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Expression of Notch1, Jagged1 and β-catenin and their clinicopathological significance in hepatocellular carcinoma

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The Notch/Jagged signaling pathway is important for cellular differentiation and proliferation. Notch1/Jagged1 can either suppress or promote tumors depending on the cell type and context. β-catenin, one of the mediators of the Wnt signalling pathway, represents a key element in one of the most important pathways of carcinogenesis. The aim of this study was to examine the expression of Notch1/Jagged1 and β-catenin in hepatocellular carcinoma and to assign clinicopathological correlations. Immunohistochemical detection of Notch1/Jagged1 and β-catenin was performed in tissue microarrays including 339 Hepatocellular carcinomas, 174 adjacent non-tumor livers and 94 normal livers. The results showed that the rate of expression was 66%, 98% and 97% for Notch1 and 36%, 85% and 92% for Jagged1 respectively in hepatocellular carcinoma, adjacent non-tumor liver and normal liver. Decreased expression of Notch1/Jagged1 was correlated significantly with Edmondson-Steiner grade. However, nuclear β-catenin was expressed in 37% of hepatocellular carcinoma tissue, which was significantly higher than its non-tumor counterparts. Increased nuclear β-catenin expression was correlated with HBs-Ag status and Edmondson-Steiner grade. Moreover, The positive expression of Notch1 was parallel with Jagged1 expression (r =0.235, p=0.000) and reduced Notch1 expression was associated with increased β-catenin expression in hepatocellular carcinoma (r =-0.125, p =0.023). In conclusion, Notch1/Jagged1 were frequently low expressed in hepatocellular carcinoma and correlated with the high expression of β-catenin suggesting that downregulation of Notch1/Jagged1 signaling may sustain tumor progression.

Key Words: Notch1, Jagged, β-catenin; hepatocellular carcinoma, tissue microarrays, immunohistochemistry.

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Asia and Africa, especially in China [1, 2]. It is responsible for approximately one million deaths every year, predominantly in the developing countries [3]. During the past decades, hepatic resection for HCC has evolved into a safe procedure with low surgical mortality [4, 5]. However, the molecular mechanisms leading to the development and progression of hepatocellular carcinoma remains unclear. Thus, the delineation of the mechanisms for hepatocarcinogenesis is important, because it provides novel opportunities for diagnosis, prognosis, and therapeutic interventions.

Notch signaling is critical for developing and maintaining tissue homeostasis [6]. Its pathway comprises a family of transmembrane receptors and their ligands, negative and positive modifiers, and transcription factors. To date, 4 mammalian receptors (Notch1 through Notch4) and at least 5 ligands (Delta 1, 3, and 4 and Jagged 1, 2) have been identified. Binding of the ligand renders the Notch receptor susceptible to metalloprotease- and γ-secretase– mediated proteolytic cleavage, which in turn results in the release of the Notch intracellular domain (NICD) from the plasma membrane and its subsequent translocation into the nucleus to mediate transcription of target genes [7].One of the ligands, Jagged1, is expressed widely in human organs including heart, placenta, kidney, lung, muscle, and pancreas. Besides, Jagged1 also plays important roles in various diseases including malignant tumors [8]. Wang et al [9] showed that Jagged1 expression in head and neck squamous cell carcinoma (HNSCC) cells triggered Notch activation in neighboring endothelial cells and promoted network formation. Aberrant Notch signaling has been linked to a wide variety of tumors [10] Notch can either suppress or promote tumors depending on the cell type and context [11, 12]. Activation of Notch signaling was observed

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in T cell acute lymphoblastic leukemias (T-ALL) [13], colon cancer [14, 16] , breast cancer [15], In primary mouse keratinocytes, Notch acts as a tumor suppressor gene, promoting exit from the cell cycle and entry into differentiation [17, 18]. Conditional ablation of Notch1 in murine epidermis results in epidermal hyperplasia, skin carcinoma, and facilitation of chemical-induced skin carcinogenesis (basal and squamous carcinomas), which implies a role of Notch1 as tumor suppressor. The antioncogenic effect of Notch1 in murine skin appears to be mediated by p21Waf1/Cip1 induction and repression of sonic hedgehog(Shh) and wingless/integration(Wnt) signaling [18] β-Catenin protein, originally identified as a submembrane component of the cadherin-mediated cell-cell adhesion system, functions as a chief downstream effector of the canonical Wnt signaling pathway.

