

# Expression of intercellular adhesive molecule-1 in liver cancer tissues and liver cancer metastasis

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**Subject headings** liver cancer; tumor metastasis; intercellular adhesion molecule-1

## Abstract

**AIM** To study the relationship between intercellular adhesive molecule-1 (ICAM-1) and liver cancer metastasis and to search for factors to predict metastasis of liver cancer.

**METHODS** ICAM-1 expression in fresh tissues of normal liver and hepatocellular cancer (HCC) was examined by immunoperoxidase staining. The expression of ICAM-1 in human hepatoma, tumor surrounding tissues and normal livers were semiquantitatively analyzed by Dot immunoblot. Tissue ICAM-1 expression at mRNA level was detected by Northern blot.

**RESULTS** All 6 cases of normal liver samples were negative in anti-ICAM-1 immunohistochemical staining, 80.0% (36/45) of HCC presented various ICAM-1 expression. The number of positive cells was a little higher in large tumors, tumors with intact capsule and metastasis, but there was no significant difference. Two cases with cancer embolus also had high ICAM-1 expression. ICAM-1 concentration in HCC ( $13.43 \pm 0.09$ ) was higher than that in tumor surrounding tissues ( $5.89 \pm 0.17$ ,  $P < 0.01$ ) and normal livers ( $4.27 \pm 0.21$ ,  $P < 0.01$ ). It was also higher in metastasis group ( $20.24 \pm 0.30$ ) than in nonmetastasis group ( $10.23 \pm 0.12$ ,  $P < 0.05$ ). Northern blot analysis revealed that ICAM-1 expression at mRNA level was also higher in HCC and cancer embolus than that in tumor surrounding tissues and normal livers.

**CONCLUSION** Tissue ICAM-1 could indicate the growth and metastasis of HCC, and may be an index that can predict liver cancer metastasis.

## INTRODUCTION

Tumor metastasis is one of the main causes of poor prognosis of hepatocellular carcinoma (HCC)<sup>[1]</sup>. The process of metastasis includes dissociation of tumor cells from primary location; arrest, adhesion and extravasation of metastatic tumor cells. Adhesive molecules on endothelial cells such as selectin cause circulating tumor cells to roll along the surface. Other molecules on tumor cells including intercellular adhesive molecule-1 (ICAM-1), vascular cellular adhesive molecule-1 (VCAM-1) and so on, subsequently stop the cells completely, then extravasate through the blood vessels into selective target tissues. During the metastatic cascade, tumor cells interact with various host cells (endothelial cells, platelets or lymphocyte), and/or extracellular matrix and basement membrane components, the homotypic and heterotypic cell clumps form a multicellular embolus, which can enhance the survival, arrest and invasiveness of tumor cells<sup>[2]</sup>. The metastatic potential of cancer cells is related to the activity of their surface adhesive molecule<sup>[3]</sup>. Recently, it was noticed that ICAM-1 plays an important role in this process. In this study, we investigated the relationship between ICAM-1 expression and the metastasis of HCC.

## MATERIALS AND METHODS

### Patients

The tissues of HCC used for immunohistochemical study were collected from 45 patients (39 males and 6 females, aged 10-82 years with a median of 51.1) who had undergone hepatectomy for HCC. All the cases were diagnosed pathologically. Eighteen of the patients were in early stage (without symptoms and signs of HCC), and 27 in moderate stage (having symptoms and signs of HCC, but no ascites, jaundice and long distance metastasis). Thirty patients had positive AFP ( $\geq 30 \mu\text{g/L}$ ) and 15 had negative AFP. The median diameter of the resected tumor was 6.7 cm (1.5-18), 30 had large tumors ( $> 5\text{cm}$ ) and 15 were had small tumors ( $\leq 5\text{cm}$ ). Patients with embolus in portal vein and its main branch or with intrahepatic and extrahepatic metastasis nodules belonged to the metastasis group, the other patients were to nonmetastasis group. Six normal livers were from the surrounding noncirrhotic liver tissues of

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pathologically proved benign tumors (4 hemangioma, 1 adenoma and 1 cyst).

