Nucleolar Organizer Regions in Meningeal Tumors

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Abstract : Tissue markers of cellular proliferation have been recently utilized as prognostic indicators in brain tumors. Nucleolar organizer regions (NORs) are loops of deoxyribonucleic acid (DNA) that transcribe to ribosomal ribonucleic acid (RNA) by RNA polymerase I. Using a silver staining technique, nucleolar organizer region-associated proteins (AgNORs) have been studied in paraffin sections of 62 meningeal tumors. The mean AgNOR number was 3.4 0.5 for typical meningioma, 5.6 1.4 for atypical meningioma and 9.2 3.4 for malignant meningeal tumors. A highly significant difference was found between the number of AgNORs in the nuclei of typical meningioma and those of atypical meningioma ($p<0.001$). It is suggested that this staining method, may be useful as an independent indicator of biological behavior in meningeal tumors.

Key Words : Argyrophilic nucleolar organizer region, Atypical meningioma, Meningeal tumors

INTRODUCTION

The biologic behavior of meningiomas is not always predictable from the histologic appearance of the tumors (2,13). This issue is of particular importance with regard to the treatment. In this regard tissue markers of cellular proliferation gained popularity in recent years (3,4,6,7,8,9,11,12,14).

NORs are loops of DNA which occur in the nucleoli of cells and possess RRNA genes. These genes correspond to portions of the acrocentric human chromosomes 13, 14, 15, 21 and 22 (1,5,16,17). An argyrophilic technique which was modified by Ploton, et al was readily applicable to paraffin sections and the AgNORs per nuclei were enumerated with ease (10). NOR numbers appear to indicate cell and nuclear activity (1,5,16,17).

MATERIALS AND METHODS

Tissues

Sixty two surgical specimens excised between 1986-1991 which had been routinely fixed in 10% formalin and processed for embedding in paraffin wax, were examined. The World Health Organization's definition for meningeal tumor was used (18). The tumor was categorized as a benign meningioma, an atypical meningioma or a malignant meningeal tumor. Benign tumors were not subdivided into histological subgroups. Malignant tumors of the meninges and related tissues included one hemangiopericytoma, one papillary meningioma, one meningeal sarcoma and one meningeal sarcomatosis. Recurrent meningiomas were not excepted as a separate group, because some of the primary tumor sections were not available and, the group was very small for a vigorous conclusion.

Nucleolar Organizer Region Staining

Deparaffinized sections of 3-4 micron in thickness, were dehydrated through an ethanol series to distilled water. The sections were then postfixed in a 3:1 ethanol-acetic acid mixture for 10 minutes and then rehydrated. The tissue sections were reacted with a solution of two parts of 50% aqueous silver nitrate solution and one part of gelatin dissolved in 1% aqueous formic acid at a concentration of 2%. The reaction was continued for 30 minutes at humidified room temperature in the dark. The sections were then washed out with distilled water, dehydrated and mounted. No counterstain was used.

Cell Counting

AgNORs were counted using a x100 oil-immersion lens in the preselected region of the tumor. In each case, 100 nuclei that contained argyrophilic staining were counted. Counting procedure was done by focusing up and down in the planes of section. The total AgNOR count was obtained by enumeration of both intra- and extra-nucleolar AgNOR dots (5). AgNORs were counted without knowledge of the histological diagnosis by one of the authors (FS).

Difference between two group means is used for the statistical analysis (15).

RESULTS

Thirty seven of the 62 cases were found to be histologically benign meningiomas, two of which were recurrent. This group of patients included 26 women and 11 men, with an average age of 47.4 years. The mean number of AgNORs per nucleus was 3.4 0.5 with a range from 2.75 to 5.04 (Table-1 and Fig. 1.A and B).

Table 1 : The Mean Number of AgNORs per Cell in Various Histologic Types of Meningeal Tumors

Group	n	Mean	S.D.	Minimum	Maximum
Typical	37	3.4	0.5	2.7	5.0
Atypical	21	5.6	1.4	3.1	14.9
Malignant	4	9.2	3.4	6.6	14.9

Twenty one cases were atypical meningiomas, 3 of which were recurrent. These patients included 10 women and 11 men, with an average age of 45.7 years. %we mean AgNORs per nucleus was 5.6 1.4, with a range of 3.07 to 10.38 (Table-1 and Fig. 2.A and B).

