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ORIGINAL PAPER

Expression and prognosis role of indoleamine 2,3-dioxygenase in hepatocellular carcinoma

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Abstract
Purpose Immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) plays an important role in immune tolerance. In some malignancies, the evidences were given to show that overexpression of IDO was related to poor prognosis of cancer patients.
Methods In this study, we investigated IDO expression in hepatocellular carcinoma (HCC) cell lines and 138 primary HCC clinical surgical specimens, and correlation of IDO expression with clinical outcomes and prognosis of HCC patients.
Results Reverse transcription-PCR analysis showed that in vitro IDO expression in HCC cell lines and non-cancerous liver cell line were dependent on IFN-γ. Immunohistochemical detection revealed that IDO was overexpressed in 49 of 138 (35.5%) tumor resection samples, whereas 89 of 138 (64.5%) cases showed weak immunostaining. IDO overexpression was significantly correlated with high metastasis rates ($P = 0.049$). Kaplan–Meier survival curves showed that overexpression of IDO resulted in significantly poor prognosis ($P = 0.017$, log-rank test). Multivariate Cox's analysis showed that IDO expression was an independent prognostic factor for overall survival of HCC patients ($P = 0.010$).
Conclusion Our data indicated that IDO may be a novel favorable prognostic indicator and candidate adjuvant therapeutic target for HCC.

Keywords Indoleamine 2,3-dioxygenase (IDO) · IDO expression · Hepatocellular carcinoma (HCC) · Prognostic indicator

Introduction

Hepatocellular carcinoma (HCC) is one of the most common and lethal malignancies worldwide and is the second leading cause of cancer death in China (Parkin et al. 2001; Tang 2000). HCC is often associated with a high potential for vacular invasion and metastasis, which contributed to a high incidence of early postoperative recurrence in the liver remnant or distant sites. The prognosis of HCC is generally poor even after surgical resection because of a high recurrence rate and the 5-year survival rate is limited to 25–39%. Recent studies have elucidated some of the underlying molecular mechanisms contributing to tumor progression, such as tumor antigen-specific immune tolerance, tumor immunosuppressive microenvironment mediating tumor immune escape (Mapara and Sykes 2004; Zou 2005). However, the exact mechanisms by which such unresponsiveness to malignant cells is generated or maintained are not fully understood.

Recently, considerable attention has been given to indoleamine 2,3-dioxygenase (IDO) for its immunosupression function. IDO degrades the essential amino acid

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tryptophan along the kynurenine pathway to form *N*-formyl kynurenine (Takikawa et al. 1986). And subsequently, cell proliferation of alloreactive T cells is thereby arrested in the G_1 phase of the cell cycle via local tryptophan deprivation and the accumulation of toxic, proapoptotic catabolites (Munn et al. 1999). It has been suggested that IDO may be a microenvironmental factor that could play a role in tumor evasion from T cell-mediated rejection (Mellor and Munn 1999; Friberg et al. 2002). Uyttenhove et al. (2003) first demonstrated that IDO was expressed in various human cancer tissues and cell lines, and that IDO was involved in protecting tumors from attack by tumor-associated antigen-specific cytotoxic T cells. In colorectal cancer, Brandacher et al. (2006) showed that IDO-high expression was associated with a significantly reduction of CD3+ infiltrating T cells as compared with tissue samples expressing low IDO, and that IDO-high immunoreactivity also correlated with frequency of liver metastases and poor prognosis of patients. In other malignancies, such as ovarian cancer, non-small cell lung cancer and endometrial cancer, evidences show that the overexpression of IDO was related to poor prognosis of cancer patients (Okamoto et al. 2005; Astigiano et al. 2005; Ino et al. 2006).

Based on above discoveries, in the present study, we investigated IDO expression in a total of 138 tissue samples of HCC by performing immunohistochemical analysis, and demonstrated that high IDO expression was a reliable indicator for disease progression and the poor prognosis of HCC.

