

Contributions of immunohistochemistry to the problem of differentiation in medulloblastoma.

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The problem of differentiation of medulloblastoma is considered. In this regard 43 medulloblastomas, showing cells with glial or neuronal features by routine histologic methods, were studied. The investigation was carried out by means of the immunohistochemical demonstration of the glial fibrillary acidic protein (GFAP) and the neuron-specific enolase (NSE). In most cases, GFAP-positive cells are preexisting astrocytes; in two cases they correspond to the transitional cells of the subependymal layer. NSE was demonstrated in areas filled with cells with neuroblastic features. The relationship between medulloblastoma with neuron-differentiation and cerebellar neuroblastoma is discussed.

Key-Words: Medulloblastoma — differentiation — GFAP — neuron-specific enolase — immunohistochemistry.

The problem of differentiation of medulloblastoma has been debated for a long time. According to one hypothesis, it is an undifferentiated neuroepithelial tumor [37, 34]. According to others it may differentiate either along the neuronal line [31, 1, 36], or the glial line [24, 20], the oligodendroglial one included [22], or both [25, 15, 4]. According to Gullotta [11, 12] it is an embryonal sarcoma.

The point at issue is whether the astrocytes and neurons found in the tumor are preexisting elements or differentiated tumor cells. Recently the immunohistochemical demonstration of the astroglial marker GFAP stimulated the discussion, as GFAP-positive cells can be found in most medulloblastomas [7, 9, 33, 22, 17, 27], but their status remains undefined. From the practical point of view, it might be wondered whether the occurrence of differentiation in medulloblastoma might be a prognostic factor, together with age [11], location spreading stage [3] and

tissue features of malignancy [16].

We considered 43 surgically excised medulloblastomas, in which we studied GFAP as marker of glial differentiation [2] and NSE (neuron specific enolase) as marker of neuronal differentiation [18].

Material and methods

Of the 180 medulloblastomas of our collection 43 tumors in which cells of glial or neuronal aspect were present in routinely stained slides were chosen for study.

GFAP was demonstrated in paraffin sections, after fixation with Carnoy at 0-4°C or with 10% formalin, by the PAP method of Sternberger et al. (1970). Rabbit antiserum to GFAP was partly supplied by Dako, S.ta Barbara, California (PAP KIT for GFA, K 507) and partly generously provided by Dr. A. Bignami (Boston,

Mass.). The latter was diluted 1:500. Substrates for peroxidase were 0.01%DAB (diaminobenzidine tetrahydrochloride) and AEC (aminocethylcarbazole), by Dako (see above).

NSE was demonstrated in formalin or Carnoy fixed material, which was postfixed in a mixture of 4% formalin and 1% glutaraldehyde in aqueous saturated solution of picric acid, at pH 6. Both PAP method and ABC technique of Hsu et al. (1980) were employed. The NSE antiserum, generously provided by Dr. P.J. Marangos, Bethesda, Md, was diluted 1:1000.

Sections were counterstained with hematoxylin, dehydrated and mounted in balsam or glycerin.

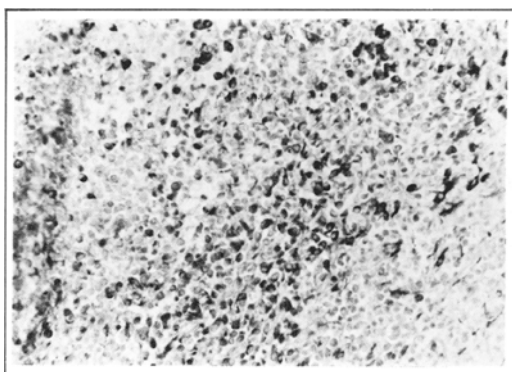
The tumors were studied also by Bodian silver impregnation and other histological methods (PTAH, toluidine blue, cresyl violet, Gomori for reticulin).