Four cases were malignant meningeal tumors, one of which were recurrent. These patients included 1 women and 3 men, with an average age of 48.2 years. The mean AgNORs per nucleus was 9.2 3.4 with a range of 6.62 to 14.86 (Table-1 and Fig.3.A and B). This group included one hemangiopericytoma, one papillary meningioma, one sarcomatous meningioma and one meningeal sarcomatosis.

A scatterplot of AgNOR number per nucleus values versus meningioma histology is seen in Fig.4.

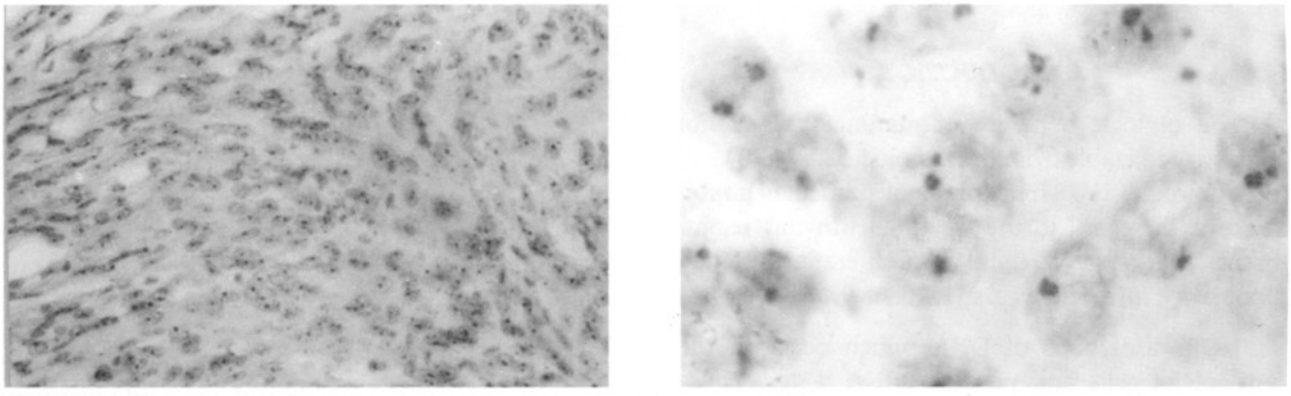

Fig. 1 : Photomicrographs of a benign meningioma. Note 2-3 small dots. AgNOR Stain. x200 (A). x1000 (B).

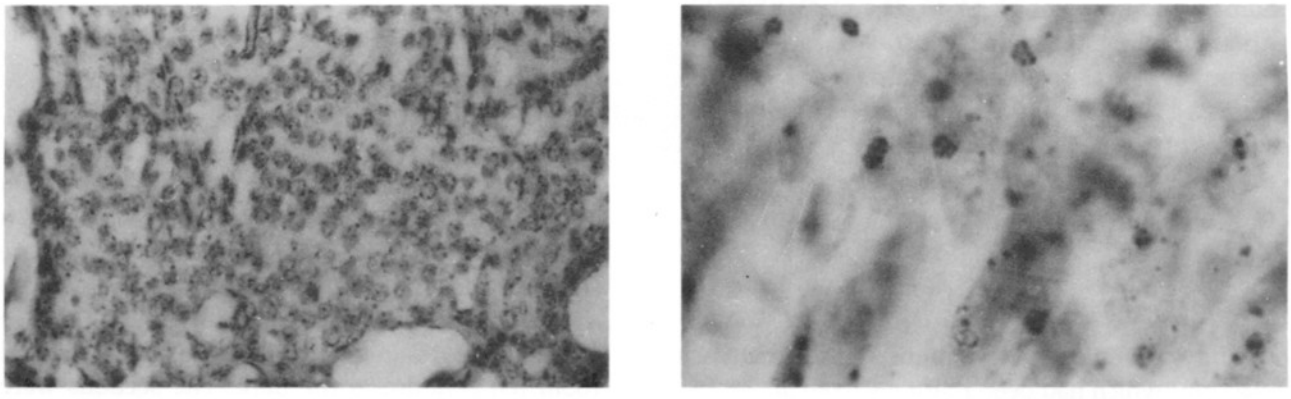

Fig. 2 : Sections from an atypical meningioma. Note the increased number of dots. AgNOR Stain. x200 (A). x1000 (B).

