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Original contribution

Nogo-A: a useful marker for the diagnosis of oligodendroglioma and for identifying 1p19q codeletion ☆

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Summary The differential diagnosis between oligodendrogliomas and other gliomas remains a critical issue. The aim of this study is to verify the diagnostic value of Olig-2, Nogo-A, and synaptophysin and their role in identifying 1p19q codeletion. A total of 168 cases of brain tumors were studied: 24 oligodendrogliomas, 23 anaplastic oligodendrogliomas, 2 oligoastrocytomas, 2 anaplastic oligoastrocytomas, 30 glioblastoma multiforme, 2 diffuse astrocytomas, 4 anaplastic astrocytomas, 10 pilocytic astrocytomas, 9 ependymomas, 12 anaplastic ependymomas, 10 central neurocytomas, 10 meningiomas, 10 choroid plexus papillomas, 10 dysembryoplastic neuroepithelial tumors, and 10 metastases. All cases were immunostained with Olig-2, Nogo-A, and synaptophysin. In 79 cases, the status of 1p/19q had already been assessed by fluorescence in situ hybridization. Thus, in selected cases, fluorescence in situ hybridization was repeated in areas with numerous Nogo-A–positive neoplastic cells. Nogo-A was positive in 18 (75%) of 24 oligodendrogliomas, 8 (80%) of 10 dysembryoplastic neuroepithelial tumors, 6 (20%) of 30 glioblastoma multiforme, and 2 (20%) of 10 pilocytic astrocytomas. Olig-2 stained 22 (91.6%) of 24 oligodendrogliomas and all dysembryoplastic neuroepithelial tumors but also 24 (80%) of 30 glioblastoma multiforme and 8 (80%) of 10 pilocytic astrocytomas. Finally, synaptophysin stained 13 (54.1%) of 24 oligodendrogliomas, 3 (10%) of 30 glioblastoma multiforme, 1 (10%) of 10 pilocytic astrocytomas, and all neurocytomas. Among the 79 tested cases, original fluorescence in situ hybridization showed 1p/19q codeletion in 12 (52.2%) of 23 oligodendrogliomas, 8 (38%) of 21 anaplastic oligodendrogliomas, and 1 (4%) of 25 glioblastoma multiforme. However, after carrying out the Nogo-A–driven fluorescence in situ hybridization, 1p/19q codeletion was observed in 8 additional cases. Nogo-A is more useful and specific than Olig-2 in differentiating oligodendrogliomas from other gliomas. Furthermore, using a Nogo-A–driven fluorescence in situ hybridization analysis, it is possible to identify a larger number of 1p19q codeletions in gliomas.

☆ Conflict of interest statement: The authors declare that they have no conflict of interest.

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1. Introduction

Differential diagnosis among astrocytomas (A), oligodendrogliomas (OL), and oligoastrocytomas (OA) is very challenging and subject to interobserver variability [1-3]. In the past, numerous studies have been carried out to find immunohistochemical markers useful for evaluating oligodendroglial differentiation and a series of molecules, such as S100 protein, Leu7, CD57, 3′-cyclic nucleotide 3′-phosphodiesterase, and tubulin polymerization promoting protein/p25, have been proposed [4,5], although their real diagnostic value is highly controversial.

More recently, it has been demonstrated that neuronal markers such as NeuN and synaptophysin are expressed in oligodendroglial tumors and are unrelated to neurocytic differentiation [6-8]. Unfortunately, the percentages reported for synaptophysin vary from 9.3% [9] to 100% of the OL studied [10], thus making this marker not very reliable.

In recent years, 2 markers, oligodendrocyte transcription factor 2 (Olig-2) and isoform A of the neurite outgrowth inhibitor (Nogo-A), have been proposed as useful markers for oligodendroglial differentiation [11-13].

Olig-2 encodes a basic helix-loop-helix transcription factor, which plays a critical role in oligodendrocyte differentiation during development [11].