Materials and methods

Cell lines and culture

Different human liver cell lines, Hep-G2, Hep-3B, Huh-7, SK-hep1 and Chang liver human normal hepatocytes were obtained from the American Type Culture Collection (ATCC). BEL-7402 and SMMC-7721 cell lines were obtained from the Committee of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Cells were grown on RPMI 1640 supplemented with 10% (v/v) FBS (fetal bovine serum) and antibiotics (50 μg/ml each of penicillin, streptomycin and gentamicin) at 37°C in a humidified 5% CO_2 atmosphere. For analysis IDO expression, 1×10^6 cells were seeded into cell culture plates and cultured for 24 h, and then the IFN-γ (final concentration 750 units/ml) were either added or not added and the cells were cultured for 48 h (Brandacher et al. 2006).

Reverse transcription-PCR

To determine the IDO transcription in human HCC cell lines, total RNA was extracted from cells with TRIzol® reagent (Invitrogen) according to the manufacturer's instructions. Total RNA (1 μg) was reverse-transcribed with 50 units of MMLV reverse transcriptase (Promega) and oligo dT as primer (Takara). The resulting cDNAs were amplified with the following oligonucleotide sequences: IDOF, 5′-TT GCTGTTCCTTACTGCCAACT-3′ (sense), IDOR, 5′-GCC TTTCCAGCCAGACAAAT-3′ (antisense). The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcription was used as an invariant endogenous control. The primer sequences were as follow: GAPDHF: 5′-CGGGAAGCTT GTCATCAATGG-3′ (sense), GAPDHR, 5′-GGCAGTG ATGGCATGGACTG-3′ (antisense).

Tissue samples

A total of 138 human primary HCC tissues were obtained from patients who underwent surgical treatment without prior radiotherapy or chemotherapy treatment at Sun Yat-sen University Cancer Center between 1999 and 2001. All patients in this study belonged to the same ethnic group. All tissue samples were fixed in 10% formalin and embedded in paraffin. The histologic cell types were assigned according to the criteria of the WHO classification.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were cut at a thickness of 4 μm. For heat-induced epitope retrieval, tissue sections were deparaffinized and rehydrated in 0.01 M, pH 6.0 citrate buffer three times at 90°C for 5 min using a microwave oven. Immunohistochemical staining was performed using the streptavidin–biotin immunoperoxidase technique. Endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 in methanol for 15 min, and nonspecific immunoglobulin binding was blocked by incubation with 5% normal rabbit serum for 10 min. Sections were incubated at 4°C overnight with primary antibody (goat anti-IDO at 1:50 dilution, Santa cruz, USA). The sections were rinsed and incubated for 30 min at room temperature with biotinylated second antibody. After washing, the sections were incubated for another 30 min with horseradish peroxidase conjugated streptavidin, and finally treated with 3,3′-diaminobenzidine tetrahydrochloride (DAB) as a substrate for 10 min. The slides were counterstained with Meyer's haematoxylin.

Semi-quantitative evaluation

Staining intensity and percentage of positive tumor cells were assessed for IDO expression by semi-quantitative evaluation of each tissue core. The intensity was scored as negative (0), weak (1+; visible at high magnification), moderate (2+; visible at low magnification), or strong (3+; strikingly

positive at low magnification). The percentage of tumor cells with positive staining were grouped and scored as negative (0, <5%), sporadic (1+, 5–25%), focal (2+, 25–50%) and diffuse (3+, 50–75%). The total score of staining intensity and percentage ranged from 0 to 9, and we defined IDO high expression as a total score >4, and low expression as a total score ≤4.

Statistical analysis

Comparisons of IDO tumor expression with clinical and pathologic features were evaluated by using χ^2 tests. Overall survival analyses were estimated by using the Kaplan–Meier method. The duration of follow-up was calculated from the date of surgery to the date of death, or last known follow-up. Univariate and multivariate analyses were performed to relate IDO expression and histopathologic parameters with HCC patients' survival by using Cox proportional hazards regression model. A two-sided *P* value of less than 0.05 was considered statistically significant. Statistical analyses were performed with the use of the Statistical Package for the Social Sciences, version 15.0 (SPSS Inc, Chicago, IL, USA).

