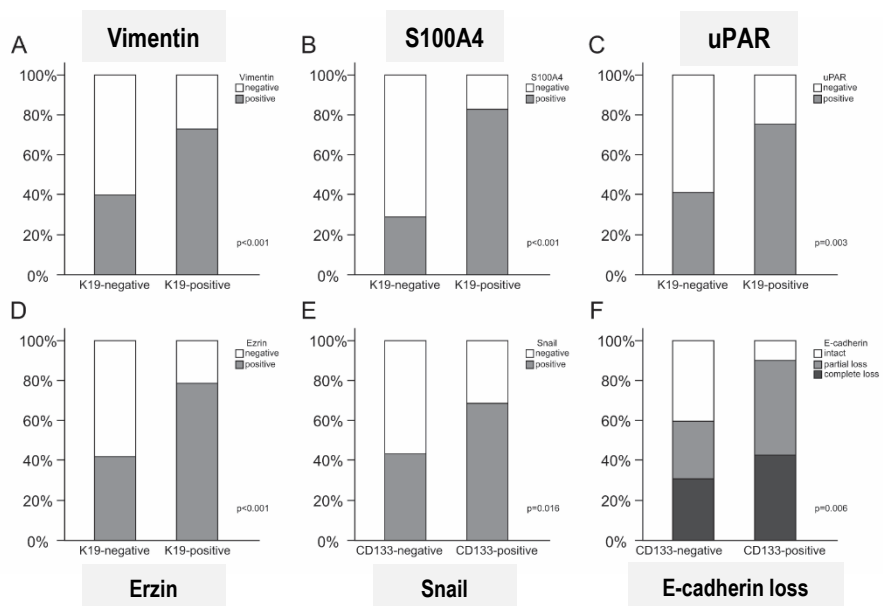
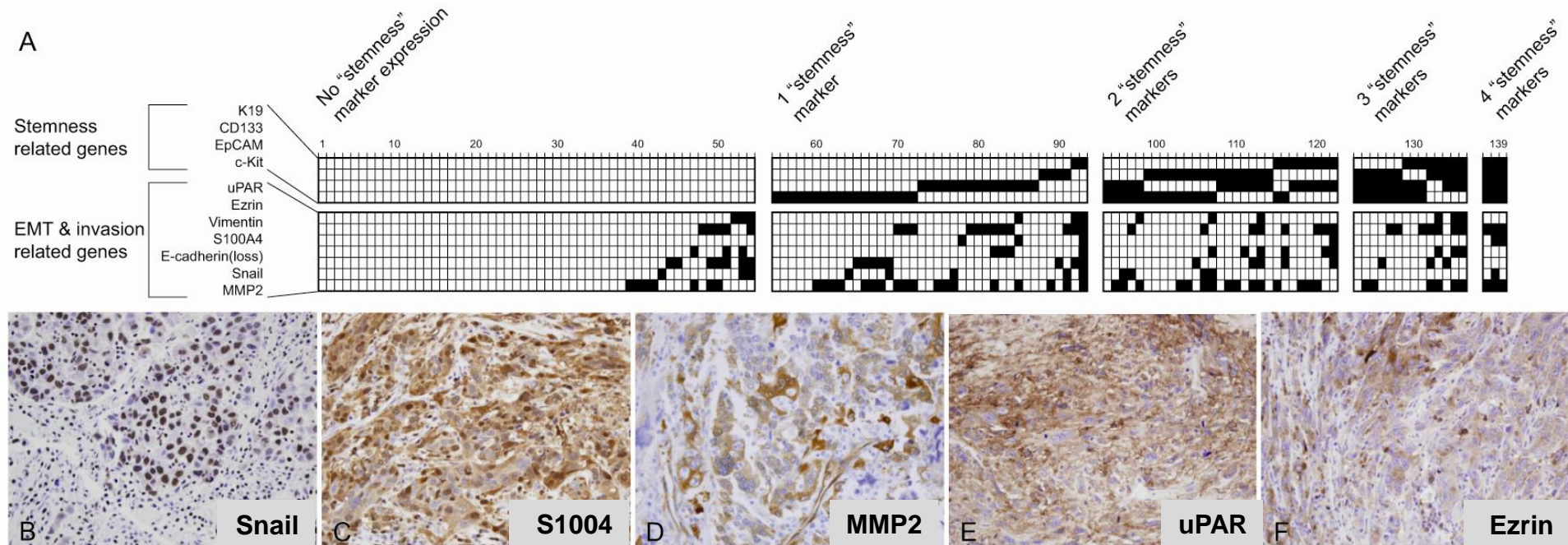


Vascular invasion



Fibrous stroma

# • Expression of EMT and invasion related genes



	Overall survival	Disease-free survival
<b>K19</b>	<b>0.018</b>	<b>0.007</b>
<b>CD133</b>	<b>0.057</b>	<b>0.552</b>
<b>EpCAM</b>	<b>0.777</b>	<b>0.302</b>
<b>c-kit</b>	<b>0.642</b>	<b>0.562</b>

# HCC Cohort 2 (n=237, HBV+ 80.2%)

## Human Hepatocellular Carcinomas With "Stemness"-Related Marker Expression: Keratin 19 Expression and a Poor Prognosis

Haeryoung Kim,<sup>1</sup> Gi Hong Choi,<sup>2</sup> Deuk Chae Na,<sup>3</sup> Ei Young Ahn,<sup>3</sup> Gwang Il Kim,<sup>3</sup> Jae Eun Lee,<sup>3</sup> Jai Young Cho,<sup>4</sup> Jeong Eun Yoo,<sup>3</sup> Jin Sub Choi,<sup>2</sup> and Young Nyun Park<sup>3,5,6</sup>

Hepatology 2011

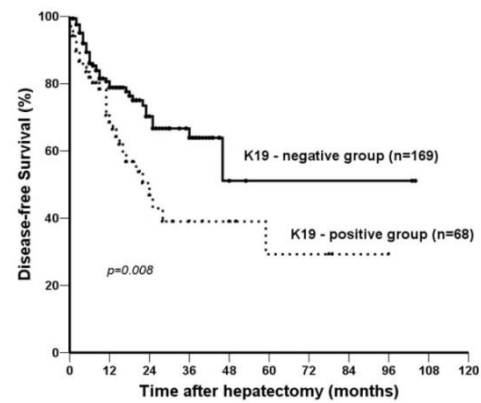
**Table 2. Comparison of Clinicopathologic Features Between K19-Positive and K19-Negative HCCs\***

Clinopathologic Features	K19-Positive Group (n = 68)	K19-Negative Group (n = 169)	P Value
Age (years, mean ± SD)	52.0 ± 10.2	56.2 ± 10.0	0.004
Gender (male:female)	42:26	147:22	<0.001
HBs antigen (%)	57 (83.8)	133 (78.7)	0.371
Serum ALT (IU/L, mean ± SD)	36.4 ± 21.3	37.3 ± 22.5	0.777
Serum AST (IU/L, mean ± SD)	46.0 ± 19.0	44.7 ± 34.6	0.780
Serum albumin (g/dL, mean ± SD)	4.3 ± 0.5	4.4 ± 0.5	0.033
Serum alpha-fetoprotein >1,000 IU/mL (%)	25 (36.8)	18 (10.7)	<0.001
Cirrhosis (%)	41 (60.3)	82 (48.5)	0.101
Tumor size (cm, mean ± SD)	3.92 ± 2.02	3.88 ± 2.58	0.911
Microvascular invasion (%)	50 (73.5)	96 (56.8)	0.017
Major vascular invasion (%)	6 (8.8)	9 (5.3)	0.317
Multiple tumors (%)	12 (17.6)	26 (15.4)	0.688
Poor differentiation* (%)	35 (51.5)	92 (54.4)	0.679
tumor-capsule formation (%)	11 (16.2)	51 (30.2)	0.027
Fibrous stroma (%)	12 (17.6)	12 (7.1)	0.015
Operation			
Minor/major resections	33/35	81/88	0.933

\*n = 237, cohort 2.

†Edmondson-Steiner grades III or IV.

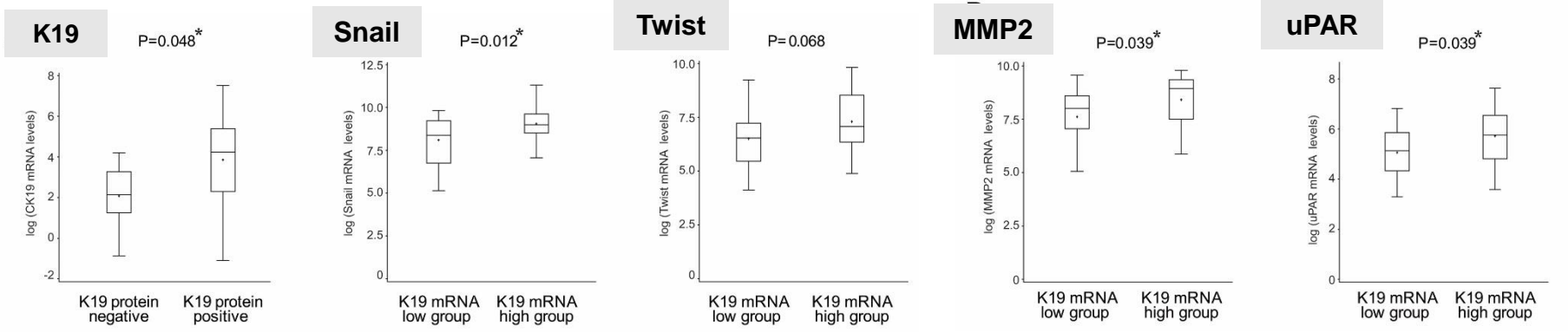
Abbreviations: K19, keratin 19; SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase.



**Table 4. Independent Prognostic Factors for Disease-Free Survival by Multivariable Analysis (Cohort 2)**

Variable	Coefficient	Standard Error	P Value	Relative Risk (95% CI)
K19 expression	0.522	0.243	0.032	1.685 (1.046-2.716)
Multiple tumors	0.883	0.278	0.002	2.417 (1.401-4.172)
Major vascular invasion	0.798	0.365	0.029	2.221 (1.085-4.545)
AST >50 IU/L	0.786	0.313	0.012	2.194 (1.187-4.054)
Tumor size >5 cm	0.672	0.263	0.010	1.958 (1.170-3.277)

Abbreviations: 95% CI, 95% confidence; K19, keratin 19; AST, aspartate aminotransferase.



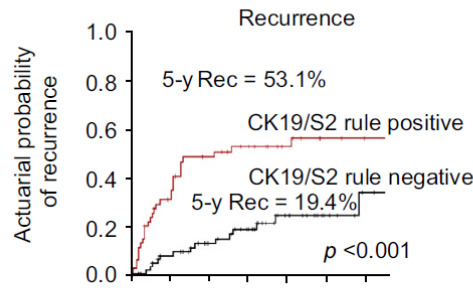
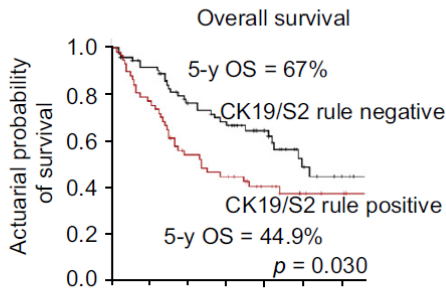
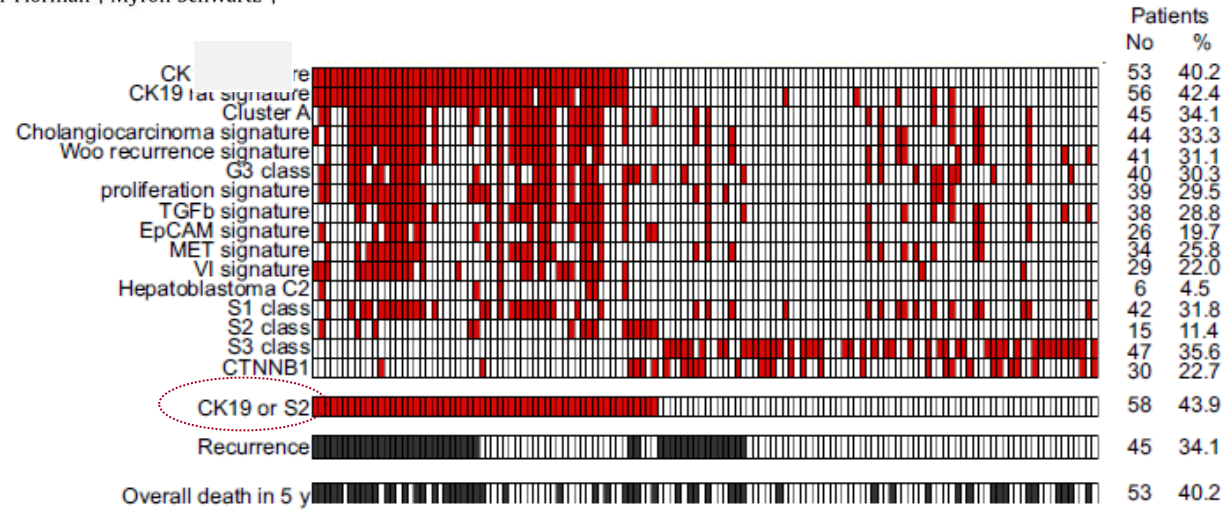