When WNT receptors are inactive, β-catenin localizes with the membrane protein E-cadherin, and kinases in the APC complex phosphorylate cytoplasmic β-catenin for its rapid degradation. When WNT receptors are activated, kinases in the APC complex are inhibited, leading to accumulation of cytoplasmic β-catenin and its translocation to the nucleus, where it facilitates the transcription of various target genes. [14] But the role of Notch signaling in the development progression of hepatocellular carcinoma including cell migration, oncogenesis, cell differentiation, invasiveness, properties, and cellular functions that propagate the malignant phenotype are not understood. In this study, Notch1/Jagged1 and β-catenin expression were examined in hepatocellular carcinoma. We try also to determined if Notch1/Jagged1 and β-catenin levels can be used to help predict the clinical course of disease by analysis the relationship between the clinicopathological features of hepatocellular carcinoma and Notch1/Jagged1 lowexpression and β-catenin overexpression.

Materials and methods

Case selection. A total of 339 patients with hepatocellular carcinoma, 22 patients with cholangiocarcinoma (CC), 82 patients with metastatic carcinoma (the sources of the liver metastases originated from intestine cancers) and 94 cases with normal liver tissue were retrieved from the archives of The first affiliated hospital, Sun Yat-Sen University from 2006 to 2007, and these cases were selected and reviewed by the authors. Selection was based on the availability of the paraffin blocks and whether there was adequate tissue in the paraffin blocks for the tissue microarray study. All patients were of Chinese origin. None of the patients had received any other therapy before operation, including chemoembolization or chemotherapy, before resection.

Construction of tissue microarrays. We used a tissue arrayer device (Beecher Instrument,Silver Spring, MD, USA) to construct the tissue microarrays (TMAs) [19]. All case samples consist of 339 hepatocellular carcinoma tissues, 22 cholangiocarcinoma tissues, 82 metastatic carcinoma tissues, 174 adjacent non-tumor livers (liver tissue surrounding hepatocellular carcinoma) and 94 normal livers were histologically reviewed and the most representative areas for each tissue or tumor type were marked in the paraffin blocks. Two selected 1-mm-diameter cylinders from two different areas were included in the TMAs. Thus, 10 different TMA blocks were constructed, two with normal tissue and adjacent non-tumor livers and eight with tumoral samples, each containing between 120 and 180 cylinders. Consecutive 4μm-thick sections were cut by a microtome and air-dried overnight. One section from the tissue microarray block was stained with hematoxylin and eosin to confirm the homogeneity of the cell populations in the tissue samples.

Immunohistochemistry. The antibody clone names, sources, dilutions, and antigen pretreatments are listed in Table 1. Sections from the tissue microarray block were deparaffinized. For antigen retrieval, the sections were immersed in citrate buffer and processed in a scientific microwave oven (Energy Beam Sciences, Agawam, MA) at 95˚**C** for 15 minutes. After pretreatment with biotin blocking, primary antibody was performed manually for 4˚**C** overnight, followed by a 20-minute incubation each in secondary antibody and streptavidin-horseradish peroxidase (Zymed Laboratories; South San Francisco, CA).The detection was performed using SP method and the positive reaction was visualized with DAB (Dako, Carpenteria, CA). Immune serum was omitted in negative controls. Tumor sections were previously determined as positive control for Notch1 (pancreas), Jagged1 (lung cancer) and β-catenin (colorectal carcinoma).

Interpretation and scoring of immunohistochemical preparations. Membranous and/or cytoplasmic expression of Notch1, membranous and/or cytoplasmic expression of Jagged1, and nuclear expression of β-catenin were considered as positive immunostaining. Expression within tumor stroma was not specifically recorded.For overall positivity, immunostaining in >5% of cells was considered positive, and ≤5% positive cells was considered negative. Additionally, both extent (on the basis of the percentage of positive cells) and intensity of immunostaining were evaluated by a semiquantitative system.Extent was scored as: 0, ≤5%; 1+ (1 point), 6% to 25%; 2+ (2 points) , 26% to 50%; 3+ (3 points), 51% to 75%; and 4+ (4 points) , 76% to 100% . Intensity was arbitrarily scored as: weak (1 point), moderate (2 points), or strong (3 points). Intensity was designated as weak when immunostaining was present but only barely detectable. To correlate extent and intensity of immunostaining,

