

Studies on mechanism of Sialy Lewis-X antigen in liver metastases of human colorectal carcinoma

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INTRODUCTION

Sialyl Lewis-X antigen, correlated with carcinoma, is a group of carbohydrate antigen containing oligosaccharide expressed both on glycolipids and glycoproteins on cell surface of embryonic tissue and tumor tissue^[1]. The SLeX antigen located on cell surface is synthesized principally by two enzymes, α 1, 3fucosyltransferase and α 2, 3sialyltransferase. In adults, SLeX antigen is expressed principally on the surfaces of granulocytic cells and some tumor cells. It is a ligand of endothelial leucocyte adhesion molecules (ELAM-1, or E-Selectin)^[2,3] and plays an important role in the adhesion of leucocyte to the vascular wall in inflammation and adhesion between tumor cells and blood endothelial cells in tumor metastases^[4,5]. This study intends to observe the expression of SLeX antigen and analyzes the correlation between the expression and metastatic potential by using cell lines of both Lovo^[6] and HT29^[7] of human colon carcinoma. Our experiments have confirmed that both cell lines possess the capability of forming tumor and having the metastatic potential. Lovo cells obviously showed a greater hepatic metastatic potential in experimental nude mice than HT29 cells^[8,9]. We employed *in situ* hybridization technique to detect the expression of SLeX antigen synthetase at mRNA level in both low and high metastatic potential of colon carcinoma cells. Meanwhile we used immunohistochemistry and flow cytometry to directly detect the expression of SLeX antigen at

intracellular protein level within Lovo and HT29 cells. We observed morphologic features of interaction between tumor cells and blood vessel endothelial cells as well as the linkage of both cells in interaction by means of scanning and transmission electron microscope. We used a method of sealed colon carcinoma cells with SLeX monoclonal antibody to observe the adhesive potential between human colon carcinoma cells and umbilical vein endothelial cells and to observe the effects of this method on hepatic metastases of experimental nude mice. We expect to find out the mechanism of how SLeX antigen functions in human colon carcinoma hepatic metastases.

MATERIALS AND METHODS

Materials

Cell lines, both Lovo and HT29 were provided by our laboratory. Endothelial cells were obtained by the perfusing and digesting human umbilical vein. Umbilical cord was provided by the Department of Gynecology and Obstetrics in Nanfang Hospital. BALB/C nude mice were provided by the Experimental Animal Center of the First Military Medical University.

Methods

Lovo, HT29 cells α 1, 3Fuc-Toligonucleotide probe was detected by *in situ* hybridization ① Cell culture and manufacture of cell patch: Lovo and HT29 cells (using RPMI-1640 complete medium) were placed in an incubate trunk that contained 5% CO₂ at 37°C. The cells grown well and were digested by pancreatin to make up a 1 × 10⁷/mL cell suspension, and then inoculated a drop of the cell suspension on cover slip of sterilized petridish and the complete medium was cultivated for 48 h, then removed the cover slip, washed thrice with 0.01 M, pH 7.2 PBS, fixed with acetone for 10 min and finally washed with PBS for use. ② *In situ* hybridization: The cell patches were digested by 10 μg/mL protease K at 37°C for 10 min and then washed with PBS buffer. 200 μL prehybridization buffer was degenerated at 95°C for 10 min, put into ice cubes to quench, then placed flat into a sealed moist case and incubated at 42°C for 3 h. Five mL of α 1, 3Fuc-T oligonucleotide probe of coragoxin with a density of 39.65 pmol/μL was added into 200 μL pre hybridization liquid to form a hybridization liquid. The hybridization liquid was degenerated at 95°C for 10 min. After being quenched in ice cubes, it was added into the cell sample, placed flat in a sealed moist case, and kept at 42°C overnight. It was washed with 6 × SSC 45% aminic acid amine, and 2 × SSC and 0.2 × SSC

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for 10 min respectively. It was added coragoxin Fab piece labeled alkaline phosphatase (1:125 dilute concentration) at 37°C for 4 h, and then was washed by buffer. NBT/BCIP was added for coloration for 2 h. It was treated with 100% alcohol and xylene and sealed by neutral gum for later use. The control was done without oligonucleotide probe. Areas that showed positive $\alpha 1, 3\text{Fuc-T mRNA}$ were dyed royal blue with royal blue grains. The deeper the color, the higher the positive degree. ③ Quantitative analysis and statistics: The quantitative analysis was done with Shen Hong's^[10] aw and Leica-Q500M image analysis system. After 10 positive cells were taken randomly, we surveyed the gray degrees ($G\alpha$) and the gray degrees of their background ($G\beta$) in the positive areas. The positive units ($\text{PU} = 100|G\alpha - G\beta|/256$) were calculated with origin 50 software. Data were analyzed statistically with the *t* test of SPSS.