The tissues used for detection of ICAM-1 were collected from 40 patients with HCC, 20 of them had large tumors and 22 encapsulated: 9 patients were in early stage and 31 in moderate stage. Positive AFP was found in 30 cases. There were 16 patients in the metastasis group, and 24 in nonmetastasis group. The 8 normal liver tissues were collected from the surrounding noncirrhotic tissues of pathologically proved benign liver tumors (5 hemangioma, 1 adenoma and 2 cyst).

### Reagents

Monoclonal antibody of ICAM-1 (1 µg/L) was purchased from R-G Company, Britain; AKP goat anti mouse IgG and biotinylated rabbit anti-mouse IgG were from Sino-American Company, China; TRIzol™ total isolation reagent from Gibco Company, USA; and megaprime™ DNA labeling systems from Amersham Company, UK.

### Methods

**Indirect immunoperoxidase staining** The fresh specimens embedded by O.C.T were frozen in liquid nitrogen, and then stored at -70°C. Serial frozen sections (6 µm) of liver specimens were fixed with acetone. After preincubated with human serum to reduce background, the slides were incubated with the primary antibody (1:50) at 4°C overnight, biotinylated rabbit anti mouse immunoglobulin (1:200) was used at 37°C for 1 h, added with avidin biotin HRP mixture (1:100 v/v), and counterstained with hematoxylin.

**Dot immuno blot** Three hundred mg tissue was homogenized in 1.5 ml suspended buffer solution (0.1 mol/L NaCl, 0.01 mol/L Tris. Cl, pH 7.6, 0.001 mol/L EDTA pH 8.9, 1% Triton-X-100), the protein concentration was determined by Hatree method<sup>[4]</sup>. The supernatant (30 µl) was spotted onto nitrocellulose membrane in a dot blot format. After non-specific blocking with 5% lipid free milk, the blots were incubated with ICAM-1 antibody (1:500) at room temperature for 2 h, and then incubated with AKP conjugated goat anti-mouse IgG (1:200) for 2 h at room temperature, then stained with NBT/BCIP (2:1 v/v). The integrated optical density (IOD) of the blot was measured by MIAS 300 automatic image analyzer. Tissue ICAM 1 = (sample IOD background IOD) × sample area / (µg protein concentration × 1000).

**Northern blot** Total RNA was extracted by TRIzol™ reagent according to the manufacturer's instruction (Gibco Life Technologies Cat No. 15596.026). Total RNA of 20 µg was denatured in

formaldehyde, electrophoresed, and transferred to nitrocellulose membrane. The plasmid containing ICAM-1 probe sequence was kindly provided by Dr. Christian Stratowa (Ernst Boehringer Institute, Vienna, Austria). Northern transfer membranes were prehybridized for 4 h at 42°C with 5×SSPE/5×SSPE/5× Denhardt's solution/1% SDS/100 mg/L Salmon sperm DNA. The 1.8 kb cDNA was labeled with 32P isotope by random primer method (Megaprime™ DNA Labeling Systems, Amersham). Northern blot analysis was performed under stringent washing conditions, 1×SSC 0.1% SDS, 30 min at room temperature and 1×SSC 0.1% SDS, 1 h at 50°C. Autoradiography was carried out at -70°C for 7 days.

**Data analysis** Significance was assessed by Student's *t* test. The results were expressed as  $\bar{x} \pm s$ .

## RESULTS

### Immunohistochemical observation of ICAM-1 in human HCC

By using anti ICAM-1 monoclonal antibody, no ICAM-1 was found in normal liver tissues (Figure 1). ICAM-1 was positive in fresh HCC (Figure 2), mainly on cell membrane, with a rate of 80% (36/45). In this group, 42.6% showed strong positive (++<sup>aa</sup>, 30%-70% positive cells), others were weak positive (+, 0-30% positive cells). ICAM 1 was also positive in 2 cases with tumor embolus in portal vein (Figure 3).

### Semi-quantitative analysis of human tissue ICAM-1

Dot immuno-blot was employed in semi-quantitative analysis of tissue ICAM-1 (Figure 4). Table 1 demonstrates that ICAM-1 content in HCC was about two-fold higher than that in the tumor surrounding liver tissues and normal controls ( $P < 0.01$ ), no significant difference was found between the latter two.