# Progenitor cell markers predict outcome of patients with hepatocellular carcinoma beyond Milan criteria undergoing liver transplantation

Oriana Miltiadous<sup>1</sup>, Daniela Sia<sup>1,2</sup>, Yujin Hoshida<sup>1</sup>, Maria Isabel Fiel<sup>1</sup>, Andrew N. Harrington<sup>1</sup>, Swan N. Thung<sup>1</sup>, Poh Seng Tan<sup>1,3</sup>, Hui Dong<sup>4</sup>, Kate Revill<sup>1</sup>, Charissa Y. Chang<sup>1</sup>, Sasan Roayaie<sup>5</sup>, Thomas J. Byrne<sup>6</sup>, Vincenzo Mazzaferro<sup>2</sup>, Jorge Rakela<sup>6</sup>, Sander Florman<sup>1</sup>, Myron Schwartz<sup>1</sup>, Josep M. Llovet<sup>1,7,8,\*</sup>

█ Positive for signature  
█ Rest

█ Positive event  
█ Negative event

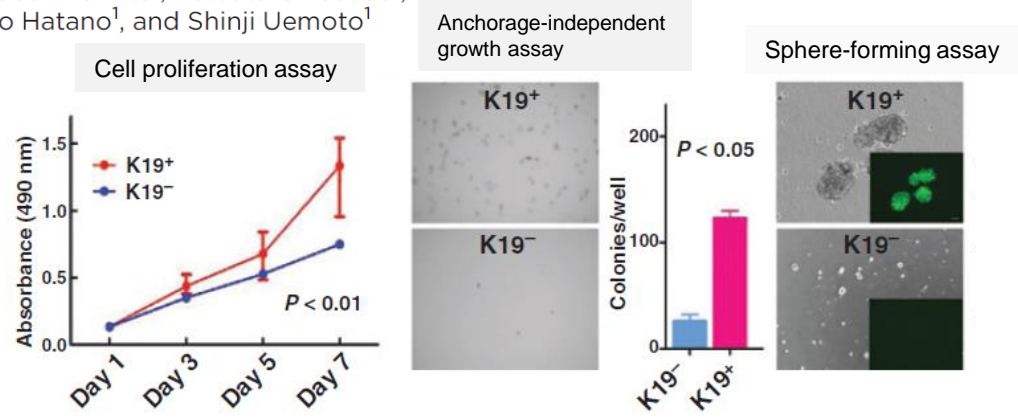




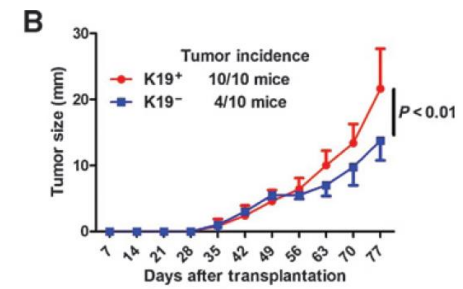
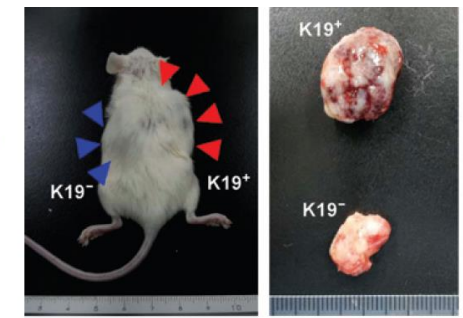
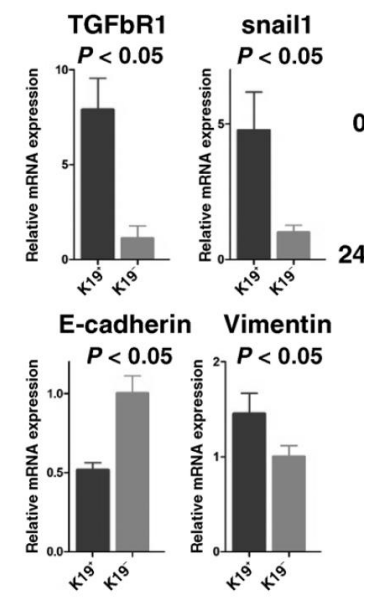
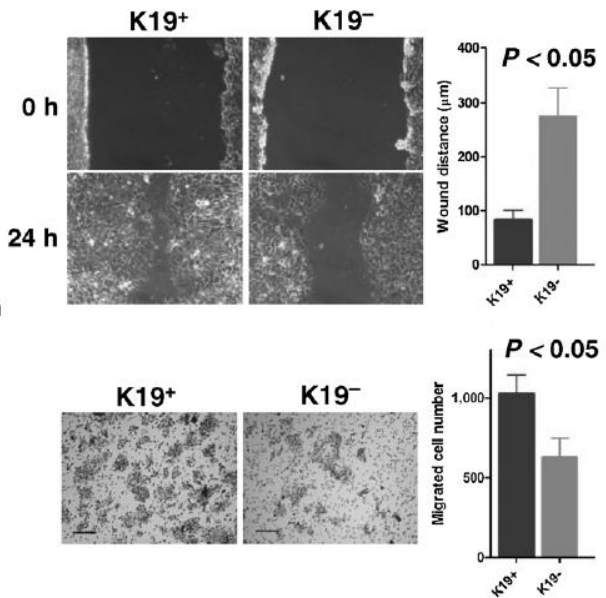
## Keratin 19, a Cancer Stem Cell Marker in Human Hepatocellular Carcinoma

Takayuki Kawai<sup>1</sup>, Kentaro Yasuchika<sup>1</sup>, Takamichi Ishii<sup>2</sup>, Hokahiro Katayama<sup>1</sup>, Elena Yukie Yoshitoshi<sup>1</sup>, Satoshi Ogiso<sup>1</sup>, Sadahiko Kita<sup>1</sup>, Katsutaro Yasuda<sup>1</sup>, Ken Fukumitsu<sup>1</sup>, Masaki Mizumoto<sup>1</sup>, Etsuro Hatano<sup>1</sup>, and Shinji Uemoto<sup>1</sup>

- HCC cell lines were transfected with a K19 promoter–driven enhanced green fluorescence protein gene
- FACS-isolated K19+/K19- cells.



### Huh7



# ✓ Stemness marker: K19

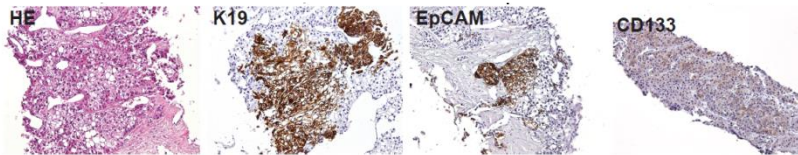


Poor prognostic marker for HCC

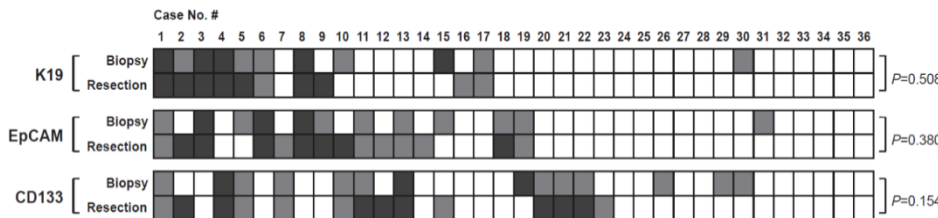
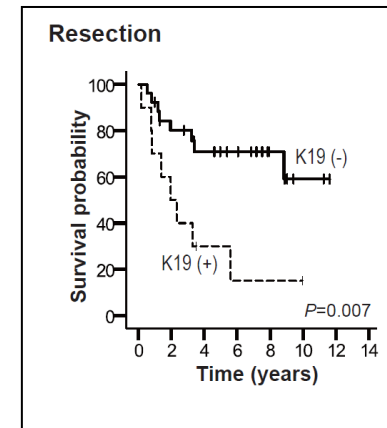
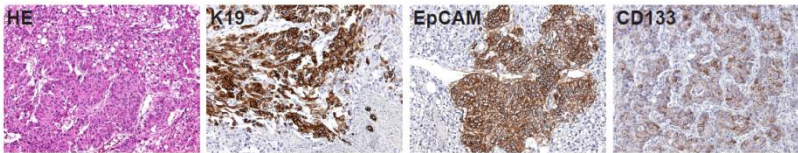
## ? Can K19 expression be useful maker in **biopsied** HCCs

### Stemness markers in matched biopsied and resected HCCs (n=36)

Biopsy

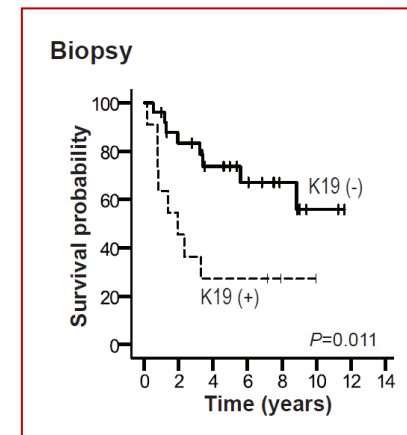


Resection



Immunomarkers	Sensitivity	Specificity	Accuracy
K19	80%	88%	86%
EpCAM	64%	86%	78%
CD133	71%	82%	78%

Immunoprofiles of resected HCCs as standard of references



# HCC with stem cell markers

1) Pathological characteristics

**2) Tumor microenvironment**

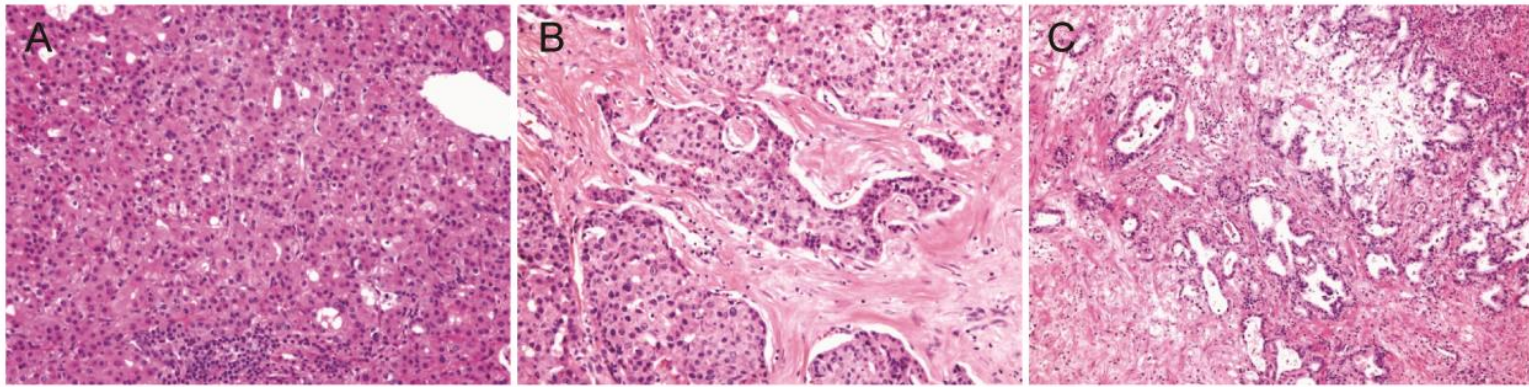
**① Fibrous stroma**

② K19 and its regulation mechanism

③ Response to locoregional treatment  
(TACE)

## 2. TME: Fibrous tumor stroma

### Scirrhous HCC (HCC with fibrous stroma)



#### HCC

Tumor cell morphology:  
resembling hepatocyte  
(polygonal cells having central  
round nuclei and abundant  
eosinophilic granular cytoplasm)  
Architectural pattern: trabecular,  
pseudoglandular, compact  
Stroma: mainly sinusoid-like  
blood spaces with no or little  
fibrosis

#### Scirrhous HCC

Tumor cell morphology:  
hepatocyte like tumor cell  
population + small, oval shaped  
tumor cell population mimicking  
liver stem/progenitor cells  
Architectural pattern: trabecular,  
nesting (small, oval shaped  
tumor cell arranged at the  
periphery of tumor nests facing  
fibrous stroma)  
Stroma: abundant fibrosis  $\geq$  50%  
of tumor area