Immunohistochemistry

① Cell culture and manufacture of cell pieces: The procedure was identical to that of *in situ* hybridization. ② Immunohistochemistry LSAB: The approaches (omitted). The negative control was incubated with PBS on the cell piece without primary antibody. The SLeX protein expression areas were dyed brown. Deep brown was defined as strongly positive, while light brown as weakly positive. ③ Quantitative analysis and statistical method were the same as those in *in situ* hybridization.

Flow cytometry

① Cell culture was identical to that of *in situ* hybridization. ② Flow cytometry measure: The Lovo and HT29 cells digested with 0.25% pancreatin, washed with PBS and centrifugated at 1000 rpm \times 5 min were made into $1 \times 10^7/\text{mL}$ cell suspension and then added SLeX monoclonal antibody (mouse anti-human IgM) (1:100 dilution). The negative control was processed with PBS instead of the primary antibody. Then the goat anti-mouse IgM labled FITC second antibody (1:60 dilution) was added at 4°C for 30 min. All the suspensions were washed with PBS at 1000 rpm \times 5 min, fixed with 1% polymeresatum at room temperature for 30 min, then re-washed with PBS and finally centrifugated at 1000 rpm \times 5 min for detection with EPICSEMTE model flow cytometry.

Adhesive test of tumor cells to human umbilical vein endothelium

① Tumor cell suspension preparation: The well-grown Lovo and HT29 cells were prepared into $1 \times 10^7/\text{mL}$ cell suspension with complete culture medium. The suspension was divided into 4 groups: Lovo cell experimental group and its control group, HT29 cell experimental group and its control group. The experimental groups were added SLeX monoclonal antibody 10 μL (5 $\mu\text{g}/\text{mL}$) and the control groups PBS instead of SLeX. They were pre-incubated at 37°C for 30 min. ② Adhesive test of

tumor cells to umbilical vein endothelium: The umbilical vein was cut open under sterile condition and was washed off red blood cells with PBS and then cut into 1 cm \times 1 cm pieces, 0.5 cm thick paraffin was put onto the bottom of the 16-well culture plate which was to be rinsed 3 times with PBS after the paraffin congealed. The plate was exposed under ultraviolet light for 30 min, added a suitable amount of culture medium for immersion for 15 min. The luminal surface of blood vessel was put upwards and fixed with stainless needles on paraffin on the bottom of wells. The TNF- α (end density 1:500U/mL) was added at 37°C for 4 h. After the culture was taken out, the prepared Lovo and HT29 cell suspensions were added into each well and incubated at 37°C for 4 h in atmosphere 5% CO₂. The umbilical vein taken out was washed with PBS and fixed with 2.5% glutaraldehyde for 30 min and then washed again, dehydrated, dried at critical point, puffed gold and detected under a S-450 scanning electronic microscope. ③ Criteria of results and statistical method: During the adhesive test between tumor cells and umbilical vein endothelium, the tumor cells, which adhered to vascular endothelium in every vision field were counted by 500 \times scanning electronmicroscope. Ten vision fields of each group were chosen randomly and their average values were calculated and statistically analyzed with *t* test.

Adhesive test between tumor cells and human umbilical vein endothelial cells

① Preparation of tumor cell suspension: as described above. ② Endothelial cell culture: 15 cm of fresh umbilical core from a healthy puerperal was washed off red blood cells with PBS. One end of the core was cramped and the 0.25% pancreatin was filled into the core from the other end. It was digested at 37°C for 15 min. The digested fluid that contained the endothelial cells was drawn out and put into a centrifugal tube and centrifugated at 1000 rpm for 15 min and processed into the cell suspension with RPMI-1640 culture medium containing 20% calf serum. The suspension was inoculated onto a plastic cell petridish, and incubated with 100 $\mu\text{g}/\text{mL}$ ECG at the atmosphere of 5% CO₂ at 37°C. The liquid was exchanged every 24 h-36 h. ③ Adhesive test of tumor cells to human umbilical vein endothelial cells: the well grown endothelial cells were digested with 0.25% pancreatin, prepared into $1 \times 10^3/\text{mL}$ cell suspension with complete culture medium, put onto 6 well petridish, added 100 $\mu\text{g}/\text{mL}$ ECG, and kept at 37°C for 20 h. After the 500U/mL TNF- α was added, it was kept at 37°C for 4 h. One mL of Lovo cells and HT29 cells processed in experimental and control groups, was added respectively into each well. The petri dish was placed into an oscillator of 120 rmp, at room temperature for 30 min and washed twice with PBS. The cells were scraped and collected into the centrifugal tubes for centrifugation of 500 rmp \times 10 min. After the upper clear liquid was removed, the cells were fixed with 2.5% glutaraldehyde

buffered, washed, fixed, dehydrated, embedded with gum, made into ultra-thin sections, colored with electronic stain for observation under a JEM1200-EX transmission electron microscope.