Table 1 List of antibodies used for immunohistochemistry

Antibody	Clone	Dilution	Source	Pretreatment
Notch1	A6	1:35	Neomarkers	Microwave, 15mM citrate (pH 6.0)
Jagged1	SC-6011	1:50	Santa Cruz	Microwave, 15mM citrate (pH 6.0)
β-catenin	14	1:200	Transduction laboratories	Microwave, 15mM citrate (pH 6.0)

Table 2 Clinicopathological characteristics of hepatocellular carcinoma in the study

Tumor characteristic	N[a]
Age	
≤50y	166
>50y	161
Sex	
Male	297
Female	31
Serum AFP	
≤20ng/ml	92
>20ng/ml	229
Tumor size	
≤5cm	112
>5cm	205
HBs-Ag status	
Negative	27
Positive	304
Edmondson-Steiner grade	
I or II	237
III or IV	73
vein or bile duct tumor thrombus	
Absent	222
Present	112
TNM stage	
I or II	164
III or IV	156

[a] total hepatocellular carcinoma samples with clinicopathological parameter analyzed in the study.

these values in positive cases were converted into composite immunohistochemical scores by multiplying the individual scores of extent by intensity (possible range of values from 1 to 12). For example, a case with 3+ extent (3 points) and moderate intensity of immunostaining (2 points) would have an immunohistochemical composite score of 3× 2 = 6 [20].

Statistical analysis. Statistical analyses were done using SPSS version 13.0 software (SPSS, Chicago, IL). The expression of Notch1, Jagged1 and β-catenin in

adjacent non-tumor liver tissue, normal liver tissue and various tumor types was compared using nonparametric statistical tests, that is, the Mann-Whitney U test or Kruskal-Wallis test. The differences between the Notch1, Jagged1 and β-catenin protein expressions and clinicopathological features of hepatocellular carcinoma were statistically analyzed using either χ^2 test or Fisher exact test; Correlation was expressed in hepatocellular carcinoma by the Pearson's correlation coefficient (two –tailed). All *P* values < 0.05 were considered statistically significant.

Results

Clinicopathologic finding in hepatocellular carcinoma. The clinicopathology features of the 339 HCC patients in the study group are summarized in Table 2. The age at presentation ranged from 9 month to 75 years (median age 50y, mean age 49y). The patients consisted of 31 females and 297 males with a ration of 1:9.6. Serum AFP concentration of most patients was more than 20ng/ml (71%; 229/321); that of the remainder were less than 20ng/ml (29%; 92/321). There are 304 patients (92%;304/331) with HBV infection; Most tumors were large (diameter range 1 to 29cm, median 7.1cm; mean 8.1cm). There are 112 patients with tumor thrombus in vein or bile duct (34%; 112/332). The HCC was graded according to the Edmondson-Steiner grade criteria. of the 330 cases with grade information available for review, 237 (76%) are grade I or II, 73(24%) are grade III or IV. These carcinomas frequently presented at advanced stage. According 2002 UICC staging criteria,121(38%; 121/322) Patients presented at stage I, 43 (13%; 43/322) at stage II, 120 (38%; 120/322) at stage III, and 36 (11%; 36/322) at stage IV.

Immunohistochemical findings. Notch1 expression. Total amount of samples, consisting of 338 HCCs, 21 CCs, 81 metastatic carcinoma spesimens, 170 adjacent nontumor liver tissue and 92 normal liver tissue was successfully analyzed in Fig 1. 227 out of 338 (66%) HCCs, 2 of 21 (10%) CCs and 16 of 81 (20%) metastatic carcinoma samples were positive for Notch1; 166 of 170 cases (98%) in adjacent non-tumor liver tissue and 89 of 92 case(97%)in normal liver tissue exhibited increased membranous and/or cytoplasmic expression of Notch1 compared with cancerous liver tissue (Fig 2). The differences between HCC and the noncancerous liver tissue were statistically significant(adjacent non-tumor liver, p=0.000; normal liver tissue, p=0.000),whereas there was no significant different of Notch1 expression in adjacent non-tumor liver tissue and normal liver tissue(p =0.871). The differences were statistically significant between CC and normal liver tissue (p=0.000), as well as metastatic carcinoma and normal liver tissue (p=0.000), but not between CC and metastatic carcinoma (p =0.313). Notch1 expression was significantly more frequent in HCC than in the other tumors(CC, p=0.000; metastatic carcinoma , p=0.000).