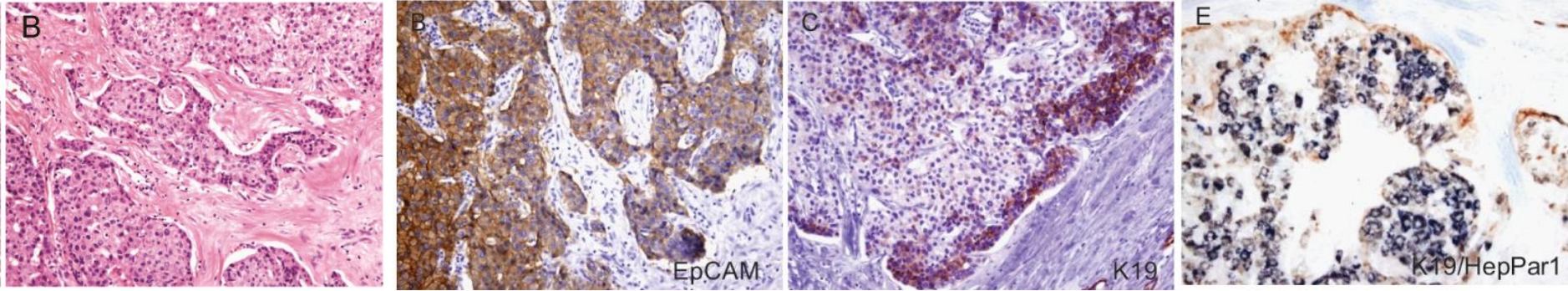
#### Cholangiocarcinoma

Tumor cell morphology:  
resembling cholangiocyte  
(cuboidal or columnar cells  
having intracytoplasmic mucin)  
Architectural pattern: glandular  
formation  
Stroma: marked fibrous stroma

Hepatocytic differentiation

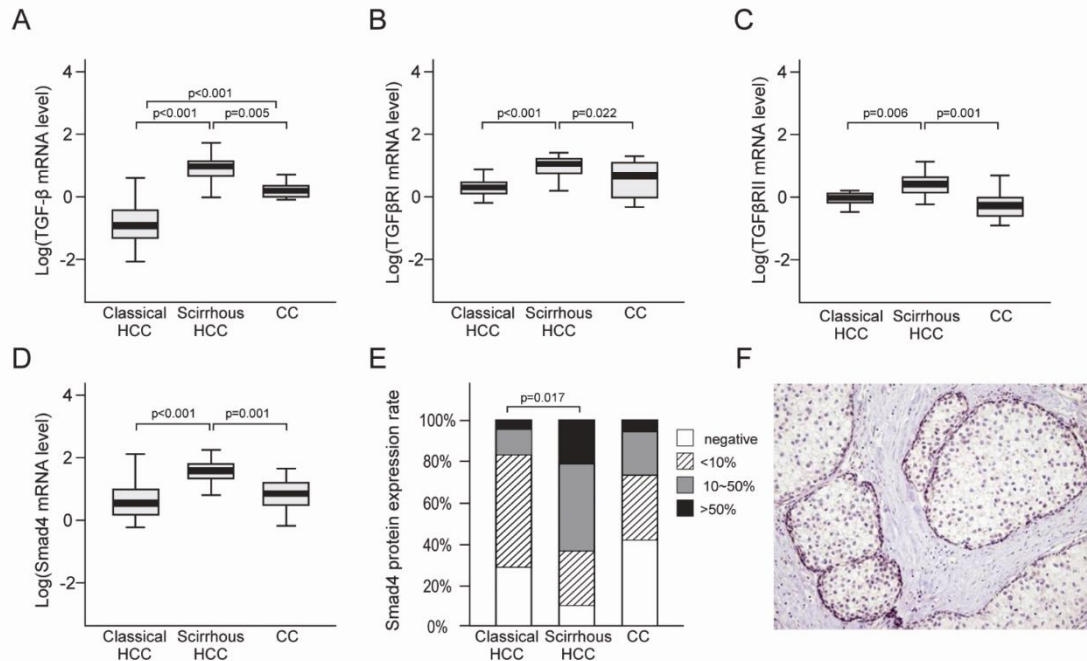
Cholangiocytic differentiation

# The expression pattern of liver stem cell markers in scirrhous HCCs

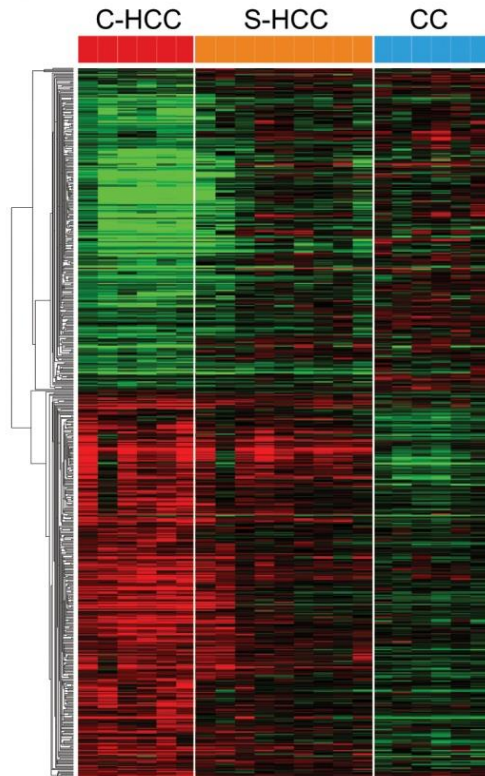


K19: brown/ Hepar1: blue

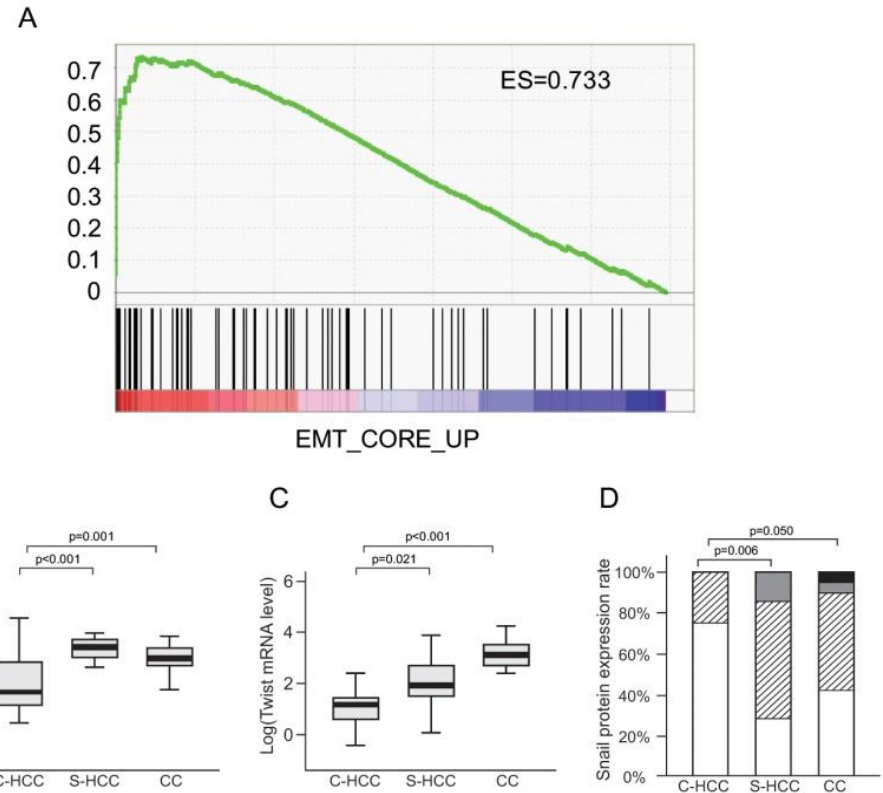
# Expression of TGF- $\beta$ signaling molecules in S-HCCs



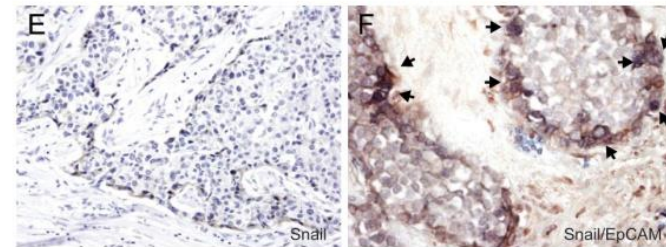
Gene expression profiles of the 617 differentially expressed gene features among C-HCCs, S-HCCs, and CCs



Expression of EMT molecules in S-HCC

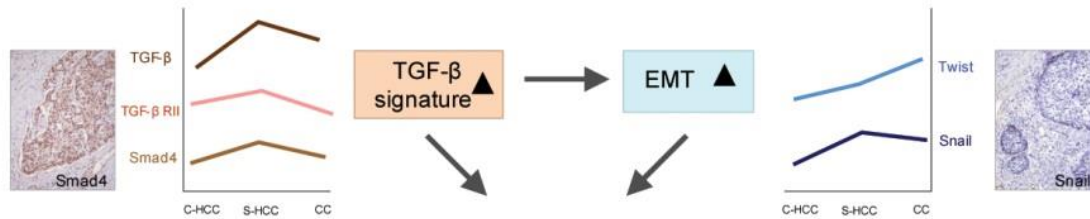
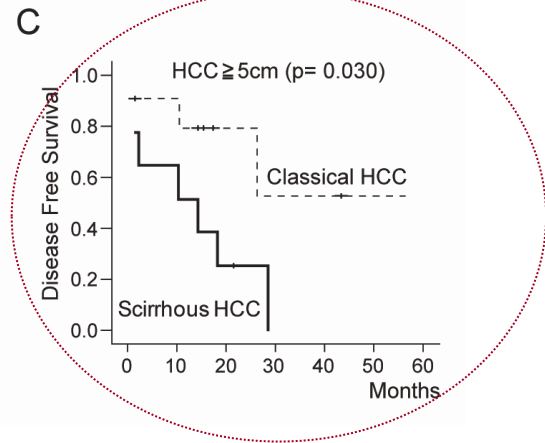
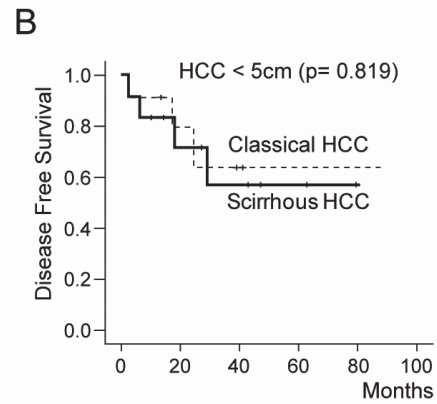
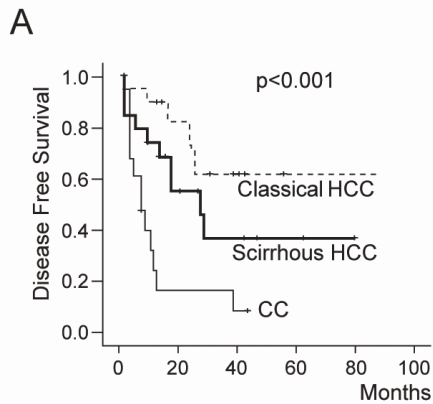


Up-regulation of cell adhesion, development, migration, and proliferation-related gene functions in S-HCC

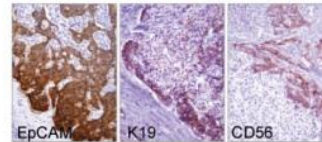


Snail

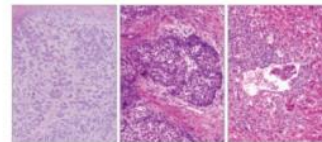
Snail: brown /  
EpCAM: blue



Tumor SPC-like cell  $\blacktriangle$   
(EpCAM, K7, K19, CD56,  
CD133, Oct3/4, cMET)



Fibrous stroma  
Vascular invasion  
Infiltrative growth



Tumor progression  
Aggressive behavior

**A Fibrous Stromal Component in Hepatocellular Carcinoma Reveals a Cholangiocarcinoma-Like Gene Expression Trait and Epithelial-Mesenchymal Transition**

Jae Yoon Seok,<sup>1\*</sup> Deuk Chae Na,<sup>1\*</sup> Hyun Goo Woo,<sup>2\*</sup> Massimo Roncalli,<sup>3</sup> So Mee Kwon,<sup>2</sup> Jeong Eun Yoo,<sup>1</sup> Ji Yong Ahn,<sup>4</sup> Gwang Il Kim,<sup>5</sup> Ju-Sub Choi,<sup>6</sup> Young Rae Kim,<sup>6</sup> and Young Nyeon Park<sup>1,6</sup>

