Histol Histopathol (2010) 25: 1269-1275

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Nuclear accumulation of glioma-associated oncogene 2 protein and enhanced expression of forkhead-box transcription factor M1 protein in human hepatocellular carcinoma

M. Lin[1]*, L.M. Guo[1]*, H. Liu[2], J. Du[1], J. Yang[1], L.J. Zhang[2] and B. Zhang[1]

[1]Department of Pathology, Peking University Health Science Center, Beijing, China and [2]Department of Pathology, Beijing Youan Hospital, Capital Medical University, Beijing, China

*Both authors contribute equally to the manuscript.

Summary. The hedgehog (Hh) signaling pathway has been reported to be crucial in human carcinogenesis and tumor progression. Glioma-associated oncogenes (Gli), are zinc finger transcription factors which mediate the transcriptional response to Hh signaling. To explore the role of Gli in the development and progression of hepatocellular carcinoma (HCC), we investigated the expression of Gli2 and FoxM1 (forkhead-box transcription factor M1) which is one of the Gli downstream target genes modulating cell cycle progression in 91 specimens of human HCCs with immunohistochemistry. These immunostaining results were compared with various clinicopathologic parameters. Immunoreactivity of Gli2 and FoxM1 was observed respectively in 84.6% (77/91) and 80.2% (73/91) cases of HCC tumor tissues, and this was considerably higher than expression in the peritumoral tissues. Distribution of Gli2 and FoxM1 proteins in tumor cells was nuclear with or without cytoplasmic staining, or cytoplasmic alone. Statistically, increased nuclear immunopositivity of Gli2 protein correlated significantly with poorer tumor differentiation ($P<0.05$), as well as with portal vein tumor thrombosis ($P<0.05$). In addition, overexpression of FoxM1 protein was significantly associated with increased tumor grade ($P<0.01$) and advanced tumor stage ($P<0.05$). Moreover, there was a significant association between the expressions of Gli2 and FoxM1 proteins in HCC ($r=0.464$, $P=0.000$). This is consistent with the concept that in human HCC, the Hh signaling pathway is involved in the differentiation and proliferation of tumor cells, in part through inducing nuclear accumulation of Gli2 protein and subsequent upregulation of FoxM1 protein.

Key words: Hedgehog pathway, Glioma-associated oncogene 2, Forkhead-box transcription factor M1, Hepatocellular carcinoma, Immunohistochemistry

Offprint requests to: Dr. Limei Guo, Department of Pathology, Peking University, Health Science Center, Xueyuan Road-38, Haidian District, Beijing 100091, China. e-mail: guolimei@bjmu.edu.cn

Introduction

The hedgehog (Hh) signaling pathway regulates body patterning and organ development during mammalian embryogenesis (Jiang and Hui, 2008; Varjosalo and Taipale, 2008). In human adults, activation of the Hh pathway has been linked to cancers of the skin, brain, lung, breast, prostate, ovary, pancreas, gastrointestinal tract and hematopoietic system (Dahmane et al., 1997; Berman et al., 2003; Pasca di Magliano and Hebrok, 2003; Thayer et al., 2003; Watkins et al., 2003; Karhadkar et al., 2004; Katoh and Katoh, 2005; Clement et al., 2007; Kim et al., 2009; Liao et al., 2009). Generally, Hh signaling occurs through the interaction of the Hh proteins, namely, Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert hedgehog (Dhh) protein, with their receptor, patched (Ptch). This interaction releases its catalytic inhibition of the G-protein-coupled receptor-like signal transducer smoothened (Smo). Smo de-repression triggers an activation cascade and nuclear translocation of the zinc finger transcription factors glioma-associated oncogenes (Gli), leading to the transcription of Hh downstream target genes resulting in modulation of cell growth and differentiation (Jiang and Hui, 2008; Varjosalo and Taipale, 2008; Scales and de Sauvage, 2009). In

mammals there are three Gli homologs. It is generally recognized that Gli1 possesses only an activator domain, and may also possess a repressor domain. Aberrant expression and amplification of Gli1 is associated with many malignancies. While Gli2 contains both activator and repressor domains, and appears to be the major nuclear effector of Hh signaling in vivo. Gli3 mostly functions as a transcription repressor (Kasper et al., 2006; Lipinski et al., 2006; Scales and de Sauvage, 2009). Down-regulation of Gli2 affects the levels of Gli1 and Ptch1, as well as other target genes, including cyclin B, N-myc, snail and bcl-2, which are involved in cell cycle progression, epithelial-mesenchymal transition, signal transduction and apoptosis (Ikram et al., 2004; Kasper et al., 2006; Kim et al., 2007; Ohta et al., 2009; Scales and de Sauvage, 2009). Recently, the forkhead-box transcription factor M1 (FoxM1) was reported to be a downstream transcriptional target gene of Hh signaling in human colorectal carcinoma and basal cell carcinoma (Teh et al., 2002; Douard et al., 2006). As a member of the Fox protein family, FoxM1 transcription factor plays a crucial role in regulating expression of the cell cycle genes, which are involved in cell proliferation, differentiation and transformation by promoting both G1-S and G2-M transition (Lehmann et al., 2003; Katoh and Katoh, 2004).

