Influence of the MACC1 Gene on Sensitivity to Chemotherapy in Human U251 Glioblastoma Cells

Chao Shang1, Yang Hong2, Yan Guo3, Yun-Hui Liu2, Yi-Xue Xue1* 

Abstract

Background: This study was conducted to determine the influence of MACC1 expression on chemotherapy sensitivity in human U251 glioblastoma cells. Materials and Methods: Expression of the MACC1 gene in 49 cases of human brain glioma was determined by quantitative real-time PCR. Silencing effects of RNA interference on MACC1 was detected by Western-blotting. Flow cytometry methods and methyl thiazolyl tetrazolium assay (MTT) were used to determine the apoptosis and growth inhibitory rates of the U251 cells with MACC1 silencing, before and after treatment with cisplatin (DDP). Results: MACC1 mRNA in gliomas was up-regulated remarkably, to 158.8% of that in peri-cancerous tissues (p<0.05). The siRNA-MACC1 could inhibit the expression of MACC1 protein significantly (p<0.05), associated with an increase in apoptosis rate from 2.57% to 5.39% in U251 cells and elevation of the growth inhibitory rate from 1.5% to 17.8% (p<0.05 for both). After treatment with DDP at various concentrations (1, 3, 5μg/ml), compared with control U251 cells, the apoptosis rate of MACC1-silenced U251 cells rose from 8.41%, 13.2% and 19.5% to 12.8%, 17.8% and 25.8%; the growth inhibitory rate increased from 16.2%, 19.3% and 24.5% to 23.7%, 28.4% and 36.3%. Conclusions: There is a notable relationship between over-expression of MACC1 and the characteristics of glioma cells. Silencing of MACC1 was found to enhance the apoptosis and growth inhibitory rates of U251 glioma cells, and thereby increase their sensitivity to DDP chemotherapy.

Keywords: Brain glioma - chemotherapy sensitivity - MACC1 gene - U251 cell

Asian Pac J Cancer Prev, 16 (1), 195-199

Introduction

Glioma is the most general primary tumors in nervous system, and show the highest mortality and mortality among endocranial tumors, because of the marked characteristics of malignant proliferation and invasion (Kuhnt et al., 2011). Gliomas are rarely curable. Treatment for a glioblastoma is customized to the individual patient and may include surgery, radiation therapy, chemotherapy, or observation. So far, surgery is the most important initial approach. Chemotherapy and radiotherapy after initial surgical resection is regarded as an effective treatment plan to prevent recurrence and metastasis (Mrugala et al., 2013). However, chemotherapy does not work for everyone with a glioma. It helps about half the people treated. For this reason, the prognosis for patients especially with high-grade glioma is still gloom, despite being given prompt and comprehensive treatment. The median survival time for adults with an anaplastic astrocytoma is about two to three years, and for those with more aggressive glioblastomas, median survival drops off to about 12-14.6 months with a two-year median survival rate of 30% (Nieder et al., 2008).

In this new era, investigating the molecular mechanisms in carcinogenesis might shed light on this deadly disease (Vlachostergios et al., 2013; Burgio et al., 2014; Ugur et al., 2014). In our previous researches, the gene chips were used to detecte the up-regulation of metastasis-associated in colon cancer-1 (MACC1) gene in human glioma. MACC1 gene was firstly identified by Stein et al in 2009 (Stein et al., 2009). MACC1 could activate the HGF/MET signal pathway and mediate the metastasis and recurrence of colorectal cancer (Wang et al., 2014). The current reports suggested that MACC1 participated in regulation of cell proliferation, apoptosis, migration and invasion (Meng et al., 2013; Zhang et al., 2014; Zhen et al., 2014). Proximally, MACC1 has been identified to act as a key biomarker for the prognosis of kinds of cancer, including colorectal cancer, gastric carcinoma and non-small cell lung cancer (Ma et al., 2013; Wang et al., 2014; Yamamoto et al., 2014). Therefore, the object of this study was to first determine the correlation between the expression abnormality of MACC1 and the carcinogenesis and development of human glioma, and then search for its possible influence on chemotherapy sensitivity of human glioblastoma U251 cells.

