DIAGNOSTIC RELEVANCE OF AgNOR IN OVARIAN SEROUS BORDERLINE TUMORS

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Abstract–Nucleolar organizer regions are loops of nuclear DNA related to ribosomal DNA, and their number in cells is believed to indicate cellular proliferative activity. Using a silver staining method to visualize these structures in paraffin embedded tissue sections, an attempt was made to determine if these AgNOR counts could be used as a diagnostic tool for serous borderline tumors of the ovary. Ten cases in each group of benign, borderline and malignant serous ovarian tumors were selected and the mean number of AgNOR was determined in all cases. A progressive increase in mean AgNOR values was noted from the benign group towards the borderline group and further to the carcinoma group; the differences between the means in each group were statistically significant. However, there was a high degree of overlap between the values of the borderline and malignant groups. These results indicate that AgNOR counts may not be very useful as a diagnostic tool in an individual case.

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Key words: ovarian tumor; nucleolar organizer regions; ovarian serous tumors; borderline tumors

INTRODUCTION

Nucleolar organizer regions (NOR) are loops of DNA which transcribe to ribosomal RNA (1). Their genes are of central importance in the formation of ribosomes and cell proteins (2). In humans, they are located on five acrocentric chromosomes, Nos. 13, 14, 15, 21 and 22 (1).

They can be visualized as black dots by a silver staining method in paraffin-embedded tissue sections (2). This argyrophil NOR (AgNOR) technique is remarkably specific as a means of detection of NORs. As ultrastructural studies, experiments using fluorescent probes and labelled rRNA, and immunoblot analysis have confirmed this issue (3). AgNOR method has been useful in assessing proliferative activity in some tumors, and in discrimination between certain benign and malignant conditions (2). Several studies have shown that increasing AgNOR counts tend to reflect increasing malignancy of a given tumor (1,4). A large number of lesions have been studied by the AgNOR technique: a clear-cut difference has been shown between the AgNOR counts in nevocellular nevi and malignant melanomas, and between low-grade and high-grade lymphomas (4,5,6). Discrimination between ovarian "borderline" epithelial tumors and carcinomas is still a problem for the surgical pathologist. Established criteria indicate that the main difference between these two groups is the presence of stromal invasion in the latter (7). However, distinction of true stromal invasion from epithelial entrapment is also difficult or subjective (8). Furthermore, introducing a subgroup of "ovarian serous borderline tumors with stromal microinvasion" further complicates the problem (9). A number of modalities have been employed to characterize more objectively this group of tumors, that is, flow cytometric analysis of DNA content of tumoral cells (10), digital image analysis (11), and most recently AgNOR counting (12). In this study we attempted to evaluate the utility of AgNOR technique in differentiation of ovarian serous borderline tumors from carcinomas.

MATERIALS AND METHODS

All cases of ovarian borderline or malignant tumors of serous / mucinous / endometrioid type from 1982 to 1994 were retrieved from the files of the Department of Pathology at Cancer Institute and the Central Department

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of Pathology at Imam-Khomeini Hospital. The cases were then reevaluated using established criteria for diagnosis of ovarian tumors (13). Eventually ten cases of serous borderline tumors with adequate numbers of slides were found. Ten cases of benign serous cystadenomas and ten cases of serous carcinomas (grade one: 7 cases; grade two: 2 cases; grade three: 1 case) were also chosen for comparison. In each case, paraffin-embedded tissue blocks were cut in 4-mm thicknesses and stained by the AgNOR method as described by Coghill et al (2). The number of AgNOR in the nuclei of one-hundred neoplastic cells of the most active areas was counted in each case under oil immersion (× 100). The counting was done by two different methods in separate sessions. In the first method, the nucleolus and any closely aggregated dots were counted as one NOR; in the second method, all discernible dots, whether intranucleolar or not, were counted separately. The mean number of AgNOR counts in each case was calculated for both methods.

Analysis of variance was performed by Student's two-tailed t-test; Fisher's linear discriminant function was done to compare the groups. Calculations were performed by "SPSS/PC" software package on IBM-PC.

RESULTS

AgNOR counting results by both methods of counting are shown in Table 1. The mean values of NORs increased in accordance with the extent of nuclear atypia. Figures 1 and 2 display actual AgNOR numbers in scattergrams.