Results

IDO expression in hepatoculluar carcinoma cell lines

First, IDO mRNA expression level was investigated in different human liver cell lines by means of RT-PCR. As shown in Fig. 1, none of HCC cell lines, non-tumor hepatic cell line (Chang liver), constitutively express IDO mRNA without IFN-γ stimulation. However, after stimulation with 750 units/ml of IFN-γ, all cell lines showed induced IDO gene expression.

Immunohistochemical staining of IDO in HCC clinical samples

In order to understand the exact state of IDO expression in vivo, we investigated IDO protein expression by immunohistochemistry in a total of 138 primary HCC specimens. High IDO expression localized in cytoplasm was found in 49 (35.5%) HCC surgical resection samples, whereas the remaining 89 cases (64.5%) displayed weak or undetectable IDO expression (Table 1). IDO expression was detected in tumor cells and adjacent nontumorous tissues. However, distant normal liver tissues did not show positive staining (Fig. 2). The analysis of relationship between overexpression

Table 1 Relationship between IDO expression and clinicopathological features in hepatocellular carcinoma

Features $n = 138$	IDO high (Total score >4) $n = 49$ (35.5%)	IDO low (Total score ≤4) $n = 89$ (64.5%)	*P* value
Age			
<50, $n = 76$	24	52	0.286
≥50, $n = 62$	25	37	
Gender			
Male, $n = 118$	47	71	0.011[a]
Female, $n = 20$	2	18	
Tumor size (cm)			
<5, $n = 66$	26	40	0.361
≥5, $n = 72$	23	49	
Histological differentiation			
Well, $n = 24$	9	15	0.878
Moderate, $n = 80$	27	53	
Poor, $n = 34$	13	21	
Liver cirrhosis			
Yes, $n = 65$	19	46	0.146
No, $n = 73$	30	43	
Metastasis			
Yes, $n = 20$	11	9	0.049[a]
No, $n = 118$	38	80	
Recurrence			
Yes, $n = 44$	19	25	0.197
No, $n = 94$	30	64	
HBSAg status			
Positive, $n = 116$	44	72	0.172
Negative, $n = 22$	5	17	
Serum AFP			
Positive, $n = 57$	17	40	0.242
Negative, $n = 81$	32	49	

[a] *P* value < 0.05

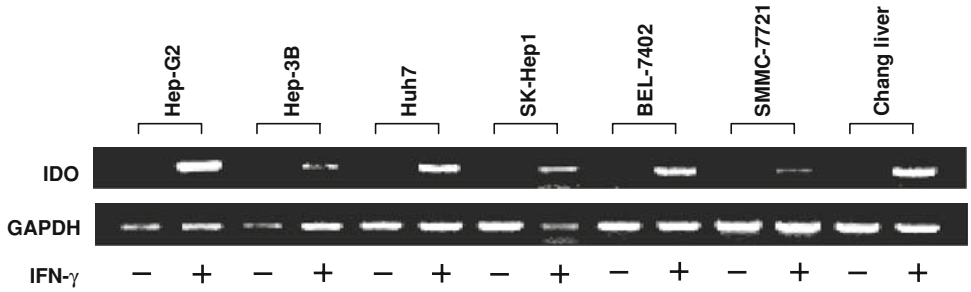


Fig. 1 IDO gene expression in human hepatocellular carcinoma cell lines Hep-G2, Hep-3B, Huh-7, SK-hep1, BEL-7402, SMMC-7721 and normal liver cells line *chang liver* with/without IFN-γ stimulating assessed by reverse transcription-PCR

