Clinicopathological Significance of Nestin Expression as a Diagnostic and Prognostic Marker in Brain Gliomas, Independent of *IDH* Mutation

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Research

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Abstract

Background: Nestin, a type VI intermediate filament, is expressed in neuroepithelial cells during embryogenesis and has been expressed in various human tumors. Recent studies have reported that expression is associated with poor prognosis in brain tumors, but the results are inconclusive. In this study, we evaluated usefulness of Nestin expression using immunohistochemistry as a diagnostic and prognostic biomarker for *IDH* mutation and the new World Health Organization (WHO) classification.

Methods: To investigate Nestin expression, immunohistochemistry was performed on 92 adult brain gliomas using tissue microarrays. We further analyzed the clinical characteristics and survival outcomes according to Nestin expression and examined its correlation with another glioma biomarker, *IDH* mutation.

Results: Sixty patients (65.2%) were Nestin-positive (weak and strong). Nestin expression and intensity were significantly correlated with age, location, diagnosis, and *IDH* mutations. Old age and high-grade gliomas showed a higher frequency and stronger intensity of Nestin expression than those of young age and with low-grade gliomas ($p < 0.001$). Gliomas with *IDH* mutations that are located in the frontal lobe showed no expression or had weak positivity. Multivariate analysis demonstrated that Nestin expression (weak, hazard ratio [HR] 5.39, $p = 0.036$; strong, HR 8.43, $p = 0.007$) and *IDH* wildtype (HR 7.63; $p = .001$) were significant independent prognostic factors. Moreover, patients with tumors expressing Nestin showed shorter survival ($p < 0.001$).

Conclusions: Nestin expression exhibits high intensity in high-grade gliomas and is a useful diagnostic marker. High expression and level of Nestin were significantly correlated with worse survival and was considered a significant marker of poor prognosis in new WHO classification, independent of *IDH* mutation.

Background

Gliomas are the most common tumors of the brain and account for the majority of cancer-related deaths (1, 2). Molecular profiling studies have reported characteristic genetic alterations related to different types of gliomas. These biomarkers were subsequently incorporated in the 2016 WHO Classification of Tumors of the Central Nervous System (revised 4th edition) (3–9). Mutations in the *IDH* gene occur in 70–80% of grade II/III gliomas and most secondary glioblastomas (10). Co-deletion of 1p/19q and isocitrate dehydrogenase (*IDH*) mutations is typically related to tumors of the oligodendroglial lineage (11). This *IDH* mutation in glial tumors predicts a more favorable outcome (12). Nestin is a member of the intermediate filament class VI and is involved in the organization of the cytoskeleton, cell signaling, organogenesis, and cell metabolism (13). During embryogenesis, it is upregulated in nervous tissues, and its expression is gradually diminished in mature neural tissues, which is a marker of neural stem and progenitor cells. In adults, Nestin expression occurs only in a small subset of cells and tissues, including the ventricular wall, central canal, and outside the subependymal tissue. Nestin, an emerging cancer stem

cell, has also been detected in various human malignancies, including neural tumor, gastrointestinal stromal tumor, pancreatic cancer, malignant melanoma, prostatic cancer, and breast cancer (14–18). Previous studies had shown that Nestin expression was higher in high-grade gliomas than in low-grade gliomas, but the results were not established and not reproducible. To date, there are few reports about clinicopathological correlation considering *IDH* mutation and the new WHO classification in brain gliomas (19, 20).

In this study, we investigated whether Nestin expression could be a prognostic biomarker independent of *IDH* mutation using immunohistochemistry (IHC) in brain gliomas designated in the new WHO classification. The diagnostic significance of Nestin was also analyzed to determine whether the application of IHC for Nestin in tumors could improve the accuracy of histopathologic diagnosis.

Methods

Patients

We retrospectively collected data from 109 patients diagnosed with glial tumors according to the 2016 WHO Classification of Tumors of the Central Nervous System between August 2013 and July 2015 from the archives of the Pathology Department at Asan Medical Center. Ninety-two patients were enrolled after excluding four cases of adult or pediatric brainstem glioma and 13 cases without sufficient tissue sample. The medical records of 92 patients were reviewed, including sex, age, tumor location, diagnosis, molecular parameters, treatments, and survival outcomes. This study adhered to the guidelines established by the Declaration of Helsinki and was approved by the Institutional Review Board of Asan Medical Center (2015-0151). Informed consent was obtained from all participants included in the study.

