Immunohistochemical staining for S-100 and GFAP proteins of spontaneous brain tumours in Wistar rats

by *C. Madsen* and *O. Ladefoged,* Institute of Toxicology, National Food Agency of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark.

Introduction

The occurrence of spontaneous tumours in the central nervous system (CNS) of rats is relatively rare. The need for a histological correct classification of rat brain tumours may, therefore, be of great importance in evaluation of chemical induced brain neoplasms in long term carcinogenicity studies.

In humans, immunohistochemistry has proved to be a valuable tool to increase the diagnostic accuracy of tumours of the CNS (*Bonnin & Rubinstein* 1984). The most important and reliable marker, so far, has been Glial Fibrillary Acid Protein (GFAP). As expected, GFAP is found in tumours of astrocytic lineage and has been of great value in the diagnosis of tumours of glial origin (*Bignami et al.* 1980). The S-100 protein, another soluble protein in the CNS, has also been applied in studies of brain tumours. It is present in glial and non-glial tumour cells as well as in tumours of non-neuroepithelial origin and is, therefore, of less diagnostic value (*Bonnin & Rubinstein* 1984, *Nakamura et al.* 1983).

The cytological distribution of GFAP and S-100 proteins in the adult rat brain has been studied by several workers using the peroxidase-labelled antibody technique. Although some conflicting results have been obtained it seems that both proteins are present in astrocytes and structures composed of astrocytic processes. The S-100 protein is found in the nucleus and cytoplasma whereas GFAP is only found in the cytoplasma (*Ludwin et al.* 1976).

The classification of rat brain tumours is almost entirely based on light microscopic morphological characteristics. However, staining for reticulin or Periodic-Acid-Shiff-stain, positive granules may improve the classification. The application of immunohistochemistry in classification of rat brain tumours so far has been disappointing (*Solleveld* 1986) and very few investigations have been performed.

The aim of the present study was to evaluate the supplementary diagnostic value of the GFAP and S-100 staining in classification of spontaneous brain tumours in Wistar rats.

Material and methods

All brain tumours found in a long term carcinogenicity study in Wistar rats with a food additive (TOSOM/TOS) were examined. The study was terminated when the rats were 132–137 weeks old. The total number of rats were 956 (*Gry et al.* 1987). Comparison between the treated rats showed no statistical evidence of a treatment-related effect and all tumours were considered as spontaneous findings.

In total the material include 32 brain tumours except pituitary gland tumours. Seven tumours were later excluded in the extended histopathological examination due to lack of tissue. For histological examination the whole brain was fixed in 4 % neutral buffered formaldehyde for up to two years. Specimens from the brain were embedded in paraffin and 4–6 µ thin sections were cut. For routine examination the slides were stained with haematoxylin and eosin. To confirm the diagnosis of granular cell tumours a PAS-staining was performed. Tumours of possible meningeal origin were stained for reticulin (*Bancroft & Stevens* 1982).

The staining for GFAP or S-100 proteins was an immunoperoxidase procedure using

Table 1. The results of staining of 25 spontaneous brain tumours in Wistar rat for PAS, reticulin, S-100 and GFAP protein.

Tumour-type	No. of	PAS	Reticulin	S-100	GFAP
Granular cell	12	12 positive	n.d.	11 negative 1 uncertain	10 negative 1 positive 1 uncertain
Astrocytoma	3	n.d.	n.d.	3 negative	3 positive
Malign meningioma	2	n.d.	2 positive	2 negative	2 negative
Meningioma syncytial	2	1 positive	2 positive	1 negative 1 uncertain	2 negative
Meningioma fibroblastic	2	n.d.	2 positive	2 negative	2 negative
Fibrosarcoma	1	n.d.	1 positive	1 negative	1 negative
Malign tumour, unclassified	1	n.d.	1 positive	1 negative	1 negative
Pineal gl. adenoma	1	1 negative	1 negative	1 negative	1 negative
Ependymoma	1	n.d.	n.d.	1 uncertain	1 negative

n.d. = not done

secondary antiserum (P217) and primary antiserum (Z311 and Z334) from Dakopatts in concentration 1:100. The peroxidase label was visualized by diaminobenzidine and the slides were counterstained with haematoxylin. The criterion for a positive result was specific staining of the tumour cells not taking into consideration reactive astrocytes in the surroundings of the tumour. Only slides which gave a positive staining for GFAP or S-100 proteins in the normal tissue outside the tumour were evaluated. If positive results or doubtful stainings were obtained, the results were controlled in a new slide including control staining without antiserum. The tumours were classified according to *Gopinath* (1986).