Hepatocellular carcinoma (HCC) is a worldwide malignancy and one of the most rapidly increasing cancers in China. Recently, Hh signaling pathway has been demonstrated to function as a critical regulator in human HCC. Increased mRNA expression of Hh pathway components, including Shh, Ptch1, Smo and Gli1 were found in HCC tumor tissues and cell lines (Huang et al., 2006; Sicklick et al., 2006). The levels of Gli2 mRNA and proteins were considerably higher in HCC cell lines than those of Gli1 and Gli3 (Kim et al., 2007). Moreover, down-regulation of the Hh pathway with cyclopamine or specific antisense oligonucleotides of Gli2 led to a significant decrease in cell growth rate, through the regulation of genes involved in cell cycle and apoptosis in HCC cell lines (Patil et al., 2006; Kim et al., 2007). These previous results suggested that Hh signaling activation, in part by increased levels and functions of Gli transcription factor and its target genes, play a predominant role in the proliferation and survival of HCC cells. However, so far little is known about the expression of Gli and its downstream target genes in HCC tissues in vivo. In the present study, we investigated the expression of Gli2 and FoxM1 proteins in human HCCs by immunohistochemical staining and compared the immunoreactivity with various clinicopathologic characteristics.

Materials and methods

Ninety-one surgical specimens of primary HCCs, resected at the Third Hospital of Peking University Health Science Center, China, between 2006 and 2009 were used in this study. Of these 91 patients, 73 were men and 18 were women. Their ages ranged from 38 to 84 with a mean of 55 years. The gross and microscopic findings were reviewed for each tumor by two pathologists in a blinded fashion. The histological type of each tumor was classified as trabecular, pseudo-glandular, solid and sarcomatous according to the World Health Organization Classification of Tumours (2000). Histological grade was assigned as well, moderately or poorly differentiated. Among these 91 specimens, 74 HCCs consisted of tumor tissues of a single grade, and 17 HCCs showed two different grades in one single tumor nodule. Tumor stage was identified as stage I ($T_1N_0M_0$), II ($T_2N_0M_0$), III ($T_3N_0M_0$; $T_{1-3}N_1M_0$) and IV (T_4 any N M_0; any T any N M_1). Patients who received preoperative therapies were excluded from this study.

Immunohistochemical staining and assessment

Formalin-fixed and paraffin-embedded 4μm tissue sections were used for immunohistochemical staining. Briefly, sections were dehydrated with graded concentrations of ethanol and immersed in 3% hydrogen peroxide for 15 minutes to inhibit endogenous peroxidase activity. Antigen retrieval was performed by heating for 2 minutes in a pressure cooker, using 0.01M citrate buffer (pH 6.0). Sections were then incubated with primary antibodies including rabbit anti-human polyclonal Gli2 antibody (1:150, Ab-26056, Abcam, UK) and rabbit anti-human polyclonal FoxM1 antibody (1:100, sc-502, Santa Cruz Biotechnology, USA) at 4°C overnight. An Envision Chem Detection Kit (DaKoCytomation, CA) was used for the secondary antibody at room temperature for 30 minutes. 3,3-diaminobenzidine-hydrogen peroxide was used as chromogen. Sections were then lightly counterstained with hematoxylin. Substitution of primary antibody with phosphate buffered saline was used as a negative control.

For evaluation of immunostaining, each tissue section was observed at low power and three representative areas with the most intense immuno-reactivity were chosen. The tumor cell staining frequency with Gli2 and FoxM1 was scored as follows: 0, none; 1, <25%; 2, 25-50%; 3, >50% positive. The staining intensity was evaluated as mild, moderate or strong. The distribution of positive staining of Gli2 and FoxM1 was classified as nuclear expression with or without cytoplasmic staining, or cytoplasmic expression alone.

Statistical analysis

Immunoreactivity of Gli2 and FoxM1 proteins was compared with various clinicopathologic characteristics in all HCC cases. The Chi-square and Fisher's exact tests were used to assess the differences in immunohisto-chemical staining levels between or among different groups. Spearman's rank correlation was applied to determine the correlation between the immunoreactions of Gli2 and FoxM1 proteins. All analyses were

performed by using SPSS 13.0 (SPSS Inc., Chicago, USA) and differences were considered significant when $P<0.05$.

