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Cooperative inhibitory effects of antisense oligonucleotide of cell adhesion molecules and cimetidine on cancer cell adhesion

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Abstract

AIM: To explore the cooperative effects of antisense oligonucleotide (ASON) of cell adhesion molecules and cimetidine on the expression of E-selectin and ICAM-1 in endothelial cells and their adhesion to tumor cells.

METHODS: After treatment of endothelial cells with ASON and/or cimetidine and induction with TNF-α, the protein and mRNA changes of E-selectin and ICAM-1 in endothelial cells were examined by flow cytometry and RT-PCR, respectively. The adhesion rates of endothelial cells to tumor cells were measured by cell adhesion experiment.

RESULTS: In comparison with TNF-α inducing group, lipo-ASON and lipo-ASON/cimetidine could significantly decrease the protein and mRNA levels of E-selectin and ICAM-1 in endothelial cells, and lipo-ASON/cimetidine had most significant inhibitory effect on E-selectin expression (from 36.37±1.56% to 14.23±1.07%, $P<0.001$). Meanwhile, cimetidine alone could inhibit the expression of E-selectin (36.37±1.56% *vs* 27.2±1.31%, $P<0.001$), but not ICAM-1 (69.34±2.50% *vs* 68.07±2.10%,$P>0.05$)and the two kinds of mRNA, either. Compared with TNF-α inducing group, the rate of adhesion was markedly decreased in lipo-E-selectin ASON and lipo-E-selectin ASON/cimetidine treated groups($P<0.05$), and lipo-E-selectin ASON/cimetidine worked better than lipo-E-selectin ASON alone except for HepG2/ECV304 group ($P<0.05$). However, the decrease of adhesion was not significant in lipo-ICAM-1 ASON and lipo-ICAM-1 ASON/cimetidine treated groups except for HepG2/ECV304 group ($P>0.05$).

CONCLUSION: These data demonstrate that ASON in combination with cimetidine *in vitro* can significantly reduce the adhesion between endothelial cells and hepatic or colorectal cancer cells, which is stronger than ASON or cimetidine alone. This study provides some useful proofs for gene therapy of antiadhesion.

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INTRODUCTION

Previous researches have shown that recurrence and metastasis of cancer are closely related to the adhesion between tumor cells and endothelial cells. A variety of cell adhesion molecules (CAM) induced by cytokines such as IL-β and TNF-α released by tumor cells, promote the adhesion. Therefore, how to inhibit the adhesion is worth of study.

Antisense oligonucleotide (ASON) technique is a new alternative of gene therapy. ASONs are short synthetic oligonucleotides (10 to 25 bases in length) designed to hybridize to RNA (sense strand) that encodes the protein of interest. On binding to an mRNA, the oligonucleotide may inhibit the expression of target protein by multiple mechanisms[1,2], exhibiting an important significance in antivirus and cancer treatment researches[3,4]. Phosphorothioate oligonucleotides have a sulfur substituting for one of the nonbridging oxygens in the phosphate backbone, markedly enhancing the stability aganist cellular and serum nucleases[5,6]. E-selectin, an early expressed CAM, and intercellular adhesion molecule-1 (ICAM-1), a late expressed CAM, are important proteins that mediate cell adhesion. Relevant studies on antiinflammation or antiadhesion using ASON have aroused increasing attention[7-9].

Cimetidine, a kind of H2R antagonist, has been shown to improve the survival of patients with colorectal cancer, melanoma, and renal cell cancer. Other H2R antagonists including ranitidine and famotidine did not have such an effect[10,11], indicating that cimetidine may exert its effect by enhancing the host immune response against tumor cells[12] or by blocking the cell growth-promoting activity of histamine[13], but not directly via histamine antagonism.

The key of anti-metastatic therapy lies in the complete inhibition of metastasis, because even if one metastatic colony is formed in an organ, it may result in death of the host eventually. Combined treatment may be an effective way to reach the goal. On the basis of previous work, we investigated the combined inhibitory effects of E-selectin or ICAM-1 ASON and cimetidine on tumor cell adhesion to provide data for further animal experiment and potential clinical application.