1Department of Neurobiology, College of Basic Medicine, 2Department of Neurosurgery, Shengjing Hospital, 3Department of Central Laboratory, School of Stomatology, China Medical University, Shenyang, China *For correspondence: xueyixue2012@163.com
Materials and Methods

Materials

The study was approved by the Ethics Committees, and we obtained patient’s permission before surgery. Total 49 glioma and corresponding paracancerous tissues provided by the Department of Neurosurgery, Shengjing Hospital of China Medical University from May 2011 to April 2014. The tumors with at least 1 cm margin from the corresponding peri-cancerous tissues were obtained from all patients through surgical resection and further histologically proven to be gliomas.

All patients had not experienced radiation or chemotherapy before surgery. The patients include 32 men and 17 women (mean age: 54.1±3.8 years, age range: 47-69 years); and included 23 cases of astroglialomas (Grade I-II), 14 of anaplastic gliomas (Grade III), 12 of glioblastomas (GBM, Grade IV). Human glioblastoma cell line U251 was obtained from Biological Sciences Cell Resource Center (China). Real-time PCR reagents were from Takara Bio (Japan), TransMessenger from Qiagen (Germen), siRNA-MACC1 and siRNA-control from Invitrogen (Carlsbad, CA, USA), DDP from Sigma (USA). The MTT Cell Proliferation Assay Kit was from Beyotime Company (Shanghai, China), Annexin V Cell Apoptosis Assay Kit from Biosea Company (Beijing, China). The PCR primers of MACC1 gene were synthesized by Takara Bio (Japan).

Quantitative real time -PCR (qRT-PCR)

After total RNA was extracted from tissue and cell samples, cDNA was synthesized and used to detect the mRNA expression (Shang et al., 2014). The MACC1 primer was designed by Primer5 as follows: forward primer, 5’- AGGAGGTCACTTGGTTCTCA -3’, reverse primer, 5’- GAGCCCAGCAGTGTCGTTCA -3’, and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as reference. The samples were normalized to 18s and the 18s<CT<30 were calculated with 2^-ΔΔCt using the Applied Biosystems 7500.

Cell transfection

24h before transfection, appropriate concentration (about 80%) resuspended U251 cells were seeded on 6-well plates. Then 1 mg of siRNA-MACC1 or siRNA-control was mixed with Enhancer R, followed by mixing with 4 μl TransMessenger, and then 900μl Serum-free medium was further added for incubating the non-transfected U251 cells. The transfected cells were incubated for 4h, and normal media was added. After 48h, the cells were harvested to detect further.

Western blot analysis

Cells were harvested and extracted protein. SDS-PAGE electrophoresis and antibody hybridization were practiced as describe previously. The ECL analysis system (Santa Cruz, USA) was used for detection in accordance with the manufacture’s protocol. Western blot quantification was performed using Image Processing and Analysis software. GAPDH was selected to be reference protein.

Flow cytometry detection

Cells (5x10^4) were harvested, and the apoptosis detection kit (Biosea, China) was used to examine the apoptosis rate in accordance with manufacturer’s instruction. Then the cells were read by flow cytometry (BD, USA) (Ex 488 nm, Em 635 nm), and the obtained numerical values were analyzed with CELLQuest 3.0 software (BD, USA). Annexin V positive cells were regarded as apoptosis cells. The cells were counted by a dual-color flow cytometric method.

Cell proliferation assay

Cells were seeded in 96-well plate with 2x10^4 cells per well. 20 μl of 0.5mg/ml MTT solution (Sigma, USA) was added to each well, and the 96-well plate was incubated at 37°C. We cleaned up the media after 4 hr, and added 0.2 ml DMSO to each well. The 96-well plate was incubated 30 min, and read on an enzyme-labeled instrument (Bio-Rad, USA) with 570 nm wavelength. The obtained numerical values were used to construct the cell growth curve.

U251 cells treated by DDP

U251 cells were treated with DDP of three different concentrations (1, 3, 5 μg/mL) (Jiang et al., 2000; Zhou et al., 2013), and the cell growth inhibitory rate and apoptosis rate was detected after 24h of incubation.

Statistical analysis

All results were obtained from independent experiments in three times. All numerical data were presented as mean±standard deviation (SD) and dealt with SPSS 13.0 software. A Student’s t-test was performed to determine the significant differences between two groups. Pearson’s correlation was analyzed between the grade of glioma and relative expression levels of MACC1 mRNA. One-way ANOVA and post hoc comparisons (LSD test) were used to determine the significant differences among multiple groups. p<0.05 was considered as significant.