Tissue microarray construction and immunohistochemistry

Tissue microarrays (TMAs) consisting of two cylindrical cores (3-mm) from formalin-fixed and paraffin-embedded tissues obtained from surgically resected or stereotactic biopsy specimens were constructed using a Quick-Ray® Manual Tissue Microarrayer (UT06; Unitma, Belrose, NSW, Australia). IHC of 4-µm paraffin sections of TMA blocks was performed using a Benchmark automatic immunostaining device (Ventana Medical Systems, Tucson, AZ, USA). The slides were incubated with primary antibodies against Nestin (rabbit anti-Nestin antibody, SP103, Abcam, 1:400). IHC for Nestin was scored as follows: negativity, no reactivity, or cytoplasmic reactivity in less than 5% of tumor cells; weak positivity, reactivity in 5% to less than half of the tumor cells and/or weak expression; and strong positivity, reactivity in more than half of the tumor cells and/or strong expression.

Statistical analysis

The Kruskal-Wallis test, chi-square test, and Fisher's exact test were performed to assess the association between Nestin expression and clinicopathological characteristics. The Cox proportional hazards regression model was used to assess the dependency of survival duration on predictor variables. Kaplan–Meier method and log-rank test were used to estimate survival rates and compare survival

distribution. All statistical analyses were performed using R software (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria). Any P value <0.05 was assumed to indicate a statistically significant difference.

Results

Clinicopathological characteristics of the patients according to Nestin expression

A total of 92 patients with adult brain gliomas were analyzed. The clinicopathological findings and relationships with Nestin expression are summarized in Table 1. IHC for Nestin is as follows: negativity (32, 34.8%), weak positivity (22, 23.9%), and strong positivity (38, 41.3%) (Figure 1). Clinicopathological characteristics of the patients were significantly different according to Nestin expression, except for the sex and status of O⁶-methylguanine-DNA methyltransferase methylation. Older patients showed strong Nestin expression ($p=0.005$). Except for the tumors located in the frontal lobe, the rest had a tendency for Nestin expression (weak or strong, $p=0.001$). The anaplastic oligodendroglioma and low-grade gliomas, including astrocytoma and oligodendroglioma, showed no strong expression for Nestin. However, the majority (84.1%, 53/63) of high-grade gliomas expressed Nestin and had a higher frequency of expression than that of low-grade gliomas. In particular, all glioblastomas with *IDH*-wildtype showed a strong positivity for Nestin. *IDH* mutation status was negatively correlated with Nestin expression.

Prognostic significance of Nestin expression in patients with brain glioma

Age, location, diagnosis, treatment, *IDH* mutation, and Nestin expression are significant predictors of survival according to the univariate Cox proportional hazard regression analyses (Table 2). Patients aged over 50 years presented an increased risk of poor prognosis (hazard ratio [HR] 2.59; 95% confidence interval [CI] 1.29 – 5.18; $P=0.008$). Gliomas located in the temporal lobe and thalamus demonstrated worse prognosis (HR 2.76; 95% CI 1.19 – 6.40; $P=0.018$, HR 5.02; 95% CI 1.38 – 18.22; $P=0.014$, respectively) than those in the frontal lobe. Of the seven pathologic diagnoses, two (anaplastic astrocytoma, *IDH*-wildtype and glioblastoma, *IDH*-wildtype) significantly presented with a poor prognosis (HR 14.89; 95% CI 2.53 – 87.45; $P=0.003$, HR 12.00; 95% CI 2.59 – 55.64; $P=0.002$, respectively), compared to diffuse astrocytoma, *IDH*-mutant (Table 2 and Supplementary figure). As established, mutant *IDH* was a significant protective factor compared to wild-type *IDH* (HR 9.82; 95% CI 3.69 – 26.13; $P<0.001$), and patients with mutant *IDH* showed significantly longer survival from the initial diagnosis ($P<0.001$, Supplementary figure). Regardless of the intensity of positivity, Nestin expression was significantly associated with decreased survival. Strong expression of Nestin (HR 18.41; 95% CI 4.24 – 80.03; $P<0.001$) had a worse prognosis, but weak expression (HR 7.81; 95% CI 1.67 – 36.15; $P=0.009$). Survival of Nestin-positive patients is significantly shorter than that of Nestin-negative patients in the log-rank test ($P<0.001$, Fig. 2A and B). In the multivariate analyses, the status of *IDH* mutation (HR 7.63; 95% CI 2.23 – 26.10; $P=0.001$) and Nestin expression (HR 5.39; 95% CI 1.11 – 26.14; $P=0.036$ for weak

positivity, HR 8.43; 95% CI 1.77 – 40.02; P=0.007 in strong positivity) were independent prognostic factors (Table 2).

