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Fucosylated Glycans Associated With Development and Metastasis of Hepatocellular Carcinoma Cells*

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Abstract Dynamic fucosylation of glycoprotein especially 80 ku which bound to UEA and LCA during the course of rat hepatocarcinogenesis was investigated. In patient hepatocellular carcinoma, more UEA- and LCA-bound proteins were also observed in patients with high metastatic potential than those with low metastatic potential. Fucosylated glycans constitute important adhesion molecules such as Lewis antigens. A differential expression pattern of Lewis antigens was further confirmed on various metastasis potential hepatocellular carcinoma cells (HCC). High metastatic hepatocellular carcinoma cell line (HMCC97H) expressed much more Lewis x and b than low metastasis HMCC97L cells. Moreover, surface Lewis x, or b expression level declined significantly after the cells were treated by retinoic acid. Not only in experimental metastasis foci, but in HCC as well, both α1,3/1,2 and α1,6 fucosyltransferase activities were quite high. After retinoic acid treatment, α1,3/1,2 fucosyltransferase activities were significantly inhibited, Lewis x on epidermal growth factor receptor reduced, and the EGFR was less phosphorylated. These results suggested that fucosylated glycans such as Lewis x played an important role in HCC development and metastasis.

Key words liver cancer, Lewis antigens, metastasis
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Like sialic acid and sulfo groups fucose is regarded as the terminal residue in the structure of oligosaccharides for the synthesis will end after the fucosylation. Fucosylated glycans can be found in many oligosaccharides and can also be seen in peptides by direct linkage in mammalian cells. In human, α1,6 fucosylation protects glycans against glycoasparaginase-catalyzed hydrolysis and is required for polysialylation [1]. Fucosylated glycans have been found biological functions such as cell recognition [2], wound repair of human airway epithelial cell monolayers [3], cell attachment or rolling [4] and growth factor receptor activation [5]. Fucosylated glycans are often altered in cancer cells compared with the normal cells. Altered carbohydrate in integrin may reduce α5β1 integrin clustering and get involved in the process of tumor cell migration [6]. Fucosylated glycans vary with tissue type and the discrepancy or microheterogeneity of the glycan depends on the tissue, development, inner condition, and cell functions. Fucosylation structure of glycans is primarily determined by the balance and availability of fucosidases and fucosyltransferases besides glycan accessibility. In patients with hepatocellular carcinoma, the activities of the fucosidase as well as fucosyltransferases [7] are usually abnormally active, which are relevant to the structure remodeling of the oligosaccharides in hepatocellular carcinoma cells (HCC). The remodeled glycans may be related to some malignant behavior of HCC. Our previous study showed that cell surface fucose was involved in cell

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migration[8] and reduced core fucose in E-cadherin[9,10] could enhance cell aggregation and impair cell migration. Fucose residues can be a key component of some important epitopes such as Lewis antigens (Le^a, Le^x, sLe^a and sLe^x, etc) and might be involved in the adhesion or migration process of hepatocellular carcinoma cells [11]. However whether fucosylated glycans are associated with metastasis of HCC remained unclear. In this paper we mainly studied the correlation between fucose moieties in glycoproteins with hepatocarcinogenesis and metastasis potential.

1 Materials and methods

1.1 Materials

Ulex europaeus agglutinin (UEA), lens culinaris agglutinin (LCA), aleuria aurantia lectin (AAL) and biotin-conjugated UEA and LCA, and alkaline phosphatase-conjugated avidin (avidin-AP) were from Vector Laboratories Inc.(USA). N-nitrosodiehtykamine (DEN), all-trans retinoic acid (ATRA), α-L-fucose, acrylamide, *p*-nitrophenyl phosphate (pNPP), o-phenylenediamine dihydrochloride (OPD) were from Sigma (USA). Goat anti mouse immunoglobulins, avidin-conjugated alkaline phosphatase or horse radish peroxidase (HRP) were from Sino-American Biotechnology Co.(China). Antibodies against Lewis a,b,x,y and sialyl Lewis x were from Calbiochem (USA) and antibody against EGF receptor was from Thermo (USA). Lab-Tek Ⅱ chamber slide was from Nalge Nunc Int. (IL,USA). GlycoTAG Reagent kit, PLASTATION Pyridylationation Reagent kit were from TAKARA (Japan). RPMI-1640, DMEM culture medium was bought from GIBCO (USA). Bicinchoninic acid (BCA) protein assay reagent kit was from PIERCE (USA). Wistar rats were from Animal Center of Shanghai Medical College, Fudan University. Nude mice Balb/c were from SLE company (Shizuoka,Japan). Hep3B, a human hepatocellular carcinoma cell line, was from ATCC (American type Culture Collection). HMCC97H (high metastasis potential hepatocellular carcinoma cells) and HMCC97L(low metastasis potential hepatocellular carcinoma cells) cells were kindly provided by Dr.Ye Li, Zhong Shan Hospital, Fudan University[12,13].

