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The expression and significance of dishevelled in human glioma

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ABSTRACT

Background: The proto-oncogene dishevelled (Dvl) is a critical component of the Wnt/β-catenin signaling pathway, and its elevated expression in various tumor types is associated with malignancy. However, a role for Dvl in glioma has not been explored.

Materials and methods: To determine whether Dvl expression is elevated in human glioma, we examined the protein levels in 67 human glioma samples and 3 normal brain specimens by Western blotting and immunohistochemistry. To investigate a possible association of Dvl with the malignant phenotype in glioma, the correlation of the Dvl immunoreactivity score (IRS) with β-catenin IRS, the tumor proliferation index (PI), and tumor invasion index (II) were determined for each sample.

Results: The Dvl IRS, β-catenin IRS, PI, and II increased significantly with the pathologic grade of glioma ($P < 0.001$) with average scores of 3.46 ± 3.45, 3.92 ± 3.28, 30.93 ± 17.92, and 20.43 ± 11.79, respectively. Furthermore, the PI and II were significantly higher for the Dvl-positive group than the Dvl-negative group ($P < 0.001$). Correlation analysis demonstrated that β-catenin IRS, PI, and II were positively correlated with Dvl IRS.

Conclusions: Dvl overexpression may contribute to the malignant proliferation and invasion of human glioma.

1. Introduction

Glioma, the most common primary brain tumors in adults, is also one of the most aggressive tumors in humans. Glioma patients with high histopathologic tumor grades often have poor prognosis, and their median survival time is <1 y even with combined treatments of surgical operations, radiotherapy, and chemotherapy [1]. As it is known to all, malignant gliomas are heterogeneous both in their cell composition and also the relative abundance of cells are capable of propagating tumor cells, though the underlying mechanism remains poorly understood [2–4]. During the past decades, wnt/β-catenin pathway, as an important role in development and in regulating adult stem-cell systems, attracts more attention. Wnt/β-catenin signaling is a conserved molecular mechanism in metazoan animals. This pathway

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broadly influence changes in gene expression that govern embryogenesis and postnatal responses, such as cell proliferation, cell-fate determination, cell survival, cell behavior, and migration during morphogenesis [5]. As one essential regulator for the development of nervous system, wnt/β-catenin signaling participates in the process of almost all aspects of neural development, including stem-cell proliferation, maintenance, and differentiation [6]. Abnormal wnt/β-catenin signaling is associated with many human diseases, including cancer, osteoporosis, aging, and degenerative disorders [7,8]. Recently, wnt/β-catenin signaling was reported to contribute to the formation of gliomas, and some proteins involved in the Wnt/β-catenin pathway were abnormally expressed in gliomas [9,10]. And more studies demonstrated that wnt/β–catenin pathway is able to increase the stem-like behavior of astrocytes and glioma cell lines, whereas the down-regulation of canonical wnt/β-catenin pathway induces apoptosis in glioma cell lines [11,12].

Dishevelled (Dvl) and β-catenin are the most important components in the canonical wnt/β-catenin pathway. Dvl is a scaffold protein with DIshevelled and aXin, basic, post synaptic density protein, Drosophila disc large tumor suppressor, and zonula occludens-1 protein and Dishevelled, Egl-10 and Pleckstrin domains, which assembles a variety of wnt signaling molecules. Some studies indicated that Dvl is highly expressed in many tumors and is involved in tumor malignancy [13–15]. On activation of wnt, the wnt ligand binds to its frizzled (Fz) receptor and transduces the signal to a cytoplasmic protein Dvl, which in turn inhibits the serine and/or threonine kinase Glycogen synthase kinase (GSK)-3β. The decreased activity of GSK-3β leads to inactivation and dissociation of the multiprotein degradation complex resulting in the accumulation and nuclear translocation of cytosolic β-catenin. The β-catenin is the key mediator of canonical wnt signaling and nuclear β-catenin is the hallmark of an active wnt pathway [16–18]. In the nucleus, β-catenin interacts with T-cell factor and/or Lymphoid enhancer-binding factor (LEF) and activates downstream target genes, including c-Myc, cyclin D1, c-Jun, c-fos, fra-1, and members of AP-1 family that are mainly involved in the regulation of cell fate and proliferation [16,17,19]. The relationship among Dvl protein expression, β-catenin protein expression, the proliferation, and invasion of glioma is unclear. In this study, the expression levels of Dvl was measured in 67 glioma samples by Western blotting and immunohistochemistry, and β-catenin was also measured by immunohistochemistry. The proliferation and invasion indexes of corresponding tumors were determined, and the relationship of Dvl immunoreactivity score (IRS) to β-catenin IRS, proliferation index (PI), and II were analyzed. Our results demonstrate for the first time that Dvl expression is elevated in glioma, and β-catenin IRS, PI, and II were positively correlated with Dvl IRS.

2. Material and methods

2.1. *Tumor specimens*

A total of 67 glioma samples and 3 normal brain samples were collected at the Department of Neurosurgery of the First Hospital of the Shanxi Medical University from September 2010–December 2012. Glioma tumors were obtained by surgical operations during tumor treatments, and normal brain tissues were collected during intracranial decompression surgery for severe traumatic brain injury. The collection of specimens was approved by the hospital ethics committee, and each patient or their families signed informed consent. All glioma patients, including 37 males and 30 females aged 14–73 y, with mean age of 43.8 ± 15.6 y were free of acute infection and did not receive any radiotherapy, chemotherapy, or hormone therapy before surgery. Only tumor samples from the first surgery of these patients were used. Each tissue sample was divided into two parts: one part was fixed in 10% formalin and then paraffin-embedded after dehydration for immunohistochemical staining; and another part was stored in liquid nitrogen for Western blotting.

2.2. *Histopathologic grading of tumor specimens*

The histopathologic grades of tumors were determined according WHO 2007 standards [20]. The pathologic classification and grading of the 67 glioma specimens were as follows: the grade I tumors included 11 cases of pilocytic astrocytomas; the grade II tumors included 12 cases of fibrillary astrocytomas and 5 cases of oligodendrogliomas; the grade III tumors included 10 cases of anaplastic astrocytomas and 4 cases of anaplastic oligodendrogliomas; and the grade IV tumors included 22 cases of glioblastomas and 3 cases of medulloblastomas.

2.3. *Western blotting*

Proteins extracted from glioma or normal brain tissues were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a nitrogen cellulose membrane. The membrane was first incubated with goat polyclonal anti–human Dvl (C-19) antibody (Santa Cruz, CA) at RT and then with HRP-labeled goat anti-rabbit secondary antibody. β-actin was also tested as an internal control. Membranes were scanned using a GDS-1 gel imaging system, and the intensity of Dvl protein was normalized by β-actin using Band-scan software. Quantification of each sample was performed in triplicate.

2.4. *Immunohistochemistry*

Goat polyclonal anti–human Dvl (C-19), rabbit polyclonal anti–human Ki-67 (H-300), mouse monoclonal anti–human matrix metalloproteinase (MMP)-9 (2C3), and rabbit polyclonal anti–human β-catenin (H-102) were purchased from Santa Cruz. An LSAB immunohistochemical kit was purchased from Maixin, Fuzhou, China. Samples were fixed in neutralized formalin, paraffin-embedded, and cut into 4-μm sections. Sections were dewaxed in xylene and dehydrated with graded alcohol. Dvl proteins were detected using streptavidin–peroxidase immunohistochemistry with phosphate-buffered saline instead of primary antibody as a negative control.

Analysis and interpretation of the immunohistochemistry results for Dvl IRS and β-catenin IRS were performed according to the method of Friedrich *et al.* [21]. From each tissue