Results

1) GFAP. In most tumors, cells with processes, resembling reactive astrocytes, localized in the peripheral parts, were strongly positive (Fig. 1). These cells were particularly evident in islets of cerebellar tissue included in the tumor. In 3 cases positive cells with less evident processes were found inside the tumoral lobules. Positive cells were never found in the meningeal growths (desmoplastic tumors). In 2 cases clusters of cells lacking evident processes and with a positive cytoplasmic ring were found; they were morphologically indistinguishable from the tumor cells (Fig. 2). These elements were mainly localized close to the fourth ventricle (Fig. 3). 2) NSE. A positive reaction was found only in 2 cases. It was evident in differentiated neurons with large cytoplasm and long processes, and in smaller cells with vesicular nucleus and evident

nucleolus (Fig. 4). One of these cases showed circumscribed, diffusely positive areas crowded with neurons (Fig. 5). The same picture was observed in islets of cerebellar tissue included in the tumor. Bodian impregnation was strongly positive in these cases. With the latter methods positive cells, not evidenced by NSE, were observed in other cases.

Fig. 2. Many cells, indistinguishable from tumor cells, showing positive cytoplasm, GFAP-PAP, 250 \times .



Discussion

In our cases we confirmed immunohistochemically the occurrence of glial and neuronal cells in medulloblastoma. In many instances they are undoubtedly preexisting cells included in the tumor. By electron microscopy, against negative observations [34, 19], others have pointed out the occurrence of glial cells and neuronal cells [4]. According to the same authors, patients with tumors differentiated in the astrocytic direction

Fig. 1. Positive astrocytes in a peripheral area of the tumor, GFAP-PAP, 250 \times .

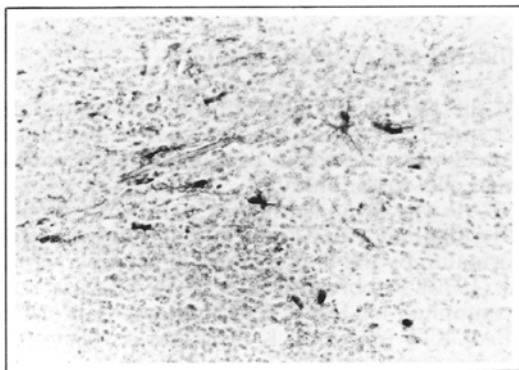


Fig. 3. The same as in Fig. 2. The cells are located close to the ventricular wall, GFAP-PAP, 250 \times .

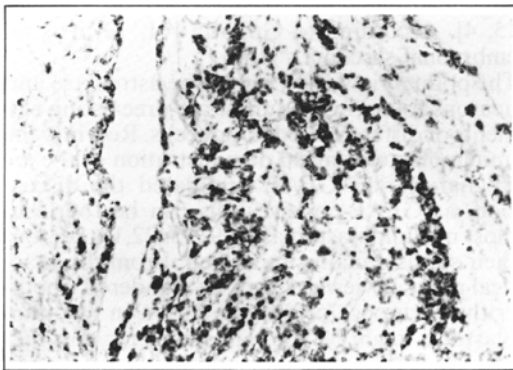


Fig. 4. Neurons and neuroblasts with positive cytoplasm, NSE-PAP, 400 \times .

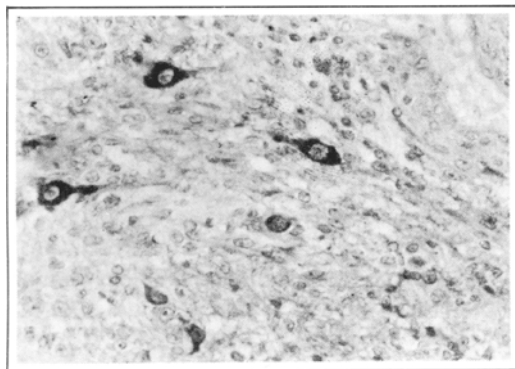
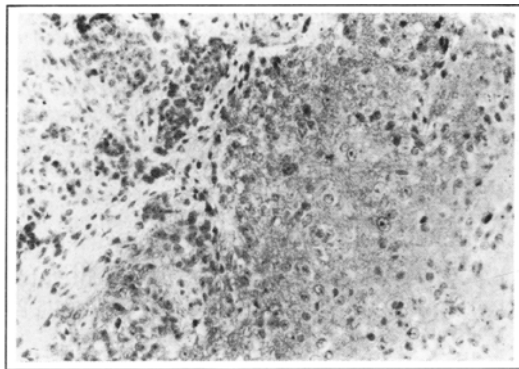


Fig. 5. Diffusely positive area with neuron resembling cells, NSE-PAP, 250 \times .



had a longer survival than those with undifferentiated tumors.