Results

MACC1 mRNA up-regulated in human brain glioma specimens

Amplification of the MACC1 gene was shown in human brain gliomas using the primer melting curve analysis. The qRT-PCR analysis showed the ΔCt of glioma and peri-cancerous tissues were 2.947±0.314 and 3.614±0.297 respectively, and the ΔΔCt was -0.667. Compared with corresponding peri-cancerous tissues, the MACC1 expression increase 158.78% in the glioma (p<0.05) (Figure1). Further, there is an positive relationship between the MACC1 expression and the pathologic grades of gliomas (Table 1).

Influence of MACC1 silencing on apoptosis and growth inhibition of U251 cell

The western blot images showed clear bands of MACC1 protein in all groups (Figure 2). The analytical results confirmed that there was no significant difference between two control groups (p>0.05). However, compared
Influence of MACC1 Expression on Chemotherapy Sensitivity in Human U251 Glioblastoma Cells

No significant difference was found between two control groups ($p>0.05$). The apoptosis rate in MACC1 silence group was significantly elevated relative to two control groups ($p<0.05$).

The growth inhibitory rate increased from 1.5% in negative control group to 17.8% in MACC1 silence group (Fig.3B), presenting significantly higher inhibition of cell proliferation in MACC1 silence group ($p<0.05$).

Impact of MACC1 gene silencing on chemotherapy sensitivity of human glioma U251 cells

In the U251 cells treated with DDP, the apoptosis rate with different DDP concentrations DDP (1, 3, 5μg/ml) were (8.41±0.41)%, (13.24±0.47)% and (19.53±0.51)%. And in the MACC1-silenced U251 cells treated with DDP, the apoptosis rates were (12.87±0.52)%, (17.75±0.46)% and (25.82±0.29)% respectively (Figure 4A). Compared with the U251 cells treated with DDP, the apoptosis rate was significantly higher than that in the MACC1-silenced U251 cells at each DDP concentration (all $p<0.05$).

Table 1. Correlation between MACC1 mRNA Expression in Brain Glioma Tissue and Pathological Differentiation

<table>
<thead>
<tr>
<th>Differentiation (Grade)</th>
<th>Number of cases</th>
<th>MACC1 mRNA expression relative quantification (gliomas/ peri-cancerous tissues)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium-well differentiated (Grade I-II)</td>
<td>23</td>
<td>1.467±0.148</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Anaplastic glioma (Grade: III)</td>
<td>14</td>
<td>1.635±0.153</td>
<td></td>
</tr>
<tr>
<td>Glioblastoma (GBM, Grade: IV)</td>
<td>12</td>
<td>1.765±0.161</td>
<td></td>
</tr>
</tbody>
</table>

*A one-way ANOVA showed that FUBP1 mRNA expression in brain glioma tissue was significantly different among the three groups with different pathological differentiation of glioma ($p<0.05$); LSD test showed significant difference between groups of medium-well differentiated glioma (Grade I-II) and anaplastic glioma (Grade: III) ($p=0.03$); as well as groups of medium-well differentiated glioma (Grade I-II) and Glioblastoma (GBM, Grade: IV) ($p=0.01$)
Following similar trends, the sensitivity of MACC1 gene in glioma.

There was not a conclusive report about chemotherapy (Hagemann et al., 2013; Yang et al., 2014). However, reported in many kinds of cancers, including glioma. Furthermore, overexpression of MACC1 gene had been identified necessary for carcinogenesis and metastasis. Met to promote HGF/Met signal pathway which had been oncogene and metastasis-inducing gene, it could activate the mechanisms of how MACC1 influences cell apoptosis and proliferation.

In conclusion, MACC1 functions as an oncogene in glioma carcinogenesis. The silencing of MACC1 could enhance the chemotherapy sensitivity of DDP in U251 cells. In this context, MACC1 would be a new potential therapeutic target for glioma patients.
Influence of MACC1 Expression on Chemotherapy Sensitivity in Human U251 Glioblastoma Cells

In this study, we investigated the role of MACC1 expression in chemotherapy sensitivity in human U251 glioblastoma cells. MACC1 was found to be a target gene for glioma treatment, or provide guidance to show chemosensitivity for chemotherapy.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (81172408, 81301862).

References