Jagged1 expression. Total amount ofsamples consisting of 329 HCCs, 22 CCs, 81 metastatic carcinoma specimens, 161 adjacent non-tumor liver tissue and 88 normal liver tissue were successfully analyzed as in (Fig 1). 118 0f 329(36%) HCCs, 9 of 22 (41%) CCs and 33 of 81 (41%) metastatic carcinoma samples were positive in Jagged1; 137 of 161 (85%) adjacent non-tumor liver tissue and 81 of 88 (92%) normal liver tissue exhibited increased membranous and/or cytoplasmic expression of Jagged1 compared with cancerous liver tissue (Fig 2). There was statistically significant difference between HCC and the noncancerous liver tissue (adjacent non-tumor liver tissue, p=0.000; normal liver tissue, p=0.000), CC and normal liver tissue (p=0.000), as well as metastatic carcinoma and normal liver tissue (p=0.000), but no significant difference between adjacent non-tumor liver and normal liver tissue(p =0.673), HCC and other tumors (CC, p=0.745; metastatic carcinoma , p=0.844), as well as CC and metastatic carcinoma (p =0.599).

β-catenin expression. Total amount of samples consisting of 333 HCCs, 22 CCs, 72 metastatic carcinoma specimens, 168

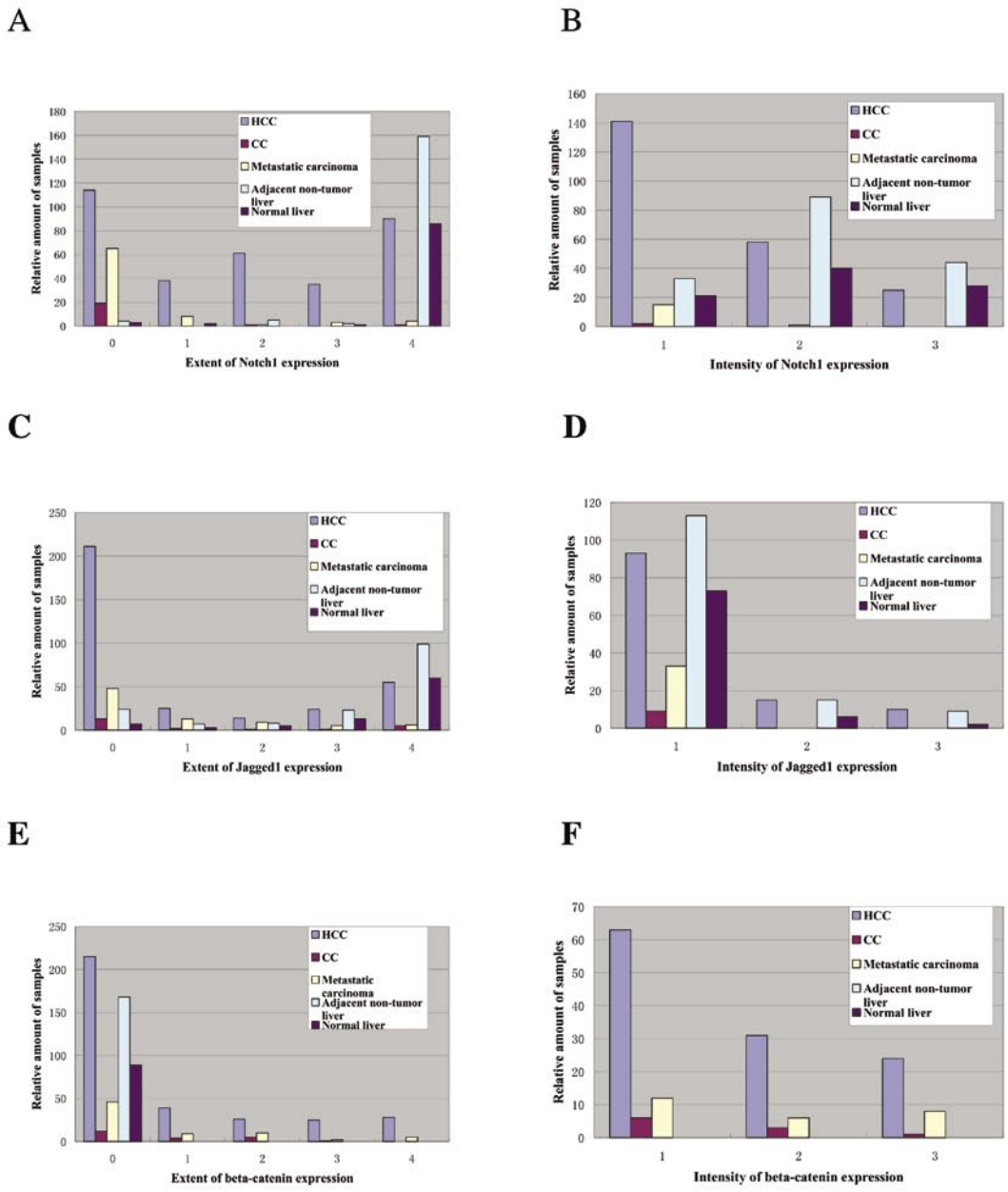

Figure 1 Expression of Notch1 (A-B), Jagged1 (C-D) and β-catenin (E-F) in HCCs, CCs, metastatic carcinoma tissues, adjacent non-tumor livers and normal livers. Relative amount of samples do not include the exfoliated cases during the experimental process.

