Evaluation of AgNOR Staining in Differentiation Between Borderline Mucinous and Serous Ovarian Tumors from Benign and Malignant Counterparts

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Abstract

Background: Differentiation between borderline serous and mucinous ovarian tumors from benign and malignant counterparts is occasionally difficult or impossible by the use of routine H & E histopathology staining. In this study differentiation capacity of Argyrophilic Nucleolar Organizer Region (AgNOR) staining was evaluated concerning these tumors.

Material and Method: For this purpose two AgNOR counts were used. The first was mAgNOR (mean number of AgNOR per nucleus) that reflects ploidy and the second pAgNOR (percentage of nuclei with greater than or equal to five AgNOR per nucleus) that correlates with proliferative activity. Forty-six serous ovarian tumors (15 benign, 13 borderline and 18 malignant lesions) and 36 mucinous ovarian tumors (15 benign, 12 borderline and 9 malignant lesions) were selected and the AgNOR and H & E staining carried out. For each tumor 100 cells were randomly examined for nuclear AgNORs.

Results: A stepwise significant increase in pAgNORS and mAgNORS from benign to borderline and malignant serous and mucinous ovarian tumors were found (P<0.001) except for two overlaps between pAgNORS of benign and borderline mucinous tumors.

Conclusion: This study concluded that the AgNOR counting is a valuable diagnostic criterion for differentiation between borderline serous and mucinous ovarian tumors from benign and malignant counterparts.

Key words: AgNOR, ovarian cancer, ovarian cystadenoma, borderline tumor

Introduction

The surface epithelial tumors account for approximately two-third of all ovarian neoplasms, and their malignant forms account for 80-90% of ovarian carcinomas. Borderline neoplasms represent an important category of ovarian common epithelial tumors usually associated with an excellent prognosis but, rarely with more aggressive and unpredictable behavior characterized by intraperitoneal seeding and frank malignant transformation.

In contrast, the ovarian cancers have very aggressive behavior and the highest fatality-to-case ratio in gynecologic malignancies.

The main criterion for differentiating ovarian cystadenocarcinomas from borderline tumors is based on stromal invasion. This criterion however can be difficult to appropriate and differential diagnosis is not always easy to establish.
Nucleolar Organizer Regions (NORs) are loops of DNA those are responsible for ribosomal RNA transcription. AgNORs are acidic proteins associated to the NORs which are selectively stained by silver colloid technique. A series of studies indicate that the quantity of AgNORs is related to the rapidity of cell proliferation, and even prognosis of tumors.

In the present study, the AgNORs method was applied to paraffin sections in a series of mucinous and serous ovarian tumors to facilitate the differential diagnosis between borderline tumors and benign and malignant counterparts. For this purpose, two AgNOR counting methods have been evaluated: 1- the mean number of AgNORs per nucleus (mAgNOR), which has been associated with ploidy and, 2- the percentage of nuclei with five or more AgNORs per nucleus (pAgNOR), which has been correlated with proliferative activity of tumors.

**Material and Method**

Formalin fixed paraffin-embedded tissue specimens from 27 ovarian carcinomas (18 serous, 9 mucinous), 25 ovarian borderline tumors, (13 serous, 12 mucinous) and 30 ovarian benign tumors (15 serous, 15 mucinous) were studied. The samples were diagnosed on the basis of hematoxyline and cosin (H & E) stains by two independent observers. Routine sections (4µm) were taken, deparaffinized in xylene and dehydrated through graded ethanol series to deionized water, then they were incubated with one part of 2% gelatine in a 1% aqueous formic acid solution mixed with 2 parts of 50% aqueous silver nitrate solution for 50 minutes at room temperature in the dark. The section were thoroughly washed in deionized water and immersed in 5% solution of sodium thiosulfate for five minutes to remove artifactual silver granules and provide permanent preparations. After washing in water, the section were dehydrated in ethanol series and cleared in xylene and then mounted. All of the solution were prepared with deionized water.

In each case, 100 epithelial cells, selected randomly, were examined with an x 100 objective lens. All of the single AgNORs and individuals AgNORs within clumps were counted, but AgNORs doublets or clusters impossible to discern by light microscopy, were considered as a single AgNOR grain.

Differences in mAgNOR and pAgNOR values between benign, borderline and malignant serous and mucinous tumor groups were assessed individually by one way analysis of variance (ANOVA). Tukey’s HSD analysis was also done if the differences were statistically significant.

**Results**

The epithelial cell areas in tissue sections were evaluated. In benign lesions the number of AgNORs per nucleus was low, increased in borderline tumors and highest in adenocarcinoma (figure 1-6). Also the irregular shaped AgNORs and clusters were mostly seen in the latter. The average of mAgNORs and pAgNORs in each group of tumors are summarized in table 1 and 2. A stepwise significant increase in pAgNORs and mAgNORs from benign to borderline and malignant serous and mucinous ovarian tumors was found (P<0.001).

There was no overlap between the groups, except for pAgNORs of two benign and borderline mucinous tumors.

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1- Serous Cystadenoma  
2- Borderline Serous Tumors  
3- Invasive Serous Cystadenocarcinoma

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<th>Table 2: mAgNOR and pAgNOR in Mucinous Ovarian Tumors</th>
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1- Mucinous Cystadenoma  
2- Borderline Mucinous tumors  
3- Invasive mucinous cystadenocarcinoma
Discussion

AgNORs counts have been reported to assist in the distinction between benign and malignant lesions\(^4,9,10,16,28\). Also, they have been correlated to grade, stage and even prognosis of several tumors\(^11,12,13,18\). There are however, a number of reports questioning the usefulness of AgNOR count. For the ovarian tumors, in few studies, it was shown that the mean AgNOR count differentiated between benign and malignant ovarian tumors and cystadeno-
carcinomas of the ovary can be well differentiated by the AgNORs counting procedure\(^9,22\).

We think that some of these differences in results are due to variation and problems inherent in the techniques and most importantly to differences in AgNOR counting methods.

In this study, we found that mAgNORs and pAgNORs demonstrated a progressive increase from adenoma to borderline tumors, and to carcinomas. These data were statistically different when assessed.
by analysis of variance. There was no overlap between the tumor groups except two, in pAgNORs between benign and borderline mucinous tumors. It is concluded that the AgNOR counting is a valuable criterion of differentiation between borderline serous and mucinous ovarian tumors from benign and malignant counterparts. Standardization of AgNOR staining and AgNOR counting by use of the most reliable method and the method which has achieved the best results, may imply a more widespread use of this staining for resolving some pathologic problems.

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References


