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Gli-2 expression was associated with poor prognosis of hepatocellular carcinoma

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곽 재 문

Abstract

Gli-2 expression was associated with poor prognosis of hepatocellular carcinoma

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Background and Aims: The Sonic hedgehog (SHH) pathway acts as a regulator of development, homeostasis, and reparative processes in endodermal tissues, including the gastrointestinal tracts. Abnormal expression or mutation of SHH components has been reported in esophageal, gastric, duodenal, and pancreatic cancers. SHH-pathway-targeted therapies have been developed and applied in the management of upper-GI tract cancer. We aimed to study the expression status of several molecules in the SHH signal pathway in hepatocellular carcinoma (HCC) and the clinicopathologic features of HCCs according to their expression status.

Methods: We assessed the expression status of the SHH ligand, Patched (PTCH), and oncogenes (Gli-1, Gli-2 and Gli-3) by immunohistochemistry in 407 HCCs blocked in 13 tissue microarrays and evaluated the clinicopathologic features, including CK19-positive biliary phenotype.

Results: The positive rates for SHH, PTCH, Gli-1, Gli-2 and Gli-3 were 49.1%, 10.1%, 1.5%, 17.0%, and 1.5%, respectively. Comparative analysis with clinicopathologic parameters revealed that the positive expression rate for SHH was higher in males than in females and was higher in patients whose preoperative alpha-fetoprotein level was higher than normal (all $p < 0.05$). Gli-2 expression was prominent in females and in patients whose tumor had a high Edmondson-Steiner nuclear grade , dedifferentiated histology (e.g., solid or spindle cell morphology), high pT stage, and CK19 positivity (all $p < 0.05$). The expression levels of PTCH, Gli-1 and Gli-3 were not associated with a significant difference in tumor features. Only positive Gli-2 expression was associated with a shorter disease-free survival (DFS) and overall survival (OS) time (10 vs 34 months, 53 vs 181 months, all $p < 0.05$). Gli-2 expression was an independent prognostic factor according to multivariate Cox analysis, including nuclear grade, vascular invasion,

multiplicity, size and extent of invasion (p = 0.015).

Conclusion: The results suggest that Gli-2 could be an independent prognostic factor and a therapeuric target for HCC.

Keywords: carcinoma, hepatocellular, hedgehogs, gli2, prognosis

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Introduction

Hepatocellular carcinoma (HCC) is the fifth- most common cancer in the world and the third-most common cause of mortality. In addition, there is a high percentage of patients with poor prognoses suffering from the intermediate or advanced stages of HCC.[1]

There are several mechanisms associated with the tumorigenesis of HCC. The Sonic hedgehog (SHH) pathway has been shown to be an important pathway in embryogenesis and a diverse variety of cancers of human cancers.[2-5] The hedgehog pathway was first identified in *Drosophila* during a screen for genes concerning early embryonic development[6] and has three ligands (Sonic, Indian, and Desert hedgehog). The SHH pathway plays a significant role in the initiation and invasion of HCC.[7]

The SHH pathway can be described as follows. The Sonic hedgehog ligand binds to the membranous receptor, patched (PTCH), leading to the activation of the suppressed smoothened(SMO) molecule and consequent

triggering of the intracellular mechanisms that activate glioma-associated-oncogenes (Gli-1, Gli-2 and Gli-3).[8]

Gli has already been proven to be an important molecule in human HCC.[9] Furthermore, the selective down-regulation of Gli-2 inhibits the proliferation of hepatocellular carcinoma cells. Therefore, we can presume that the SHH pathway is important in HCC tumorigenesis. [9] However, there are only a few studies that have examined the correlations between SHH pathway molecule expression and HCC prognosis, including disease-free or overall survival.

Thus, there is a growing need to study the SHH pathway molecules in association with prognostic factors to develop more specific targeted therapies for human HCC.

Objectives

The goal of this study was to investigate the expression frequency and correlation with clinicopathologic features of the SHH pathway molecules in HCC tissues and to analyze their influence on the disease-free survival and overall survival.

Materials and Methods

1. Patients

The specimens used in this study were surgically resected at Seoul National University Hospital, from 1998 to 2005, and diagnosed as hepatocellular carcinoma. We collected cases by slide review after searching pathologic reports and medical records, and selected 407 tissues that were well-fixed and contained a sufficient amount of tumor cells.

2. Analysis of clinicopathologic features

All cases were reviewed by a pathologist. In addition, the patient medical records were examined to confirm clinicopathologic features such as age, sex, and type of preoperative treatment. We also re-examined pathologic features such as pathologic stage, histologic type, cell type, Edmondson-Steiner nuclear grade, gross type, and vascular invasion. The tumors were classified according to the WHO classification. To analyze overall survival and follow-up, we took advantage of the date of surgery for the beginning of the follow-up period. Follow-ups were continued until December 31, 2005.

3. Manufacturing tissue microarrays

We chose formalin-fixed paraffin-embedded blocks containing representative tumor sections of 407 cases of hepatocellular carcinoma, and manufactured slides with tissue microarrays (4 μm), including up to 60

tissue cores in one slide.

