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Antiproliferative Effect of Multiple Autocrine Loop Blockade in Human Malignant Glioma Cell Lines

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Abstract

The effects of specific antibodies against growth factors and receptors on deoxyribonucleic acid (DNA) synthesis in two established human glioma cell lines, A172 and TM-1, were examined. Anti-platelet-derived growth factor (PDGF), anti-basic fibroblast growth factor (bFGF), and anti-epidermal growth factor receptor (EGF-R) antibodies inhibited thymidine incorporation by both cell lines in serum-free medium. Antibody specific to transforming growth factor-α only slightly suppressed DNA synthesis by both cell lines. Although the antiproliferative effects of anti-PDGF and anti-bFGF antibodies decreased in serum-supplemented medium, the effect of anti-EGF-R antibody was little changed. The combination of anti-PDGF, anti-bFGF, and anti-EGF-R antibodies significantly inhibited thymidine incorporation by the two cell lines even in serum-supplemented medium. This preliminary study suggests that simultaneous blockade of multiple autocrine loops may provide a new approach to the treatment of human malignant gliomas.

Key words: multiple autocrine loops, platelet-derived growth factor, basic fibroblast growth factor, epidermal growth factor receptor, transforming growth factor-α, glioma cells

Introduction

Most human glioma cells produce platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and transforming growth factor-α (TGF-α).[8,10,17,20] Glioma cells also have specific receptors for these growth factors.[8,10,17] These growth factors enhance the growth of glioma cells *in vitro*, suggesting the involvement of multiple autocrine loops in the proliferation of human malignant gliomas.[5,8,12,19] Various methods based on this concept have been investigated for inhibiting glioma growth. Specific anti-PDGF and anti-bFGF antibodies inhibit glioma growth *in vitro* and *in vivo*.[7,13,15,16] Anti-epidermal growth factor receptor (EGF-R) antibody has an antiproliferative effect for human gliomas *in vitro*,[18] and this antibody has been conjugated to a radioisotope for *in vivo* targeting therapy for human gliomas.[4] However, whether pharmacological blockade of a single autocrine loop can effectively suppress glioma growth is unclear. In addition, the multiplicity of the growth factor-mediated pathways requires more effective therapeutic approaches.

Our present study examined the effect of specific antibodies against various growth factors and receptors on deoxyribonucleic acid (DNA) synthesis in two established human malignant glioma cell lines.

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Materials and Methods

I. Cell culture

Two human glioma cell lines, A172 and TM-1, were used in the experiments. A172 was a generous gift from the Japanese Cancer Research Resources Bank (Tokyo). TM-1 was established and characterized in our laboratory.[1,8] The cells were grown in a monolayer in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS) and maintained at 37°C under 20% O_2 and 5% CO_2 in air with 100% humidity. The cells were subcultured every 3–5 days by treatment of the monolayers with 0.25% trypsin and 0.02% ethylene-

diamine-tetra-acetic acid.

II. Specific antibodies against growth factors and receptors

Mouse monoclonal antibody specific to the human PDGF-B chain (PGF007) was a generous gift from Mochida Pharmaceutical Company (Tokyo). Mouse monoclonal anti-human bFGF antibody (3H3) was kindly provided by Takeda Pharmaceutical Company (Osaka). Goat anti-human TGF-α antibody was purchased from Biotope (Washington, U.S.A.). Mouse monoclonal anti-EGF-R antibody (clone 528) was purchased from Oncogene Science (New York, U.S.A.). Control mouse immunoglobulin G (IgG) was purchased from Japan Dako (Kyoto). The antibodies were diluted for use with serum-free Dulbecco's modified Eagle's medium (DMEM) containing 0.3% bovine serum albumin.

III. ^{3}H-thymidine incorporation assay

A172 or TM-1 cells (1×10^4 cells) growing in the logarithmic phase were plated onto a Costar 24-well tissue culture dish in DMEM supplemented with 10% FBS. The cells were incubated for 24 hours to allow attachment. The medium was then aspirated, and the culture washed twice with serum-free DMEM. The medium was then replaced with DMEM with or without 10% FBS. ^{3}H-thymidine (0.5 μCi) and specific antibodies were added at the same time. Mouse IgG was used as a control. The cells were harvested after 24 hours for labeling. DNA was extracted with salt-saturated phenol and the incorporated radioactivity was determined with a spectrometer. Each experiment was done in quadruplicate.

