ELSEVIER

Archives of Medical Research 43 (2012) 395–401

Archives of Medical Research

PRELIMINARY REPORT

Effect on the Expression of MMP-2, MT-MMP in Laryngeal Carcinoma Hep-2 Cell Line by Antisense Glucose Transporter-1

Ying-Ying Xu,[a] Yang-Yang Bao,[a] Shui-Hong Zhou,[a] and Jun Fan[b]

[a]*Department of Otolaryngology,* [b]*State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Received for publication March 8, 2012; accepted June 28, 2012 (ARCMED-D-12-00130).

Background and Aims. Glucose transporter protein-1 (Glut-1) is correlated with biological behaviors of malignant tumors. However, there was no evidence that overexpression of Glut-1 mechanistically lead to invasion or metastasis of cancer cells. We hypothesized that Glut-1 regulates the expression of membrane type 1-MMP (MT1-MMP) and matrix metalloproteinase-2 (MMP-2).

Methods. Analysis of the expression of Glut-1, MMP-2, β-actin, and MT1-MMP was performed using RT-PCR. Expression of Glut-1 protein, MMP-2, and MT1-MMP was detected by Western blotting.

Results. At mRNA and protein levels, Glut-1 and MMP-2 were co-expressed in the Hep-2 laryngeal carcinoma cell line. After transfection, Glut-1 antisense oligodeoxynucleotide (AS-ODN) decreased the expression of MMP-2 mRNA and protein as well as Glut-1 mRNA and protein. Glut-1 AS-ODN also decreased the expression of MT1-MMP mRNA.

Conclusions. Co-expression of Glut-1 and MMP-2 in Hep-2 laryngeal carcinoma cells and Glut-1 may regulate MMP-2 and MT1-MMP expression.

Key Words: Glucose transporter protein-1, Matrix metalloproteinases, Laryngeal carcinoma, Antisense oligodeoxynucleotide, Targeted therapy.

Introduction

Many studies have shown relationships between overexpression of glucose transporter protein-1 (Glut-1) and certain biological behaviors of malignant tumors (1–3). In previous studies we found that the *Glut-1* gene expression level and protein expression were correlated with lymph node metastasis, poor survival, and clinical stages of head and neck carcinomas (2). Furthermore, a significant correlation was found between increased uptake of 2-fluorodeoxyglucose and Glut-1 mRNA or Glut-1 protein (4). However, high expression of Glut-1 only indicated increased malignant potential and cancer cell proliferation (1–6); there was no evidence that overexpression of Glut-1 mechanistically lead to invasion or metastasis of cancer cells.

Metastasis involves degradation of the basement membrane and stromal extracellular matrix (ECM) and migration into adjoining blood vessels, resulting in tumor growth at distant organ sites (7,8). This invasive process is thought to involve multiple proteolytic enzymes including matrix metalloproteinases (MMPs), which are secreted by tumor cells (9). Recent studies showed that MMPs are an important factor in the invasion and metastasis of malignant cells (10–12). MMPs may degrade the basement membrane and ECM. At least 22 members of the human MMP gene family have now been identified. Among these, the gelatinases, gelatinase A (MMP-2) and gelatinase B (MMP-9), are associated with the malignant tumor cell phenotype because of their unique ability to degrade type-IV collagen, a major component of the basement membrane. MMP-2 has been shown to be activated by

Address reprint requests to: Shui-Hong Zhou, Department of Otolaryngology, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou City, Zhejiang Province 310003, China; Phone: 86-13957125581; FAX: 86-57187236895; E-mail: zhouyunzhoush@163.com

0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2012.07.003

membrane type 1-MMP (MT1-MMP). MT1-MMP has been studied most extensively among the MT-MMPs (9,13,14).

Because the invasion and metastasis of cancer indicate accelerated growth of cancer cells, and because the uptake and use of glucose is increased accordingly, we suggest that there may be some correlations between Glut-1, MT1-MMP, and MMP-2 involving their common participation in cancer metastasis and invasion. However, the exact mechanism of invasion and metastasis of Glut-1, MT1-MMP, and MMP-2 in cancer is not yet clear. There are few studies on the mechanisms (7,15) and, to our knowledge, there is no report on laryngeal carcinoma. We hypothesized that Glut-1 may regulate the expression of MT1-MMP and MMP-2, which leads to invasion and metastasis of laryngeal carcinoma.

