THE EFFECT OF TRANSFECTED CX43 GENE ON THE GJIC AND PROLIFERATION OF GLIOMA CELLS

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ABSTRACT

Objective: To evaluate the effect of Cx43 gene on gap junction intercellular communication (GJIC) and proliferation of glioma cells. Methods: Cx43 cDNA was transfected into TJ905 human glioblastoma cells using lipofectamine. The expression of Cx43 was identified by Northern blot analyses, in situ hybridization and immunohistochemistry. MTT assay and average number of AgNORs (Argyrophlic nuclear organizer regions) were used to determine the cell proliferation. TUNEL method was used for detection of cell apoptosis, and scrape loading and dye transfer method for examination of GJIC. Results: The Cx43 expression was greatly upregulated when Cx43 gene was transfected into TJ905 glioma cells. The cell proliferation was inhibited while the cell apoptosis was not increased and GJIC was significantly restored in the glioma cells transfected with Cx43 gene. Conclusion: Cx43 gene has an inhibitory effect on the glioma cell proliferation, but no effect on induction of cell apoptosis. The restoration of GJIC may be the major mechanism involved in its effect. Cx43 gene can be the candidate for gene therapy of gliomas.

Key words: Malignant glioma cells, Cx43 gene, Gene transfection, GJIC, Cell proliferation

Cell to cell communication via gap junction plays an important role in the maintenance of normal cell growth. Its disruption may lead to aberrant cell growth and eventually development of tumors. Gap junctions (GJ) are specialized intercellular channels between plasma membrane of two adjacent cells. Each GJ channel is composed of two connexons contributed by each of two communicating cells and each connexon is a hexameric assembly of protein subunits known as connexins (Cx). Cx genes function as a family of tumor suppressor genes. At least 14 different members in Cxs family have been identified from mammalians and they are expressed in tissue specific manner. For instance, Cx43 is the predominant connexin in the brain expressed in astrocytes, while oligodendrocytes and some neurons express Cx32.

In our previous study, it was found that Cx43 gene expression was significantly downregulated with the ascending of tumor grade. Sorocanu, et al also reported an inverse correlation between the amount of Cx43 protein and malignancy grade of gliomas by Western blot and immunohistochemical analyses.

To further study the role of Cx43 gene in the development of glioma, we have transfected Cx43 cDNA into TJ905 human glioblastoma cell line without endogenous Cx43 expression, the effect of Cx43 on glioma cell proliferation as well as the gap junction intercellular communication (GJIC) were investigated in the present study.

MATERIALS AND METHODS

Plasmid Constructs

Plasmid PCMV-Cx43cDNA carrying the entire coding region of Cx43 gene (1.39Kb in length) and the empty vector PcDNA3.1 (5.4Kb) used in this experiment were kindly provided by Dr. David Kiang (University of Minnesota).
Cell Culture and Transfection

Human glioblastoma cell line TJ905 established by our laboratory was grown in DMEM supplemented with 10%FCS. 3x10^5 glioma cells were plated in 35mm petri dishes and grown overnight at 37°C in 5%CO2 when reaching 80% confluent the cells were transfected with plasmid PCMVCx43cDNA and PcdNA3.1 using lipofectamine (Life Technologies, USA) and positive transfectants were selected as previously described\textsuperscript{11}. Three stably transfected clones expressing different levels of Cx43mRNA as determined by Northern blot analysis were used for study.

RNA Isolation and Northern Blot Analysis

Total RNA was extracted from glioma cells using TRIzol reagent (Life Technologies, USA). The RNA was loaded on 0.9% formaldehyde agarose gels at 20 μg/lane, separated by electrophoresis, transferred to a positive charged nylon membrane, and hybridized with 32P labeled Cx43cDNA. The blots were stripped and rehybridized with 32P labeled β-actin cDNA probe as an internal control. The images were quantified by densitometry.

In situ Hybridization

Using Cx43cDNA as probe, in situ hybridization was performed with DIG DNA labeling Kit and Digoxigenin nucleic acid detection Kit (Boehringer Mannheim, Germany) according to the manufacturer’s instruction.

Immunohistochemistry

For immunostaining of Cx43, the ABC-peroxidase method was used. Coverslips plated with different groups of glioma cells were washed 3 times with Tris buffer, covered with 1:50 dilution of primary anti-Cx43 monoclonal antibody (Santa Cruz, USA) overnight at 4°C, then biotinylated secondary antibody in a dilution 1:200 was added at room temperature for 1h. After washing with Tris buffer, the cells were incubated with 3,3’ diaminobenzidine (30mg dissolved in 100 ml Tris buffer containing 0.03% H2O2) for 5 min, rinsed in water and counterstained with Haematoxylin.

Growth Rate Determination

The MTT assay was used. Briefly, 4x10^5 glioma cells in 200μl were added to each well of 96 well plate in triplicate. On each day of consecutive 6 days, 20 μl MTT (5mg/ml) was added, and the cells were incubated at 37°C for 4h. The assay was stopped by lysing the cell with 200 μl of DMSO (Dimethyl sulfoxide) for 5 min and quantification (optical density) measurements were obtained at 570nm and expressed as a percentage of control.

The proliferation activity of glioma cells was examined by counting the average number of AgNORs per cell. The coverslips bearing glioma cells were incubated for 45 min with freshly prepared Silver colloid solution, containing one volume of 2% gelatin in 1% aqueous formic acid and two volumes of 50% aqueous Silver nitrate solution, under safelight condition at room temperature and then rinsed quickly in the distilled water. The positively stained AgNORs appeared as brown-black dots in the cell nuclei. The number of AgNORs in 100-200 cell nuclei were counted under the light microscope at a magnification of x1000 (oil immersion) and the average number of AgNORs was determined for each cells.

Detection of Apoptosis

Apoptosis was detected by TUNEL method using in situ cell death Kit (Boehringer Mannheim, Germany) according to supplier’s instruction.

Scrape Loading and Dye Transfer (SLDT) Assay

Cultured cells in 35mm petri dishes were grown to confluency. After rinsing with PBS, monolayer cells were immersed in 0.05% Lucifer Yellow (MW 457.2) and 0.05% Rhodamine-Dextran (MW 10,000) in PBS. Scrape loading was performed using a sharp knife to draw several clear straight lines on the petri dishes. Cells were incubated in dye solution for an additional 3 min at room temperature, then washed with PBS and observed under inverted fluorescent microscope. Cells competent in GJIC showed transfer of Lucifer Yellow to neighboring cells from the border of scraped line, while the high molecular weight Rhodamine-Dextran dye stayed in situ in the loaded cells. Cells incompetent in GJIC did not show dye transfer, both Lucifer Yellow and Rhodamine-Dextran were retained in the original loaded cells at the injured border.

RESULTS

Cx Gene Expression in Transfected Glioma Cells

As shown by Northern blot analysis, in situ hybridization and immunohistochemical staining, the Cx43 mRNA and protein expression were upregulated.