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Nucleolar Organizer Regions in Stromal Tumors of the Stomach

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ABSTRACT

The number and the largest particle size of the nucleolar organizer regions (NORs) in 18 stromal tumors of the stomach were counted and measured, respectively, using the one-step silver staining technique (AgNOR) with an image analysis system. The purpose of this study was to evaluate the possible contribution and feasibility of this simple method as a useful adjunct in the diagnosis of malignancy, in view of the difficulty encountered in distinguishing between benign and malignant gastric stromal cell tumors.

The mean number of AgNOR in 9 benign tumors was 2.41 (SD 0.96) and 8.71 (SD 2.72) in 9 malignant ones, without any overlap between the two ranges. The mean size of the largest AgNOR particle was 1.61 μm^2 (SD 0.68) in the benign lesions and 2.55 μm^2 (SD 1.02) in the malignant lesions, but with some overlapping between the two ranges. There was no correlation found between the number and size of the AgNOR particles.

Therefore, it is suggested that the number of AgNOR particles could be used as an additional parameter in distinguishing malignancy from benignancy. In contrast, the size of the largest AgNOR particle does not appear to be so useful in distinguishing malignant tumors from benign tumors, due to considerable overlapping of size in these two different conditions.

Key words: nucleolar organizer regions, stromal tumor, stomach

Nucleolar organizer regions (NORs) are structures consisting of clusters of ribosomal DNA (rDNA) encoding for ribosomal RNA (rRNA) production. NORs can be demonstrated by the binding of their associated proteins to silver (Ag+) ions[1].The major silver staining protein is C23 protein (nucleolin)[2], which probably controls rDNA transcription[3]. In humans, the presence of NORs on the short arm of the five acrocentric chromosome pairs has been utilized by cytogeneticists for more than a decade for the evaluation of chromosomal aberrations. This

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silver-binding reaction has been recently applied in histopathology[4]. Much interest has been shown in the diagnostic[5-9] and prognostic[10-13] value of demonstrating NORs in tissue specimens, and studies have been performed on nearly every system of the body.

A close correlation between the proliferative behavior and the quantitative change in AgNORs has been found in many tumor types. As a result, we measured both the number of NOR and the NOR size in cases of gastric stromal (mostly smooth muscle) tumor to determine their usefulness as additional parameters in distinguishing between benign and malignant tumors, since distinction histologically is difficult regardless of the tumor's microscopic pattern.

MATERIALS AND METHODS

1. Specimens

Nine cases each of benign and malignant gastric stromal tumors, taken from the routine files at Taipei Medical College Hospital, were examined. Epithelioid tumors were not included.

2. Staining for NORs

Sections were cut at a thickness of 3 μm from routinely processed paraffin blocks, and were taken to water via xylene and graded ethanols. The sections were then submitted to the AgNOR procedure[1,14] at room temperature for 30 minutes. The AgNOR staining solution comprised 2% gelatin in 1% aqueous formic acid. This solution was then mixed in a proportion of 1:2 volumes with 50 aqueous silver nitrate under dark room conditions. After staining, the mixture was poured from the slides, and the slides were then washed with distilled water for 10 minutes and incubated in 5% sodium thiosulphate for a further 10 minutes,to avoid a nonspecific reaction by elimination of chelated silver ions. Counterstaining was not performed, and tne sections were dehydrated to xylene and mounted in synthetic medium.

3. Enumeration and Measurement

For each specimen, one hundred cells in randomly selected fields were examined,using a x100 oil immersion lens. The AgNORs were seen as dots within the nuclei of the cells. Individually discernible dots were outlined by an electronic cursor and the number and the size of the largest dot were counted and measured respectively by the microprocessor by means of an image analysis system (Flovel, JAPAN). The mean number and size of AgNORs per cell were then calculated.

Each specimen was examined for mitotic figures, and the number in 50 high-power fields (HPF) was counted.

RESULTS

In all the specimens examined, well-defined black silver-stained dots were observed in the nuclei and were arranged in one or more clusters, or occurred as individual satellites. The overall results, including the mitotic rates and tumor sizes, are summarized in Tables 1 and 2.

Numbers of NORs

The number of AgNORs in benign gastric stromal tumors were found to be less than the numbers in malignant lesions. The range of the mean AgNOR count per cell in the former was 1.40-3.81 (mean 2.41; SD 0.96) and 6.00-13.86 (mean 8.71; SD 2.72) for the latter (significance of difference $p < 0.001$ Student's t test).

Table I -AgNOR numbers and sizes and mitotic counts in benign gastric stromal tumors

Specimen number	AgNOR number	largest AgNOR size (μm^2	mitotic count /50HPF
1	3.81	0.72	2
2	1.40	1.54	2
3	1.84	2.84	0
4	2.20	1.50	0
5	3.43	1.79	0
6	3.73	0.64	1
7	1.66	2.25	0
8	1.67	1.63	0
9	1.99	1.56	0
Mean	2.41	1.61	
SD	0.96	0.68	
SEM	0.91	0.64	

Table II -AgNOR numbers and sizes and mitotic counts in maliganant gastric stromal tumors

Specimen number	AgNOR number	largest AgNOR size (μm^2	mitotic count /50HPF
1	13.86	1.67	7
2	6.00	3.53	6
3	8.66	1.83	11
4	6.45	3.23	12
5	8.16	0.98	7
6	8.59	2.11	21
7	7.23	2.53	9
8	6.92	4.27	15
9	12.51	2.78	29
Mean	8.71	2.55	
SD	2.72	1.02	
SEM	2.56	0.97	

Size of NORs

The largest AgNOR particles in cells of benign gastric stromal tumors were smaller than those in the malignant tumors. The range of the mean largest AgNOR particle size per cell for the former was 0.64-2.84 μm^2 (mean 1.61; SD 0.68) and 0.98-4.27 μm^2 (mean 2.55; SD 1.02) for the latter (significance of difference $0.05 > P > 0.02$ Student's t test).