adjacent non-tumor liver tissue and 89 normal liver tissue were successfully analyzed as in (Fig 1). Focal or generalized nuclear accumulation of β-catenin was observed in 123 of 333(37%) HCCs, 10 of 22 (45%) CCs and 28 of 71 (39%) metastatic carcinoma samples (Fig 2), but not in adjacent non-tumor liver tissue or normal liver tissue. There was statistically significant difference of β-catenin nuclear expression between HCC and the noncancerous liver tissue (adjacent non-tumor liver, p =0.000; normal liver tissue, p =0.000), CC and normal liver tissue (p =0.000), as well as metastatic carcinoma and normal liver tissue (p =0.000), but no significant difference between adjacent non-tumor liver and normal liver tissue(p =1.000), HCC and other tumors (CC, p =0.682; metastatic carcinoma, p =0.535), as well as CC and metastatic carcinoma (p =0.984).

Clinicopathologic correlations. The results of Notch1 staining intensity and proportion of stained tumor cells compared with clinicopathological characteristics of hepatocellular carcinoma are summarized in Table 3. Notch1 overall stain-

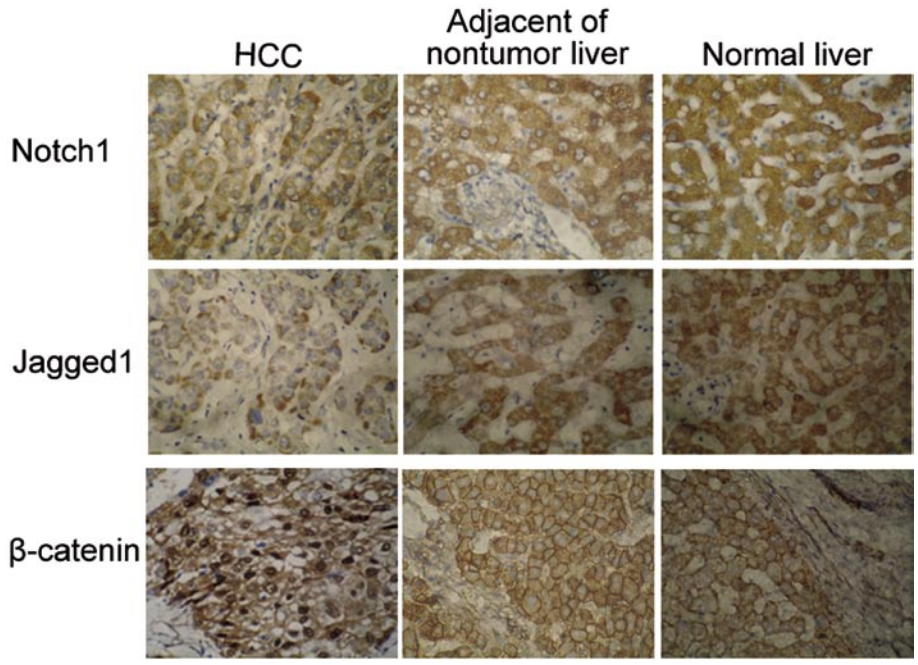

Figure 2 Biomarker expression in HCC, Adjacent non-tumor liver and Normal liver. The parameters assessed were Notch1 membranous and/or cytoplasmic expression; Jagged1 membranous and/or cytoplasmic expression; β-catenin nuclear expression.