Results

In table 1 the results of the stainings are shown. Four tumours were positive in the GFAP protein staining and one tumour showed a doubtful reaction. Three of the four positives were astrocytomas.

In the GFAP-positive astrocytomas only some of the tumour cells stained for GFAP-

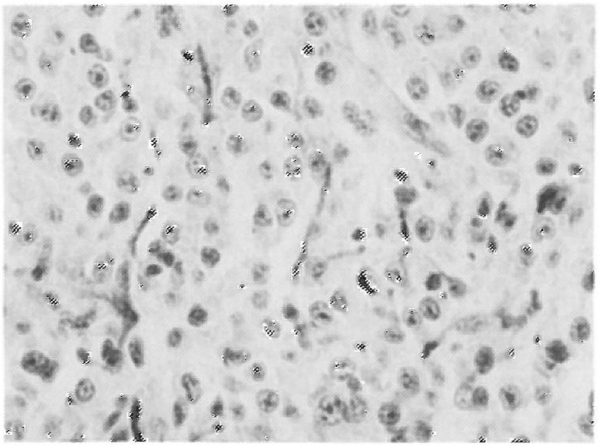

Figure 1. Tumour cells from the brain of rat diagnosed as an astrocytoma. The red-brown colour indicates GFAP-like immunoreactivity (immunperoxidase with haematoxylin, × 130).

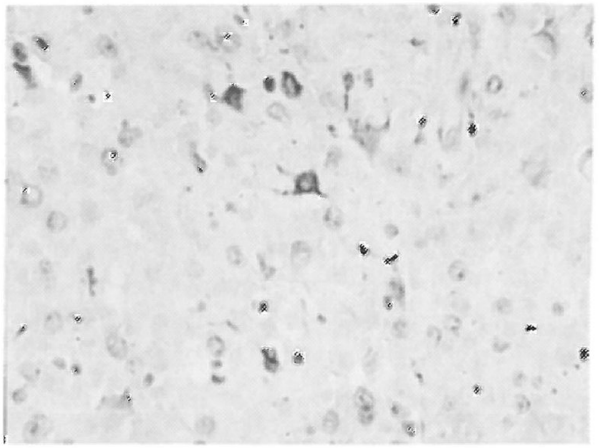

Figure 2. Tumour cells from the brain of rat diagnosed as granular mixed cell tumour. The red-brown colour indicates GFAP-like immunoreactivity (immunoperoxidase with haematoxylin, × 130).

protein (fig. 1). In one tumour classified as granular cell tumour the GFAP-staining revealed a population of clearly GFAP-positive cells beside the PAS-positive and GFAP-negative tumour cells (fig. 2).
No tumours were stained strongly for S-100. Three tumours were weakly stained and 22 tumours were clearly negative. The weak positive tumours were classified as one granular cell tumour, a meningioma and an ependynoma.

Discussion
The results of the present investigation indicate that staining for GFAP may be a valuable additional criterion for classification of astrocytomas. The conclusion should, however, be taken with reservation since the material considered of only 3 tumours. Other investigators have reported (preliminary results) that some tumours diagnosed as astrocytomas in the rat were negative for GFAP (*Solleveld et al.* 1986, *Krinke et al.* 1985). After staining for GFAP, one of the granular cell tumours was diagnosed as a mixed tumour. In such cases the additional staining for GFAP may, therefore, be of diagnostic value.
In the staining for S-100 protein almost all tumours were negative in agreement with other reports (*Solleveld et al.* 1986). The few doubtful reactions or weakly positive stainings for S-100 protein were found in one granular cell tumour, in one meningioma and in one ependymoma.
It is known that tissue fixation and handling can reduce the antigenic response but in our experience staining for GFAP and S–100 proteins in normal rat astrocytes was not a problem even after very long fixation and the normal brain tissue was stained strongly. Quantitatively the expression of GFAP was less in tumour cells than in normal astrocytes. This may also hold true for the expression of S-100. In rat brain tissue the expression of S-100 was less than that for GFAP according to our experience. The quantitative expression of the two markers is less in rat brain tumours than in human brain tumours, and less than in reactive astrocytes of rats (*Solleveld et al.* 1986). The explanation for that may be that the specificity of the S-100 antiserum is less than the specificity of GFAP antiserum because of antigenically different activities of the protein subunit (*Takahashi et al.* 1984).
The result of staining of this relatively small number of tumours do not allow a firm conclusion but indicate that GFAP-staining may be of additional diagnostic value in rat brain tumour classification whereas staining for S-100 protein in tumours found in this study does not seem to have any value.