Cancer stem cell



TME

# HCC with stem cell markers

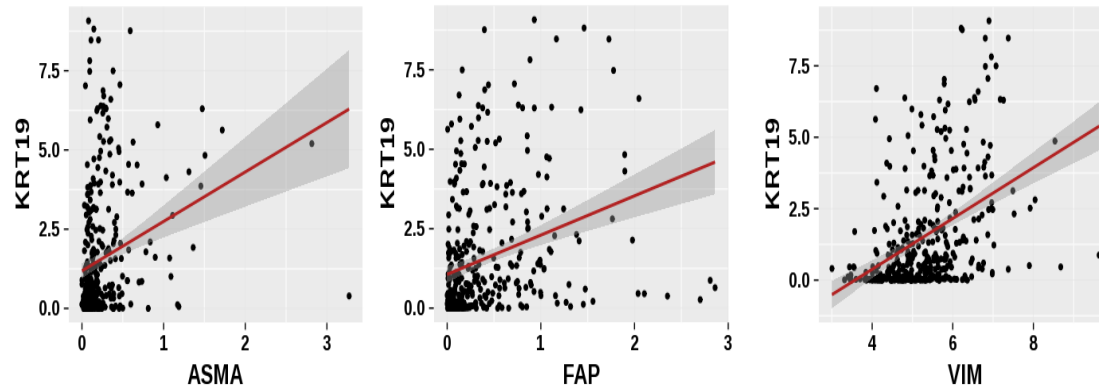
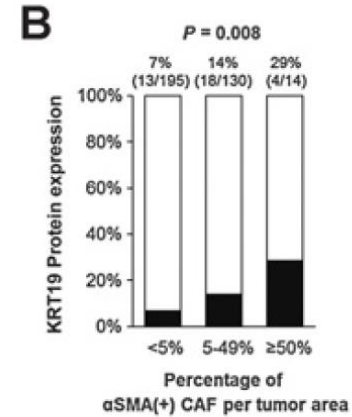
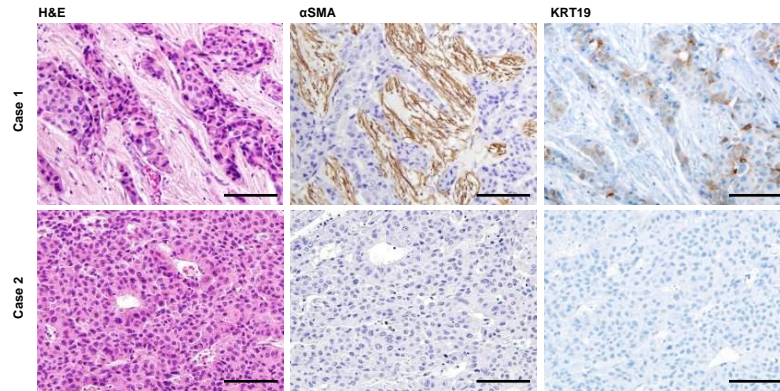
1) Pathological characteristics

2) Tumor microenvironment

- ① Fibrous stroma
- ② **K19 and its regulation mechanism**
- ③ Response to locoregional treatment (TACE)

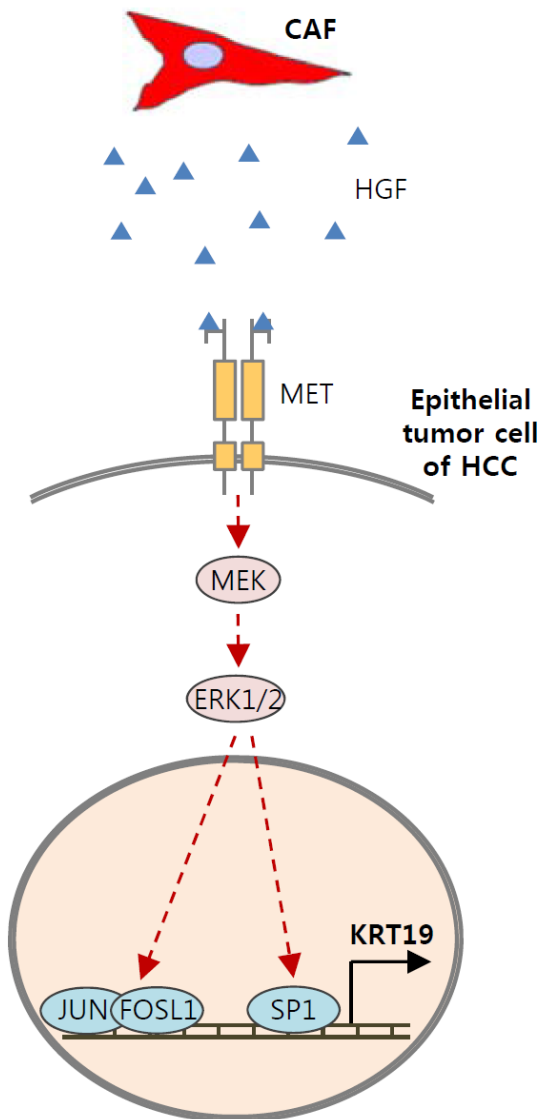


✓ Correlation between the  $\alpha$ SMA-positive CAFs and KRT19 protein expression.

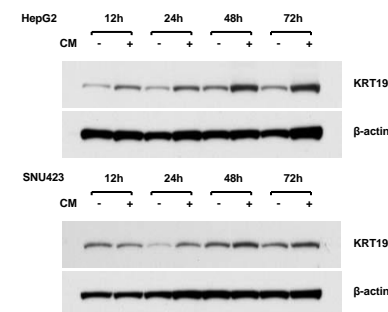
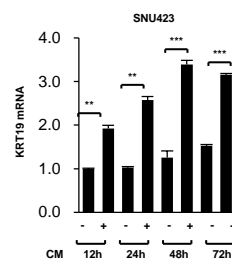
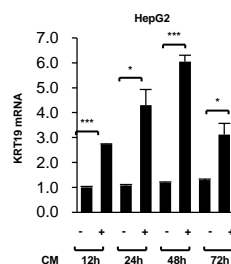


Correlation between the gene expression level of KRT19 and fibrous stromal markers including ASMA, FAP, and VIM in HCC data from TCGA (n = 371).

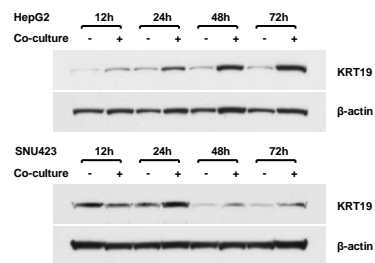
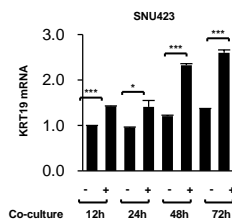
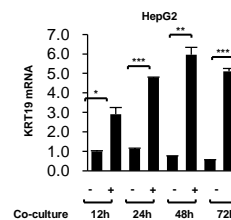
# Keratin 19 expression in hepatocellular carcinoma is regulated by fibroblast-derived HGF via MET-ERK1/2-AP1 and SP1 axis



✓ KRT19 expression in HCC is upregulated by paracrine factors from hepatic stellate cells

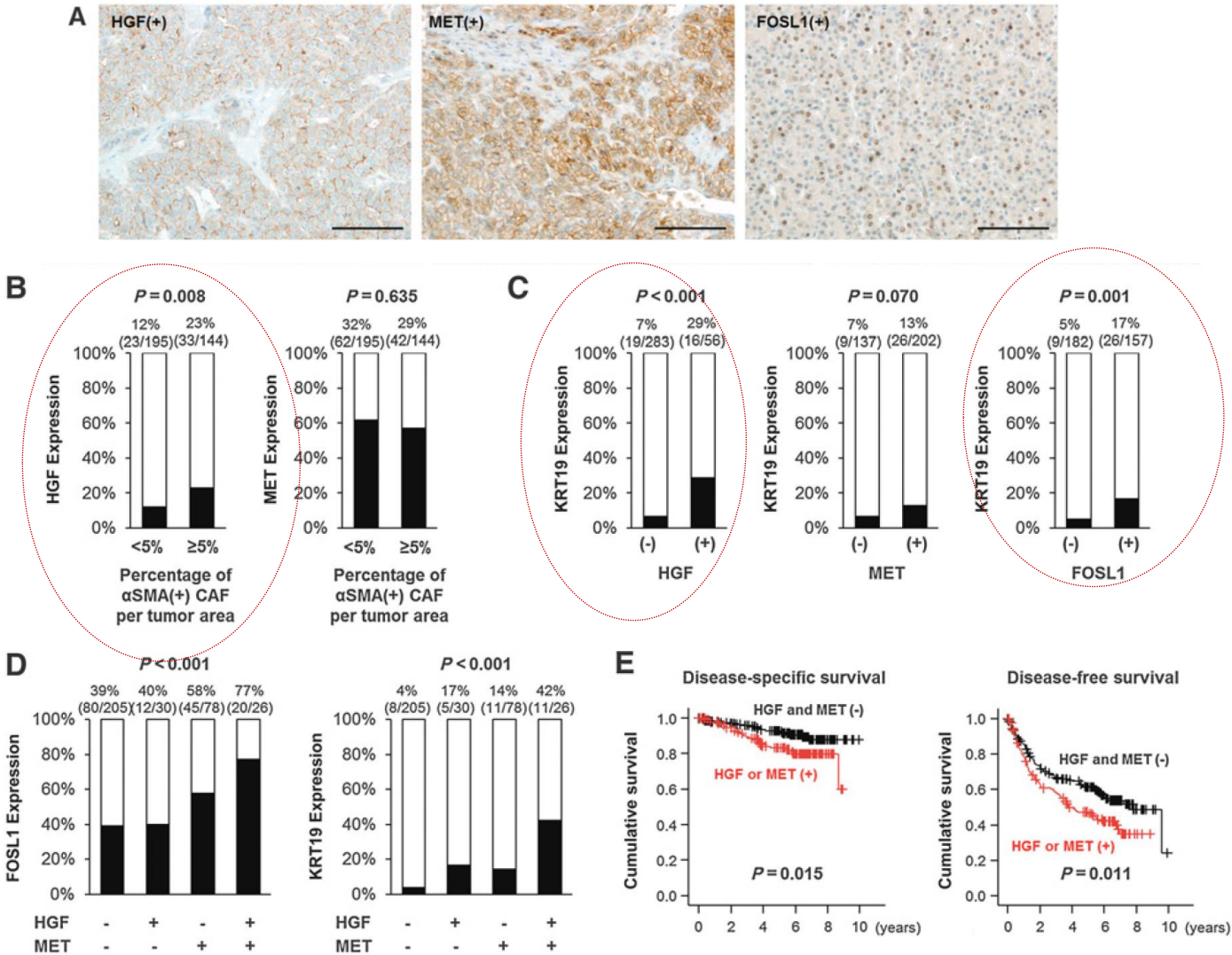


mRNA and protein expression levels of KRT19 after treatment with hTERT-HSC CM.

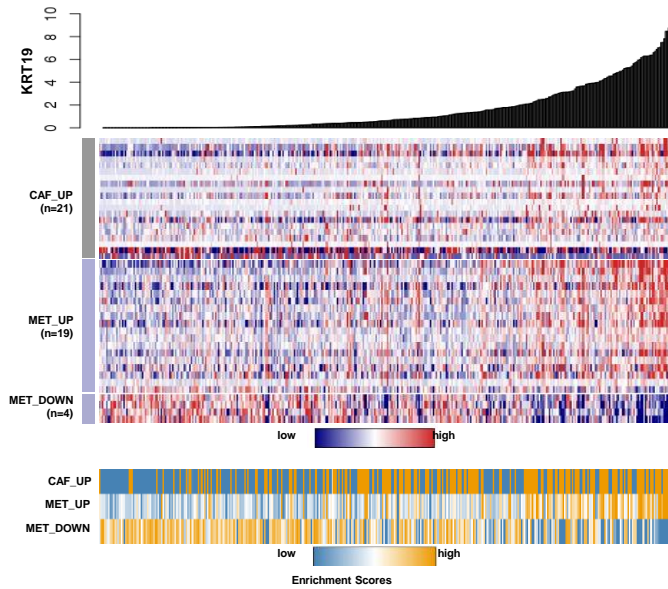


mRNA and protein expression levels of KRT19 after co-culture with hTERT-HSC

- ✓ In clinical specimens of human HCC (n=339), HGF and/or MET expression correlates with a higher KRT19 and FOSL1 expression.

