Expression of neuronal and glial markers in so-called oligodendroglial tumors induced by transplacental administration of ethyl-nitrosourea in the rat

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Summary. A series of 18 tumors with histological features of oligodendrogliomas, induced in the rat by means of transplacental ethyl-nitrosurea administration were studied for immunohistochemical demonstration of neuronal (synaptophysin and neurofilament protein) and glial (gliofibrillar acidic protein and vimentin) markers. Most of the tumors showed cells with strong positivity to synaptophysin and to a lesser degree, to neurofilament protein, suggesting the neuronal character of these neoplasms. In 10 tumors, cells with strong positivity to vimentin were found, and in three cases, tumoral cells expressed gliofibrillar acidic protein. The observation that ENU-induced oligodendroglial tumors express neuronal and, to a minor degree, glial markers, suggests their interpretation as primitive neuroectodermal tumors with clear neuronal differentiation.

Key words: Oligodendroglioma, ENU, Experimental brain tumors, Synaptophysin, NF, GFAP, Vimentin, Immunohistochemistry

Introduction

The ethyl-nitrosurea (ENU)-induced tumor of the nervous system is an experimental model widely used in neuro-oncology. Injection of a single sufficient dose of ENU on the 16th to 21st days of gestation in rats produces nervous system tumors in the offspring with a mean latency of between 5 and 6 months (Schiffer, 1991).

Although ENU-induced brain tumors have been mainly classified on the basis of their light-microscopic similarities to certain human neurogenic tumors, there exist discrepancies in different reports about incidence of types of tumors, perhaps because there is inadequate criteria for a precise classification (Mandybur and Alvira, 1982).

At present, it is accepted that most intra-parenchymatous brain tumors induced by ENU show morphological features of oligodendrogliomas, with variable degree of differentiation (Grossi-Paoletti et al., 1970, 1972; Jones et al., 1973; Janisch and Schreiber, 1977; Schiffer et al., 1978; Conley, 1979; Lantos, 1980; Mauro et al., 1983; Reifenberger et al., 1989; Schiffer, 1991). Nevertheless, the lack of accurate immuno-histochemical markers for oligodendroglial tumors makes it difficult to confirm their exact nature (Koestner, 1990).

On the other hand, it is now recognized in human neuropathology that some brain tumors showing morphological features of oligodendrogliomas should now be reclassified as central neurocytomas, the neuronal character of their cells having been recognized by electron microscopy and immunohistochemistry (Hassoun et al., 1982; Pearl et al., 1985; Towsend and Seaman, 1986; Nishio et al., 1988; Poon et al., 1988; Barbosa et al., 1990; Patil et al., 1990; Vaquero et al., 1992).

Given the possibility that so-called oligodendroglial rat tumors induced in the brain after ENU administration, might be in reality tumors of neuronal nature or showing signs of neuronal differentiation, we have studied the expression of neuronal and glial markers in 18 such tumors.

Materials and methods

The 18 tumors studied were induced by trans-placentary administration of ENU (50 mg/kg) to gestating Wistar rats at day 17 of gestation. The location of such tumors were intracerebral in 16 cases and within the spinal cord in 2 cases. All of them were clinically manifest in the offspring by appearance of neurological signs after a mean latency of 9 months. At necropsy, the brains or spinal cords were fixed in formol-saline at 10% sliced in order to delimit the accurate location of tumors, and regions showing macroscopical tumors were embedded in paraffin. In addition to histological studies

