Molecular Classification of HCC

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Molecular classification of HCC

1. Brief itroduction of molecular classification besed on gene expression signature

2. β-catenin activated typical subclass

3. TGF-β activated subclass

4. Hepatic progenitor/biliary marker positive subclass
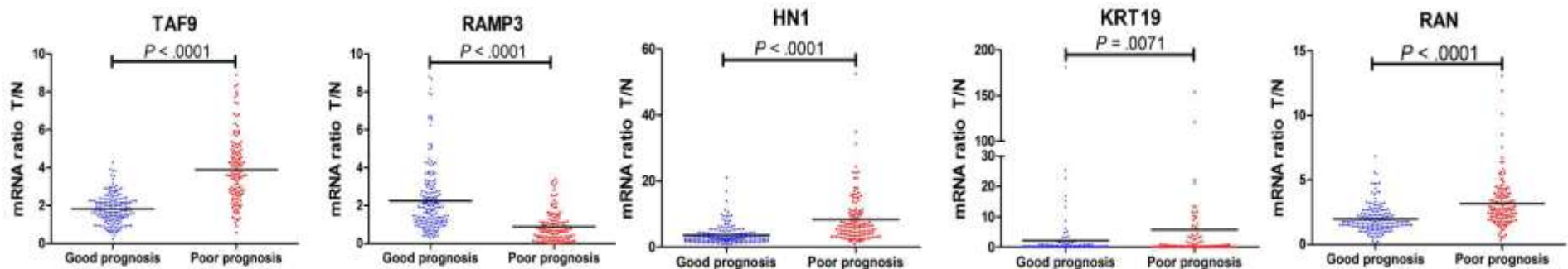
molecular classification of HCC
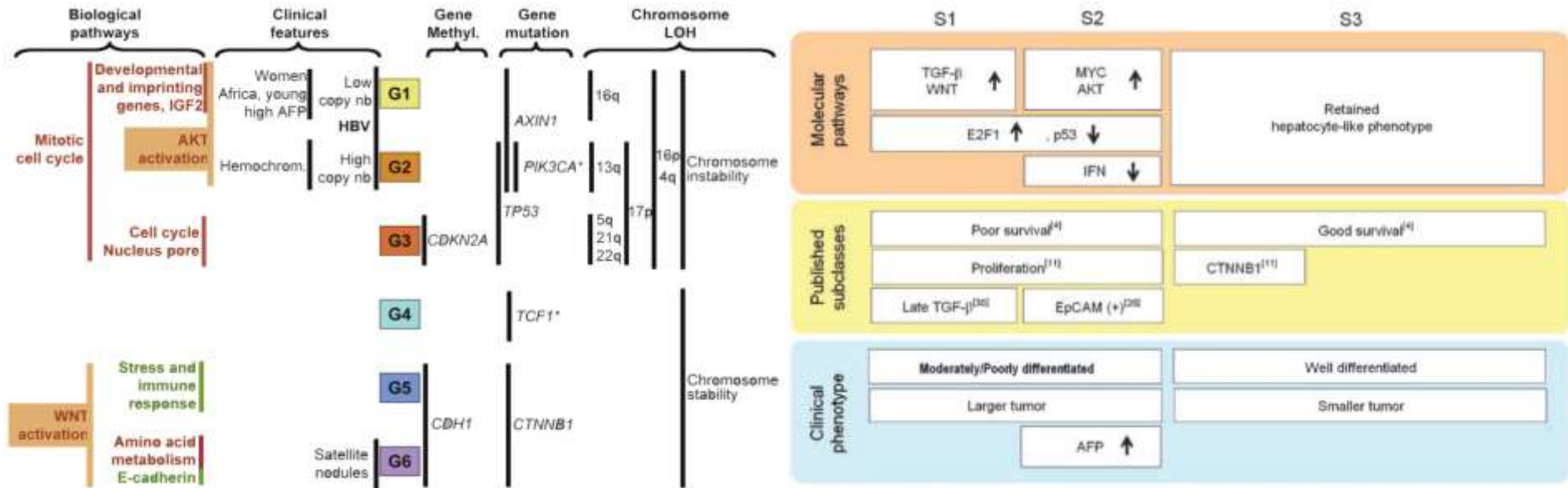
Robust subgroups of HCC (G1-G6) associated with clinical and genetic characteristic. The G3 signature was independent predictors of HCC recurrence

Boyault S, et al. Hepatology 2007;45:42-52
Villanueva A, et al. Gastroenterology 2011;140:1501-12

Three class structure of HCC (S1-S3) correlated with clinical parameters (tumor size, cell differentiation, AFP level)
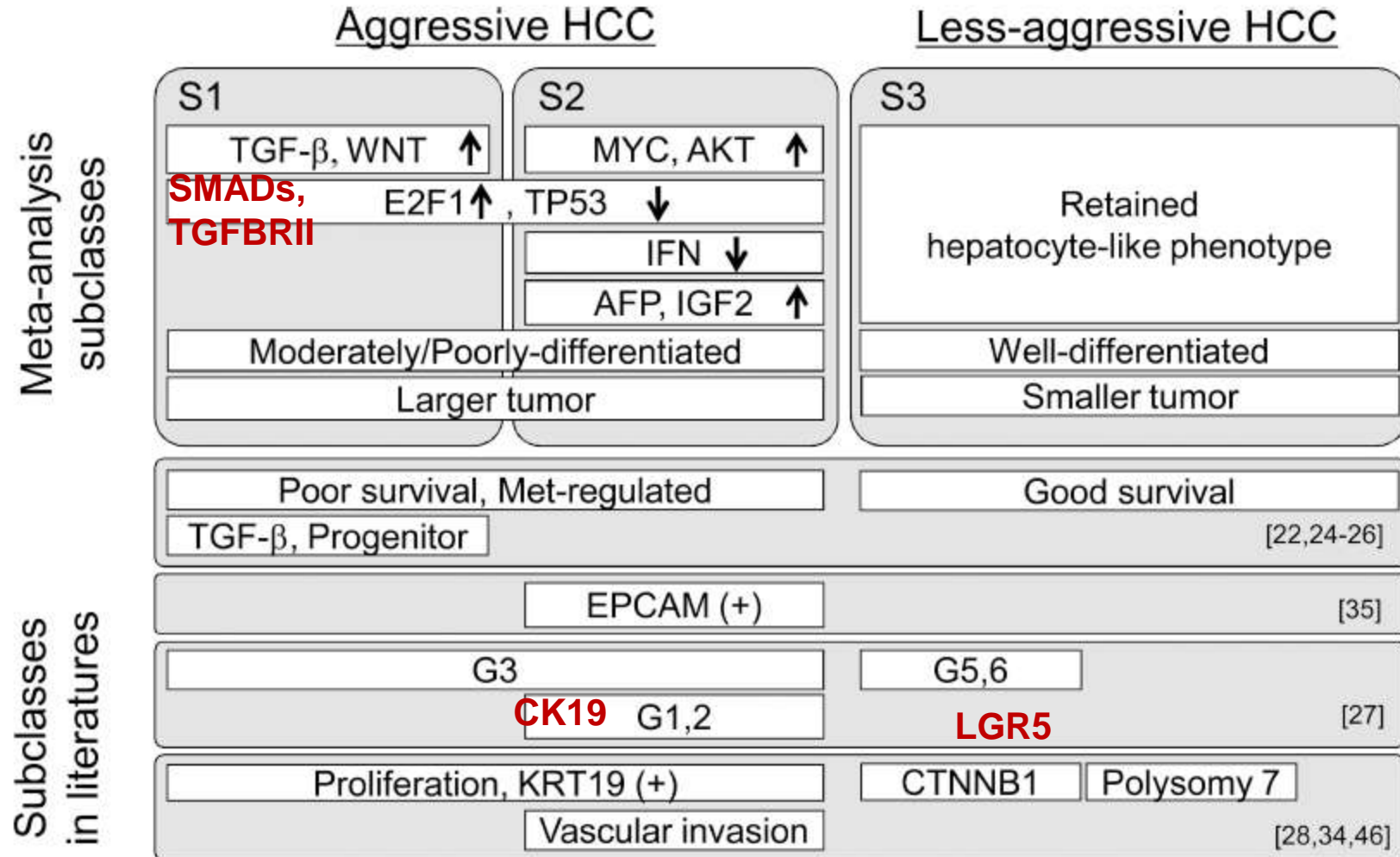
Hoshida Y, et al. Cancer Res 2009;69:7385-92

Nault, et al. Gastroenterology 2013;145:176-187

5-gene score was significantly associated with prognosis, independent of tumor stage, etiology, or presence of cirrhosis

# Global overview of molecular classification of HCC

Aggressive HCC | Less-aggressive HCC

Meta-analysis subclasses

S1: TGF-β, WNT ↑; SMADs, TGFBRII
S2: MYC, AKT ↑; E2F1 ↑, TP53 ↓; IFN ↓; AFP, IGF2 ↑
S1/S2: Moderately/Poorly-differentiated; Larger tumor
S3: Retained hepatocyte-like phenotype; Well-differentiated; Smaller tumor

Poor survival, Met-regulated; TGF-β, Progenitor | Good survival [22,24-26]

Subclasses in literatures

EPCAM (+) [35]

G3; CK19 G1,2 | G5,6; LGR5 [27]

Proliferation, KRT19 (+); Vascular invasion | CTNNB1; Polysomy 7 [28,34,46]

Hoshida Y, Toffanin S, Lachenmayer A, et al. Molecular Classification and novel targets in hepatocellular carcinoma: recent advancement. Senin Liver Dis. 2010; 30(1): 35-51

GPR49/LGR5

✓**Orphan G protein-coupled seven-transmembrane receptor; leucine-rich-repeat-containing G-protein-coupled receptor 5**

✓**Adult stem cell marker: Clevers H et al**

✓**Down-stream target of Wnt and Hedgehog signaling and overexpressed in cancer**

LGR5 is a target gene of WNT signaling -1-

Overexpression of LGR5 in HCCs with CTNNB1 mutations

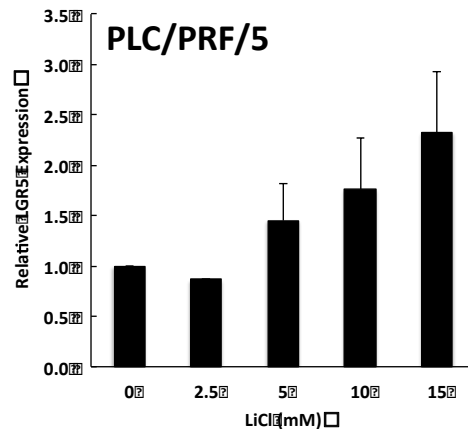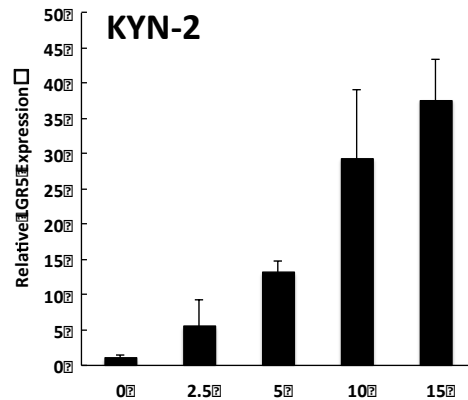**CTNNB1 mutation activates WNT signaling**
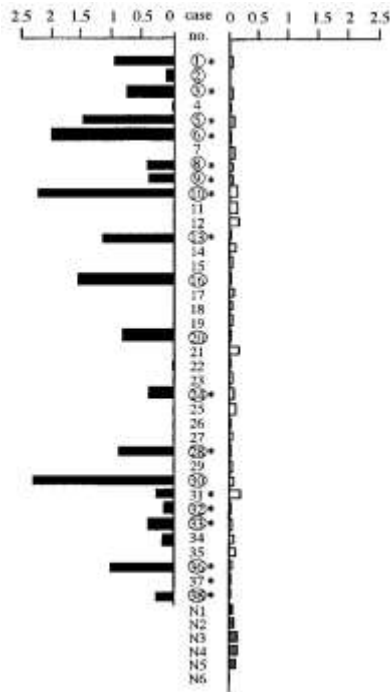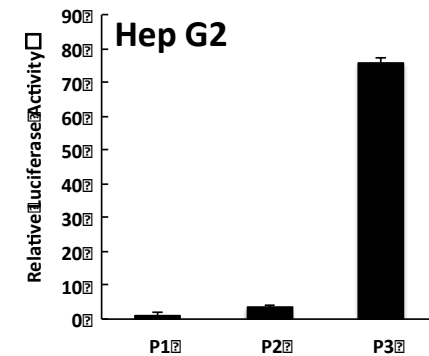

Hepatology (2003) 37, 528-533

* Case with CTNNB1 mutation
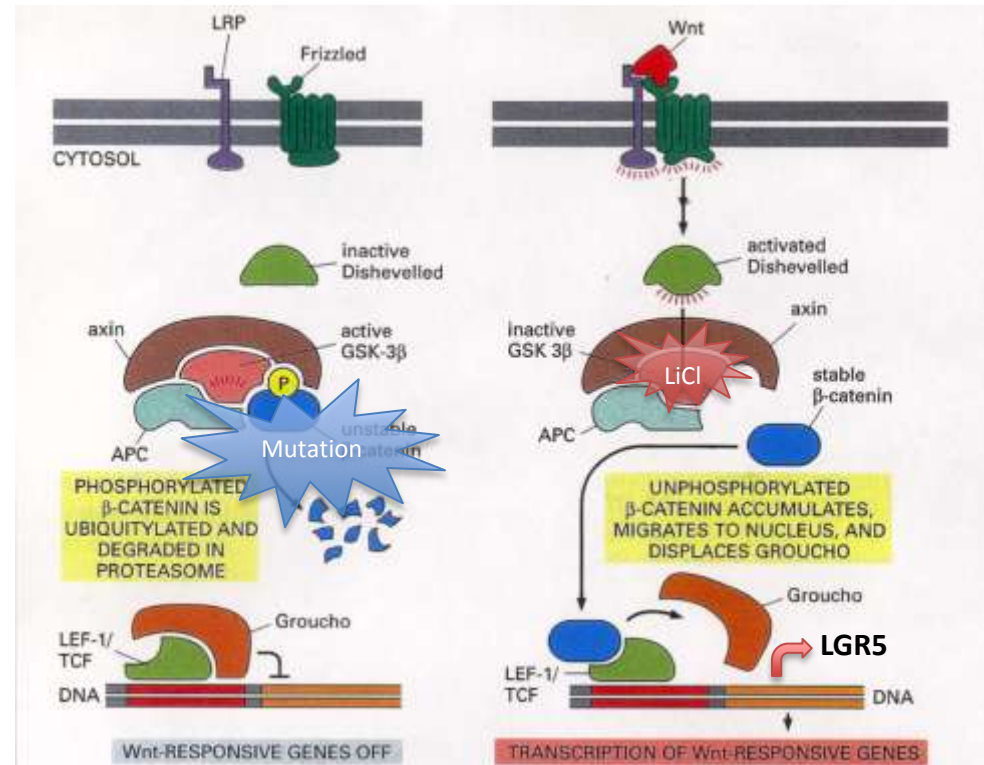
Molecular Biology of the Cell 4th edition

