Prognostic Significance of Nuclear DJ-1 Expression in Astrocytoma

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Abstract. The present study was conducted to determine whether any correlation exists between the expression of DJ-1 and WHO grading of the tumor or patient prognosis, and to analyze the function of this oncogene in astrocytomas. Twenty-nine formalin-fixed and paraffin-embedded glioblastomas (grade IV), 21 anaplastic astrocytomas (grade III), and 14 diffuse astrocytomas (grade II) were immunohistochemically studied to identify the expression of DJ-1 protein. The expression of DJ-1 was detected both in the nucleus and cytoplasm of tumor cells; however, such expression varied from case to case. While there was no difference in the cytoplasmic expression of DJ-1 among astrocytomas, its nuclear expression was inversely correlated with the WHO grading of astrocytomas. Moreover, the overall survival of patients with maintained (group 1) or mixed (groups 2 and 3) was significantly longer than those with decreased (group 3) expression (p=0.0063). The present study demonstrated that the survival of patients with astrocytomas was inversely correlated with the nuclear DJ-1 status of the tumor. We herein demonstrated for the first time that the DJ-1 molecule might therefore play an important role as a tumor suppressor in astrocytomas.

DJ-1 is a 20-KDa protein whose sequence is conserved among prokaryotic and eukaryotic cells (1, 2). DJ-1 expression in Parkinson’s disease (PD) has been well-documented. In 2003, mutations of the DJ-1(PARK7) gene were initially discovered in two pedigrees of patients with inherited PD. A 14-kb homozygous deletion in the DJ-1 gene, which removes exons 1 through 5, was identified to segregate with PD in a large Dutch family, and a homozygous 497T to G transition, which results in a missense mutation (L66P), was detected to segregate with PD in an Italian family (3). At present, the exact function of DJ-1 is unclear. However, there are several reports of the DJ-1 molecule being associated with the central nervous system (CNS). In adult mice, DJ-1 transcripts and proteins are strongly and homogenously expressed in all regions of the central nervous system (CNS) and expressed in both neurons and glial cell types (4). Furthermore, Shang et al. detected the expression of DJ-1 mRNA in neuronal and non-neuronal populations of several structures of the motor system, such as the substantia nigra, red nucleus, caudate putamen, globus pallidus, and deep nuclei of the cerebellum (5). In the human brain, DJ-1 protein seems to be localized in neurons (6) as well as non-neural cells (7). Regarding the function of DJ-1, Yokota et al. reported that it is an essential component of the oxidative stress response in neurons and neuroblastoma cells (8, 9).

In regard to its relationship with cancer, DJ-1 was first identified as an oncogene and showed a cooperative transforming activity with H-ras, more than three times as strong as the activity of the ras/myc combination (10). Therefore, DJ-1 may play an important role as an oncogene, thus suggesting that it may be a possible prognostic indicator in patients with astrocytoma. To analyze the actual role of DJ-1 in astrocytomas, the present study immunohistochemically investigated the relationships between the expression levels, their localization, clinicopathological factors, and the prognosis in astrocytoma patients.

Materials and Methods

Cases. Sixty-four formalin-fixed and paraffin-embedded primary astrocytomas that were surgically resected at Kitasato University Hospital from 1998 to 2006 were selected from the neurosurgery files. They were classified by WHO grading into 29 glioblastomas (grade IV), 21 anaplastic astrocytomas (grade III), and 14 diffuse
astrocytomas (grade II). The patients included 26 men and 38 women, with a mean age of 52.2 years (range: 16-89) at the time of the initial diagnosis. Eighteen of these patients are currently alive and well, and 32 patients died of the disease with a mean follow-up of 19.4 months (range: 1-112 months). Fourteen patients were lost to follow-up.

This study was approved by the Ethical Committee of Kitasato University School of Medicine. All patients were informed of the aim of the study and consented to the use of their samples.

**Immunohistochemical staining (IHC).** Three-μm-thick sections were deparaffinized in xylene, and rehydrated with a descending ethanol series and tap water. The sections were treated with 3% hydrogen peroxide for 10 min and autoclaved in 0.01 M citrate buffer (pH 6.0) with 0.1% Tween 20 at 121°C for another 10 min. After blocking with 0.5% casein for 10 min, the sections were incubated with mouse monoclonal anti-human DJ-1 antibody (diluted 1:4000, MBL, Nagoya, Japan). After rinsing in TBS (0.01 M Tris-HCl, 150 mM NaCl, pH 7.5) three times for 5 min each, the sections were incubated with indirect polymer reagent (ChemMate ENVISION, Dako, Glostrup, Denmark) for 30 min at room temperature. After rinsing in TBS three times for 5 min each, the sections were visualized using Stable Diaminobenzidine solution (Invitrogen, Carlsbad, CA, USA), counterstained with Mayer’s hematoxylin and mounted.