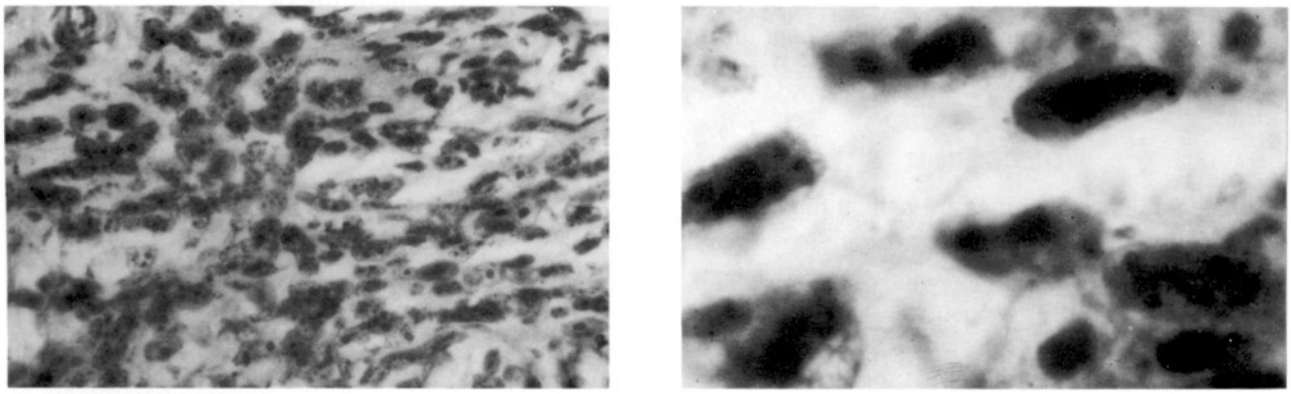

Fig. 3 : Photomicrographs of a highly cellular malignant meningeal tumor. Note the increased no. of AgNOR dots, as well as presence of atypical nucleoli. AgNOR Stain, x200 (A), x1000 (B).

