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B7-H6 expression is induced by lipopolysaccharide and facilitates cancer invasion and metastasis in human gliomas

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ABSTRACT

Although great progress has been made in treatment regimens, gliomas are still incurable and the 5-year survival remains poor. Studies focusing on molecules that regulate tumorigenesis or tumor immunity may provide potential therapeutic strategies for patients with glioma. B7-H6 is selectively expressed in tumor cells and plays vital roles in host immune responses. In this study, we demonstrated that B7-H6 was expressed in glioma cell lines, including CRT, U251, SHG-44, SF-295, TG-905 and U373, and tumor tissues isolated from glioma patients. Moreover, the expression levels of B7-H6 were significantly correlated with glioma grade. Previous studies reported that inflammatory mediators and cytokines induced the expression of B7 family members including programmed death-ligand 1, B7-H2 and B7-H4. Therefore, we explored the regulation of B7-H6 expression in gliomas and showed that lipopolysaccharide induced the expression of B7-H6 in glioma cells. To further analyze the roles of B7-H6 in gliomas, the expression of B7-H6 in glioma cells was knocked down. The results of cell counting kit-8, colony formation, wound healing, and transwell migration and invasion assays demonstrated that the proliferation, migration and invasion of glioma cells were inhibited after knocking down B7-H6. To elucidate the specific mechanisms of B7-H6 function in cancer progression, we examined the expression levels of proteins involved in cell apoptosis, migration and invasion. We demonstrated that the expression levels of E-cadherin and Bcl-2 associated X protein increased, and the expression levels of vimentin, N-cadherin, matrix metalloproteinase-2, matrix metalloproteinase-9 and survivin decreased after knocking down B7-H6. In conclusion, B7-H6 plays important roles in glioma, and targeting B7-H6 may provide a novel therapeutic strategy for glioma patients.

1. Introduction

Malignant glioma is the most frequent and aggressive primary brain neoplasm in adults [1,2]. The median age at diagnosis of malignant gliomas is 40 years [3]. According to the World Health Organization histologic classification, gliomas are designated grades I–IV [4]; grades I and II are classified as low grade whereas grades III and IV are classified as high grade [5,6]. The median survival time of patients with high-grade glioma is no more than 1 year even after comprehensive therapies [1]. Although great progress has been made in the biology of glioma in the past years, the mechanism of gliomagenesis remains unclear. Research on the molecules that regulate gliomagenesis may provide insights into new therapeutic strategies for glioma patients.

B7-H6, also known as natural cytotoxicity triggering receptor 3

Abbreviations: Bax, Bcl-2 associated X protein; Bcl-2, B-cell lymphoma-2; CCK-8, Cell counting kit-8; DMEM, Dulbecco's modified Eagle's medium; EMT, Epithelial-mesenchymal transition; FBS, Fetal bovine serum; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, Granulocyte-macrophage colony stimulating factor; IFN, Interferon; IL, Interleukin; LPS, Lipopolysaccharide; MMP-2, Matrix metalloproteinase-2; MMP-9, Matrix metalloproteinase-9; NCR3LG1, Natural cytotoxicity triggering receptor 3; NKp30, Natural killer cell P30-related protein; PD-L1, Programmed death-ligand 1; qPCR, Real-time quantitative polymerase chain reaction; siRNA, Short interfering RNA; TNF-α, Tumor necrosis factor-α; VEGF, Vascular endothelial growth factor; wt, Wild-type

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Table 1
Primers and siRNA oligonucleotides sequence.

Primers or siRNA	Sequence
B7-H6-Forward	5′-GAC CGA AGG TGA TCT GAA AGT-3′
B7-H6-Reverse	5′-GTC GGA ATG CCT CTT GGT GA-3′
GAPDH-Forward	5′-CCA AGG TCA TCC ATG ACA AC-3′
GAPDH-Reverse	5′-AGG GAT GAT GTT CTG GAG AG-3′
siRNA-B7-H6	5′-CCA CAA AGU CUG AGA AAC A (dTdT)-3′
	5′-UGU UUC UCA GAC UUU GUG G (dTdT)-3′

(NCR3LG1), is a newly identified member of the B7 family [7]. Recent studies have demonstrated that B7-H6 is detected in human tumors. Moreover, B7-H6 expression levels are associated with various clinical parameters. B7-H6 protein expression is correlated with differentiation, overall survival, disease-free survival, and prognosis of patients with oral squamous cell carcinoma [8]. Breast cancer patients with genomic changes in *B7-H6* show worse overall survival than those without [9]. B7-H6 is expressed on the cellular membrane and in the cytoplasm in tumor tissues from patients with ovarian cancer [10], and B7-H6 expression is significantly associated with cancer metastasis, cancer progression, and overall survival of human ovarian cancer [10]. Silencing of B7-H6 inhibits tumor growth, colony formation, migration, and invasion, and induces cell apoptosis and sensitivity to chemotherapeutic drugs in B-cell lymphoma [11]. In addition, B7-H6 on the surface of tumor cells binds to natural killer cell P30-related protein (NKp30) on natural killer cells, leading to cytokine production and triggering cytolysis of tumor cells [12]. Thus, B7-H6 may represent a novel tumor biomarker and potential target for therapy.