**Table 1** ICAM-1 expression in different liver tissues [ $(\bar{x} \pm s)/1000$ ]

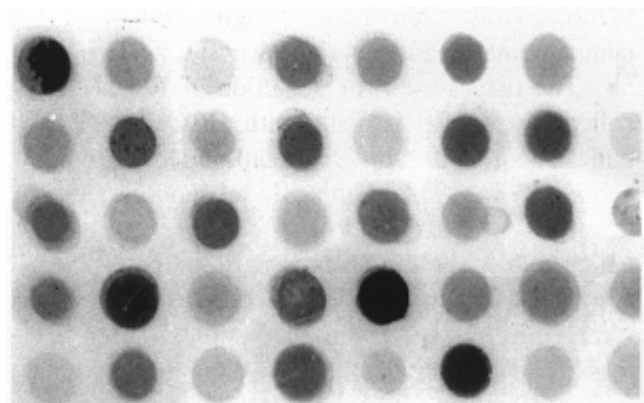
Groups	Cases	ICAM-1
HCC	40	13.43 ± 0.09
Tumor surrounding liver tissues	40	5.89 ± 0.17
Normal liver	8	4.27 ± 0.21

$P < 0.01$ , compared with HCC group

The expression of tissue ICAM 1 was also compared with tumor biological characters (Table 2), ICAM-1 in cancer tissues was only related to tumor metastasis, and there was no relationship with tumor size and capsule formation.

**Table 2 The relationship of ICAM 1 content with some tumor biocharacters ( $\bar{x} \pm s$ )**

Groups	Cases	ICAM-1	P- value
Metastasis	16	20.24±0.30	<0.05
Nonmetastasis	24	10.23±0.12	
Metastasis tumor surrounding liver	16	6.80±0.08	>0.05
Nonmetastasis tumor surrounding liver	21	4.78±0.04	
Intact capsule	22	13.55±0.20	>0.05
Nonintact capsule	15	12.93±0.32	
Large HCC	20	14.45±0.26	>0.05
Small HCC	14	10.96±0.47	



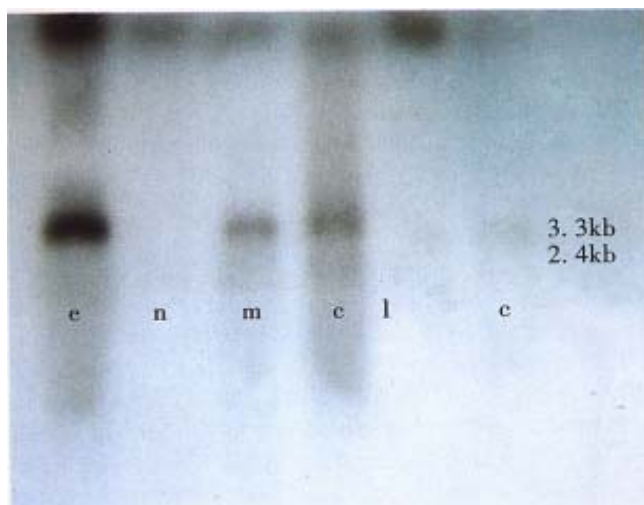
**Figure 4** Tissue ICAM-1 expression by Dot immunoblot.



**Figure 1** ICAM-1 expression in normal liver tissues by immunohistochemical staining.  
**Figure 2** ICAM-1 expression in liver cancer tissues by immunohistochemical staining.  
**Figure 3** ICAM-1 expression in tumor embolus by immunohistochemical staining.

**ICAM-1 expression at transcriptional level**

In this study, special DNA probe was used in Northern blot for detecting ICAM-1 mRNA, two transcripts with 3.3 kb and 2.4 kb in length was defined.  $\beta$ -actin was acted as internal standard. The data obtained from image analysis indicated that ICAM-1 mRNA expression in tumors was higher than that in tumor surrounding liver tissues and normal controls as well as cancer embolus (Figure 5).



