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MORPHOMETRIC STUDY ON AgNORs OF ESOPHAGEAL SQUAMOUS CELL CARCINOMA AND PARANEOPLASTIC EPITHELIAL CELLS

Chen Mao-huai, Wu Ming-yao

Department of Pathology, Shantou University Medical College
12 Raoping Road, Shantou, Guangdong Province, P.R. China

ABSTRACT

The nuclei and the silver-stained nucleolar organizer region-associated proteins(AgNORs) in the normal, the dysplastic and the cancer cells of 38 cases of esophageal squamous cell carcinoma were measured by an image analysis system. The results were shown as follow: (1)The nuclear area and the AgNOR counts in the normal cells were similar to those in the dysplastic cells($P>0.5$); both variables of the above cells were significantly lower than those in cancer cells($P<0.05$). (2)The AgNOR area per cell and the AgNOR/nuclear area ratio increased significantly from the normal through the dysplastic to the cancer cells($P<0.005$). (3) No significant differences were found in the mean AgNOR dot area and the mean AgNOR dot shape between the dysplastic and the cancer cells ($P>0.05$), both parameters of the two kinds of cells were significantly higher than those in the normal cells($P<0.05$). The authors suggest that the morphometric study of AgNORs may be valuable in further studies on the change of AgNORs during the progression of the esophageal epithelial lesions towards cancer.

Key words: nucleolar organizer region-associated proteins(AgNORs), esophageal carcinoma, morphometry.

INTRODUCTION

Nucleolar organizer regions(NORs) are the DNA segments in the nucleoli, which are associated with the transcription of ribosomal RNA and nucleolar formation (Underwood and Giri,1988, Crocker and Paramjit, 1987, Smith et al., 1988). Some investigators differentiate the benign and the malignant tumors on the basis of the silver-stained nucleolar organizer region-associated proteins(AgNORs) morphometry(Hansen and Ostergard, 1990, Giri et al.,1989). We have analyzed the morphometric AgNORs in esophageal carcinoma with an image analysis system to investigate the change of AgNORs during the changes leading to cancer. Our idea is to provide objective variables for making the discrimination between benign and maligant lesions of the esophagus.

MATERIALS AND METHODS

Samples

Thirty eight lesions of the esophagus, comprising 5 normal esophageal epithelia, 25 dysplastic epithelia and 32 invasive squamous cell carcinomas, were selected from the histopathology files of the Shantou University Medical College. Tissue samples were cut at 4 μm thickness and stained with a standard haematoxylin and eosin.

AgNORs staining

Sections were cut at 4 μm onto glass slides. The sections were dewaxed in xylene, rehydrated through descending ethanol concentrations and washed in running deionized, distilled water. The silver colloid solution for staining of AgNORs was prepared by dissolving gelatin in 1 per cent aqueous formic acid at a concentration of 2 per cent, this solution was mixed, 1:2 volumes, with 50 per cent aqueous silver nitrate to obtain the final working solution. This was dropped onto the sections and left for 60 minutes in the dark, at room temperature, after which the sections were washed with deionized water and rehydrated through descending ethanol concentrations, mounted in a synthetic medium.

Morphometric study

Nuclear and AgNORs morphometry were performed with a commercially available automatic image analysis system(Quantimet 520, Cambrige). The sections were examined using a 100 × immersion lens. The images were accepted from a TV camera and displayed on a separate monitor and edited using the cursor and the grey level. The cells in the intermediate

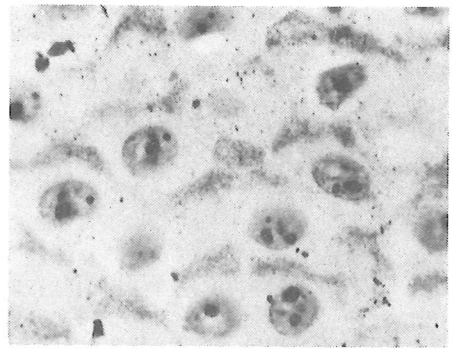

Fig.1. AgNOR dots in the normal epithelial cells of the esophagus. (argyrophil stain) ×1000