Results

Immunoreactivity of Gli2 and FoxM1 in human HCCs

Gli2 and FoxM1 proteins showed positive staining in 84.6% (77/91) and 80.2% (73/91) cases of HCC. In the peritumoral liver tissues, staining of Gli2 protein was uniformly negative. FoxM1 expression was found in the peritumoral hepatocytes in only 6.6% (6/91) cases. Gli2 and FoxM1 immunopositivity was present in the tumor parenchymal cells and not in the stromal cells. Subcellular localization of Gli2 and FoxM1 proteins in the tumor cells was nuclear with or without cytoplasmic staining, or cytoplasmic only. Neither staining frequency nor intensity of Gli2 protein showed variation with tumor grade, whereas the localization of positive signals in the nucleus appeared to increase.

In the well differentiated HCCs, Gli2 protein was remarkably observed in the cytoplasm alone. As shown in one tumor with well differentiation, expression of Gli2 protein was predominantly cytoplasmic, with an even dot-like distribution (Fig. 1A). In the moderately differentiated HCCs, expression of Gli2 was about equally distributed in the cytoplasm and nucleus. As

Table 1. Correlation of expression of Gli2 and FoxM1 proteins in HCC with clinicopathologic parameters.

Factors		Gli2 expression								FoxM1 expression							
			Positive (n=77)								Positive (n=73)						
	Total (n=91)	Negative (n=14)	1 (n=17)	2 (n=29)	3 (n=31)	P†	Nucleus (n=39)	Cytoplasm (n=38)	P†	Negative (n=18)	1 (n=36)	2 (n=26)	3 (n=11)	P†	Nucleus (n=15)	Cytoplasm (n=58)	P†
Age						0,878			0,307					0,808			0,165
≤55yo	48	7	8	17	16		23	18		11	17	14	6		10	27	
>55yo	43	7	9	12	15		16	20		7	19	12	5		5	31	
Sex						0,376			0,591					0,223			0,882
Male	73	10	16	22	25		31	32		16	31	19	7		11	46	
Female	18	4	1	7	6		8	6		2	5	7	4		4	12	
Occurrence						0,934			0,389					0,171			0,149
Simple	58	9	10	18	21		23	26		8	25	16	9		11	39	
Complex	33	5	7	11	10		16	12		10	11	10	2		4	19	
Tumor size						0,960			0,273					0,721			0,480
<1.5cm	7	1	1	3	2		3	3		1	3	3	0		1	5	
1.5-3.0cm	24	4	6	6	8		7	13		5	11	4	4		2	17	
>3.0cm	60	9	10	20	21		29	22		12	22	19	7		12	36	
Histological type						0,975			0,764					0,161			0,838
Trabecular	35	6	8	10	11		14	15		9	17	7	2		5	21	
Pseudoglanular	28	3	5	11	9		13	12		4	13	9	2		4	20	
Solid	15	2	2	5	6		8	5		2	4	6	3		3	10	
Sarcomatous	13	3	2	3	5		4	6		3	2	4	4		3	7	
Differentiation						0,010			0,000					0,006			0,193
Well	19	6	8	4	1		1	12		8	6	5	0		0	11	
Well + Mod	7	0	1	4	2		1	6		3	3	0	1		0	4	
Mod	41	7	7	10	17		17	17		5	22	11	3		10	26	
Mod + Poor	10	0	1	5	4		7	3		1	3	4	2		3	6	
Poor	14	1	0	6	7		13	0		1	2	6	5		2	11	
PVTT						0,017			0,004					0,388			0,123
Positive	37	2	4	13	18		24	11		6	12	13	6		9	22	
Negative	54	12	13	16	13		15	27		12	24	13	5		6	36	
fc-inf ‡						0,180			0,953					0,805			0,953
Positive	17	1	1	8	7		8	8		2	8	5	2		3	12	
Negative	74	13	16	21	24		31	30		16	28	21	9		12	46	
Peritumoral						0,365			0,267					0,548			0,691
Cirrhosis	58	8	11	22	17		23	27		11	24	18	5		9	38	
No-cirrhosis	33	6	6	7	14		16	11		7	12	8	6		6	20	
Stage						0,417			0,052					0,044			0,027
T1-2	50	10	10	16	14		16	24		12	24	11	3		4	34	
T3-4	41	4	7	13	17		23	14		6	12	15	8		11	24	

† Chi-Square and Fisher's Exact test. P value <0.05 was considered statistically significant. Mod: moderate; PVTT: portal vein tumor thrombosis; ‡ fc-inf: microscopic infiltration of cancer cells into tumor capsule.