4. Immunohistochemistry process and evaluation

4.1. Antibodies

For this study, we used SHH-, PTCH-, Gli-1-, Gli-2- and Gli-3-specific antibodies to target SHH pathway members. Information about each antibody is listed in Table 1.

4.2. Immunostaining process

The specimen sample sections were prepared using a Bond Polymer Detection Kit and Leica Bond-Max Autostainer (Leica Microsystems GmbH, Wetzler, 35578, Germany). First, the samples were deparaffinised and hydrated. The samples were placed in pH 6.0 citrate buffer or pH 9.0 Tris-EDTA buffer at 100℃ for 15 minutes. For blocking endogenous peroxidase activity, the samples were incubated in 3% hydrogen peroxide for 6 minutes and incubated for 30 minutes for protein blocking. The

samples were incubated with the relevant primary antibodies for 1 hour at room temperature. The samples were then incubated with a secondary antibody biotinylated anti-goat IgG for 30 minutes. Next, the samples were incubated with ABC reagent for 30 minutes. Finally, the samples were incubated with DAB substrate kit reagents for 2 minutes and then counterstained.

4.3. Evaluation of immunohistochemical results

Positive expression rate was assessed using two methods. For SHH and PTCH, only diffuse cytoplasmic positivity was considered positive. The assessment of Gli transcription factors (Gli-1, Gli-2 and Gli-3) was considered positive when the percentage of positive cells was more than 10% of tumor cells.[10] The cut-off values for the immunohistochemical scores are listed in Table 2.

5. Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 19.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.) was used for statistical analysis. The assessment of the relationship between the positive SHH-pathway molecule expression and patient's clinicopathologic factors was performed using the chi-square test. Survival analysis for disease-free survival and overall survival was conducted and verified using the Kaplan-Meier product-limit method. Differences that were meaningful in the univariate analysis were also used in a Cox proportional hazards regression model. We defined a positive correlation as being present when the p-value was less than 0.05.

Results

1. Expression frequency of SHH pathway molecules in HCC

Out of 407 cases, SHH, PTCH, Gli-1, Gli-2 and Gli-3 were expressed in the following number of samples: 49.1% (200), 10.1% (41), 1.5% (6), 17.0% (69), 1.5% (6), respectively.

2. Relationship between the SHH pathway and clinicopathologic features

We investigated correlations of age, sex, operational method, tumor size, tumor multiplicity, gross type, vascular invasion, Edmondson-Steiner nuclear grade, histologic pattern, cell type, co-expression with biliary phenotype, invasion to adjacent tissue, pathologic T stage, etiology of underlying hepatitis, fibrosis extent of background liver, and preoperative treatment with the expression of SHH pathway molecules. The median values of preoperative alpha-fetoprotein level, disease-free survival and overall survival were determined both for high-expression and no-

expression cases. There were distinct clinical correlations of Gli-2 positivity with clinicopathologic factors such as Edmondson-Steiner nuclear grade, cell type, biliary phenotype, and pathologic T stage. Gli-2 expression was also well correlated with disease-free survival and overall survival (Table 3).

3. Analysis of survival curve according to Gli-2 expression

The average follow-up period of the total 407 patients was 181 months. We analyzed the correlation of the SHH pathway molecules and disease-free survival of patients with Kaplan-Meyer univariate analysis, and only Gli-2 presented a meaningful relationship (positive vs negative: 10 vs 34 months, p = 0.008). In contrast, the other examined pathway molecules had no relation with prognosis (Table 3). The correlation with overall survival of patients was also significant only in the case of positive Gli-2-expressing HCC patients (positive vs negative: 53 vs 181 months, p =

0.003).

Among the clinicopathologic features of HCC, Edmondson-Steiner nuclear grade, vascular invasion, tumor multiplicity, tumor size, and extent of invasion were well correlated with disease-free survival and overall survival. Therefore, we analyzed these factors with a multivariate analysis using a Cox proportional hazards regression model. As a consequence, Gli-2 was determined to be an independent prognostic factor, as were all the variables mentioned above (Table 5, all $p < 0.05$).

Discussion

The hedgehog signaling pathway plays an important role in various human tumor types, including gastric cancer, prostate cancer, lung cancer, esophageal cancer, and breast cancer.[11-15] Among the molecules of the SHH pathway, Gli-1, Gli-2 and Gli-3 participate as important transcription factors. Previous studies have demonstrated that the molecules of the SHH pathway are prognostic parameters of human gliomas, especially the Gli transcription factors.[10] Similarly, another study found that the overexpression of Gli-2 is responsible for the progression of HCC, and selective down-regulation of Gli-2 inhibits the proliferation of HCC tumor cells.[9] These results suggest Gli-2 may possibly be an HCC prognostic factor. Lin *et al*[16] examined the downstream target genes of Gli-2 and reported that FoxM1 (forkhead-box transcription factor M1) is up-regulated by Gli-2 and modulates cell cycle progression, which consequently increases tumor grade and stage.