Statistical analysis showed that there is a significant difference in the mean number of AgNOR between the cystadenomas and borderline tumors ($p \sim 0.001$), and between the borderline and carcinoma groups ($p < 0.05$) by the first method of counting. Considering the second method, this difference between the borderline and carcinoma group ($p < 0.01$) was even more significant. However, discriminant analysis of the results of the first method (Table 2) revealed an unacceptably high degree of overlap between the second and third groups. Discriminant analysis of the results of the second method of counting (Table 3) was more encouraging, correctly classifying all members of the first two groups in their proper places. But, 40% of carcinomas were classified as borderline tumors by this method.

DISCUSSION

In this study we tried to examine the utility of AgNOR counting as a diagnostic tool for ovarian serous tumors. Our results show a progressive increase in the mean number of AgNORs from cystadenomas to borderline tumors and to carcinomas. These results are generally similar to those of Praised and Ray (12). Analysis of variance indicates that the differences between the means in these groups are statistically significant, more so by the second method of counting. This was not unexpected, as the proliferative activity of neoplastic cells increases from the benign group towards the "borderline" and further towards the carcinoma group. The counting method obviously has a significant effect on results. Other authors have used the first or the second method in their studies of ovarian serous tumors (14,12,8). We examined both methods and showed that counting all discernible AgNOR dots—although more difficult to perform and more tiring to the eyes—clearly gives superior results for differentiation of borderline and malignant serous tumors of the ovary. Our result confirms the results obtained by Crocker et al (13) and Prasad (12).

Although previous reports show significant results in differentiation of ovarian serous borderline tumors from carcinomas by AgNOR technique (8,12,14), they do not mention tumor grades in their carcinoma group cases.

Apparently, if the number of intermediate-and high-grade carcinomas in the study is high, the difference between mean AgNOR counts of the borderline and malignant groups would increase, with concomitant lesser degrees of overlapping in the results. This may explain why our results are not as satisfactory as those reported by others, since 7 of our 10 cases of carcinomas were grade I. The selection of more low-grade carcinomas was done deliberately because we believe that the main problem for the surgical pathologist lies in differentiating this very subgroup of carcinomas from borderline tumors. Apparently, most carcinomas of higher grades are obviously invasive, and easy to diagnose. Khattech et al (8) also pointed out that they found no difference in AgNOR numbers between borderline tumors and grade I carcinomas. Misclassification 4 of our 10 cases of malignant tumors as "borderline" tumors, (i.e., under diagnosis) by discriminant analysis of final results further supports the point.

In conclusion, despite these overall satisfactory results, the problem does not seem to be totally solved. When facing a certain case with an AgNOR mean number in the upper limits of borderline group (and overlapping with the lower limits of carcinoma group) the dilemma seems to persist. This should not be surprising, since some borderline tumors and low grade carcinomas may have identical histologic and cytologic appearances. Theoretically it can be argued that proliferative activity and invasive potential are different characteristics of tumoral cells that are not always of the same degree. This

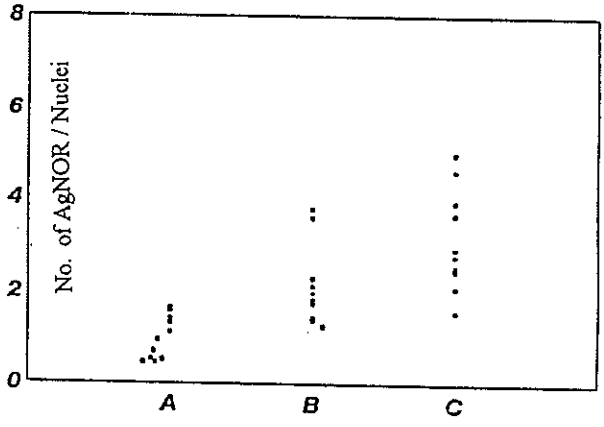


Fig. 1. Actual AgNOR counts of ovarian serous tumors, as performed by the first method of counting. A: Adenoma, B: Borderline, and C: Carcinoma.