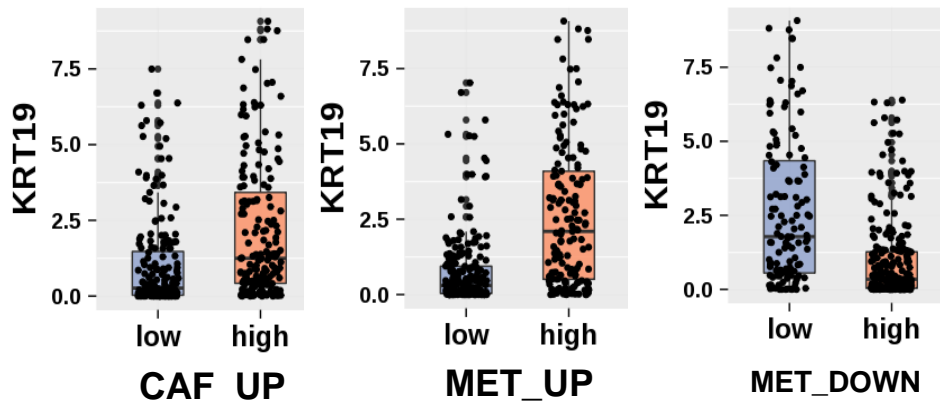


✓ Analysis of HCC data from TCGA revealed KRT19 expression was closely associated with CAF and MET-mediated signaling activities.



Expression heatmap of CAF\_UP (n=21), MET\_UP (n=19), and MET\_DOWN gene signatures (n=4) according to the KRT19 gene expression in the HCC data of TCGA (n=371).

✓ These findings reveal KRT19 expression in HCC is regulated by cross-talk between cancer-associated fibroblasts and HCC cells, illuminating new therapeutic targets for this aggressive disease.



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Molecular Cell Biology

Cancer Research

Keratin 19 Expression in Hepatocellular Carcinoma Is Regulated by Fibroblast-Derived HGF via a MET-ERK1/2-AP1 and SP1 Axis

Hyungjin Rhee<sup>1</sup>, Hye-Young Kim<sup>1</sup>, Ji-Hye Choi<sup>2,3</sup>, Hyun Goo Woo<sup>2,3</sup>, Jeong Eun Yoo<sup>1</sup>, Ji Hae Nahm<sup>1</sup>, Jin-Sub Choi<sup>4</sup>, and Young Nyun Park<sup>1,5</sup>



# HCC with stem cell markers

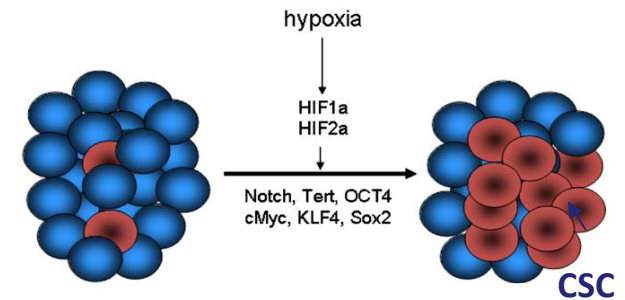
1) Pathological characteristics

2) Tumor microenvironment

- ① Fibrous stroma
- ② K19 and its regulation mechanism
- ③ **Response to locoregional treatment (TACE)**

# Hypoxic tumor microenvironment

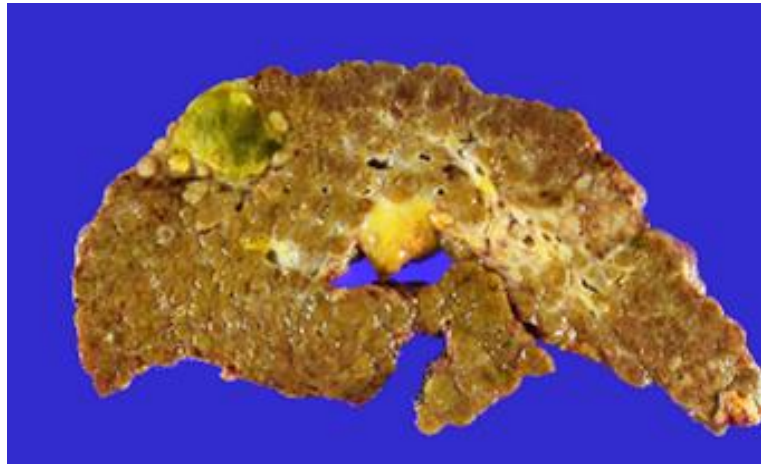
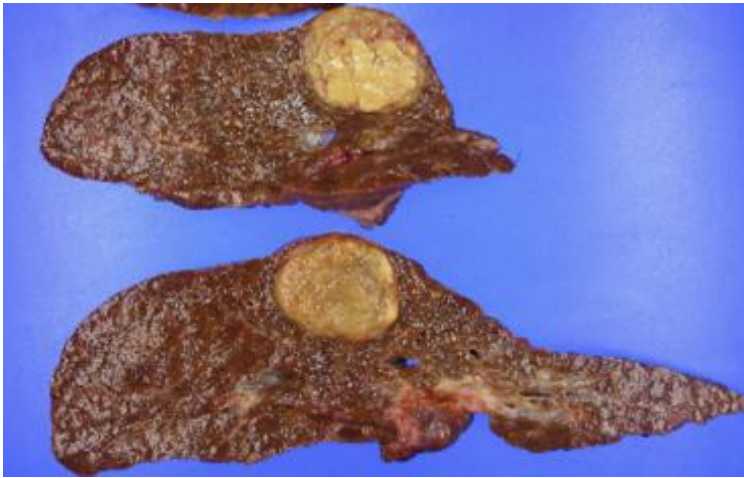
- Hypoxia
  - Most solid tumors
  - Rapid tumor growth and relative lack of blood supply
- The cancer stem cell population is expanded through HIF1/HIF2 pathway under hypoxia (*in vitro* study).



Nat Rev Cancer. 2011 Jun;11(6):393-410

## ✓ TACE (Transarterial chemoembolization)

- One of most popular loco-regional therapies in HCC
- TACE induces tumor hypoxia.
- Response to TACE is various and a significant number of HCC (50-86%) recurs.



The hypoxic TME may serve as a niche for cancer stem cell.

? Does TACE induced hypoxia affect outcome of HCC patients with stemness marker expression

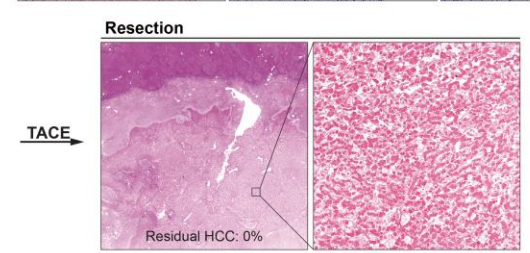
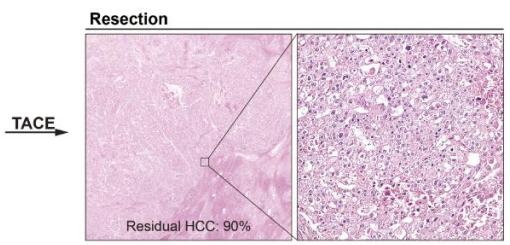
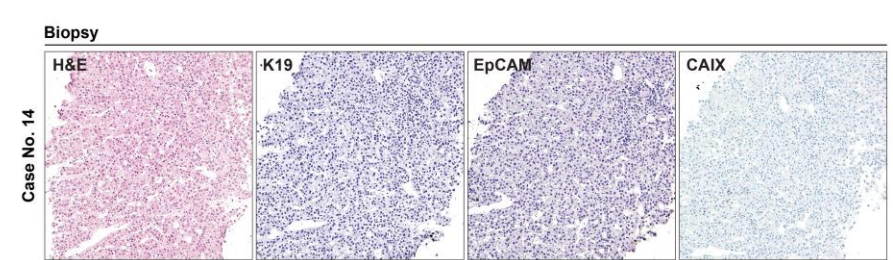
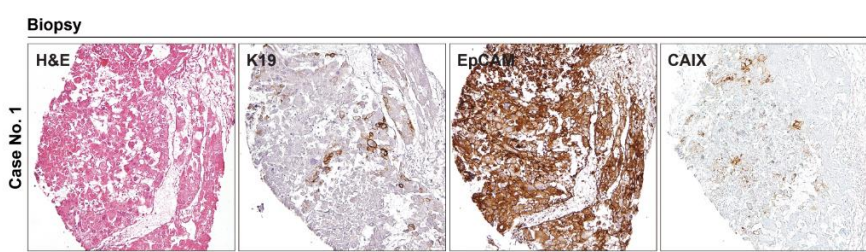
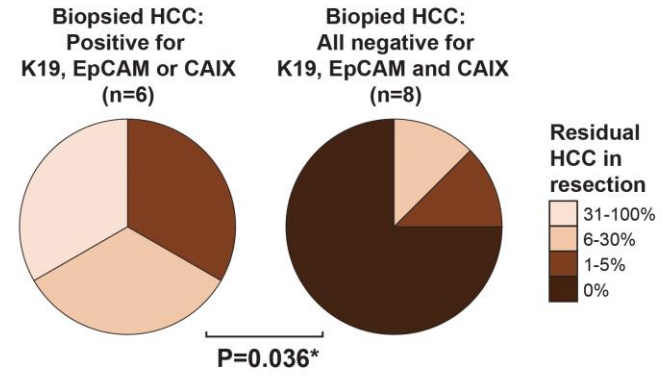
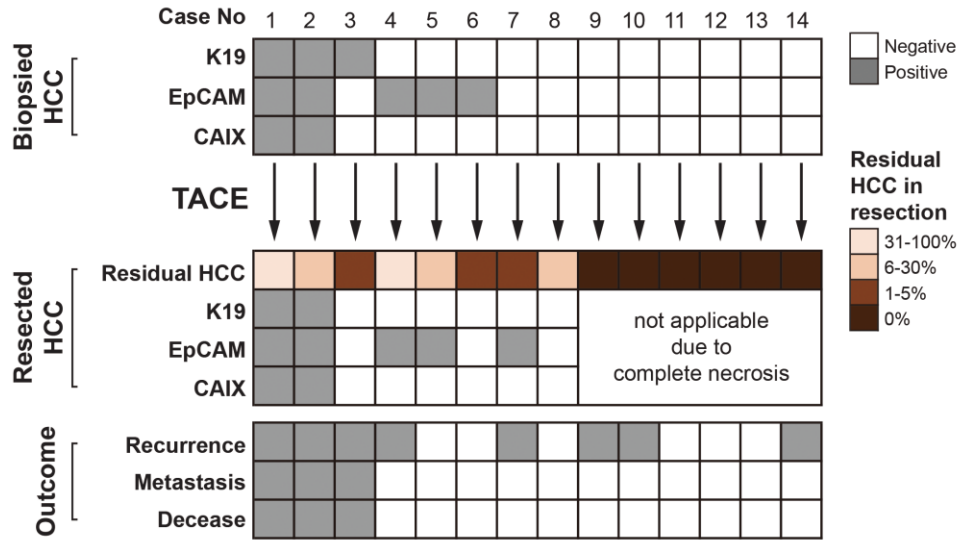
Cohort 1: Biopsy-TACE (1 session)-curative resection matched HCCs (n=14)

Cohort 2: Explanted HCCs with preop locoregional Tx (60%) (n=68)

Cohort 3: Resected HCCs without preop locoregional Tx (n=100)

# Cohort 1: Biopsy-TACE (1 session) - curative resection matched HCCs (n=14)

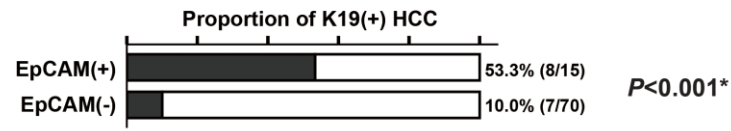
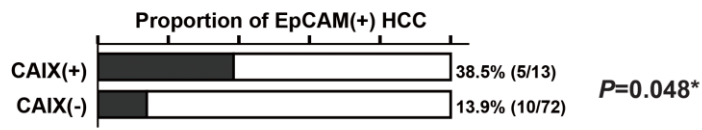
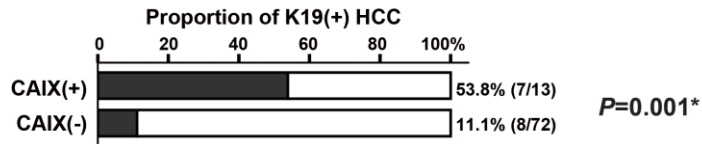
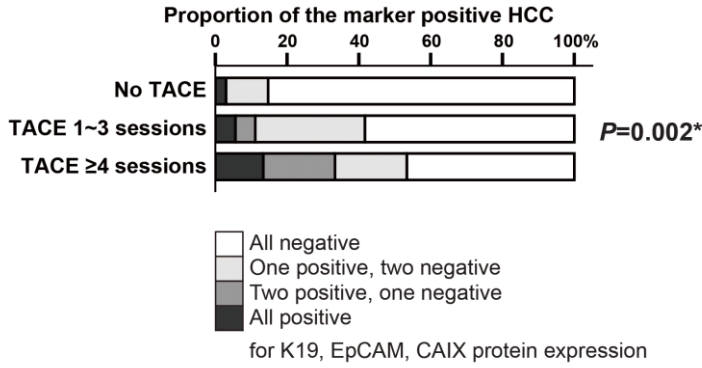
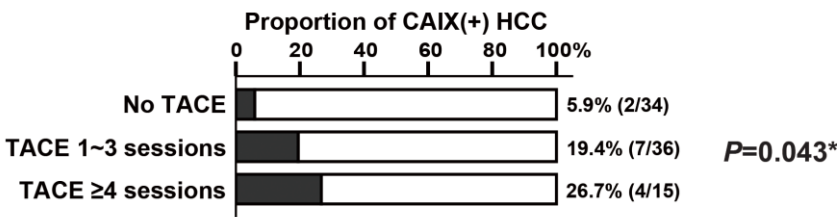
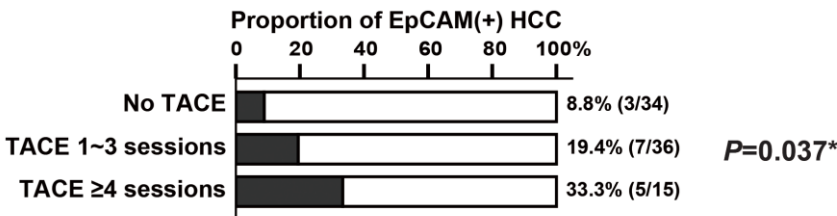
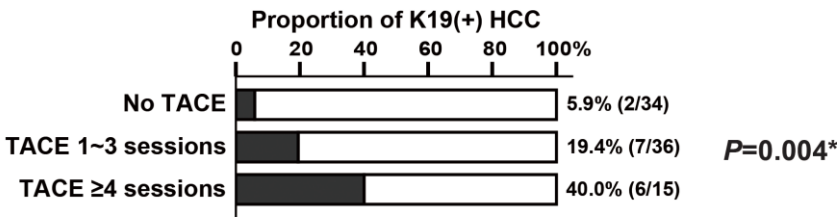
## ✓ Single nodular HCC