Effects of SLeX monoclonal antibody on liver metastases of experimental nude mouse

① Preparation of tumor cell suspension: The well-grown Lovo cells and HT29 cells were digested with 0.25% pancreatin, washed twice and diluted into the cell suspension with PBS after 1000 rpm \times 5 min. The cell suspension concentration was regulated to 1×10^7 /mL. The cell suspension was divided into 4 groups: Lovo cell, HT29 cell and their corresponding control groups. The experimental groups were added 10 μ L SLeX monoclonal antibody while the control groups were added the same volume of PBS instead of SLeX. The cell suspensions were incubated at room temperature for 30 min. ② Influences of SLeX monoclonal antibody on liver metastases of experimental nude mouse: 365-week-old BALB/C healthy male nude mice were divided into 4 groups as described above. After the nude mice were anesthetized with 4% amine ketone intraperitoneal injection, their abdominal cavities were opened at 0.5 cm below left rib edge under sterile condition to expose their spleens. The cell suspension (0.3 mL) prepared was injected to the capsule below in the spleen and the abdominal cavity was closed. The mice were fed under SPF condition and killed after 4 weeks. Their spleens, livers, hearts, kidneys and lungs were taken out, dehydrated, transparented and paraffin-embedded. The tumor tissues visible were sliced while the tissues of which tumors were not found with naked eyes made serial sections before being HE dyed and observed under a light microscope. ③ Criterion of results and statistical methods: The liver metastases in each group was observed with naked eyes and under light microscope. The data obtained from the observation were tested with χ^2 -test with SPSS software, with $P < 0.05$ being significant.

RESULTS

In situ hybridization

$\alpha 1$, 3Fuc-T mRNA expressions in Lovo and HT29 cells showed negative results in the control groups while those were positive in the experimental groups. In the experimental groups, $\alpha 1$, 3Fuc-T mRNA in the Lovo cells were strongly positive, and their cytoplasm showed deep blue with visible royal blue fine particles (Figure 1), while $\alpha 1$, 3Fuc-T mRNA in the HT29 cells were weakly positive, and their cytoplasm showed light blue with visible royal blue fine particles (Figure 2). $\alpha 1$, 3Fuc-T mRNA expressions in Lovo cells were stronger than those HT29 cell, and their difference was significant ($P = 0.002$, Table 1).

Table 1 Expressions of $\alpha 1$, 3Fuc-TmRNA in two cell lines

	<i>n</i>	$\bar{x} \pm s$	<i>t</i>	<i>P</i>
Lovo cells	10	21.2055 \pm 7.7472	3.537	0.002
HT29 cells	10	10.7922 \pm 5.1653		

Immunohistochemistry

The SLeX expressions in Lovo cells and HT 29 cells were negative in the control groups but positive in experiment groups. The SLeX antigen expressions in Lovo cells were strongly positive, and their cytoplasm showed brown or deep brown (Figure 3); The SLeX antigen expressions in HT29 cells were weakly positive, and their membranes were brown (Figure 4). There was a significant difference between the expressions of the two ($P = 0.025$, Table 2).

