Nestin, an Important Marker for Differentiating Oligodendroglioma from Astrocytic Tumors

Abstract
Background: Nestin is an acronym for neuroepithelial stem cell protein. It is an intermediate filament protein expressed in proliferating cells during the developmental stages in a variety of embryonic and fetal tissues. It is also expressed in some adult stem/progenitor cell populations, such as newborn vascular endothelial cell. Differentiation between astrocytic tumors and oligodendroglioma tumor is of paramount importance because of different lines of treatment and different prognosis.

Design: We performed Nestin immunostaining on paraffin blocks of 16 cases of astrocytomas of various grades (3 Glioblastoma, 3 anaplastic astrocytoma, 3 fibrillary astrocytoma and 7 Pilocytic astrocytoma) and on 12 oligodendroglioma (6 grade II, and 6 grade III). All cases of oligodendroglioma has confirmation by FISH for 1p 19q.

Result: Nestin staining was seen in all astrocytic tumors. The strongest staining was in glioblastomas and in anaplastic astrocytomas. Pilocytic astrocytomas show mostly focal and weak staining with strong staining of Rosenthal fibers. Grade II astrocytoma shows weak but more intense staining than pilocytic astrocytoma. No Nestin immunostaining was seen in any of the oligodendroglioma tumor cells, but Nestin stained the endothelial cells in oligodendroglioma as a positive internal control.

Conclusion: Nestin is an important immunohistochemical marker in differentiating oligodendroglioma from astrocytic tumors. Nestin, also, is helpful in grading astrocytoma

Keywords: Nestin; Astrocytoma Grading; Oligodendroglioma; Immunohistochemistry

Abbreviations: IF: Intermediate Filament; GFAP: Glial Fibrillary Acidic Protein; CNS: Central Nervous System; GBM: Glioblastomas Multiforme

Introduction

Nestin is an intermediate filament (IF) protein. These intermediate filament proteins are expressed mostly in neural stem cells [1-3]. Nestin is also expressed by many other stem cells and it is considered a primitive marker [4-7]. Upon differentiation, Nestin becomes down-regulated and is replaced by tissue-specific intermediate filament proteins [8]. During neuro- and gliogenesis, Nestin is replaced by cell type-specific intermediate filaments, e.g. neurofilaments and glial fibrillary acidic protein (GFAP) [9]. One instance of Nestin expression in adult organisms, and perhaps that for which Nestin is best known, are the neuronal precursor cells of the subventricular zone [8,10]. Interestingly, Nestin expression is reinduced in the adult during pathological situations, such as the formation of the glial scar after CNS injury and during regeneration of injured muscle tissue [11]. Nestin expression has been extensively used as a marker for central nervous system (CNS) progenitor cells in different contexts [4]. Nestin has recently received attention as a marker for detecting newly formed endothelial cells [3,12].

Material and Methods

We performed Nestin Immunostaining on paraffin blocks of 16 cases of astrocytomas of various grades (3 Glioblastoma, 3 anaplastic astrocytoma, 3 fibrillary astrocytoma and 7 Pilocytic astrocytoma) and on 12 oligodendroglioma (6 grade II, and 6 grade III) (Table 1). All cases of oligodendroglioma has confirmation by FISH for 1p 19q. In immunohistochemical staining, deparaffinized sections underwent heat induced antigen retrieval [autoclaved in 10-mM citrate buffer (pH 6.0) for 10 min] and were subjected to a reaction with 3 % hydrogen peroxidase for 10 min to remove endogenous peroxidase. Then, the reaction was conducted using a 250-fold diluted solution of rabbit polyclonal anti-human nestin antibody (Biocare concentrated polyclonal antibody dilution 1:250, rabbit polyclonal).