In the present study, we examined the expression status of some of the molecules of the SHH signaling pathway. SHH protein, PTCH and Gli-2 were well expressed in our samples, but the Gli-1 and Gli-3 expression levels were relatively low. Accordingly, high SHH protein, PTCH and Gli-2 expression levels can be considered important factors in human HCC.

In particular, high Gli-2 expression was associated with dedifferentiated histologic features, represented by high Edmondson-Steiner grade, pathologic T stage and CK19 expression. These results can augment the possibility of Gli-2 serving as a prognostic factor because CK19 positivity has been associated with a poor HCC prognosis. Zhuang *et al* [17] found that CK19 positivity is an independent risk factor of lymph node metastasis for HCC and also an independent prognostic factor. Chung *et al*[18] demonstrated that CK19 is more expressed in hypovascular hepatocellular carcinomas than in hypervascular carcinomas. Accordingly CK19 is a useful prognostic marker of early recurrence and poor survival

of HCC patients.

We found that a poor prognosis was more common in patients having tumors co-expressing Gli-2 and CK19. On the other hand, these results have the potential of aiding with the development of specific and combined targeted HCC therapies. However, there are few studies on the actual relationship between CK19 positivity and Gli-2 activity nor their co-expression and prognostic influence. Thus, additional studies on this subject need to be performed.

Cox-2 multivariate regression analysis revealed that among the SHH pathway molecules, only Gli-2 was an independent prognostic factor. In addition, high Edmondson-Steiner nuclear grade, vascular invasion, tumour multiplicity, tumour size and extent of tumour invasion were related to poor prognosis. Our results indicated the same results in regards to disease-free survival and overall survival. Therefore, even after disease recurrence, Gli-2 can be a meaningful therapeutic target.

Vascular invasion and tumour multiplicity are components of a high pathologic T stage. Gli-2 expression and high T stage were well-correlated, however, each individual component, such as vascular invasion and tumour multiplicity, did not present a correlation with Gli-2 expression. Thus, further studies are needed on the pathological changes associated with vascular invasion accompanying tumour multiplicity in HCC.

There are various possible mechanisms through which Gli-2 activates and facilitates a poor prognosis. One of these mechanisms was revealed by Lin *et al*[16]. Gli-2 can also accelerate cell cycle progression via transition through the G(2)-M cycle, in the case of prostate cancer.[12] Another cellular mechanism is necessary explain the results found in this study.

The hedgehog pathway is associated not only with HCC, but also with various types of liver diseases, including primary biliary cirrhosis.[19]

Therefore, additional studies are required to fully elucidate the relationship of the hedgehog pathway with other liver carcinomas and severe liver diseases, such as cholangiocarcinoma and primary biliary cirrhosis. Naturally, the goal of these studies should be the elaboration and development of targeted molecular anti-HCC therapies.

Conclusion

Several studies are in progress with a goal of elucidating the molecules and pathways that play an important role in the carcinogenesis of HCC.

We studied the relationships of selected SHH pathway molecules: SHH protein, PTCH, Gli-1, Gli-2 and Gli-3. Gli-2 demonstrated relationship with the clinicopathologic factors of HCC patients and was an independent prognostic factor.

CK19, a known indicator of poor HCC prognosis, was co-expressed with Gli-2 in our specimens. Therefore, Gli-2 should be considered as an important target for molecular therapy, especially in the case of HCC patients with a poor prognosis.

Table 1. Antibodies used in this study

Molecule	Host	Retrieval method	Dilution	Localization	Cat#/Company
SHH	Rabbit monoclonal	pH6.0 Bond epitope retrieval solution 1	1:500	Cytoplasm	1843-1, Epitomics, Burlingame, U.S.A.
PTCH	Rabbit polyclonal	pH9.0 Bond epitope retrieval solution 2	1:30	Cytoplasm	sc-9016, Santa Cruz, Dallas, U.S.A.
Gli-1	Rabbit polyclonal	pH6.0 Bond epitope retrieval solution 1	1:300	Nuclear	PAI-22557, Thermoscientific, Yokohama, Japan
Gli-2	Rabbit polyclonal	pH6.0 Bond epitope retrieval solution 1	1:200	Nuclear	18-732-292462, Genway, San Diego, U.S.A.
Gli-3	Rabbit polyclonal	pH6.0 Bond epitope retrieval solution 1	1:300	nuclear	LS-B73/15300, Lifespan, Seattle, U.S.A.