In this study, we demonstrated that B7-H6 was expressed in glioma cells and tissues isolated from glioma patients. The expression levels of B7-H6 in glioma patients were correlated with glioma grade. B7-H6 expression in glioma cells was induced by lipopolysaccharide (LPS). B7-H6 knockdown inhibited the -9 proliferation, migration and invasion of glioma cells. B7-H6 knockdown led to downregulation of vimentin, N-cadherin, matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase9 (MMP-9) and survivin, and upregulation of E-cadherin and Bcl-2 associated X protein (Bax).

Table 2
Clinicopathological characteristics of 79 glioma patients.

Characteristic	Number (%)
Age (years)	
Median	43.52 (55.09)
Range	3–77
Sex	
Male	39 (49.37)
Female	40 (50.63)
WHO grade	
I–II	38 (48.1)
III–IV	41 (51.9)

Table 3
Associations between B7-H6 expression and clinicopathological factors in glioma patients.

Clinicopathological factors	Low expression	High expression	*P* value
Gender			0.69
Male	15	24	
Female	9	31	
Age, years			0.895
< 40	13	35	
> 40	11	20	
Grade			0.002
I–II	18	20	
III–IV	6	35	

2. Materials and methods

2.1. Cell lines, primary cells and cell culture

Human glioma cell lines U251, CRT, SHG-44, SF-295, TG-905 and U373 were cultured in Dulbecco's modified Eagle's medium (DMEM) (Hyclone, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) and maintained in a humidified cell culture incubator at 37 °C with 5% CO_2/95% air. To obtain glioma primary cells, fresh surgically resected glioma tissues were collected from patients at the Department of Neurosurgery, Linyi People's Hospital (Linyi, Shandong Province, China). All patients

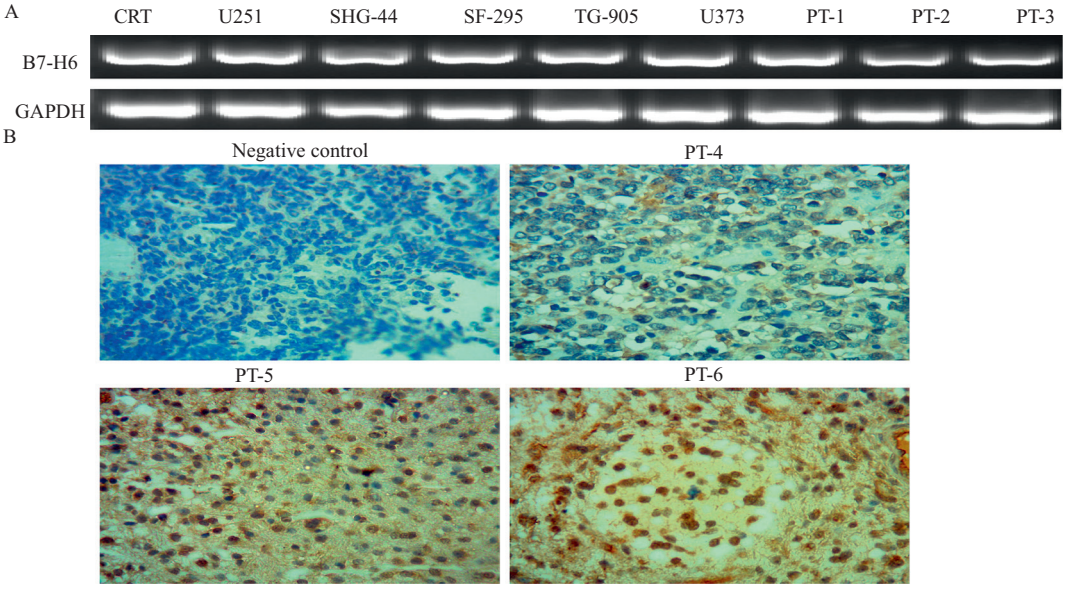

Fig. 1. B7-H6 expression in human gliomas. (A) B7-H6 mRNA expression in six human glioma cell lines CRT, U251, SHG-44, SF-295, TG-905, U373, and three primary cell lines isolated from fresh human surgical glioma tissue samples. (B) Representative immunohistochemistry staining of B7-H6 expression in glioma patients. An isotype control antibody was used for negative control staining. Original magnification ×400.