QUANTITATIVE ASSESSMENT OF THE DEGREE OF MORPHOLOGICAL DEDIFFERENTIATION OF GASTRIC CANCER CELLS

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Quantitative assessment of the nucleic acid content in preparations stained with gallocyanin and chrome alum was carried out in 20 cases of gastric carcinoma. Coefficients representing the ratios between DNA+RNA of the nucleus and RNA of the cytoplasm and between DNA of the nucleus and the total RNA in the nucleus and cytoplasm, and the index of DNA accumulation were compared in adenocarcinomas and in undifferentiated carcinomas of the stomach. The ratio between the area of nucleus and cytoplasm and the ratio of DNA+RNA of the nucleus to RNA of the cytoplasm were statistically significantly higher in the adenocarcinomas than in the undifferentiated gastric carcinomas. This suggests a lower level of differentiation of the adenocarcinoma of the cells than of tumor cells from undifferentiated forms of gastric carcinoma.

KEY WORDS: gastric carcinoma; nucleic acids; microspectrophotometry.

In the morphological diagnosis of tumors assessment of the degree of maturity of the tumor cells is used, and usually the intensity of polymorphism, the ratio between the volumes of the nucleus and cytoplasm of the tumor cells, and the hyperchromatism of the nuclei are usually determined visually. However, this approach is not sufficient in all cases for the objective evaluation of the degree of morphological dedifferentiation of tumor cells and, in particular, to judge changes in their ploidy. The quantitative study of the content of genetic material in the nuclei of tumor cells, according to data in the literature, involves determination of the DNA content in the nucleus, stained by the Feulgen reaction. Staining with gallocyanin and chrome alum, which is also specific and suitable for quantitative investigations [13, 14], can be used to determine the content of both DNA and RNA. Very few investigations using this method have been published in connection with the morphology of cancer, and yet it can provide new information with respect to the objective study of criteria of the degree of dedifferentiation of tumor cells. Great importance also has been attached to the nucleocytoplasmic ratio for assessment of the degree of differentiation [5, 8, 9]. The object of the present investigation was the objectivization of criteria of the degree of dedifferentiation of gastric cancer cells.

Certain quantitative indices were used in order to assess cell dedifferentiation: coefficients representing the ratios between the area of cross-section of nucleus and cytoplasm, and indices of the nucleic acid content in the nucleus and cytoplasm of tumor cells from adenocarcinomas and undifferentiated forms of gastric carcinoma.

EXPERIMENTAL METHOD

Material from 20 patients with gastric carcinoma (7 with adenocarcinoma and 13 with undifferentiated tumors) was examined. Sections cut after fixation in Carnoy's fluid and embedded in celloidin and paraffin wax in the same way for all cases were stained with gallocyanin and chrome alum; one of two serial sections was treated before staining with protease-free crystalline ribonuclease.

In each case the volume of the nucleus and cytoplasm was measured with an integrating microspectrophotometer [1], the DNA and RNA content in the nucleus and the RNA content in the cytoplasm of 30 tumor cells in the section without ribonuclease treatment and the DNA content in the nucleus of 30 tumor cells and of 30 lymphocytes in a section treated with ribonuclease were determined. The ratio between the areas of cross section of the nucleus and cytoplasm in 30 cells from each patient was calculated. The results of the measurements were subjected to statistical analysis. The level of probability of the significance of differences adopted was 0.95.

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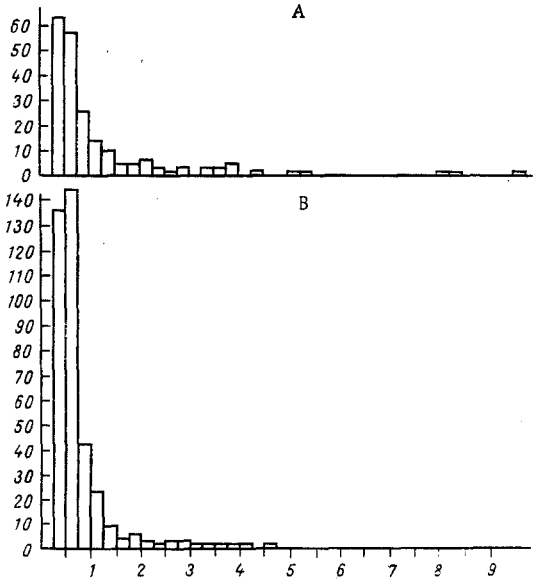

Fig. 1. Distribution of cells by nucleo-cytoplasmic ratio in adenocarcinomas (A) and undifferentiated carcinomas (B) of the stomach. Abscissa, nucleo-cytoplasmic ratio; ordinate, number of cells.