Table 2. Immunohistochemical score cut-off values

Antibody	Cut-off value
Gli-1	≥10%
Gli-2	≥10%
Gli-3	≥10%

SHH, PTCH : cytoplasmic staining / Gli : nuclear staining

Table 3. Association of SHH molecule expression with clinicopathologic characteristics of hepatocellular carcinoma

Clinicopathologic Factors	SHH (+)	*p value*	PTCH (+)	*p value*	Gli-1 (+)	*p value*	Gli-2 (+)	*p value*	Gli-3 (+)	*p value*
Age (year, mean) (+) vs (-)	54.7 vs 54.3	*0.500*	57.3 vs 54.2	*0.149*	55.7 vs 54.5	*0.779*	55.7 vs 54.3	*0.416*	56.7 vs 54.5	*0.593*
Gender										
male (n=344)	183 (53.2)	*<0.001*	33 (9.6)	*0.452*	5 (1.5)	*0.935*	53 (15.4)	*0.052*	6 (100.0)	*0.596*
female (n=63)	17 (27.0)		8 (12.7)		1 (1.6)		16 (25.4)		0 (0.0)	
Operation method										
partial hepatectomy (n=379)	187 (49.3)	*0.245*	36 (9.5)	*0.133*	6 (1.6)	*1.000*	64 (16.9)	*0.793*	6 (1.6)	*1.000*
total hepatectomy (n=27)	13 (48.1)		5 (18.5)		0 (0.0)		5 (18.5)		0 (0.0)	
Size (cm, mean) (+) vs. (-)	5.4 vs 5.4	*0.730*	6.1 vs 5.3	*0.397*	4.9 vs. 5.4	*0.617*	5.7 v 5.4	*0.092*	4.3 vs 5.4	*0.435*
Multiplicity										
single lesion (n=307)	153 (49.8)	*0.622*	39 (9.8)	*0.723*	5 (1.6)	*1.000*	50 (16.3)	*0.530*	5 (1.6)	*1.000*
muliple lesions (n=100)	47 (47.0)		11 (11.0)		1 (1.0)		19 (19.0)		1 (19.0)	
Gross type										
vaguely nodular (n=5)	3 (60.0)	*0.946*	1 (20.0)		0 (0.0)	*0.844*	1 (20.0)	*0.637*	0 (0.0)	*0.418*
expanding nodular (n=150)	73 (48.7)		16 (10.7)		1 (0.7)		31 (20.7)		5 (3.3)	
Multi nodular (n=100)	46 (46.0)		8 (8.0)		3 (3.0)		12 (12.0)		1 (1.0)	
perinodular extension (n=127)	63 (49.6)		15 (11.8)		2 (1.6)		22 (17.3)		0 (0.0)	

others (n=24)	14 (58.3)		0 (0.0)		0 (0.0)		3 (12.5)		0 (0.0)	
Vascular invasion										
absent (n=213)	101 (47.4)	*0.466*	19 (8.9)	*0.418*	4 (1.9)	*0.479*	31 (14.6)	*0.176*	4 (1.9)	*0.687*
present (n=194)	99 (51.0)		22 (11.3)		2 (1.0)		38 (19.6)		2 (1.0)	
ES grade										
I (n=36)	17 (47.2)	*0.230*	4 (11.1)	*0.981*	0 (0.0)	*0.506*	5 (13.9)	*0.004*	0 (0.0)	*0.418*
II (n=216)	100 (46.3)		23 (10.6)		3 (1.4)		20 (13.9)		2 (0.9)	
III (n=144)	77 (53.5)		11 (7.6)		3 (2.1)		28 (19.4)		4 (2.8)	
IV (n=11)	6 (54.5)		3 (27.3)		0 (0.0)		6 (54.5)		0 (0.0)	
Histologic pattern										
Trabecular/ acinar (n=387)	198 (51.2)	*0.131*	41 (10.6)	*0.462*	6 (1.6)	*1.000*	63 (16.3)	*0.111*	5 (1.3)	*0.262*
others (n=20)	10 (50.0)		0 (0.0)		0 (0.0)		6 (30.0)		1 (5.0)	
Cellular type										
hepatic (n=384)	188 (49.0)	*0.282*	41 (10.7)	*0.604*	6 (1.6)	*1.000*	63 (16.4)	*0.036*	5 (1.3)	*0.296*
non-hepatic (n=23)	12 (52.2)		0 (0.0)		0 (0.0)		6 (26.1)		1 (4.3)	
Biliary phenotype (CK19)										
absent (n=357)	173 (48.5)	*0.383*	38 (10.6)	*0.325*	5 (1.4)	*0.540*	55 (15.4)	*0.021*	4 (1.1)	*0.156*
present (n=49)	27 (55.1)		3 (6.1)		1 (2.0)		14 (28.6)		2 (4.1)	
Extent of invasion										
confined parenchyma (n=319)	160 (50.2)	*0.894*	33 (10.3)	*0.647*	5 (83.3)	*0.978*	52 (16.3)	*0.267*	5 (1.6)	*0.978*
invasion to Glisson capsule/ perihepatic tissue (n=74)	34 (45.9)		7 (9.5)		1 (1.4)		13 (17.6)		1 (1.4)	
Invasion to Other organ	6 (42.9)		1 (7.1)		0 (0.0)		4 (28.6)		0 (0.0)	