LGR5 is a target gene of WNT signaling -2-
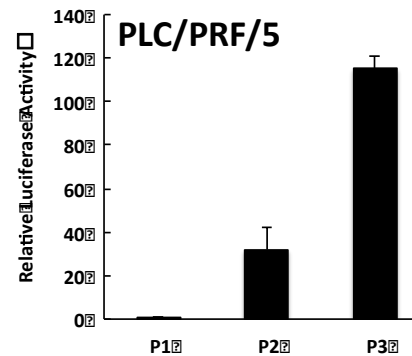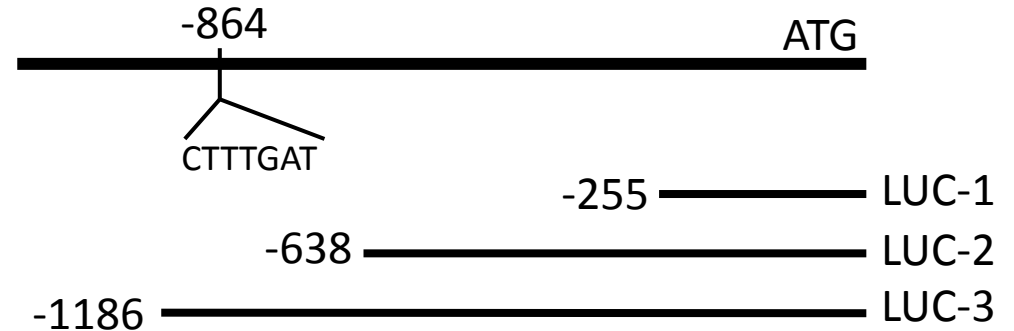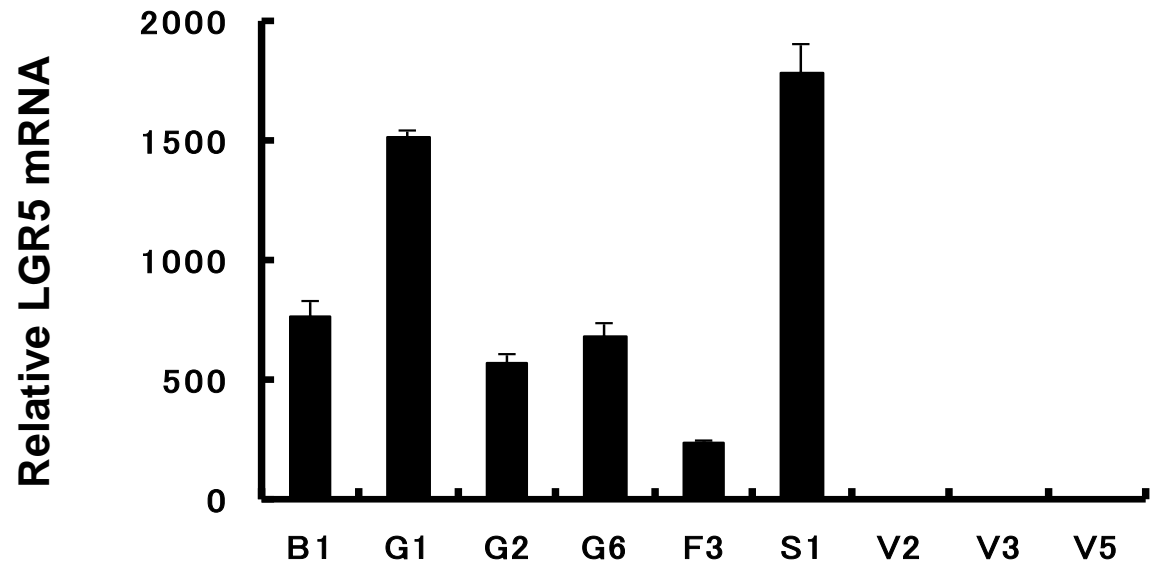
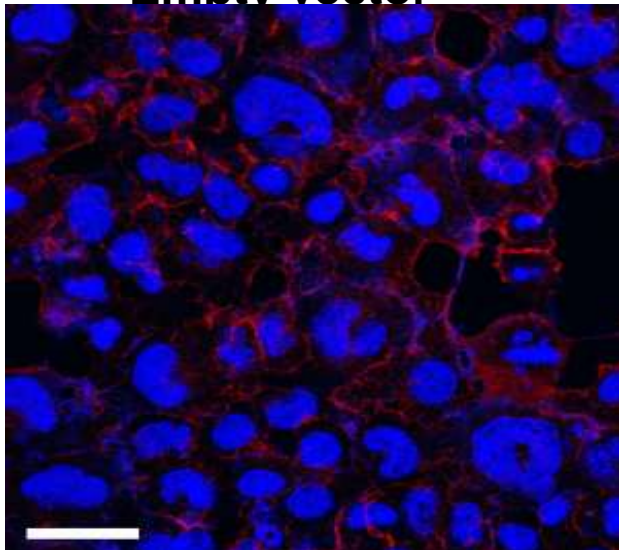# High expression of GPR49 in Wnt activated HCC cell-lines (qRT-PCR)
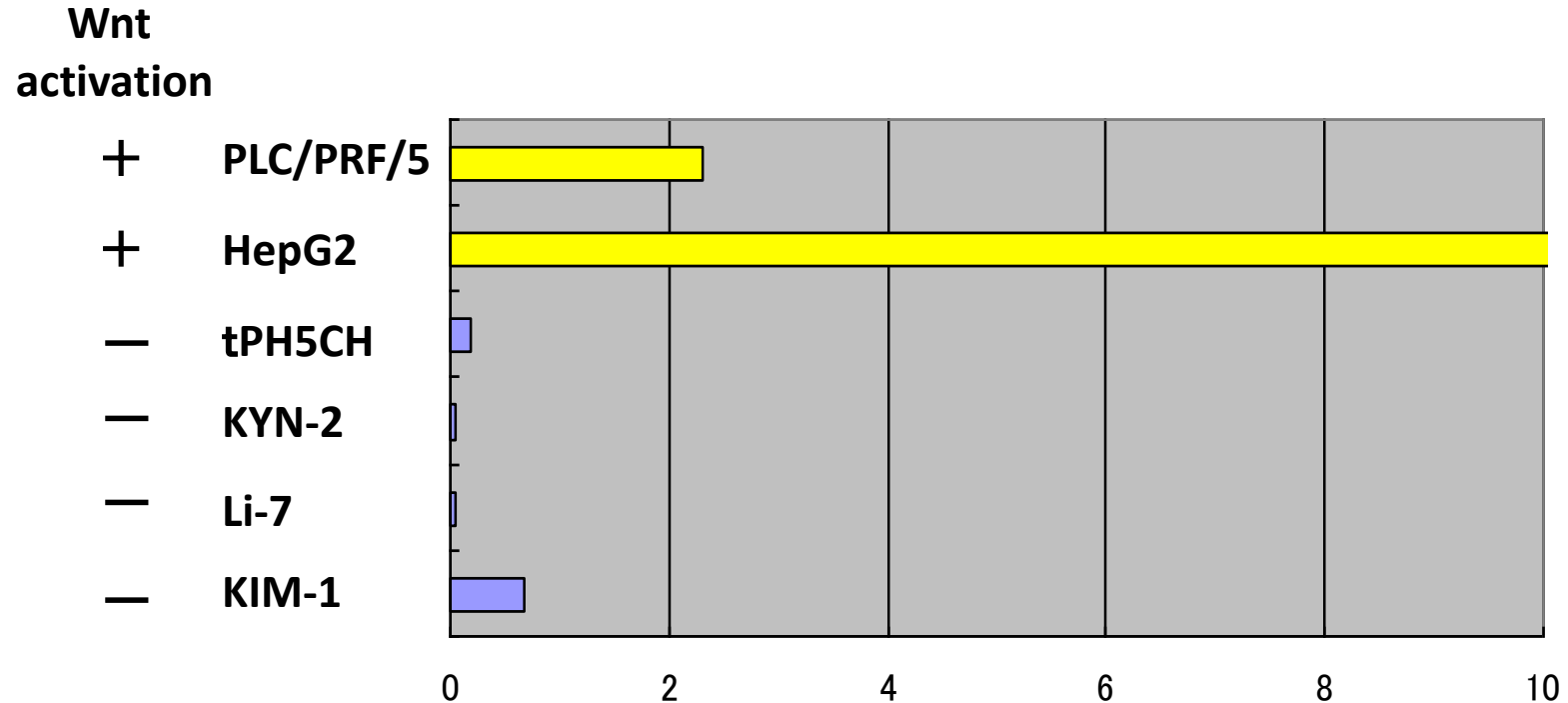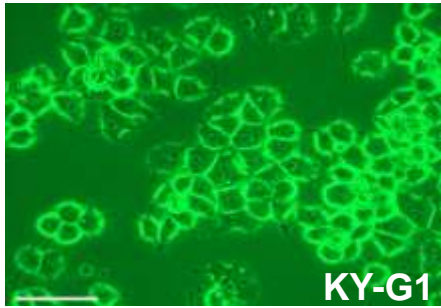
Establishment of LGR5-overexpressing clones.
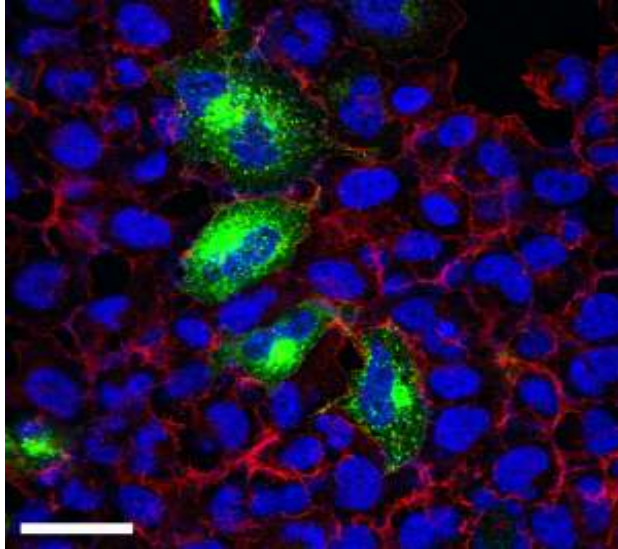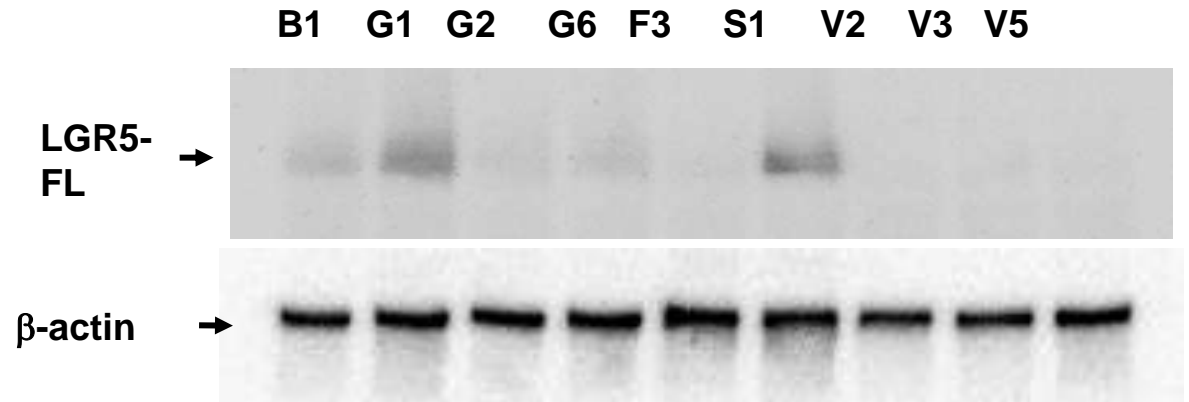
Morphology of clones containing LGR5-FL or empty vector.
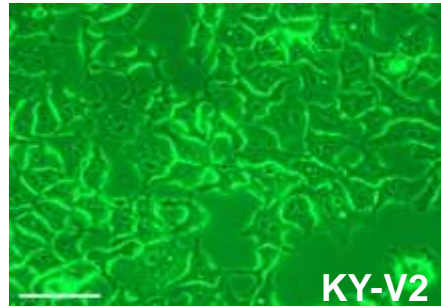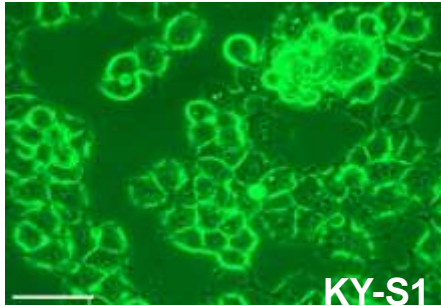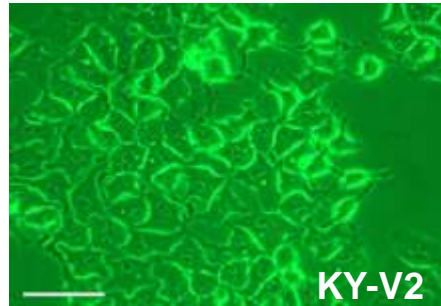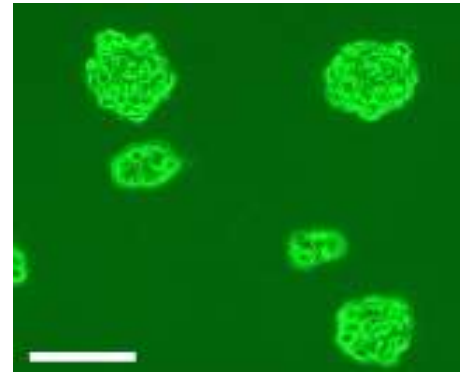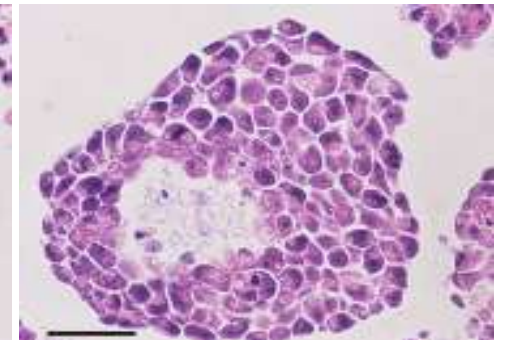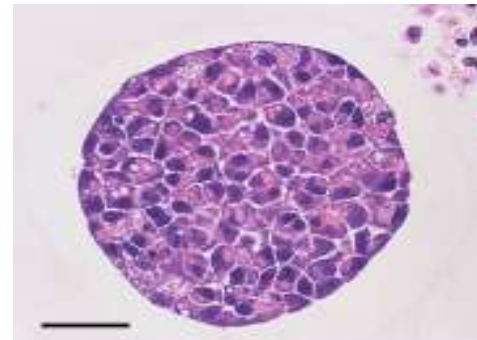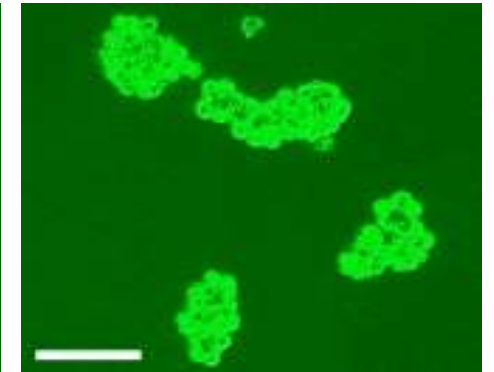
Localization of F-actin, E-cadherin and β-catenin in LGR5-overexpressing or empty vector clones

Growth and survival of KY-G1 and KY-V2 cells.