**Evaluation.** The immunohistochemical staining score of the nucleus was categorized into three different groups. Group 1 (maintained expression) was defined as a level of DJ-1 equivalent to the nuclear staining of normal cells as an internal control. Group 3 (reduced expression) showed reduced nuclear or no obvious nuclear staining. These were not clearly classified as group 1 or 3 were classified as group 2.

**Statistical analysis.** The χ² test was used to assess the correlation among tumor grades and levels of DJ-1 expression. Overall survival of the patients among the groups was analyzed using the log-rank test. P<0.05 was considered significant. The data were analyzed on a personal computer using the StatView-J 5.0/PC+ software package (SAS Institute Inc., Cary, NC, USA).

**Results**

Expression of DJ-1 was observed both in the nucleus and cytoplasm of glial cells in normal brain tissues (Figure 1a). In astrocytomas, the expression of DJ-1 was observed in both the nucleus and cytoplasm of tumor cells, and their levels varied from case to case. Representative nuclear staining of each group is summarized in Figure 1b-d. Nuclear staining of tumor cells showed a tendency to decrease with a reduction in the aggressiveness of the tumor. Of 29 glioblastomas, nuclear DJ-1 staining placed 16 in group 3 (55.2%), 12 in group 2 (41.4%) and 1 in group 1 (3.4%). Similarly, for 21 anaplastic astrocytomas, nuclear DJ-1 staining placed 1 in group 3 (4.8%), 8 in group 2 (38.1%), and 12 in group 1 (57.1%). In 14 diffuse astrocytomas, the same staining placed 2 in group 2 (14.3%) and 12 in group 1 (85.7%); no group 3 cases were observed. The nuclear DJ-1 status in the tumor cells was significantly different among glioblastomas, anaplastic astrocytomas, and diffuse astrocytomas while no significant relationship between the tumor aggressiveness and staining intensity was noted for cytoplasmic DJ-1 expression. In diffuse astrocytomas, nuclear DJ-1 expression was categorized into group 1 or 2 in all cases, but about a half of all glioblastomas cases fell into group 3. A significant difference in nuclear DJ-1 expression between glioblastomas and anaplastic astrocytomas was observed (p<0.001; Figure 2).

In astrocytomas, the overall survival of patients in group 3 was significantly shorter than in groups 1 and 2 (p=0.0063; Figure 3a). No significant difference was detected between the level of cytoplasmic DJ-1 expression and the overall survival of patients with astrocytomas. In glioblastomas, the overall survival of patients in group 3 also tended to be shorter than in groups 1 and 2. In anaplastic astrocytomas, there was only one group 1 patient, and this patient also had the shortest survival time (Figure 3b). Moreover, these cases in group 3 tended to have a poorer prognosis at the same grade.

**Discussion**

In breast cancer, elevated levels of circulating DJ-1 protein and anti-DJ-1 autoantibody were detected, and 37% of newly diagnosed patients were found to show DJ-1 protein in sera, which may thus indicate its clinical utility as a novel tumor biomarker (11). Proteomics studies revealed that the level of DJ-1 protein was increased in primary non-small cell lung carcinoma samples, while it decreased after the induction of drug-induced apoptosis (12). In addition, Pardo et al. demonstrated that DJ-1 was overexpressed in uveal malignant melanoma cells, and soluble DJ-1 protein was also detected in the culture medium (13). However, there are no previous reports concerning the expression of the DJ-1 molecule and its clinicopathological significance in brain tumors including astrocytomas. As a result, the present study was conducted to clarify the relationship between DJ-1 protein expression and tumor aggressiveness and prognosis in astrocytomas.