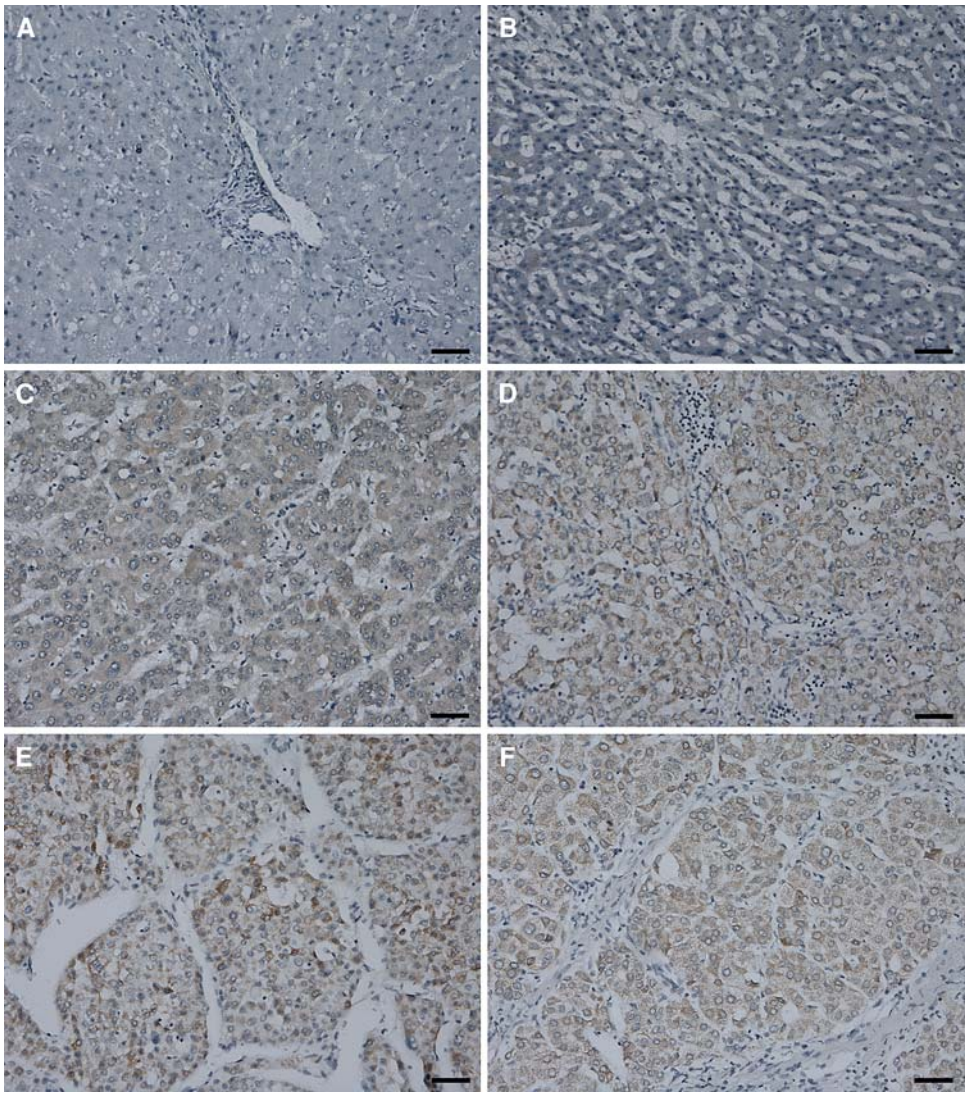


Fig. 2 IDO protein expression in primary hepatocellular carcinoma surgical specimens shown by immunohistochemistry. **a**, **b** distant normal liver tissue; **c**, **d** well differentiated HCC; **e**, **f** moderately differentiated HCC; **g**, **h** poorly differentiated HCC; **I, J**, surrounding noncancerous tissue (original magnification, ×200)

of IDO and various clinicopathological parameters was listed in Table 1. IDO expression was only significantly correlated with gender and metastasis ($P = 0.049$), but not with age, tumor size, tumor grade, cirrhosis, serum AFP, HBSAg and recurrence.

