• BRIEF REPORTS •

ADAM17 mRNA expression and pathological features of hepatocellular carcinoma

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Abstract

AIM: To study the expression of a disintegrin and metalloproteinase 17 (ADAM17) mRNA in hepatocellular carcinoma (HCC) and to evaluate the relationship between ADAM17 mRNA expression and clinicopathological features of HCC.

METHODS: Hepatocellular carcinomas (HCC) from 31 cases were divided into small HCC (SHCC), nodular HCC (NHCC) and solitary large HCC (SLHCC) according to tumor diameter and the number of nodes. ADAM17 mRNA expressions were compared among those groups by means of semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). The relationship between ADAM17 mRNA expression level and clinicopathological features of HCC was evaluated.

RESULTS: NHCC had lower differentiation and was more frequently of microvascular invasion (10/12) than SHCC (3/11) and SLHCC (3/8) ($P < 0.05$), but no statistical difference was observed between SHCC and SLHCC comparing their clinicopathological features. ADAM17 mRNA expression was detected in 77.4% (24/31) of HCC tissues and was significantly higher than that in paired non-cancerous liver tissues in which only 35.5% (11/31) of the samples were detected of the expression ($P < 0.05$). The expression of ADAM17 mRNA was much higher in NHCC than in SHCC and SLHCC ($P < 0.05$), while no significant difference was discovered between SHCC and SLHCC. The quantities of ADAM17 mRNA were significantly higher in poorly differentiated HCC than in well or moderately differentiated HCC, but no statistical difference was found concerning liver cirrhosis, tumor capsule formation or microvascular invasion of the cancer.

CONCLUSION: The increased expression of ADAM17 may play a key role in the development of HCC. The expression levels of ADAM17 mRNA varied among different pathological types of HCC. Lower mRNA expression of ADAM17 mRNA in SLHCC may be associated with the better molecular pathological features of SLHCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases in the world. Approximately 560 000 new cases of HCC are diagnosed each year, constituting 6% of all new human cancers[1]. The HCC mortality rate in China is approximately 20.4/100 000 and is the second leading cause of cancer death among Chinese males[2]. The prognosis of large hepatocellular carcinoma (LHCC) is generally considered to be worse than small hepatocellular carcinoma (SHCC), but long-term survival of some LHCC, especially solitary large hepatocellular carcinoma (SLHCC), could be frequently observed after curative resection[3-5]. From the clinical observation and our research, we hypothesized that SLHCC was of relatively better biological behavior[2]. Furthermore, we have preliminarily proved our hypothesis in a series of researches by showing that SLHCC was of relatively better pathological features and surgical prognosis[2,3,6]. Although many systemic studies have been performed, the unique clinical and molecular pathological features of SLHCC are still far from a clear deep understanding and need further investigation.

A disintegrin and metalloproteinase 17 (ADAM17) is a kind of transmembrane metalloproteinase[7], which can cleave the ectodomain of many transmembrane proteins. The substrates of ADAM17 mediated cleavage include tumor necrosis factor-α (TNF-α), tumor necrosis factor receptor type I, (TNFR I) and tumor necrosis factor receptor type II (TNFR II), interleukin1 receptor type II (IL1R II), Notch receptor, L-selectin, mucin1 (MUC1), CD30, tumor necrosis factor-related activation-induced cytokine (TRANCE) and many ligands of epidermal growth factor receptor (EGFR), such as transforming growth factor-α (TGF-α), heparin-binding epidermal growth factor-like growth factor (HB-EGF), amphiregulin and so on[8-19]. Hassan reported that the oxygen radicals generated by smoke stimulated ADAM17 to cleave transmembrane amphiregulin. The binding of amphiregulin to EGFR then promoted proliferation of lung cancer cells[20]. The cleavage function of ADAM17 was required for the activation of EGFR by TGF-α in Hela cells, and the high expression level of ADAM17 in mammary tumors was correlated with high activation rate of EGFR[21]. Another experiment showed that single metastatic lung cancer cell in bone marrow presented overexpression of ADAM17[22]. All these suggested that ADAM17 played a key role in the development of some cancers. It is well known that the EGFR mediated pathway played an important role in HCC development[23,24]. The overexpression of EGFR and its ligands were observed in HCC tissue and were related to the prognosis of HCC patients[25,26]. However, much less is known about the mechanisms involved in signal transduction, which activate the EGFR in HCC. It is even not sure whether there is an increased activation rate of EGFR in HCC besides the overexpression of EGFR and its ligands. As ADAM17 played an important role in the activation of EGFR[27] and up to now, there is no report about the expression and function of ADAM17 in HCC, so to have a better understanding of the molecular mechanisms involved in HCC development and the unique molecular pathological features of SLHCC, we studied the expression of ADAM17 in HCC and paired non-cancerous liver tissues.