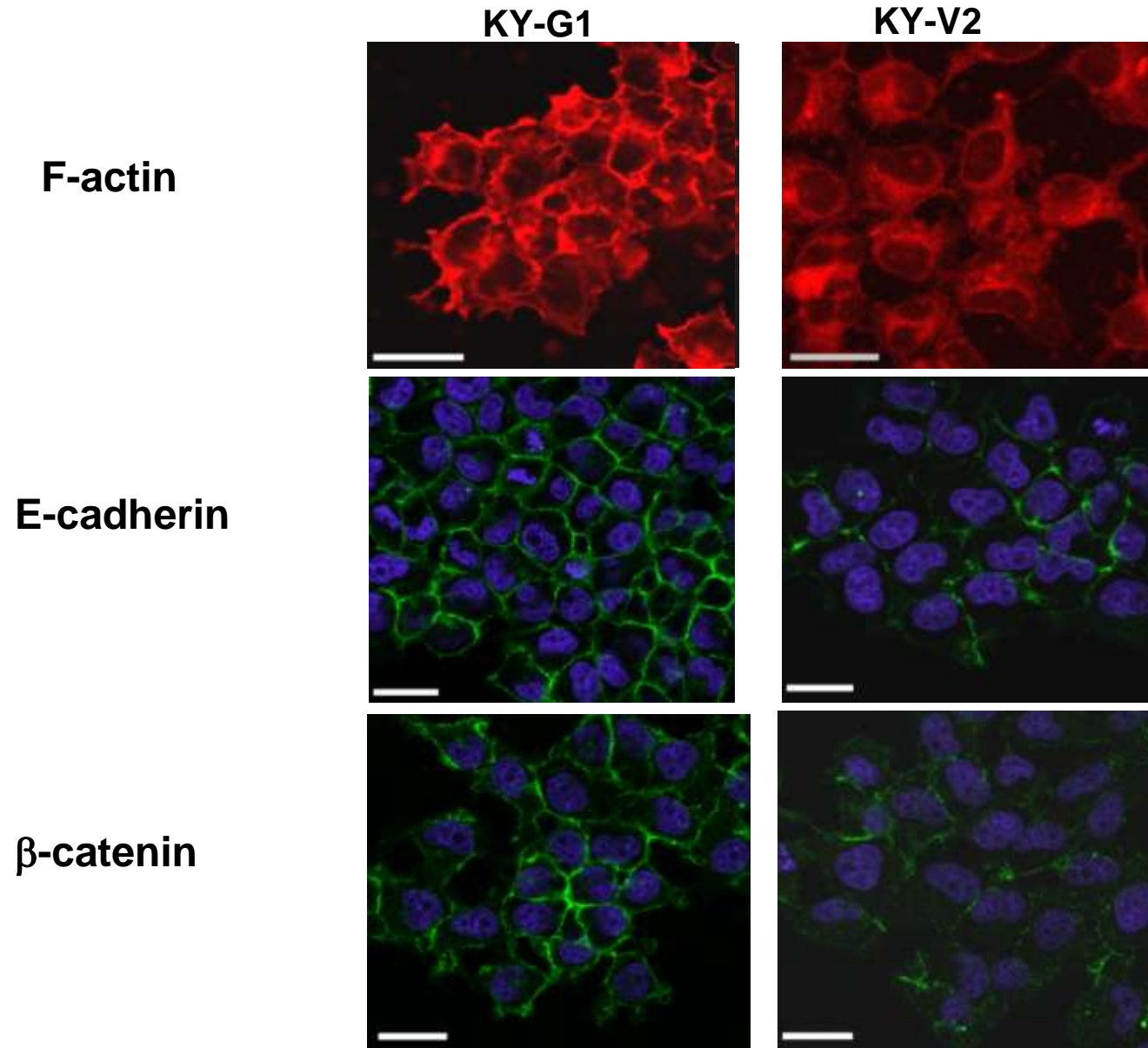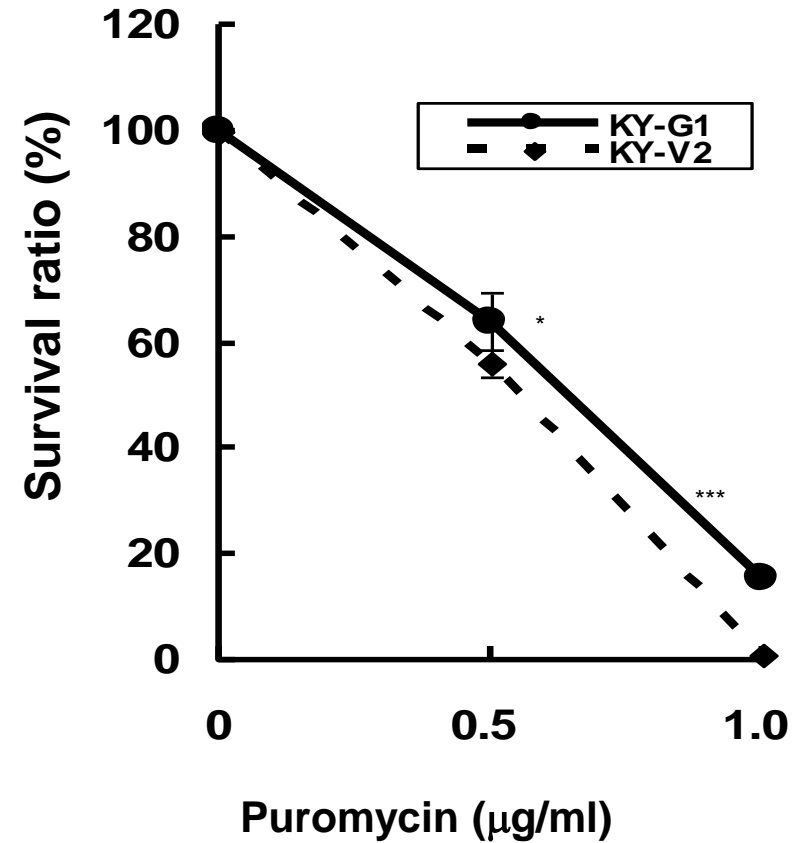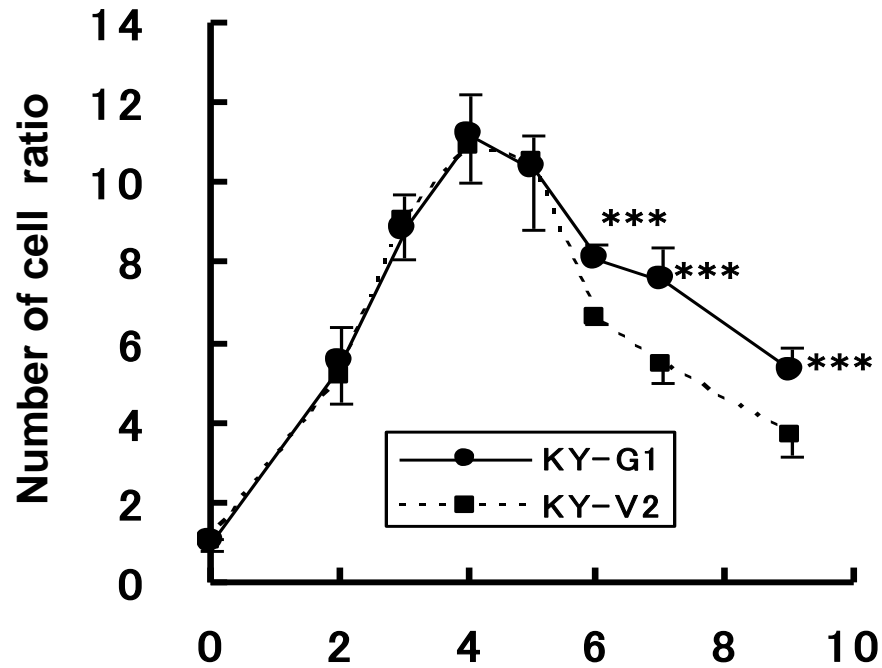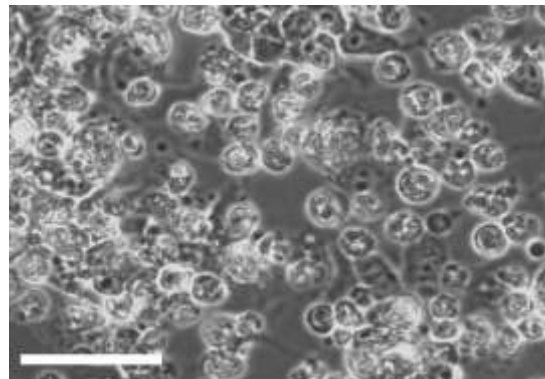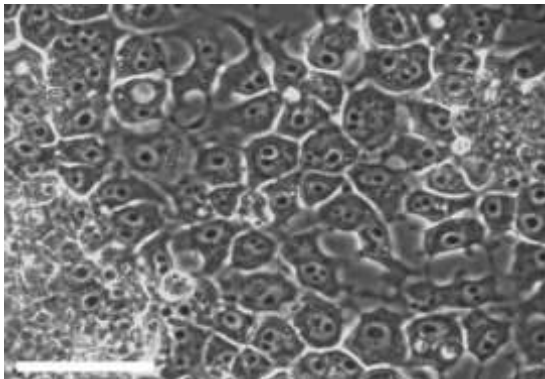# Colony formation and Motility assay
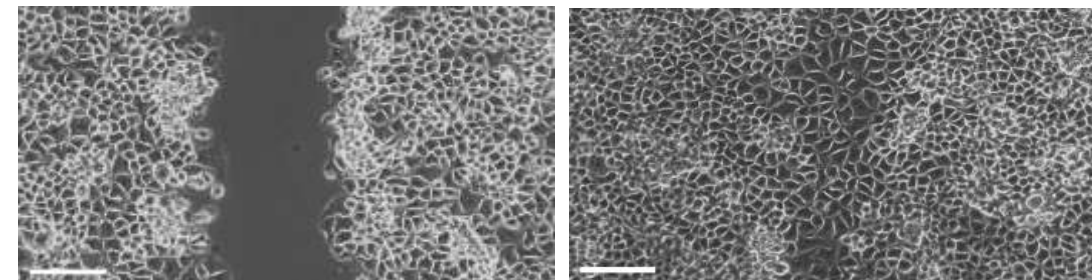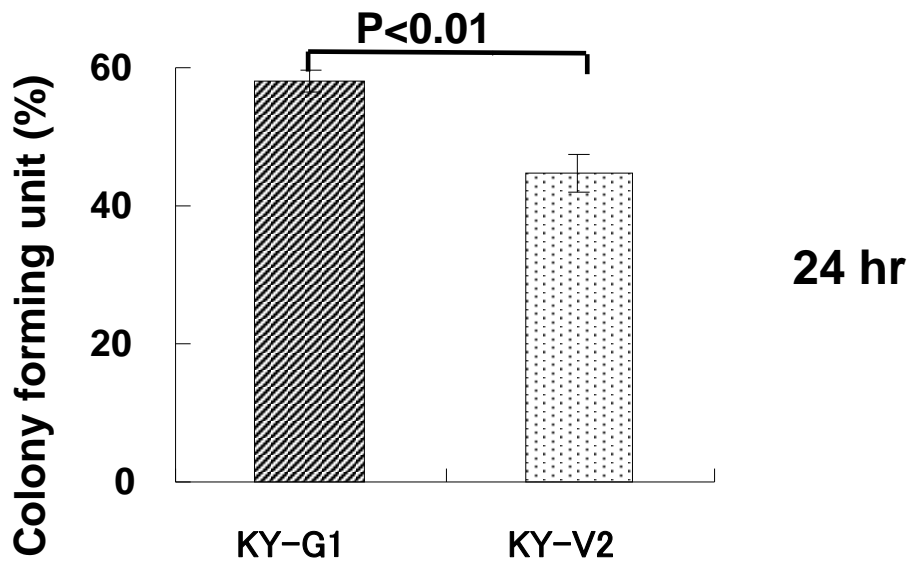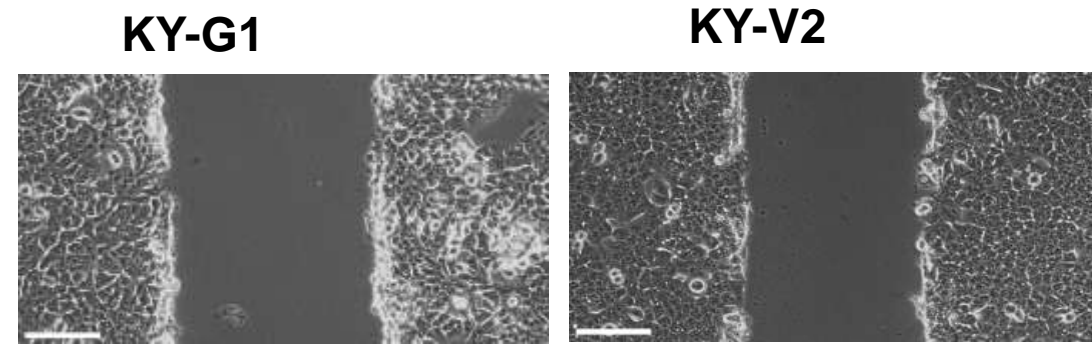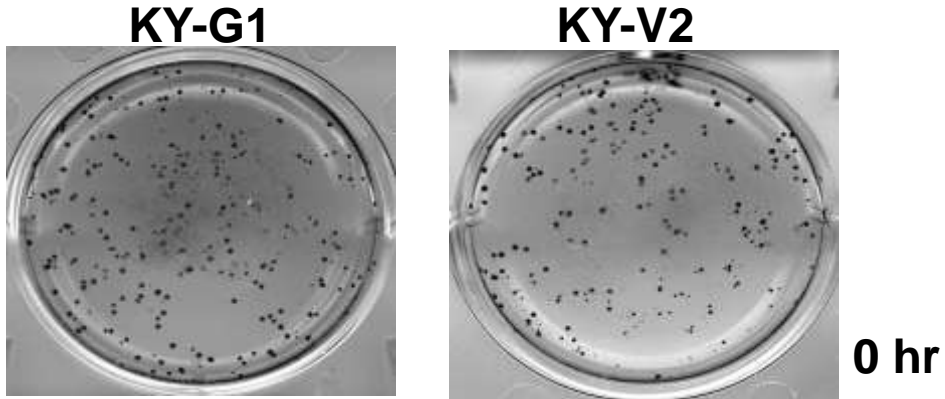
Histological analysis of tumors formed by KY-G1 or KY-V2 clones in the livers of NOG mice.