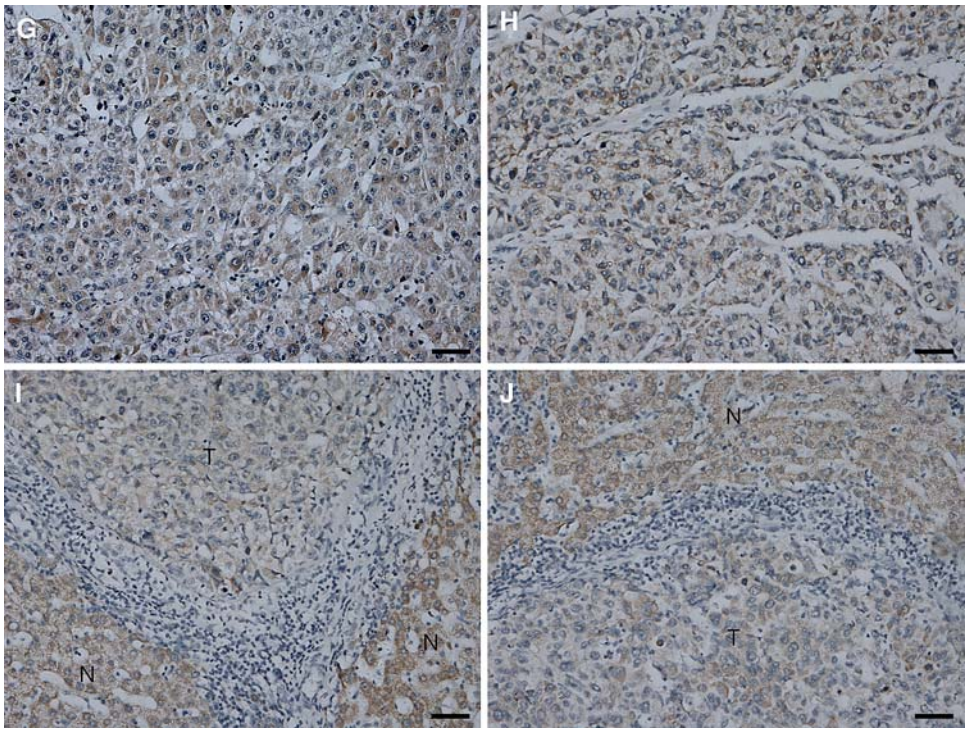
Prognostic significance of high IDO expression

To elucidate the prognostic role of IDO in HCC, overall survival rates were estimated by Kaplan–Meier survival curves (Fig. 3). High IDO expression was significantly associated with the poor prognosis of patients with HCC ($P = 0.017$, log-rank test). Overall survival rate and 5-year survival rate of high-IDO group were significantly lower than low-IDO group [46.1 vs. 22.4% (overall survival rate) and 44 vs. 21% (5-year survival rate), respectively, $P < 0.05$, Fig. 3]. On the other hand, the recurrence rate tends to be higher in the high-IDO group (19/30 cases, 63.3%) than in the low-IDO group (25/64 cases, 39.0%) although the difference was not significant ($P = 0.197$, Table 1).

Cox proportional-hazard model analysis

Univariate and multivariate analysis was further carried out using Cox proportional-hazard model to compare the impact of IDO expression and other clinical and pathological parameters on prognosis of 138 HCC patients. Univariate analysis showed IDO expression, tumor size, serum AFP, metastasis and recurrence which were significant prognostic factors (Table 2). IDO expression was one of the independent predictors of survival ($P = 0.01$, Table 2), as were tumor size ($P = 0.01$) and serum AFP ($P = 0.002$) on multivariate analysis. The relative risk in patients with IDO-high was 1.804 times greater than that of patients with IDO-low (Table 2).

Fig. 2 continued



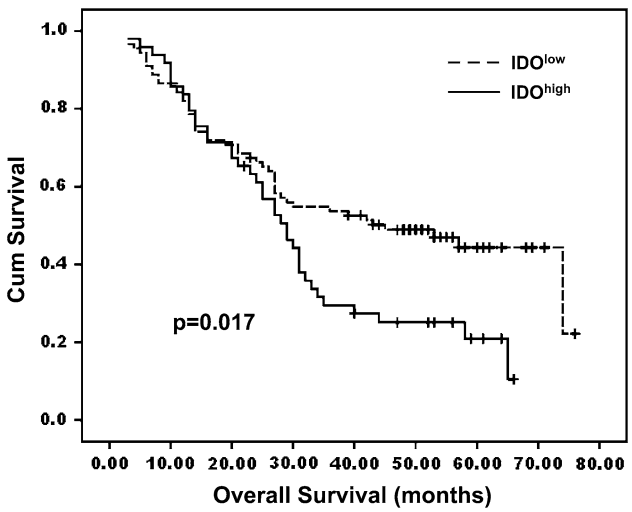


Fig. 3 Kaplan–Meier survival curves for overexpression-IDO group versus low-IDO group in 138 patients with HCC showed a highly significant separation ($P = 0.017$, log-rank test)