MATERIALS AND METHODS

Materials

Thirty-one fresh HCC specimens (26 from males and 5 from females) were obtained by surgical resection in Xiangya Hospital, Central South University between May 2002 and February 2003. Tissues 1 cm away from the edge of the cancer were used as the paired non-cancerous control. The tissues used for RNA extraction were immediately snap-frozen in liquid nitrogen and stored at -70 °C. The specimens used for pathological study were fixed with 10% formalin, dehydrated by conventional methods, embedded in paraffin, cut into slices of 5 μm thick and stained by haematoxylin and eosin.

The specimens were divided into SHCC group (largest diameter less than 5 cm for single tumor nodule or the sum of diameters less than 5 cm for two tumor nodules, $n = 11$), SLHCC group (single tumor nodule with largest diameter more than 5 cm, $n = 8$) and NHCC group (two or more tumor nodules in the liver, only two tumor nodules and the sum of diameters less than 5 cm were excluded, $n = 12$).

Clinicopathological study

All specimens were examined under a microscope after haematoxylin-eosin staining by two pathologists. Four aspects of clinicopathological features including liver cirrhosis, Edmondson classification, capsule formation and microvascular invasion were studied.

RT-PCR

The total RNA was extracted from HCC tissue and paired non-cancerous liver tissue by using the TRIzol (Invitrogen, USA). The quality of RNA was checked through the ribosomal RNA bands on the gel. Two μg of each intact total RNA sample was reverse-transcribed to complementary DNA (cDNA) by using RT-PCR kit (MBI, USA). The PCR primer sequences used were as follows: sense: 5'-GCACAGGTAATAGCAGTGAGTGC-3' and antisense: 5'-CACACAATGGACAAGAATGCTG-3' for ADAM17; sense: 5'-TTCCAGCCTTCCTTCCTGG-3' and antisense: 5'-TTGCGCTCAGGAGGAGC AAT -3' for β-actin. The sizes of PCR products were 440 bp for ADAM17 and 218 bp for β-actin. The procedure was as follows: denaturation at 94 °C for 5 min, then 40 cycles of denaturation at 94 °C for 50 s; annealing at 52 °C for 1 min and extension at 72 °C for 1 min. The PCR products were electrophoresed in 20 g/L agarose gels, and visualized under ultraviolet light. The expression ratio of ADAM17 mRNA to β-actin was determined by Eagle Eye II photo-analysis system.

Statistical analysis

The χ^2 test was used for quantitative enumeration data. The student's t test and one-way analysis of variance were used for qualitative data. The statistic analysis was performed by statistical software SPSS11.0. P value less than 0.05 was considered significant.

RESULTS

Clinicopathological features of HCC in three groups

According to the results of pathological study (shown in Table 1), the NHCC group had higher incidences of microvascular invasion (10/12) compared with SLHCC (3/8) and SHCC (3/11) ($P<0.05$). Only 8.3% NHCC (1/12) was classified as Edmondson I-II, while 62.5% SLHCC (5/8) and 63.6% SHCC (7/11) were classified as Edmondson I-II. The differentiation of NHCC was significantly poorer than SLHCC and SHCC ($P<0.05$). The other two pathological features of SLHCC and SHCC were also better than NHCC but the difference did not reach statistical significance. No statistical difference of the four pathological features was observed between SLHCC and SHCC.

Table 1 Pathological features of HCC in three groups

Pathological features	SHCC ($n = 11$)	SLHCC ($n = 8$)	NHCC ($n = 12$)
Liver cirrhosis			
Present	7	6	9
Absent	4	2	3
Microvascular invasion			
Present	3	3[a]	10
Absent	8	5[a]	2
Capsule formation			
Present	8	6	4
Absent	3	2	8
Edmondson classification			
I-II	7	5	1
III-IV	4	3	11

[a]$P<0.05$ *vs* NHCC.