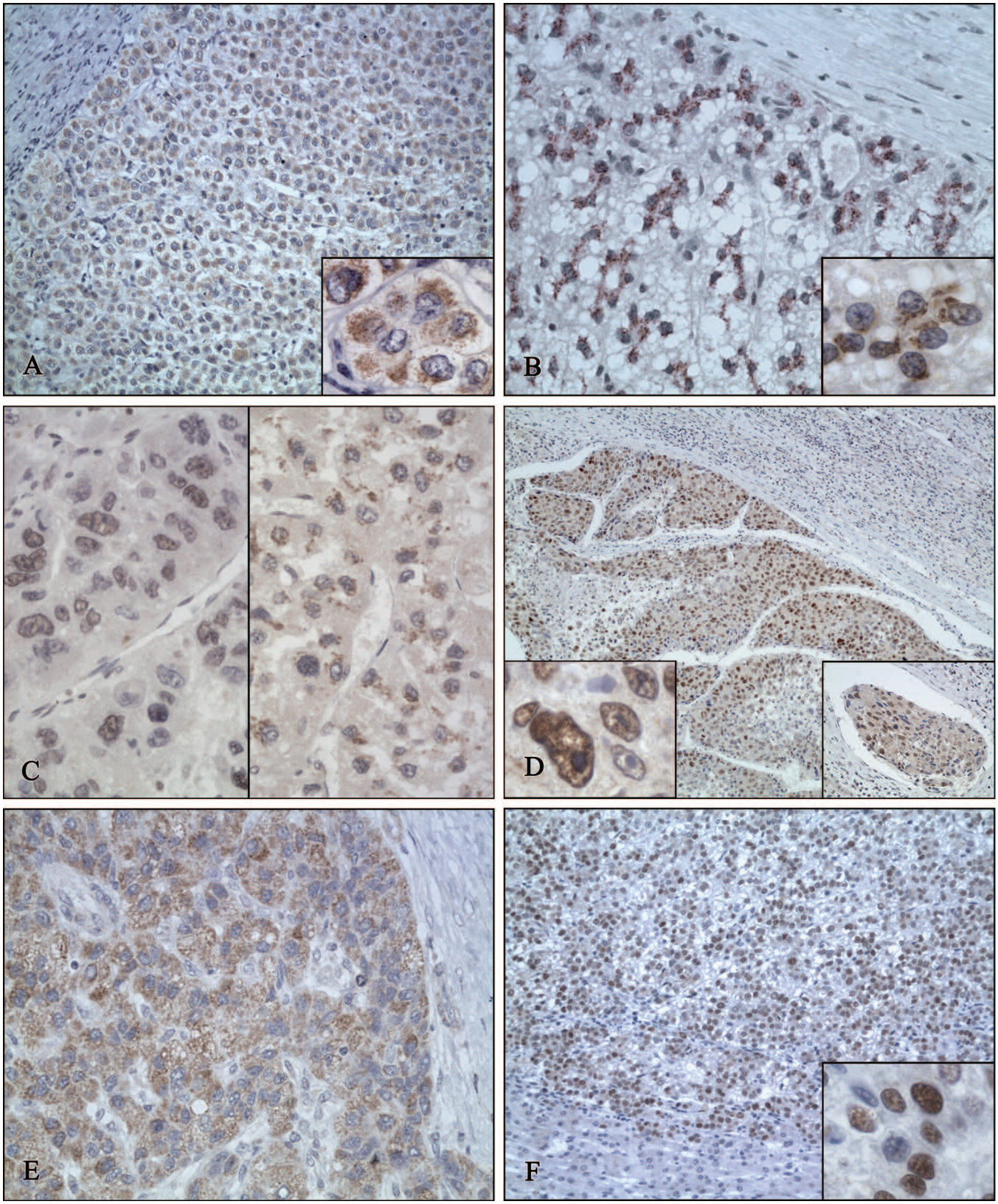

Fig. 1. A. Well differentiated HCC. Gli2 protein was expressed in the cytoplasm of most tumor cells. At high magnification (insert), the expression shows an even dot-like pattern. x 200. Insert: x 1000. **B.** Moderately differentiated HCC with fatty degeneration. Gli2 protein was strongly concentrated around the nuclear membrane and probably was localized in the Golgi apparatus. x 400. Insert: x 1000. **C.** Moderately differentiated HCC. In one single tumor nodule, some tumor cells (right-hand) showed sparse nuclear accumulation of Gli2 protein, while others (left-hand) showed homogeneous cytoplasmic dot-like Gli2 expression. x 400. **D.** Poorly differentiated HCC. Gli2 expression was diffusely and strongly nuclear in cells of the primary tumor and tumor within venous thrombus (left-hand corner insert). x 100. Right-hand corner insert: x 1000. Left-hand corner insert: x 100. **E.** Moderately differentiated HCC. Most tumor cells showed homogeneous FoxM1 expression in the cytoplasm. x 400. **F.** Moderately differentiated HCC. Diffuse and moderate nuclear staining of FoxM1 protein was found. x 400