MATERIALS AND METHODS

Materials

Human endothelial cell line ECV304, hepatic cancer cell lines HepG2 and BEL7404 and colorectal cancer cell line Ls-174-t were purchased from the Cellular Biology Institute of Chinese Academy of Sciences and grown in DF medium (DMEM: Ham's F12=3:1) containing 100 mL· L^{-1} fetal calf serum, penicillin 1×10^5U· L^{-1} and streptomycin 100 mg· L^{-1}. Transfection kit TransFast™ liposome, Taq enzyme, RT-PCR kit and marker were from Promega. Mouse anti-human ICAM-1 mAb and E-selectin mAb were from Lab Vision. RNA extract (Trizol) was purchased from GIBCO BRL. Human recombinant TNF-α was purchased from Jingmei Biological Engineering Co., Shenzhen. ^{3}H-TdR was purchased from the Atomic Energy Institute of Shanghai. Cimetidine was the product of Smithkline d Beecham Pharmaceutical Co., Germany.

ASON and primers

As previously described[14,15], phosphorothioate oligoribonucleotides of ICAM-1, E-selectin and control ASON were synthesized by Shenggong Bio-Engineering Company, Shanghai. The sequences of ICAM-1, E-selectin and control ASON, and the primers for ICAM-1, E-selectin and β-actin are shown in Table 1.

Table 1 Sequences of ASON and primers

Name		Sequence	Length
ASON			
E-selectin		5'-TTCCCCAGATGCACCTGTTT-3'	
ICAM-1		5'-CCCCCACCACTTCCCCTCTC-3'	
Control primer		5'-GCCGAGGTCCATGTCGTACGC-3'	
E-selectin	Forward:	5'-AAAATGTTCAAGCCTGGCAGTTCC-3'	
	Reverse:	5'-GTGGTGATGGGTGTTGCGGTTTCA-3'	509 bp
ICAM-1	Forward:	5'-CACAAGCCACGCCTCCCTGAACCTA-3'	
	Reverse:	5'-TGTGGGCCTTTGTGTTTTGATGCTA-3'	458 bp
β-actin	Forward:	5'-CTGTCTGGCGGCACCACCAT-3'	
	Reverse:	5'-GCAACTAAGTCATAGTCCGC-3'	250 bp

Methods

Preparation of lipo-ASON In order to improve the efficiency of ASON uptake of cells and prevent degradation in cultured cells and human serum, we used a commercial liposome that was comprised of synthetic cationic lipid and neutral lipid. It enhanced ASON' s biofunction by improving its merging with cell membrane of eukaryotic cells and entering cell plasma to combine with mRNA[16]. At a lower final concentration of 1/10 the nude ASON, lipo-ASON worked more effectively on inhibition in our previous report. According to the manufacturer' s protocol of TransFast™ liposome, 200 μl of serum-free medium per well should contain 3.6 μl(1.5 μg) ASON and 4.4 μl liposome. So 10 minutes before the experiment, ASON was first mixed with serum-free medium, then liposome was added to make lipo-ASON medium (1 μmol/L) at room temperature.

Treatment of endothelial cells A total of 5×10^4 ECV304 endothelial cells (3 to 6 generations) per well were put into a 24-well plate and incubated at 37 ℃ in 5 mL/L CO_2 humidified atmosphere for 48 hours, when cells grew to a confluence of 80%. E-selectin and ICAM-1 were divided into 5 groups according to the following different treatments, respectively. GroupI (Basal): Treatment with TNF-α without lipo-ASON or cimetidine, and the cultivation time was identical to the other groups. Group II (TNF-α): After cultivation for 48 hours, the supernatants were discarded, endothelial cells were washed with PBS and replaced with 200 μl of serum-free medium. Group III (cimetidine): After cultivation for 24 hours, 200 μl of serum-free medium containing cimetidine (10^{-8}M) was added and cultured for another 24 hours, then the supernatants were discarded, cells were washed with PBS and replaced with 200 μl of serum-free medium. Group IV (lipo-ASON): After cultivation for 48 hours, the supernatants were discarded, cells were washed with PBS and replaced with 200 μl of serum-free medium containing lipo-ASON. Group V (lipo-ASON/ cimetidine): After cultivation for 24 hours, 200 μl of serum-free medium containing cimetidine (10^{-8}M) was added and cultured for another 24 hours, then the supernatants were discarded, cells were washed with PBS and replaced with 200 μl of serum-free medium containing lipo-ASON.