(n=14)										
pT stage by AJCC 7th ed.										
pT1 (n=171)	83 (48.5)	*0.929*	15 (8.8)	*0.578*	4 (2.3)	*0.564*	25 (14.6)	*0.019*	4 (2.3)	*0.362*
pT2 (n=161)	80 (49.7)		19 (11.8)		2 (1.2)		23 (14.3)		1 (0.6)	
pT3 (n=50)	24 (48.0)		6 (14.6)		0 (0.0)		14 (28.0)		1 (2.0)	
pT4 (n=24)	12 (50.0)		1 (4.2)		0 (0.0)		7 (29.2)		0 (0.0)	
Underlying aetiology of hepatitis										
HBV (n=290)	144 (49.7)	*0.075*	28 (9.7)	*0.715*	4 (1.4)	*0.979*	54 (18.6)	*0.732*	3 (1.0)	*0.963*
HCV (n=24)	12 (50.0)		3 (12.5)		0 (0.0)		3 (12.5)		0 (.0)	
HBV and HCV (n=5)	2 (40.0)		0 (0.0)		0 (0.0)		1 (20.0)		0 (0.0)	
alcohol (n=2)	2 (100.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
nonB and nonC (n=43)	14 (32.6)		5 (11.6)		1 (2.3)		5 (11.6)		1 (2.3)	
Fibrosis of background liver										
no or portal fibrosis (n=55)	26 (47.3)	*0.704*	7 (12.7)	*0.062*	0 (0.0)	*0.577*	9 (16.4)	*0.808*	0 (0.0)	*0.672*
periportal fibrosis (n=49)	21 (42.9)		10 (20.4)		2 (4.1)		7 (14.3)		0 (0.0)	
septal fibrosis (n=79)	42 (53.2)		7 (8.9)		1 (1.3)		16 (20.3)		2 (2.5)	
cirrhosis (n=194)	93 (47.9)		14 (7.2)		3 (1.5)		34 (17.5)		3 (1.5)	
Preoperative treatment										
done (n=163)	83 (50.9)	*0.584*	15 (9.2)	*0.719*	1 (0.6)	*0.409*	31 (19.0)	*0.316*	3 (1.8)	*0.620*
not-done (n=243)	117 (48.1)		25 (10.3)		5 (2.1)		37 (15.2)		3 (1.2)	
Preoperative α-fetoprotein (μl/ml, mean) (+) vs (-)	2541.7 vs 11515.8	*0.018*	8388.7 vs 6956.0	*0.428*	12565.3 vs 7012.7	*0.280*	9460.9 vs 6634.9	*0.115*	530.2 vs 7196.9	*0.959*

Disease free survival (month, median) (+) vs (-)	29.0 vs 33.0	*0.608*	16.0 vs 32.0	*0.788*	58.0 vs 30.0	*0.836*	10.0 vs 34.0	*0.008*	112.0 vs 30.0	*0.938*
Overall survival (month, median) (+) vs (-)	NA vs 181.0	*0.747*	NA vs 181.0	*0.311*	NA vs 181.6	*0.844*	53.0 vs 181.0	*0.003*	23.0 vs 181.0	*0.203*

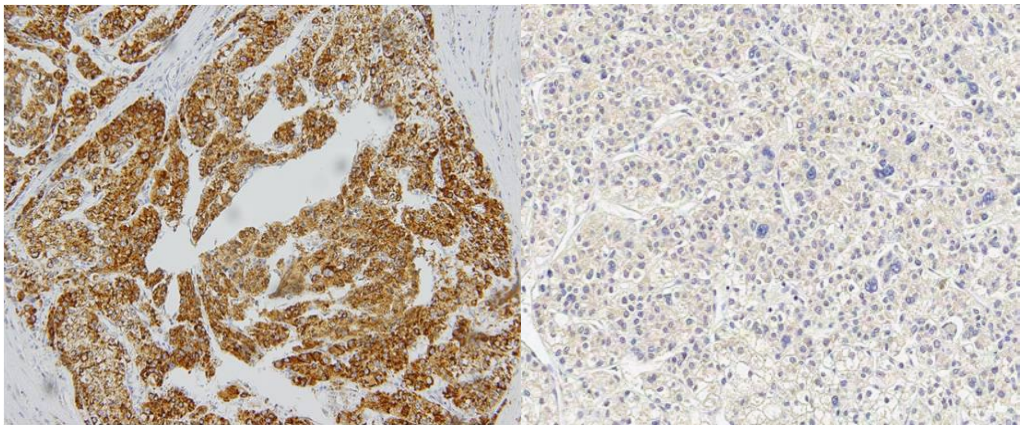
Table 4. Kaplan-Meier survival analysis of clinicopathologic factors of HCC patients

Clinicopathologic factor	p value
Gender	0.479
Multiplicity	0.001
Gross type	0.073
Vascular invasion	<0.001
ES nuclear grade	<0.001
Histologic pattern	0.149
Cellular type	0.520
Extent of invasion	<0.001
Underlying hepatitis	0.319
Fibrosis of background liver	0.363

