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ORIGINAL PAPER

Down-regulation of β-centractin might be involved in dendritic cells dysfunction and subsequent hepatocellular carcinoma immune escape: a proteomic study

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Abstract
Aims Proteomic study was used to clarify the mechanism of hepatocellular carcinoma (HCC) immune escape concerning Dendritic cells (DCs') dysfunction and their association with HCC invasion.
Methods Human peripheral blood mononuclear cells (PBMCs) derived DCs from healthy donors were pulsed with soluble cell lysates prepared from different metastatic potential human HCC cell lines. The total protein of these DCs was analyzed by two-dimensional electrophoresis and Electro-Spray Mass Spectrometry. The allostimulatoy capacity and phenotype of these DCs were also evaluated. The clinical significance of β-centractin, one of the largest quantitative changed spot, down-regulation in DCs was further evaluated in autologous PBMCs derived DCs pulsed with auto-tumor lysates in 26 HCC patients.
Results The expression of β-centractin was found to be considerably lower either in DCs pulsed with HCCLM6 (high metastatic potential HCC cell line) lysates, accompanied by down-regulation of CD86 molecule and impaired allostimulatory capacity, than those of DCs pulsed with lysates from HCC cell lines with low or without metastatic potential or in DCs pulsed with lysates from HCC with invasiveness than those without invasiveness.
Conclusions The down-regulation of β-centractin in DCs pulsed with high metastatic potential HCC lysates might associate with DCs dysfunction and HCC invasiveness.

Keywords Hepatocellular carcinoma (HCC) · Dendritic cells (DCs) · β-centractin · Proteomics

Abbreviations

HCC	Hepatocellular carcinoma
DCs	Dendritic cell
PBMCs	Peripheral blood mononuclear cells
AFP	Alpha-fetoprotein
APC	Antigen presenting cell
TCR	T-cell receptor

Introduction

The mechanism of hepatocellular carcinoma (HCC) immune escape is intricate, including escape from immune attack by down-regulation or loss of expression of tumor antigen and MHC molecules (Seliger et al. 2000; Gabrilovich 2004), down-regulation of IFN-γ receptor expression (Nagao et al. 2000) and others. Moreover, HCC can suppress host immune system by promoting the expression of Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) in hepatoma cells and enhancing the expression of Fas and TRAIL receptor (TRAILR) in lymphocytes to kill lymphocytes (Li et al. 2005), exerting immunosuppressive and tolerogenic effects on immunocompetent cells through excretion of immunosuppressive factors like IL-10 and TGF-β (Beckebaum et al. 2004), inhibiting differentiation and maturation of dendritic cells

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(DCs) while inducing DCs apoptosis (Kiertscher et al. 2000), inducing DCs dysfunction by down-regulation of CD40 and CD86 molecules, and impairing allostimulatory capacity and decreased production of IL-12 and TNF-α (Um et al. 2004).

The available results of HCC immune escape were mainly originated from in vitro studies (Dunn et al. 2004), however, these studies are different from in vivo studies and cannot reflect the true immune state in the host thoroughly (Srivastava 2000). To elucidate the mechanism of tumor immune escape, the host immune response and their cross-talk are therefore important issues to be understood. DCs' function in initiating, regulating, and maintaining immune responses has been well established (Steinman et al. 2000, 2002). Proteomics' role in describing the complete protein inventory and the dynamics of a living cell also has been well understood. It is of great importance to study the assignment of protein identities which are linked to key biological mechanisms associated with disease processes (Friedman and Fox 2004; Posadas et al. 2005; Feng et al. 2006). This study was aimed to get a further understanding of the mechanism of HCC immune escape concerning DCs and their association with tumor characteristics using proteomic study. To this end, human peripheral blood mononuclear cells (PBMCs) derived DCs from healthy donors were pulsed with soluble cell lysates prepared from human HCC cell lines with different metastatic potential, that were HCC cell line with high metastatic potential (HCCLM6) (Li et al. 2004), with lower metastatic potential (MHCC97L) (Li et al. 2001), and without metastatic potential (Hep3B). The comparison of protein profiles among different DCs indicated that β-centractin expression was considerably lower in DCs pulsed with HCCLM6 lysates. This finding was further confirmed in 26 HCC patients, which mean down-regulation of β-centractin in autologous DCs pulsed with auto-tumor cell lysates was found to be related to invasive characteristics like tumor thrombus and nonintact tumor capsule.