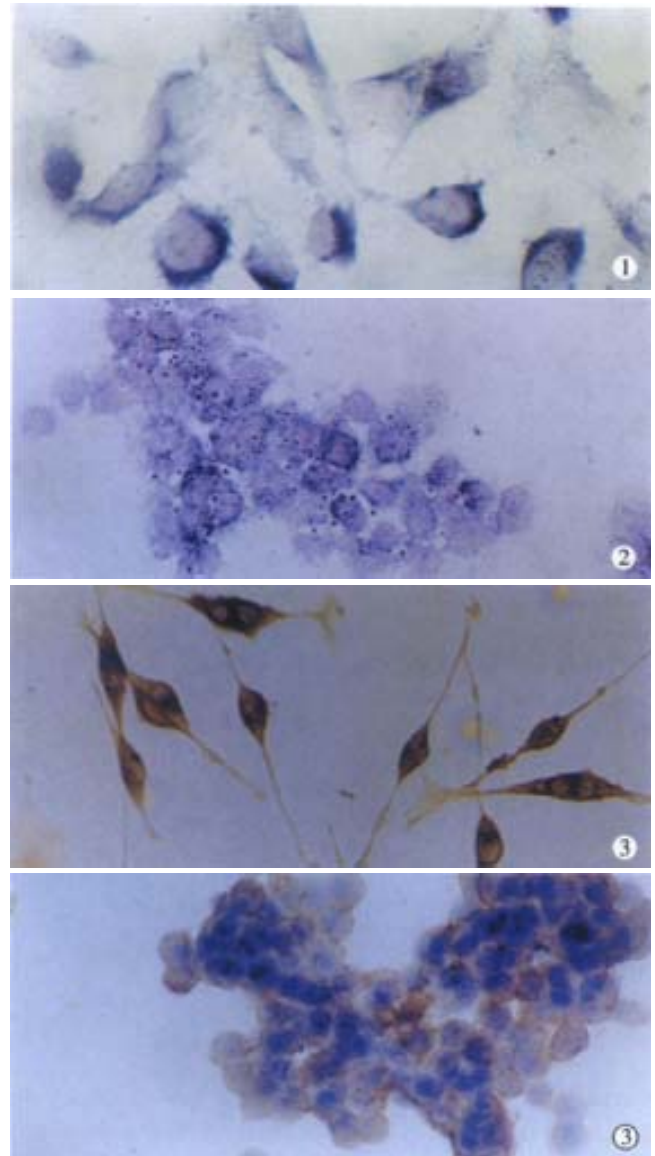


Figure 1 The expression of $\alpha 1$, 3Fuc-TmRNA in Lovo cells shows strongly positive staining, with deep royal blue cytoplasm and visible royal blue beads. *In situ* hybridization. $\times 400$

Figure 2 The expression of $\alpha 1$, 3Fuc-TmRNA shows weakly positive staining, light royal blue with royal blue bead in cytoplasm of HT29 cells. *In situ* hybridization. $\times 400$

Figure 3 The expression of SLeX antigen in Lovo cells shows strongly positive staining, with deep brown in cytoplasm. Immunohistochemistry. $\times 400$

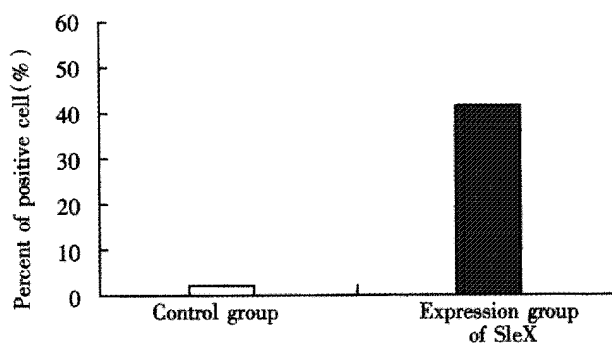
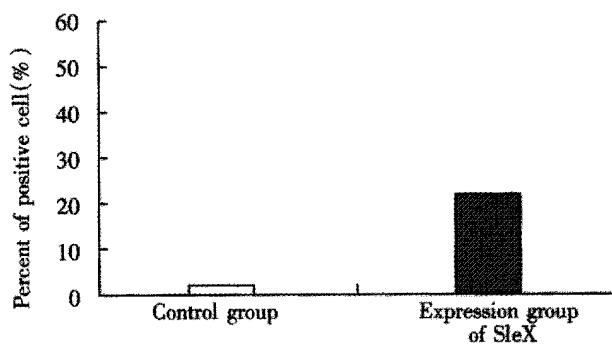
Figure 4 The expression of SLeX antigen shows positive or weakly positive staining, brown in membrane of HT29 cells. Immunohistochemistry. $\times 400$

Table 2 Expressions of SLeX antigens in the two cell lines

	<i>n</i>	$\bar{x} \pm s$	<i>t</i>	<i>P</i>
Lovo cells	10	32.7633±10.8716	2.452	0.025
HT29 cells	10	21.9188±8.8016		

Flow cytometry quantitative detection

The Lovo cell and HT29 cell SLeX antigen expressions were positive. The ratio of positive SLeX antigen expressions in the Lovo experimental groups was 42.9% while that in the control groups was 2.3%. The positive rates in the HT29 cell line were 23.3% and 2.1% respectively in the experimental groups and their control groups. The percentage was greater in the experiment groups of Lovo cell line than that in the experiment groups of the HT29 cell line (Figures 5,6).