KY-G1
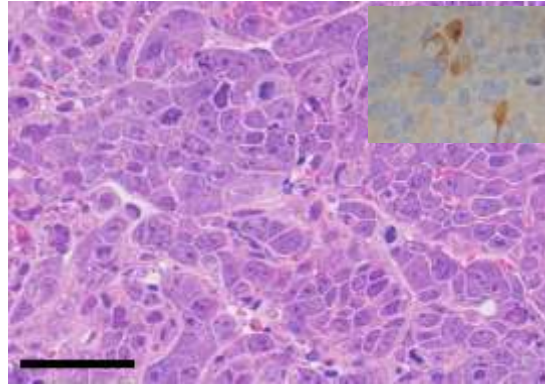
KY-V2
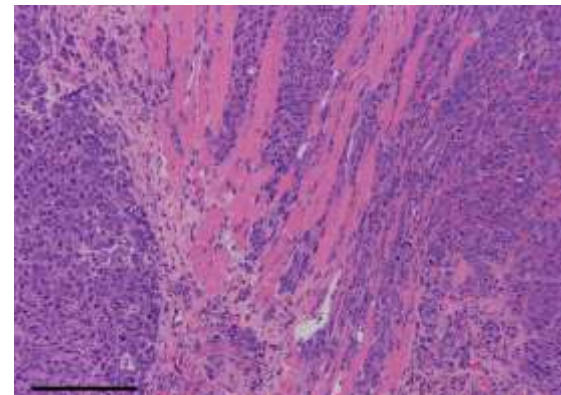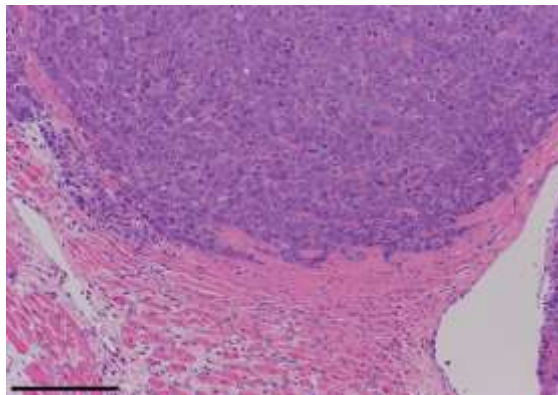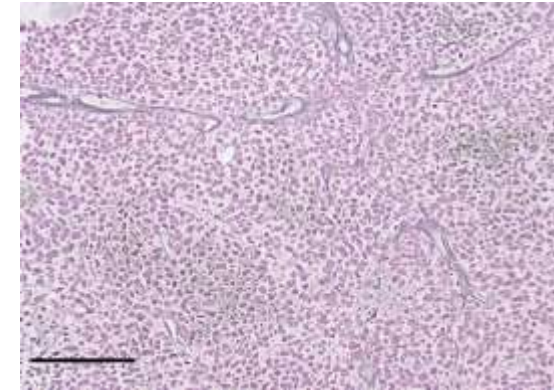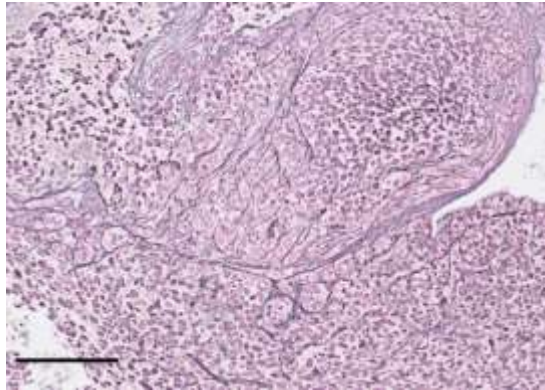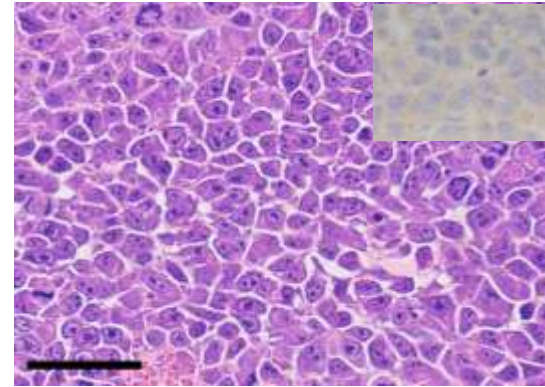
Metastasis of LGR5-overexpressing or empty vector clone in the liver after implantation into the spleen of NOD mice.

KY-G1
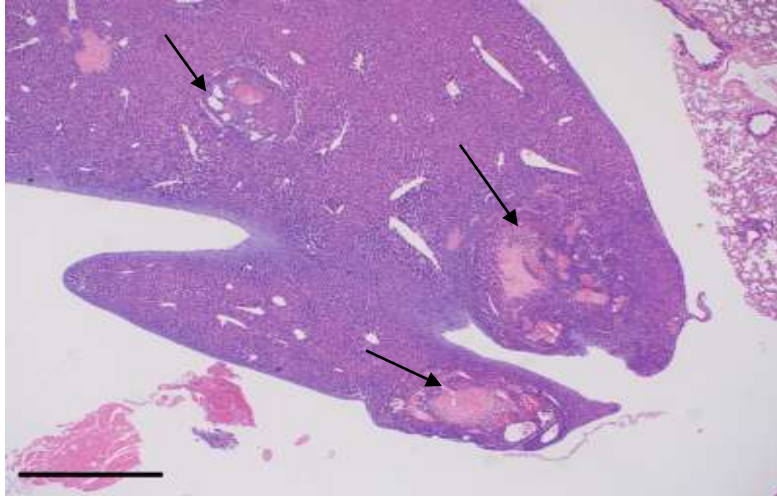
KY-V2
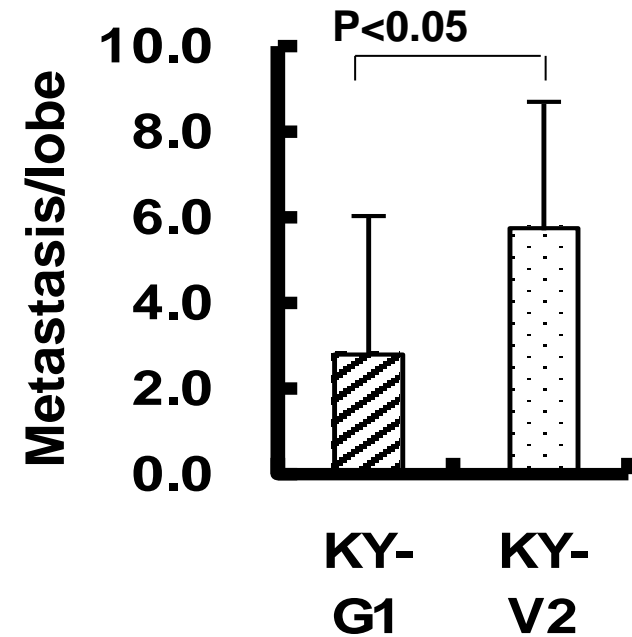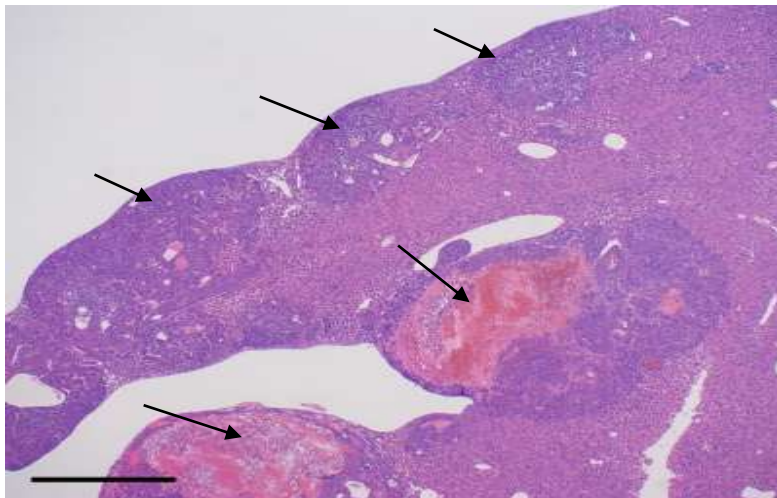
P<0.05

Down-regulation of LGR5 in HCC cell lines by treatment with siRNAs.
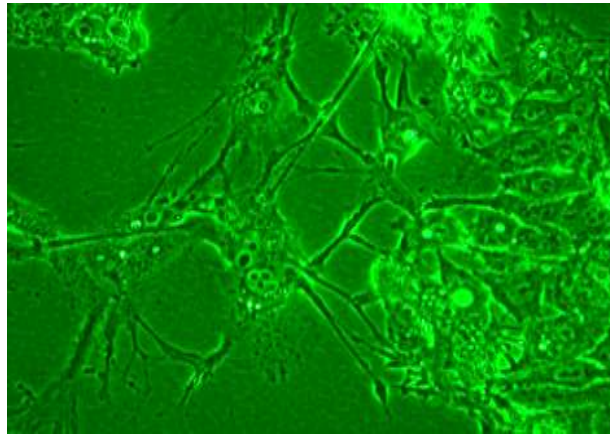
HepG2 | siControl | si585 | si662
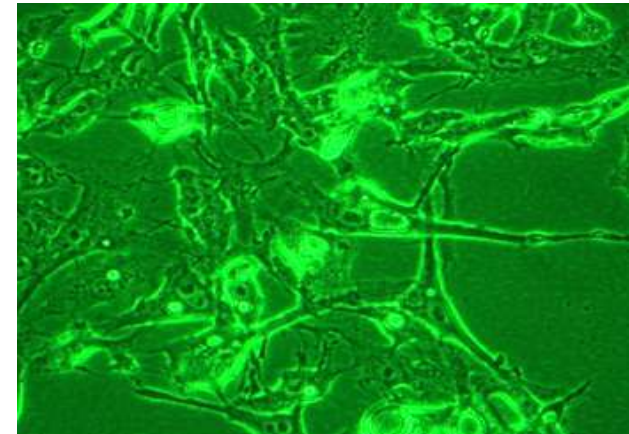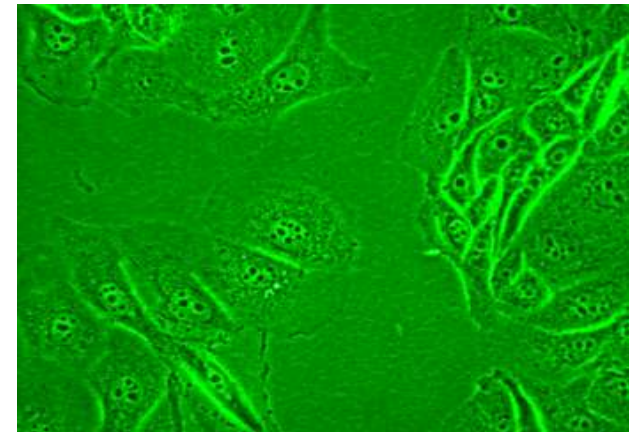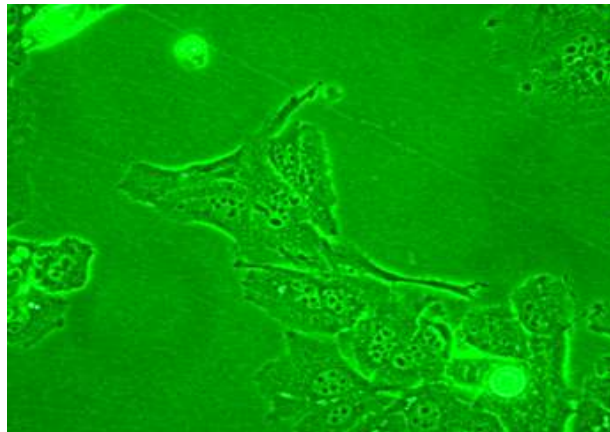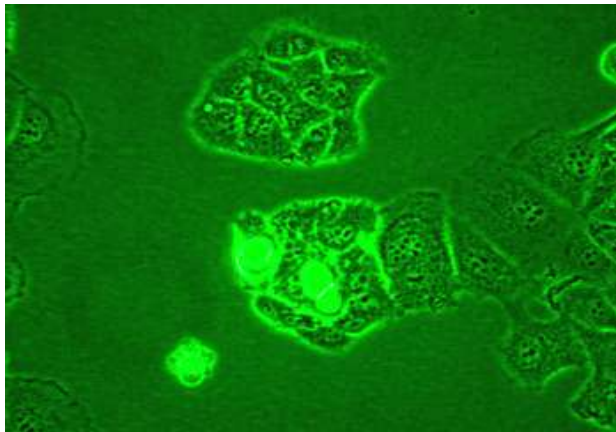
PLC/PRF/5