Groups II to V were cultured for 4 hours at 37 ℃. For E-selectin expression groups, the supernatants were discarded, 200 μl medium containing 5 ng TNF-α was added and continuously cultured for 4 hours at 37 ℃. For ICAM-1 expression groups, the supernatants were discarded, 200 μl medium was added and continuously cultured for 4 hours at 37 ℃, then 5 ng TNF-α was added and cultured for 16 hours.

Flow cytometry Endothelial cells, treated with ASON or cimetidine and TNF-α as described above, were removed from the plate by brief trypsinization with 0.25% trypsin. Cells were washed with DEME plus 10% FCS, stained with primary mAb (2 μg/μl) diluted in D-PBS containing 2% BSA and 0.2% sodium azide, followed by fluorescein-conjugated goat anti-mouse IgG. Each step was performed at 4 ℃. Cells were analyzed by flow cytometry using a Bryte HSFACScan (BID-RAD, US).

RT-PCR 2.5×10^5 of ECV304 endothelial cells per well were placed into a 6-well plate and then performed as described above. Total cellular RNAs were extracted and quantitated by a spectrophotometer. 2 μg of RNA was used to perform reverse transcription as recommended by the supplier. E-selectin gene was amplified 30 cycles by half-quantitation under the following conditions: denaturation at 94 ℃ for 5 min followed by at 94 ℃ for 30 s, at 64 ℃ for 45 s and at 72 ℃ for 45 s, then extension at 72 ℃ for 7 min. ICAM-1 gene was amplified 30 cycles by half-quantitation under the following conditions: denaturation at 94 ℃ for 5 min followed by at 94 ℃ for 30 s, at 60 ℃ for 45 s and at 72 ℃ for 45 s, then extension at 72 ℃ for 7 minutes. For analysis, 10 μL of the amplified product was tested in 20 g/L agarose gel with ethidium bromide staining, then photographed and scan-analysed. The ratio of average integrated density value (IDV) of E-selectin and ICAM-1 to IDV of β-actin was expressed as the relative intensity of E-selectin and ICAM-1, respectively.

Monolayer cell adhesion assays 1×10^4 ECV304 endothelial cells per well were placed into a 96-well plate and then performed as described above. Human hepatic cancer cell lines HepG2 and BEL7404 and colorectal cancer cell line Ls-174-t were subcultured for 24 hours, then incubated with medium containing ^{3}H-TdR(9 μCi/ml)for 12 hours at 37 ℃. The three kinds of cells (2.5×10^5) were added to ECV304 monolayer in a final volume of 0.1 ml respectively, and incubated for 1 h at 37 ℃. Nonadherent cells were removed from the plate by gentle washing with warm PBS. By brief trypsinization with 0.25% trypsin, the adherent cells were removed onto a piece of fiberglass paper (49#), washed with 5% trichorolacetic acid and dried at 80 ℃. The number of adherent cells was determined by measuring flash value using a flash-detecting device (Tri-Carb 2300TR, Packard Co Lit).

Statistics analysis

Data were presented with $\bar{x}\pm s$, statistical analysis was performed using ANOVA. Differences were judged to be statistically significant when the *P* value was less than 0.05.

RESULTS

Effect of ASON and/or cimetidine on expression of E-selectin and ICAM-1

Using cytofluorometry, we examined the effect of lipo-ASON or cimetidine on the level of E-selectin and ICAM-1 protein in ECV304 cells stimulated by TNF-α (Figure 1). Maximum expression of E-selectin or ICAM-1 was observed in TNF-α inducing group with no other treatments. Compared with TNF-α inducing group, the expression of E-selectin in all treated groups was differently decreased ($P<0.001$), most markedly in lipo-ASON/cimetidine treated group ($P<0.001$, *vs* lipo-ASON alone group), showing that lipo-ASON in combination with cimetidine had a better inhibitory effect. Similarly, the expression of ICAM-1 in lipo-ASON and lipo-ASON/cimetidine treated groups decreased significantly ($P<0.001$), but the change of ICAM-1 expression in cimetidine treated group was not significant ($P=0.296$), suggesting that cimetidine did not affect ICAM-1 expression in endothelial cells.