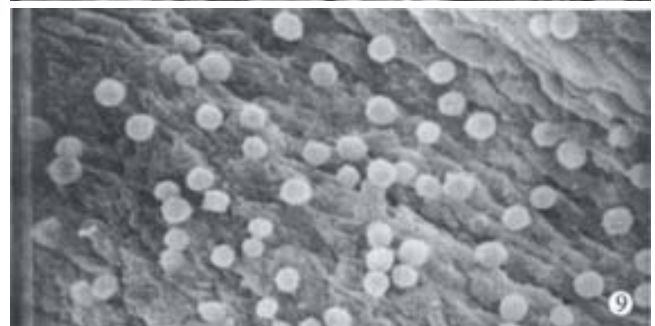
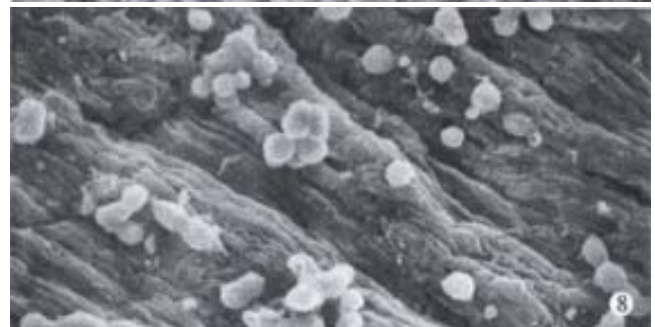
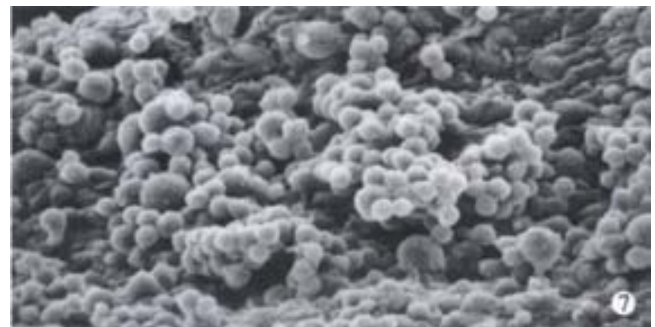
**Figure 5** The detective result of Lovo cells. flow cytometry**Figure 6** The detective result of HT29 cells. flow cytometry**Observation of the adhesion of tumor cells to umbilical vein endothelial cells under scanning electronmicroscope**

Both Lovo cell and HT29 cell line had adhesive potential to umbilical vein endothelial cells in the control groups. The Lovo cell line showed greater adhesive potential to umbilical vein endothelial cells than the HT29 cell line. It could be observed under a 500× scanning electronmicroscope after co-incubation for 4h, a great deal of Lovo cells adhered to umbilical vein endothelial cells, and majority of them were clustered (Figure 7); and some of the HT29 cells adhered only to the surface of the umbilical vein endothelial cells, and only a part of them clustered (Figure 8). The adhesive potential was obviously even lower in the

experiment groups. Under a 500 × scanning electronmicroscope after co-incubation for 4 h, the Lovo cells adhered to the umbilical vein endothelial cells were scattered and no clustering could be observed (Figure 9). The adhesive potential of the HT29 cells was weaker than that of the Lovo cells. Only a small amount of the HT29 cells adhered to the endothelial cells, and did not cluster (Figure 10). The cells adhered to the surface of the umbilical vein endothelial cells were fewer in the experiment groups than in the control groups. There was a significant difference between them ($P = 0.0001$, Table 3).

Table 3 The different adhesive potentials of tumor cells to umbilical vein endothelial cells with the tumor cells sealed

	<i>n</i>	Control groups $\bar{x} \pm s$	Experimental groups $\bar{x} \pm s$	<i>t</i>	<i>P</i>
Lovo cells	10	213±15.6702	124±16.7319	12.167	0.0001
HT29 cells	10	147±4.5522	69±7.3786	28.632	0.0001

**Figure 7** In control groups, a lot of Lovo cells adhered to the surface of umbilical vein endothelium, and tumor cells clustered. SEM, ×500**Figure 8** In control groups, The HT29 cells adhered to the surface of umbilical vein endothelium, a part of them clustered. SEM, ×500**Figure 9** In experimental groups, a small amount of the Lovo cells adhered to the umbilical vein endothelium, no clustering. SEM, ×500

