Nestin Expression in Meningiomas of Different Grades

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Summary

Objective: Nestin has been shown to be expressed in some tumor tissues such as glioma, melanoma, ependymoma, rhabdomyosarcoma, gastrointestinal stromal tumor and testicular stromal tumor. Nestin expression patterns may suggest important relation with the degrees of cancer. In this study, the aim was to demonstrate the expression of nestin in the tumor tissues of patients with different grades meningioma and compare to patients with glioblastoma.

Methods: In the study, the tumor tissues of 28 meningioma (10 grade I, 10 grade II and 8 grade III, respectively) and 10 glioblastoma patients were used. Nestin immunohistochemistry staining method was applied to the sections taken from paraffin blocks of tumor tissue to determine the nestin immunoreactivity intensity.

Results: Nestin immunoreactivity intensity in the tumor tissues of grade II and grade III meningiomas was higher than grade I meningioma group.

Conclusion: In conclusion, it was determined that high-grade meningiomas exhibited higher nestin immunoreactivity than the low-grade meningioma (grade I). Thus, we suggest that the nestin expression may have a contribution to the diagnosis and grading of meningioma tumor tissues.

Key words: Nestin, meningioma, glioblastoma, immunohistochemistry

Farklı Gradelerdeki Meningiomlarda Nestin Ekspresyonu

Özet


Yöntem: Çalışmada, yirmi binyedek meningiomi (10 grade I, 10 grade II ve 8 grade III) ve 10 glioblastom tanısı almış hastaya ait tümör dokuları kullanıldı. Tümör dokularına ait parafin bloklardan alınan kesitlere, nestin ekspresyonunun belirlenmesi için nestin immunohistokimya boyama yöntemi uygulandı.

Bulgular: Meningiom derece II ve meningiom derece III gruplarına ait tümör dokularında, meningiomi derece I grubunda göre nestin immünreaktivite yoğunluğunu daha yüksekti.

Sonuç: Sonucu, yüksek dereceli meningiomların düşük dereceli meningiomlardan daha çok nestin ekspresyonu gösterdikleri belirlendi. Dolayısıyla, meningiom tümör dokularının teşhisinde ve sınıflandırılmasında nestin ekspresyonunun katkı sağlayacağı kanıtladık.

Anahtar Kelimeler: Nestin, meningiomi, glioblastom, immünhistokimya
INTRODUCTION

Nestin is a marker for progenitor cells that can mostly be found in tissues during the embryonic and fetal periods, but also in adult tissues and in tumors\(^{(14,16)}\). There is very little information about nestin function even though it is a marker of proliferating and migrating cells. This protein has been identified as a marker of stem or progenitor cells throughout the development of the central nervous system (CNS)\(^{(3,21)}\). Nestin is also expressed in some stem/progenitor cell populations such as new formation of vascular endothelial cells\(^{(2,28)}\), skeletal muscle precursor cells\(^{(26)}\), hair follicle precursor cells\(^{(16)}\) and pancreatic stem cells\(^{(20,27)}\) as well as expressed in proliferating cells in various embryonic and fetal. It has been shown that nestin, which is known as an important marker of neural stem/progenitor cell, is expressed in different types of tumors such as glioma, ependymoma, melanoma, rhabdomyosarcoma, gastrointestinal stromal tumor (GIST), testicular stromal tumor and adrenocortical tumors\(^{(1,5,17,22,33)}\). In addition, some studies have shown that nestin is strongly expressed in some malignant tumors such as high-grade angiosarcoma, GIST\(^{(35)}\) and malignant glioma\(^{(24)}\). Based on these observations, nestin has been suggested to be a diagnostic and prognostic indicator of the malignant grade of tumors.

In previous studies, nestin expression in tumor tissues of different grades has been studied in association with malignant process, however no study has been conducted to compare the intensity of nestin expression in different grades of meningioma. The aim of the present study was to determine the expression of nestin and the correlation between malignant grades in the meningioma tumor tissues with different grades and also, to compare them to glioblastoma tumor tissues which exhibited intense nestin expression by using immunohistochemical staining method.

MATERIAL AND METHODS

**Tissues**

In the study, samples of tumor tissues were taken from the patients diagnosed with meningioma and glioblastoma by two expert pathologists at Erciyes University, Faculty of Medicine, Department of Pathology. A total of 38 samples were used, 10 samples for grade I, 10 for grade II and 8 for grade III were taken from the meningioma group by checking the patients' pathology reports and 10 samples from the patients with glioblastoma were included as a control group in the study. Information types of the tumors and ages, genders of the patients were obtained from the pathology reports of the patients. Basic pathological data of the patients are given in Table 1. Tissues obtained by surgery in the Department of Neurosurgery were fixed in 10% neutral formalin, dehydrated in graded ethanols and embedded in paraffin wax. Samples serially sectioned in sagittal plane at 5 µm and mounted on slides covered with poly-L-lysine. Each section was stained with hematoxylin-eosin (H&E) to determine the morphological appearance of each part of the tumor tissues. Paraffin sections underwent nestin immunohistochemical staining.