# Cohort 2 : Liver transplantation HCC cohort with/without preoperative treatment (n=85)

- Total necrotic HCCs were excluded as immunoprofiles could not be evaluated.
- Stemness markers (K19, EpCAM) and hypoxia marker (CAIX) expression were frequently found in HCCs underwent multiple TACE sessions.



# Cohort 2 : Liver transplantation HCC cohort with/without preoperative treatment (n=85)

## Clinicopathological features of hepatocellular carcinomas in cohort 2 according to the CAIX protein expression status

<i>Clinicopathologic features</i>	<i>CAIX positive n = 13 (15%)</i>	<i>CAIX negative n = 72 (85%)</i>	<i>P-value</i>
Age (year)	52 (45–56)	54 (50–60)	0.067
Gender (male/female)	10 (77%)/3 (23%)	59 (82%)/13 (18%)	0.670
Etiology (hepatitis B/hepatitis C/alcohol/unknown)	11 (85%)/1 (8%)/1 (8%)/0 (0%)	59 (82%)/8 (11%)/4 (6%)/1 (1%)	0.941
Cirrhosis	13 (100%)	70 (97%)	1.000
Serum aspartate transaminase (IU/l)	42 (35–75)	46 (35–68)	0.779
Serum alanine transaminase (IU/l)	31 (13–58)	32 (20–40)	0.883
Serum albumin (g/dl)	3.0 (2.6–3.8)	3.3 (2.8–3.6)	0.399
Serum platelet (1000/ $\mu$ l)	66 (41–101)	79 (54–118)	0.173
Serum alpha-fetoprotein (IU/ml)	29.0 (2.9–246.2)	10.9 (4.8–60.9)	0.502
Serum PIVKA-II (AU/ml) <sup>a</sup>	76.0 (24.0–402.3)	37.5 (18.3–96.0)	0.131
<i>Tumor pathology</i>			
Diameter of largest tumor (cm)	3.0 (2.3–3.5)	2.2 (1.6–3.5)	0.319
Total tumor diameter (cm)	3.5 (2.4–5.5)	3.6 (2.4–6.4)	0.665
Differentiation (Edmonson–Steiner grades I/II/III)	1 (8%)/11 (85%)/1 (8%)	27 (38%)/40 (56%)/5 (7%)	0.104
Capsule formation (absent/partial/complete)	0 (0%)/9 (69%)/4 (31%)	22 (31%)/32 (44%)/18 (25%)	0.063
<b>Microvascular invasion</b>	8 (62%)	13 (18%)	<b>0.002<sup>b</sup></b>
Number of the lesion (1/2 or 3/ $\geq$ 4)	6 (46%)/6 (46%)/1 (8%)	23 (32%)/35 (49%)/14 (19%)	0.466
Milan status (in/out)	9 (69%)/4 (31%)	48 (67%)/24 (33%)	1.000
<b>K19 protein expression</b>	7 (54%)	8 (11%)	<b>0.001<sup>b</sup></b>
<b>EpCAM protein expression</b>	5 (39%)	10 (14%)	<b>0.048<sup>b</sup></b>

Variables are expressed as median (interquartile range) or number (%).

<sup>a</sup>Serum PIVKA-II levels not available for five patients.

<sup>b</sup>Indicates  $P < 0.05$ .

# Cohort 2 : Liver transplantation HCC cohort with/without preoperative treatment (n=85)

## Univariate and multivariate analyses of disease-free survival in cohort 2

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	P-value <sup>a</sup>	Hazard ratio (95% confidence interval)	P-value <sup>a</sup>
Age (≥60 years)	0.03 (0.0–8.1)	0.222		
Gender (male)	0.8 (0.2–2.8)	0.677		
Etiology (hepatitis B virus)	2.4 (0.3–18.7)	0.400		
Serum alpha-fetoprotein (≥400 IU/ml)	8.4 (2.6–26.6)	< 0.001 <sup>b</sup>	6.8 (1.8–26.1)	0.005 <sup>b</sup>
Serum PIVKA-II (≥400 AU/ml)	6.0 (1.8–20.0)	0.004 <sup>b</sup>	2.0 (0.5–8.7)	0.358
Microvascular invasion (positive)	2.4 (0.8–7.5)	0.138		
Differentiation (Edmonson–Steiner grade III)	1.4 (0.2–10.6)	0.769		
Milan status (out of Milan)	2.1 (0.7–6.4)	0.209		
Pre-transplantation transarterial chemoembolization (one or more sessions)	7.7 (1.0–59.5)	0.051		
CAIX protein expression (positive)	7.4 (2.4–22.9)	0.001 <sup>b</sup>	6.4 (1.9–22.0)	0.003 <sup>b</sup>
K19 protein expression (positive) <sup>c</sup>	3.5 (1.4–8.7)	0.007 <sup>b</sup>		
EpCAM protein expression (positive) <sup>c</sup>	3.6 (1.4–8.8)	0.006 <sup>b</sup>		

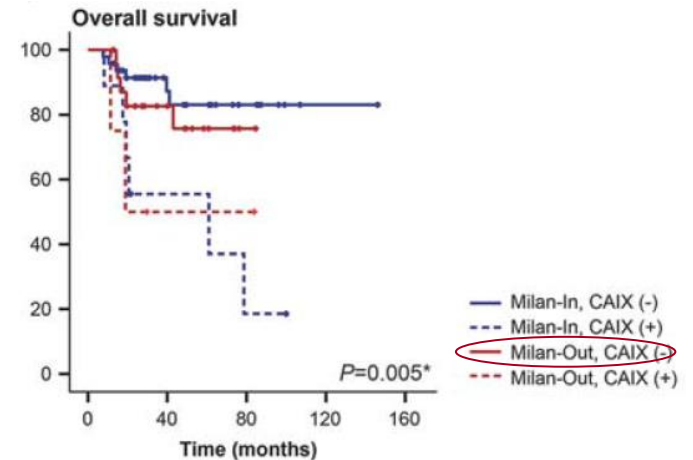
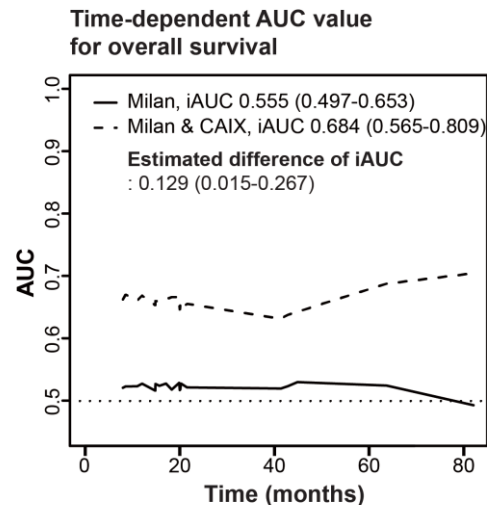
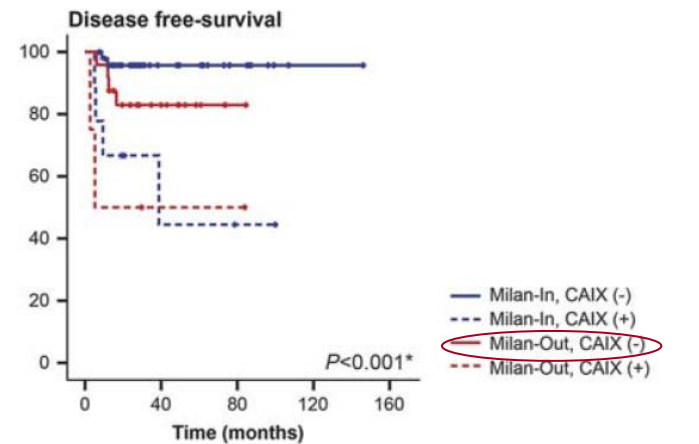
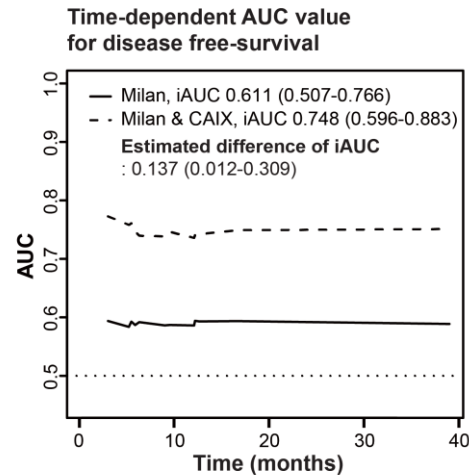
<sup>a</sup>Univariate and multivariate analyses were performed using the Cox proportional hazards model.

<sup>b</sup>Indicates  $P < 0.05$ .

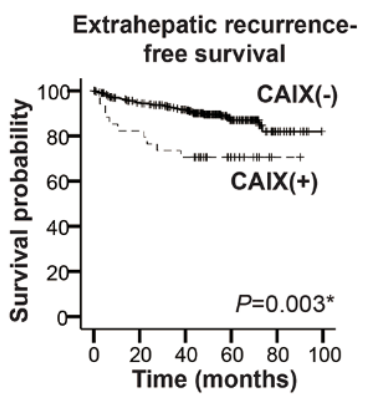
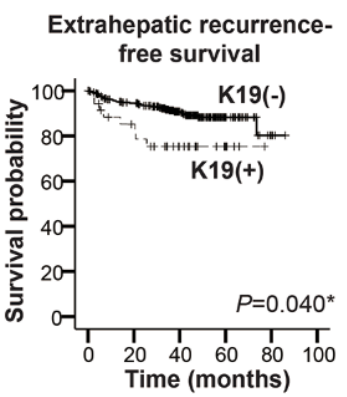
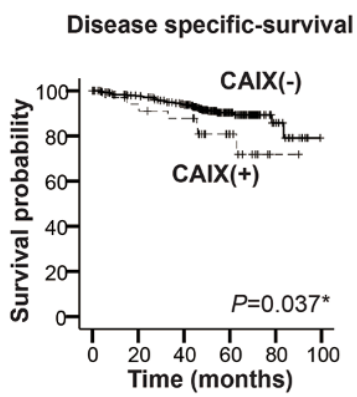
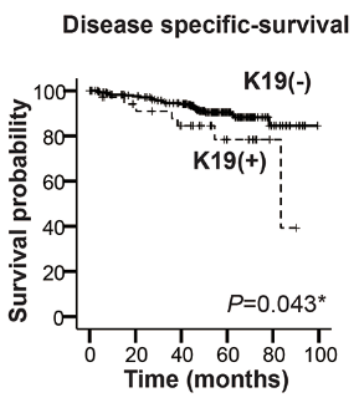
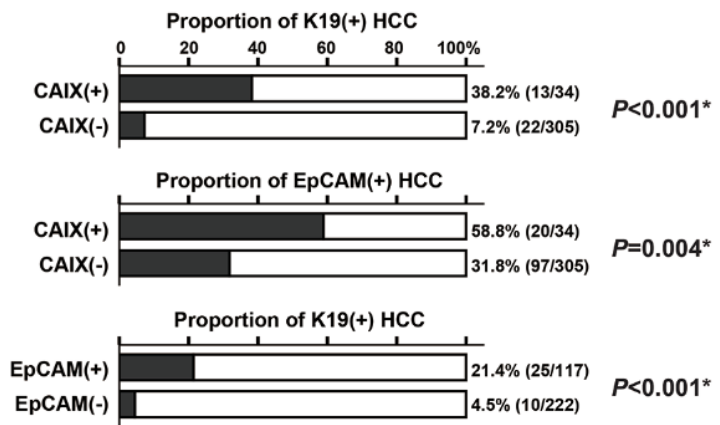
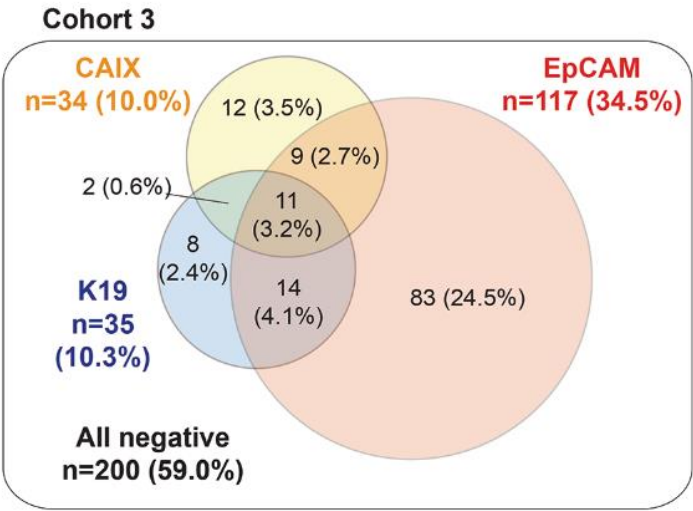
<sup>c</sup>K19 and EpCAM were not included in the multivariate analysis because of significant associations with CAIX.