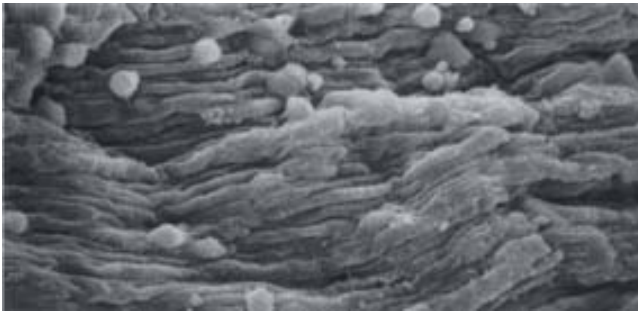


Figure 10 In experimental groups, a small amount of the HT29 cells adhered to the umbilical vein endothelium, no clustering. SEM, $\times 500$

Observation of adhesive potential of tumor cells to umbilical vein endothelial cells under transmission electron microscope

In the control groups, there were a great deal of microvilli or interdigitations on the Lovo cell membrane, but few interdigitations on the HT29 cell membrane. The Lovo cells were linked to umbilical vein endothelium via interdigitations on the cell membrane (Figure 11). Some of the apophyses stretched into endothelial cell membrane; but the HT29 cells connected endothelial cell membrane directly via the cell membrane (Figure 12). No significant difference in the way of linking between the experimental groups and the control groups was observed.

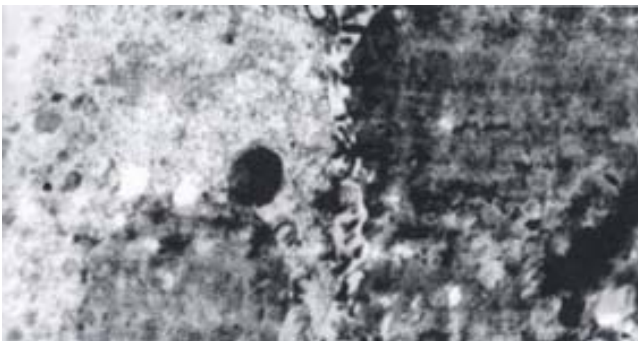


Figure 11 The Lovo cells were linked to umbilical vein endothelium via interdigitations on the cell membrane. TEM $\times 20K$



Figure 12 The HT29 cells connected endothelial cell membrane directly via the cell membrane. TEM $\times 24K$

Effects of SLeX monoclonal antibody on the liver metastases model of experimental nude mice

The ratio of liver metastases was 9/9 (100%) in the control groups of Lovo cells, while it was 2/9 (22%) in the experimental groups. The ratio of liver metastases in the experimental groups was less than that in the control groups with a significant difference between the two groups ($P < 0.001$). The ratio of liver metastases was 4/9 (44.4%) in the control groups of HT29 cells, but 1/9 (11.1%) in the experimental groups. The ratio of liver metastases in the experimental groups was less than that in the control groups with significant difference between the two groups ($P < 0.002$, Table 4).

Table 4 Effects of SLeX monoclonal antibody on the liver metastases model of experimental nude mice

	Lovo cells		HT29 cells	
	Metastases	No metastases	Metastases	No metastases
Control groups	9	0	4	5
Experimental groups	2	7	1	8

Lovo cell groups $\chi^2 = 11.45$, $P < 0.001$; HT29 cell groups $\chi^2 = 2.498$, $P < 0.002$.

DISCUSSION

SLeX antigens are mostly expressed on the cell surface of epithelial tumors such as lung cancer, gastric cancer, colon cancer, ovary cancer^[11,12]. The positive ratio in carcinoma of colon is 76%-90%^[1,13]. The expression of SLeX antigen is mostly determined by two kinds of enzyme, $\alpha 1$, 3Fuc-T and $\alpha 2$, 3ST. The successive activities of the two enzymes existing in N-acethylactosamine synthesize the SLeX antigens. $\alpha 1$, 3Fuc-T is an important material to produce SLeX antigens as well as to adjust and control the expression of SLeX antigens^[14]. The increase of $\alpha 1$, 3Fuc-T in matrix plasma results in the increase of SLeX antigens on the cell surface^[15]. The $\alpha 1$, 3Fuc-T and $\alpha 2$, 3ST synthesize the SLeX antigens which possess a strong affinity to Lectin in E-selectin and epidermal growth factor resulted with adhesion of tumor cells to the endothelial cells^[3]. The SLeX antigens on the cell surface in the colon carcinoma as a kind of ligands, their identification from and adhesion to E-selectin recipients on the endothelial cell surface of liver vasculature are considered as the molecular basis of liver metastatic of colon carcinoma, and as a critical step in the liver metastatic process of colon carcinoma^[5]. Modification of carbohydrate antigens on the tumor cell surface, such as SLeX antigen, will affect the ways of tumor cell metastases^[4,5,16], such as adhesive potential^[17], mobility^[18,19], invasion^[20], immunogenicity and other immune identification process^[21,22] or other unknown cell behaviors which will affect the