with haematoxylin-eosin technique, an immuno-histochemical study for demonstration of gliofibrillar acidic protein (GFAP), vimentin, neurofilament protein (NFP), and synaptophysin was performed. Polyclonal rabbit anti GFAP, and monoclonal mouse anti vimentin, synaptophysin and NFP, that included the subunits of 70, 160 and 210 kd, were used as primary antisera. All antibodies, except synaptophysin (Euro-Diagnostic, Holland, dilution 1:50) were obtained pre-diluted from Bio-Genex laboratories (San Ramon, CA, USA). In brief, the staining procedure was as follows: 5 μm-thick sections were deparaffinized in xylene, rehydrated in a graded ethanol series, and rinsed in phosphate-buffered saline (PBS), pH 7.6. They were placed in 3% H_2O_2 in water for 10 minutes to block endogenous peroxidase activity, and immersed in PBS for 30 minutes. The subsequent incubations (all at 37° C in humid chamber) were as follows: a) normal serum from the species in which the secondary antibody was made for 10 minutes; b) primary antibody 30 minutes, three 5- minute washes in PBS; c) biotin-labelled goat anti-mouse antibody (in monoclonal primary antibodies) or biotin-labelled goat anti-rabbit antibody (in polyclonal antibodies) for 20 minutes, three 1 minute washes in PBS; and d) avidin-biotin-peroxidase or streptavidin-alkaline phosphatase complex (Bio-Genex, San Ramon, CA, USA) 20 minutes, three 1 minute washes in PBS. Incubation in substrate then took place, using 3-amino-9-ethyl-carbazole or fast Red TR. A nuclear counterstaining with haematoxylin was applied after substrate reaction. In all cases, positive stain control was done with cerebellum for GFAP, synaptophysin and NFP, and with skin for vimentin. Negative controls were done substituting primary antibodies for 1-2 drops of normal serum during the procedure.

Results

All tumors of the present series showed morphological features of oligodendrogliomas in haematoxylin-eosin-stained samples (Fig. 1). Table 1 shows the pattern of immunohistochemical positivity

Table 1. Pattern of immunohistochemical positivity in the tumors of the series.

Case	Synaptophysin	NFP	GFAP	Vimentin
1	+++(S)	+++(S)	+++(S)	+++(S)
2	+++(S)	+++(S)	+++(S)	+++(S)
3	+++(S)	+++(S)	+++(S)	+++(S)
4	+++(S)	+++(S)	+(L)	+++(S)
5	+++(S)	+++(S)	-	+++(S)
6	+++(S)	+++(S)	-	+++(S)
7	+++(S)	+++(S)	-	+++(S)
8	+++(S)	+++(S)	-	+++(S)
9	+++(S)	+++(S)	-	+++(S)
10	+++(S)	++(S)	-	+++(S)
11	+++(S)	++(S)	-	+(L)
12	+++(S)	++(S)	-	+(L)
13	+++(S)	++(S)	-	+(L)
14	+++(S)	++(S)	-	+(L)
15	+++(S)	++(S)	-	-
16	+++(S)	+(L)	-	-
17	+(S)	+(L)	-	-
18	+(S)	+(L)	-	-

-: absence of positive cells; +: <25% of tumor cells showing positivity; ++: 25 to 75% of cells showing positivity; +++: >75% of cells showing positivity. S: strong positivity; L: low positivity; NFP: neurofilaments protein; GFAP: gliofibrillar acidic protein.
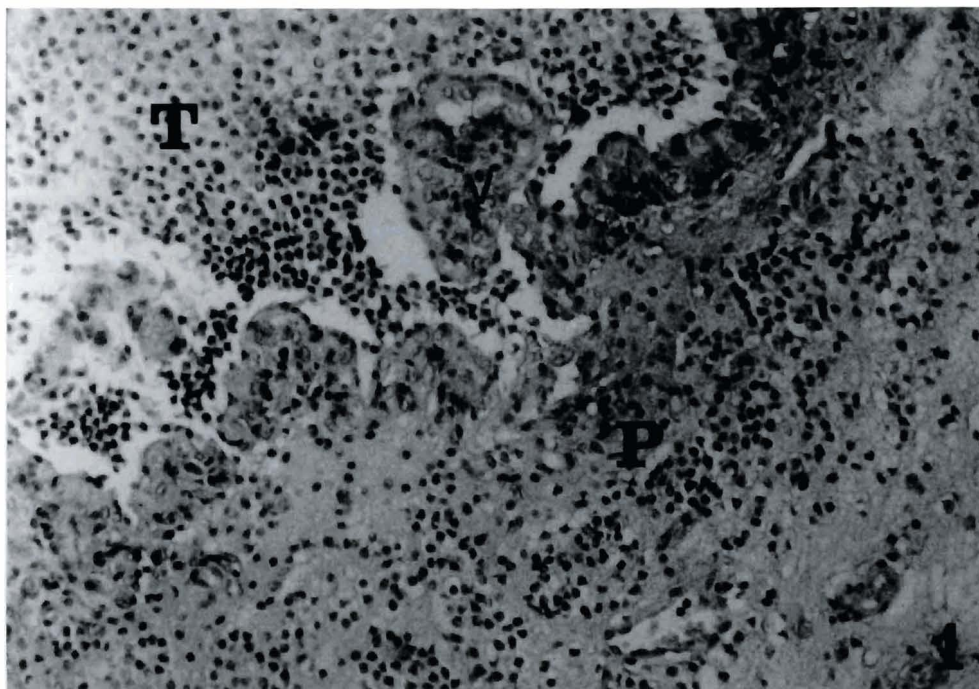