Results

Thymidine incorporation was inhibited in a dose-dependent manner by anti-PDGF, anti-bFGF, anti-TGF-α, and anti-EGF-R antibodies (Figs. 1 and 2). Anti-PDGF (30 μg/ml), anti-bFGF (1 μg/ml), and anti-EGF-R (20 μg/ml) antibodies suppressed thymidine incorporation by A172 cells to 74%, 49%, and 68% of the control value, respectively, in serum-free medium (Table 1). These antibodies at the same concentrations suppressed thymidine incorporation by TM-1 cells to 46%, 25%, and 42% of the control value, respectively, in serum-free medium (Table 2). Anti-TGF-α antibody only slightly suppressed thymidine incorporation by both cell lines in serum-free medium. Although the inhibitory effects of anti-PDGF and anti-bFGF antibodies decreased in medium supplemented with 10% FBS, anti-EGF-R

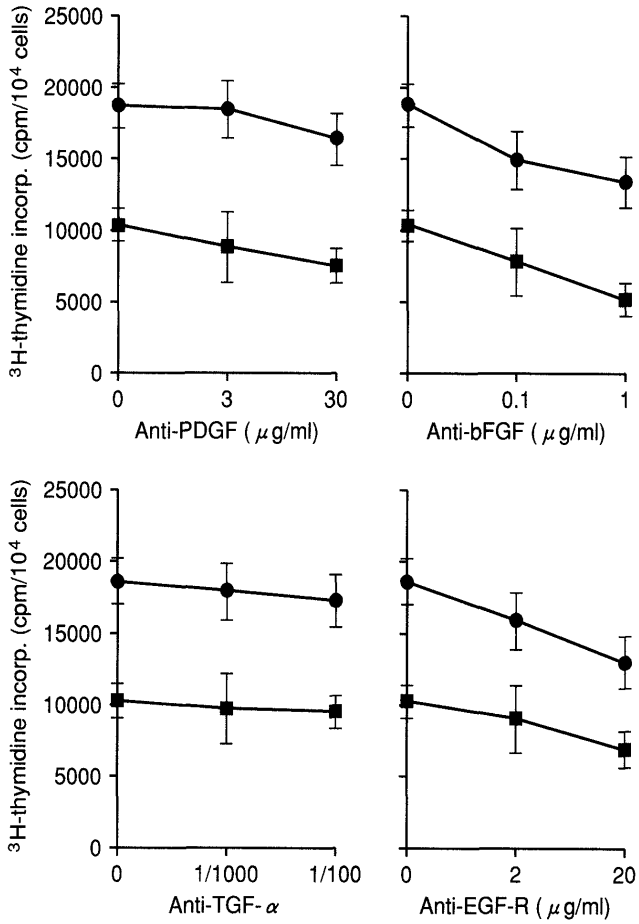

Fig. 1 Effect of anti-PDGF, anti-bFGF, anti-TGF-α, and anti-EGF-R antibodies on thymidine incorporation by A172 cells in 10% serum-supplemented medium (*circles*) and serum-free medium (*squares*).

antibody had a similar inhibitory effect on thymidine incorporation by both cell lines even in serum-supplemented medium. Anti-TGF-α antibody did not affect DNA synthesis by either cell line in the serum-supplemented medium.

The antiproliferative effect of a combination of anti-PDGF, anti-bFGF, and anti-EGF-R antibodies significantly inhibited thymidine incorporation by A172 and TM-1 cells in both serum-free and serum-supplemented medium (Tables 1 and 2).

Discussion

This study demonstrated the antiproliferative effects of anti-PDGF, anti-bFGF, and anti-EGF-R antibodies in two established human glioma cell lines. The inhibition of the DNA synthesis by each antibody differed in the glioma cell lines. Similar observations have been described previously by several in-

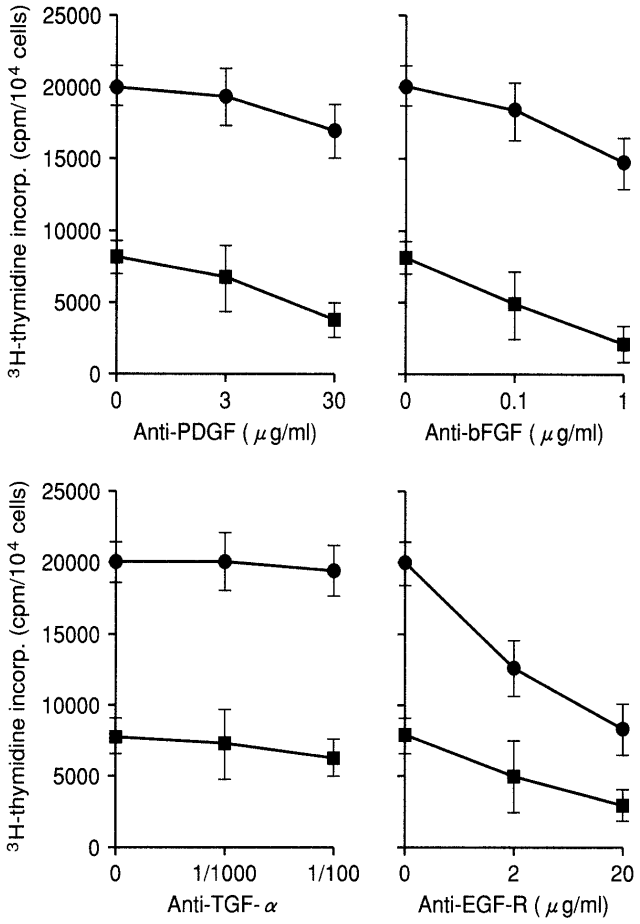

Fig. 2 Effect of anti-PDGF, anti-bFGF, anti-TGF-α, and anti-EGF-R antibodies on thymidine incorporation by TM-1 cells in 10% serum-supplemented medium (*circles*) and serum-free medium (*squares*).