Expression of ADAM17 mRNA in HCC and paired non-cancerous liver tissue

As shown in Figure 1, RT-PCR products of ADAM17 and β-actin presented at the same site as previously designed. The expression of ADAM17 mRNA was detected in 77.4% (24/31) of HCC tissues, much higher than that in paired non-cancerous liver tissues in which only 35.5% (11/31) tissues were detected of the expression ($P<0.05$).
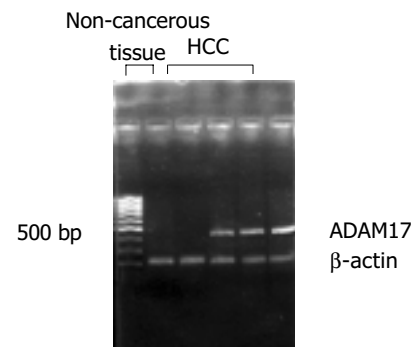

Figure 1 ADAM17 transcription was detected in HCC but no obvious transcription was observed in paired non-cancerous tissues.

Transcription level of ADAM17 in SLHCC, SHCC and NHCC

As shown in Figures 2, 3, the transcription level of ADAM17 was 0.8±0.7 (mean±SD) in SLHCC and 0.9±0.6 in SHCC. Both were significantly lower than that in NHCC (1.6±0.5) ($P<0.05$). No statistical difference in transcription level of ADAM17 was observed between SLHCC and SHCC.
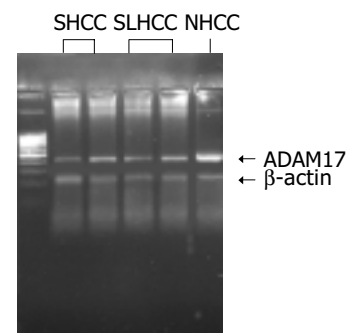

Figure 2 The expression of ADAM17 mRNA was higher in NHCC compared with SHCC and SLHCC.
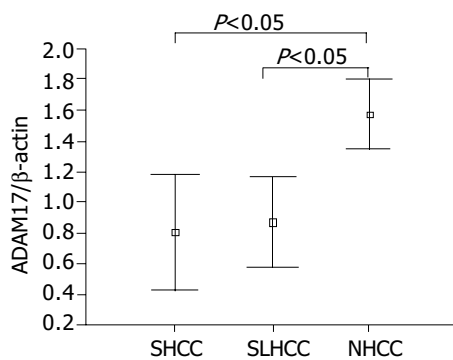
Figure 3 Transcription level of ADAM17 in three groups of HCC.

Relationship between transcription level of ADAM17 and clinicopathological features of HCC

The transcription level of ADAM17 was compared between different pathological types of HCC. The ADAM17 transcription level was much higher in HCC samples classified as Edmondson I-II compared with those classified as Edmondson III-IV (*P*<0.05). While no statistical difference in transcription level of ADAM17 was observed when capsule formation, liver cirrhosis, and microvascular invasion were concerned (Table 2).

Table 2 Relationship between ADAM17 transcription and pathological features of HCC

	Sample (*n*)	Transcription level of ADAM17 (mean±SD)	*P*
Liver cirrhosis			
Present	22	1.141±0.743	>0.05
Absent	9	0.996±0.662	
Microvascular invasion			
Present	16	1.279±0.648	>0.05
Absent	15	0.893±0.750	
Capsule formation			
Present	18	1.058±0.157	>0.05
Absent	13	1.187±0.800	
Edmondson classification			
I-II	13	0.768±0.204	<0.05
III-IV	18	1.349±0.157	

DISCUSSION

HCC ranks fifth in frequency worldwide among all malignancies[1]. Surgery is the only potential curative treatment of HCC[28]. The post-operative survival of SHCC was generally considered to be better than LHCC, but long-term disease-free survival of some LHCC was also frequently observed after curative resection. Furthermore, some clinical investigation showed that the 5-year survival rate of LHCC after curative resection was not statistically different from SHCC[4,5]. This kind of LHCC was named SLHCC because it was of some unique characters such as: isolated lesion, expanding growth and relatively integrated fibrous capsule formation *et al.*[2]. Previous studies showed that SLHCC had relative better prognosis after curative resection[2,3,6]. Consistent with this, the relatively better pathological features of SHCC were found in this study. The differentiation of SLHCC was much better than that of NHCC, and NHCC was more frequently of microvascular invasion compared with SLHCC. No statistic difference in pathological features was observed between SHCC and SLHCC.