Bader et al. described DJ-1 protein as being strongly and homogenously expressed in almost all cells throughout the CNS in mice (4). However, in humans, Kotaria et al. reported that DJ-1 expression was mainly observed in astrocytes rather than neurons (14). The present study demonstrated that DJ-1 expression was observed both in the nucleus and cytoplasm of glial cells in normal brain tissues. We confirmed the results of Kotaria et al. Since the DJ-1 gene was initially identified as an oncogene, several studies have reported a correlation between the expression of DJ-1 and malignant potential of tumors. These findings may reveal one of the molecular mechanisms involved in both cancer cell survival and the aggressiveness of such tumors (15). However, no study concerning the precise localization of DJ-1 protein and tumor aggressiveness has yet been reported. This is the first report to show that DJ-1 protein is localized in the cytoplasm in
addition to the nucleus of tumor cells, and that the nuclear DJ-1 status is correlated with the patient outcome. The present study demonstrated that the nuclear DJ-1 status was inversely correlated with the WHO grading and patient outcome in astrocytomas. Moreover, even in the same grade, cases in group 3 tended to exhibit a poorer prognosis.

In 2005, DJ-1 was reported to be a negative regulator of PTEN (16). Mutation of PTEN has been detected in 30 to 44% of high-grade astrocytomas (glioblastomas and anaplastic astrocytomas) (17). Specifically, the inactivation of PTEN plays an important role in the progression of gliomas. PTEN is a dephosphorylation enzyme that is involved in cell growth and survival (18). Moreover, it is well-known that in mammalian cells, the increased expression of DJ-1 inactivates PTEN and promotes cell survival (16).

In breast cancer, an increased expression of cytoplasmic DJ-1 was observed, thus suggesting that it plays a role as an oncogene (16). In this study, the accumulation of cytoplasmic DJ-1 protein in tumor cells was observed, but no correlation between its levels, tumor aggressiveness, and prognosis in astrocytomas was detected. On the other hand, because nuclear DJ-1 staining in malignant astrocytomas was reduced compared to that in benign tumors and normal astrocytes, the role of nuclear DJ-1 in gliomas may therefore differ from its previously reported function as an oncogene in other tumor types. There are a few reports concerning the down-regulation of DJ-1 protein in tumors (11, 19). Naour et al. (11) reported that the normal breast epithelium adjacent to carcinomas showed more intense and diffuse cytoplasmic and nuclear immunoreactivity for (RNA-binding regulatory subunit/DJ-1) (RS/DJ-1) compared with the carcinomas, and the decreased expression of RS/DJ-1 protein in the nucleus and cytoplasm of tumor cells with a concomitant increase of secreted RS/DJ-1 in breast cancers. A similar result was reported by Zhang et al. (19), whereby the down-regulation of DJ-1 in HBV-infected, well-differentiated tumors compared with adjacent non-tumor tissues was detected by two-dimensional gel electrophoresis and immunohistochemistry in hepatocellular carcinomas. RS/DJ-1 has been shown to bind to RS and inhibit its RNA-binding activity (1). RNA-binding proteins play an important role in the control of gene expression through a variety of post-transcriptional mechanisms (20). Naour et al. (11) speculated that the low expression levels of RS/DJ-1 may affect cell transformation by increasing the RNA-binding activity of RNA-binding subunit. Subcellular localization studies showed DJ-1 to be present in the cytosol, mitochondria, and nucleus (21, 22); however, it is not clear whether this compartmentalization of DJ-1 has the same function. Junn et al. (23) demonstrated that DJ-1 is present mostly in the cytoplasm and to a lesser extent in mitochondria and the nucleus, but it translocates to mitochondria and the nucleus on oxidative challenge. They reported that mitochondrial DJ-1 appears to be primarily responsible for protection against oxidative stress; nuclear DJ-1 also protects against oxidative damage with different mechanisms. The present and previous studies suggest the potential role of DJ-1 as a tumor suppressor. Recently, Tillman et al. (24) reported a novel
mechanism for DJ-1, whereby the DJ-1-mediated regulation of androgen receptors may promote the progression of prostate cancer to androgen independence. To clarify the role of nuclear DJ-1 in astrocytomas, further genomic and proteomic studies are required. However, in this study, the observed correlation between the status of nuclear DJ-1 expression and tumor aggressiveness and prognosis may indicate its clinical usefulness as a prognostic marker. Finally, this is the first report to demonstrate nuclear DJ-1 status to be an important indicator of the patients outcome in astrocytoma.

**Conclusion**

DJ-1 has been reported to be an oncogene in several tumor types, including breast and lung cancer. We herein confirmed that it may play a potential role as a tumor suppressor in astrocytoma. In this study, a significant association between the nuclear status of DJ-1 protein and both WHO grading and the patient prognosis and was demonstrated in astrocytomas. This differs from the previously reported function of DJ-1 as an oncogene. This study is therefore the first to show that the DJ-1 molecule might play a potential role as a tumor suppressor in astrocytoma.

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