shown in Fig. 1B, in one example of HCC with moderate differentiation and fatty degeneration of tumor cells, positive signals were strongly concentrated around the nuclear membrane and appeared to be localized in the Golgi apparatus. In another example of HCC with moderate differentiation, in the same tumor nodule, some tumor cells showed sparse nuclear accumulation of Gli2 protein, while others showed homogeneous cytoplasmic Gli2 expression (Fig. 1C). In the poorly differentiated HCCs expression of cytoplasmic Gli2 protein was noticeably reduced, and there was enhanced expression of nuclear Gli2 protein. As shown in Fig. 1D, in one HCC with poor differentiation Gli2 expression was diffusely nuclear in cells of the primary tumor and in tumor within venous thrombus.

Positive FoxM1 staining was observed in 11 of 19 (57.8%) well differentiated, in 36 of 41 (87.8%) moderately differentiated, and in 13 of 14 (92.9%) poorly differentiated HCCs. In HCCs with FoxM1 staining positivity, 79.5% (58/73) cases showed predominantly cytoplasmic staining with no indication of nuclear staining, and the remaining 20.5% (15/73) of HCCs showed nuclear staining, with or without cytoplasmic staining. As shown in Fig. 1E, in one HCC with moderate differentiation FoxM1 expression was homogeneously cytoplasmic in most tumor cells. Diffuse nuclear distribution of FoxM1 protein was found in another HCC with moderate differentiation (Fig. 1F).

Correlation between expression of Gli2 and FoxM1 and clinicopathologic characteristics

Increased nuclear immunopositivity of Gli2 protein was significantly correlated with poorer tumor differentiation ($P<0.05$), as well as with portal vein tumor thrombosis ($P<0.05$) (Summarized in table 1). Overexpression of FoxM1 protein was significantly associated with increased tumor grade ($P<0.01$) and advanced tumor stage ($P<0.05$). However, there was no significant association between Gli2 and FoxM1 immunostaining or any of the other clinicopathologic characteristics. Moreover, for rank correlation analysis, Gli2 expression positively correlated with FoxM1 immunoreactivity in HCCs ($r=0.464$, $P=0.000$).

Discussion

Recently, Gli2 has been reported to be involved in human tumorigenesis and development. In non-tumorigenic prostate epithelial cells, ectopic expression of Gli2 resulted in accelerated cell cycle progression and augmented proliferation (Thiyagarajan et al., 2007). Specific knockdown of the Gli2 gene with small hairpin RNA or antisense oligonucleotides in prostate carcinoma cells reduced tumor cell cluster formation and delayed tumor xenograft growth (Thiyagarajan et al., 2007; Narita et al., 2008). Similarly, the important role of Gli2 in regulating epidermal proliferation and basal cell carcinoma formation has been detected (Ikram et al., 2004; Regl et al., 2004). Together, these findings suggest a possible common mechanism whereby tumor formation and growth may require the up-regulation of Gli2 to maximize transcriptional output. In human HCC, although the activation of Hh pathway has been previously documented, the role of Gli2 protein in modulating tumor cell proliferation and differentiation in vivo has not been well characterized. In our study, a majority of HCCs exhibited Gli2 protein immunopositivity. Moreover, it was observed and statistically confirmed that in well differentiated or less aggressive HCCs, Gli2 expression was predominantly cytoplasmic only. In contrast, with increasing tumor grade or advanced invasion of tumor cells into the portal vein, expression of Gli2 protein showed more pervasive distribution in the nuclei of tumor cells. Nuclear localization of Gli protein is generally recognized as a hallmark of its transcriptional activity (Kasper et al., 2006; Scales and de Sauvage, 2009). Therefore, our results firstly confirmed the association of Gli2 protein with HCC differentiation in vivo. Overexpression of nuclear Gli2 protein may both reflect tumor differentiation and aggressive behavior.

Moreover, the regulation of subcellular localization and translocation of Gli2 protein may be complex and dependent on the nature of the pathway involved. The function of cytoplasmic Gli2 protein and the mechanism modulating cytoplasmic-nuclear shuttling of the Gli transcription factors should be further studied. In addition, although Gli2 seems to play the dominant role in regulating HCC differentiation presently, we cannot exclude the possible important function of Gli1 and Gli3 in the proliferation of some HCCs, as observed in other types of cancers. On the other hand, in samples of liver fibrosis induced by toxin or bile duct ligation and of primary biliary cirrhosis, stromal cells such as activated hepatic stellate cells and bile ductular cells expressed Hh components (Sicklick et al., 2005; Jung et al., 2007). These findings can be extended to some other studies. In human pancreatic and colorectal carcinomas, tumor-derived Hh stimulated expression of Gli1, Gli2 and Ptch1 in the infiltrating stroma but not in the tumor parenchymal cells (Scales and de Sauvage, 2009). Together, these results in part implied a paracrine modulation of Hh signaling. However, in our HCC samples, we did not find expression of Gli2 protein in stromal cells in tumoral or peritumoral tissues. The expression and role of the Hh pathway components in the tumor stroma of HCC warrants further attention.