Fig. 1. Histological features of the oligodendroglioma-like tumors studied in the present series. In each case, the tumor (T) is constituted by cells showing small dark nuclei, lying within a network of lightly-stained vacuolated spaces. Tumor cells infiltrate the surrounding brain parenchyma (P). Prominent vascular hyperplasia (V) can be seen. Haematoxylin-eosin. x 75

that each of the tumors expressed in the series.

In all cases, GFAP was detected on reactive astrocytes trapped in the tumor tissue, and in reactive astrocytes of the surrounding nervous tissue (Fig. 2). Nevertheless, in three tumors of the series, a clear positivity was found on tumor cells (Fig. 3). In these cases, tumor cells expressed vimentin, also. Generally, vimentin was positive on reactive astrocytes, with a pattern similar to that obtained with GFAP (Fig. 4), but in 10 tumors of the series, most of the tumor cells showed strong positivity for vimentin (Fig. 5).

In 16 tumors of the present series, a clear, strong

Neuronal and glial markers in ENU-induced oligodendrogliomas
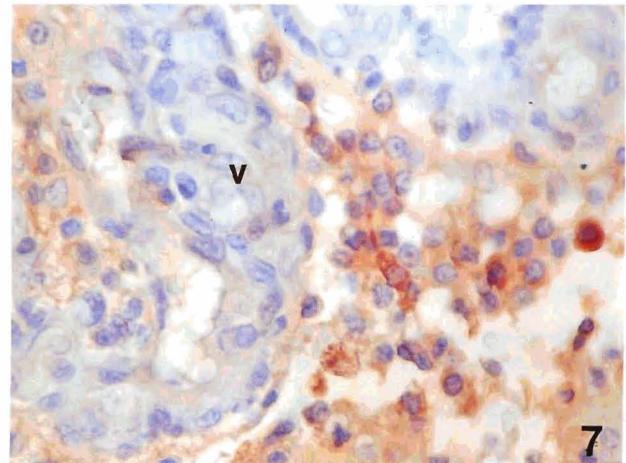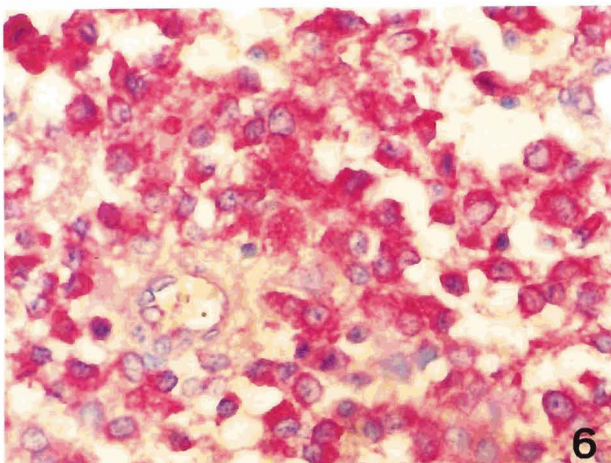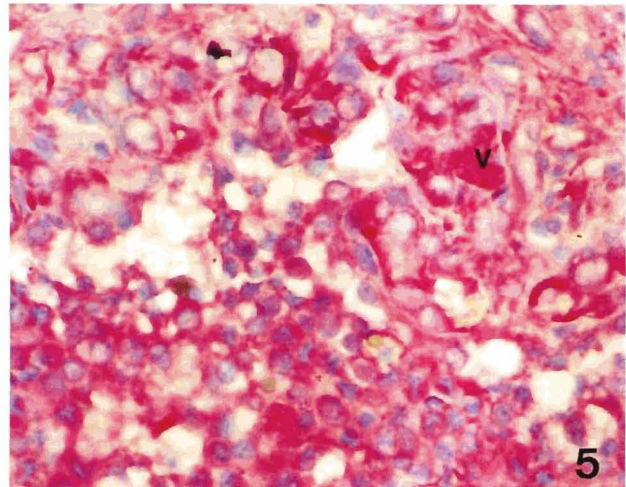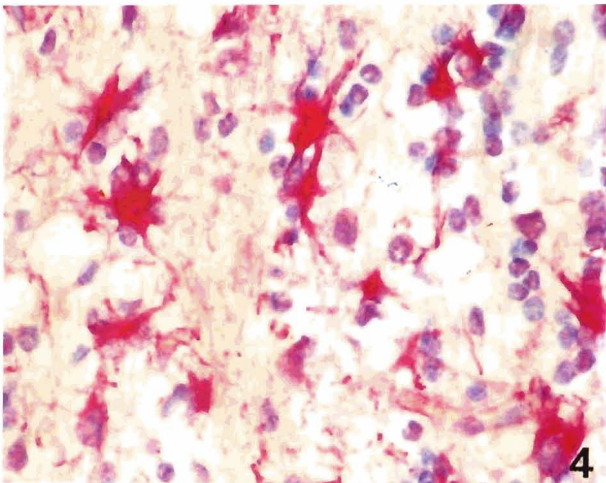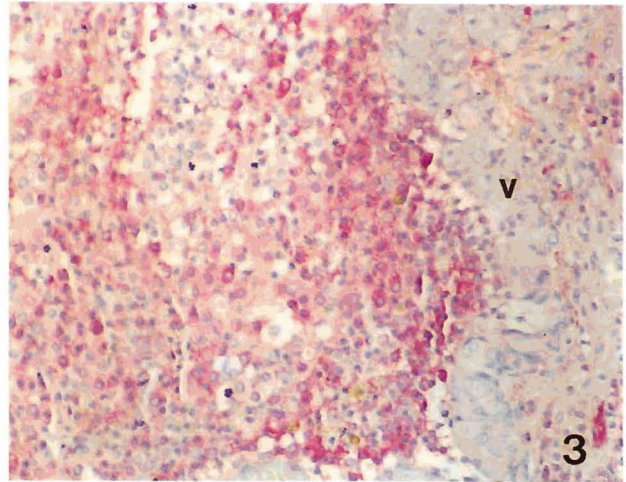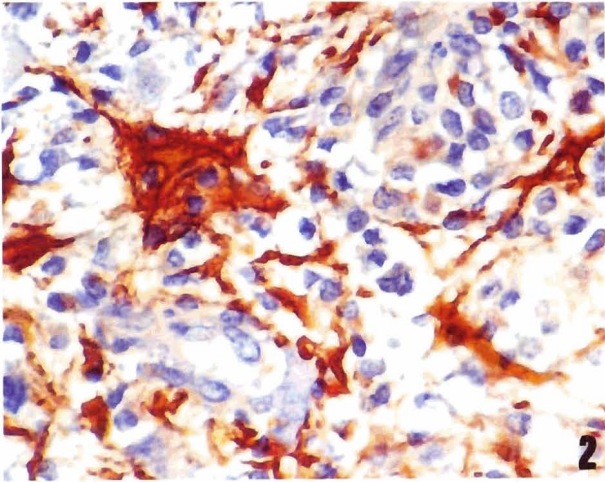
positivity for synaptophysin was noted, both in tumor cells and in tumor neuropil (Fig. 6). These tumors also showed positivity for NFP, but the immunostained cells were lesser in number (Fig. 7).

