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World J Gastroenterol 2005;11(31):4916-4917
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Expression of heparanase mRNA in anti-sense oligonucleotide-transfected human esophageal cancer EC9706 cells

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Supported by the Natural Science Foundation of Henan Province, No. 0311043700 and the Foundation for Young Mainstay Teachers in Colleges and universities of Henan Province, No.100(2003) and the Building Foundation for 211 Key Fields during the 15th Five-year Plan Period of Ministry of Education, No. 2(2002)
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Received: 2005-02-17 Accepted: 2005-04-02

Abstract

AIM: To investigate the effects of anti-sense oligonucleotides (ASODNs) on mRNA expression of heparanase in human esophageal cancer EC9706 cells.

METHODS: One non-sense oligonucleotide (N-ODN) and five ASODNs against different heparanase mRNA sites were transfected into EC9706 cells, then the expression of heparanase mRNA in EC9706 cells was studied by *in situ* hybridization.

RESULTS: The expression of heparanase mRNA could be inhibited by ASODNs.There was no significant difference among five ASODNs ($P>0.05$), but there was a significant difference between ASODNs and N-ODN or non-transfected group (ASODN1: 2.25±0.25, ASODN2: 2.21±0.23, ASODN3: 2.23±0.23, ASODN4: 2.25±0.24 *vs* N-ODN: 3.47±2.80 or non- transfected group: 3.51±2.93 respectively, $P<0.05$).

CONCLUSION: The expression of heparanase mRNA in EC9706 cells can be inhibited by ASODNs *in vivo*, and heparanase ASODNs can inhibit metastasis of esophageal squamous cell carcinoma or other tumors by inhibiting the expression of heparanase.

Key words: Esophageal cancer EC9706 cells ;Heparanase; Anti-sense oligonucleotides; *In situ* hybridization

Chen KS, Zhang L, Tang L, Zhang YH, Gao DL,Yan L, Zhang L. Expression of heparanase mRNA in anti-sense oligonucleotide-transfected human esophageal cancer EC9706 cells. *World J Gastroenterol* 2005; 11(31): 4916-4917
http://www.wjgnet.com/1007-9327/11/4916.asp

INTRODUCTION

Heparanase (HPA) is closely associated with cell proliferation, differentiation, adhesion and exudation, and may play a crucial role not only in tumor development and progression but also in tumor invasion and metastasis[1] Studies indicate that HPA is expressed in tumor tissues, such as liver carcinoma, ovarian adenocarcinoma, cervical squamous carcinoma, colonic adenocarcinoma, lymphoma, fibrosarcoma and melanoma[1,2]. We detected the expression of HPA mRNA in anti-sense oligonucleotide (ASODN) transfected by *in situ* hybridization, so as to provide the basic theory for esophageal carcinoma target therapy by heparanase ASODN technique.

MATERIALS AND METHODS

Materials

Human esophageal carcinoma EC9706 cell line was provided by Chinese Academy of Medical Sciences Heparanase ASODNs1-4, N-ODN and biotin-labeled HPA cDNA probe were synthesized by DaLian Bao Bio Co., Ltd.SA-Bio-AP and BCIP/NBT were purchased from Promega Company, USA RPMI-1640 was obtained from Gibco Company, USA.

Cell culture

Human esophageal carcinoma EC9706 cells (a adherent cell line) were cultured in RMPI-1640 and divided into six groups until formation of monolayer on the bottle wall. Groups 1 to 4 were treated with 20 μmol/L ASDON1, ASDON2, ASDON3, ASDON4, respectively. Control group 1 was treated with N-ODN at the same concentration. Neither ASDON nor N-ODN was added to control group 2. All groups were cultured for another 48 h, and then the cells were harvested after trypsinization The cells were adjusted to a concentration of 10^6/mL and added to the prepared slides for hybridization.