Discussion

In the present study, we explored the diagnostic capability and prognosis of adult brain gliomas according to Nestin expression and its association with clinicopathological characteristics. Nestin is a type VI intermediate filament that is expressed in neural stem cells, cancer stem cells, and poorly differentiated cancer cells. In addition, Nestin contributes to aggressive behaviors, including invasiveness and metastasis, in various tumors. These studies raised the question of whether Nestin can act as a prognostic marker for high-grade gliomas, and previous studies have examined its viability as a potential marker for glioma stem cells. However, the results of studies focusing on different populations were inconclusive. To date, there is no study considering the correlation of *IDH* mutation and the new WHO classification.

Our study showed no relationship between Nestin expression and patient sex; however, an older age was associated with stronger Nestin expression. High-grade gliomas, specifically glioblastomas, are common in the elderly. Although, gliomas located in the frontal lobe tend not to express Nestin, as such expression is no longer present after differentiation into mature neural cells. Considering that adult neural stem cells are located in the subventricular zone, fully-differentiated glial cells and glial tumor cells in the cortex can be expected not to express Nestin. Higher-grade gliomas are mainly developed in the deep portion and in continuity with the lateral ventricle.

Diffuse astrocytoma, oligodendroglioma, and anaplastic oligodendroglioma showed no strong expression of Nestin in IHC. Less than half of diffuse astrocytoma (42.8%, 6/14), anaplastic oligodendroglioma (46.2%, 6/13), and a case of oligodendroglioma (6.7 %, 1/15) weakly highlighted Nestin expression. Anaplastic astrocytoma showed weak Nestin-positivity in more than half (57.1% in *IDH*-mutant, 66.7% in *IDH*-wild) and strong positivity in each case. All glioblastomas with wild-type *IDH* demonstrated strong Nestin expression. Both the expression rate and intensity of Nestin tended to increase with the degree of WHO grade. However, Nestin expression was negatively correlated with *IDH* mutation. Given these results, Nestin expression and intensity were significantly correlated with pathologic diagnosis. In daily clinical practice, IHC for Nestin could be very useful for pathologic diagnosis. Difficulty in conducting the biopsy of brain parenchymal lesions and small amount of biopsy specimens contributed to diagnostic challenges. Moreover, differentiating glioblastomas from other gliomas is very difficult if the specimen has no necrosis or microvascular proliferation. In our analysis, all glioblastomas, *IDH*-wildtype, showed strong Nestin expression. Considering expression and intensity of Nestin at the time of diagnosis, glioblastomas will not be overlooked. Nestin could be a useful marker for diagnosis. This result concurs with that of previous studies (21–23).

Nestin has been associated with cell growth, migration, invasion, and adhesion to the extracellular matrix. Therefore, gliomas with strong Nestin expression are expected to have a poor prognosis (24), while other

studies have shown that Nestin expression has an effect on prognosis in some subgroups of patients or showed no relationship with prognosis (12, 25, 26). In our study, patients with Nestin-positive tumors had significantly worse survival than those without Nestin expression, regardless of clinicopathological characteristics. In terms of prognostic utility, Nestin expression was found to be comparable to *IDH* mutation status. The multivariate analysis for the survival of patients with brain glioma according to clinicopathological parameters revealed that Nestin expression is a significant molecular marker, independent of *IDH* mutation.

In this study, we used IHC for Nestin in brain gliomas to verify its feasibility as a diagnostic and prognostic marker in new WHO classification, independent of *IDH* mutation. Nestin expression has a negative prognostic effect and serves as an independent marker of survival. Clear correlation between the expression rate and intensity of Nestin and pathologic diagnosis makes an accurate patient diagnosis.

Abbreviations

IDH: isocitrate dehydrogenase

IHC: immunohistochemistry

TMA: tissue microarray

HR: hazard ratio

CI: confidence interval

Declarations

Ethics approval

This study adhered to the guidelines established by the Declaration of Helsinki and was approved by the Institutional Review Board of Asan Medical Center (2015-0151).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The author declares that they have no competing interests.

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Authors' contributions

Woo CG analyzed and interpreted the patient data. Woo CG performed the histological examination, and was a major contributor in writing the manuscript. Woo CG read and approved the final manuscript.

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Tables

Due to technical limitations, table 1 and 2 xlsx are only available as a download in the Supplemental Files section.

Figures

Figure 1

Nestin expression in the adult brain gliomas (scale bar, 100um). Oligodendroglioma, IDH-mutant and 1p/19q-codeleted (A, HE, x200) showed no Nestin expression in immunohistochemistry (B, Nestin, x200). Diffuse astrocytoma, IDH-mutant (C, x200) had a weak expression for Nestin (D, x200). Glioblastoma, IDH-wildtype (E, x200) was strongly positive (F, x200).

Figure 2

Kaplan-Meier survival curves of patients with brain gliomas. Overall survival (A) and progression free survival (B), according to Nestin expression in immunohistochemistry. Log-rank tests for (A) and (B) yielded $P < 0.001$ and $P < 0.001$, respectively.

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