Discussion

Our present study shows that, in the rat, most of the ENU-induced tumors with morphological features of oligodendrogliomas, express strong positivity for synaptophysin, suggesting their neuronal character. Synaptophysin is an integral membrane glycoprotein originally isolated and characterized by Wiedenmann and Franke (1985) from presynaptic vesicles in bovine brain neurons, and, to date, its presence, demonstrated immunohistochemically, has been used as a marker for neuroendocrine differentiation in many neoplasms (Hachitanda et al., 1989), in primitive neuroectodermal tumors (Molenaar and Trojanowski, 1991) and in central neurocytomas (Barbosa et al., 1990; Vaquero et al., 1992). In our series, the neuronal character of the studied tumors is supported by their positivity to NFP, a marker specifically expressed by neuron or neuron-like cells (Molenaar and Trojanowski, 1991).

The pattern of positivity that we have found for GFAP and for vimentin agree with previous observations on this type of experimental tumor (Conley, 1979; Mauro et al., 1983; Yoshino et al., 1985; Reifenberger et al., 1989; Mennel and Dreyhaupt, 1990; Raju et al., 1990). While GFAP is considered an accurate marker for glial tumors, vimentin is not considered a specific marker because it can be expressed in virtually all cell types, often being coexpressed with intermediate filaments of other classes, and it is well known that in the course of embryogenesis, it appears first in immature glial cells, but rapidly decreases as GFAP becomes expressed. Likewise, vimentin expression predates that of NFP in neuronal cells. In any case, the demonstration of vimentin-positive, and to a lesser degree, GFAP-positive tumor cells, suggests that in so-called ENU-induced oligodendrogliomas, tumor cells can express glial markers in addition to neuronal markers, a possibility that has been reported in primitive neuroectodermal human tumors (Molenaar and Trojanowski, 1991).

In view of these data, we think that most ENU-induced oligodendrogliomas could be considered

primitive neuroectodermal tumors, showing a tendency to differentiate toward a neuronal phenotype.

It is possible that in the past the oligodendroglioma-like appearance of most ENU-induced intra-parenchymatous brain neoplasms has impeded their correct interpretation, similar to that which has happened in human neuropathology with the central neurocytoma, a tumor generally misdiagnosed as oligodendroglioma on the basis of its histological aspect, the neuronal character of which may now be suggested by the immunohistochemical detection of synaptophysin (Barbosa et al., 1990; Vaquero et al., 1992).

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Fig. 2. GFAP positivity on reactive astrocytes trapped in the tumor tissue. In this case, tumor cells were GFAP negative. x 250

Fig. 3. Oligodendroglioma-like tumor showing prominent immunoreactivity for GFAP. On the right, proliferative blood vessels (v) can be seen. x 75

Fig. 4. Reactive astrocytes within an oligodendroglioma-like tumor, showing strong immunoreactivity for vimentin. x 250

Fig. 5. Strong positivity for vimentin in the tumor cells of an oligodendroglioma-like tumor. In the upper field of the figure, proliferative vessels (v) can be seen. Cells of the vascular walls showed positivity for vimentin also. x 250

Fig. 6. Strong positivity for synaptophysin in the tumor cells of an oligodendroglioma-like tumor. x 250

Fig. 7. Immunoreactivity for NFP in the tumor cells of an oligodendroglioma-like tumor. v: proliferative vessels. x 250

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