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Original contribution

Caveolin-1 expression in diffuse gliomas: correlation with the proliferation index, epidermal growth factor receptor, p53, and 1p/19q status☆

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Summary Caveolin-1 (cav-1) has been proposed as an immunohistochemical marker able to distinguish astroglial from oligodendroglial tumors. In addition, it has been suggested that the reduction of caveolin-1 expression in glioblastoma cells increases their proliferative and invasive potential. Accordingly, the present study investigates caveolin-1 immunoexpression and correlation with the 1p/19q status, histologic grade, proliferation index, epidermal growth factor receptor, and p53 expression in a series of 73 diffuse gliomas. A membranous and cytoplasmic immunolabeling for caveolin-1 was detected in neoplastic cells of 60% of cases. No significant differences in terms of caveolin-1 expression were observed between astrocytomas, oligodendrogliomas, and oligoastrocytomas. In addition, caveolin-1 expression was not correlated with 1p/19q status in oligodendrogliomas and mixed oligoastrocytomas. Caveolin-1 was expressed in most high-grade (World Health Organization III and IV) gliomas. Low caveolin-1 expression correlated with a higher Ki-67 labeling index and the absence of p53 overexpression in glioblastomas, and it was significantly associated with epidermal growth factor receptor overexpression in anaplastic astrocytomas. In conclusion, the present study indicates that caveolin-1 is not useful as diagnostic marker to differentiate grade II astrocytomas from oligodendrogliomas.

1. Introduction

Caveolin-1 (cav-1) is a 22-kd protein mainly expressed by the endothelial cells, adipocytes, fibroblasts, and smooth muscle cells [1]. In the nervous system, its expression has been documented in normal rat astroglial cell cultures [2] as well as in dorsal root ganglion [3] and Schwann cells [4].

There is evidence that Cav-1 exerts an ambivalent role in tumorigenesis. Indeed, it may exercise tumor suppressor activity by inhibiting the signaling products of several proto-oncogenes [5]; on the other hand, its tyrosine-14 phosphorylation results in growth stimulation [6], suggesting that cav-1 may also behave as a protumorigenic factor. Given its increased levels in comparison to the corresponding non-neoplastic tissues, a protumorigenic role has been attributed

☆ Dedicated to my son Alessandro on the occasion of his birth.

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to cav-1 in several neoplasias [7-9], including meningiomas [10,11] and gliomas [12-15]. Cassoni et al [13] have recently shown that cav-1 is expressed in astroglial tumors but not in oligodendrogliomas and have proposed cav-1 immunohistochemical detection as a novel diagnostic tool to differentiate astroglial tumors from oligodendroglial ones. These authors also reported that cav-1 immunoexpression in the astroglial-derived tumors is variable, with populations of cav-1–positive and cav-1–negative cells coexisting in half of the glioblastomas studied [13]. How cav-1–positive cells contribute to glioblastoma phenotype has not been yet fully clarified. Nonetheless, it has been recently reported that the reduction of cav-1 expression in U87 MG glioblastoma cells increases their proliferative and invasive potential, suggesting a higher biological aggressiveness of cav-1–negative glioblastoma cells [16].

In the present study, we investigated cav-1 immunoexpression in a series of diffuse gliomas in an attempt to verify the usefulness of cav-1 immunostaining in the differential diagnosis of astrocytoma versus genetically proven oligodendroglioma and mixed oligoastrocytoma. Moreover, to seek a correlation, if any, between the biologic features and cav-1 expression, the latter was correlated with histologic grade, proliferation index, epidermal growth factor receptor (EGFR), and p53 expression.

2. Materials and methods

Seventy-three cases of surgically resected gliomas were taken from the files of the Unit of Neuropathology, La Sapienza University, Rome, and of the Department of Pathology, University of Messina, Italy. For each case, the hematoxylin-eosin sections were reviewed and all cases were classified according to the World Health Organization (WHO) classification system [17]. Thus, the study cohort comprised 44 astrocytomas, including 7 diffuse astrocytomas (WHO grade II), 17 anaplastic astrocytomas (WHO grade III), with 2 anaplastic gemistocytic astrocytomas and 20 glioblastomas (WHO grade IV); 11 oligodendrogliomas (WHO grade II); 5 anaplastic oligodendrogliomas (WHO grade III); 8 oligoastrocytoma (WHO grade II) and 5 anaplastic oligoastrocytomas (WHO grade III). Table 1 summarizes the clinicopathologic characteristics of all studied cases. All specimens had been fixed in 10% neutral formalin for 24 hours at room temperature and embedded in paraffin at 55°C. Cav-1, EGFR, p53, and Ki-67 expression was evaluated by immunohistochemistry in serial sections cut from the paraffin blocks. In some surgical specimens (8 cases), a fragment of normal brain tissue was also present; thus, in these cases, cav-1 immunoexpression was evaluated in the normal tissue as well.

2.1. Immunohistochemistry

Paraffin blocks were cut into 4-μm-thick serial sections for the immunohistochemical procedures. Briefly, the intrinsic endogenous peroxidase activity was blocked with 0.1% H_2O_2 in methanol for 20 minutes, then normal sheep serum was applied for 30 minutes to prevent unspecific adherence of serum proteins. EGFR and Ki-67 antigens were unmasked by antigen retrieval procedures (10 mmol/L, pH 6.0, sodium citrate buffer heated in a microwave oven for 3 cycles ×5 minutes).

Consecutive sections were successively incubated at 4°C overnight with the following antibodies: the polyclonal rabbit antibody against cav-1 (Santa Cruz Biotechnology, Inc, Santa Cruz, CA; working dilution 1:500), the monoclonal mouse antibody against EGFR (clone H11, Dako Cytomation, Glostrup, Denmark; working dilution 1:200), the monoclonal mouse antibody against p53 (clone DO-7, Dako Cytomation; working dilution 1:50), and the antibody against Ki-67 (clone MIB-1, DAKO, Glostrup, Denmark; working dilution 1:50).

The bound primary antibodies were visualized by avidin-biotin-peroxidase detection using the Vectastain Rabbit/Mouse Elite Kit, according to the manufacturer's instructions. To reveal the immunostaining, the sections were incubated in darkness [18] for 10 minutes with 3-3′ diaminobenzidine tetrahydrochloride (Sigma Chemical Co, St Louis, MO), 100 mg in 200 mL 0.03% hydrogen peroxide in phosphate-buffered saline. Nuclear counterstaining was performed by Mayer hemalum. Specimens of adipose tissue as well as endothelium and smooth muscle of the vessels

Table 1 Clinicopathologic characteristics of the 73 analyzed glial tumors

No. of cases and cell of origin	Tumor type and grade	No. of cases and subtypes		Age range
Astroglial tumors (n = 44)	Astrocytoma II	7		44-70
	Astrocytoma III	17		
		15 anaplastic	2 gemistocytic	42-58
	Glioblastoma IV	20		33-74
Oligodendroglial tumors (n = 16)	Oligodendroglioma II	11		38-64
	Oligodendroglioma III	5		45-70
Mixed oligo-astroglial tumors (n = 13)	Oligoastrocytoma II	8		28-48
	Oligoastrocytoma III	5		25-62