To test this we examined the effects of antisense oligodeoxynucleotides (AS-ODNs) against Glut-1 expression on the activities of MT1-MMP and MMP-2 and the invasion of laryngeal carcinoma Hep-2 cells in the present study. Our results may again confirm the feasibility of Glut-1 as a potential therapeutic target for laryngeal carcinoma *in vitro.*

Materials and Methods

Cells, Antibodies, and Plasmids

The laryngeal carcinoma Hep-2 cell line was purchased from the Cell Research Institute of the Chinese Academy of Sciences (Shanghai, China). Chloroform, isopropyl alcohol, and anhydrous alcohol were purchased from Hangzhou Changzhen Chemical Plant (Hangzhou, China). Agarose was purchased from Biowest (Madrid, Spain). Trizol was purchased from Invitrogen (Carlsbad, CA). Reverse transcriptase MMLV and TAQ enzyme were purchased from Promega (Madison, WI). DNA Marker DL2000, pcDNA3.1 vector, restriction endonucleases *Hind*III and *Xba*I, and T_4 DNA ligase were purchased from TaKaRa Co (Osaka, Japan). Tween 20 and Ponceau S were purchased from Sigma (St. Louis, MO). Primary antibodies against Glut-1, MMP-2, and MT1-MMP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies donkey anti-rabbit and donkey anti-mouse, cell lysis, Supersignal West Femto Kit, and PMSF were purchased from Pierce (Rockford, IL). Primers were synthesized by Invitrogen. Sequences of the entire coding regions of Glut-1, MMP-2, and MT1-MMP were obtained from GenBank, and primers were designed using ClustalX and Omega 2.0 Applied Software.

Cell Culture

Hep-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO-BRL, Gaithersburg, MD) containing 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT), 2 mM L-glutamine, 100 U/mL penicillin, and 100 g/mL streptomycin at 37°C in a 5% CO_2 atmosphere. Cells were trypsinized and harvested after reaching 80–90% confluence.

Generation of Glut-1 cDNA, Construction of Glut-1 Expression Vector and Antisense Oligonucleotides

Construction of the pcDNA3.1 Glut-1 antisense and sense vectors has been described previously (15). Briefly, total RNA was isolated from Hep-2 cells. Reverse transcription (RT)-PCR was used to generate Glut-1 complementary DNA (cDNA) from 800 ng of total RNA. The product of the antiGlut-1 or Glut-1 pcDNA3.1 vector was digested with *Hind*III and *Xba*I, and cDNA was extracted from an agarose gel. After digestion, cDNA was then subcloned into the vector pcDNA3.1 with T_4 DNA ligase. The linked products were named pcDNA3.1-anti-Glut-1(+) and pcDNA3.1-Glut-1(+). The transforming mixture was selected on LB agar plates with ampicillin (100 mg/L). To confirm positive clones, the recombinant plasmids pcDNA3.1-anti-Glut-1(+) and pcDNA3.1-Glut-1(+) were digested with *Hind*III and *Xba*I. The sequences of the amplified PCR products were then verified.

Cell Culture and Transfection with Glut-1 Antisense Oligonucleotides or Empty Expression Vector

When Hep-2 cells were in good condition and the numbers of cells were adequate, Hep-2 cells were seeded in six-well plates at a density of 3.12×10^5 cells/well. After 24 h, empty pcDNA3.1 vectors (mock transfection), pcDNA3.1-anti-Glut-1(+), or pcDNA3.1-Glut-1(+) was transfected into Hep-2 cells using the Lipofectamine 2000 reagent (Invitrogen). The transfected quantity of each plasmid was 4 μg. Each group had three wells/plate according to the manufacturer's protocol. After 24 h, the medium was replaced with 1200 μg/mL G418 medium. The cells in two of three nontransfected wells were replaced with 1200 μg/mL G418-selective medium. The medium was then replaced every 2 days until it was full of transfected cells in each of the six-well plates. After cultured cells were propagated to passage to a 25 cm^2 cell culture flask, the cells were amplified. After the cells were amplified to some degree, some tubes of Hep-2 cells with different transfected plasmids were frozen, and then some cells were assayed.

DNA Extraction and Identification of Transfection by PCR

DNA from each group was extracted using a TIANamp Genomic DNA Kit (Tiangen Biotech, DP304, Beijing, China) according to the manufacturer's instructions. Briefly, cells were harvested and transferred to a 1.5 mL microcentrifuge tube. They were centrifuged (12,000 rpm, 30 sec) to pellet the cells. The supernatant was then