

ESTIMATION OF NUCLEOLAR ORGANIZER ACTIVITY IN TUMOR CELLS  
FROM PATIENTS WITH ESOPHAGEAL AND GASTRIC CARCINOMA

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The number, shape, and functional activity of nucleoli of interphase cells can be studied by impregnating them with silver [1, 4-7, 10]. The number of grains of silver in the nucleoli reflects activity of their nucleolar organizers (NO) in ribosomal RNA synthesis [6, 8].

The writers' investigations have shown that the size of the nucleoli and the number of grains of silver contained in them decrease in the course of hematopoiesis in normal subjects and patients with leukemia with maturation of the cells [3], and the number of actively functioning NO in human tumor cells may be considerably increased [2, 9].

On the basis of these findings, increased NO activity in tumor cells could be postulated, so that this feature could be used both to detect tumor cells in mixed cell populations and to diagnose malignant neoplasms in various situations. This paper describes a study of the estimation of NO activity of tumor cells in patients with esophageal and gastric cancer.

EXPERIMENTAL METHOD

Experiments were carried out on biopsy material with verified histopathological diagnosis of esophageal carcinoma in 16 patients, gastric carcinoma in 14 patients, gastritis with reduced secretory activity in three patients, leiomyoma of the esophagus in one patient, and esophageal varices in one patient.

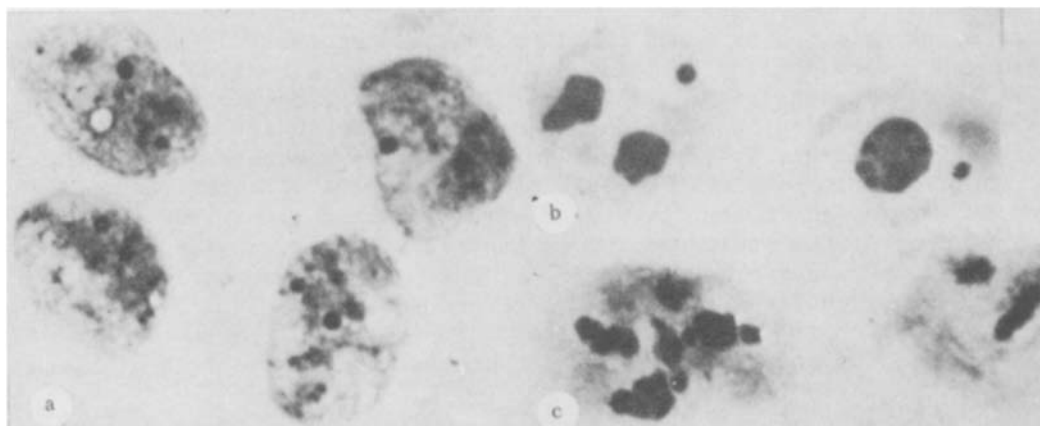


Fig. 1. Cells with nucleoli stained with silver nitrate from esophageal and gastric biopsy material from noncancerous patients (a) and from patients with tumors (b, c): Large nucleoli of tumor cells, containing more than the normal number of grains of silver, can be seen (1200  $\times$ ).

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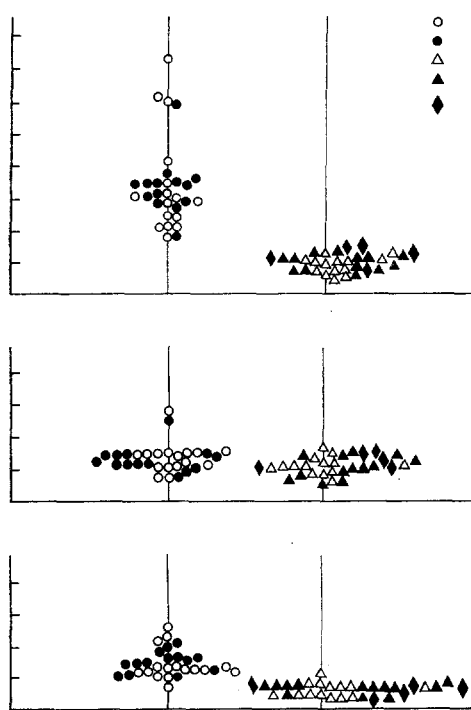


Fig. 2. Results of quantitative estimation of number and activity of nucleoli, stained with silver nitrate, from malignant (I) and normal (II) esophageal and gastric cells. A) Mean number of grains of silver per nucleus; B) mean number of nucleoli in nuclei of cells examined; C) mean number of grains of silver per nucleolus. Empty circles — esophageal cancer cells; filled circles — gastric cancer cells; empty triangles — morphologically unchanged esophageal cells in biopsy material from cancer patients; lozenges — morphologically unchanged cells in biopsy material from noncancerous patients.