ing proportion was significantly correlated with age, level of serum concentration of AFP and Edmondson-Steiner grade (p =0.001, p =0.001and p =0.005, respectively); The intensity of expression of Notch1 was significantly correlated with Edmondson-Steiner grade and vein or bile duct tumor thrombus (p =0.034, p =0.047). We also sought to investigate the relationship between both the extent and intensity of Jagged1 expression and clinicopathological parameters (Table 4). Jagged1 staining proportion was significantly correlated with Edmondson-Steiner grade and TNM stage (p =0.033, p =0.007); The intensity of expression of Jagged1 was significantly correlated with vein or bile duct tumor thrombus (p =0.024). Moreover, nuclear β-catenin staining proportion and intensity were significantly correlated with HBs-Ag status (p =0.043, p =0.029) and higher overall staining proportion in poor differentiated HCC tissues (26%) compared with well differentiated HCC tissues (14%) (p =0.018) (Table 5).

Association analysis. The positive expression of Notch1 was parallel with the Jagged1 expression in the hepatocellular carcinoma (r =0.235, p =0.000). However, Negative expression of Notch1 in tumors is correlated with nuclear coexpression of β-catenin; tumors positive in Notch1 expression were negative in nuclear β-catenin expression (r =-0.125, p =0.023).

Discussion

The Notch1 gene is located on chromosome 9q [34]. An interesting aspect of Notch is its apparently opposite functions in tumor development, since it can act as an oncogene or as a tumor suppressor. For example, in B lymphocytes development, Notch1 induces growth arrest and apoptosis [21] whereas in T cells, it induces cell proliferation [13] Notch signaling acts as a growth antagonist or tumor suppressor not only in murine basal cell carcinoma but also in late disease stage of cervical cancer [22]. The result of altered Notch signaling depends on its normal function in a given tissue. In this study, we investigated the expression of the Notch1 as well as expression of Jagged1 in hepatocellular carcinoma, adjacent non-tumor liver and normal liver. The results show that the rate of expression is 66%, 98% and 97% for Notch1 and 36%, 85% and 92% for Jagged1 respectively in hepatocellular carcinoma, adjacent non-tumor liver and normal liver. Notch1/Jagged1 was low expressed in hepatocellular carcinoma in contrast to its high level of expression in non-neoplastic livers. Decreased expression of Notch1 in hepatocellular carcinoma was correlated significantly with age, level of serum concentration of AFP, Edmondson-Steiner grade and vein or bile duct tumor thrombus. Also, that of Jagged1 was correlated significantly with Edmondson-Steiner grade, vein or bile duct tumor thrombus and the TNM stage of patients. Notch1 and Jagged1 expression were high in well-differentiated HCC but decreased in poorly differentiated HCC (Table 4, Table5), thus Notch1 signaling could play a role as tumor suppressor in HCC. Notch1 expression was parallel with the Jagged1 expression in the hepatocellular carcinoma, So low-level Notch1 and Jagged1 co-expression

Table 3 Notch1 overall staining proportion and intensity related to clinicopathological variables

Tumor characteristic	Proportion			Intensity			
	≤50%	>50%	*p*	1	2	3	*p*
Age							
≤50y	97(60%)	64(40%)		71(66%)	26(24%)	11(10%)	
>50y	64(41%)	91(59%)	0.001	64(56%)	29(25%)	21(19%)	NS
Sex							
Male	146(49%)	151(51%)		120(59%)	52(26%)	30(15%)	
Female	17(57%)	13(43%)	NS	15(75%)	3(15%)	2(10%)	NS
Serum AFP							
≤20ng/ml	32(35%)	60(75%)		35(51%)	23(33%)	11(16%)	
>20ng/ml	125(55%)	103(45%)	0.001	97(65%)	32(21%)	21(14%)	NS
Tumor size							
≤5cm	54(49%)	57(51%)		48(62%)	18(23%)	12(15%)	
>5cm	101(49%)	104(51%)	NS	85(61%)	35(25%)	19(14%)	NS
HBs-Ag status							
Negative	17(63%)	10(37%)		11(73)	1(7%)	3(20%)	
Positive	148(49%)	155(51%)	NS	126(60%)	54(26%)	29(14%)	NS
Edmondson-Steiner grade							
I or II	106(45%)	131(55%)		95(57%)	45(27%)	27(16%)	
III or IV	45(63%)	26(37%)	0.007	33(80%)	5(12%)	3(7%)	0.034
vein or bile duct tumor thrombus							
Absent	106(47%)	116(53%)		86(55%)	43(28%)	26(17%)	
Present	59(54%)	50(46%)	NS	51(73%)	12(17%)	7(10%)	0.047
TNM stage							
I or II	76(46%)	88(54%)		64(55%)	34(29%)	18(16%)	
III or IV	81(53%)	73(47%)	NS	69(68%)	20(20%)	13(12%)	NS

[a] total hepatocellular carcinoma samples analyzed in the study.