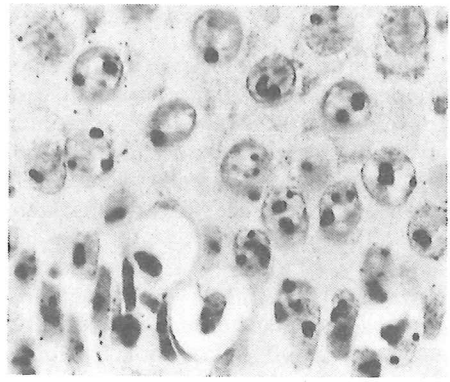

Fig.2. AgNOR dots in the dysplastic epithelial cells of the esophagus. (argyrophil stain) ×1000

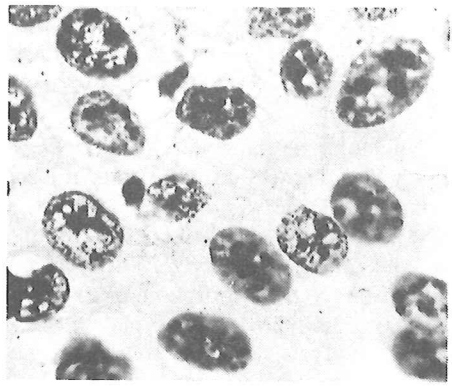

Fig.3. AgNOR dots in the squamous cell carcinoma of the esophagus. (argyrophil stain) ×1000

layer of the normal and the dysplastic epithelia in the esophagus were selected. Those cells in the basal and the superficial layer were not included. The poorly differentiated cancer cells in either well or poorly differentiated squamous cell carcinoma were selected. Those cancer cells with keratin pearl were not included. More than 50 cells in each case were measured randomly by program-controlled system. Individually discernible or separable dots, some of which were aggregated AgNORs, in each nucleus of the above cells were measured and recorded. The nuclear area, the AgNOR area per cell, the AgNOR/nuclear area ratio, the mean AgNOR counts per cell, the AgNOR dot area and its shape were assessed. The shape of the AgNOR dot is a measurement whose value is given by the simple formula: shape = $P^2/4\pi A$ (P = perimeter of the dot , A = area of the dot). For a perfect circle, shape =1.0, deviation from a circle, whether elliptical or for example, dendritic or spindle shaped, resulted in a shape value of more than 1.0. For each, the mean and standard deviation (Mean±SD) were calculated.

The differences between the groups were submitted to analysis by the student's t-test.

RESULTS

The nuclei were light yellow and the AgNOR dots brown in the section after the argyrophil staining method.

The nuclear morphometric results were displayed in table 1.

The nuclear area and the AgNOR counts per cell(Mean±SD) in the normal cells (99.09±11.31μm^2, 2.53±0.63, respectively, Fig.1) were similar to those in the dysplastic cells (102.50±21.73μm^2, 2.86±0.91, respectively, Fig.2) ($P>0.5$), both variables of the above cells were significantly lower than those in the cancer cells(126.11±26.38μm^2 , 3.84±1.18, respectively, Fig.3)($P<0.05$). The differences in the mean AgNOR area per cell(Mean±SD) between the normal (12.32±4.92μm^2), the dysplastic (30.97±11.51μm^2) and the cancer cells (46.37±13.69μm^2) were significant ($P<0.005$). The differences in the AgNOR /nuclear area ratio between the normal(0.14±0.06), the dysplastic (0.33±0.11)

Table 1. Morphometric results on AgNORs of esophageal carcinoma.

	no of patient	nuclear area (μm^2)	AgNOR counts per cell	AgNOR area per cell(μm^2)	AgNOR /nuclear area ratio	AgNOR dot area (μm^2)	AgNOR dot shape
normal cells	5	99.09 ±11.31	2.53 ±0.63	12.32 ±4.92	0.14 ±0.06	4.55 ±1.35	2.31 ±1.50
dysplastic cells	25	102.50 ±21.73	2.86 ±0.91	30.97 ±11.51	0.33 ±0.11	11.38 ±6.63	3.19 ±0.57
cancer cells	32	126.11 ±26.38	3.84 ±1.18	46.37 ±13.69	0.43 ±0.13	13.14 ±7.36	3.31 ±0.60

and the cancer cells (0.43±0.13) were also significant (P<0.005). No significant differences were found in the mean AgNOR dot area and in the mean AgNOR dot shape between the dysplastic (11.38±6.63μm^2, 3.19±0.57, respectively) and the cancer cells (13.14±7.36μm^2, 3.31±0.60, respesctively) (P>0.05), both features of the two kinds of cells were significantly higher than those in the normal cells (4.55±1.35μm^2, 2.31±1.50, respectively) (P<0.05).