Nogo-A is an oligodendrocyte-specific member of the reticulon family, strongly expressed in oligodendrocytes and, to a weaker extent, in myelin sheaths and neurons [14]. It is a known myelin-associated inhibitor of axonal regeneration after spinal injury [15]. Nogo-A expression in glial central nervous system tumors has been already reported, being expressed in approximately 80% of OL and in more than 20% of glioblastoma multiforme (GBM) in contrast to other glial tumors, such as A, anaplastic A, and ependymomas [12,13].

OL are frequently characterized by loss of chromosomes 1p and 19q, a feature which predicts a response to treatment and a significantly longer survival curve [16]. A 1p/19q codeletion is the consequence of an unbalanced translocation between the entire arm of 19p to 1q [17,18].

Therefore, detection of a 1p/19q codeletion by fluorescence in situ hybridization (FISH) analysis represents a very important information for the management of patients with OL [19]. However, FISH results may be influenced by the neoplastic areas selected to carry out the test. Thus, an immunohistochemical marker of oligodendroglial lineage could be helpful both in reaching a correct histologic diagnosis and in carrying out a FISH analysis.

The purposes of this study were to verify the use of Nogo-A, Olig-2, and synaptophysin in providing a correct histologic diagnosis of OL and to investigate a possible complementary role in selecting the more informative areas suitable for detecting 1p/19q codeletion using FISH analysis.

2. Materials and methods

2.1. Case selection

A total of 168 randomly selected cases of central nervous system tumors were retrieved from the files of the Section of Anatomic Pathology of the Department of Haematology and Oncology of the University of Bologna at Bellaria Hospital (Bologna). All the tumors were restaged and graded at the time of the immunohistochemical analysis by 2 pathologists (G.M. and M.P. F.) according to the 2007 World Health Organization criteria [6]. Any disagreement was discussed using a double head microscope, and a consensus was reached.

The series included 24 OL and 23 anaplastic OL: the large amounts of OL (19/24) and of anaplastic OL (17/23) show the classic oligodendroglial features (honeycomb appearance, microcalcifications, microcysts, and branching capillaries), whereas remaining cases present areas with microgemistocytes, eosinophilic granular cells, and reactive astrocytes. Furthermore, we enrolled 2 OA, 2 anaplastic OA, 30 GBM, 2 A, 4 anaplastic A, 10 pilocytic A (PA), 9 ependymomas, 12 anaplastic ependymomas, 10 central neurocytomas, 10 meningiomas, 10 choroid plexus papillomas, 10 dysembryoplastic neuroepithelial tumors (DNT), and 10 metastases to the brain having various primary origins (1 melanoma, 3 mammary carcinomas, 1 urothelial carcinoma, 1 gastric adenocarcinoma, 2 squamous carcinomas, 1 adenocarcinoma of the lung, and 1 ovarian carcinoma).

2.2. Immunohistochemistry

From selected blocks, 4-μm-thick sections were immunohistochemically stained with 3 different antibodies: (1) anti–Nogo-A (polyclonal, diluted 1:500; Chemicon-Millipore Corp, Billerica, MA, USA), (2) anti-Olig-2 (polyclonal, dilution 1:300; Chemicon-Millipore Corp), and (3) anti-synaptophysin (polyclonal, dilution 1:40; Neomarkers, Thermo Fisher Scientific, Fremont, CA, USA).

Immunohistochemical reactions were performed using the UltraVision LP Large Volume Detection System HRP Polymer (Thermo Fisher Scientific, Fremont, CA) as follows: dewaxing and antigen retrieval were achieved by pretreatment with W-CAP TEC buffer solution pH6 (Bio-Optica, Milan, Italy) at 95°C for 25 minutes, and inhibition of the endogenous peroxidases was obtained in 3% H_2O_2. After rinsing the slides in distilled water and in buffer solution (PBS-Tween 20, 1×; Bio-Optica), the sections were incubated in a humid chamber at room temperature (RT) for 5 minutes with Ultra V Block solution (Ultravision LP; Lab Vision Corp, Thermo Fisher Scientific). The sections were then washed in buffer solution 3 times for 2 minutes each and incubated with primary antibody for 1 hour at RT in a humid chamber. The sections were then washed in buffer solution and incubated with Primary Antibody Enhancer solution