**H&E staining**

The samples were deparaffinized and rehydrated with distilled water. They were then ablated in 1% hydrochloric acid alcohol solution for 30 seconds after staining with hematoxylin for 7 minutes and then washed with distilled water. Samples were stained with eosin for 2 minutes, dehydrated and immersed in xylene for 15 minutes. Finally, the samples were mounted and analyzed, using an Olympus microscopy (Olympus BX51, Tokyo, Japan).

**Nestin immunohistochemistry**

The tumor specimens were deparaffinized in xylene and rehydrated in ethanol and water. Immunohistochemistry was
performed with the avidin-biotin-peroxidase method and applied by a streptavidin-biotin kit. Briefly, each tissue section was deparaffinized, rehydrated and then incubated with 3% fresh hydrogen peroxide (H2O2) in methanol for 10 minutes. After rinsing with phosphate-buffered saline (PBS), antigen retrieval was carried out by microwave treatment in 0.01 M sodium citrate buffer (pH 6.0) at 100°C for 15 minutes. Anti-Polyvalent HRP kit (Thermo Scientific, USA) was used for the following steps. Sections were incubated with normal goat serum for 15 minutes at room temperature in order to block non-specific binding and followed by incubation with rabbit polyclonal anti-human nestin antibody (Rb X nestin, AB 5922, Millipore, final dilution 1:1000) overnight at 4°C. After rinsing with PBS, the slides were incubated for 10 minutes at room temperature with biotin-conjugated secondary antibodies. This step was followed by the addition of streptavidin-conjugated peroxidase working solution that binds to the biotin present on secondary antibody for 10 minutes. Subsequently, the sections were incubated with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Thermo Scientific, USA) for 10 minutes, rinsed in distilled water and counterstained with Mayer's hematoxylin. Finally, the sections were dehydrated in ethanol and xylene. Negative controls were prepared by substituting PBS for primary antibody. All applications were performed at room temperature in a moist ambient to prevent samples from drying. The prepared samples were analysed by a search microscope (Olympus BX51, Tokyo, Japan) with a DMP digital camera.

Quantitative immunohistochemistry

In total, 38 samples from 38 patients were stained with nestin. Quantitative immunohistomorphometry were performed with Image J software. For tumor tissue immunohistomorphometric analyses, 8-10 different areas (per visual fields) from groups were randomly defined for each tumor tissues. The mean of immunoreactivity intensity calculated by using Image J software (x400 magnification).

Statistical analysis

To evaluate the statistical significance of the statistical analysis, the program Graph Pad Prism was used. A two-tailed student's t-test for unpaired samples was applied to compare the groups with the control groups to search for statistically significant differences. Statistical significance was set as P-values< 0.05.

Controls

In this study, tissue samples belonging to the glioblastoma group were used for positive controls. Negative control samples for the glioblastoma group performed by dropping or using PBS instead of primary antibody were applied in other immunohistochemical staining steps.

RESULTS

Basic Demographic and Histological Information

In this study, tissue samples of 38 patients were included. Of the patients; 28 had meningioma, the meningioma grade I group included 4 males and 6 females; 7 patients with meningioma grade I were below 60 years, and 3 were above 60 years, respectively. Meningioma grade II group included 5 males and 5 females; the age of 6 patients were below 60 years and 4 patients were exactly at 60 years. The meningioma grade III group included 1 male and 7 females; 4 patients were below 60 and 4 patients were above 60 years. Glioblastoma group included 4 males and 6 females with 5 of them below 60 years, the remaining 5 patients above 60 years old, respectively (Table 1). The patients in grade I meningioma received the following diagnoses: 8 had transitional-type meningioma, 1 had the secretory meningioma, and 1 had squamous meningioma. The patients in grade II meningioma group received the following
diagnoses; 8 had atypical meningioma, 1 had fibroblastic meningioma, and 1 had chordoid meningioma. In the grade III meningioma, their diagnoses were as follows: 5 had malignant meningioma, 2 had rhabdoid meningioma and 1 had the papillary type of meningioma.