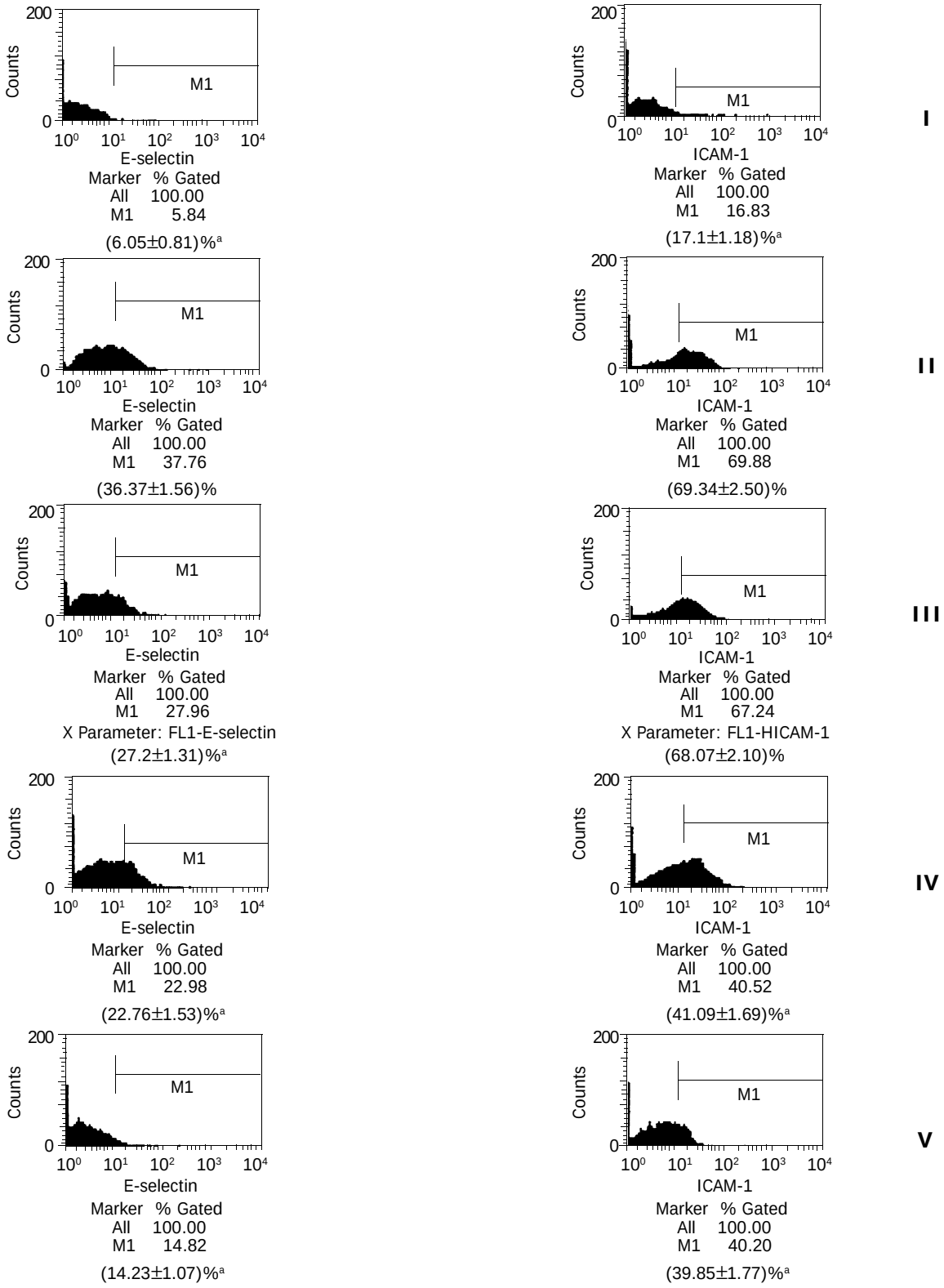

Figure 1 Effect of ASON on expression of E-selectin and ICAM-1 in endothelial cells ($^aP<0.01$, *vs* group II, $n=5$). I: Basal, II: TNF-α, III: cimetidine, IV: lipo-ASON, V: lipo-ASON/cimetidine.