# Cohort 2 : Liver transplantation HCC cohort with/without preoperative treatment (n=85)

- CAIX was an independent predictor of recurrence/survival after transplantation.
- When CAIX is combined to Milan criteria, prediction of recurrence/survival could be improved.

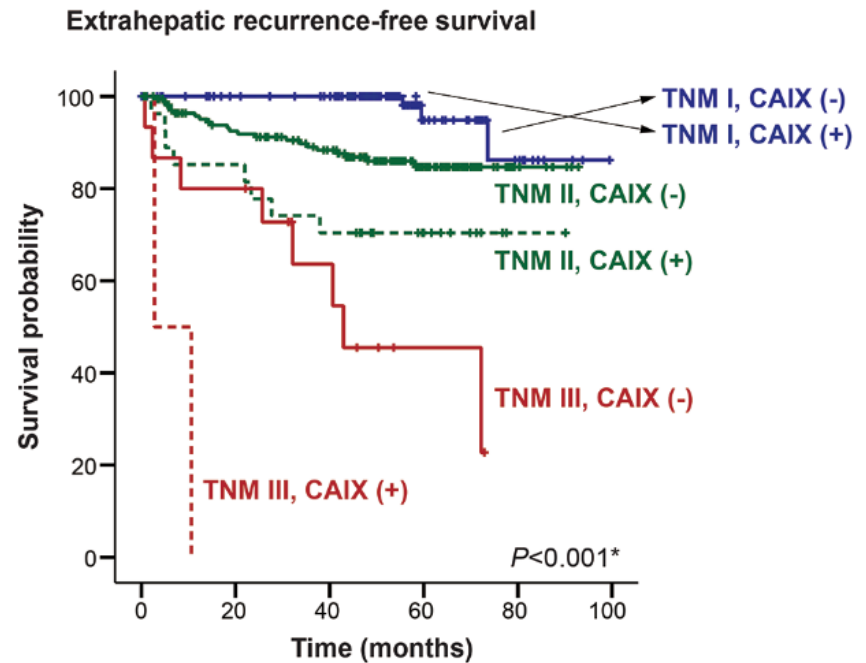
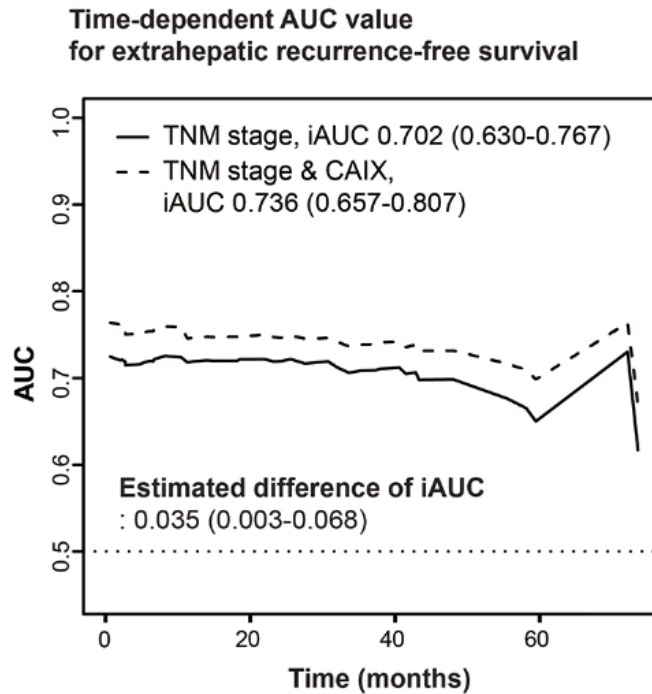


# Cohort 3 : Hepatic resection HCC cohort without preoperative treatment (n=339)



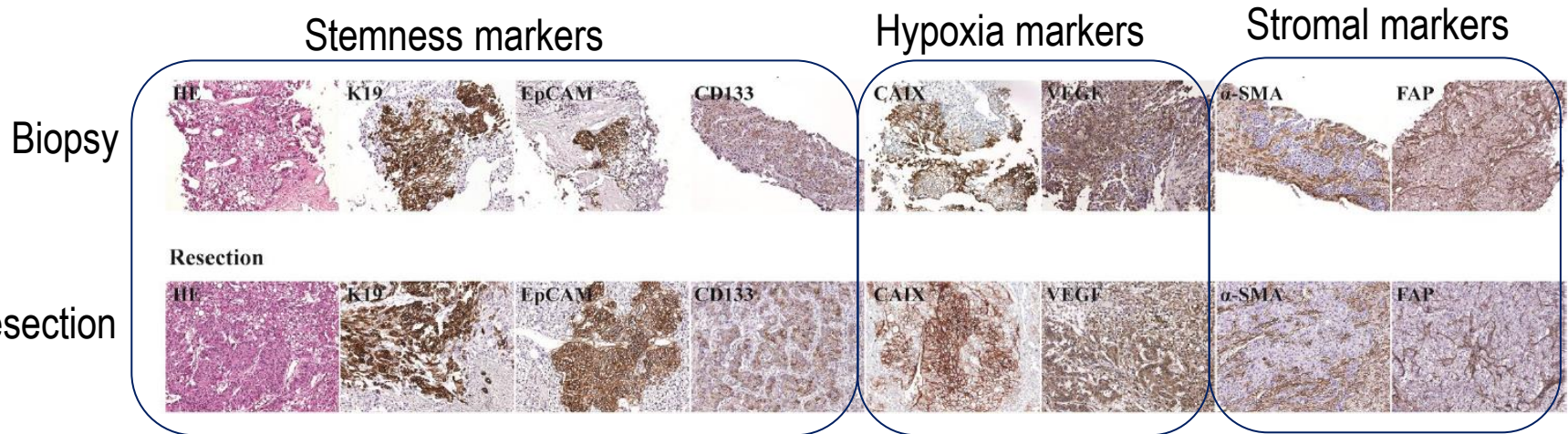
## Cohort 3 : Hepatic resection HCC cohort without preoperative treatment (n=339)

- Stemness markers (K19) and hypoxia marker (CAIX) were poor prognostic indicator.
- CAIX expression was independent prognostic factor for extrahepatic recurrence.



# What happens in cancer stem cell and TME during TACE ?

HCC TACE group: Bx-**TACE**- Resection (n=10)  
HCC Non-TACE group: Bx ----- Resection (n=36)



HCC TACE group:

Bx-TACE- Resection (n=10)

HCC Non-TACE group: Bx

----- Resection (n=36)

**Immunomarker expression in biopsied specimens**

K19	5 (50%)	11 (31%)	0.283
EpCAM	6 (60%)	12 (33%)	0.157
CD133	3 (30%)	9 (25%)	0.706
CAIX	10 (100%)	10 (28%)	>0.999
VEGF	28 (100%)	28 (78%)	0.682
$\alpha$ -SMA	6 (60%)	25 (69%)	0.573
FAP	5 (50%)	19 (53%)	0.876

No difference

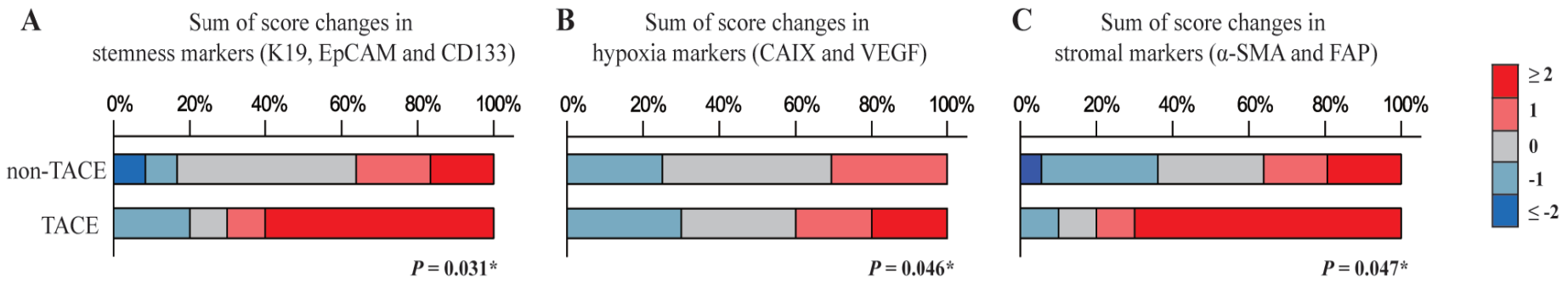
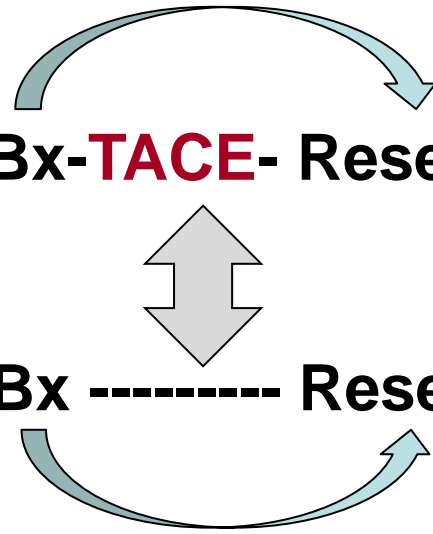
**Immunomarker expression in resected specimens**

K19	5 (50%)	10 (28%)	0.257
EpCAM	8 (80%)	14 (39%)	<b>0.032*</b>
CD133	6 (60%)	13 (36%)	0.277
CAIX	6 (60%)	10 (30%)	0.074
VEGF	6 (60%)	29 (81%)	0.220
$\alpha$ -SMA	9 (90%)	15 (42%)	<b>0.011*</b>
FAP	9 (90%)	19 (53%)	0.064