1.2 Methods

1.2.1 DEN-induced rat hepatocarcinogenesis. Studies had been previously approved and monitored by the local ethics committee before the animal experiment and all the performance of operation on the mice was according to the guide line of National Institute of Health, USA. The method of hepatocarcinogenesis induction and histological confirmation was according to our previous report[14]. Wistar rats, female, 4 week age, were fed with 70 mg/kg DEN once a week until the sixteenth week. Four rats were put to death weekly under anesthesia. Liver tissues and the suspected carcinomatous tissue were selected for histological examination and protein sample preparation as our previous report[15].

1.2.2 Preparations of membrane protein. Cultured hepatocellular carcinoma cells or rat hepatic tissues or samples from patients with hepatocellular carcinoma (from Liver Cancer Institute, Zhong Shan Hospital, Shanghai Medical College, Fudan University) were homogenated and lysed with 1 ml buffer (0.1 mol/L Tris-HCl pH 7.2, 0.32 mol/L sucrose, 1 mmol/L DTT, PMSF and EGTA) after being washed. These homogenates were centrifuged at 800 *g* for 15 min at 4℃. The supernatant was centrifuged at 100 000 *g* for 1 h at 4℃. The pellets were dissolved in 0.1 ml buffer containing 0.1 mol/L Tris-HCl pH 7.2, 2 mmol/L DTT, 1 mmol/L PMSF, and 1 mmol/L EGTA, which was used as membrane fraction protein source.

1.2.3 Lectin blot. The membrane proteins were quantified using a BCA kit at 562 nm and loaded onto 10% SDS-PAGE at 100 μg per lane. The proteins in gel were blotted onto a nitrocellulose (NC) membrane after electrophoresis. The NC membrane was then blocked in TBST (10 mmol/L Tris-HCl pH 8.0, 150 mmol/L NaCl, 0.05% Tween-20) containing 3% BSA at room temperature for 2 h. Then the blot was incubated in TBST containing 100 μg biotin-conjugated UEA or LCA for 2 h at room temperature and was washed 3 times with 50 ml of TBST. After being incubated with avidin-HRP (1∶100 dilutions) for 1 h at room temperature, the membrane was washed 3 times with TBST, incubated with ECL and exposed to an X-ray film.

1.2.4 Cell ELISA. Hepatocellular carcinoma cell lines were treated with 10×10^{-6} mol/L retinoic acid for 48 h. Then, the cells were transferred to 96-well plates at the concentration of 3×10^4/well for further 12 h incubation. The plate was washed twice with PBS and fixed with 4% paraformaldehyde in PBS. Interior H_2O_2 or alkaline phosphatase was saturated and consumed by their substrate pretreatment respectively. For lectin binding, the plate was incubated with biotin-conjugated UEA or LCA for 1 h after blocking with 1% blocking

reagent. Washed with PBS, the cells were incubated with avidin-AP for 1 h at room temperature. Chromogen pNPP was added into the wells after the cells were washed. The plate was incubated at 37℃ 1 h and the *A* value was read at 450 nm. For monoclonal antibody binding, blocked with 1% blocking reagent in PBS overnight at 4℃, the plates were incubated with primary antibodies against Lewis a, b, x, y, or sialyl Lewis x for 2 h at room temperature, and then with horse radish peroxidase-conjugated goat anti-mouse immunoglobulins after washed with PBS containing 0.05% Tween 20 3 times. The color was developed by HRP substrate OPD and the value was read at 490 nm. All the data were expressed as mean and standard deviation. The experiment was repeated independently 4 times.