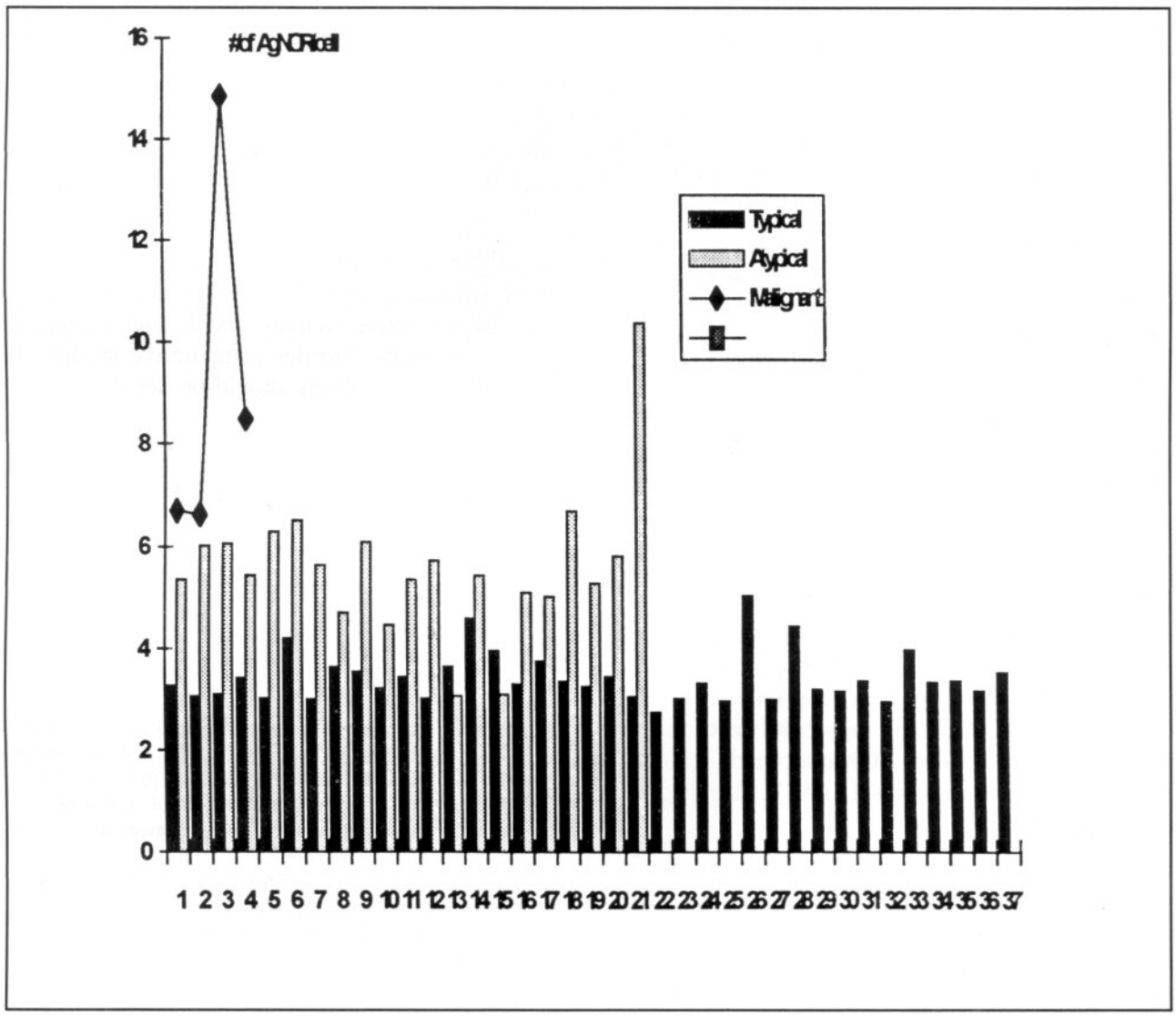

Fig. 4 : Scattergram of AgNOR numbers in histologic types of meningeal tumors.

The differences in the number of AgNORs between benign, atypical meningiomas were significant ($p<0.001$). Malignant group is small for the statistical analysis.

DISCUSSION

In general meningiomas are benign tumors with an excellent prognosis following complete resection (2,13). Though the most important predictor of recurrence is the extent of the surgical resection, some meningiomas have a worse clinical outcome with a higher recurrence rate and rapid progression in spite of total removal (2,13). Features such as degree of anaplasia, cellularity, mitotic rate, tumor necrosis, brain invasion and sheeting roughly correlate with the growth rate and frequency of recurrence (2). There is still no agreement on the histological criteria for predicting the aggressive biologic behavior of the tumor, though it is important to define the follow-up period of patients and to make a decision on adjunctive therapy such as radiotherapy.

The indices for the proliferative activity of tumors gained popularity in recent years. The labeling index for BUdR, Ki-67, DNA polymerase 'SYMBOL 97 f "Symbol"' or flow cytometric DNA analysis provide information on cell kinetics (4,6,7,9,11). Most of the above technique are expensive and necessitate the use of fresh tissue or image analysis systems. AgNOR method is applicable to paraffin sections without the need of image analysis systems and can be performed in any surgical pathology laboratory (1,5,10,16,17).

NORs are chromosomal segments in which rRNA is encoded. In human beings five acrocentric chromosomes bear NORs. Several proteins, such as RNA polymerase I, C23 protein, B23 protein, 100-kDa protein and 80-kDa protein are known to be associated with NORs.The argyrophil method for NORs, identifies those NOR-associated proteins rather than NORs themselves (1,5,16,17). This method is highly specific (1,5,16,17).

Though the exact significance of changes in the number and distribution of AgNORs is not fully understood, it has been decided that an increased number of AgNORs might reflect an increased rDNA transcription, and might indicate increased nucleolar and cellular activities (1,5,16,17). Therefore AgNORs have been utilized as an index for the proliferative activity of cells. There have been several reports that higher AgNOR count correlate with grade of the malignancy in various tumor types (3,8,12,14). And there is also correlation between AgNOR count and cell proliferation indices such as, BUdR labelling, Ki-67 and, DNA flow cytometer (6,9).

We found highly significant differences ($p<0.001$) between benign and atypical meningioma in terms of mean of AgNOR counts. The results of our AgNOR study were considered to reflect well the histological grading and possibly recurrence of meningeal tumors. The higher the AgNOR number, higher the histological grade. It has been previously shown that there is a close relationship between parameters of cellular proliferation and grade in meningioma and its related tumors, using a variety of methods for analysis of tumor cell kinetics (4,6,7,9,11). In our study we came to a conclusion that, when the AgNOR number was > 4.00, histological grade did not indicate a benign nature. In such cases, adjunctive therapy after surgery, such as radiotherapy may be considered.

In summary, AgNOR technique seems to be a useful method for evaluation of proliferative activity of various tumors. It is cheap, simple and applicable to conventionally fixed and processed paraffin sections. Further comparative studies of AgNOR in brain tumors should be done.

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