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Evaluation of argyrophilic nucleolar organizer region staining in predicting the behavior of meningiomas

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BACKGROUND: The number of argyrophilic nucleolar organizer regions (AgNOR) correlates with cellular proliferative activity. Comparing non-recurrent, recurrent, atypical and malignant meningiomas we studied the value of the routine applicability of the AgNOR count in the prognostication of this tumor.

PATIENTS AND METHODS: Two hundred and thirty-eight meningiomas were reviewed blindly and graded using WHO grading schema. Eighty-one cases were selected and arranged in six groups according to clinical data and grading: 14 benign non-recurrent meningiomas; 14 primary benign recurrent meningiomas and their subsequent benign recurrences; 14 atypical; 11 malignant and 14 spinal meningiomas. Silver-stained slides were prepared and mean, median and standard deviation of AgNOR dots determined.

RESULTS: There was a proportionate increase of AgNOR dots with increasing tumor grade. There was a significant difference between benign non-recurrent tumors versus benign recurrent (P<0.0001) and atypical or malignant (P<0.0001) tumors. A difference was also noted between the recurring tumors versus malignant ones (P=0.002) but no significant difference was seen between recurrent and atypical; atypical and malignant; intracranial and intraspinal; and primary of recurring meningiomas with their subsequent recurrences. A mean AgNOR count of <2.3 could separate benign tumors from atypical or malignant meningiomas with 93% specificity; and 93% of tumors with benign histology had no recurrence potential if their mean AgNOR count was less than 1.8.

CONCLUSION: This study indicates that in meningioma, the AgNOR count has a good correlation with tumor grading and recurrence, which may aid pathologists and clinicians in predicting tumor behavior.

Meningiomas are the most frequently encountered primary nonglial tumors of the central nervous system. They are benign in most instances and may be cured with gross total resection; however, approximately 9% to 22% of patients experience recurrence depending on tumor location, vascular or neural extension, the extent of surgical resection and the inherent proliferative potential of the primary tumor. Rarely, they are frankly malignant, leading to metastasis. The correlation between histologic appearance and clinical behavior of meningiomas has remained imprecise since a histologically benign tumor may recur following apparently gross total resection. Evaluation of proliferative indices may help conventional histology in the prognostication of recurrence, prediction of outcome and malignant potential of meningiomas. The concept of rapidly growing benign
meningioma was introduced in 1997. These tumors are histologically indistinguishable from benign non-recurrent tumors, but have higher proliferative cell indices as determined by various methods of cell kinetic studies, including BUDR, PCNA, Ki-67, flow cytometric DNA analysis, and nuclear argyrophilic nucleolar organizer region (AgNOR) quantitation. These studies are important because their results may enhance adjunctive radiotherapy following surgery. Nucleolar organizer regions (NORs), which have been recognized for more than a decade, are chromosomal segments that contain ribosomal genes and a set of acidic non-histone proteins that become visualized by silver stains by selective binding to silver ions. The AgNOR protein quantity represents a valuable parameter in cell kinetics, being significantly associated with the rapidity of cell duplication. The aims of this study were 1) to evaluate the relationship between grading of meningiomas and the AgNOR count, particularly regarding discrimination of benign and malignant tumors, 2) to determine the predictive value of the AgNOR count for anticipation of tumor recurrence, and 3) to compare the proliferative potentials of intraspinal and intracranial meningiomas.

Table 1. The age and sex of patients within each category of meningioma.

| Category No. | Category definition                        | Mean age (years) | Age range (years) | Male | Female | Total |
|--------------|-------------------------------------------|------------------|-------------------|------|--------|-------|
| 1            | Benign non-recurrent intracranial meningiomas | 52.0             | 39-68             | 4    | 10     | 14    |
| 2            | Benign recurrent intracranial meningiomas* | 41.1             | 16-56             | 6    | 8      | 14    |
| 3            | Recurrent cases of group 2 **             | 47.9             | 22-55             | 6    | 8      | 14    |
| 4            | Atypical intracranial meningiomas         | 41.4             | 13-72             | 10   | 4      | 14    |
| 5            | Malignant intracranial meningiomas        | 51.3             | 33-65             | 9    | 2      | 11    |
| 6            | Spinal meningiomas                        | 49.1             | 16-76             | 7    | 7      | 14    |
| -            | Total                                     | 47.0             | 13-76             | 42   | 39     | 81    |

* Primary of histologically benign tumors that recurred in the study period following gross total resection
**All were histologically benign and recurred during the 6 years after resection of the primary tumor

Patients and methods
We selected 238 meningioma specimens excised between 1997 and 2002 in Sina hospital, Tehran. All had been routinely fixed in 10% buffered formalin and processed for embedding in paraffin wax. All of the slides were reviewed and scored according to the latest WHO classification (2000). Meningeal hemangiopericytomas were excluded. Age, sex, extent of surgical resection and history of recurrence until the end of 2003 were recorded. Cases with incomplete data or a questionable total resection were excluded. Among 238 evaluated meningiomas, 81 cases were selected and divided into 6 categories according to predefined criteria (Table 1). Fourteen atypical and 11 malignant meningiomas matched the criteria. In other groups, cases were randomly selected if the total number exceeded 14.