Down-regulation of LGR5 in HCC cell lines by treatment with siRNAs.
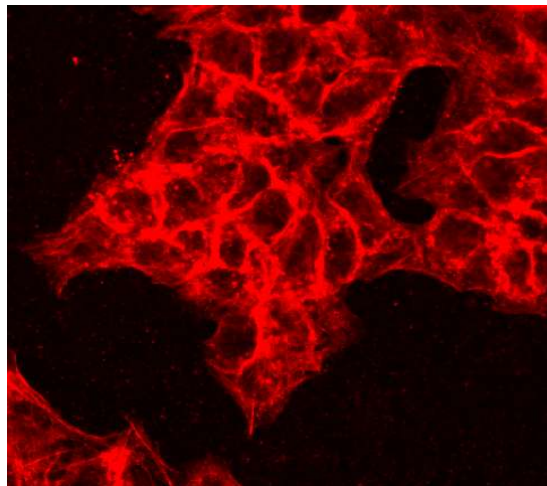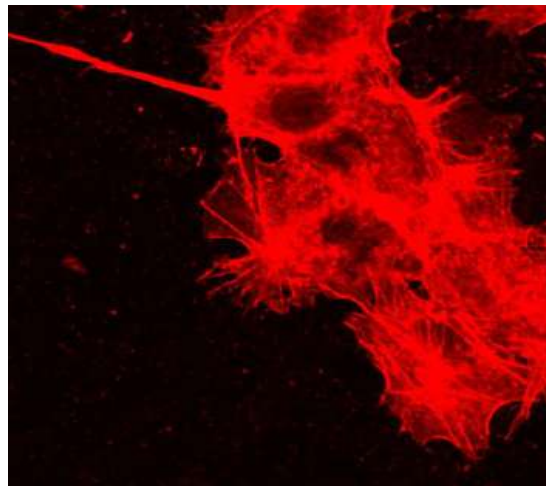
HepG2   siControl   si585   si662
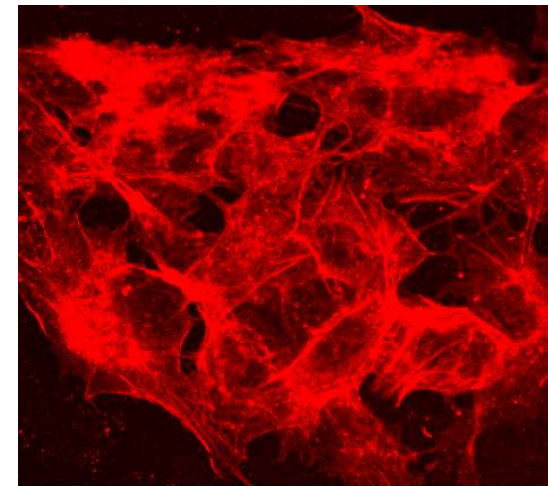
PLC/PRF/5
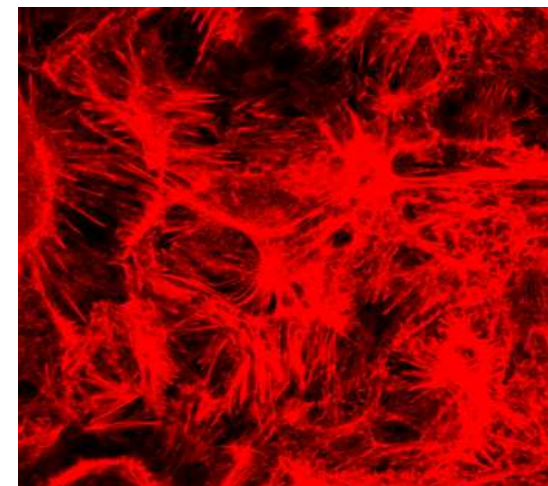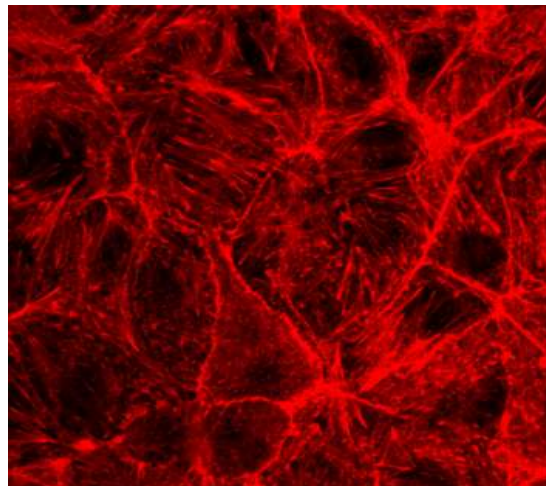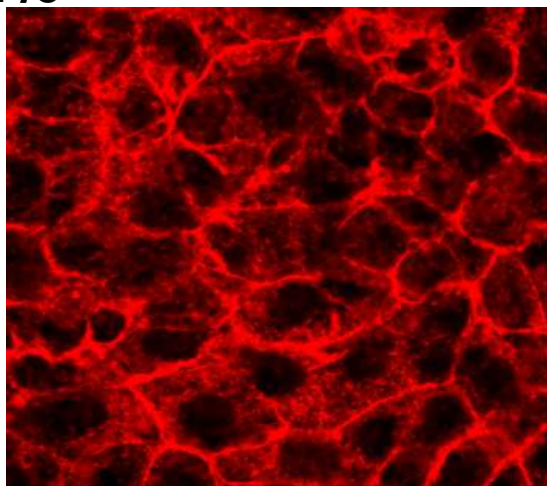
Motility of HCC cells after down-regulation of LGR5.
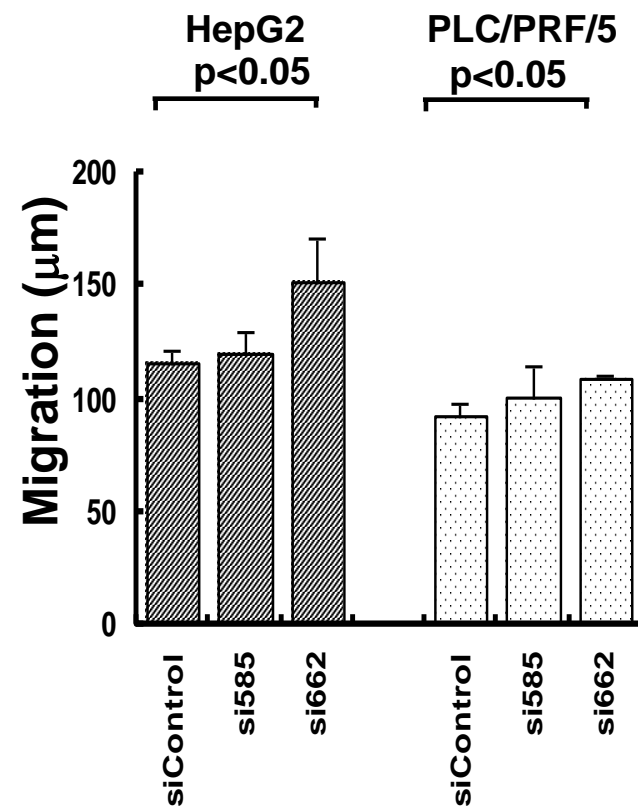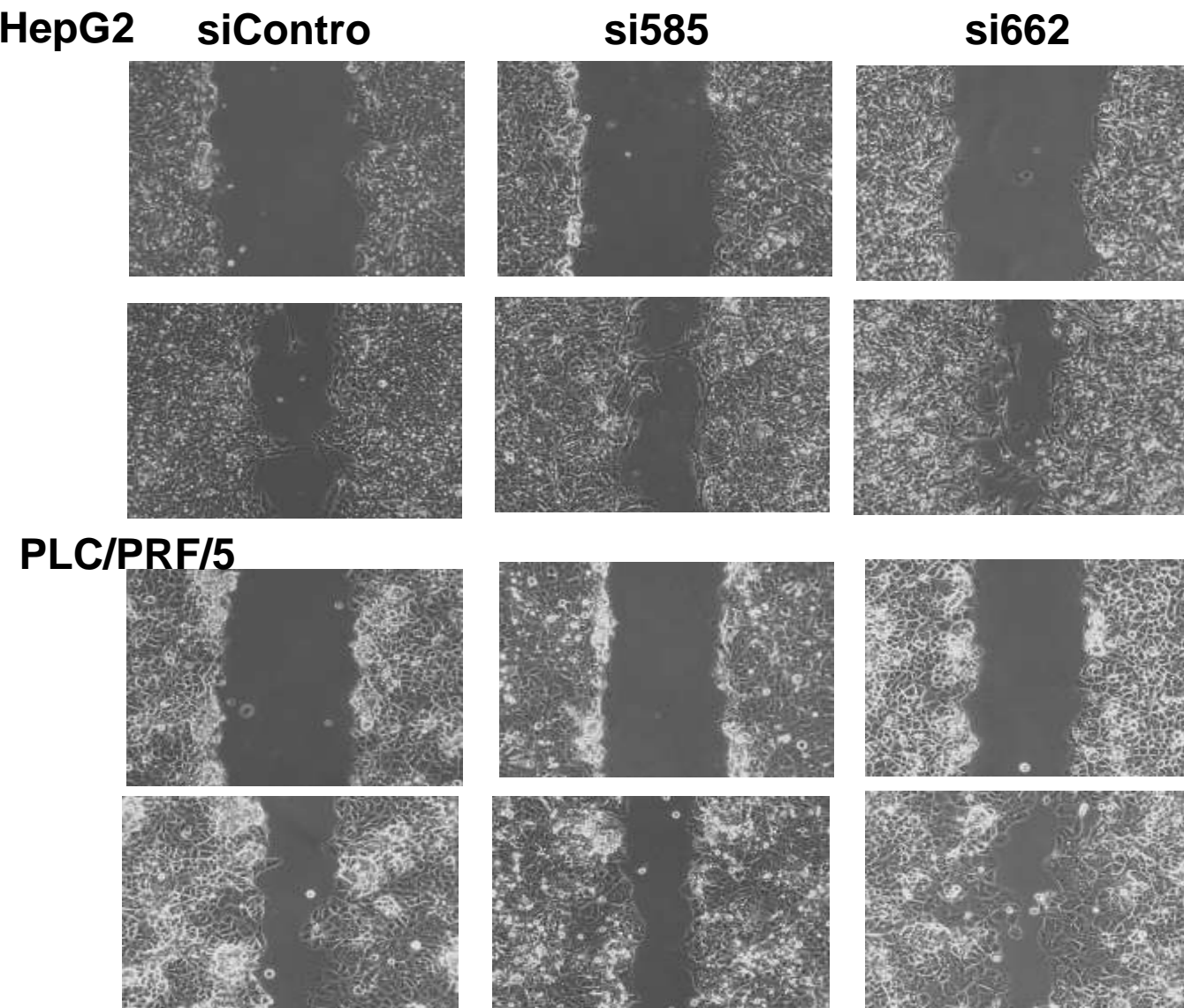
Relationship between Gpr49 mRNA expression and clinicopathologic features

Well to mod > poor
F > M
Non-LC > LC
   although no statistical siginificance

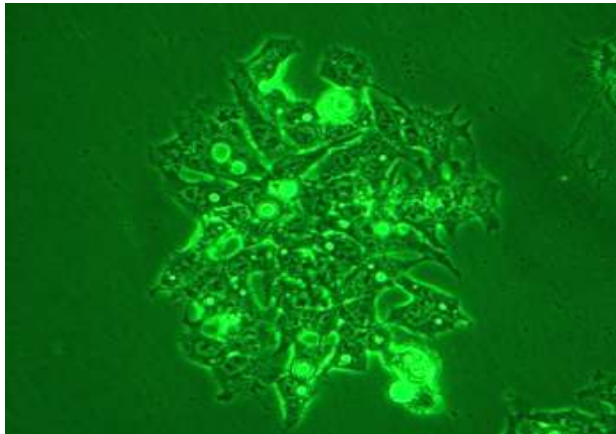| Features	n	Gpr49 Overexpression* (%)	P Value
Age (y)			.76
≤ 65	18	8 (44.4)	
> 65	20	10 (50)	
Sex			.22
Male	31	13 (41.9)	
Female	7	5 (71.4)	
Virus			.58
HBs Ag	9	3 (33.3)	
HCV Ab	24	12 (50)	
HBs Ag (−), HCV Ab (−)	5	3 (60)	
Serum AFP (ng/mL)			.74
≤ 100	23	10 (43.5)	
> 100	15	8 (53.3)	
Tumor size (cm)			.75
≤3	14	6 (42.9)	
>3	24	12 (50)	
Differentiation			.23
Well	3	2 (66.6)	
Moderate	29	15 (51.7)	
Poor	6	1 (17.7)	
Macroscopic type†			.63
1	11	4 (36.4)	
2	18	9 (50)	
3	8	4 (50)	
Massive	1	1 (100)	
Vascular tumor spread (and or intrahepatic metastasis)			.74
Present	24	12 (50)	
Absent	14	6 (42.9)	
Noncancerous liver tissue			.21
Normal	4	3 (75)	
CH	16	9 (56.3)	
LC	18	6 (33.3)	
β-catenin mutation in HCC			<.001
Positive	16	14 (87.5)	
Negative	22	4 (18.2)	

GPR49/LGR5 in HCC

✓Frequent overexpression of GPR49/LGR5 (47%) in advanced HCC

✓GPR49/LGR5 seems to be involved in maintenance of cell polarity and making typical structure of HCC, increased survival potential and resistance to chemotherapy: Typical features of HCC

✓LGR5 may represent β-catenin activated typical subclass of HCC biologically, and also serve as a biomarker of the subclass.

TGF-β activated subclass ?
Two Major *Opposite* Role of TGF-β Signaling in Cancer

- Growth arrest

    CDKN1A (p21) expression

- Malignant progression

    EMT

    Angiogenesis

    Immunosuppression

TGFβ signaling in HCC

- **Levels of TGFβ1 are high in HCC and LC compared with normal liver.**

- **Mutations in TGFβR2 or smad4 are very rare in HCC.**

  Microsattelite instability associated with hepatocarcinogenesis. Kondo Y et al, J Hepatol 1999

- **Transforming Growth Factor-β Gene Expression Signature in Mouse Hepatocytes Predicts Clinical Outcome in Human Cancer.**

  Thorgeirsson S et al. Hepatology 2008

  Early and Late TGF-β Signatures

Reduced TGFBR2 Expression in Metastatic Liver Cancer Cells by Two-way Clustering Analysis of TGF-β Signaling-related Genes

TABLE 1. Tumorigenicities and Metastatic Abilities of Human HCC Cell Lines in SCID Mice

	Cell Line				
	Li7	KYN-2	KIM-1	PLC/PRF/5	HepG2
No. of mice with local tumor growth	12/12	5/5	4/5	8/8	8/8
No. of mice with intrahepatic metastasis	6/12	5/5	0/4	0/8	0/8

NOTE. Male SCID mice, 5 or 6 weeks old, were given a single intrahepatic injection of 2.0×10^6 cells. Six to 7 weeks later, the mice were killed, and tumor formation was estimated macroscopically and microscopically. The data are the number of mice with local tumor growth or metastasis, followed by the number of mice evaluated.
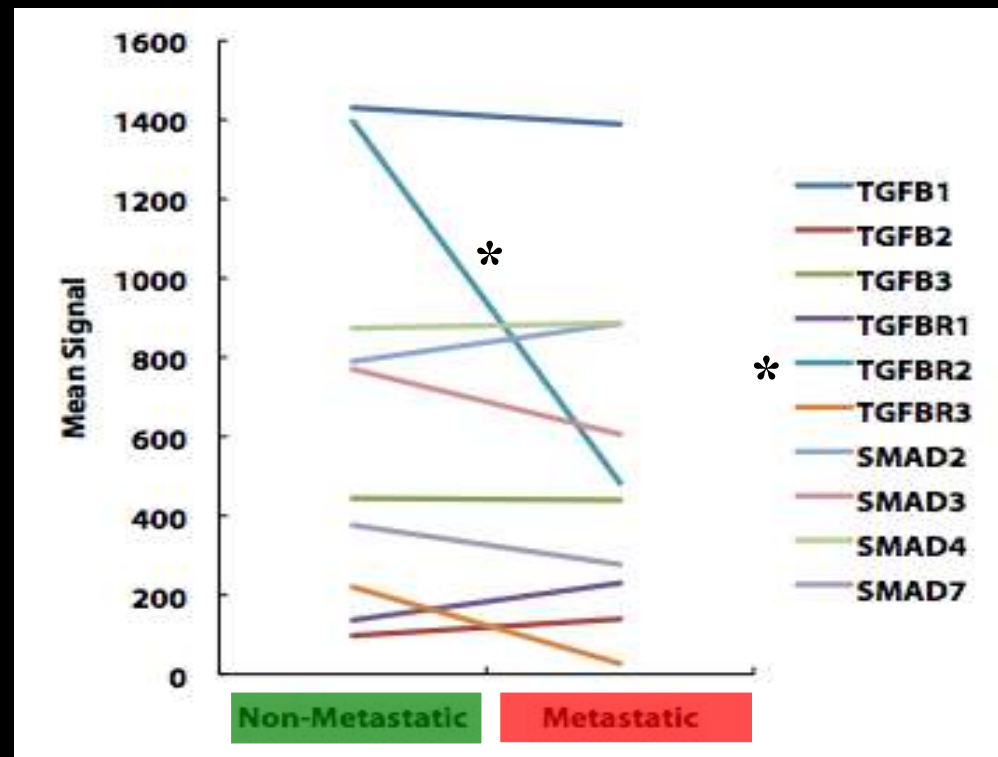
Primary Focus

Intrahepatic Metastasis

Lab Invest 2010
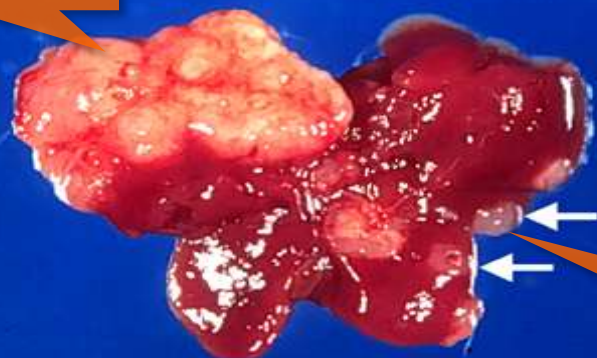
Responses to TGF-β

Immunohistochemical expression of TGFBR2 in HCC cases

TGFBR2 Expression in HCC Cases (n = 136)
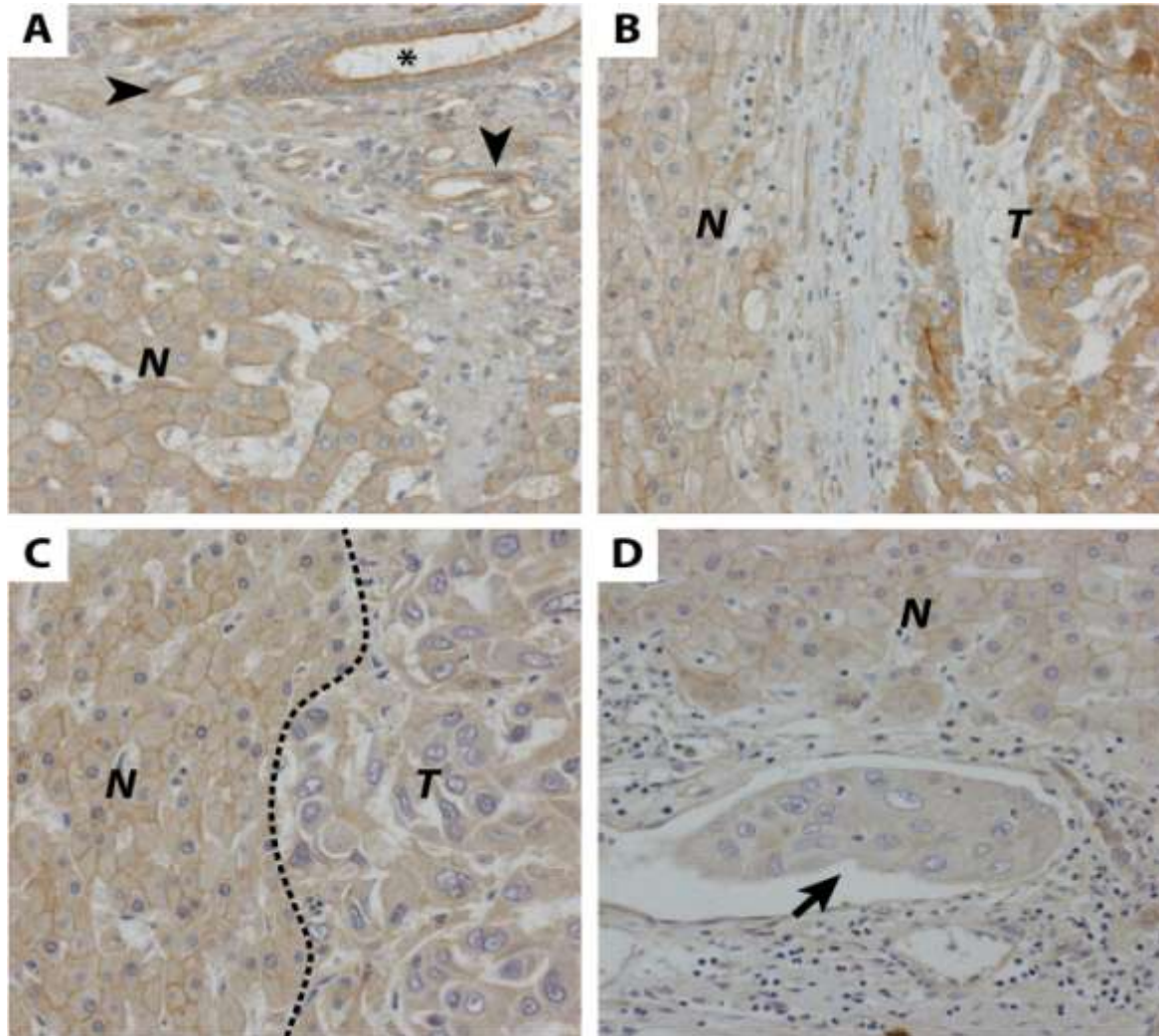
Table 1 Correlations between clinicopathological characteristics and TGFBR2 expression in patients with HCC.