Mitotic frequency

The range of the number of mitotic figures for the benign group of lesions was 0-2 /50 HPF and for the malignant group 6-29 /50 HPF

DISCUSSION

Predicting the biologic behavior of gastric

stromal tumors is not straightforward and requires multiple features to determine malignancy. The presence of mitotic figures in appreciable numbers is generally a reliable indication of malignancy. However, in counting the number of mitotic figures one should realize that the size of a high-power field varies considerably, depending on the characteristics of the microscope[15,16]. Other cellular features such as cellularity and cytologic atypia must also be considered,although, sometimes completely benign tumors may show marked cellularity and nuclear pleomorphism including even the presence of bizarre giant cells. The gross size of a tumor is also a valuable discriminant of metastatic potential,although it is possible that a tumor as small as 1 cm in size may metastasize[17] and a very large tumor may behave in a benign fashion. The criteria for the diagnosis of malignancy in our institution is based largely,but not solely, on the mitotic rate. Other features, such as tumor size, dense cellularity, nuclear pleomorphism, tumor necrosis and microinvasion, are also taken into account.

The number and the size of the AgNORs are thought to reflect the degree of cellular differentiation, activation and malignancy of the cell. Many types of tumor have demonstrated that the AgNOR number rises with decreasing differentiation and increasing malignancy[4-8,18,19]. However, in other types of tumor, the results have been less discriminating[19-20,22]. Correlation between the NOR size and number in some types of tumor has been observed and an inverse relationship between AgNOR number and size has been clearly demonstrated[23,24]. This means that AgNORs are smaller in tumors of higher grade malignancy than in tumors of lower grade malignancy, and also smaller in malignant tumors than in benign ones, although in some studies[25,26] the findings have not been so conclusive.

This study was morphometric, with a relatively limited number of cases due to the comparative rarity of gastric stromal tumors. The number and size of the largest AgNOR particle in benign and malignant gastric stromal tumor cells were determined. We found that AgNOR particles were more numerous in the nuclei of malignant cells. This is in agreement with the study done by Yu et al[27]. In their series, most gastrointestinal stromal tumors graded as benign had an AgNOR number of less than 3 per nucleus whereas the majority of tumors graded as malignant had a higher AgNOR count. In this study, all benign tumors had an AgNOR number of less than 4 and all malignant tumors had AgNOR counts of 6 or more. Since the mitotic rate plays a role in the discrimination between benign and malignant stromal tumors, it is of interest that no linear relationship was observed between the AgNOR count and the mitotic rate either in benign or in malignant lesions. This may be due to the fact that these two methods quantify different aspects of proliferative activity of cells.

In measuring the size of the largest AgNOR particles, instead of calculating the summed areas of dots in one nucleus, we chose only to measure the largest one, since if this method is applicable in helping in the diagnosis of malignancy, it would be easier to perform and more routinely useful. However, although the average size of the largest AgNOR particle in malignant stromal cells was significantly larger that in benign ones, relying on the size of the largest AgNOR particle could be a misleading discriminator, because of a wide overlap of values

in individual cases.

Some features, such as cellularity, anaplasia and mitotic count, used as criteria for the diagnosis of malignancy in gastric stromal tumors, are subjective and suffer from considerable inter- and intra-observer variation. As diagnostic guidelines,these drawbacks also apply to AgNOR counting. However, such problems could be at least partially resolved by establishing an intra-laboratory standardization for the enumeration of the AgNOR dots. We conclude that with this easily performed AgNOR method, which is readily applicable to formalin-fixed and paraffin embedded sections, and with a good cut-off between benign and malignant cells,the AgNOR count can be used as an additional helpful criteria in the determination of malignancy, especially for those tumors of uncertain malignant potential.

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胃基質腫瘤之核仁機化質區

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利用 18 個胃基質腫瘤，以銀染色法並藉著影像分析系統來計算其細胞核內 nucleolar organizer region (NOR)之數目及測量其最大顆粒之面積。其目的是要評估，該實驗之結果能否用來幫助此類腫瘤之良性及惡性之診斷，基本上良性及惡性之胃基質腫瘤在組織形態上不易作鑑別診斷。

九個良性腫瘤中，NOR 之平均數目爲 2.41 (SD 0.96)，而九個惡性腫瘤則爲 8.71 (SD 2.72)，兩類數值其範圍並無重疊。最大 NOR 顆粒面積之平均值，良性腫瘤爲 1.31 μm^2 (SD 0.68)，而惡性腫瘤爲 2.55μm^2 (SD 1.02)，兩類數值之間有重疊現象。每個案例其 NOR 之數目多少和顆粒大小並無關聯。

實驗結果發現，NOR 之數目可成爲另一項之參數以用來幫助區分此類腫瘤之良、惡性，而測量 NOR 顆粒之大小則無法達到此目的。

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