Abbreviations: NS, not significant; AFP, indicates α-fetoprotein; HBs-Ag, hepatitis B surface antigen. AFP (μg /L).

may have a synergistic effect on tumor suppression. Our data does not support the opinion of Cantarini MC et al.[23] l reporting higher levels of Notch1 immunoreactivity were detected in the HCC containing tissue relative to the adjacent tumor-free liver. However, Consistent with our observation is the report that intracellular forms of all 4 Notch receptors, including Notch1, are down-regulated in 13 of 20 HCCs . [24] Runzi Qi et al[25]demonstrated that Notch1 signaling results in significant growth inhibition of HCC cells both in vitro and in vivo, which is related to growth arrest and apoptosis induction. Using Notch1 knockout mouse model, Adrien G et al[26] found that deletion of Notch1 did not result in bile duct paucity, but, surprisingly, resulted in a continuous proliferation of liver cells. These results are consistent with our observation and supports our assertion that inactive Notch1 may promote hepatocarcinogenesis.

We also investigated the expression of Notch1/Jagged1 in cholangiocarcinoma and metastatic carcinoma. We found that immunohistochemical expression of Notch1 was frequent in HCC (66% of cases) compared with CC (10% of cases) and metastatic carcinoma (20% of cases). In contrast, immunohistochemical expression of Jagged1 in HCC (36%) showed no difference compared with CC (41%) and metastatic carcinoma (41%). Our results showed that the expression levels of Notch1/Jagged1 differed greatly in hepatocellular carcinoma, cholangiocarcinoma and metastatic carcinoma and were correlated with different clinicopathological parameters in hepatocellular carcinoma. For Notch signaling can be modulated at several levels [27].

In the present study, immunohistochemical nuclear expression of β-catenin was frequent in HCC compared with adjacent non-tumor liver and normal liver tissue. Inagawa et al found that upregulation of nuclear expression of the β-catenin, which is consistent with our observation and supports our assertion that β-catenin is activated in HCC [28]. 20% to 90% of HCCs display β-catenin activation because of diverse mechanisms that include mutation in genes encoding for β-catenin or CTNNB1 [29, 30], AXIN-1, and AXIN-2 [31],as well as frizzled-7 upregulation [32] and GSK3β inactivation [33].Also, nuclear expression of β-catenin was frequent in CC and metastatic carcinoma compared with normal liver tissue. Our results showed that β-catenin plays important roles in tumor development and progression. Examination of the clinicopathological parameters for HCC patients indicated nuclear β-catenin expression was correlated with HBs-Ag status and histological grade. Nuclear β-catenin accumulation significantly increased in the poor differentiated tumors and decreased in the well differentiated. The result was similar to the previous study [34] nuclear β-catenin expression was higher in the less-differentiated cancer tissues. This shows that the abnormal accumulation of β-catenin progresses

Table 4 Jagged1 overall staining proportion and intensity related to clinicopathological variables

Tumor characteristic	Proportion			Intensity			
	≤50%	>50%	*p*	1	2	3	*p*
Age							
≤50y	123(77%)	37(23%)		46(84%)	5(9%)	4(7%)	
>50y	119(76%)	38(24%)	NS	45(76%)	9(15%)	5(9%)	NS
Sex							
Male	221(77%)	66(23%)		81(71%)	13(12%)	7(6%)	
Female	22(71%)	9(29%)	NS	9(75%)	1(8%)	2(17%)	NS
Serum AFP							
≤20ng/ml	64(72%)	25(28%)		26(72%)	6(17%)	4(11%)	
>20ng/ml	176(79%)	46(21%)	NS	61(82%)	8(11%)	5(7%)	NS
Tumor size							
≤5cm	84(76%)	27(24%)		37(84%)	5(11%)	2(5%)	
>5cm	152(77%)	45(23%)	NS	50(77%)	9(14%)	6(9%)	NS
HBs-Ag status							
Negative	20(74%)	7(26%)		5(63%)	3(37%)	0(0%)	
Positive	226(77%)	68(23%)	NS	86(81%)	11(10%)	9(8%)	NS
Edmondson-Steiner grade							
I or II	172(74%)	59(26%)		72(80%)	11(12%)	7(8%)	
III or IV	61(87%)	9(13%)	0.033	10(71%)	3(21%)	1(7%)	NS
vein or bile duct tumor thrombus							
Absent	160(74%)	56(26%)		60(74%)	15(19%)	6(7%)	
Present	86(81%)	20(19%)	NS	30(88%)	0(0%)	4(12%)	0.024
TNM stage							
I or II	111(70%)	47(30%)		50(75%)	12(18%)	5(7%)	
III or IV	126(83%)	25(17%)	0.007	38(90%)	2(5%)	2(5%)	NS

