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Pigment epithelium-derived factor inhibits glioma cell growth in vitro and in vivo

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Abstract

Glioblastoma multiforme is the most common malignant brain tumor in adults, and it is among the most lethal of all cancers. Recent studies have shown that pigment epithelium-derived factor (PEDF) can induce differentiation and inhibit angiogenesis of several tumors. This study was designed to determine whether gliomas angiogenesis and tumor growth could be inhibited by PEDF. We found that PEDF down-regulated expression levels of vascular endothelial growth factor and up-regulated the expression of thrombospondin-2 and augmented apoptosis in a dose-dependent manner in both A172 and U87 glioma cells lines after 48 h of treatment. Analysis of the cell cycle showed arrest in the G_1 phase and block in S phase of the cell cycle. Meanwhile PEDF induced apoptosis was associated with increases of p53 and Bax and inhibition of Bcl-2. Conditioned medium with PEDF showed a significantly reductive effect on migration in vitro accompanied with a significant reduction of matrix metalloproteinase-9 expression. PEDF suppressed glioma cell migration in vitro and tumor burden in athymic nude mice. These results demonstrate for the first time inhibitory effects of PEDF on the growth and migration of human gliomas via induction of apoptosis and blocking of migratory-related factors. PEDF activation can be a novel approach for future therapeutic purposes against gliomas.

Keywords: Pigment epithelium-derived factor; Gliomas; Angiogenesis; Apoptosis; Invasion

Introduction

Gliomas are diffuse and highly invasive brain tumors accounting for about 50% all primary brain tumors (Alvord and Sahaw, 1991). Malignant gliomas are characterized by a high proliferation rate, marked local invasion and extensive angiogenesis (Bjerkvig et al., 1997). The malignant progression from astrocytoma to glioblastoma multiforme is often accompanied by up-regulation of vascular endothelial growth factor (VEGF) and increased angiogenesis (Liu et al., 2006).

Pigment epithelium-derived factor (PEDF), a 50 KDa glycoprotein and a member of the serine protease inhibitor gene family, was initially confirmed as a potential neurotrophic and neuroprotective factor that promotes the survival of cerebellar granule cells as well as spinal motor neurons (Folkman, 1995) from damage caused by increased intraocular pressure from transient ischemic reperfusion (Conway et al., 2001). Recently, PEDF has been shown to potently inhibit endothelial cell migration in a dose-dependent manner, placing it among the most potent natural inhibitors of angiogenesis (Dawson et al., 1999). In addition, PEDF can completely block granulocyte-macrophage colony stimulation factor stimulated cell division of microglia in rats, which is called "gliastatic" effect (Mahtabifard et al., 2003). But so far, no study evaluating the role of exogenous PEDF on gliomas has been performed.

In view of the neurotrophic, anti-angiogenic and "gliastatic" effect of PEDF, we considered that it might be a potential therapeutic agent against diseases of the central nervous system, especially gliomas. In this study, we explored whether gliomal angiogenesis and tumor growth could be inhibited by PEDF. The data presented here show for the first time that PEDF could

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Table 1
Real-time nucleotide primer sequence

Gene	Forward primer	Reverse primer	Product length (bp)
VEGF	ACATCTTCCAGGAGTACCCTGATGAG	GCATTCACATTTGTTGTGTGCTGT	212
TSP-2	GTACAAGTGCGAGTGCCAGA	TCATTGTCATCGTCATC	216
P53	TGCGTGTGGAGTATTTGGATG	GAGGTTGGCTCTGACTGTACCA	90
Bcl-2	CAGCTGCACCTGACGCCCTTCA	ATGCACCTACCCAGCCTCC	259
Bax	AAGCTGAGCGAGTGTCTCAAGC	ACTCGGAAAAAGACCTCTCGG	113
GAPDH	GAAGGTGAAGGTCGGAGTCA	GAAGATGGTGGTGATGGGATTTC	226

significantly reduce tumoral neoangiogenesis and tumor growth in vitro and in vivo.