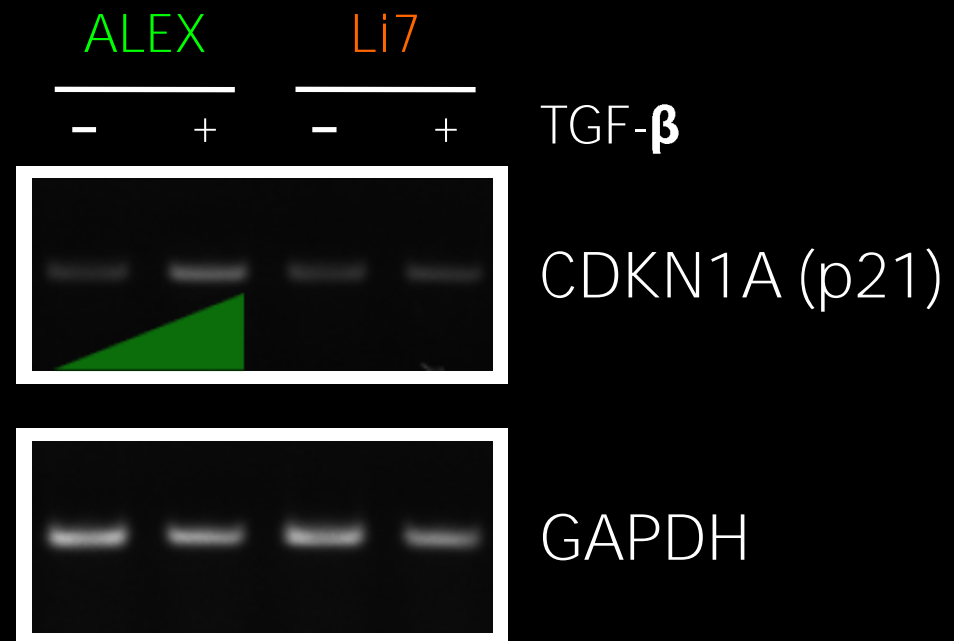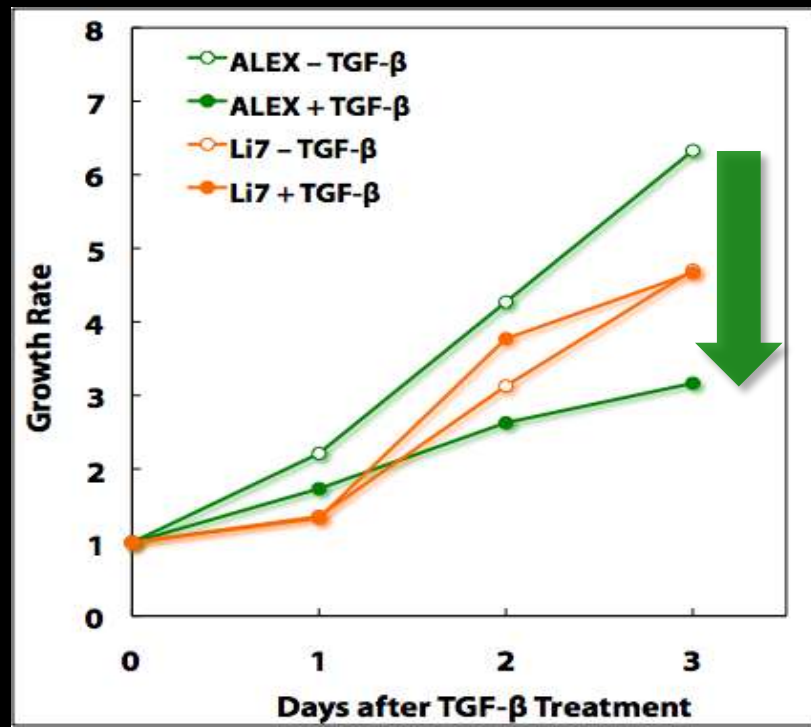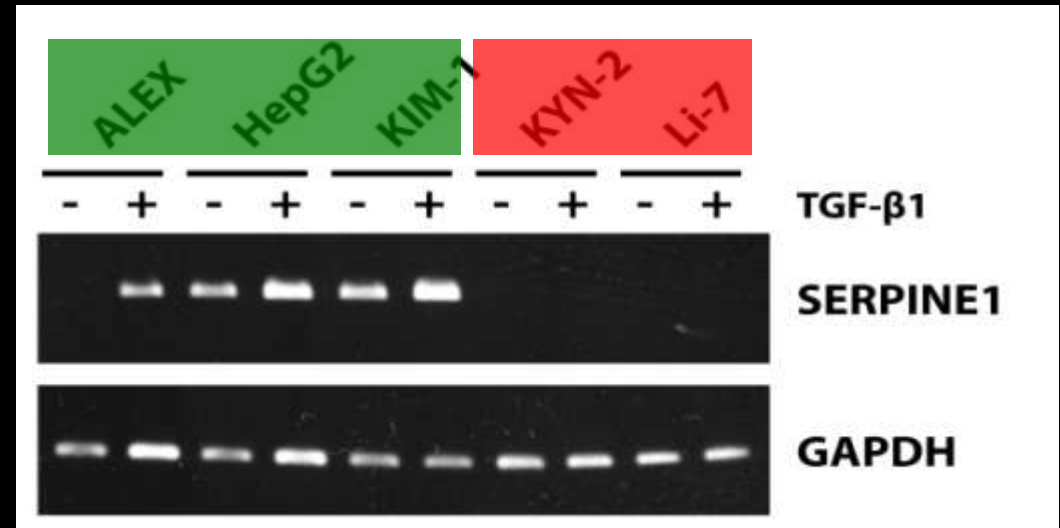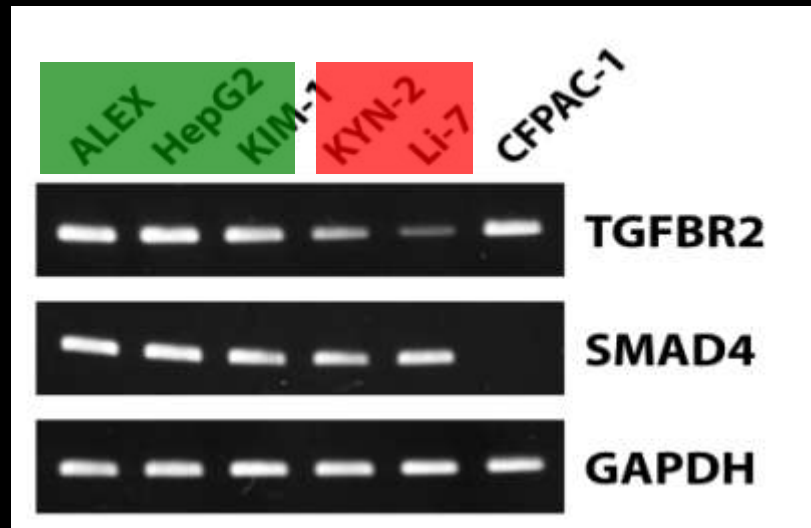| Characteristics	TGFBR2 expression Unchanged (n = 102)	Reduced (n = 34)	χ2 test P value
Mean Age (± SD)	62.2 ± 9.4	60.2 ± 12.8	0.421[†]
Gender			0.475
Male	89	28	
Female	13	6	
Etiology*			0.023
HBV	17	13	
HCV	68	15	
NBNC	16	6	
AFP serum level			0.202
< 20 ng/mL	34	13	
≥ 20 ng/mL	25	17	
Tumor size			< 0.001
≤ 2 cm	54	6	
> 2 cm	48	28	
Differentiation			< 0.001
Well	34	2	
Moderately	62	18	
Poorly	6	14	
Portal involvement			0.002
−	62	10	
+	40	24	
Intrahepatic metastasis			< 0.001
−	90	17	
+	12	17	

Small HCC (2 cm or less) with reduced TGFBR2 expression

Case	Diffe	fc-inf	s	vp	vv	va	b	im	NoT	BC	AFP	Px
58M	por	+	0	1	0	0	0	0	LC	C	55	Rec (6M) (LTx)
33F	mod	+	0	1	0	0	0	0	LC	B	4399	No rec (50M)
71F	por	+	0	1	0	0	0	0	CH	C	1280	No rec (14M)
61M	mod	+	0	1	0	0	0	0	CH	B	4	Rec (16M)
63M	well	+	0	1	0	0	0	0	LC	C	14	No rec
	mod	-	0	0	0	0	0	0	LC	C	14	(27M, LTx)
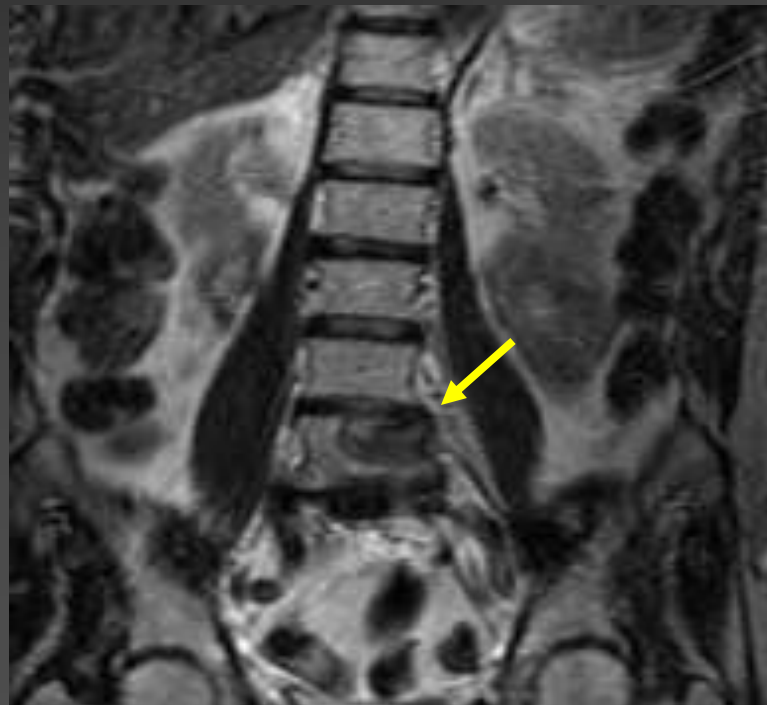
**Bone Meta
(6 mo post LTx)**
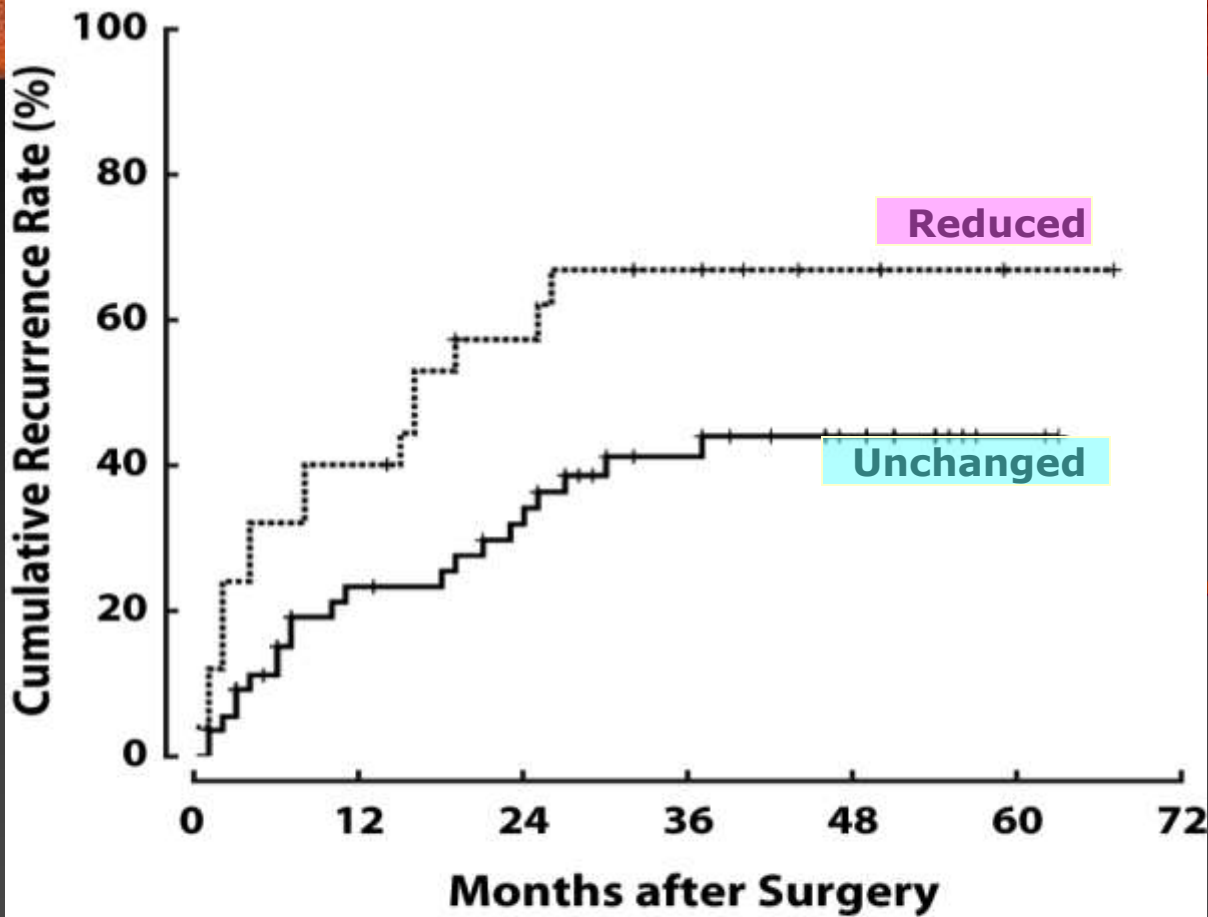
Time-to-recurrence
(n=80)

TGF-β in HCC

- Down-regulation of TGFBR2 in late progression of HCC

- Decreased expression of TGFBR2 can serve as immunohistochemical marker for aggressive HCC

- Canonical TGF-β signaling may play a negative role or non-canonical TGF-β signaling may be activated and play a positive role in liver cancer progression.