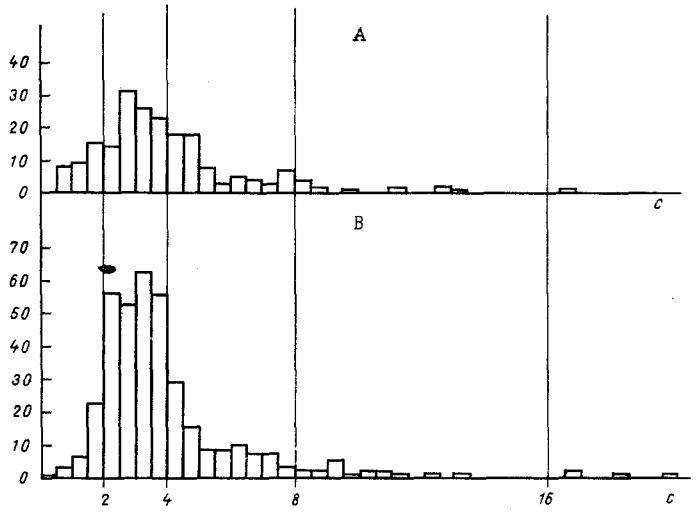

Fig. 2. DNA content in tumor cells of adenocarcinomas (A) and undifferentiated carcinomas (B) of stomach. Abscissa, DNA content (in ploidy units); ordinate, number of cells.

EXPERIMENTAL RESULTS

The distribution of the tumor cells by the values of the nucleo-cytoplasmic ratio obtained in cases of adenocarcinomas (210 cells) and undifferentiated carcinoma (390 cells) is shown in histograms in Fig. 1. In the adenocarcinomas no tumor cells were found with a nucleus whose area of cross section amounted to 0.25-0.5 of the area of the cytoplasm, whereas in the undifferentiated carcinomas more than 20 such cells were found. In adenocarcinomas single cells were found whose nucleus had an area 8-15 times greater than the area of the cytoplasm, whereas no such cells were found in the undifferentiated carcinomas. The number of tumor cells with a nucleo-cytoplasmic ratio of over 1 was 30.5% of the total number of cells in the adenocarcinomas but only 13.8% in the undifferentiated carcinomas. The mean values of the nucleo-cytoplasmic ratio also differed statistically significantly, being 2.09 ± 0.23 for adenocarcinomas and 0.87 ± 0.09 for undifferentiated carcinomas ($t = 5.09$, $P < 0.01$).

Tumor cells of adenocarcinomas thus had a higher nucleo-cytoplasmic ratio than undifferentiated carcinoma cells. The fact that the lower the degree of differentiation of the cells the higher their nucleo-cytoplasmic ratio has been established and confirmed in recent years by morphometric methods [5, 8, 9]. Consequently, tumor cells of undifferentiated carcinomas are more mature, according to this index, than adenocarcinoma cells. This is in agreement with the more manifest functional differentiation of the tumor cells of the undifferentiated carcinoma than of the adenocarcinoma [6].

Comparison of the content of DNA+RNA in the nucleus with the RNA content in the cytoplasm in each tumor cell showed that the mean ratio of nuclear DNA+RNA to cytoplasm RNA was 7.0 ± 1.06 for adenocarcinomas and 3.53 ± 0.47 for undifferentiated carcinomas. The difference was significant ($t = 3.01$, $P < 0.01$). Predominance of the nuclear nucleic acid content over the cytoplasmic RNA was thus much more marked in the adenocarcinomas.

To obtain the DNA/RNA ratio for the nucleus and cytoplasm the mean values of nuclear DNA+RNA, cytoplasmic RNA, and nuclear DNA were calculated for the tumor cells in each case. From the mean nuclear DNA+RNA content the mean nuclear DNA content was subtracted to give the mean RNA content in the nucleus, and this was added to the mean cytoplasmic RNA content for that case. In this way the ratio between the mean nuclear DNA and the total nuclear+cytoplasmic RNA could be calculated. This index, of course, is less accurate than the previous one, for the nuclear DNA+RNA and DNA were found in different cells of different sections from the same patient. The mean value of this ratio for adenocarcinomas was 0.76 ± 0.26 and for undifferentiated carcinomas 0.44 ± 0.04. Although the same tendency was found as for the ratio of nuclear DNA+RNA to cytoplasmic RNA, i.e., it was higher for adenocarcinomas and lower for undifferentiated carcinomas, the difference was not significant ($t = 1.28$, $P > 0.2$).