**Figure 5** Detection of ICAM-1 mRNA by Northern blot. E: embolus; N; tumor surrounding liver; C: liver cancer; normal liver

**DISCUSSION**

Intercellular adhesive molecule-1 (ICAM-1, CD54), a transmembrane glycoprotein, is a member of the immunoglobulin superfamily and functions as counterreceptor for leukocyte function associated antigen-1 (LFA-1, CD11a/CD18) and complement receptor type 3 (MAC-1, CD11b/CD18)<sup>[5]</sup>. It mediates homotypic and heterotypic cell interaction, not only plays an important role in the tumor genesis, but also can reflect tumor curative

effect<sup>[6]</sup>, and may be a critical factor in the process of bloodborne metastasis of cancer<sup>[7]</sup>. Recently it was found that ICAM-1 plays an important role in tumor recurrence and metastasis. Hung<sup>[8]</sup> *et al* found that anti-ICAM-1Ab can prevent the growth of APH-77 tumors in SCID mice by altering the homing and growth of tumor cells in certain anatomical sites. Most of normal tissues do not express ICAM-1, but ICAM-1 expression has been observed in many tumors, such as renal cancer, uterine cancer, lung cancer, gastric cancer, bladder cancer, etc. Santarosa<sup>[9]</sup> *et al* demonstrated that renal cancer recurrence after operation was related to ICAM-1 expression in cancer tissues and patients with <50% of ICAM-1 positive cells in cancer tissues had prolonged disease-free survival after a median follow-up of 60 months.

In this study, all 6 cases of normal liver sample were negative in anti ICAM-1 immunohistochemical staining, 36/45 HCC presented various ICAM-1 expression. The rate of positive cell was a little higher in large tumors, tumors with intact capsule and tumors with metastasis, but there was no significant difference. It has been noticed that 2 cases with embolus also had high ICAM-1 expression. As indicated in semiquantitative analysis of tissue ICAM-1, ICAM-1 concentration in liver cancer was higher than in tumor surrounding liver tissues and normal livers. Tissue ICAM-1 was higher in metastasis group than that in nonmetastasis group. Northern blot analysis revealed that ICAM-1 expression at mRNA level was also higher in HCC and cancer embolus than in tumor surrounding liver tissues and normal controls. So it was suggested that ICAM-1 might be one of the characters of liver cancer, and could be a diagnostic indicator for metastatic status of liver cancer.

The deduced ICAM-1 amino acid sequence analysis revealed the presence of C<sub>3</sub>/C<sub>4</sub> binding protein consensus sequence between residues 341 and 404, so ICAM-1 may be involved in C<sub>3b</sub> binding. Since C<sub>3b</sub> binding protein generally acts to accelerate the dissociation of active complement complexes, ICAM-1 may help the tumor escape immune destruction, providing an important property needed for successful metastasis. Functioning as a ligand of LFA-1, ICAM-1 can establish the heterotypic adhesion of tumor cells with migratory and invasive leukocytes present in the tumor cells, enabling individual

cell to dissociate from primary tumor and go into circulation<sup>[10]</sup>. There is soluble ICAM-1 (sICAM-1) in serum, which comes from proteolytic cleavage of membrane associate ICAM-1. Since LFA-1 could also be blocked with soluble ICAM-1, sICAM-1 may also block the attachment of cytotoxic T cell and/or NK cells to cancer cells. This could be one of the mechanisms of cancer cells escaping from the immunosurveillance system of the host, and can inhibit lymphocyte mediated cytotoxicity, therefore a favorable environment for tumor cell proliferation would be created<sup>[11]</sup>. Spontaneous regression of human liver tumor was quite rare and the tumors were unusually resistant to various immunotherapy, probably due to the high content of sICAM-1 in those patients.

In our experiment, we also found that there was no statistically significant difference in ICAM-1 expression between tumor surrounding liver tissues and normal livers, and between tumor metastasis group and nonmetastasis group. So we think that ICAM-1 reflects the metastatic ability of cancer cells but not the cancer environment.

In conclusion, tissue ICAM-1 could reflect the growth and metastatic status of HCC and may be a factor to predict liver cancer metastasis.

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