metastasis potential of tumor cells. In other words, the structural changes of carbohydrates on tumor cell surface play a key role in the metastases of tumor cells^[23]. The well known metastatic steps of tumor cells are: proliferation of tumor cells accompanied by angiogenesis of tumor → intrusion of tumor cells into basement membrane and then into blood vessels and lymphatic vessels → into blood circulation → detection in the target organ and adhesion to the capillaries → breaking through the capillaries and formation of minute metastatic spots → formation of new blood vessels and proliferation of metastatic spots^[24]. There are different metastasis potential tumor cells in the primary area, for example, in colon carcinoma, SLeX antigens with high expression in tumor cells show high metastatic potential while the antigens with low expression in tumor cells show weak metastatic potential. The tumor cells with strong metastatic potential are more likely to fall off from the primary area and enter blood vessels and thus adhere to hepatic vascular endothelial cells so as to grow and form liver metastasis^[25]. The reason is that SLeX antigens on the tumor cell surface as ligands of E-selectin on the endothelial cell surface of the capillaries induce adhesion between tumor cells and blood vessel endothelial cells of the target organ^[26] and promote orientating tendency in motion and result in metastasis.

Our study demonstrated that the Lovo cell line with strong metastatic potential has high expression of SLeX antigen and high expression of SLeX antigen synthase, α 1, 3Fuc-T mRNA while the HT29 cells line with weak metastatic potential has low expression of SLeX antigen and low expression of SLeX antigen synthase α 1, 3Fuc-T. Our finding that SLeX antigen expression is closely correlated with colon carcinoma metastatic potential.

Our study also showed that Lovo cells with high expression of SLeX antigen had strong adhesive ability to vascular endothelial cells while the HT29 cell with low expression of SLeX antigen had weak adhesive potential. The connecting way to vascular endothelial cells of the Lovo cells with high expression of SLeX antigen was quite different from that of the HT29 cells with low expression of SLeX antigen. The SLeX monoclonal antibodies could effectively restrain the adhesion of tumor cells to vascular endothelial cells and well reduce the formation of liver metastases of experimental nude mice. We speculate that the reason for the decrease of adhesion is that SLeX monoclonal antibodies may have sealed carbohydrate antigens on the tumor cell membrane → site of SLeX ligand and result in lower adhesive potential of tumor cells to vascular endothelial cells^[27]. The results suggest that SLeX antigens play an important role in liver metastases of colon carcinoma. SLeX monoclonal antibody or its analogs may prevent the formation of liver metastases of colon carcinoma.