DISCUSSION

The histological assessment of AgNORs can have diagnostic value in defined situations. There are two methods for enumerating AgNORs: method A, an attempt was made to resolve nucleolar cluster of AgNORs into their discernible number of discrete dots, method B, nucleolar clusters were counted as a single AgNOR irrespective of the number of dots within the nucleolus(Suresh et al.,1990, Giri et al.,1989). However, counting of AgNOR dots in tissue sections may be time-consuming and led to observer fatigue and consequent error. We performed a morphometric study of AgNORs using an image analysis system standardized for the AgNORs analysis. The method B was adopted for our study. AgNOR dots, some of which were aggregated AgNORs, were measured without subjective judgement.

Our experiment showed that the AgNOR area per cell and the AgNOR/nuclear area ratio increased gradually from the normal through the dysplastic to the cancer cell in the esophagus. This result suggested the increased AgNORs was associated with the cellular atypism.and supported the dysplastic cell was an important step through which the normal cell became cancer cell. In addition, the AgNOR counts in cancer cells were higher than those in dysplastic and normal cells. The results that the increased AgNOR counts per cell in malignant cell by comparison with their normal counterparts were also reported by Giri(1989) and Crocker and Mcgovern(1988). There has, however, been some debate as to whether a high number of AgNORs is a reflection of hyperdiploidy or an indication of a high cellular proliferation rate. Thus, a study of breast lesions suggested that AgNOR counts were at least partially , related to ploidy (Giri etal., 1989). Suresh(1990) reported that AgNOR counts were more clearly related to cell ploidy rather than to the degree of proliferative activity. Whilsh, by contrast, it had been claimed that AgNOR counts tended to reflect the rate of cellular proliferation and were unrelated to ploidy (Crocker et al., 1988). As we know, the high cellular proliferation rate was seen in either dysplastic cells or cancer cells. From our morphometric result of AgNORs, the AgNOR dot in the dysplastic cells was irregular and its area was larger than that in the normal cells but the AgNOR counts did not increase in dysplastic cells. The cancer cells also had larger AgNOR dot with its shape irregular. The results supported the increased AgNOR dot area reflected the increase of cell prolilferation. It was well established that many human solid tumors exhibited an abnormally high content of cellular DNA(Merkel et al., 1987). DNA aneuploidy was identified in 70.1% of the patients(Ruol et al., 1990) and 30 of 31 patients (Stephens et al., 1989) with squamous cell carcinoma of the esophagus. The increase of AgNOR counts which was only occured in cancer cells in our experiment might related to cellular ploidy. Underwood(1988) considered a quantifiable apparent

increase in the mean AgNOR count could result if either cell proliferation and transcriptional activity increases or cell ploidy increases. Our study concluded that the increased AgNOR area per cell including increases of AgNOR counts and/or AgNOR dot area reflected the cell proliferation and cell ploidy. The change of AgNOR size will be useful in evaluating the cell proliferation and predicting the patient's prognosis and the AgNOR counts seem to represent the transformation or malignancy, and could be helpful for diagnosing the neoplasm at the light microscopic level.

Our experiment also showed that the nuclear area, the AgNOR counts, the AgNOR area per cell and the AgNOR/nuclear area ratio were useful in discriminating the squamous cell carcinoma and the non-carcinoma (including the normal and the dysplastic cell). The dysplasia is the precancerous lesion of esophageal carcinoma and it could be distinguished from the normal cell by the AgNOR area per cell, and the AgNOR/nuclear area ratio, the mean AgNOR dot area and its shape but the nuclear area and the AgNOR counts had no discriminating significance. On the whole, AgNORs morphometry was a discriminating parameter of the esophageal carcinoma and its precancerous lesions. Setting up the discriminating model combined with these and other histological features may be able to provide new objective diagnostic criteria of the esophageal carcinoma.

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