Immunohistochemical Expression of Nestin as Cancer Stem Cell marker in Gliomas

Abstract

Background: Gliomas represent the most frequent primary tumors of central nervous system (CNS), contributing to more than half of the incidence of brain tumors. Cancer stem cell markers (CSC) identify a group of patients at high risk for progression. Nestin is an intermediate filament (IF) protein was first described as a neural stem cell/progenitor cell marker. Nestin-positive neuroepithelial stem cells are detected in the subventricular zone of the human adult brain and they remain mitotically active throughout adulthood. The expression of Nestin in gliomas has been suggested to be related to dedifferentiation, improved cell motility, invasive potential and increased malignancy. This study aims to investigate Nestin immunohistochemical expression in different types of glioma and its correlation with different clinicopathological parameters.

Materials and Methods: Nestin immunostaining was studied in 60 specimens of glioma using avidin-biotin peroxidase method.

Results: Nestin was strongly expressed in 11/60 (18.33%), moderately expressed in 29/60 (48.33%) and weekly expressed in 15/60 (25%) of studied gliomas. A significant positive correlation was found between Nestin expression and histologic type (p< 0.001) and increasing grade of gliomas (p< 0.001).

Conclusion: Increased Nestin expression is correlated with tumor progression, increasing grade, and poor prognostic parameter of glioma. Nestin is a useful marker for detection of CSC in high-grade glioma which is responsible for resistance to chemo-radiotherapy and may serve as a predictor for patient outcomes.
The CSCs are responsible for resistance of glioblastomas multiforme (GBM) to radiotherapy and chemotherapy, thereby contributing to poor survival of these patients [9].

The importance of CSCs in estimating the prognosis of patients with gliomas has therefore been widely investigated using several markers closely related to the presence of these cells [10].

Nestin is a class VI intermediate filament (IF) protein that was first described as a neural stem/progenitor cell marker [11]. Neuroepithelial stem cells can differentiate into neurons, oligodendrocytes, and astrocytes, and Nestin has been shown to be down-regulated completely disappeared during such differentiation [12]. An investigation of Nestin as a cell surface marker of CSCs showed that the efficiency of diagnosis and treatment of brain malignancies can be ameliorated by determining these cell surface markers [13].

Patients and Methods

Tissue samples

Formalin-fixed, paraffin-embedded brain tumor tissue blocks from sixty patients were selected prospectively from specimens that were delivered to Pathology Laboratory from Neurosurgery Department, Faculty of Medicine, Sohag University during the period from January 2016 to July 2018. The research was approved from the ethical committee. Gliomas were graded in accordance with the WHO, 2016 grading system, based on specific histopathological characteristics such as cellularity, nuclear atypia, mitotic activity, microvascular proliferation and necrosis.

Immunohistochemistry

After evaluating Hematoxylin and Eosin stained slides, serial sections from each block were used for Immunohistochemistry (IHC) of Nestin using avidin biotin peroxidase complex method. A dilution of 1:150 from Mouse monoclonal antibody against human Nestin (Clone 10C2, Cat. # GTX30671, Gene Tex) was used.

Scoring of immunoreactions and statistical analyses

Nestin expression appeared as brownish cytoplasmic staining. The immunoreactive score (IRS) was determined by multiplying an estimate of the percentage of the immunoreactive cells (Proportion score; PS) with an estimate of the staining intensity (Intensity score; IS) according to Arai et al, 2012 [14]. A proportion score was defined as the percentage of positively stained cells: 0= negative, <30% positive tumor cells (+), 30%-60% positive tumor cells (++), >60% positive tumor cells (+++). An intensity score was defined as the staining intensity of positive tumor cells: No staining=0, Weak staining=1, Medium staining=2, Strong staining=3. An IRS of 1-3 was considered weak, 4 and 6 was moderate and 9 was considered strong.