1.2.5 Immunological staining. Cells were incubated in the Lab-Tek chamber slides for 1～3 days, washed with PBS 3 times and incubated with 1% blocking reagent in PBS for 2 h at room temperature. Then the cells were sequentially incubated with primary antibody (anti-Lewis x, y, a, b, sialyl Lewis x monoclonal mouse IgGs in 1 ∶10 dilution) and FITC-conjugated Goat anti-mouse IgGs and DAPI (4′, 6-diamindino-2-phenyindole, dilactate). The slide was loaded with mounting medium and observed under an Olympus fluorescent microscope to count the positive cells out of 200 cells or a laser scanning confocal microscope (MPL LSM 510) to acquire the image with the software of LSM 510 version 2.5 SP2.

1.2.6 Flow cytometric analysis. After culture, cells were collected by a rubber policeman and washed 3 times with PBS (pH 7.4) containing EDTA and filtered through a fine mesh to remove cell clumps. For the analysis of cell surface antigen expression, cells were incubated with a primary antibody (mouse against Lewis x, y, a, b, and sialyl Lewis x) first after washing and blocking. Then the cells were incubated with FITC-conjugated goat against mouse immunoglobulins, or FITC-conjugated lectin (AAL, LCA, UEA). At least 20 000 cells were acquired and analyzed for the fluorescent signal. The data were expressed as means fluorescence intensity.

1.2.7 Animal metastasis. Studies had been previously approved by the local ethics committee and all the performance of operation on the mice was according to NIH guidelines. The animal metastatic model was established according to the previous report [16,17]. All mice were maintained in a germ-free room with air and temperature control. Careful consideration was taken not to expose the animals to unmotivated suffering. Cell suspension containing Hep3B 5×10^6 was injected into the spleens of Balb/c nude mice under direct sight. 70 days later, the mice were killed under anesthesia and the livers, tumor mass, and lymphoid nodules were collected for sample preparation.

1.2.8 α1,6 fucosyltransferase assay. The assay of α1,6 fucosyltransferase was according to the reference[18]. Briefly, all sample cells were lysed in the buffer consisted of 20 mmol/L Hepes pH 7.4, 0.5 mmol/L DTT, 0.2 mmol/L PMSF, and 1% Tween-20 by sonication on ice for 10 min after collection and washing with PBS three times. The substrate oligosaccharide-asparagine (GnGn-bi-Asn) was pre-labeled with 4-(2-pyridylamino)butylamine, which was a gift kindly provided by professor Taniguchi, Department of Biochemistry, Osaka University, Japan. GDP-fucose was used as the donor substrate. After the reaction at 37℃ for 1 h, the substrate and product were separated by the column of TSK gel ODS 80T in HPLC. The fluorescent signal was monitored with the excitation wavelength at 320 nm and emission wavelength at 400 nm.

1.2.9 α1,3/1,2 fucosyltransferase assay. The sample enzyme was obtained by the same method as used in α1,6 fucosyltransferase assay. The reaction system consisted of 50 mmol/L MES buffer (pH 6.5), 5 mmol/L ATP, 25 mmol/L $MnCl_2$, 10 mmol/L L-fucose, 75 μmol/L GDP-fucose, 25 μmol/L Galβ1-4GlcNAcβ1-3Galβ1-4Glc-pyridylamine. The substrate Galβ1, 4GlcNAcβ1, 3Galβ1, 4Glc used in this system was prelabeled with aminopyridine fluorescence by GlycoTAG reagent kit. The reaction was carried out in a total volume of 10 μl at 37℃ for 1 h, and then terminated by boiling for 3 min after adding 20 μl Milli Q water. All the samples were centrifuged at 15 000 r/min for 15 min. The supernatant 10 μl was injected onto a column of TSK gel ODS 80 TM (1.5 mm × 250 mm, Tosoh, Japan) with elution of 20 mmol/L ammonium acetate (pH 4.0), 1 ml/min. The peak of the product was measured by HPLC (Shimadzu SPC-6AV).