Materials and methods

Generation and phenotype evaluation of DCs

Ethical approval for this study was obtained from research ethics committee of Zhongshan Hospital of Fudan University. Leukocyte concentrate of healthy donors was got from Shanghai Centre Blood Bank, Shanghai, People's Republic of China. A group of 26 consecutive patients with pathologically confirmed HCC at Zhong Shan Hospital, Fudan University was also involved in the study. All patients gave consent for explant tissue and blood sample to be used for research. Briefly, peripheral venous blood samples were isolated by Lymphoprep (Axis-Shield ProC As, Norway, density 1.077 g/ml) after 30 min centrifugation at 800×*g*. Thereafter, DCs were generated and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco Bal, Paisley, UK), 100 ng/ml recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) and 10 ng/ml interleukin-4 (IL-4) (Pepro Tech EC, London, UK) at 37°C in a humidified 5% CO_2 atmosphere. Culture medium containing cytokines was refreshed every 2 days. On day 7, soluble tumor cell lysates and TNF-α 10 ng/ml (R&D system, Minneapolis, MN, USA) were added for another 3-day culture. On day 10, DCs were resuspended, washed thoroughly and incubated with anti-CD1a, CD80, CD83, CD86, and HLA-DR monoclonal antibodies (Becton Dickinson, San Diego, CA, USA) at 4°C for 45 min, followed by incubated with secondary antibodies (Becton Dickinson) for another 45 min. Finally, DCs were harvested and data acquisition and analysis were performed on a FACS Calibur flowcytometer (Becton Dickson).

Preparation of cell lysates and antigen pulsing

Different metastatic potential human HCC cell line HCCLM6 (high) and MHCC97L (low) were established from the same parental cell line (Li et al. 2001, 2004). Non-metastatic HCC cell line (Hep3B) was purchased from ATCC, Manassas, VA, USA. Cell lysates were prepared as described (Kotera et al. 2001; Schnurr et al. 2001; Gregoire et al. 2003; Bohnenkamp et al. 2004). Briefly, confluent cells from different cell lines were first detached, followed by washing three times with Hank's balanced saline solution (HBSS) thoroughly to eliminate supernatant which may contain secreted proteins during tumor cell culture and resuspended at 1×10^7 cells/ml. These cell suspensions were prepared by freeze-thaw cycle that repeated three times in rapid succession, followed by being treated with Branson Sonifier ultrasonic cell disruptor (Branson Ultrasonics Corporation, Danbury, CT, USA) in a 4°C ice bath for 15 s four times. Thereafter, samples were centrifuged at 6,500×*g* for 10 min and the supernatant was filtered using 0.22 μm pore-size filters to rule out contamination of sedimentable particles. The protein concentration was determined by BCA protein assay (Pierce, Rockford, IL, USA). On day 7, tumor cell lysates were added to DCs cultures at a final concentration of 10 ng/ml and DCs were continued to be incubated for 3 days and resuspended, washed thoroughly to minimize contamination of soluble tumor cell lysates for further study.

Twenty-six fresh sterile tumor samples from surgical resections were processed to tumor cell lysates. Sample digestion was completed and the resulting cell suspension was passed through a 70-μm cell strainer. Autologous

tumor cells were collected and cultured at a cell density of 2×10^6 cells/ml for 24 h. Then tumor cells were harvested for preparation of lysates using the same protocol described above.

Generation of T lymphocytes and MLR assay

Nonadherent PBMCs were employed to generate T lymphocytes by passing through a T-Cell Enrichment Column (R&D system). DCs pulsed with different cell lysates, together with nonpulsed DCs, were used for MLR test. In assay of Ag-specific proliferation, T lymphocytes were incubated with allogeneic DCs at a ratio of 20:1(With the least amount of DCs while yielding significant results among different ratios of 4:1, 10:1, and 20:1). The cells were cultured for 4 days and pulsed with 1 μCi/well [^{3}H]-thymidine (New England Nuclear, Boston, MA, USA) for the last 16 h of incubation. Radioactivity was measured by scintillation counting in cpm. All assays cultures were performed in triplicate in round-bottom 96-well plates.