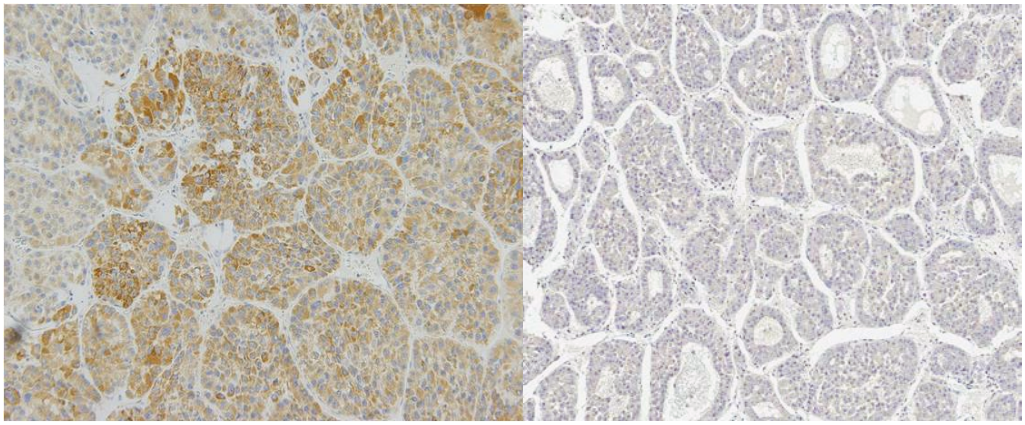
Table 5. Gli-2 expression as an independent prognostic factor by according to multivariate Cox analysis

Factor	p value	Exp(B)	95% CI for Exp(B)	
ES nuclear grade	0.038	1.24	[1.01	1.52]
Vascular invasion	0.029	1.36	[1.03	1.79]
Multiplicity	0.022	1.39	[1.04	1.86]
Size	0.003	1.05	[1.01	1.09]
Extent of invasion	<0.001	1.36	[1.17	1.58]
Gli-2 positivity	0.015	1.49	[1.07	2.06]

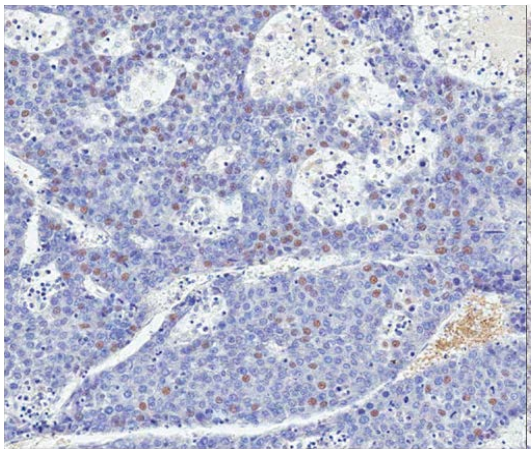
Figure 1



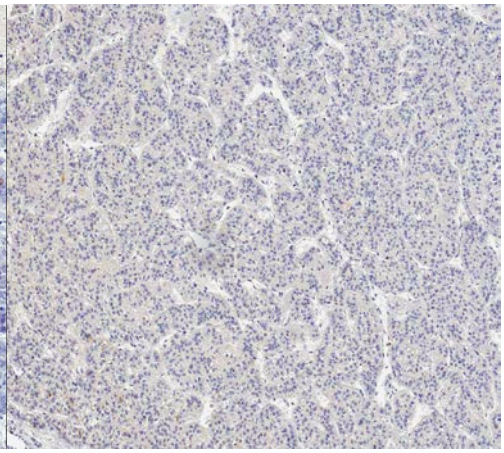
SHH(+)　　SHH(-)



PTCH(+)　　PTCH(-)



Gli-1(+)



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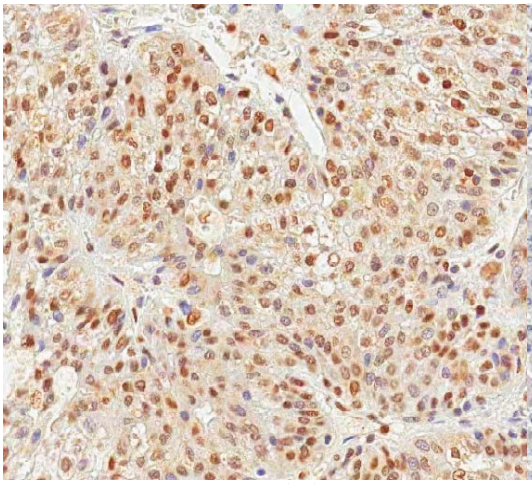

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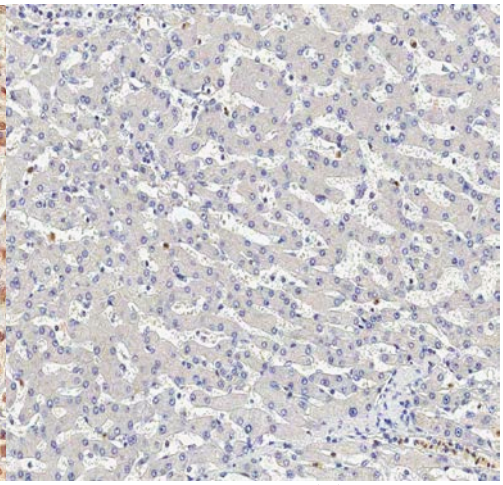