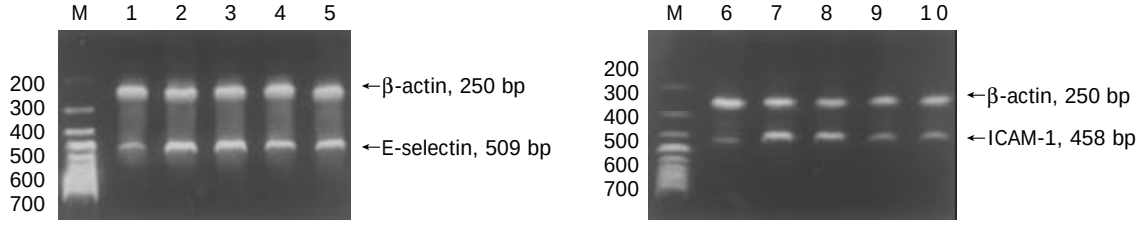

Figure 2 Agarose gel electrophoresis of RT-PCR products. M:Marker, 1: E/basal, 2: E/TNF-α, 3: E/cimetidine, 4: E/lipo-ASON, 5: E/lipo-ASON/cimetidine, 6: I/basal, 7: I/TNF-α, 8: I/cimetidine, 9: I/lipo-ASON, 10: I/cimetidine.

Agarose gel electrophoresis of RT-PCR products

To investigate the effects of lipo-ASON and/or cimetidine on the level of E-selectin and ICAM-1 mRNA, RT-PCR analysis was carried out. As shown in Figures 2 and 3, TNF-α could induce E-selectin and ICAM-1 gene expression. Lipo-ASON or lipo-ASON/cimetidine could significantly reduce the level of E-selectin and ICAM-1 mRNA, but cimetidine did not.

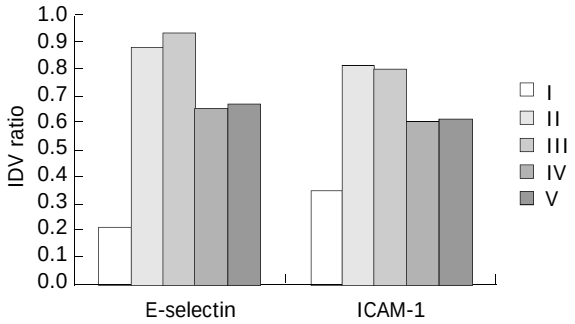

Figure 3 Relative expression value of ICAM-1 and E-selectin to β-actin by RT-PCR. I: Basal, II: TNF-α, III: Cimetidine, IV: Lipo-ASON, V: Lipo-ASON / Cimetidine.

Effect of ASON and/or cimetidine on adhesion

To examine the effects of lipo-ASON and/or cimetidine on the adhesion between tumor cells and ECV304 cells, a monolayer cell adhesion assay was carried out. As shown in Table 2, the adhesions of HepG2, BEL7404 and Ls-174-t cells to ECV304 cells were strongly induced by stimulation with TNF-α. Compared with TNF-α inducing group, the rate of adhesion was markedly decreased in lipo-E-selectin ASON and lipo-E-selectin ASON/cimetidine treated groups (P<0.05), and lipo-E-selectin ASON/cimetidine worked better than lipo-E-selectin ASON alone except for HepG2/ECV304 group (P<0.05). However, the decrease of adhesion was not significant in lipo-ICAM-1 ASON and lipo-ICAM-1 ASON/cimetidine treated groups except for HepG2/ECV304 group (P>0.05).