Proteomic analysis of the whole cell extract of DCs

Dendritic cells, nonpulsed or pulsed with lysates from HCCLM6, MHCC97L, and Hep3B were harvested and washed three time with HBSS. Then 2×10^6 DCs were treated with 150 μl cell lysis solution (9 M urea, 4% w/v CHAPS, 40 mM Tris, 40 mM DTT, 2% w/v Pharmylte, and 1 mM PMSF) in an ice bath, followed by being centrifuged at 13,000 rpm 4°C for 45 min and the protein concentration of the supernatant was determined. About 80 μg total cell extracts were used for isoelectric focusing which was performed on 18 cm immobilized pH gradient (IPG) strips (Amersham Pharmacia, San Francisco, CA, USA) using an IPGphor (Amersham Pharmacia) for 60–80 kVh. After focusing, IPG strips were sequentially equilibrated in buffer A (6 M urea, 50 mM Tris, 30% v/v glycerol, 2% SDS, and 1% DTT) and buffer B (6 M urea, 50 mM Tris, 30% v/v glycerol, 2% SDS, and 2.5% iodoacetamide) for 15 min each. The second dimensional separation was done under the Hoefer SE 600 system (Amersham Pharmacia). Two-dimensional gel electophoresis (2-DE) was performed in triplicate for every sample. The silver-stained 2-DE gels were scanned (GS710 scanner, Bio-Rad, Hercules, CA, USA), digitalized and analyzed using PDquest Software (Bio-Rad). Spot detection, quantitation and standardization were carried out for all matched spots. The experiments were repeated three times with similar results.

Interested spots and unstained gel were cut out and the pieces were totally immersed in 100 μl, 100 mM NH_4HCO_3 for 20 min. After being freezing-dried, enzymatic digestion was initiated and the resulting peptides were recovered. The extracts were desalted with ZipTip C_{18} (Millipore, Bedford, MA, UK) and were run through mass spectrometry (MS) analysis. Proteins were identified via automated database searching of all tandem mass spectra against the nonredundant Human International Protein Index protein sequence database (IPI, European Bioinformatics Institute, http://www.ebi.ac.uk/IPI/) using the TurboSEQUEST program in the BioWorks™ 3.1 software suite. For spots identification, an accepted SEQUEST result should have a ΔCn score of at least 0.1 (regardless of charge state). Peptides with a + 1 charge state and a cross-correlation (Xcorr) of at least 1.9 or peptides with a + 2 charge state and an Xcorr ≥2.2 or peptides with a + 3 charge and an Xcorr ≥3.75 were accepted.

Western blot analysis

About 60 μg of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane (Millipore Corp.) at 90–100 mA for 1.5–2 h. Immunostaining was performed with goat polyclonal primary antibodies specific for human *β*-centractin at a dilution of 1:100 (Abcam, Cambridge, MA, UK) for 2 h, followed by incubation with rabbit polyclonal anti-goat IgG-conjugated to horseradish peroxidase (HRP) at a dilution of 1:50 (Abcam) for 45 min. Immunoreactive bands were visualized using ECL reagents (Amersham, Arlington Heights, IL, USA) and exposed to Kodak X-Omat film for 2–10 min.

Immunocytochemistry analysis

Dendritic cells were adjusted to a concentration of 5×10^6 cells/ml and samples of 100 μl were used for immunocytochemistry analysis. The cells were seeded on glass coverslips and fixation was done with acetone at −20°C for 30 min. After rinsed with phosphate buffered saline, sections were permeabilized in 0.1% Triton X-100 for 10 min. Nonspecific sites were blocked with rabbit serum for 1 h. Goat polyclonal primary antibodies specific for human *β*-centractin at a dilution of 1:1,000 (Abcam) were applied overnight at 4°C. Thereafter, sections were incubated with fluorescene isothiocynate (FITC)-labeled rabbit anti-goat secondary antibodies at a dilution of 1:500 (Jingmei BioTech Co. Ltd., Shenzhen, People's Republic of China) for 45 min at 4°C. After final wash, sections were examined under fluorescence microscope (Olympus Japan Co. Ltd., Tokyo, Japan).

Statistical analysis

The expression levels of *β*-centractin were evaluated by the ratio of gray values of *β*-centractin and *β*-actin. Statistical

analyses were performed on SPSS 12.0 analysis software (SPSS, Chicago, IL, USA). The Student–Newman–Keuls test was used for comparison between groups and Paired *T*-test for pairing data. $P < 0.05$ were considered to be significant.