Abbreviations: NS, not significant; AFP, indicates α-fetoprotein; HBs-Ag, hepatitis B surface antigen. AFP (μg /L).

Table 5 β-catenin overall staining proportion and intensity related to clinicopathological variables

Tumor characteristic	Proportion			Intensity			
	≤50%	>50%	*p*	1	2	3	*p*
Age							
≤50y	138(85%)	24(15%)		29(55%)	13(25%)	11(20%)	
>50y	131(82%)	28(18%)	NS	32(52%)	18(30%)	11(18%)	NS
Sex							
Male	247(85%)	44(15%)		54(54%)	28(28%)	18(18%)	
Female	22(71%)	9(29%)	NS	7(47%)	3(20%)	5(33%)	NS
Serum AFP							
≤20ng/ml	78(85%)	14(15%)		11(38%)	10(34%)	8(28%)	
>20ng/ml	186(83%)	37(17%)	NS	49(59%)	21(25%)	13(16%)	NS
Tumor size							
≤5cm	94(85%)	17(15%)		21(53%)	9(22%)	10(25%)	
>5cm	168(84%)	32(16%)	NS	36(53%)	21(31%)	11(16%)	NS
HBs-Ag status							
Negative	17(68%)	8(32%)		1(11%)	5(56%)	3(33%)	
Positive	255(85%)	45(15%)	0.043	60(57%)	26(25%)	20(18%)	0.029
Edmondson-Steiner grade							
I or II	200(86%)	32(14%)		41(55%)	21(28%)	13(17%)	
III or IV	53(74%)	19(26%)	0.018	14(44%)	9(28%)	9(28%)	NS
vein or bile duct tumor thrombus							
Absent	186(85%)	33(15%)		45(58%)	18(23%)	15(19%)	
Present	87(81%)	20(19%)	NS	15(41%)	13(35%)	9(24%)	NS
TNM stage							
I or II	139(85%)	24(15%)		34(59%)	16(28%)	8(714%)	
III or IV	124(83%)	26(17%)	NS	23(45%)	15(29%)	13(25%)	NS

Abbreviations: NS, not significant; AFP, indicates α-fetoprotein; HBs-Ag, hepatitis B surface antigen. AFP (μg /L).

along with the decrease in differentiation. β-catenin has been suggested as a functional target gene for Notch1 signaling that mediates the tumor-suppressive effect in murine skin where removal of Notch1 results in the generation of tumors associated with an increase in the levels of active β-catenin [18]. While some of the elevation of β-catenin in these cells might be a secondary consequence of activation of Wnt signalling, Hayward´s [35] observations suggest that the loss of Notch1 can also contribute to the elevation by allowing the activation of β-catenin. In this study, We also investigated the correlation between β-catenin in nuclear expression and Notch1 immunoreactivity, which has never been reported on large scale of HCC cases. Patients with nuclear β-catenin positive expression tumors showed markedly decreased Notch1 expression. This correlation between β-catenin and Notch1 expression suggests that Notch1 could exert a negative modulation on the level of β-catenin.

In summary, our findings indicate that (a) Notch1/ Jagged1 expression decreased in hepatocellular carcinoma, cholangiocarcinoma and metastatic carcinoma; (b) hepatocellular carcinoma, cholangiocarcinoma and metastatic carcinoma frequently expressed β-catenin; (c) in hepatocellular carcinoma, the expression of these several biomarkers correlated with clicopathological parameters; (d) the positive expression of Notch1 was parallel with the Jagged1 expression in HCC; reduced Notch1 expression was associated with increased β-catenin expression in HCC. Together, these findings also suggested that Notch1/Jagged1 signaling inaction possibly have an effect on high level of β-catenin and downregulation of Notch1 signaling may sustain tumor development and progression. Further investigation is required to define the role of the Notch signaling in neoplasms to delineate the exact molecular pathways leading to hepatocarcinogenesis.

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