REFERENCES

- Fukushima K, Hirota M, Terasaki PI, Wakisaka A, Togashi H, Chia D, Suyama N, Fukushi Y, Nudelman E, Hakomori SI. Characterization of sialosylated Lewisx as a new tumor-associated antigen. *Cancer Res*, 1984; 44:5279-5285
- Sell S. Cancer-associated carbohydrates identified by monoclonal antibodies. *Hum Pathol*, 1990;21:1003-1019
- Majuri ML, Niemel- R, Thsala S, Renkonen O, Renkonen R. Expression and function of α 2, 3-sialyl- and α 1, 3/1, 4-fucosyltransferases in colon adenocarcinoma cell lines: role in synthesis of E-selectin counter-receptors. *Int J Cancer*, 1995;63:551-559
- Lowe JB, Stoolman LM, Nair RP, Larsen RD, Berhend TL, Marks RM. ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell*, 1990;63:475-484
- Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B. Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. *Science*, 1990;250:1132-1135
- Drewinko B, Romsdahl MM, Yang LY, Ahearn MJ, Trujillo JM. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. *Cancer Res*, 1976;36:467-475
- Mitchell BS, Horny HP, Schumacher U. Immunophenotyping of human HT29 colon cancer cell primary tumours and their metastases in severe combined immunodeficient mice. *Histochem J*, 1997;29:393-399
- Li ZG, Ding YQ, Deng YJ, Guo JQ, Zhu MG. Mechanism of CD44V6 in human colorectal carcinoma metastasis. *Zhonghua Yixue Zazhi*, 1998;78:729-732
- Deng YJ, Li ZG, Qiu HM, Huang ZY, Ding YQ, Zhu MG. Comparison of metastatic potentials of human colorectal carcinoma cell lines and their features related to metastasis. *Huaren Xiaohua Zazhi*, 1998;6:33-35
- Liu HM, Gao TM, Tong ZQ. Expression of p53 mRNA and its protein in hippocampal CA1 region after reperfusion following global ischemia in rats. *Zhonghua Yixue Zazhi*, 1999;79:67-68
- Zenita K, Kirihata Y, Kitahara A, Shigetani K, Higuchi K, Hirashima K, Murachi T, Miyake M, Takeda T, Kannagi R. Fucosylated type 2 chain polylectosamine antigens in human lung cancer. *Int J Cancer*, 1988;41:344-349
- Singhal AK, Qrntoft TF, Nudelman E, Nance S, Schibig L, Stroud MR, Clausen H, Hakomori S. Profiles of lewisx-containing glycoproteins and glycolipids in sera of patients with adenocarcinoma. *Cancer Res*, 1990;50:1375-1380
- Hanski K, Drechsler K, Hanisch FG, Sheehan J, Manske M, Ogorek D, Klussmann E, Hanski ML, Blank M, Xing PX, McKenzie IFC, Devine PL, Riecken EO. Altered glycosylation of the MUC-1 protein core contributes to the colon carcinoma-associated increase of mucin-bound sialyl-lewisx expression. *Cancer Res*, 1993;53:4082-4088
- Weston BW, Smith PL, Kelly RJ, Lowe JB. Molecular cloning of a fourth member of a human α (1,3) fucosyltransferase gene family. *J Bio Chem*, 1992;267:24575-24584
- Izumi Y, Kawamura YJ, Irimura T. Carbohydrate antigens in carcinoma invasion and metastasis. *Nippon-Geka-Gakkai-Zasshi*, 1996;97:140-144
- Phillips ML, Nudelman E, Gaeta FCA, Perez M, Singhal AK, Hakomori SI, Paulson JC. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. *Science*, 1990;250:1130-1132
- Tiemeyer M, Swiedler SJ, Ishihara M, Moreland M, Schweingruber H, Hirtzer P, Brandley BK. Carbohydrate ligands for endothelial-leukocyte adhesion molecule 1. *Proc Natl Acad Sci USA*, 1991;88:1138-1142
- Takada A, Ohmori K, Takahashi N, Tsuyuoka K, Yago A, Zenita K, Hasegawa A, Kannagi R. Adhesion of human cancer cells to vascular endothelium mediated by a carbohydrate antigen, sialyl lewis A. *Biochem Biophys Res Commun*, 1991;179:713-719
- Berg EL, Robinson MK, Mansson O, Butcher EC, Magnani JL. A carbohydrate domain common to both sialyl Le^x and sialyl Le^a is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. *J Biol Chem*, 1991;266:14869-14872
- Gong EC, Hirohashi S, Shimamoto Y, Watanabe M, Ino Y, Teshima S, Kodaira S. Expression of carbohydrate antigen 19-9 and stage-specific embryonic antigen 1 in nontumorous and tumorous epithelia of the human colon and rectum. *J Natl Cancer Inst*, 1985;75:447-454
- Tyrrell D, James P, Rao N, Foxall C, Abbas S, Dasgupta F, Nashed M, Hasegawa A, Kiso M, Asa D, Kidd J, Brandley BK. Structural requirements for the carbohydrate ligand of E-selectin. *Proc Natl Acad Sci USA*, 1991;88:10372-10376
- Atkinson BF, Ernst CS, Herlyn M, Stepelwski Z, Sears HF, Koprowski H. Gastrointestinal cancer-associated antigen in immunoperoxidase assay. *Cancer Res*, 1982;42:4820-4823
- Raz A, Lotan R. Endogenous galactoside-binding lectins: a new class of functional tumor cell surface molecules related to metastasis. *Cancer Metastasis Rev*, 1987;6:433-452
- Wang RN. Advance at the mechanism study of invasion and metastases of stomach carcinoma. *Xin Xiaohuabingxue Zazhi*, 1994;2:162-163
- Yamada N, Chung YS, Maeda K, Sawada T, Ikebara T, Nishino H, Okuno M, Sowa M. Increased expression of sialyl lewis A and sialyl lewis X in liver metastases of human colorectal carcinoma. *Invasion Metastasis*, 1995;15:95-102
- Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Kabuto T, Iwanaga T, Matsushita Y, Irimura T. Increased expression of sialyl lewisx antigen correlates with poor survival in patients with colorectal carcinoma: clinicopathological and immunohistochemical study. *Cancer Res*, 1993;53:3632-3637
- Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Izumi Y, Irimura T. Involvement of carbohydrate antigen sialyl lewisx in colorectal cancer metastasis. *Dis Colon Rectum*, 1997;40:420-431