Results

Changes of protein profiles in DCs pulsed with different lysates

The match ratios of the 2-DE gels varied from 84.4 to 90.5%, averaging 85.7% and the correlation coefficients between any two ranged from 0.82 to 0.90 with the average of 0.85, which suggested that the results were well comparable. There were 21 spots exhibited quantitative changes over threefold among DCs pulsed with different lysates and nonpulsed, 12 proteins related to cytoskeleton, signal transduction, cytoplasmic movement, energy metabolism, and some others of which were identified by electrospray ionization mass spectrometry (ESI-MS) (Table 1). It was found that the expression level of *β*-centractin (spot 21) was down-regulated obviously in DCs pulsed with lysates from HCCLM6, a high metastatic HCC cell line, as compared with DCs pulsed with lysates from MHCC97L and Hep3B. The result was representative of three separated experiments.

Western blot and immunocytochemical results of *β*-centractin in DCs pulsed with different lysates

There was nearly no detectable *β*-centractin expression in nonpulsed DCs by Westen blot, with the ratio of gray scale value 0.074. The expression level of *β*-centractin in DCs pulsed with lysates from HCCLM6 was the lowest among the three pulsed groups, while the expression levels of *β*-centractin were significantly high in the other two groups (Fig. 1). Comparing between DCs pulsed with high metastatic potential HCCLM6 and low metastatic potential MHCC97L, the ratio of gray scale values were 0.213 and 1.014, respectively (Fig. 1).

Immunocytochemisty was similar. No fluorescence can be detected in control group and weak staining was found in DCs pulsed with lysates from HCCLM6, while the

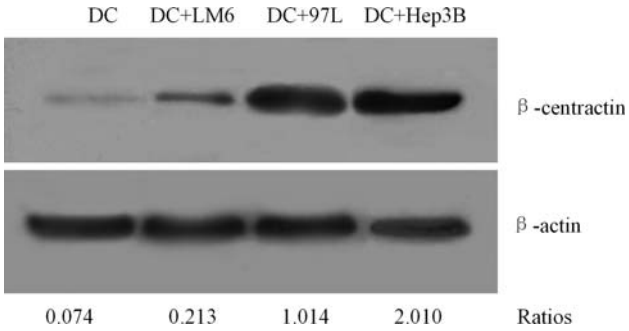

Fig. 1 Western blot analysis of *β*-centractin expression in different DCs, pulsed and nonpulsed. About 60 μg of cellular protein of DCs was transferred to polyvinylidene difluoride membrane. Immunostaining was performed with goat polyclonal primary anibodies specific for human *β*-centractin (at a dilution of 1:100), followed by incubation with rabbit polyclonal anti-goat IgG-conjugated to HRP (at a dilution of 1:50). Immunoreactive bands were visualized using ECL reagents. The expression levels of *β*-centractin were evaluated by the ratio of gray values of *β*-centractin and *β*-actin. There was nearly no detectable *β*-centractin expression in nonpulsed DCs. The expression level of *β*-centractin in DCs pulsed with lysates from HCCLM6 was the lowest among the three pulsed groups, while the expression levels of *β*-centractin were similar between the other two pulsed groups

Table 1 Twelve identified proteins via IPI database searching

Numbers	Estimated MW (Kd)/pI	IPI accession ID	Percent overlap (%)	Products
3	59/5.1	00009856.1	29.5	Keratin type 1 cytoskeletal 10
6	52/5.9	00013847.1	18.4	Ubiquinol-cytochrome C reductase complex core protein I, mitochondrial precursor
8	41.3/6.5	00031411.1	32.2	Cadherin-related tumor suppressor homolog precursor
9	33.4/6.0	00294158.1	26.9	Biliverdin reductase A precursor
10	50/6.2	00470657.1	20.9	Anti-colorectal carcinoma heavy chain
11	38.6/7.6	00455315.2	21.6	Annexin A2 Isoform 2
13	36.4/6.9	00291005.4	6.9	Cytosolic malate dehydrogenase
16	30/4.7	00382894.1	36.2	TPMsK3
17	14/5.3	00032409.1	13.5	Late endosomal/Lysosomal Mp 1 interacting precursor
18	27/7.5	00024057.1	22.4	Transgelin-2
20	25/7.2	00024990.6	19.8	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial precursor
21	43/6.0	00029469.1	28.6	*β*-centractin

cytoplasm, intracellular organelles, cell membrane, and dendrites were strongly positively stained in the other two pulsed groups (Fig. 2).