2 Results

2.1 Fucosylated glycoproteins during hepatocarcinogenesis

Fucosylation was analyzed in membrane proteins

from DEN-induced rat hepatocarcinoma which was confirmed by histological examination (data not shown). UEA and LCA lectins were both employed for the detection of fucosylated glycoproteins. Compared with the samples from the rats week 0 (before DEN induction), UEA blot results showed that the fucosylated glycoprotein at band of 80 ku increased remarkably and got to its summit at week 17(Figure 1a), while other bands decreased. LCA blot also showed that glycoprotein 80 ku increased along with the hepatocarcinogenesis and got to its summit at weeks 15～17, about 2.34 times higher than that of week 0, while the protein bands decreased after the induction, especially during 2～9 weeks, and increased again during 15～17 weeks.

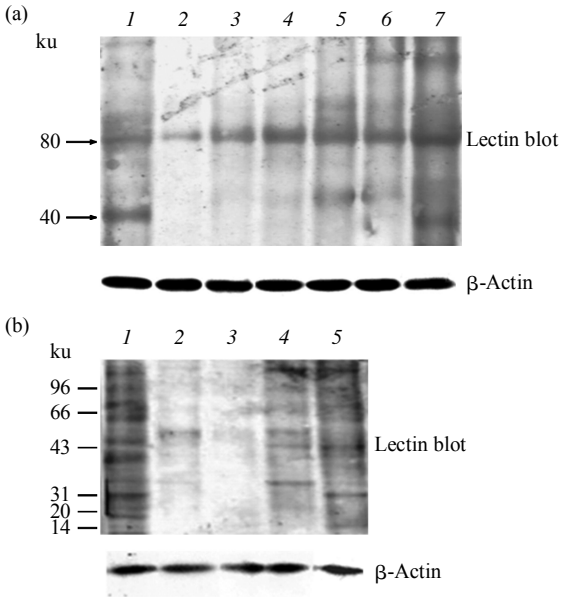

Fig. 1 Fucosylated glycoproteins in rat hepatocarcinogenesis induced by DEN

(a) UEA lectin blot analysis of membrane proteins of rat hepatocarcinoma tissues. Samples were from the rat liver in every 2 weeks. *1*, *2*, *3*, *4*, *5*, *6* and *7*: Samples from week 0, 3, 5, 7, 9, 11 and 17 respectively. Standard molecular mass was indicated by arrows. (b) LCA lectin blot analysis of the membrane proteins. *1*: Liver mass from a patient with highly metastatic hepatocellular carcinoma; *2*: Adjacent liver tissue in the same patient as lane *1*; *3*: Liver mass from the patient with low metastatic hepatocellular carcinoma; *4*: Adjacent liver tissue in the same patient as lane *5*; *5*: Liver mass from another patient with high metastatic hepatocellular carcinoma.

2.2 Fucosylated glycoprotein in patient HCC with various metastasis potentials

Glycoprotein fucosylation of cell membrane fraction was also investigated in patients with hepatocellular carcinoma with various metastasis potentials. The results of LCA blot was shown in Figure 1b and indicated that more LCA binding protein bands were seen in hepatocellular carcinoma with high metastatic potential than in those with low metastatic potential. Moreover some bands (68 ku, 85 ku) were expressed higher in high metastasis HCC samples than in low metastatic potential samples. The adjacent liver tissues had fewer bands and lacked the bands at about 80 ku as compared with the hepatoma samples. UEA-blot also showed a similar result which indicated that the samples from patients with high metastatic potential had much more bands than those from low metastasis potential. The peripheral tissues surrounding hepatocellular carcinoma had only a few bands. UEA lectin mainly binds α1,2 fucose residues and LCA lectin binds to core α1,6 fucose residues in N-glycan. These results suggested there were abundant α1,2 and α1,6 fucosylated glycans in hepatocarcinoma with high metastatic potential.

2.3 Fucosylation after ATRA treatment

Retinoid acid was found to inhibit hepatocellular carcinoma cells in multiple pathways. In this study we treated hepatocellular carcinoma cell lines with ATRA and investigated the surface fucosylation. In result of cell ELISA assay, the surface fucosylation of hepatocellular carcinoma cells decreased significantly after 24 and 48 h treatment. The lectin binding of either UEA or LCA decreased significantly in a time-dependent manner (data not shown). We further investigated the means fluorescence intensity of lectin binding on individual cell by a flow cytometer. The data in Figure 2a showed the means fluorescent intensity of UEA binding was significantly lower than control after the treatment on the average cell.