Table 2 Adhesion rate of endothelial cells transfected by E-selectin and ICAM-1 ASON to tumor cells ($\bar{x}$±s)%

Group	I	II	III	IV	V
E-selectin / HepG2	17.7±0.9[a]	80.4±6.2	58.9±2.7	38.0±5.0[a]	24.1±1.5[a]
E-selectin / BEL7404	8.1±1.0[a]	75.9±0.6	52.2±2.4[a]	26.7±1.6[a]	17.4±1.1[a,b]
E-selectin / Ls-174-t	16.0±1.6[a]	77.9±5.7	53.9±2.6[a]	43.1±1.7[a]	29.5±2.6[a,b]
ICAM-1 / HepG2	5.0±1.3[a]	71.0±2.4	68.6±1.8	69.0±1.4	67.3±2.2 [a]
ICAM-1 / BEL7404	3.0±0.9[a]	65.9±4.1	63.6±5.0	63.5±4.0	64.7±1.7
ICAM-1 / Ls-174-t	5.8±0.8[a]	63.0±2.0	63.4±1.2	64.5±1.8	62.8±1.3

(1) I: Basal, II:TNF-α, III: Cimetidine, IV: Lipo-ASON, V: Lipo-ASON / Cimetidine. (2) [a]P<0.05, *vs* groupII; [b]P<0.05, group V *vs* group IV.

DISCUSSION

ICAM-1 cDNA with 2.98 Kb in length consists of 5' -untranslated region, 57 bp, a continuous open reading frame, 1 598 bp, and 3' -untranslated region, 1 330 bp. E-selectin cDNA with 3.85 Kb in length consists of 5' -untranslated region, 116 bp, a continuous open reading frame, 1 830 bp, and 3' -untranslated region, 1 898 bp. Bennett' s research[14] showed that among all the designed ASONs, ASONs hybridized to 3' -untranslated region were more potent than those hybridized to AUG transcription-initiation site or 5' -untranslated region. In our previous experiment, we found that each selected ASON of ICAM-1 and E-selectin hybridized to 3' -untranslated region could reduce the expression of objective gene by nearly 40% respectively, demonstrating that their effects were sequence specific. This loss in mRNA might be due to the ASON targeted objective pre-mRNA in the nucleus, which resulted in activation of endogenous RNase H or a related enzyme and mediated hydrolysis of mRNA-ODN at the hybridization site[17,18].

With the presence of cytokines such as IL-1β and TNF-α secreted by tumor cells, vascular endothelial cells can produce different adhesive molecules at different times. After stimulation of cytokines, E-selectin shows its features in 4 to 5 hours, which mediate the early adhesion, and ICAM-1 shows its features in 12 to 48 hours, which mediate the late adhesion. In this article, through the treatment with E-selectin or ICAM-1 ASON before the induction by TNF-α, the expressions of objective molecules were blocked, but the adhesion between endothelial cells and tumor cells was only significantly inhibited by E-selectin ASON. The reasons were as follows: 1) Hepatic and colorectal cancer cells could highly express sle^x Ag[19], the ligand of E-selectin. It is the major molecule that mediates the adhesion to endothelial cells directly. 2) ICAM-1 also appeared in the membrane of hepatic or colorectal cancer cells. The adhesion between cancer cells and vascular endothelial cells *in vitro* resorted to LFA-1[20], the ligand of ICAM-1, which expresses on the membrane of white blood cells. However, there were no white blood cells to bridge *in vivo*. 3) Hepatic or colorectal cancer cells like HepG2 could also express affluent integrin $\alpha_2\beta_1$, which directly mediated the adhesion between tumor cells and endothelial cells[19]. It is this kind of molecules that work in ICAM-1 ASON treated group.

Cimetidine, a kind of H2 receptor antagonist, has been used to inhibit the secretion of gastric acid in clinic. It has also been used to prolong the survival of patients with various forms of cancer in recent years[21,22]. Even at the highest noncytotoxic concentration (10^{-4}M), cimetidine has been shown not to influence the expression of ICAM-1 protein, ICAM-1 mRNA and E-selectin mRNA, but could obviously reduce the expression of E-selectin protein[22,23]. We proposed that cimetidine' s inhibitory action on metastasis was due to blocking the adhesion between endothelial cells and tumor cells, especially colorectal cells with higher expression of sle^x Ag[24]. Since other H2 receptor antagonists, such as ranitidine and famotidine, did not play the same role under the same experimental conditions[22], it has become obvious that the mechanism of cimetidine' s inhibiting function is involved in a step after transcription. p38 MAPK[25], for instance, might be the regulatory molecule that activates the expression, of a number of genes at the level of posttranscription[26-28].