Additionally, in the present study, we found a more pronounced FoxM1 protein induction in human HCCs. Previous studies have shown that a variety of human cancers, such as lung, pancreas and cervical cancer, required FoxM1 for tumor growth and progression in vitro or in vivo (Kim et al., 2006; Wang et al., 2007; Chan et al., 2008). In murine hepatocytes, nuclear translocation of FoxM1 seemed to be regulated by mitogenic signaling induced by partial hepatectomy, and this translocation was critical for transcriptional

activation of FoxM1 target genes modulating the G1-S transition (Wang et al., 2002). In rat hepatocarcinogenesis, FoxM1 up-regulation was followed by a very prominent rise in FoxM1 targets, including some proteins involved in G2-M transition (Calvisi et al., 2009). These previous studies suggested the possibility of FoxM1 in modulating human hepatocarcinogenesis. In our study, FoxM1 protein showed highest expression in poorly differentiated HCCs, followed by moderate and well differentiated tumors. In addition, overexpression of FoxM1 was significantly correlated with advanced tumor stage. Taken together, our findings may provide support for the concept that FoxM1 protein is essential for HCC proliferation, differentiation, and progression.

The close and intricate interactions between Hh signaling and the Fox transcription family regulate the development and maturation of some organs in human embryogenesis (Jeong et al., 2004; Maeda et al., 2007). In adults, such interactions seemed to be well conserved and made a difference in tumor formation. Teh et al. (2002) found that in human basal cell carcinoma, Shh signaling, via Gli1, up-regulated FoxM1 expression and induced its transcriptional activity. Similarly, Douard et al. (2006) demonstrated an increased expression of Shh mRNA in human colonic adenocarcinomas and in a colorectal cell line, with increased downstream expression of Gli1 and FoxM1 mRNA known to promote cell proliferation. On the other hand, it should be noted that Hh signaling may directly regulate cell cycle gene expression without the involvement of FoxM1. To confirm this, it has been shown that stimulation of esophageal cancer cells with Shh ligand or Gli-1 overexpression resulted in up-regulation of G1-cyclin activity and increased proliferation (Sims-Mourtada et al., 2006). Furthermore, Gli1 consensus DNA-binding sequences were identified in the 5-regions of cyclin D2, suggesting that these genes represent immediate downstream targets (Yoon et al., 2002). In our study, a significant association of the expression of Gli2 and FoxM1 proteins was identified. This suggested that aberrant activation of Gli2 protein in HCC may lead to tumor proliferation through the deregulated cell cycle control, probably modulated by FoxM1. Moreover, in hepatocarcinogenesis, cellular transformation resulting in invasive cancer must involve multiple cellular genetic changes affecting oncogenes, tumor suppressor genes or the signal transduction pathway (El-Serag and Rudolph, 2007). It is possible that the effect of Gli2 and FoxM1 proteins on cell differentiation and proliferation may reflect changes in different genes and signaling pathways in HCC.

In conclusion, our results suggested that in human HCC, the Hh pathway may be involved in the differentiation, proliferation and invasion of tumor cells through inducing nuclear accumulation of Gli2 protein, and subsequent stimulation of the downstream target gene, FoxM1. Gli2 may be a therapeutic target that, in association with other targets, may contribute to create networked biological treatments for HCC patients.

Acknowledgements. We greatly appreciate Professor Michael A McNutt, Department of Pathology, Peking University Health Science Center, China, for his critical suggestions and English revision. The present study was supported by two research grant from the National Natural Science Foundation of China (NSFC, 30700349 and 30440012).

References

Berman D.M., Karhadkar S.S., Maitra A., de Oca R.M., Gerstenblith M.R., Briggs K., Parker A.R., Shimada Y., Eshleman J.R., Watkins D.N. and Beachy P.A. (2003). Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature 425, 846-851.

Calvisi D.F., Pinna F., Ladu S., Pellegrino R., Simile M.M., Frau M., De Miglio M.R., Tomasi M.L., Sanna V., Muroni M.R., Feo F. and Pascale R.M. (2009). Forkhead box M1B is a determinant of rat susceptibility to hepatocarcinogenesis and sustains ERK activity in human HCC. Gut 58, 679-687.