2.4 Fucosyltransferase activities after ATRA treatment

Fucosyltransferases were assayed in Hep3B cell to evaluate the enzyme activity regulation after the cells were treated with ATRA for various times. Within half an hour of ATRA treatment, both α1,6 FucT and α1,3 FucT activities in Hep3B cells were significantly inhibited as compared with the control. However after 48 h treatment, the activity of α1,6 FucT in Hep3B cells returned to the previous level, while α1,3/1,2 FucT activities which were responsible for Lewis antigen synthesis were still significantly lower than the control (Figure 2b).

2.5 Fucosyltransferase activities in metastatic tissues

In the metastatic animal models, 4 out of 12 nude mice had intrahepatic metastasis and 5 had abdominal lymph nude metastasis. All these metastatic foci were isolated and prepared as the enzyme source for fucosyltransferase assays. The results (Figure 2c) showed that α1,6 FucT activity in metastasis foci of the liver were significantly higher than the tissue without metastasis($P < 0.05$), and the activity in metastatic lymph nudes significantly higher than normal tissues ($P < 0.01$). The activities of α1,3/1,2 FucT in liver metastasis foci as well as in lymph nude metastasis tissues were significantly higher than those without metastasis ($P < 0.05$) (Figure 2c). These suggested that the metastasis foci had high activities of both α1,3/1,2 and α1,6 FucT.