Biopsy material obtained by means of fiberoptic endoscopes (Olympus), using cytological brushes and forceps, were applied to thin, defatted slides, air-dried, fixed with a mixture of methanol and glacial acetic acid (3:1) for 10 min, and treated with 2N formic acid solution (10 min). After washing in distilled water and drying in air the preparations were impregnated with silver nitrate by the method in [5], taking account of recommendations on standardization of silver solutions with the aid of silver oxide [7]. At the end of impregnation the preparations were again thoroughly rinsed with distilled water, and stained for 15 sec with a 1% solution of Giemsa stain made up in phosphate buffer, pH 6.8. Cells were studied in the light microscope under a magnification of 1200, and the number and size of nucleoli and the number of argyrophilic granules in them were estimated (Fig. 1). When the silver grains were confluent, their number was determined approximately on the basis of the area of the nucleolus occupied by one grain, followed by comparison of the area of blocks of aggregated granules in the region.

From each patient 50 tumor cells and 50 morphologically unchanged cells were analyzed, bearing in mind that the most important features of tumor cells are atypia of their morphology, a high nucleo-cytoplasmic ratio, and the presence of large, hypertrophied or modified nucleoli in their nuclei. On the basis of the results the mean number of nucleoli in the cells and the mean number of grains of silver in the nuclei and nucleoli were determined.

#### EXPERIMENTAL RESULTS

The dimensions of the nucleoli of most cells from patients with esophageal and gastric cancer were considerably larger than in material from patients with noncancerous diseases.

TABLE 1. Number of Nucleoli and Grains of Silver in Cells of 30 Patients with Esophageal and Gastric Cancer and Five Subjects of Control Group ( $M \pm m$ )

Material	Number of nucleoli	No. of grains of silver	
		in nucleus	in nucleolus
Control	$2,6 \pm 0,2$	$11,4 \pm 1,9$	$5,3 \pm 0,5$
Tumors:			
tumors cells	$2,7 \pm 0,1^{a, b}$	$35,3 \pm 2,4^{a, b}$	$13,3 \pm 0,7^{a, b}$
morphologically unchanged cells	$2,0 \pm 0,2^a$	$9,8 \pm 0,5^a$	$5,1 \pm 0,4^a$

Legend. a, b) Differences between mean values significant at  $P < 0.01-0.0001$ .

Meanwhile the number of nucleoli in the nuclei and the number of grains of silver in them varied considerably both in individual patients and within the same cell population (Fig. 1).

The investigation conclusively demonstrates high activity of NO in human tumor cells; this could be due, first, to their immaturity and their relatively high proliferative activity, and also to an increase in their number of chromosomes, with actively functioning NO. The possibility that the tumor cell can make use of the ribosomal apparatus for realizing the program of oncogene activation and oncoprotein synthesis, likewise must not be forgotten. Whether these changes in NO function are characteristic of tumors in other situations, and the extent to which they can be used in diagnosis and prognosis of malignant tumors, only further research can show.

These results show that the presence of cells with large nucleoli, active in ribosomal RNA synthesis, in cell preparations enabled the diagnosis of cancer to be made before the end of the histopathological investigation in all 30 patients. In two cases only repeated histological investigation confirmed the preliminary conclusion of the presence of esophageal and gastric cancer, based on the results of silver impregnation of NO. On the other hand, the absence of cells with active NO in preparations from four patients with the presumptive diagnosis of malignant gastric ulcer (in two), esophageal cancer (in one), and atrophic gastritis (in one) made the diagnosis of cancer questionable, and its absence was subsequently confirmed histologically.

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