To establish whether it is the DNA content or the RNA content in the tumor cells that is responsible for the differences between these ratios, the DNA content was analyzed in the nuclei of adenocarcinomas and undifferentiated carcinomas after reduction to ploidy units (Fig. 2). The histograms show no difference in distribution of adenocarcinoma and undifferentiated carcinoma cells: Cells with a DNA content greater than tetraploid (4c) or octaploid (8c) constituted the same percentage of the total number of tumor cells.

On average, therefore, nuclei of adenocarcinomas contain a little more DNA than nuclei of undifferentiated carcinoma cells. The higher values of the ratio of the nuclear nucleic acid content to the cytoplasmic RNA content in adenocarcinomas than in undifferentiated carcinomas is due to the higher RNA content and to differences in its distribution between the nucleus and cytoplasm.

Synthesis of specific RNAs on the DNA template and their transport into the cytoplasm are essential for differentiation and maturation of cells. As was shown in a survey by Dustin [12], the cytoplasmic factor plays the decisive role in cell differentiation. Undifferentiated and embryonic cells are characterized by a high nuclear RNA content and a relatively lower cytoplasmic RNA content [4]. Data in the literature on quantitative determination of RNA in tumors are few in number and contradictory in nature: Tumor cells with a low level of differentiation contain less RNA in their cytoplasm than highly differentiated tumor cells [3, 10]. Consequently, the sharp predominance of the nuclear nucleic acid content over the cytoplasmic RNA content in adenocarcinomas must be regarded as evidence of the lower level of differentiation of their cells than of undifferentiated carcinoma cells.

It has been emphasized [7, 11] that proliferative activity "interferes" with cell maturation. In fact, in the period of DNA synthesis preceding cell division, synthesis of the RNA necessary for differentiation on DNA is impossible. For that reason, sharp stimulation of DNA synthesis leads to some decrease in the RNA content in the cells. Consequently, the tendency for sharper predominance of the DNA content over RNA in adenocarcinomas than in undifferentiated carcinomas observed in the present investigation can be regarded as the result of the greater intensity of proliferation and the lower level of differentiation of adenocarcinoma than of undifferentiated carcinoma cells.

It can thus be concluded that adenocarcinomas and undifferentiated carcinomas differed significantly from each other in their nucleo-cytoplasmic volume ratio, indices of DNA accumulation, and the ratio between the total content of nucleic acids in their nucleus and cytoplasm. The higher values of these ratios in the adenocarcinomas than in undifferentiated carcinomas can be regarded as evidence of the lower level of differentiation of adenocarcinoma cells than of undifferentiated carcinoma cells and of their greater proliferative activity.

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GROWTH OF SYNGENEIC TRANSPLANTABLE TUMORS IN SYRIAN HAMSTERS IMMUNIZED WITH EMBRYONIC HAMSTER TISSUE

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The effect of immunization with embryonic tissue on growth of two syngeneic transplantable tumors was investigated in Syrian hamsters. Immunization of inbred hamsters with embryonic hamster tissue followed by transplantation of continuous lines of syngeneic tumors was shown neither to inhibit nor to stimulate growth of the tumors. It is suggested that embryonic antigens do not participate in the mechanism of transplantation antitumor immunity.

KEY WORDS: embryonic antigens; immunization.

The possibility of using embryonic antigens for immunization against tumors is a topic for discussion. Evidence that immunization with embryonic antigens can prevent both the formation of primary induced tumors and growth of transplanted tumors has been obtained [1, 2]. Other workers have described the unsuccessful results of such immunization [4, 5]. Some workers who obtained positive results by immunization with embryonic cells against tumors induced in Syrian hamsters with SV_{40} virus state in their later communications that several conditions must be closely observed if immunization with embryonic tissue is to be successful [3]. These conditions are as follows: 1) the embryos must be of a certain age (in particular, 9-10 days for Syrian hamster embryos); the suspension of embryonic cells must be prepared by mechanical dispersion without the use of trypsin or versene; 3) the embryos must be from females pregnant for the first time; 4) triple immunization of the animals with embryonic tissue is essential; 5) the embryonic tissue must be irradiated to prevent its differentiation in vivo and the formation of embryos; 6) the most sensitive modification of the transplantation test must be used.

The effect of the first and subsequent gestations on the frequency of origin of primary tumors induced by virus SV_{40} in Syrian hamsters was investigated previously. The results showed that the significant decrease in the frequency of tumor formation in multiparous females is not an immunologic phenomenon.

The object of the present investigation was to study the effect of immunization with embryonic hamster tissue on growth of two transplantable tumors in inbred hamsters.

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