Our research demonstrated that E-selectin played a primary role in initiating the adhesion of cancer cells to vascular endothelial cells through its interaction with its specific ligand, sialyl Lewis antigens[29,30]. Lipo-E-selectin ASON in combination with cimetidine could inhibit the expression of E-selectin more effectively and reduce the adhesive rate of endothelial cells to tumor cells in early stage, reaching the goal of double benefits from outside and inside of cells. It not only guarantees the major aim of inhibiting adhesion, but also lowers the negative influence of cells subjected to drug *ab extra*. In short, this study provides some useful proofs for gene therapy of antiadhesion, which would become a new therapeutic alternative to inhibit high recurrence and metastasis of hepatic or colorectal cancer.

REFERENCES

1 **Giles RV**. Antisense oligonucleotide technology: from EST to therapeutics. *Curr Opin Mol Ther* 2000; **2**: 238-252

2 **Capiati DA**, Vazquez G, Tellez Inon MT, Boland RL. Antisense oligonucleotides targeted against protein kinase c alpha inhibit proliferation of cultured avian myoblasts. *Cell Prolif* 2000; **33**:

307-315
3 **Zhoug S**, Wen SM, Zhang DF, Wang QL, Wang SQ, Ren H. Sequencing of PCR amplified HBV DNA pre-c and c regions in the 2.2.15 cells and antiviral action by targeted antisense oligonucleotide directed against sequence. *World J Gastroenterol* 1998; **4**: 434-436
4 **Wang XW**, Yuan JH, Zhang RG, Guo LX, Xie Y, Xie H. Antihepatoma effect of alpha-fetoprotein antisense phosphorothioate oligodeoxyribonucleotides *in vitro* and in mice. *World J Gastroenterol* 2001; **7**: 345-351
5 **Acosta R**, Montanez C, Gomez P, Cisneros B. Delivery of antisense oligonucleotides to PC12 cells. *Neurosci Res* 2002; **43**: 81-86
6 **Agrawal S**, Kandimalla ER, Yu D, Hollister BA, Chen SF, Dexter DL, Alford TL, Hill B, Bailey KS, Bono CP, Knoerzer DL, Morton PA. Potentiation of antitumor activity of irinotecan by chemically modified oligonucleotides. *Int J Oncol* 2001; **18**: 1061-1069
7 **Guo F**, Li Y, Wu S. Antisense IRAK-2 oligonucleotide blocks IL-1-stimulated NF-kappaB activation and ICAM-1 expression in cultured endothelial cells. *Inflammation* 1999; **23**: 535-543
8 **Stepkowski SM**, Wang ME, Condon TP, Cheng-Flournoy S, Stecker K, Graham M, Qu X, Tian L, Chen W, Kahan BD, Bennett CF. Protection against allograft rejection with intercellular adhesion molecule-1 antisense oligodeoxynucleotides. *Transplantation* 1998; **66**: 699-707
9 **Cheng Q**, Chen X, Ye Y. Antisense oligonucleotide attenuates renal tubulointerstitial injury in mice with unilateral ureteral obstruction. *Zhonghua Yixue Zazhi* 1999; **79**: 533-537
10 **Lawson JA**, Adams WJ, Morris DL. Rantidine and cimetidine differ in their *in vitro* and *in vivo* effects on human colonic cancer growth. *Br J Cancer* 1996; **73**: 872-876
11 **Hahm KB**, Kim WH, Lee SL, Kang JK, Park IS. Comparison of immunomodulative effects of the histamine-2 receptor antagonist cimetidine ranitidine,and famotidine on peripheral blood mononuclear cells in gastric cancer patients. *Scand J Gastroenterol* 1995; **30**: 265-271
12 **Adams WJ**, Morris DL, Rose WR, Lubowski DZ, King DW. Cimetidine preserves non-specific immune function after colonic resection for cancer. *Aust N Z J Surg* 1994; **64**: 847-852
13 **Adams WJ**, Lawson JA, Morris DL. Cimetidine inhibits *in vivo* growth of human colon cancer and reverses histamine stimulated *in vitro* and *in vivo* growth. *Gut* 1994; **35**: 1632-1636