Results

No Nestin immunostaining was seen in any of the oligodendroglioma tumor cells, but Nestin stained the endothelial cells in oligodendroglioma as a positive internal control (Figure 1,2).
Table 1: This shows the Grades of Astrocytoma.

|                     | Grade I Pilocytic Astrocytoma | Grade II fibrillary astrocytoma | Grade III Anaplastic astrocytoma | Grade IV Glioblastoma multiforme | Grade II Oligodendroglioma | Grade III Anaplastic oligodendroglioma |
|---------------------|-------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------|----------------------------------------|
| No. of cases        | 7                             | 3                               | 3                               | 3                               | 6                         | 6                                      |
| Gender              | M:4, F:3                      | M:1, F:2                        | M:2; F:1                        | M:2, F:1                        | M:3, F:3                  | M:4, F:2                              |
| Average Age         | 11                            | 35                              | 44                              | 52                              | 42                        | 46                                     |
| Nestin expression   | Negative-very weak            | weak                            | strong                          | Very strong                     | negative                  | Negative                               |

Nestin staining was seen in all astrocytic tumors. Pilocytic astrocytomas show mostly focal and weak staining with strong staining of Rosenthal fibers (Figure 3). Grade II astrocytoma shows weak but more intense staining than pilocytic astrocytoma (Figure 4). The strongest staining was in glioblastomas multiforme (GBM) and in Anaplastic astrocytomas (Figure 5,6).
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Figure 3:
A: Pilocytic astrocytoma.
B: negative-weak staining in the astrocytes, but strong staining in Rosenthal fibers and eosinophilic.

Figure 4:
A: low grade astrocytoma.
B: weak staining in astrocytes.

Figure 5:
A: Anaplastic astrocytoma, Grade III.
B: strong Immunostaining for Nestin.
Discussion and Conclusion

This study has clarified that the importance of nestin over-expression in glial tumor in two parts: The first one, in helping grading astrocytomas from Grade I-IV. The second part in helping differentiating astrocytomas from oligodendroglioma.

Since Nestin is an important protein in immature neural crest cells, its presence indicate that the cells are of neural crest origin [1-3]. Brain tumor that originate from neural crest and high grade tumors are expected to over express nestin.

Differentiating astrocytomas into different grades depends on morphology, mitotic figures and Ki-67. For example, differentiating grade II from anaplastic astrocytoma relies on finding mitotic figures and on Ki-67. Immunohistochemistry for Ki-67 has shown that a very good correlation with glioma grading and behavior [13,14]. Ki-67 is usually less than 4% in diffuse astrocytoma grade II [13]. Ki-67 is a proliferation factor that is expressed in cells in cell cycle during G1, S, G2/M phase [9]. Nestin is expressed in glioma tissue in astrocytoma lineage cells. The degree of nestin expression increases as the degree of malignancy increases, that is, as differentiation decreases. Now with nestin over-expression, we have another objective marker helping us to differentiate and grade astrocytomas. The higher grades of astrocytomas: Glioblastoma multiform and anaplastic astrocytoma, shows strong expression of Nestin (Figures 5&6), [4,6, 11,15-18]

Differentiating oligodendrogliomas from astrocytomas is very important because of two main reasons. The first one is that the prognosis of oligodendroglioma is better than astrocytoma, grade for grade. For example Oligodendrogloma grade II, has a median survival of 11.6 years with 10-year survival rate of 51% [14,18]. While the mean survival for fibrillary astrocytoma WHO grade II, is in the range of 6-8 years [18,19]. The same thing is applied for anaplastic oligodendrogloma grade II with a mean survival of 4-5, while it is of 2 year duration in anaplastic astrocytoma [18]. Nestin is an important immunohistochemical marker in differentiating oligodendrogloma from astrocytic tumors [4,11,20, 21]. Nestin is expressed only in tumors with some components of astrocytoma. Nestin is not expressed at all in any of the pure oligodendrogloma cases and only expressed in the vascular endothelium.

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Nestin, an Important Marker for Differentiating Oligodendroglioma from Astrocytic Tumors

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