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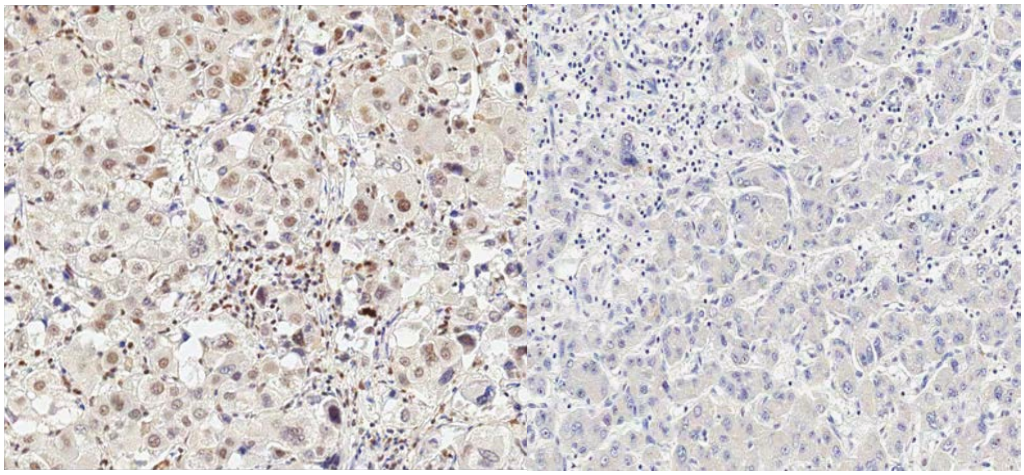
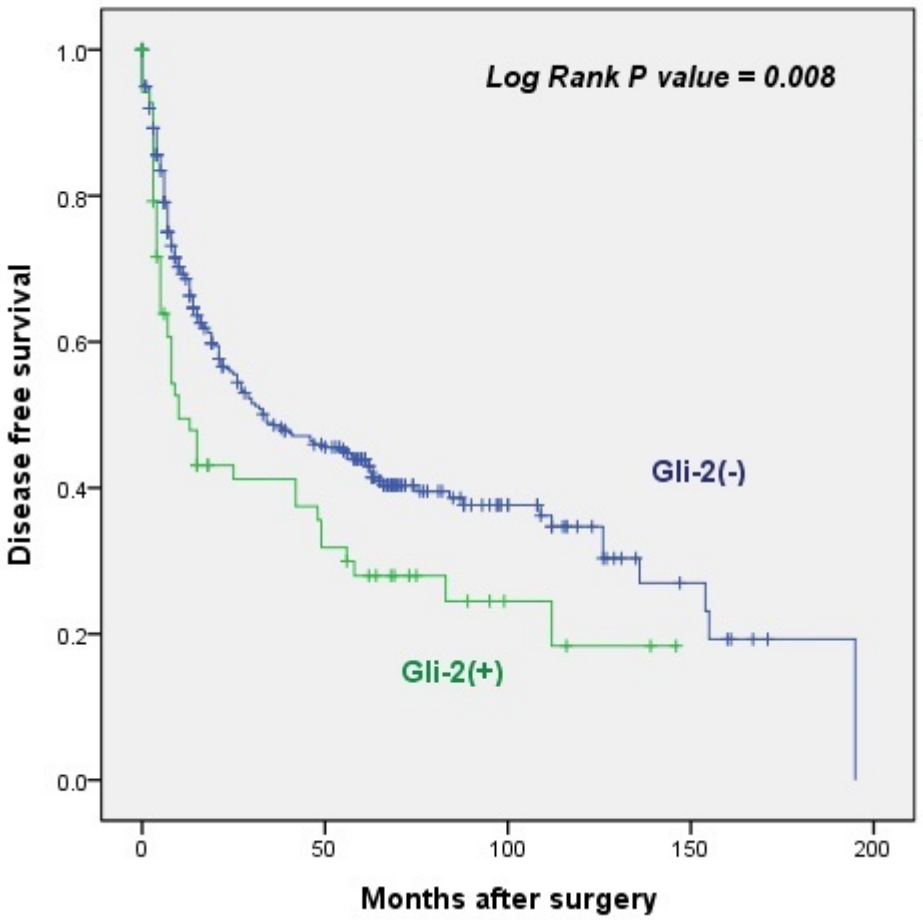


Figure 1. Expression of SHH pathway molecules in HCC. SHH and PTCH were considered positive when more than 50% of tumour cells were stained. Gli-1, Gli-2 and Gli-3 were considered positive when more than 10% of tumour cells were stained. SHH, sonic hedgehog; HCC, hepatocellular carcinoma; PTCH, membrane receptor patched.