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Sammendrag
Spontane hjernetumorer forekommer relativt sjældent hos rotter. Det er derfor at stor betydning ved evalueringen af kemisk inducerede tumorer, at klassifikationen foretages så korrekt som muligt. Værdien af at anvende immunohistokemiske metoder (GFAP og S-100) som supplement til PAS-farvning og reticulinfarvning blev undersøgt på 25 spontane hjernetumorer fra et langtidsforsøg på Wistar rotter. Astrocytomer farvedes positivt for GFAP, mens ingen tumorer havde en tydelig positiv reaktion for S-100. Den hyppigst forekommende tumortype var granular celle tumor, der ikke farvedes for indhold af GFAP of S-100. Der blev dog påvist en tumor af denne type, der viste sig at have en population af celler, der var GFAP-positive.
På baggrund af de relativt få astrocytomer i materialet kan der ikke drages nogen sikker konklusion om værdien af GFAP-farvningen, men undersøgelsen tyder på, at GFAP er af værdi ved diagnosticering af astrocytomer. Ingen tumor udtrykte klart aktivitet for S-100, og det kan konkluderes, at den anvendte S-100 farvning ikke havde nogen reel værdi ved klassifikationen af hjernetumorerne i denne undersøgelse.

Summary
The occurrence of spontaneous brain tumours in rats is relatively rare. The need for a histological correct classification of rat brain tumours is of great importance in evaluation of chemical induced brain neoplasms in long term carcinogenicity studies.

The value of additional immunohistochemical staining for GFAP and S-100 proteins were investigated in a long term carcinogenicity study. Astrocytomas were positive for the GFAP protein but no tumours had a clear positive reaction for the S-100 protein. The most common tumour type was granular cell tumour which did not stain for the GFAP and S-100 proteins. However one tumour of this type had a subpopulation of cells that stained for GFAP protein. In the light of the relatively few astrocytomas in the material, no firm conclusion on the value of staining for GFAP protein can be drawn. However the investigation suggests that staining for GFAP protein is of value in the diagnosis of astrocytomas.
Staining for S-100 protein did not show a positive reaction in any of the tumours.
In conclusion staining for S-100 protein in this study did not have any diagnostic value.

Yhteenveto / K. Pelkonen
Spontaanien aivokasvainten esiintyminen on rotassa suhteellisen harvinaista. Rotan aivokasvainten histologisesti oikea luokittaminen on hyvin tärkeätä pitkäaikaistoksisuustutkimuksissa kemiallisesti aiheutettujen kasvainten yhteydessä.
Työssä selvitettiin GFAP:n ja S-100-proteiinien immunohistokemiallisen lisävär jäyksen merkitystä pitkäaikaiskarsinogeenisyystutkimuksen yhteydessä. Astrosytoomat olivat positiivisia GFAP-proteiinin suhteen, mutta mitkään tuumorit eivät selvästi olleet positiivisia S-100-proteiinin suhteen. Yleisin kasvaintyyppi oli jyvässolukasvain, joka ei värjäytynyt GFAP ja S-100-proteiinien suhteen. Yhdessä tämän tyypin kasvaimessa oli kuitenkin soluryhmiä, jotka värjäytyivät GFAP-proteiinin suhteen. Astrosytoomia oli aineistossa niin niukasti, että värjäyksen arvosta GFAP-proteiinin suhteen ei voi tehdä varmoja johtopäätöksiä. Tutkimus viittaa kuitenkin siihen, että GFAP-proteiinivärjäyksellä on merkitystä astrosyytoomien diagnosoinnissa.
S-100-proteiinivärjäys ei antanut positiivista tulosta missään kasvaimessa.
Yhteenveto todetaan, että S-100-proteiinin värjäyksellä ei tässä tutkimuksessa ollut minkäänlaista diagnostista arvoa.

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