Chan D.W., Yu S.Y.M., Chui P.M., Yao K.M., Liu V.W.S., Cheung A.N.Y. and Ngan H.Y.S. (2008). Over-expression of FOXM1 transcription factor is associated with cervical cancer progression and pathogenesis. J. Pathol. 215, 245-252.

Clement V., Sanchez P., de Tribolet N., Radovanovic I. and Altaba A. (2007). Hedgehog-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr. Biol. 17, 165-172.

Dahmane N., Lee J., Robins P., Heller P. and Altaba A. (1997). Activation of the transcription factor GLI1 and the sonic hedgehog signaling pathway in skin tumors. Nature 389, 876-881.

Douard, R., Moutereau S., Pernet P., Chimingqi M., Allory Y., Manivet P., Conti M., Vaubourdolle M., Cugnenc P.H. and Loric S. (2006). Sonic hedgehog-dependent proliferation in a series of patients with colorectal cancer. Surgery 139, 665-670.

El-Serag H.B. and Rudolph K.L. (2007). Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterol. 132, 2557-2576.

Huang S., He J., Zhang X., Bian Y., Yang L., Xie G., Zhang K., Tang W., Stelter A.A., Wang Q., Zhang H. and Xie J. (2006). Activation of the hedgehog pathway in human hepatocellular carcinomas. Carcinogenesis 27, 1334-1340.

Ikram M.S., Neill G.W., Regl G. and Eichberger T. (2004). GLI2 is expressed in normal human epidermis and BCC and induces GLI1 expression by binding to its promoter. J. Invest. Dermatol. 122, 1503-1509.

Jeong J., Mao J., Tenzen T., Kottmann A.H. and McMahon A.P. (2004). Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. Genes Dev. 18, 937-951.

Jiang J. and Hui C.C. (2008). Hedgehog signaling in development and cancer. Dev. Cell 15, 801-812.

Jung Y., McCall S.J., Li Y.X. and Diehl A.M. (2007). Bile ductules and stromal cells express hedgehog ligands and/or hedgehog target genes in primary biliary cirrhosis. Hepatology 45, 1091-1096.

Karhadkar S.S., Bova G.S., Abdallah N., Dhara S., Gardner D., Maitra A., John T., Isaacs J.T., Berman D.M. and Beachy P.A. (2004). Hedgehog signaling in prostate regeneration, neoplasia and metastasis. Nature 431, 707-712.

Kasper M., Regl G., Frischauf A.M. and Aberger F. (2006). Gli transcription factors: mediators of oncogenic hedgehog signaling. Eur. J. Cancer 42, 437-445.

Katoh M. and Katoh M. (2004). Human FOX gene family. Int. J. Oncol. 25, 1495-1500.

Katoh Y. and Katoh M. (2005). Hedgehog signaling pathway and gastric cancer. Cancer Biol. Ther. 4, 1050-1054.

Kim I.M., Ackerson T., Ramakrishna S., Tretiakova M., Wang I.C., Kalin T.V., Major M.L., Gusarova G.A., Yoder H.M., Costa R.H. and Kalinichenko V.V. (2006). The Forkhead box m1 transcription factor stimulates the proliferation of tumor cells during development of lung cancer. Cancer Res. 66, 2153-2161.

Kim J.E., Singh R.R., Cho-Vega J.H., Drakos E., Davuluri Y., Khokhar F.A., Fayad L., Medeiros L.J. and Vega F. (2009). Sonic hedgehog signaling proteins and ATP-binding cassette G2 are aberrantly expressed in diffuse large B-cell lymphoma. Mod. Pathol. 22, 1312-1320.

Kim Y.S., Yoon J.W., Xiao X.K., Dean N.M., Monia B.P. and Marcusson E.G. (2007). Selective down-regulation of glioma-associated oncogene 2 inhibits the proliferation of hepatocellular carcinoma cells. Cancer Res. 67, 3583-3593.

Lehmann O.J., Sowden J.C., Carlsson P., Jordan T. and Bhattacharya S.S. (2003). Fox's in development and disease. Trends Genet. 19, 339-344.

Liao X., Siu M.K.Y., Au C.W.H., Wong E.S.Y., Chan H.Y., Ip P.P.C., Ngan H.Y.S. and Cheung A.N.Y. (2009). Aberrant activation of hedgehog signaling pathway in ovarian cancers: effect on prognosis, cell invasion and differentiation. Carcinogenesis 30, 131-140.

Lipinski R.J., Gipp J.J., Zhang J., Doles J.D. and Bushman W. (2006). Unique and complimentary activities of the Gli transcription factors in Hedgehog signaling. Exp. Cell Res. 312, 1925-1938.

Maeda Y., Davé V. and Whitsett J.A. (2007). Transcriptional control of lung morphogenesis. Physiol. Rev. 87, 219-244.