ADAM17 is a sheddase of many transmembrane proteins. The substrates of ADAM17 mediated shedding include many

ligands of EGFR such as TGF-α, HB-EGF, amphiregulin *et al.*[14]. It has been well known that the activation of EGFR is essential for the carcinogenesis and metastasis of many cancers, while ADAM17 mediated shedding is a key mechanism of sending signals to activate EGFR[19-21]. Overexpression of ADAM17 has been observed in gastric tumor, mammary cancer, leukemia cell lines and prostate cancer cell lines[20,21,29-32]. In this study the transcription of ADAM17 was detected in 77.4% (24/31) HCC samples and was statistically higher than that in paired non-cancerous samples in which only 35.5% (11/31) were detected (*P*<0.05). This suggests that ADAM17 may play a key role in HCC development.

Previous studies showed that SLHCC had relative better prognosis after curative resection. To understand the underlying molecular mechanism, the transcription levels of ADAM17 in SLHCC, SHCC and NHCC were also studied. As expected, the transcription level of ADAM17 was much higher in NHCC than in SLHCC and SHCC, while no statistical difference was observed between SLHCC and SHCC. The transcription level of ADAM17 mRNA was also detected to be statistically higher in samples classified as Edmondson III-IV compared with those classified as I-II. The higher expression of ADAM17 mRNA in NHCC as well as in poorly differentiated HCC suggested that it might facilitate tumor invasiveness. The lower transcription level of ADAM17 in SLHCC was probably associated with the relatively better molecular pathological features of SLHCC.

The relationship between ADAM17 mRNA expression and the other two tumor features: microvascular invasion and tumor capsule formation was also studied in this study. Although much higher ADAM17 mRNA level was detected in tumors with microvascular invasion and those without integrated capsule formation, the difference did not reach statistical significance. This maybe due to the relatively small sample size in this study. As only 31 samples were used in the study, especially after the samples were divided into three groups, the significance of the research results may be impaired. The role of ADAM17 in HCC invasiveness needs further investigation with a larger sample size.

The increased transcription of ADAM17 may facilitate the growth and invasiveness of HCC in several ways. (1) Overexpressions of EGFR and its ligands were observed in HCC and were related to the prognosis. However, ADAM17 mediated shedding is a key mechanism of sending signals to activate EGFR. ADAM17 is required for the activation of EGFR by TGF-alpha or amphireglin in several cancer cells and the high expression of ADAM17 in mammary tumors was correlated with a high activation rate of EGFR[20,21]. As ADAM17 regulates the ligands production and activity of EGFR, the overexpression of ADAM17 is probably as important as the increased expression of EGFR and its ligands for HCC development; (2) ADAM17 can function as an effector of G protein-coupled receptor (GPCR) -mediated signaling. Activation of GPCR specifically results in ADAM17 cleavage and release of amphiregulin, which could activate EGFR and regulate the proliferation and motility of squamous cell carcinoma[32]. Overexpression of GPCR was also observed in HCC and was strongly correlated with carcinogenesis of HCC[33]. ADAM17 may be a key element of communication between GPCR and EGFR in HCC and facilitates HCC development; (3) TRANCE could activate osteoclast and help cancer cells metastasize to bones. Overexpression of TRANCE was found in bone metastatic lesion of several cancers[34]. ADAM17 may play a role in the bone metastasis of HCC by cleaving TRANCE; (4) The shedding of TNFR by ADAM17 may cause disorder of host immune system as the soluble form of TNFR could bind to the TNF and block its attack to cancer cells[35].

Other transmembrane proteins associated with HCC

metastasis such as Fas ligand, CXCL12, E-cadherin, interleukin-6α[36-39] were also the suspected substrates of ADAM17 mediated shedding, so it is of particular significance to study the sheddase role of ADAM17 in HCC development. The ADAM metalloproteinase family includes more than 30 members by now[40]. Many of them not only function as metalloproteinases to shed transmembrane proteins[41-43] in cancer but also can work as adhesion molecules[44,45]. In contrast to the matrix metalloproteinases, relatively few data on expression of ADAMs in cancer tissue are available. The expression of ADAM12 and ADAM9 were recently studied in HCC tissue and were of particular importance[46]. To study the role of other ADAM family members may help us better understand HCC development.

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