Statistical analysis

Data was analyzed using SPSS program version 17.0. Quantitative data was expressed as means ± standard deviation, median and range. Qualitative data was expressed as number and percentage. The data were tested for normality using Shapiro–Wilk test. The nonparametric Mann–Whitney test, Kruskal–Wallis test and Spearman’s correlation were used for data which wasn’t normally distributed. P value <0.05 was considered statistically significant and <0.001 was considered highly significant.

Results

The age range of the studied patients was wide 1.5–72 years, with mean±SD was 38.23±22.41 years, the median age was 44 years. The male to female ratio was 1.14:1. The representative H&E stained sections of the collected 60 specimens of gliomas were evaluated according to the WHO classification [15], into the following histological types:

Seven 7/60 (11.7%) cases were pilocytic astrocytoma, twenty-one 21/60 (35%) cases diffuse astrocytoma, five 5/60 (8.3%) cases ependymoma, five 5/60 (8.33%) cases oligodendroglioma, nine 9/60 (15%) cases anaplastic astrocytoma, two 2/60 (3.33%) cases anaplastic ependymoma, and eleven 11/60 (18.33%) cases glioblastoma multiforme. Glioma was graded according to the WHO grading system [15], into four grades according histological finding

Seven cases (7/60; 11.7%) grade I; with low cellularity and no mitosis or necrosis. Thirty-one cases (31/60; 51.67%) grade II; with moderate cellularity, pleomorphism and nuclear atypia. Eleven cases (11/60; 18.33%) grade III; featuring high cellularity, nuclear atypia and brisk mitotic activity. Eleven cases (11/60; 18.33%) grade IV; featuring high cellularity, pleomorphism, brisk mitosis, microvascular proliferation and geographic necrosis. Nestin expression appeared as brownish cytoplasmic staining. Nestin expression varied among different types and grades of gliomas (Table 1). Nestin was expressed in 55/60 (91.7%) of studied glioma; being weakly expressed in 15/60 (25%), moderately expressed in 29/60 (48.3%) and strongly expressed in 11/60 (18.33%) of the studied cases. There was significant correlation between Nestin expression and both histological type (p <0.001), and tumor grade (p <0.001) (Graphs 1,2). However, there is no statistical correlation between Nestin expression and age, sex of the patients, tumor size or tumor site (Figure 1).

Discussion

Our study included 60 specimens of gliomas investigated for Nestin expression. The IRS of Nestin was studied in relation to clinicopathological variables (age, sex, tumor site, size, histological types and tumor grade). This study revealed that Nestin was expressed in about 55/60 (91.7%) of gliomas. This expression was strong in 11/60 (18.33%); 8/60 (13.33%) glioblastoma multiform, and 3/60 (5%) anaplastic astrocytoma. Nestin was moderately expressed in 29/60 (48.3%) of studied gliomas; 15/60 (25%) diffuse astrocytoma, 2/60 (3.33%) ependymoma, 6/60 (10%) anaplastic astrocytoma, 2/60 (3.33%) anaplastic ependymoma, 3/60 (5%) glioblastoma multiform and 1/60 (1.7%) pilocytic astrocytoma. Finally, week Nestin expression was noticed in 15/60 (25%) of studied gliomas; 3/60...
Table 1: Correlation between Nestin expression and the studied clinicopathological parameters.