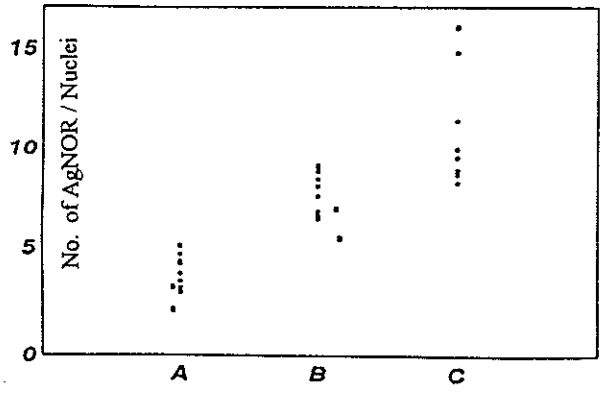


Fig. 2. AgNOR counts of ovarian serous tumors as performed by the second method of counting. A: Adenoma, B: Borderline, and C: Carcinoma.

Table 1. Average number of AgNOR counts in ovarian serous tumors.

Histology	Number of case	Mean (±SD)[a]	Range[a]	Mean (±SD)[b]	Range[b]
Serous cystadenomas	10	1.42±0.16	1.12-1.66	4.43±0.73	2.94-5.13
Serous borderline tumors	10	2.21±0.83	1.40-3.59	7.74±1.09	6.44-9.18
Serous carcinoma	10	3.53±1.79	1.57-5.00	11.17±3.23	8.06-16.06

[a]AgNOR counting by the first method.
[b]AgNOR counting by the second method.

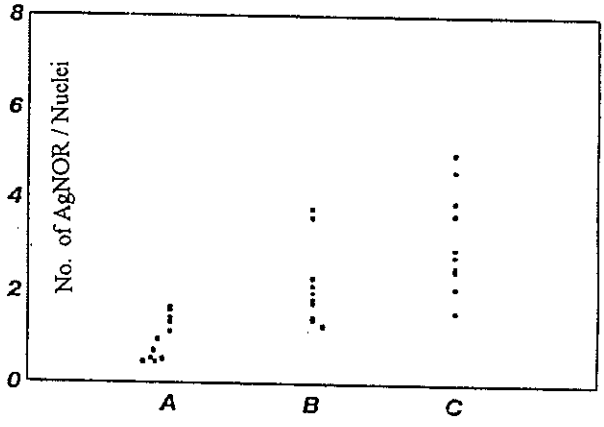
Table 2. Discriminant analysis of AgNOR counts in ovarian serous tumors as performed by the first method [Coghill et al (2)].

Type of tumor	Number of case	AD	BL	CA	Correct discrimination (%)
Adenoma	10	9	1	0	90
Borderline	10	5	3	2	30
Carcinoma	10	1	4	5	50

AD = Adenoma
BL = Borderline
CA = Carcinoma

Table 3. Discriminant analysis of AgNOR counts in ovarian serous tumors as performed by the second method. [Crocker J et al (3)].

Type of tumor	Number of case	AD	BL	CA	Correct discrimination (%)
Adenoma	10	10	0	0	100
Borderline	10	0	10	0	100
Carcinoma	0	0	4	6	60



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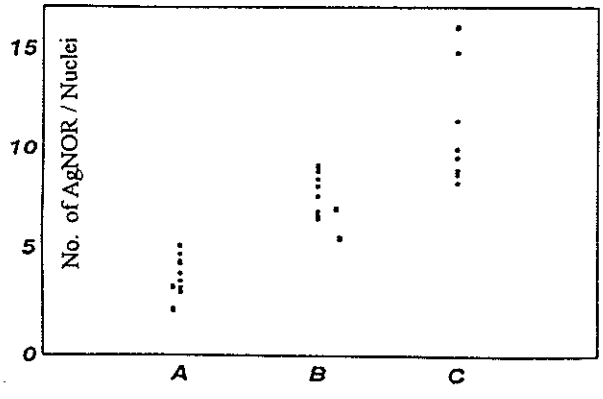

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may explain the discrepancy between certain tumors with very close mean AgNOR numbers but different biologic activity and invasion potential. Obviously, further large scale studies are needed to address this issue. However, the ideal method of differentiation of these two groups would be one that specifically detects the presence or absence of invasion potential in tumoral cells, and this is yet to be developed.

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