Discussion

Recently, IDO overexpression has been showed to contribute to tumor-induced tolerance, depending on its ability of local depletion of tryptophan and increase of its downstream metabolites, causing suppression of cytotoxic T lymphocyte proliferation (Munn and Mellor 2007). There are two potential action sites for IDO-mediated tolerance to tumors. One is that IDO is expressed by host antigen-presenting cells (APCs) in tumor-draining lymph nodes, suppressing T cells responses and inducing antigen-specific T cells energy (Munn et al. 2002; von Bergwelt-Baildon et al. 2006; Lee et al. 2003, 2005; Munn et al. 2004a, b). A second potential site is that IDO is expressed by tumor cells itself, creating local immune tolerogenic microenvironment (Uyttenhove et al. 2003; Brandacher et al. 2006; Astigiano et al. 2005). Furthermore, it has been implicated that IDO might cross talk with T regulatory cells (Tregs), forming a closely interactive network, with Tregs inducing IDO and IDO driving differentiation of new Tregs (Munn et al. 2004a, b; Munn and Mellor 2007; Mellor and Munn 2004; Grohmann et al. 2002; Orabona et al. 2006; Fallarino et al. 2006, 2003; Finger and Bluestone 2002; Curti et al. 2007; Nakamura et al. 2007). These complicated factors result in IDO inducing tumor immune escape.

In the present study, we investigated the IDO mRNA expression in HCC cell lines by RT-PCR analysis and protein expression in primary HCC tissue by immunohistochemical analysis, and evaluated the correlation of IDO expression level and clinical outcome of HCC patients.

From in vitro cell lines study, we found that IDO expression can be induced in HCC cell lines and non-tumor hepatic cell line with IFN-γ stimulation (Fig. 1), suggesting that IDO expression in malignant and nonmalignant liver cells might be responsive to inflammatory environment, because IFN-γ has been shown as an effector cytokine released by tumor-associated antigen-specific T cells (Coussens and Werb 2002). Our results are in line with the findings of previous colorectal cancer studies by

Table 2 Univariate and multivariate analysis of overall survival in hepatocellular carcinoma

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
IDO	1.668	1.085–2.565	0.020[a]	1.804	1.154–2.819	0.010[a]
Age	0.990	0.644–1.521	0.962			
Gender	0.547	0.264–1.133	0.104			
Tumor size	1.978	1.275–3.068	0.002[a]	1.810	1.150–2.848	0.010[a]
Histologic grade	1.383	0.987–1.937	0.059			
Cirrhosis	0.907	0.593–1.387	0.653			
HBSAg status	1.384	0.734–2.610	0.315			
Serum AFP	2.035	1.326–3.124	0.001[a]	2.003	1.290–3.111	0.002[a]
Metastasis	1.753	1.014–3.029	0.044[a]	1.039	0.567–1.904	0.902
Recurrence	1.582	1.024–2.444	0.039[a]	1.383	0.866–2.209	0.174

HR Hazard ratio, *CI* confidence interval

[a] *P* value < 0.05

Brandacher et al. (2006), but not with those of Uyttenhove et al. (2003), indicates that IDO activation with induced way or continual feature might depend on the type of tumor. IDO protein expression in vivo in human HCC tissue samples were further tested by immunohistochemistry; the results showed IDO positive staining was present not only in tumor cells, but also in surrounding noncancerous liver cells. This result further confirmed that both tumor cells and noncancerous liver cells will express IDO protein, which is accorded with cell line results. However, IDO expression was not found in distant normal liver tissues. Thus, increasing IDO expression might be limited in local HCC microenvironment, both in tumor cells and surrounding nontumorous cells, possibly due to the active inflammation.