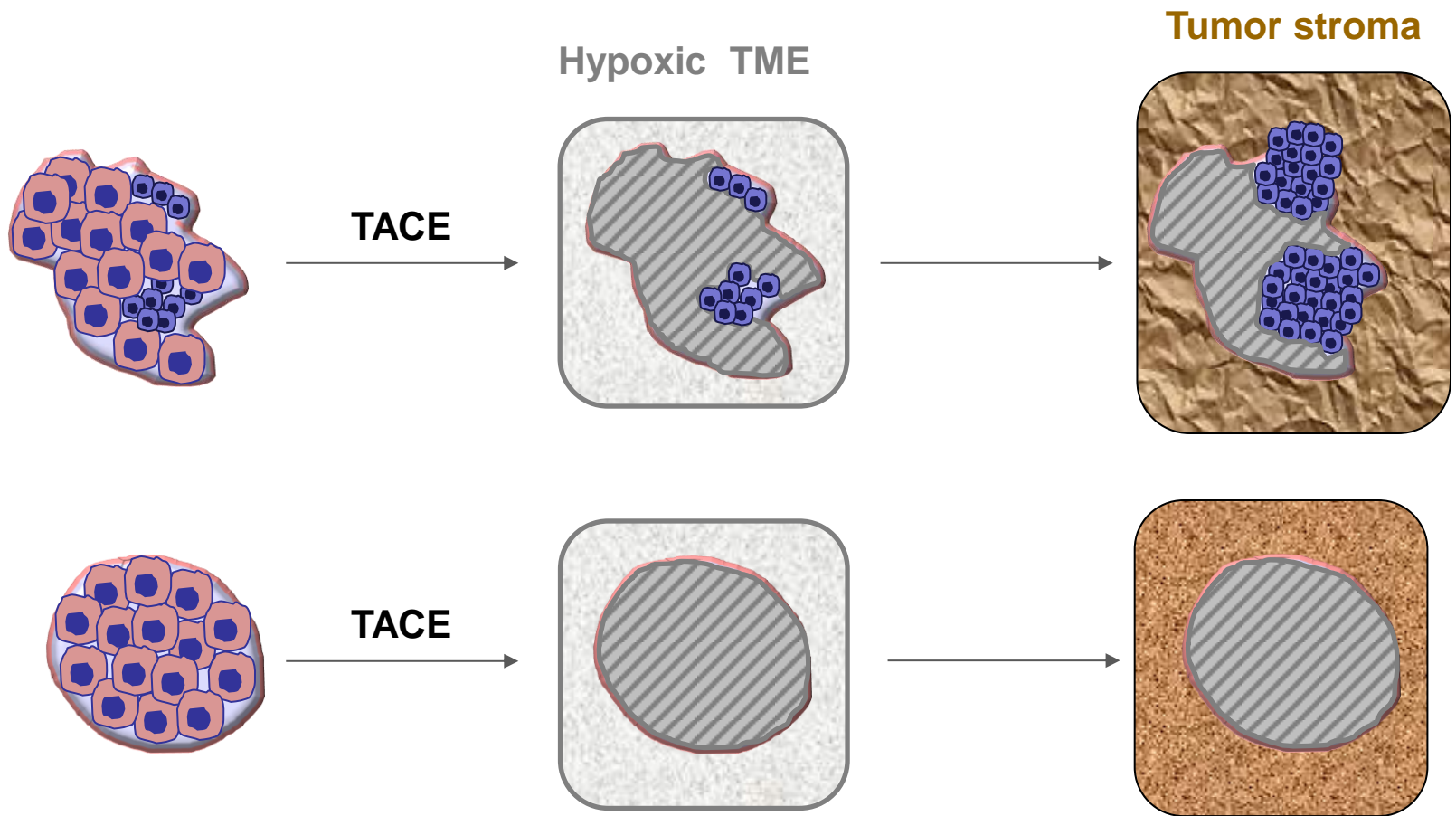


HCC TACE group: Bx-TACE- Resection (n=10)

HCC Non-TACE group: Bx ----- Resection (n=36)



**Increased expression of stemness markers and altered tumor stroma in HCC under TACE-induced hypoxia : A biopsy and resection matched study**



 HCC cells without stemness

 HCC cells with stemness

- ✓ The expression of stemness (K19, EpCAM) and hypoxia (CAIX) related markers were correlated each other.
- ✓ HCC with these markers showed resistance to TACE and poorer outcome.
- ✓ Stemness marker expression is considered to be increased along with tumor stromal alteration under TACE-induced hypoxia, which might promote the aggressive biology of HCC.
- ✓ Evaluation of stemness and hypoxia markers may have an additional value in predicting HCC outcome, especially for TACE-treated HCCs.

### Poor outcome of hepatocellular carcinoma with stemness marker under hypoxia: resistance to transarterial chemoembolization

Hyungjin Rhee<sup>1</sup>, Ji Hae Nahm<sup>1</sup>, Haeryoung Kim<sup>2</sup>, Gi Hong Choi<sup>3</sup>, Jeong Eun Yoo<sup>1</sup>, Hye Sun Lee<sup>4</sup>, Myoung Ju Koh<sup>1</sup> and Young Nyun Park<sup>1,5</sup>

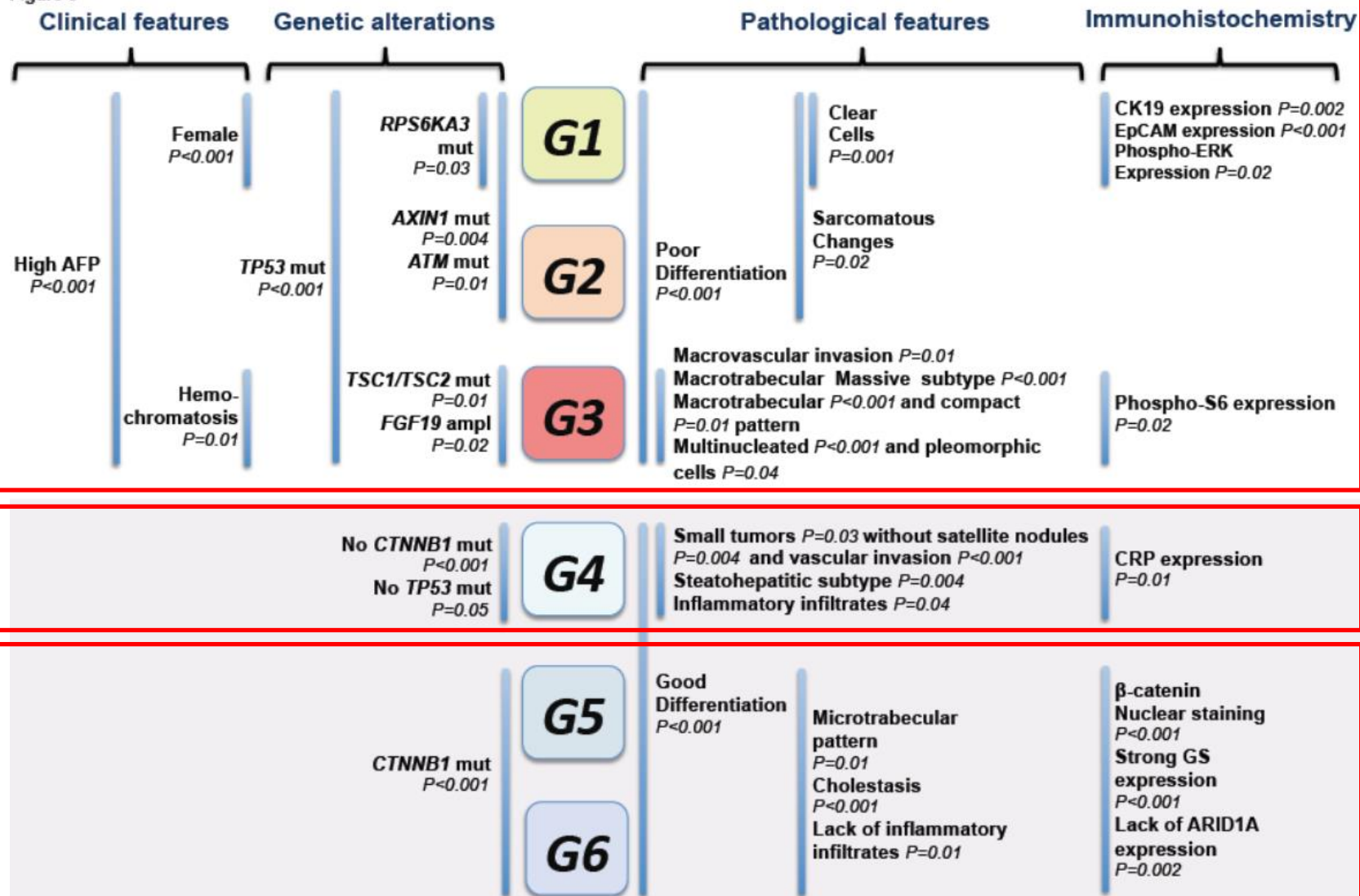
**Increased expression of stemness markers and altered tumor stroma in hepatocellular carcinoma under TACE-induced hypoxia: A biopsy and resection matched study**

Ji Hae Nahm<sup>1,\*</sup>, Hyungjin Rhee<sup>2,3,4,\*</sup>, Haeryoung Kim<sup>5</sup>, Jeong Eun Yoo<sup>1,4,7</sup>, Jee San Lee<sup>1,3,4</sup>, Youngsic Jeon<sup>1,3,4</sup>, Gi Hong Choi<sup>6</sup> and Young Nyun Park<sup>1,3,4,7</sup>

# Histological Subtypes of HCC Are Related To Gene Mutations and Molecular Tumour Classification

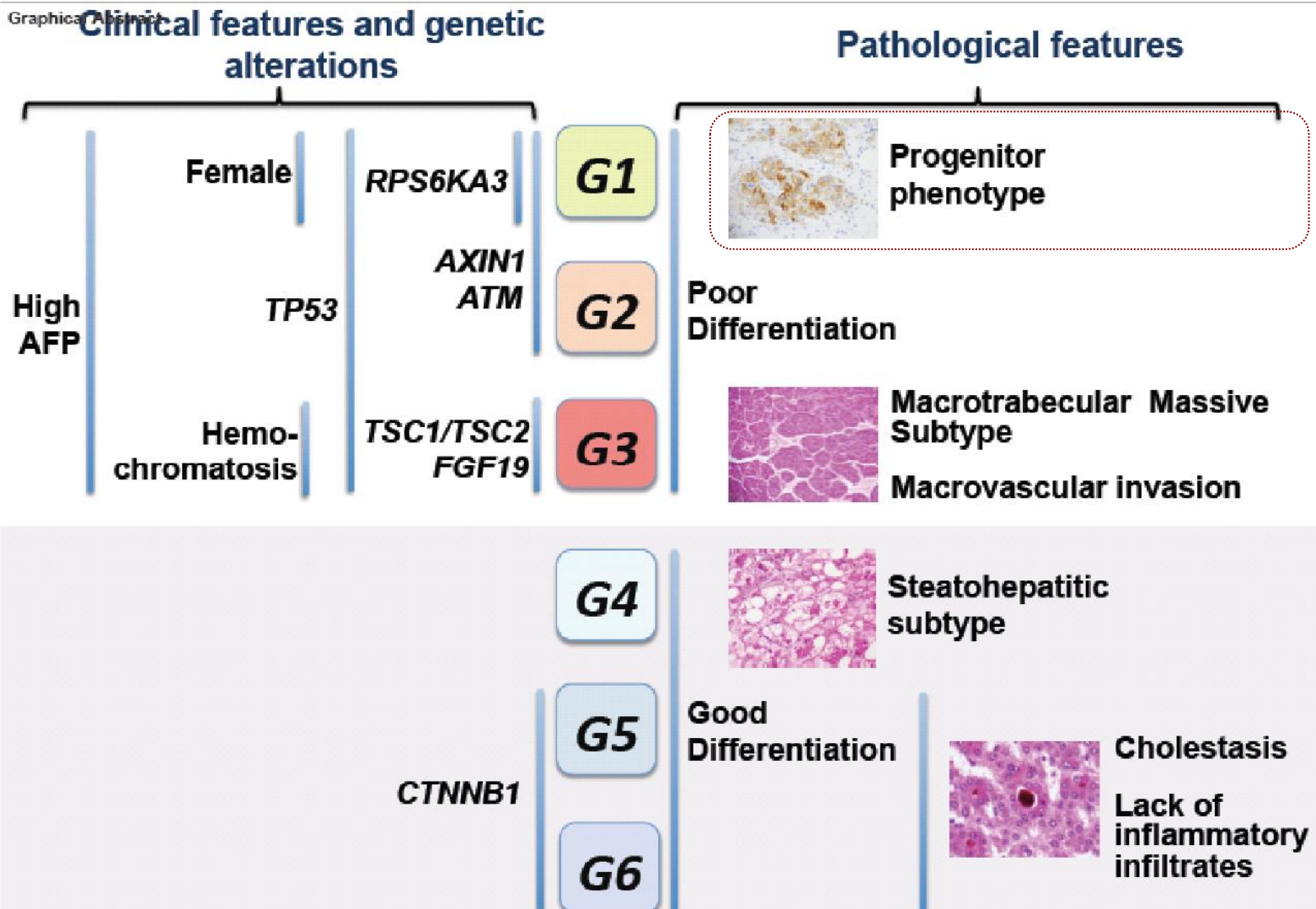
Calderaro J, et al, J Hepatol 2017

Figure 5



# Histological Subtypes of HCC Are Related To Gene Mutations and Molecular Tumour Classification

Graphical Abstract



# Celebrating Dr. Jae Ro's 50<sup>th</sup> Anniversary of Pathology : Do your BeST!



1971년 교실 야유회, 경기도 대성리



2008, Seoul



# KOPANA meeting, 2010 Washington DC

## Article Citation:

Young Nyun Park (2011) Update on Precursor and Early Lesions of Hepatocellular Carcinomas. Archives of Pathology & Laboratory Medicine: June 2011, Vol. 135, No. 6, pp. 704-715.

**SPECIAL SECTION—KOREAN PATHOLOGISTS ASSOCIATION OF NORTH AMERICA 9TH SPRING SEMINAR**

## Update on Precursor and Early Lesions of Hepatocellular Carcinomas

Young Nyun Park, MD, PhD



139 citation