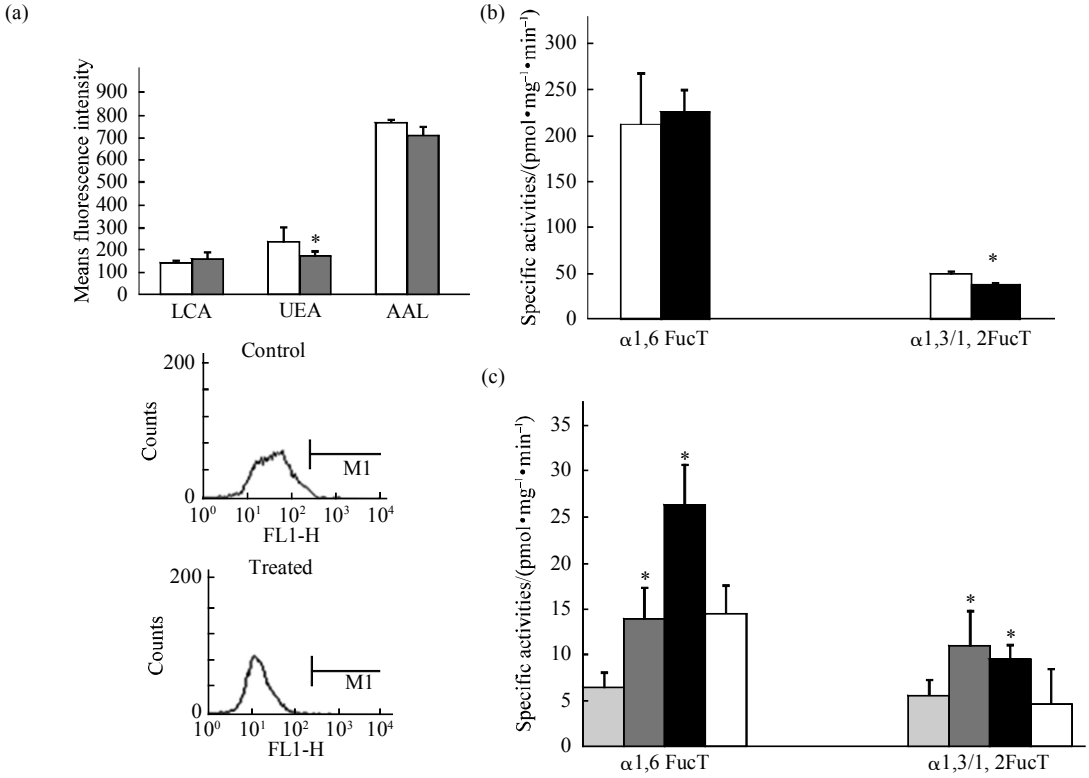

Fig. 2 Fucosyltransferase activities

(a) Expression inhibition of fucosylated sugar chains on HCC by retinoic acid. Hep3B cells were treated with 10 mol/L ATRA and analyzed by FACS for cell surface binding in means fluorescent intensity. Four independent experiments were repeated. Comparison was made by Student t test between treated cells and control cells. * $P < 0.05$. Low panel was the result of UEA staining analyzed by FACS. □: Control; ▩: Treated. (b) Fucosyltransferase activities in Hep3B cells treated by ATRA for 48 h were compared with control. The activities of α1,3/1,2 fucosyltransferase decreased significantly. Four independent experiments were repeated.* $P < 0.05$. □: Control; ■: ATRA. (c) Fucosyltransferase activities in the tissues with and without metastasis were observed compared. The activity of α1,6 fucosyltransferase in metastatic liver tissues was significantly higher than that in tissues without metastasis ($P = 0.022$, $n = 4$). The activities of α1,3 /1,2 fucosyltransferase in liver tissues with metastasis were also much higher than those without metastasis ($P = 0.0169$, $n = 4$). ▩: Liver without metastasis; ▩: Liver with metastasis; ■: LN with metastasis; □: LN without metastasis.

2.6 Lewis antigens on HCC with various metastasis potentials

Lewis antigen contains fucosylated tri or pentasaccharides in α1,2, α1,3, or α1,4 fucose structure. The expression of Lewis antigen is considered to be important adhesion epitope. We therefore investigated Lewis antigens on hepatocarcinoma cells with various metastasis potentials, and observed Lewis antigen expression differentially on various metastasis potential tumors (Figure 3). On the surface of MHCC97 cells, mainly Lewis x and y were expressed. The expressional levels of Lewis x or b were much higher on MHCC 97H cells (high metastasis HCC) than those of MHCC97L cells (low metastasis HCC) (Figure 3b). Lewis x and b contain a common α1,3 fucose although their backbone is different in structure.

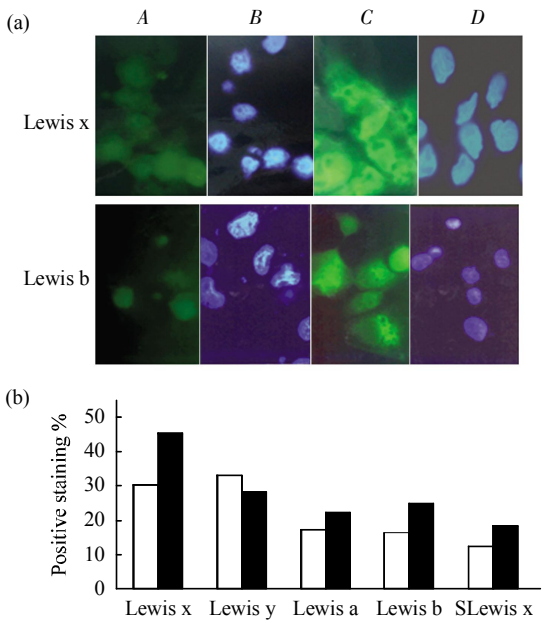

Fig. 3　Differential expression of Lewis antigens on HCC with various metastasis potentials

(a) Lewis x and b on both MHCC 97L and MHCC 97H cells. *A*: FITC staining of Lewis x ,b on MHCC 97L cells; *B*: DAPI staining on MHCC 97L cells; *C*: FITC staining of Lewis x, b on MHCC-97H cells; *D*: DAPI staining on MHCC 97H cells. (b) Positive staining of Lewis antigens was counted as percentage on HCC with various metastasis potentials. The experiment was repeated independently for 3 times. □: MHCC 97L; ■: MHCC 97H.