We further analyzed the interrelation of IDO expression and the clinicopathological parameters in HCC. The IDO expression was significantly higher with metastasis samples (Table 1). Consistently, Ino et al. (2006) and Brandacher et al. (2006) showed that high IDO expression also correlated with metastasis in endometrial cancer and colorectal cancer. That mechanism of IDO expression contributes to tumor metastasis remains to be further elucidated. Brandacher et al. (2006) reported that high IDO expression was associated with a significant reduction of CD3+ infiltrating T cells in colorectal cancer. Recently, Nakamura et al. (2007) demonstrated that some of Foxp3+ Treg cells in metastatic lymph node contacted IDO-expression APC in uterine cervical cancer. These findings suggested that IDO mediating immune suppression activity might contribute to the disease progression of cancer.

Kaplan–Meier survival analysis showed that high IDO expression significantly correlated with shorter survival time of HCC patients (Fig. 3, $P = 0.017$). The 5-year survival rate and overall survival rate of high-IDO group were significantly lower than low-IDO group (Fig. 3, $P < 0.05$). More ever, high IDO expression also showed a tendency to correlate with high recurrence rate (Table 1). This is in keeping with recent reports on other human epithelial tumors such as colorectal cancer, ovarian cancer and endometrial cancer (Brandacher et al. 2006; Okamoto et al. 2005; Ino et al. 2006). Furthermore, univariate analysis showed IDO expression was one of significant risk factor for overall survival of HCC, and multivariate analysis showed IDO expression was an independent predictor of survival for HCC patients (Table 2). These results suggested that the HCC patients with IDO overexpression very probably have a relapse and have poor prognosis, and IDO may become a newly useful prognostic indicator for the HCC. Thus, inhibiting IDO activity might contribute to the application of adjuvant therapy intervention for HCC.

In summary, we demonstrated in this study that both HCC cancer cells and adjacent surrounding noncancerous cells showed increased IDO expression, and this may create an immunosuppressive system, helping tumor cells to avoid immune attack. Overexpression of IDO in HCC patients correlated with disease progression and poor clinical outcomes, and IDO was shown to be an independent prognostic factor for overall survival of HCC patients. Our findings indicate that IDO is a novel prognostic marker for HCC, and may become a new candidate molecular target for therapeutic intervention.

References

Astigiano S, Morandi B, Costay R, Mastracciz L, D'Agostino A, Rattob GB, Melioli G, Frumento G (2005) Eosinophil granulocytes account for indoleamine 2,3-dioxygenase-mediated immune escape in human non-small cell lung Cancer. Neoplasia 7:390–396

Brandacher G, Perathoner A, Ladurner R, Schneeberger S, Obrist P, Winkler C, Werner ER, Werner-Felmayer G, Weiss HG, Gobel G, Margreiter R, Konigsrainer A, Fuchs D, Amberger A (2006) Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. Clin Cancer Res 12:1144–1151

Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867

Curti A, Pandolfi S, Valzasina B, Aluigi M, Isidori A, Ferri E, Salvestrini V, Bonanno G, Rutella S, Durelli I, Horenstein AL, Fiore F,

Massaia M, Colombo MP, Baccarani M, Lemoli RM (2007) Modulation of tryptophan catabolism by human leukemic cells results in the conversion of CD25− into CD25+ T regulatory cells. Blood 109:2871–2877

Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P (2003) Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 4:1206–1212

Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, Orabona C, Bianchi R, Belladonna ML, Volpi C, Santamaria P, Fioretti MC, Puccetti P (2006) The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J Immunol 176:6752–6761

Finger EB, Bluestone JA (2002) When ligand becomes receptor-tolerance via B7 signaling on DCs. Nat Immunol 3:1056–1057

Friberg M, Jennings R, Alsarraj M, Dessureault S, Cantor A, Extermann M, Mellor AL, Munn DH, Antonia SJ (2002) Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. Int J Cancer 101:151–155

Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, Puccetti P (2002) CTLA-4-Ig regulates tryptophan catabolism in vivo. Nat Immunol 3:1097–1101

Ino K, Yoshida N, Kajiyama H, Shibata K, Yamamoto E, Kidokora K, Takahashi N, Terauchi M, Nawa A, Nomura S, Nagasaka T, Takikawa O, Kikkawa F (2006) Indoleamine 2,3-dioxygenase is a novel prognostic indicator for dendometrial cancer. B J Cancer 95:1555–1561

Lee JR, Dalton RR, Messina JL, Sharma MD, Smith DM, Burgess RE, Mazzella F, Antonia SJ, Mellor AL, Munn DH (2003) Pattern of recruitment of immunoregulatory antigen presenting cells in malignant melanoma. Lab Invest 83:1457–1466

Lee JH, Torisu-Itakara H, Cochran AJ, Kadison A, Huynh Y, Morton DL, Essner R (2005) Quantitative analysis of melanoma-induced cytokine-mediated immunosuppression in melanoma sentinel nodes. Clin Cancer Res 11:107–112

Mapara MJ, Sykes M (2004) Tolerance and cancer mechanisms of tumor evasion and strategies for breaking tolerance. J Clin Oncol 22:1136–1151

Mellor AL, Munn DH (1999) Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? Immunol Today 20:169–473

Mellor AL, Munn DH (2004) IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 4:762–774

Munn DH, Mellor AL (2007) Indoleamine 2,3-dioxygenase and tumor-induced tolerance. J Clin Invest 117:1147–1154

Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL (1999) Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med 189:1363–1372

Munn DH, Sharma MD, Mellor AL (2004a) Ligation of B7-1/B7-2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. J Immunol 172:4100–4110

Munn DH, Sharma MD, Hou D, Baban B, Lee JR, Antonia SJ, Messina JL, Chandler P, Koni PA, Mellor AL (2004b) Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. J Clin Invest 114:280–290

Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R Jr, Slingluff CL, Mellor AL (2002) Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 297:1867–1870

Nakamura T, Shima T, Saeki A, Hidaka T, Nakashima A, Takikawa O, Saito S (2007) Expression of indoleamine 2,3-dioxygenase and the recruitment of Foxp3-expressing regulatory T cells in the development and progression of uterine cervical cancer. Cancer Sci 98:874–881

Okamoto A, Nikaido T, Ochiai K, Takakura S, Saito M, Aoki Y, Ishii N, Yanaihara N, Yamada K, Takikawa O, Kawaguchi R, Isonishi S, Tanaka T, Urashima M (2005) Indoleamine 2,3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells. Clin Cancer Res 11:6030–6039

Orabona C, Puccetti P, Vacca C, Bicciato S, Luchini A, Fallarino F, Bianchi R, Velardi E, Perruccio K, Velardi A, Bronte V, Fioretti MC, Grohmann U (2006) Toward the identification of a tolerogenic signature in IDO-competent dendritic cells. Blood 107:2846–2854

Parkin DM, Bray F, Ferlay J, Pisani P (2001) Estimating the world cancer burden: Globocan 2000. Int J Cancer 94:153–156

Takikawa O, Yoshida R, Kido R, Hayaishi O (1986) Tryptophan degradation in mice initiated by indoleamine 2,3-dioxygenase. J Biol Chem 261:3648–3653

Tang ZY (2000) Hepatocellular carcinoma. J Gastroenterol Hepatol 15(Suppl):1–7

Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, Boon T, Van den Eynde BJ (2003) Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 9:1269–1274

von Bergwelt-Baildon MS, Popov A, Saric T, Chemnitz J, Classen S, Stoffel MS, Fiore F, Roth U, Beyer M, Debey S, Wickenhauser C, Hanisch FG, Schultze JL (2006) CD25 and indoleamine 2,3-dioxygenase are up-regulated by prostaglandin E2 and expressed by tumor-associated dendritic cells in vivo: additional mechanisms of T-cell inhibition. Blood 108:228–237

Zou W (2005) Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer 5:263–274