In situ hybridization

The samples were fixed in 40 g/L paraformaldehyde for 30 min. After being washed in distilled water, samples were pre-treated with fresh 0.5% H_2O_2/formaldehyde to block endogenous peroxidases for 30 min at room temperature. The samples were treated with 30 g/L proteinase k diluted freshly by citric acid for 10 min at 37 ℃, pre-hybridized in pre-hybridized solution without probe for 4 h, and then for an additional 12 h at 42 ℃. The final concentration of the labeled heparanase probe was 1 μg/L. After hybridization, excess probes were removed through rinsing in 0.1×SSC at 42 ℃, and samples were treated with SA-Bio-AP for 10 min

at 37 ℃, and rinsed again. Color reaction was performed with new BCIP/NBT in dark for 2-4 h. The samples without probe were used as negative control.

Result assessment

Ten fields of each section were observed under oil microscope and 100 cells of each field were counted. Scores were obtained according to the staining intensity and count degree of *in situ* hybridization [3].

Statistical analysis

The SPSS 10.0 statistical package was used for all analyses. Data were expressed as mean±SD, and analyzed by ANOVA $P<0.05$ was considered statistically significant.

RESULTS

Expression of heparanase mRNA in EC9706 cells

Heparanase mRNA (blue-purple granule) was located in cytoplasm. There was no positive signal in the cell sample without probe (Figure 1).

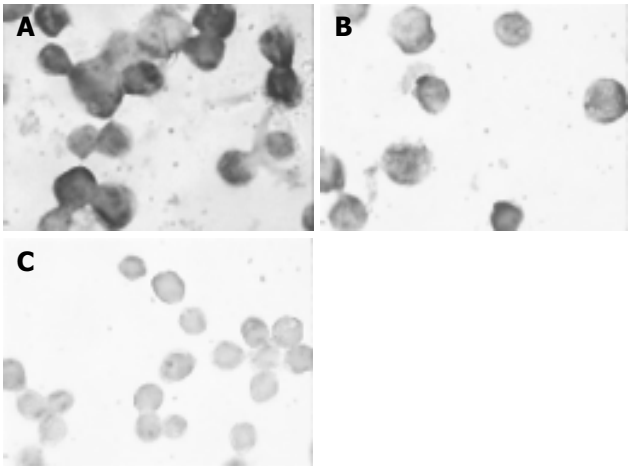

Figure 1 Expression of heparanase mRNA in human esophageal cancer EC9706 cells. **A:** non- transfected group; **B:** ASODNs-transfected group; **C:** control group without probe.

Influence of ASODN on expression of heparanase mRNA in EC9706 cells

The level of heparanase mRNA expression in ASODNs1-4-transfected EC9706 cells was 2.25±0.25, 2.21±0.23, 2.23±0.23, 2.25±0.24, respectively There was a significant difference between experimental group and control group 1 (3.47±2.80, $P<0.05$). But there was no significant difference between control groups 1 and 2 (3.51±2.93). There was no significant difference in different ASODNs.

DISCUSSION

Heparanase is a kind of endoglycosidase. It can degrade haparan sulfate glyoprotein, damage the basement membrane and promote invasion and metastasis of tumors. It expresses in tissues of spleen, lymph nodes, peripheral blood, bone marrow and infant liver, but not in tissues of heart, encephalon, lung, liver, kidney and pancreas of normal adults[1-4]. Studies indicate that the level of heparanase expression in liver carcinoma, ovarian adenocarcinoma, colonic adenocarcinoma and melanoma is high[4-5]. Its activity correlates with the metastatic potential of mouse lymphoma, fibrosarcoma and melanoma cells. The level of heparanase expression in tumor cells with high metastatic potential is higher than that in tumor cells with little or no metastatic potential[6-9], suggesting that heparanase gene expression is closely associated with metastasis of tumors.

To explore new methods of inhibiting the metastasis of tumor we transfected human esophageal carcinoma EC9706 cells with four heparanase anti-sense oligodeoxynucleotides (ASODNs1-4) and observed the effect of ASODN on the expression of HPA gene in EC9706 cells. The results indicate that heparanase can express in human esophageal carcinoma EC9706 cell line and the expression of heparanase mRNA in EC9706 cells were effectively depressed by transfected ASODNs1-4 suggesting that ASODNs may inhibit the invasion and expression of heparanase gene. These results may provide the basic theory for preventing the invasion and metastasis of esophageal carcinoma.

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Science Editor Wang XL and Guo SY **Language Editor** Elsevier HK