Table 1 Suppression of thymidine incorporation by A172 cells with specific antibodies

Antibodies added	Mean thymidine incorporation (cpm/10^4 cells)	
	Serum-free	10% FBS
Normal mouse IgG (50 μg/ml)	10348	18689
Anti-PDGF Ab (30 μg/ml)	7604 (73.5%)*	16391 (87.7%)
Anti-bFGF Ab (1 μg/ml)	5068 (49.0%)**	13226 (70.8%)*
Anti-TGF-α Ab (1/100)	9723 (94.0%)	17378 (93.0%)
Anti-EGF-R Ab (20 μg/ml)	7078 (68.4%)*	13269 (71.0%)*
Anti-PDGF, anti-bFGF, and anti-EGF-R Abs	2763 (26.7%)**	7972 (42.7%)**

Statistical significance (*$p < 0.05$, **$p < 0.01$) using Student's unpaired t-test comparing thymidine incorporation in the presence of specific antibodies to that with normal mouse IgG (control). Ab: antibody.

Table 2 Suppression of thymidine incorporation by TM-1 cells with specific antibodies

Antibodies added	Mean thymidine incorporation (cpm/10^4 cells)	
	Serum-free	10% FBS
Normal mouse IgG (50 μg/ml)	8156	20119
Anti-PDGF Ab (30 μg/ml)	3729 (45.7%)**	16981 (84.4%)
Anti-bFGF Ab (1 μg/ml)	2028 (24.9%)**	14747 (73.3%)*
Anti-TGF-α Ab (1/100)	6689 (82.0%)	19736 (98.1%)
Anti-EGF-R Ab (20 μg/ml)	3409 (41.8%)**	8691 (43.2%)**
Anti-PDGF, anti-bFGF, and anti-EGF-R Abs	1639 (20.1%)*	7053 (35.1%)*

Statistical significance (*$p < 0.05$, **$p < 0.01$) using Student's unpaired t-test in comparison with thymidine incorporation in the presence of specific antibodies to that with normal mouse IgG (control).

vestigators.[7,13,15] Considerable variation in growth factor production and receptor expression in glioma cell lines and glioma subclones has also been reported.[9,10,17] The amount of endogenously produced growth factor and the number of receptors is considered to be related to the response of the glioma cell lines to the specific antibody. The observation that anti-TGF-α antibody only slightly suppressed DNA synthesis of both glioma cell lines can be explained as follows. First, the specific polyclonal anti-TGF-α antibody used in this study had less specificity and affinity compared to the other monoclonal antibodies. Second, both cell lines only secrete small amounts of TGF-α. Derynck *et al.*[3] reported that A172 cells did not express any messenger ribonucleic acid for TGF-α. In contrast, we observed that TM-1 cells produced TGF-α, overexpressed EGF-R, and responded to exogenously added TGF-α.[8] In addition, exogenous TGF-α stimulated thymidine incorporation by A172 cells (data not shown). These findings suggest that responsiveness to TGF-α in human glioma cells is increased by EGF-R,[20] although the production of TGF-α might be negligible.

The combination of anti-PDGF, anti-bFGF, and anti-EGF-R antibodies had an additive inhibitory effect on DNA synthesis in the two glioma cell lines, indicating that multiple autocrine loops involved PDGF, bFGF, and EGF-R control DNA synthesis in the two glioma cell lines. The pathways mediated by multiple growth factors have both autocrine and paracrine roles in inducing angiogenesis.[14,17,20] The simultaneous blockade of multiple autocrine loops may provide an effective therapeutic tool for controlling glioma growth.

The inhibitory effect of anti-PDGF and anti-bFGF antibodies decreased in serum-supplemented medium, as found previously by Pollack *et al.*[13] We believe that serum-supplemented medium is more similar to the situation *in vivo*, where an impaired blood-brain barrier results in leakage of serum factors into the tumor. Serum contains a large amount of PDGF of platelet origin.[2] Serum and plasma also contain bFGF and EGF.[6,11] Therefore, attempted blockage of an autocrine loop by a ligand-neutralizing antibody may require a large quantity of the specific antibody for effective *in vivo* suppression of glioma growth. In contrast to ligand-neutralizing antibodies, anti-EGF-R antibody suppressed DNA synthesis in both serum-free and serum-supplemented medium. Anti-EGF-R monoclonal antibody inhibits both EGF and TGF-α binding to EGF-R.[18,20] Because multiple ligands interact with EGF-R, therapeutical efforts should be directed toward the EGF-R in the EGF-R-mediated pathway and not toward ligand neutralization.[20]

The simultaneous blockade of multiple autocrine loops produced a significant antiproliferative effect on human glioma cells *in vitro*. This preliminary study suggests that pharmacological blockade of multiple autocrine loops may provide a new therapeutic approach for human malignant gliomas.

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