Even so, the suspicion that these cells are preexisting included cells cannot be removed. We found no GFAP-positive cells in the meningeal growths, as did Deck et al. (1978), Mannoji et al. (1981), and Palmer et al. (1981). According to Gullotta and Schindler (1982) even these cells however could be interpreted as preexisting elements.

It is, therefore, necessary to be very cautious about accepting GFAP-positive elements in medulloblastoma as tumoral cells.

However, in two cases of ours the aspect of positive cells was clearly different from that of reactive included astrocytes. They could be assimilated to the type 1 cells of Mannoji et al. (1981). The problem is whether these cells are still preexisting astrocytes, or differentiated tumoral cells or non astroglial GFAP-positive cells. In this regard two remarks are in order: the cases containing such cells are few, i.e. 2 out of 43 for us and 3 out of 25 for Mannoji et al. (1981). In our cases the areas containing such cells were located near the fourth ventricle; therefore, the positive cells might be "transitional" cells of the subependymal layer showing characteristics resembling those of ependymocytes [30]. They might have been included in the progressing tumoral growth. Their GFAP-positivity depends on their nature, either astrocytic or ependymal. In this regard, in fact, the frequent positive reaction in ependymomas [6, 8, 33, 26] and subependymomas [32, 5, 26] must be recalled.

The problem of neuronal differentiation is even more complicated. Our findings demonstrate, on the one hand, that included neurons may occur and be revealed by the NSE technique

and, on the other hand, that in two cases these neurons or neuroblastic cells are so densely packed together that their preexisting nature can be excluded. In no part of a cerebellar lamella do neuronal cells morphologically so different from granule cells reach such a density. Our impression is that the identification of neuronal cells might be more frequent, if the demonstration of the neuronal nature of a cell were easier. In the rat and monkey NSE characterizes completely differentiated neurons [18]. As a matter of fact, the relationship between NSE and differentiation is demonstrated by its higher concentration in ganglioneuroblastoma than in neuroblastoma [21]. Neurons not attaining a certain stage of differentiation may thus be missed. To these limiting factors it must be added that the reliability of immunohistochemical demonstration of NSE in human tumoral material, routinely processed for histology, is modest and needs further investigation. Biochemical evaluation of NSE in medulloblastomas yielded negative results [18].

The existence of neuronal differentiation in medulloblastoma opens up the problem of the occurrence of a cerebellar neuroblastoma [26]. In the literature undifferentiated cerebellar tumors with areas of neuronal differentiation have been reported as medulloblastoma or neuroblastoma [10, 28, 14, 35].

The problem has been resolved by Pearl and Takei (1981) by considering cerebellar neuroblastoma as one form of medulloblastoma. To date the number of cases with these characteristics is too small for the biological behavior of this tumor group to be ascertained. It remains to be seen whether this morphologic aspect represents a prognostic factor.

Sommario

Gli Autori affrontano il problema della differenziazione del medulloblastoma. Allo scopo sono stati studiati medulloblastomi in cui all'esame istologico routinario risultavano presenti elementi di aspetto gliale e neuronale. Lo studio è stato condotto mediante la dimostrazione immunoistochimica della proteina acida gliofibrillare (GFAP) e dell'enolasi specifica neuronale (NSE). Nella maggior parte dei casi gli elementi GFAP-positivi sono astrociti preesistenti inclusi; in due casi invece corrispondono ad elementi "di passaggio" dello strato subependimale. La NSE è dimostrata positiva in aree contenenti cellule di aspetto neuroblastico addensate. Il rapporto fra il medulloblastoma a differenziazione neuronale ed il neuroblastoma in fossa posteriore è discusso.

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