2.7　Lewis antigen expression after ATRA treatment

We then further observed the cell surface expression of Lewis antigens after ATRA treatment by both cell ELISA assay and flow cytometry. Hepatocarcinoma cells, Hep3B were treated with ATRA for 48 h and assayed by cell ELISA. The binding results indicated that Lewis x and y were the main epitopes on Hep3B and the binding levels of Lewis x or Lewis b were significantly suppressed on the surface (Figure 4a) after ATRA treatment. The binding level of Lewis a was also lower than the control although no statistics significance, but Lewis y or sialyl Lewis x binding did not change significantly after the treatment. By flow cytometer analysis, the results also indicated that Lewis x and y were higher than other Lewis antigens. After ATRA treatment mainly Lewis x and Lewis b expression reduced (Figure 4b). Reduced Lewis x unevenly distributed on the membrane of Hep3B (Figure 4c, d). Lewis x epitope as well as core fucose was further observed on epidermal growth factor receptor (EGFR) in HCC through immunoprecipitation analysis (Figure 4e), but Lewis b not. After ATRA treatment Lewis x on EGFR reduced remarkably and did the phosphorylated EGFR

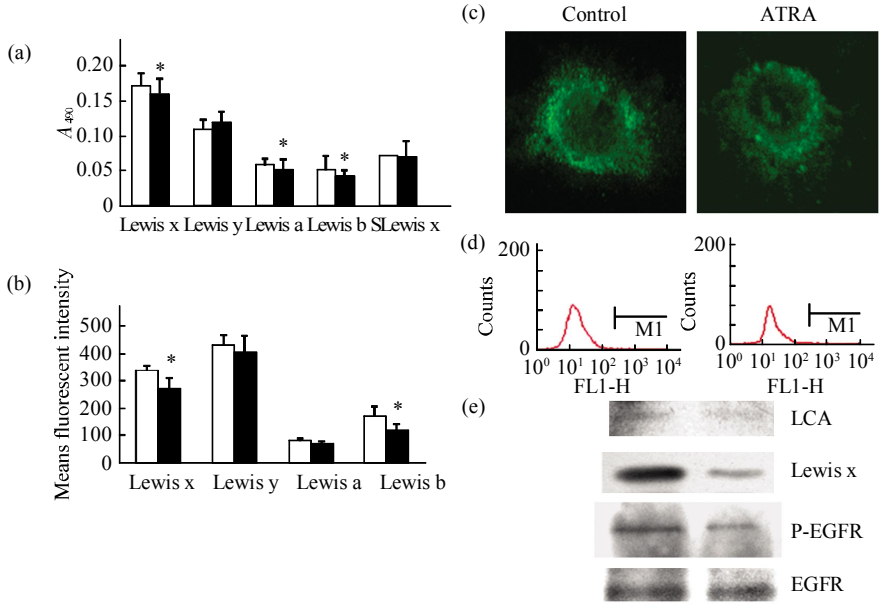

Fig. 4　Lewis antigen expression on HCC was regulated by retinoic acid

(a) Hep3B cells were treated with ATRA and seeded in 96-well plate and then stained with monoclonal antibodies against Lewis x, y, a, b, and sialyl Lewis x and the data were expressed as A_{490} ($\bar{x} \pm s$). Four independent experiments were repeated. □: Control; ■: Treated. (b) To observe Lewis antigen expression on individual cells, HCC were analyzed by the flow cytometer to indicate means fluorescent intensity on average cells after the treatment. □: Control; ■: Treated. (c) Hep3B cells were stained with anti-Lewis x monoclonal antibody and subsequently with FITC-conjugated goat anti-mouse Ig M. The stained cells were then observed under a laser scanning confocal microscope. (d) The cells were further analyzed by flow cytometry for the surface Lewis x expression and the result showed that the fluorescence signal declined in ATRA-treated cells. (e) The cell lysates were immunoprecipitated by the antibody against EGFR and blotted by antibodies against phosphorylated tyrosine and Lewis x and biotinylated LCA respectively.

(Figure 4e) which was the activated form of the receptor.