Deparaffinized sections were hydrated through an ethanol series to distilled water. The sections were covered for 30 minutes at room temperature in a dark place with a freshly prepared solution consisting of 1 volume of 1% aqueous formic acid containing 2% (W/V) gelatin and of 2 volumes of 50% aqueous silver nitrate. The slides were rinsed three times with deionized distilled water, differentiated in 5% sodium thiosulfate, mounted routinely and examined under light microscopy. Silver-stained nucleolar organizer regions were counted blindly in 200 cells in each slide under oil immersion at x1000 magnification in at least 4 HPFs and the mean, median and standard deviation of counts in each slide was determined. In heterogeneous tumors, as in some atypical meningiomas, counting was performed in those tumor areas that showed the highest degree of atypia, although counts from less atypical areas did not significantly differ when tumors were examined for this purpose. In all cases, counting was performed independently by both authors. Interobserver variation was less than 5%. The mean of two counts was used in the statistical analysis, which was a nonparametric analysis...
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(Kruskal-Wallis test). Comparison between groups was carried out by the Mann-Whitney test (calculated U). All statistical analyses were performed on SPSS 11.5 software.

Results

In all specimens, clearly defined silver-stained dots were observed in 200 tumor cell nuclei (Figure 1). The variable-sized AgNOR dots were dispersed throughout the nucleus. In benign non-recurrent tumors most nuclei contained only one large AgNOR dot. In other groups the number of dots per nuclei increased in number but decreased in size as tumor grade became higher. The mean AgNOR number and 95% confidence interval were 1.37 (0.30) for benign non-recurrent tumors; 1.87 (0.38) for recurrent meningiomas; 2.01 (0.36) for atypical meningiomas, 2.39 (0.74) for malignant meningiomas and 1.24 (0.14) for spinal tumors, and 1.63 (0.31) for the recurrent cases of the second group (Figure 2).

There was a proportionate increase of AgNOR count from benign to atypical to malignant. Although there was a significant difference between benign versus atypical (U=19.5, P<0.0001) and benign versus malignant (U=9, P<0.0001), no meaningful difference could be shown between atypical and malignant forms. AgNOR counts were significantly higher in benign recurrent tumors compared to non-recurrent meningiomas (U=23, P<0.0001). This difference was also noted between the recurring tumors versus malignant ones (U=23, P=0.002) but no significant difference was seen between recurrent versus atypical, intracranial versus intraspinal and primary tumors of recurring meningiomas with their subsequent recurrences.

Figure 1. Photomicrograph of AgNOR staining (×1000): a) benign non-recurrent meningioma: most nuclei contain only one dot. b) cells contain more than one dot. d) malignant meningioma (with small cell morphology): many nuclei contain more than one dot.
**Discussion**

Although AgNOR scoring is one of the earliest methods used and suffers from some technical variations, it may still be of value in tumor pathology. Ki-67, PCNA and BUDR represent the growth fraction, but actual proliferative activity is affected not only by the growth fraction but also by cell cycle duration. It is reported that AgNOR quantity can be a marker of cell cycle duration.\(^\text{12,13}\) Moreover, as a necessary adjunct to histology, AgNOR may still be the simplest and most inexpensive method applicable to paraffin sections even in small regional laboratories in developing countries. As previously described by Maier\(^\text{1}\) and Capelle\(^\text{14}\) we found that the AgNOR count in meningioma correlates with tumor grade, although with some overlap between groups, particularly between atypical and malignant tumors (Figure 2). The latter finding may be due to a high proliferative index in both tumors.

Using the Receiver Operating Characteristic (ROC) curve and calculation of the area under curve with 95% confidence interval, the cut-off values for discrimination between benign versus malignant and atypical meningiomas and also for recurring versus non-recurring meningiomas were determined. Using the above mentioned method and setting a cut-off value of 2.3 for the AgNOR count, benign meningiomas can be distinguished from atypical or malignant ones with a sensitivity, specificity, positive predictive value (PPV) and Youden index of 32%, 93%, 67% and 0.25, respectively. We also found that the presence of nuclei with AgNOR dots >6 may separate benign meningiomas from atypical or malignant ones with a sensitivity, specificity, PPV and Youden index of 45%, 99%, 92% and 0.44, respectively. In our study, which was similar to that of Capelle\(^\text{14}\) and Scarpelli,\(^\text{15}\) a significant positive relation was noted between the AgNOR count and recurrence potential; however; Orita\(^\text{6}\) could not show this correlation, probably due to unequal sample size. In an attempt to find an objective criteria to estimate the recurrence potential of histologically benign meningiomas (groups 1 and 2), a cut-off value of 1.8 was established for the AgNOR mean. The recurrence potential of benign meningioma after gross total resection was estimated as having a sensitivity, specificity, PPV and Youden index of 60%, 93%, 82% and 0.53, respectively. It could also be noted that benign non-recurring meningiomas most often have no more than 4 AgNOR dots (sensitivity 80%; specificity 88%; ppv 92% ; Youden index 0.68).

Our study was also similar to that of De Stefano\(^\text{16}\) and Maier,\(^\text{4}\) in which no difference was identified between the primary of recurrent tumors and their recurrences, possibly indicating their recurrence potential from the outset. The mean AgNOR of benign recurring meningiomas showed a significant difference from malignant ones, but was not different from atypical meningiomas. This is similar to the study of Kudoh,\(^\text{5}\) using other proliferative indices, and probably points to the close relationship between tumor proliferative indices and clinical behavior that is not necessarily compatible with histologic appearance. Spinal meningiomas, as shown in this study, are mostly benign, bearing low proliferative indices; however; unlike Arumi,\(^\text{17}\) we found no difference between benign cranial and spinal tumors. Further studies may be required to validate these results, selecting more cases, longer follow-up periods and using multiple proliferative markers.

This study suggests that evaluation of cell proliferation markers such as AgNOR, integrated with standard histopathology can provide for better histopathologic grading of meningiomas and at the same time can help to predict the clinical behavior of a tumor regardless of its benign histologic appearance.
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