Morphological, phenotypic, and allostimulatory capacity changes of DCs pulsed with different lysates

On day 10 of culture, these cells formed long, fine cytoplasmatic protrusions typical of mature DCs development under scanning electron microscopy and yielded characteristic DC phenotype over 90% under flow cytometry, which were suitable for subsequent analysis (Supplement data, Table S1). No morphological and cytoskeleton changes were detected in any groups. For DCs pulsed with lysates from HCCLM6, both the expression level of CD86 molecule and the MLR-stimulating capacity were significantly lower than those pulsed with lysates from MHCC97L and Hep3B ($P < 0.05$) (Fig. 3).

Beta-centractin expression in patients' autologous DCs pulsed with auto-tumor lysates

In 26 patients with HCC, the mean of the ratios of gray values (β-centractin/β-actin) was 0.226 ± 0.193 (range from 0.02 to 0.79). The expression levels of β-centractin in DCs pulsed with lysates from HCC with intact capsule or without tumor thrombi were significant higher than those of HCC without capsule or with tumor thrombi, respectively ($P = 0.022$, and 0.045, respectively). But the expression of β-centractin showed no relation to daughter nodules, serum alpha-fetoprotein (AFP) level and tumor size (Table 2).

Discussion

Proteomics describes the complete protein inventory and the dynamics of a living cell and helps to clarify the relationship