3 Discussion

The fucose residue, a part of oligosaccharides which has important bioactivities, is regarded as one of the terminal structures of oligosaccharides[19]. As long as the fucose residue is transferred, the oligosaccharides synthesis will usually stop. Cooperation between fucosidase and fucosyltransferase can alter the structure of glycans and remodel oligosaccharide moieties of tumor cells to adapt to the functional need of the carcinoma behaviors. Patients who suffered from hepatocellular carcinoma often have high activities of fucosidase and fucosyltransferase[20, 21]. We took advantage of the rat hepatocarcinogenesis model induced by DEN to observe the expression of the membrane glycoproteins containing fucose of liver tissue and the result showed that the fucosylated glycoproteins dynamically changed along with the hepatocarcinogenesis course both in UEA and LCA blot. For LCA mainly binds to core fucose of N-glycan, while UEA mainly binds to α1,2 fucose residues. The oligosaccharides of glycoprotein at 80 ku may contain either outer chain fucose or core fucose residue of N-glycan. In patients with hepatocellular carcinoma, there also was a relationship between fucosylated glycans and HCC metastasis, and those with highly metastatic potential had much more fucose-containing glycoproteins expressed. To know the fucosylation epitopes on cell surface further, we investigated cell surface Lewis antigens and observed a differential expression pattern of Lewis antigens on various metastasis potential cancer cells. Lewis x and b were more highly expressed on high metastasis HMCC97H than those on low metastasis HMCC97L. After inhibition by retinoic acid, cell surface lectin binding of UEA and Lewis x or Lewis b expression level declined significantly.

Fucose residues constitute some important oligosaccharides[22] such as Lewis antigens which can recognize selectin and other ligands and induce the adherence between leucocytes or platelets and endothelial cells so that they play an important role in cell adherence with endothelial cells. Although the backbones of Lewis x and Lewis b { Fucα1,2Galβ1,3 (Fucα1,4)GlcNAc-R} are different, there is a common enzyme α1,3FucTⅢ as the key enzyme responsible for the synthesis of either α1,3 fucose or α1,4 fucose. The activities of α1,3/1,2 FucT were not only found an elevation in liver metastatic foci, but also significantly inhibited by ATRA treatment in HCC, which agreed with the result above. The activities that we measured in current study reflected both α1,3 FucT (Ⅲ, Ⅳ, Ⅴ, Ⅵ) and α1,2 FucT. The enzyme α1,3 FucTⅢ was able to synthesize either α1,3 or α1,4 fucose residues. Meanwhile, α1,6 FucT activity in metastatic liver tissue was as well much higher than the control. The enzyme α1,6 FucT was responsible for the synthesis of core α1,6 fucose, which implied that both outer and core fucose residues were associated with HCC development and the metastasis. Fucose residues can be in many glycoproteins, like α fetal protein (AFP), integrin, etc. Previous study proved that α1,6 FucT could glycosylate E-cadherin, but over expression [23] resulted in exceedingly fucosylating integrin and AFP, which could prevent tumor cells from metastasis [24]. Core fucose residue was recently reported to be important in cytokine receptor activation such as TGF receptor and epidermal growth factor receptor[25, 26]. The results in this study showed that fucose residues resided on EGFR and after ATRA treatment Lewis x on EGFR reduced and the tyrosine phosphorylation of the receptor was inhibited. The phosphorylation of EGFR is considered as the activated receptor and hence suppression of the phosphorylation suggested the negative regulation of EGFR downstream signaling, but the detail needs further study.

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岩藻糖链与肝癌细胞发生和转移的相关性研究 *

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摘要 研究观察了大鼠诱发肝癌过程中，与 UEA、LCA 凝集素相结合的含岩藻糖糖蛋白尤其是 80 ku 蛋白的动态变化．在肝癌病人标本中，也观察到了高转移性肝癌细胞比低转移性肝癌细胞表达更多的 UEA、LCA 相结合的岩藻糖蛋白．岩藻糖寡糖可以构成一些非常重要的黏附分子的结构，如 Lewis 抗原．继而进一步观察了不同转移潜能的肝癌细胞中 Lewis 抗原的表达差异，发现高转移性肝癌细胞 (HMCC97H) 比低转移性肝癌细胞(HMCC97L)表达更高的 Lewis x 和 b．在肝癌转移动物模型中，转移灶组织中的 Lewis 抗原合成关键酶 α1,3/1,2 以及 α1,6 岩藻糖转移酶活性远比对照组高．当肝癌细胞在维甲酸作用以后，细胞表面的 Lewis x 或 b 的水平显著下降，α1,3/1,2 岩藻糖转移酶活性也显著下降．同时我们观察到 Lewis x 可以存在于表皮细胞生长因子受体(EGFR)分子上，在维甲酸作用以后，EGFR 上的 Lewis x 抗原和磷酸化水平都显著性下降．上述结果提示岩藻糖化的糖链如 Lewis x 在肝癌细胞的发生和转移过程中起重要的作用．

关键词 肝癌，Lewis 抗原，转移

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