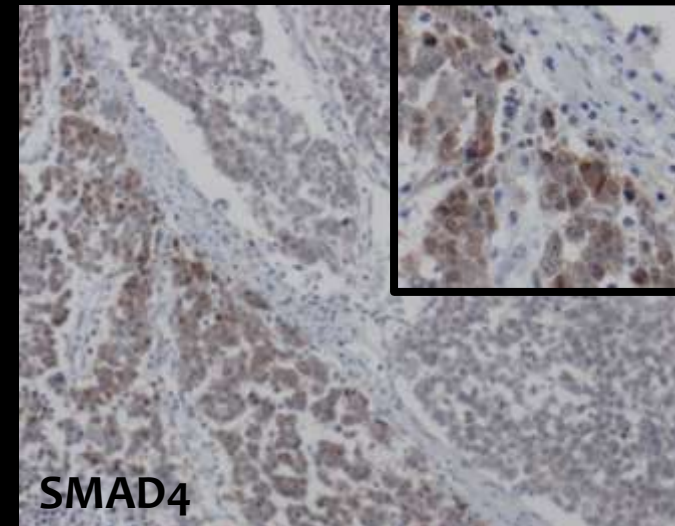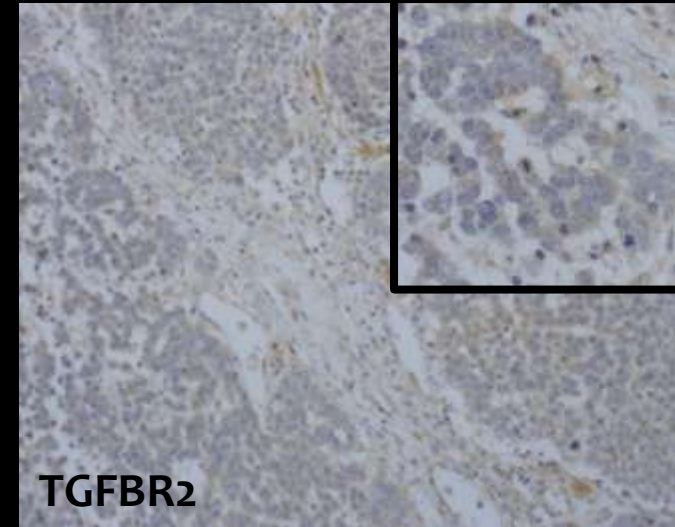- We need further study to clarify TGF-β activated subclass

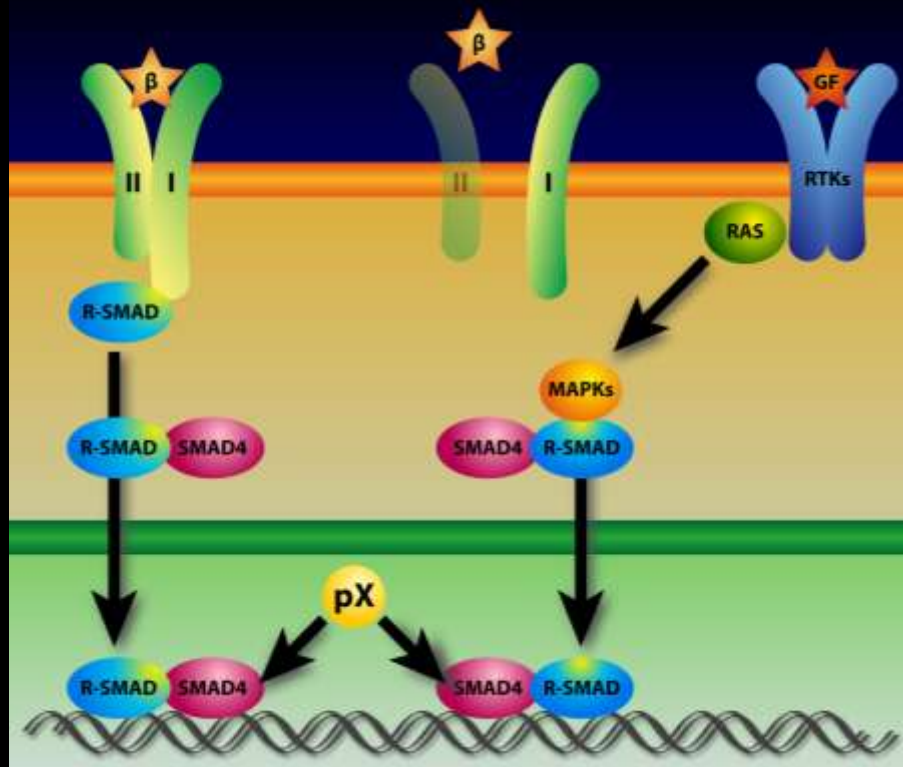# TGFBR2-independent signaling pathway in pancreas cancer
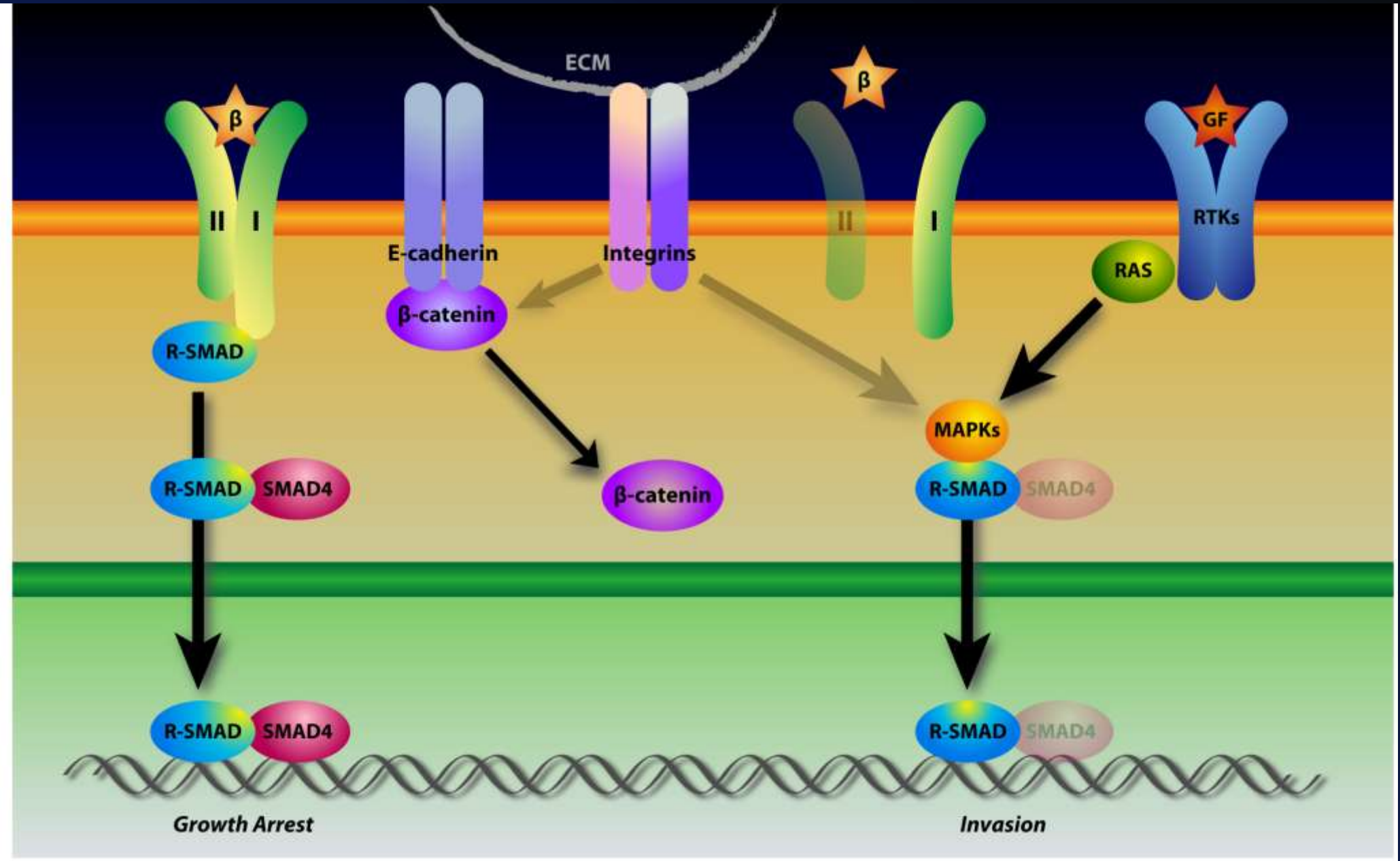
TGFBR2-Independent SMAD4 Translocation to Nucleus and HBV

TGFBR2 & Etiology (P = 0.023)		
	TGFBR2+	TGFBR2–
HBV	17	13
HCV	68	15
NBNC	16	6

Hepatic progenitor/biliary marker positive subclass

A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells

Ju-Seog Lee et al. Nature Medicine 12;410-416, 2006

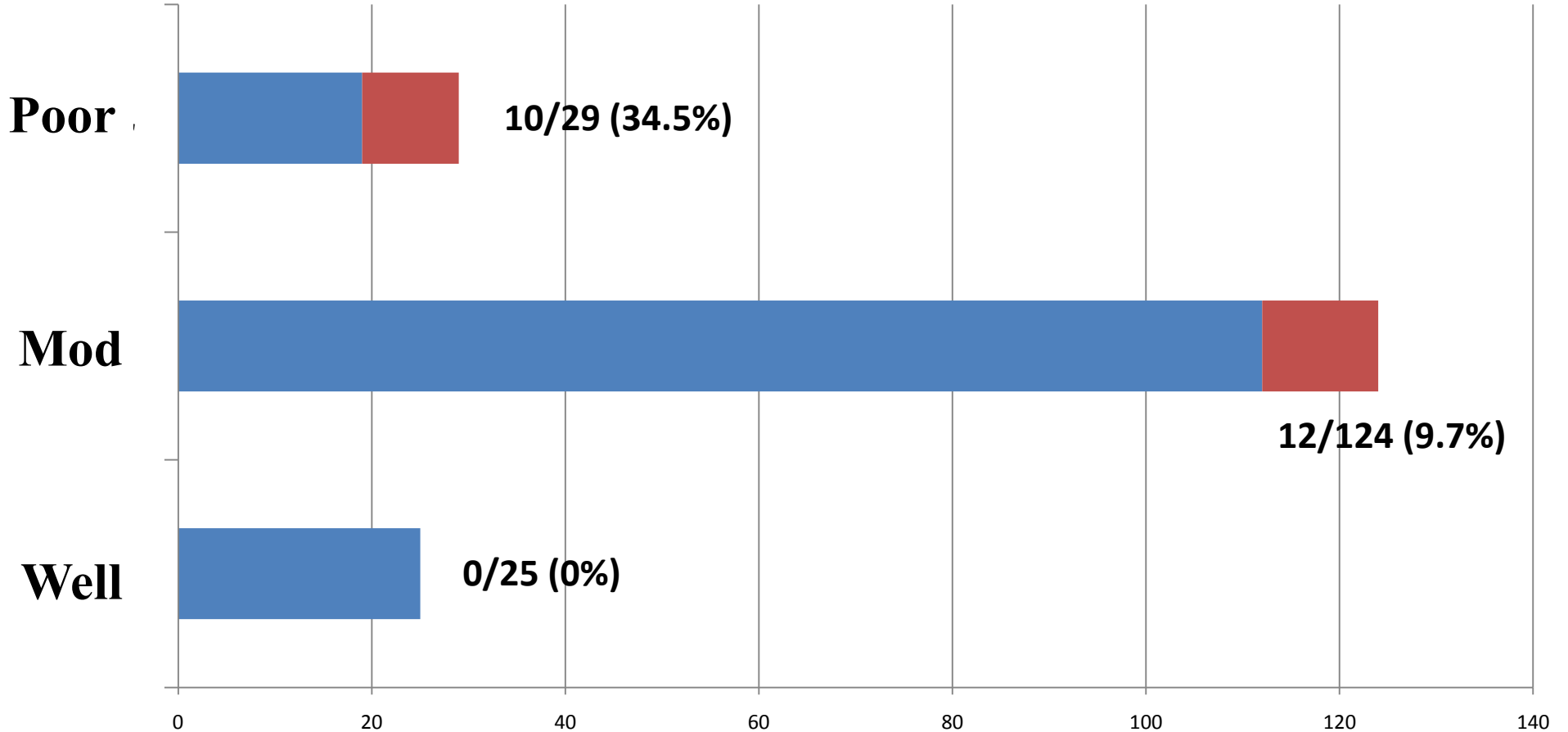**Hepatoblastic signatures: CK7, CK19, Vimentin etc.**

Cytokeratin 19 expression in hepatocellular carcinoma predicts early postoperative recurrence

Uenishi T et al. Cancer Science 94;851-857, 2003

CK7/CK19   -/- : 93 cases   +/- : 49 cases
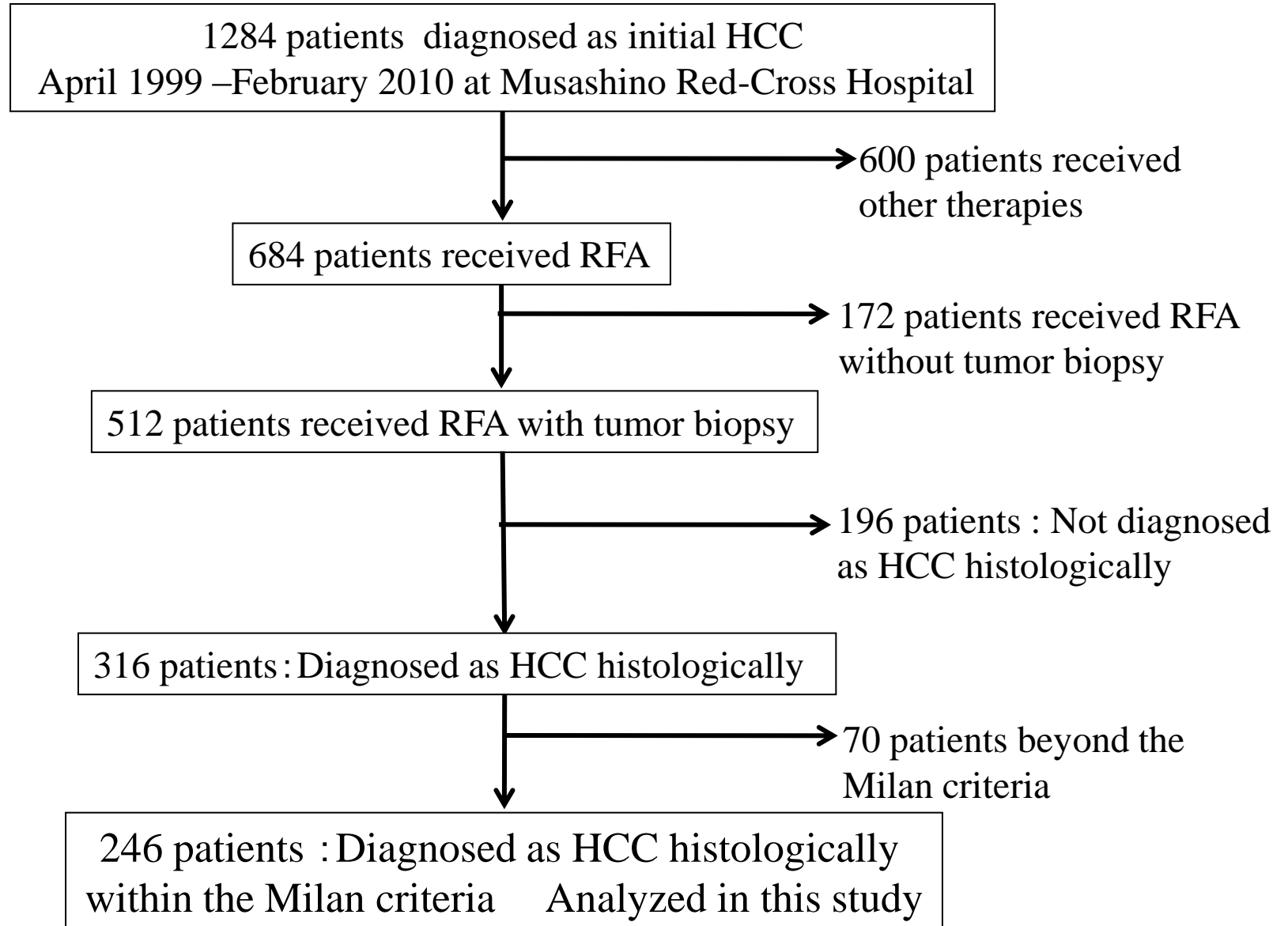   -/+ : 1 case   +/+ : 15 cases