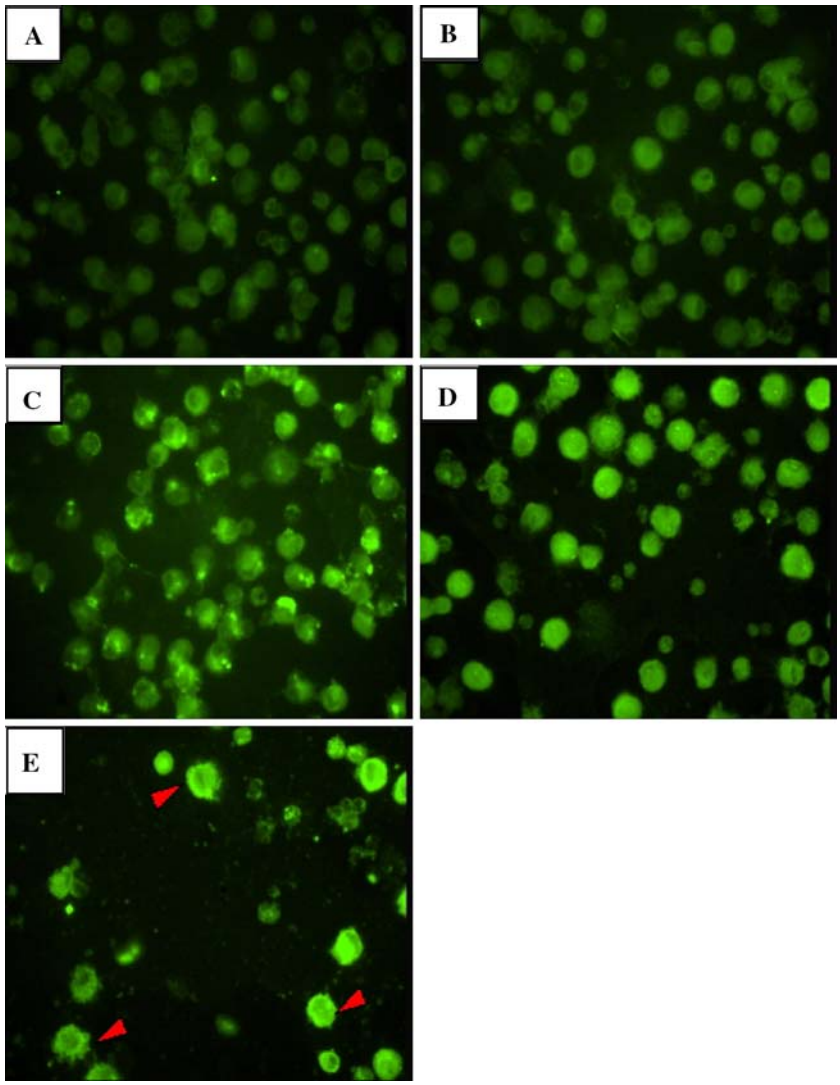

Fig. 2 Immunocytochemistry analysis of β-centractin expression in different DCs, pulsed and nonpulsed. Goat polyclonal primary anibodies specific for human β-centractin (at a dilution of 1:1,000) were applied overnight, followed by incubation with FITC-labeled rabbit anti-goat secondary antibodies (at a dilution of 1:500). Sections were mounted and examined under fluorescence microscope. No fluorescence can be detected in control group and weak staining was found in DCs pulsed with lysates from HCCLM6, while the cytoplasm, intracellular organelles, cell membrane and dendrites were strongly positively stained in the other two pulsed groups. **a** control group (nonpulsed DCs) (×200). **b** DCs pulsed with lysates from HCCLM6 (×200). **c** DCs pulsed with lysates from MHCC97L (×200). **d** DCs pulsed with lysates from Hep3B (×200). **e** DCs pulsed with lysates from Hep3B (×400). *Red arrows* indicate β-centractin expression in dendrites of DCs

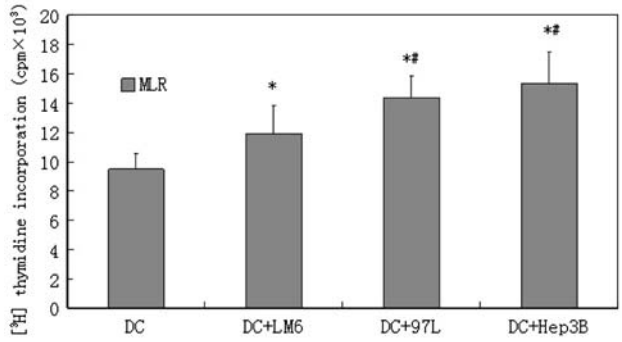

Fig. 3 Impaired allostimulatory capacity of DCs pulsed with lysates from HCCLM6. After antigen pulsing, the MLR-stimulating capacity of the three pulsed groups was greatly enhanced as compared with the control group ($P < 0.05$). DCs pulsed with lysates from HCCLM6 was lowest among the three pulsed groups ($P < 0.05$). *$P < 0.05$ (compared with DC), #$P < 0.05$ (compared with DC + LM6)

Table 2 The relationship between β-centractin expression and clinicopathological characteristics in HCC patients

Characteristics	Numbers	Mean ± SD[a]	*T*-value	*P*-value
Tumor capsule*				
Intact	9	0.342 ± 0.260		
Not intact	17	0.165 ± 0.113	−2.442	0.022
Spreading nodules				
Yes	7	0.173 ± 0.106		
No	19	0.246 ± 0.216	0.850	0.404
Tumor thrombi*[,b]				
Yes	12	0.148 ± 0.104		
No	14	0.294 ± 0.228	2.151	0.045
AFP level				
>20 μg/l	15	0.223 ± 0.157		
≤20 μg/l	11	0.231 ± 0.243	0.105	0.917
Tumor size				
>5 cm[c]	17	0.258 ± 0.187		
≤5 cm	9	0.166 ± 0.200	−1.173	0.252

*$P < 0.05$

[a] The ratios of gray values (β-centractin/β-actin) by Western blot study

[b] Microscopic or macroscopic tumor thrombi

[c] Including cases with over two nodules

between protein expression profiles and their function changes (Friedman and Fox 2004; Posadas et al. 2005; Feng et al. 2006). This study showed that the expression of β-centractin was down-regulated in DCs pulsed with lysates from high metastatic potential HCC cell line HCCLM6, which were phenotypically and functionally defective, that is, low expression of CD86 molecule and low allostimulatory capacity. Furthermore, the expression of β-centractin in DCs pulsed with auto-tumor lysates is negatively correlated to the invasiveness of HCC, that means the expression levels of β-centractin in DCs pulsed with lysates from HCC with intact capsule or without tumor thrombi were significant higher than those without capsule or with tumor thrombi. Generally, in patients with HCC, the generation, differentiation and maturation of DCs were inhibited, CD40 and CD86 molecules of DCs were down-regulated, production of IL-12 and TNF-α of DCs was impaired, and apoptosis of DCs was induced (Kiertscher et al. 2000; Beckebaum et al. 2004; Um et al. 2004). Previous proteomic study on DCs mainly focused on their protein profiles changes before and after differentition or maturation (Richards et al. 2002; Pereira et al. 2005). But none have reported mechanisms of HCC immune escape involving changes in DCs' protein expression profiles. It's interesting to note that in our study the expression difference was particularly obvious between DCs pulsed with lysates from HCCLM6 and MHCC97L HCC cell line (Li et al. 2001, 2004). So, we suggested that the higher the invasiveness of the cancer was, the lower the expression of β-centractin and the higher inhibitory degree of DCs would be.