14 **Bennett CF**, Condon TP, Grimm S, Chan H, Chiang MY. Inhibition of endothelial cell adhesion molecule expression with antisense oligonucleotides. *J of Immunol* 1994; **152**: 3530-3540
15 **Zhang XP**, Kelemen SE, Eisen HJ. Quantitative assessment of cell adhesion molecule gene expression in endomyocardial biopsy specimens from cardiac transplant recipients using competitive polymerase chain reaction. *Transplantation* 2000; **70**: 505-513
16 **Gao X**, Huang L. Cationic liposome mediated gene transfer. *Gene Ther* 1995; **2**: 710-722
17 **Condon TP**, Bennett CF. Altered mRNA splicing and inhibition of human E-selectin expression by an antisense oligonucleotide in human umbilical vein endothelial cells. *J Biol Chem* 1996; **271**: 30398-30403
18 **Vickers TA**, Koo S, Bennett CF, Crooke ST, Dean NM, Baker BF. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. A comparative analysis. *J Biol Chem* 2003; **278**: 7108-7118
19 **Kawakami-Kimura N**, Narita T, Ohmori K, Yoneda T, Matsumoto K, Nakamura T, Kannagi R. Involvement of hepatocyte growth factor in increased integrin expression on HepG2 cells triggered by adhesion to endothelial cells. *Br J Cancer* 1997; **75**: 47-53
20 **Tanabe K**, Alexander JP, Steinbach F, Campbell S, Novick AC, Klein EA. Retroviral transduction of intercelluar adhesion molecule-1 enhances endothelial attachment of bladder cancer. *Urol Res* 1997; **25**: 401-405
21 **Matsumoto S**. Cimetidine and survival with colorectal cancer. *Lancet* 1995; **346**: 115
22 **Kobayashi K**, Matsumoto S, Morishima T, Kawabe T, Okamoto T. Cimetidine inhibits cancer cell adhesion to endothelial cells and prevents metastasis by blocking E-selectin expression. *Cancer Res* 2000; **60**: 3978-3984
23 **Leonardi A**, DeFranchis G, De Paoli M, Fregona I, Plebani M, Secchi A. Histamine-induced cytokine production and ICAM-1 expression in human conjunctival fibroblasts. *Curr Eye Res* 2002; **25**: 189-196
24 **Matsumoto S**, Imaeda Y, Umemoto S, Kobayashi K, Suzuki H, Okamoto T. Cimetidine increases survival of colorectal cancer patients with high levels of sialyl Lewis-X and sialyl Lewis-A epitope expression on tumour cells. *Br J Cancer* 2002; **86**: 161-167
25 **Read MA**, Whitley MZ, Gupta S, Pierce JW, Best J, Davis RJ, Collins T. Tumor necrosis factor α-induced E-selectin expression is activated by the nuclear factor-κB and c-JUN N-terminal kinase / p38 mitogen-activated protein kinase pathways. *J Biol Chem* 1997; **272**: 2753-2761
26 **Pietersma A**, Tilly BC, Gaestel M, de Jong N, Lee JC, Koster JF, Sluiter W. p38 mitogen activated protein kinase regulates endothelial VCAM-1 expression at the post-transcriptional level. *Biochem Biophys Res Commun* 1997; **230**: 44-48
27 **Caivano M**. Role of MAP kinase cascades in inducing arginine transporters and nitric oxide synthetase in RAW264 macrophages. *FEBS Lett* 1998; **429**: 249-253
28 **Miyazawa K**, Mori A, Miyata H, Akahane M, Ajisawa Y, Okudaira H. Regulation of interleukin-1 β-induced interleukin-6 gene expression in human fibroblast-like synoviocytes by p38 mitogen-activated protein kinase. *J Biol Chem* 1998; **273**: 24832-24838
29 **Majuri ML**, Niemela R, Tiisala S, Renkonen O, Renkonen R. Expression and function of α2,3-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
30 **Kunzendorf U**, Kruger-Krasagakes S, Notter M, Hock H, Walz G, Diamantstein T. A sialyl-Le(x)-negative melanoma cell line binds to E-selectin but not to P-selectin. *Cancer Res* 1994; **54**: 1109-1112

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