Table 1: Basic pathological data of the patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Meningioma Grade I</th>
<th>Meningioma Grade II</th>
<th>Meningioma Grade III</th>
<th>Glioblastoma</th>
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<td>Age</td>
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<td>&gt; 60.6</td>
<td>&gt; 60.4</td>
<td>&gt; 60.5</td>
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<td>E/5</td>
<td>E/2</td>
<td>E/4</td>
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<td></td>
<td>K/6</td>
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**Nestin expression upregulated in high-grade meningiomas**

Different cross-sectional areas of the glioblastoma group also presented dense cellularity (numerical aspects of morphology and growth of cells), vascular endothelial proliferation and a typical characteristic feature of glioblastoma with areas of coagulative necrosis. Glioblastoma tumor tissues exhibited strong expression of nestin known as an intermediate filament protein (mean of nestin IR intensity: 149,827). Nestin was mainly expressed in the cytoplasm of the tumor cells, as well as in the nucleus (Figure 1a). Nestin was not expressed in the glioblastoma tumor cells used as a negative control (Figure 1b).

The histopathology of grade I meningiomas is usually characterized by the same-shaped tumor cells with oval-shaped nuclei and with cytoplasmic-nuclear inclusions. Grade I meningiomas have spiral-shaped tumor cells and are divided into several regions with thin collagen septa containing blood vessels (Figure 2a). There was no or weak nestin immunoreactivity in tumor cells with grade I meningiomas. Nestin was mainly expressed in the cytoplasm of tumor cells. When compared to nestin expression of glioblastoma tumor cells with grade I meningioma cells, grade I meningioma cells exhibited less nestin immunoreactivity intensity than glioblastoma tumor cells (Figure 2b). Whereas tumors cells exhibited no immunoreactivity for nestin, nestin expression was only confined with vascular endothelial cells in the some tumor areas of all samples (Figure 2c). This difference between grade I meningioma and glioblastoma was statistically significant (mean of nestin IR intensity: 123,629) (Figure 3f) (P < 0.001).

The histopathology of atypical meningioma was different from grade I group meningiomas. Grade II meningiomas are characterized by pleomorphism and hypercellularity, as well
as by pattern loss and occasional mitotic figures.

Additionally, the grade II atypical meningioma with increased mitotic activity of three or more of the following histologic features: increased cellularity, uninterrupted patternless or sheet-like growth. The nuclei of tumor cells are larger than cytoplasm and even the nucleolus is easily distinguished in some tumor cells (Figure 3a). Nestin expression was only focal in all grade II meningioma samples. Nestin immunoreactivity was clear in the vascular endothelial cells (Figure 3b). The nestin immunoreactivity intensity of all the grade II meningioma was usually a little closer to nestin expression in glioblastoma group (Figure 3c). On the other hand, there was an intense expression in some tumor areas or no stained-negative tumor areas for all grade II meningioma group sample. In meningioma grade II group, nestin immunoreactivity intensity was determined to be nearly the same as nestin expression of glioblastoma tumor cells (mean of nestin IR intensity: 145,923). Besides, the all meningioma grade II tumor cells had more nestin immunoreactivity intensity than the tumor cells of meningioma grade I groups (Figure 3f) (P <0.001).

In grade III rhabdoid type meningioma, there was a marked pleomorphism and an increase in mitotic activity. Tumor cells had large eosinophilic cytoplasm and eccentric nuclei (Figure 3d). With regard to the nestin immunoreactivity of the meningioma grade III group, two samples in this group didn't express nestin whereas the other tumor samples exhibited nestin immunoreactivity as intense as glioblastoma tumor cells (Figure 3e). Nestin immunoreactivity of grade III meningioma was nearly closer to the expression pattern of grade II meningioma group samples. This nestin expression intensity pattern was higher than grade I meningioma group, however it was lower when the intensity of the nestin expression was compared to grade II meningioma group (mean of nestin IR intensity: 137,009) (Figure 3f). There was no statistically significant difference between grade III meningioma and the glioblastoma groups (P> 0.01).

Figure 1: (A) Negative control tissue with glioblastoma (WHO grade IV). (B) Nestin was strongly expressed in the positive control glioblastoma. Nestin was mainly expressed only in the cytoplasm of some tumor cells (→), while the other tumor cells expressed nestin in both the cytoplasm (↔) and nucleus (↔) (Nestin immunohistochemistry, X40).
Figure 2: Appearance of tumor cells in the grade I meningioma tissue under normal light microscopic. (A) Tumor cells have oval-shaped nuclei (→) (Hematoxylin-Eosin, X40). (B) The group of grade I meningioma showed weak nestin immunoreactivity in the tumor cells. (C) Whereas tumors cells showed no immunoreactivity for nestin, nestin expression was confined to vascular endothelial cells in some tumor areas (→) (Nestin immunohistochemistry, X40).