Narita S., Ettinger S., Hayashi N., Muramaki M., Fazli L., Kim Y. and Gleave M.E. (2008). GLI2 knockdown using an antisense oligonucleotide induces apoptosis and chemosensitizes cells to paclitaxel in androgen-independent prostate cancer. Clin. Cancer Res. 14, 5769-5777.

Ohta H., Aoyagi K., Fukaya M., Danjoh I., Ohta A., Isohata N., Saeki N., Taniguchi H., Sakamoto H., Shimoda T., Tani T., Yoshida T. and Sasaki H. (2009). Cross talk between hedgehog and epithelial-mesenchymal transition pathways in gastric pit cells and in diffuse-type gastric cancers. Br. J. Cancer 100, 389-398.

Pasca di Magliano M. and Hebrok M. (2003). Hedgehog signaling in cancer formation and maintenance. Nat. Rev. Cancer 3, 903-911.

Patil M.A., Zhang J., Ho C., Cheung S.T., Fan S.T. and Chen X. (2006). Hedgehog signaling in human hepatocellular carcinoma. Cancer Biol. Ther. 5, 111-117.

Regl G., Kasper M., Schnidar H., Eichberger T., Neill G.W., Ikram M.S., Quinn A.G., Philpott M.P., Frischauf A.M. and Aberger F. (2004). The zinc-finger transcription factor GLI2 antagonizes contact inhibition and differentiation of human epidermal cells. Oncogene 23, 1263-1274.

Scales S.J. and de Sauvage F.J. (2009). Mechanisms of hedgehog pathway activation in cancer and implications for therapy. Trends Pharmacol. Sci. 30, 303-312.

Sicklick J.K., Li Y.X., Choi S.S., Qi Y., Chen W., Bustamante M., Huang J., Zdanowicz M., Camp T., Torbenson M.S., Rojkind M. and Diehl A.M. (2005). Role for hedgehog signaling in hepatic stellate cell activation and viability. Lab Invest. 85, 1368-1380.

Sicklick J.K., Li Y.X., Jayaraman A., Kannangai R., Qi Y., Vivekanandan P., Ludlow J.W., Owzar K., Chen W., Torbenson M.S. and Diehl A.M. (2006). Dysregulation of the hedgehog pathway in human hepatocarcinogenesis. Carcinogenesis 27, 748-757.

Sims-Mourtada J., Izzo J.G., Apisarnthanarax S., Wu T.T., Malhotra U., Luthra R., Liao Z., Komaki R., van der Kogel A., Ajani J. and Chao K.S. (2006). Hedgehog: an attribute to tumor regrowth after chemoradio therapy and a target to improve radiation response. Clin. Cancer Res. 12, 6565-6572.

Teh M.T., Wong S.T., Neill G.W., Ghali L.R., Phipott M.P. and Quinn A.G. (2002). FOXM1 is a downstream target of Gli1 in basal cell carcinoma. Cancer Res. 62, 4773-4780.

Thayer S.P., di Magliano M.P., Heiser P.W., Nielsen C.M., Roberts D.J., Lauwers G.Y., Qi Y.P., Gysin S., Fernández-del Castillo C., Yajnik V., Antoniu B., McMahon M., Warshaw A.L. and Hebrok M. (2003). Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 425, 851-856.

Thiyagarajan S., Bhatia N., Reagan-Shaw S., Cozma D., Thomas-Tikhonenko A., Ahmad N. and Spiegelman V.S. (2007). Role of GLI2 transcription factor in growth and tumorigenicity of prostate cells. Cancer Res. 67, 10642-10646.

Varjosalo M. and Taipale J. (2008). Hedgehog: functions and mechanisms. Genes Dev. 22, 2454-2472.

Wang X.H., Kiyokawa H., Dennewitz M.B. and Costa R.H. (2002). The Forkhead Box m1b transcription factor is essential for hepatocyte DNA replication and mitosis during mouse liver regeneration. Proc. Natl. Acad. Sci. USA 99, 16881-16886.

Wang Z.W., Banerjee S., Kong D., Li Y. and Sarkar F.H. (2007). Down-regulation of forkhead box M1 transcription factor leads to the inhibition of invasion and angiogenesis of pancreatic cancer cells. Cancer Res. 67, 8293-8300.

Watkins D.N., Berman D.M., Burkholder S.G., Wang B., Beachy P.A. and Baylin S.B. (2003). Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 422, 313-317.

Yoon J.W., Kita Y., Frank D.J., Majewski R.R., Konicek B.A., Nobrega M.A., Jacob H., Walterhouse D. and Iannaccone P. (2002). Gene expression profiling leads to identification of Gli1-binding elements in target genes and a role for multiple downstream pathways in Gli1-induced cell transformation. J. Biol. Chem. 277, 5548-5555.

Accepted April 12, 2010