<table>
<thead>
<tr>
<th>Clinico-pathological Parameter</th>
<th>NO of cases</th>
<th>Nestin expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative IRS (5 cases)</td>
<td>Weak IRS (15 cases)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td>2 (7.1%)</td>
<td>8 (28.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (46.7 %)</td>
<td>3 (9.4%)</td>
<td>7 (21.8%)</td>
</tr>
<tr>
<td>Male</td>
<td>32 (53.3 %)</td>
<td>3 (7.5%)</td>
<td>10 (25%)</td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td>2 (10%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>Supra-tentorial</td>
<td>40 (66.7 %)</td>
<td>3 (7.5%)</td>
<td>10 (25%)</td>
</tr>
<tr>
<td>Infra-tentorial</td>
<td>20 (33.3 %)</td>
<td>2 (10%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td>2 (28.6%)</td>
<td>11 (35.5%)</td>
</tr>
<tr>
<td>I</td>
<td>7 (11.7%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>31 (51.7%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>11 (18.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>11 (18.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
<td>2 (28.6%)</td>
<td>4 (57.1%)</td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>7 (11.7%)</td>
<td>1 (4.8%)</td>
<td>5 (23.8%)</td>
</tr>
<tr>
<td>Diffuse astrocytoma</td>
<td>21 (35%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>9 (15%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glioblastoma multiform</td>
<td>11 (18.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>5 (8.3%)</td>
<td>0</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>2 (3.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>5 (8.3%)</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>P- Value was calculated by Mann-Whitney test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* P- value was calculated by Kruskal Wallis Test.</td>
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</tbody>
</table>
(5%) oligodendroglioma, 3/60 (5%) ependymoma, 4/60 (6.7%) pilocytic astrocytoma and 5/60 (8.33%) diffuse astrocytoma.

Nestin was strongly expressed in 11/60 of the studied gliomas (3/11 WHO grade III and 8/11 WHO grade IV) and moderately expressed in 29/60 (1/29 WHO grade I, 17/29 WHO grade II, 8/29 WHO grade III and 3/29 WHO grade IV). Week Nestin expression were noticed in 15/60 cases (4/15 WHO grade I and 11/15 WHO grade II).

Our results showed a significant positive correlation between Nestin expression and histological type (p < 0.001) and pathological grade of gliomas (p< 0.001). These results are in agreement with those previously reported by Strojnik et al., (2007) who found in their study that nestin was expressed in 95.8% of 87 patients which was significantly higher in high- than in low-grade tumors (P < .0001). and it was shown to be a strong prognostic marker for decreased overall survival (P = .0001) [16].

Ma et al., who confirmed that Nestin was correlated with tumor grade of the tumor in 72 cases [17], Kitai et al., found that 33 glioblastoma /65 different brain tumors cases were positive for Nestin [18], Wan et al., used tissue microarray in 283 cases of brain tumor and concluded that Nestin expression was correlated with tumor WHO Grade [19], Arai et al. 2012. studied 257 cases of brain tumors and Nestin was correlated with worse overall survival (14) as well as Hatanpaa et al., [20], in 50 cases, and Lin et al., in 41 cases [21].

In contrast; a previous study by Rani et al., showed that Nestin expression was noted in both low- and high-grade gliomas and did not correlate with the grade of tumor in 16 cases of brain tumors. We suggest their results may be due to application of Rabbit polyclonal anti-human Nestin in their study in contrast to monoclonal antibody used in ours which is more specific [22].

However, contradictory to our study, Chinnaiyan et al., evaluated Nestin by tissue microarray of 156 cases of glioblastoma multiform and they found no statistically significant difference in either overall survival or progression-free survival (PFS) [23] and Kim et al., concluded that Nestin is not a prognostic factor in 88 cases of glioblastoma multiform using immunohistochemical technique [24].

Conclusion

Increased Nestin expression is correlated with tumor progression, increasing grade, and poor prognosis of glioma. Nestin is a useful marker for detection of CSC in high-grade glioma and may serve as a predictor for patient outcomes.

Recommendations

Studying the expression of Nestin on a large number of cases, different varieties of gliomas and on surgical resection margins.

Prospective studies properly powered based on this study should be undertaken to determine the significance of these findings; follow up of patients and getting enough information about response to treatment to emphasize the correlation between Nestin expression and the patient overall survival and disease outcome.

Studying the change in expression of Nestin in correlation with other CSC markers (e.g CD133) to clarify their role in glioma tumorigenesis.

References


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