Beta-centractin, a subgroup of centractin, was first identified by Clark and Meyer (1992) and Clark et al. (1994). Centractin, involved in many pathological and physiological processes, is a major component of the dynactin complex and constitutes most of its functional domains (Way and Weeds 1990; Lees-Miller et al. 1992; Plamann et al. 1994; Schroer 1994; Schafer et al. 1994; Vallee and Sheetz 1996; Holleran et al. 1996; Bingham and Schroer 1999; Grakoui et al. 1999; Al-Alwan et al. 2001, 2003; Eaton et al. 2002; Cuadrado-Tejedor et al. 2005; Dustin et al. 2006). Available data on centractin and dynactin are generally concerning their structure (Clark et al. 1994; Holleran et al. 1996), gene location (Elsea et al. 1999), and physiological function (Clark and Meyer 1999; Aspengren and Wallin 2004). We are unaware of any publication demonstrating its relationship with tumor. Based on our study, almost no expression of β-centractin was detected in nonpulsed DCs and the expression level of β-centractin in DCs pulsed with lysates from HCCLM6 was the lowest, while the cytoplasm, intracellular organelles, cell membrane, and dendrites were strongly positively stained in the other two pulsed groups. This result indicated that down-regulation of β-centractin might affect transport of substances in DCs. As for allostimulatory capacity, although response was induced after being pulsed with lysates from HCCLM6, it still was the lowest. These suggested that HCC can escape from immune surveillance through causing either phenotypical or functional defective in DCs. The mechanism underlying this is still far from being understood, we hope that down-regulation of β-centractin in DCs may be an important factor.

Immunological synapse is recognized as the structural base for immunological responses (Dustin et al. 2006). Activation of the adaptive immune occurs as a result of the

interaction of T-cell receptors (TCRs), an important part of immunological synapse (Grakoui et al. 1999; Bromley et al. 2001; Creusot et al. 2002). The immunological synapse serves to enhance and sustain signaling through the TCRs for long periods of time (Lanzavecchia and Sallusto 2001). As an actin-related protein, β-centractin has an important role in the formation and stabilization of immunological synapse (Andrews et al. 2005). In neurons, it was reported that alterations of centractin expression could compromise the cytoskeletal superstructures at postsynaptic size (Cuadrado-Tejedor et al. 2005). Furthermore, costimulatory molecules, such as CD80 and CD86 are essential for immunological synapse and T-cell activation (Pardoll 2002). In addition to TCRs signaling, T-cell activation also requires accessory molecules such as actin cytoskeleton and plasma membrane lipid rafts (Pizzo and Viola 2005). We suggested that down-regulation of β-centractin could interfere with the formation of immunological synapse between DCs and T-cells through B7 molecules and their receptor on T-cells. In addition, the identified 12 proteins exhibited in 2-DE were related to cytoskeleton, signal transduction, cytoplasmic movement, and energy metabolism, which was in accordance with others (Richards et al. 2002; Pereira et al. 2005). Using proteomic analysis, Richards et al. (2002) and Pereira et al. (2005) also found protein profiles changes involving signal pathways, cell adhesion, cytoskeleton, motility, and metabolism during DCs differentiation and maturation. So, these expression changes would inevitably have impacts on DCs function and do bring us the speculation that down-regulation of β-centractin may cause dysfunction of DCs pulsed with HCC.

In conclusion, we described for the first time that DCs pulsed with lysates from high metastatic HCC cell line were phenotypically and functionally defective, with the concurrent down-regulation of β-centractin expression. In patients with HCC, we also found that down-regulation of β-centractin in DCs pulsed with auto-tumor lysates was negatively correlated to invasiveness of HCC. Considering DCs' crucial roles in tumor immunology (Slingluff Jr et al. 2006), HCC might impair DCs through down-regulation of β-centractin expression.

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