Figure 2


Log Rank P value = 0.008
Gli-2(-)
Gli-2(+)
Disease free survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
Months after surgery

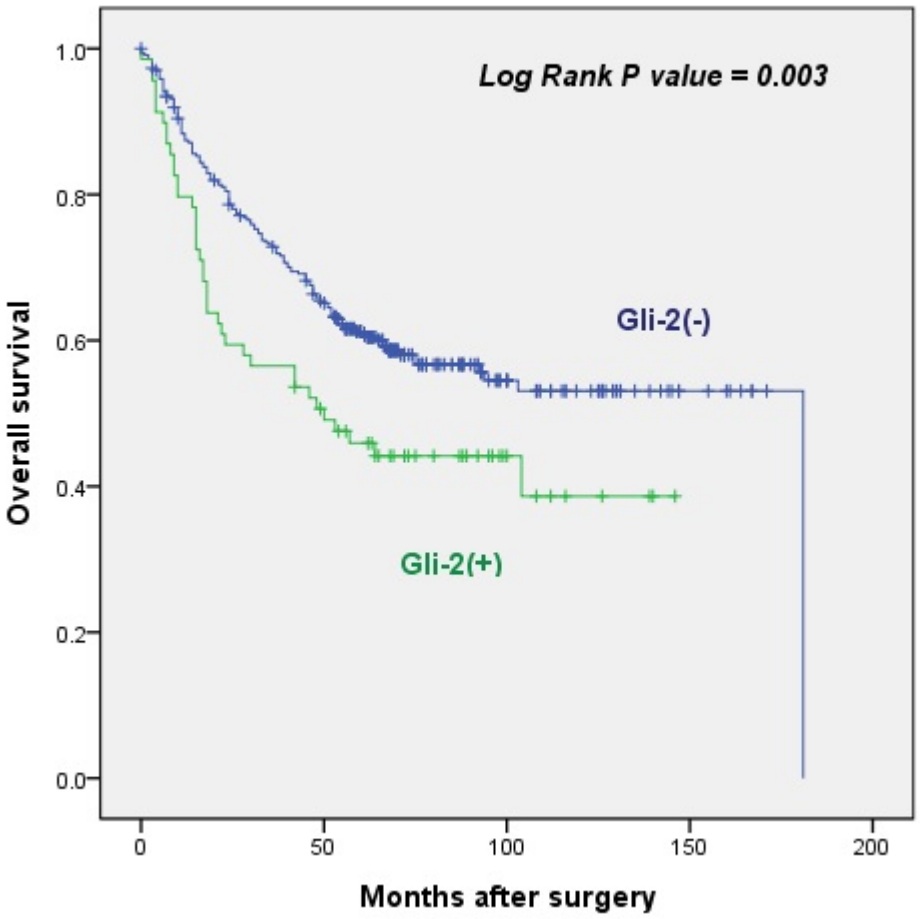


Figure 2. Disease free survival & overall survival according to Gli-2 expression. HCC patients with Gli-2 expression presented significantly shorter disease-free survival and overall survival

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국문초록

Sonic hedgehog(SHH) 신호전달계는 위장관 계통을 포함한 여러 내배엽 기원의 조직에서 발육, 항상성 유지, 치유활동 등에 조절인자로서 관여한다. SHH 신호전달 관련 물질들의 비정상적인 발현 및 돌연변이가 식도, 위장, 십이지장, 췌장암 등에서 보고되었다. 이러한 SHH 신호전달 관련 물질을 표적으로 하는 치료법이 개발되어 현재 상부 위장관암의 치료에 응용되고 있다. 본 연구에서는 간세포암에서 SHH 신호전달계에 관여하는 인자들의 발현 양상과 임상-병리학적 특성과의 상관 관계를 알아보고자 하였다.

외과적으로 절제되어 얻은 407개의 간 세포암 조직을 이용하여 13개 블럭의 미세조직배열 슬라이드를 제작하고 SHH 신호전달계에 관여하는 요소인 SHH 리간드 (SHH), Patched (PTCH), Gli-1, Gli-2, Gli-3에 대한 면역조직화학염색을 시행하였다.

간세포암 조직에서 SHH, PTCH, Gli-1, Gli-2, Gli-3의 발현율은 각각 49.1%, 10.1%, 1.5%, 17.0% 및 1.5%였으며 임상-병리학적 양상을 분석한 결과 SHH의 발현은 남성 환자, 담관암이 동반된 경우, 섬유주 양상의 조직학적 특성을 가진 조직에서 높게 나타났다. Gli-2 발현은 여성 환자, 높은 Edmonson-Steiner 핵 등급, 고형성 또는 방추형 세포 형태와 같은 미분화된 조직학적 특성, 높은 T 스테이지, CK19 양성인 경우와 관련성이 높았다. PTCH, Gli-1, Gli-3도 비슷한 양상을 보였다.

SHH 신호전달계에 관련된 여러 인자 중 Gli-2의 양성 발현만이 무병 생존률 및 전체 생존률과 연관이 있었으며, 콕스 다변량 분석상 독립적인

예후인자로 작용하였다.

따라서 Gli-2는 간세포암의 예후를 예측할 수 있는 독립적 인자이며, 표적 치료의 대상이 될 가능성이 있음을 시사하였다.

주요어: 간세포암, 헤지혹, Gli2, 예후

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