Figure 3: (A) The light microscopic appearances of atypical meningioma (grade II). Tumor cells with prominent nucleolus (→) and showed mitotic activity (→) (Hematoxylin-Eosin, X40). (B) Immunohistochemical staining of nestin in grade II meningiomas. Nestin immunoreactivity was detected predominantly in the walls of blood vessels in some tumor areas (→). (C) The some tumor cells exhibited intense nestin immunoreactivity in the meningiomas grade II group (Nestin immunohistochemistry, X40). (D) Light microscopic appearance of rhabdoid type meningioma grade III group. Rhabdoid cells have amphophilic cytoplasm and wide eccentric nuclei (→) (Hematoxylin-Eosin, X40). (E) Tumor cells demonstrated intense nestin immunoreactivity of meningiomas in grade III group (Nestin immunohistochemistry, X40). (F) Quantification of the nestin immunoreactivity (IR) intensity in glioblastoma and meningioma groups with different grades. *p<0.05, ** p<0.01, *** p<0.001
DISCUSSION

Nestin is an intermediate filament protein that was initially identified during the studies involving cellular organization of the developing nervous system (12). Multipotential neuroepithelial stem cells temporarily express nestin during embryonic development (11). Nestin expression in those cells subsequently decreased at later developmental stages and finally disappeared in terminally differentiated and mature cells (4, 34). The nestin expression in the final stage of the differentiation of the multipotent stem cells to neurons and astrocytes is reduced as parallel with the expression initiation of other intermediate filament proteins, such as GFAP. Interestingly, in adults nestin expression re-induction has been reported in the fully differentiated organism after CNS damage and under pathological conditions, such as the renovation of the damaged muscle tissue (32). Nestin is an intermediate protein which is produced mainly in the developing CNS and developing somites of rodents in the embryonic stage (7, 14). The brains of developing mouse embryos have high nestin expression, peaking on the 15th day of the development and nestin expression decreases with growth (6). Less nestin expression is demonstrated in the normal adult brain, but more expression is found in angiogenic endothelial cells in brain tumors (6, 31). Recent studies have shown that nestin exhibits intense expression in both cultured and endothelial cells growing within neuroepithelial tumors in humans (6) indicating that nestin is a potential marker for endothelial cells during the rapid growth of endothelial cells (10, 23, 30). Although initially identified in glioma, nestin expression has been demonstrated in several other malignancies including angiosarcoma, gastrointestinal stromal tumors (35), hemangioblastomas (30), melanoma (12, 13) and basal epithelial breast cancer (15). Interestingly, nestin expression in many of these tumors, including glioma has been shown to correlate with advanced grade (6, 9, 19, 25, 28, 29), supporting the role of nestin as a marker for differentiation. Ehrmann et al. (9) discovered the expression of nestin in glioma and ependymoma tumors in nervous system which was positively correlated with the malignancy of the tumor.

Despite the fact that studies associated with nestin expression in tumor tissues are quite limited, in several studies nestin expression was demonstrated to be intense in glioblastoma known as the most malignant astrocytic tumor (18, 24). In the current study, it is important in terms of the inclusion of glioblastoma patients as control group whose tissues exhibit such intense nestin expression. Additionally, in the present study, nestin was not only expressed in vascular endothelial cells, but also in tumor cells of different grades of meningiomas as well. Nestin may be a diagnostic and prognostic indicator in grades of malignant tumors. For instance, Ehrmann et al. (9) discovered that among nervous system tumors, the expression of nestin in glioma and ependymoma was positively correlated with the malignancy of the tumors. Klein et al. (13), showed that protein seemed to be correlated with the high proliferative and migrational activity of primitive neuroectodermal tumors of the CNS and metastatic melanoma suggest that nestin might play a role in the proliferation and invasion of angiosarcoma cells, the expression of nestin was more intense in poorly differentiated angiosarcoma than in well-differentiated angiosarcoma (9, 13). Yang et al. (35) showed that there was a statistically significant difference in the more intense expression of nestin in high malignant GIST than in low malignant GIST and this may be correlated with malignancy in GIST and angiosarcoma. This relationship has been studied with malignant degrees of nestin expression in different tumor tissues. However, up today
the expression of nestin in different grades of meningioma has not been studied. On the other hand, only one study was conducted on nestin expression in grade I meningothelial meningiomas(24). Nestin in the WHO grade I meningothelial meningiomas was expressed predominantly in the walls of blood vessels, but also in the surrounding tumor cells in some parts of the tumor. In our study, similarly, nestin in the grade I meningiomas was expressed primary in the endothelial cells of blood vessels and some tumor cells. Nestin immunoreactivity intensity in grade II and grade III meningiomas were higher than grade I meningioma. However, the most intense nestin-immunoreactive cells were detected in the tumor cells of the glioblastomas, control group.

CONCLUSION

In conclusion, we suppose that the increase of nestin expression in grade II and grade III meningiomas when compared to grade I meningioma, may have a contribution to the diagnosis and grading of meningioma tumor tissues. Therefore, we believe that presence of nestin in clinical diagnosis of CNS tumors may constitute a potential mean to classify other intermediate filaments, such as GFAP and neurofilaments.

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