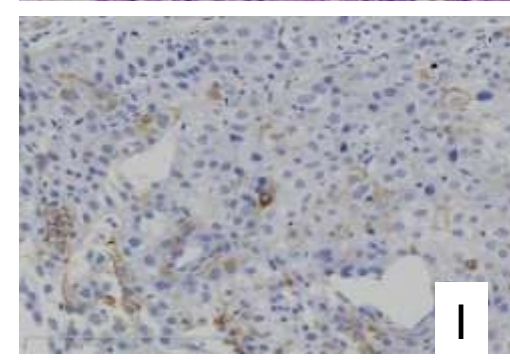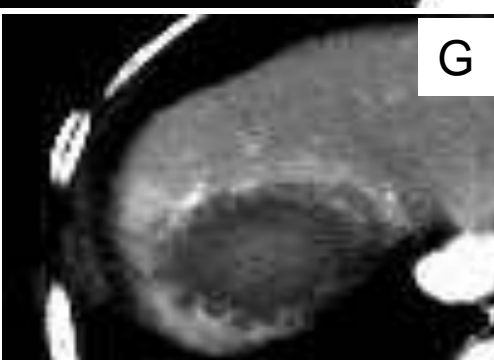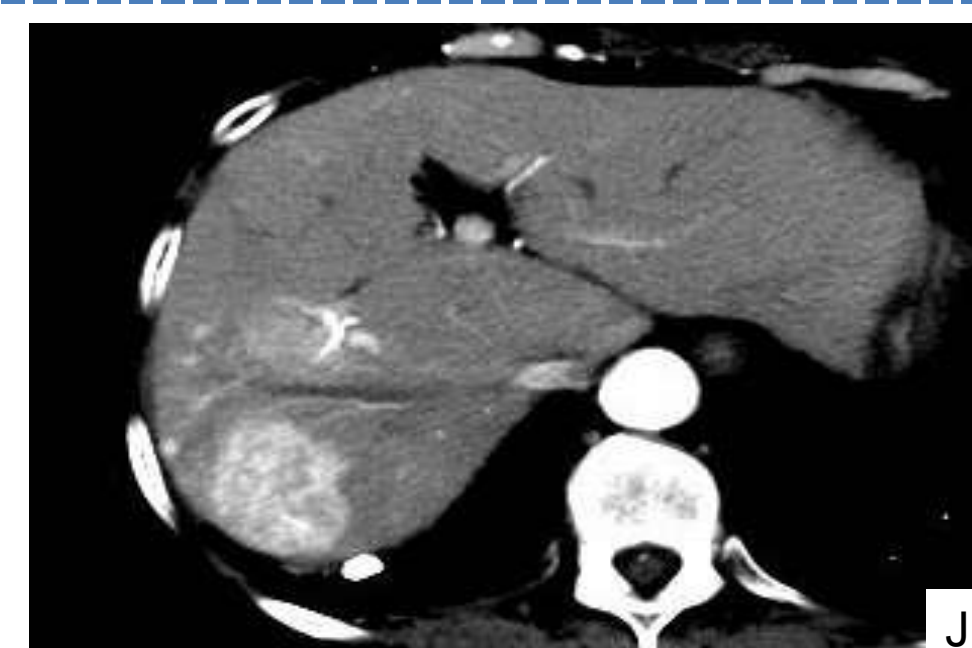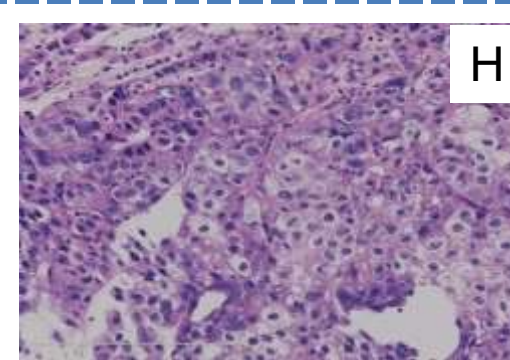# Incidence of CK19 positive HCC in Tissue Microarray

22/178 (12.4%)

Poor — **10/29 (34.5%)**

Mod — **12/124 (9.7%)**

Well — **0/25 (0%)**

Grants from MHLW, chaired by Prof Arii

Expression of Keratin19 is Related to High Recurence of HCC after RFA

1284 patients diagnosed as initial HCC
April 1999 –February 2010 at Musashino Red-Cross Hospital

→ 600 patients received other therapies
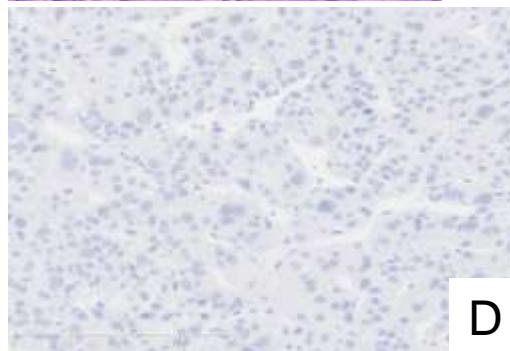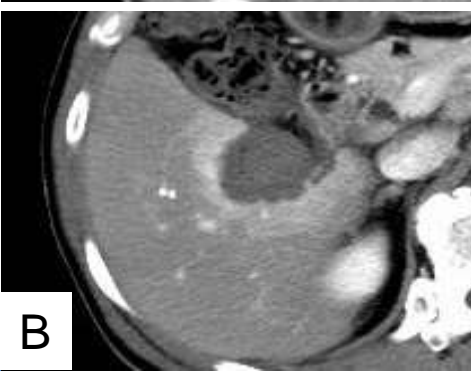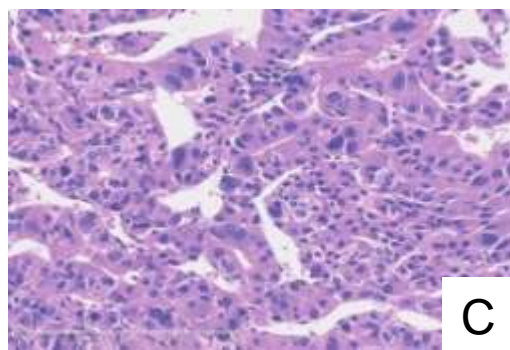
684 patients received RFA

→ 172 patients received RFA without tumor biopsy

512 patients received RFA with tumor biopsy

→ 196 patients : Not diagnosed as HCC histologically

316 patients : Diagnosed as HCC histologically

→ 70 patients beyond the Milan criteria

246 patients : Diagnosed as HCC histologically within the Milan criteria   Analyzed in this study

Tsuchiya K, Komuta M et al, Oncology 2011

A

B

C

D

4Y 10M, Rec (-)
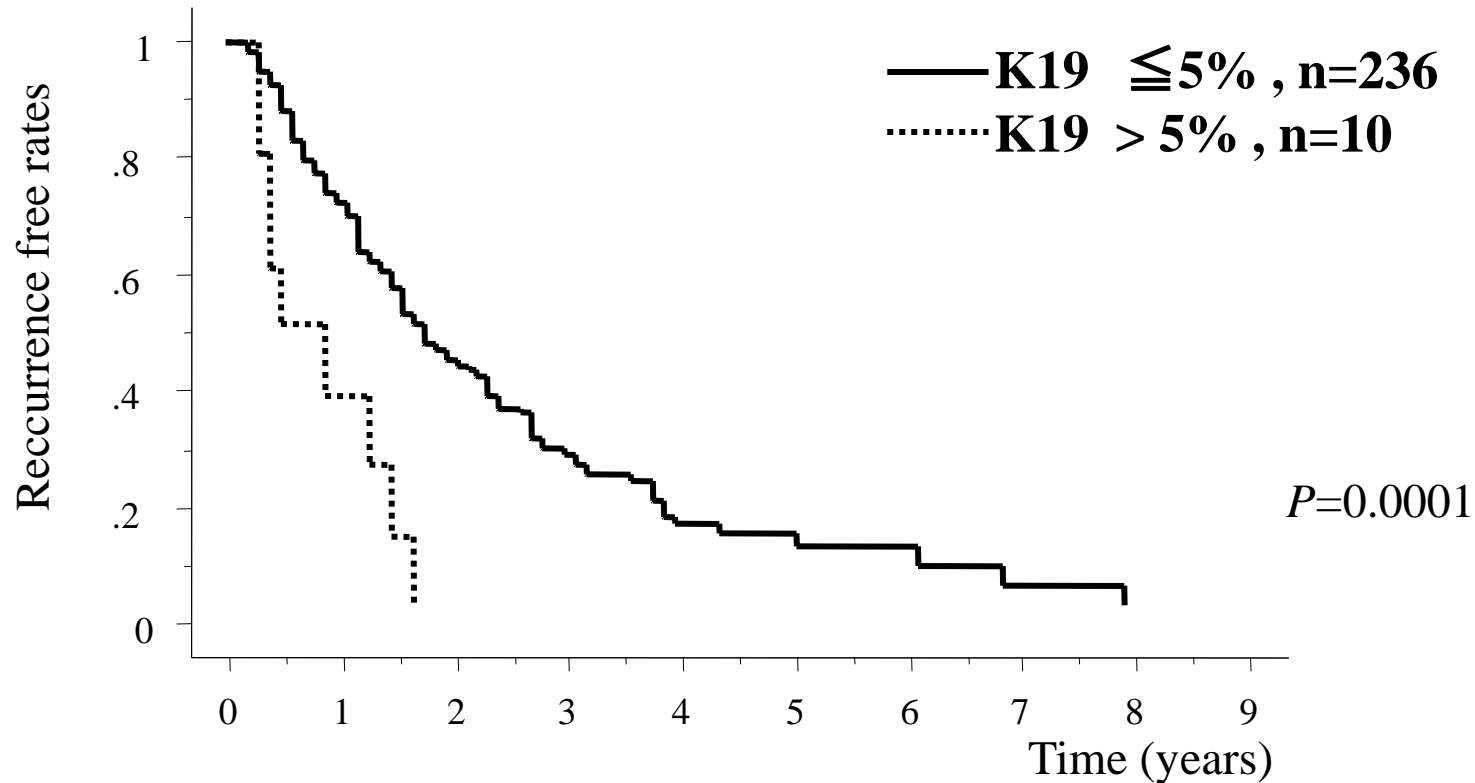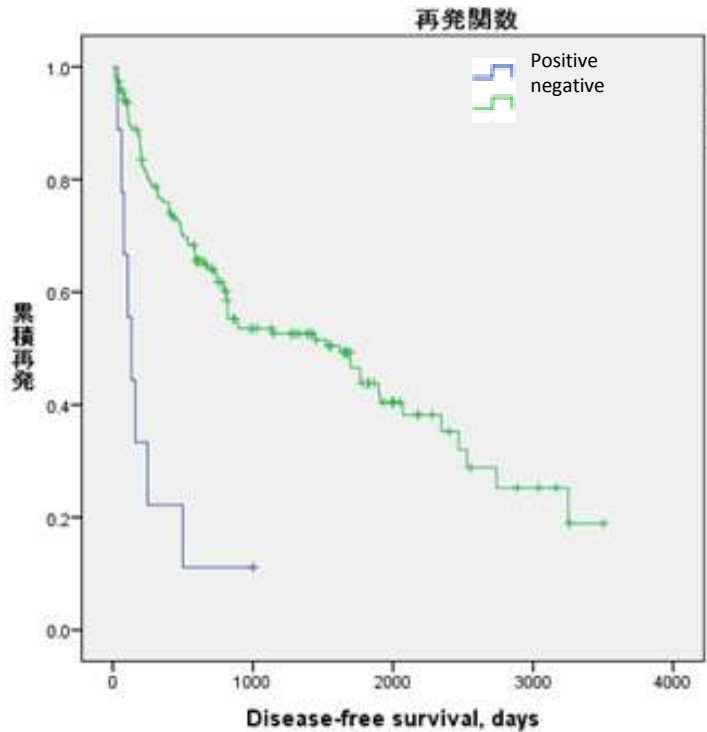
E

F

G

H

I

J

5M, Rec (+)   Tsuchiya K, Komuta M et al

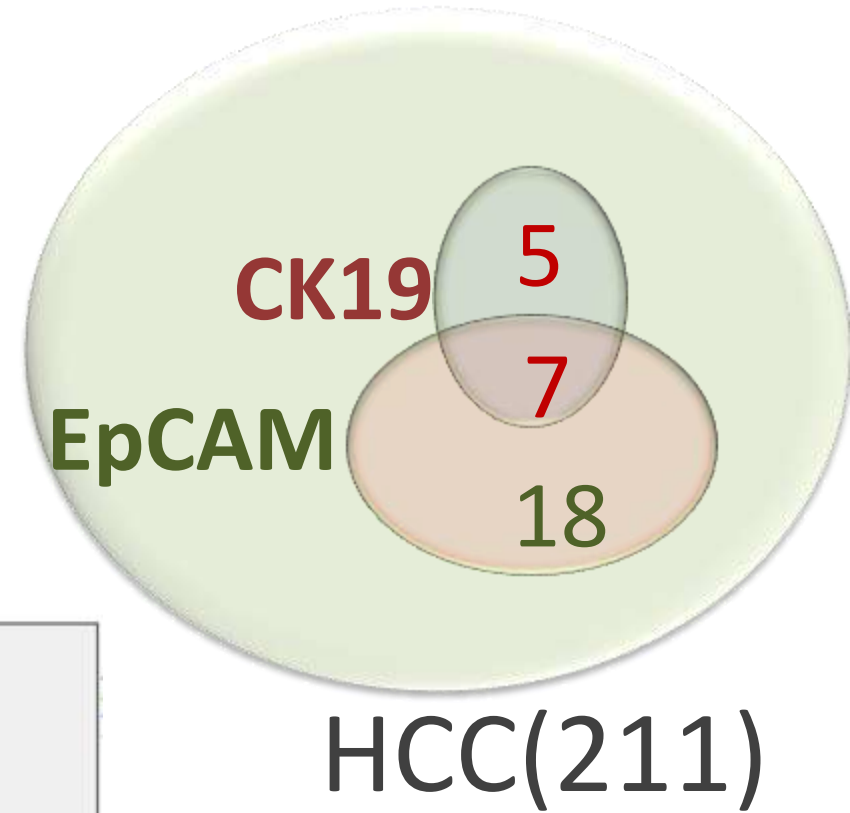# Recurrence free rates, in patients treated by RFA, according to the keratin (K) 19 expression in the tumor

Tsuchiya K, Komuta M et al

Expression of CK19 and EpCAM in surgically resected HCC

CK19

EpCAM

HCC(211)

Global overview of molecular classification of HCC

Meta-analysis subclasses

S1
- TGF-β, WNT ↑
- E2F1↑ , TP53 ↓
- Moderately/Poorly-differentiated
- Larger tumor

S2
- MYC, AKT ↑
- IFN ↓
- AFP, IGF2 ↑

S3
- Retained hepatocyte-like phenotype
- Well-differentiated
- Smaller tumor

Subclasses In literatures
- Poor survival, Met-regulated
- EPCAM (+)
- G3
- G1,2
- Proliferation, KRT19 (+)
- Vascular invasion
- Good survival
- G5,6
- CTNNB1 | Polysomy 7

IHC marker histopathiology
- **TGFßR2-SMADs**
- **Ki-67** **CK19**
- *LGR5*

Molecular diagnosis and IHC-based subclassification of HCC

Acknowledgement