Materials and methods

Cell lines and culture

Human malignant U87 glioma cells, A172 glioma cells and human umbilical vein endothelial cells (HUVECs) were obtained from American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI1640 with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

Tube formation assay

HUVECs (5×10^4) were cultured on ECMatrix™ in 0.5 ml RPMI1640 containing recombinant PEDF (rPEDF; Zhang et al., 2005). Endothelial cell tubes were allowed to form overnight at 37 °C. After 12 h, the capillary formation induced by different concentrations of rPEDF (0, 20, 100, 500 ng/ml) was analyzed by measuring the number of capillary structures according to the protocol (Chemicon).

Cell viability assay

The U87 cell ($U87_{con}$), A172 cell ($A172_{con}$) alone and with rPEDF of 100 ng/ml ($U87_{PEDF}$, $A172_{PEDF}$) were used for following experiments. U87 and A172 cells were seeded in 96-well plates and incubated in serum-free medium for 24 h. Then, the cells were changed to the medium containing 1% FBS. After 3 days, 20 μl MTT solution (5 mg/ml, Sigma) was added to each well and the plate were further incubated for 3 h. The crystal was dissolved in 0.04 M HCl in isopropanol. Absorbance at 490 nm was measured with a microplate reader (Bio Rad, Hercules, CA). The value of the treated cells was calculated as percent of the untreated control.

Apoptosis assay

$U87_{PEDF}$ and $U87_{con}$ cells were plated on glass coverslips and fixed with 4% paraformaldehyde in PBS for 20 min. Cells were washed in PBS and treated with 0.3% H_2O_2 for 30 min to eliminate endogenous peroxidases and then incubated in 50 mmol/l Tris–HCl buffer (pH 7.5). Apoptosis was measured by in situ TUNEL analysis. Apoptosis was quantified by determining the percentage of positively stained cells in all of the nuclei in 10 randomly chosen fields taken at 400 magnification.

Cell cycle analysis

Cells were diluted with culture medium to the seeding density (10^6 cells/ml), suspended in 6-well plates and grown in serum-free medium for 24 h. Cells were trypsinized, washed in PBS, fixed in 70% ethanol, and treated with 10 μg/ml RNase (Roche Diagnostics Corp). Cells were then stained with PI (Sigma; 5 μg/ml), and cell cycle profile was determined using

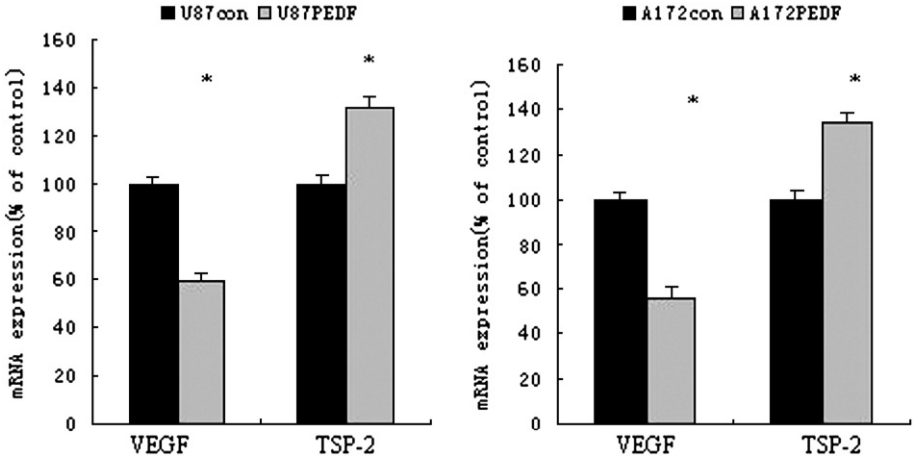

Fig. 1. PEDF-mediated shift in VEGF and TSP-2 expression levels. VEGF and TSP-2 Real-time RT PCR revealed the down-regulation of VEGF and the up-regulation of TSP-2 in $U87_{PEDF}$ and $A172_{PEDF}$ cells. *$P<0.05$, compared with $U87_{con}$ and $A172_{con}$ cells.