3. S. Bazin and A. Delaunay, Ann. Inst. Pasteur, 106, 543 (1964).
4. S. Bazin, A. Delaunay, N. Briquelet, and J. C. Allain, Ann. Inst. Pasteur, 110,347 (1966).
5. M. C. Burleigh, A. J. Barrett, and G. S. Lazarus, Biochem. J., 137, 387 (1974).
6. J. L. E. Ericksson and B. F. Trump, Histochemie, 4, 470 (1964).
7. D. M. Frankland and C. H. Wynn, Biochem. J., 85, 276 (1962).
8. K. Maruyama, I. Okazaki, K. Kashiwazaki, et al., Biochem. Exp. Biol., 14, 191 (1978).
9. I. Montfort and R. Perez-Tamayo, J. Histochem. Cytochem., 23, 910 (1975).
10. I. Montfort and R. Perez-Tamayo, Am. J. Pathol., 92, 411 (1978).
11. T. G. Morrione and J. Levine, Arch. Pathol., 84, 59 (1967).
12. Y. Murawaki and C. Hirayama, Clin. Chim. Acta, 108, 121 (1980).
13. I. Okazaki and K. Maruyama, Nature, 252, 49 (1974).
14. I. Ikazaki, M. Oda, K. Maruyama, et al., Biochem. Exp. Biol., 11, 15 (1974).
15. C. H. Wynn, Nature, 215, 1191 (1967).

ACTIVITY OF NUCLEOLAR ORGANIZERS OF EPITHELIAL TUMOR CELLS OF THE HUMAN LARGE INTESTINE

N. N. Mamaev, A. A. Mikhailov, S. I. Irzhanov, and I. V. Bykhovets

UDC 616.348-006.66-018.13-076.5

KEY WORDS: nucleolar organizers; silver impregnation; mitoses; adenomas and adenocarcinomas of the large intestine.

The introduction of the method of silver impregnation of nucleoli into cytology has provided the investigator with a unique opportunity of assessing activity of ribosomal cistrons in single cells actually in cytologic preparations; moreover, the number of silver granules in the nucleoli corresponds approximately to the number of actively working ribosomal cistrons (rC) or of RNA-polymerases-1 in the cells analyzed [11-14].

High activity of rC of human tumor cells, discovered recently with the aid of silver nitrate [3, 6], may be linked with their low differentiation, their relatively high proliferative activity, and also a possible increase in the number of acrocentric chromosomes in the cells with actively functioning nucleolar organizers (NO).

To test these hypotheses, it was decided to compare the character of silver staining of NO and mitotic activity of cells of epithelial tumors of the human large intestine, characterized by different degrees of disturbance of cell differentiation.

EXPERIMENTAL METHOD

Material for investigation consisted of preparations of 48 adenomas of different histological types (14 tubular, 34 villous and tubulo-villous), and 13 adenocarcinomas, obtained as a result of local and radical operations for tumors of the large intestine, and also biopsies performed in the Leningrad City Oncologic Dispensary. Regions of macroscopically unchanged mucosa from segments of large intestine, resected mainly for cancer, taken as far away as possible from the tumor, were used for the control. Pieces of tumor were placed on defatted slides and ground to a paste. The smears were fixed, treated with formic acid, and stained with silver by the method described previously [5, 6, 12]. From 50 to 100 cells were analyzed in the smears and the number of nucleoli and argentophilic granules in them determined. The mean number of nucleoli and argentophilic granules in all the cells studied was then calculated. Ability of the cells to start mitosis was studied in paraffin sections stained with hematoxylin and eosin, using Alov's classification to evaluate pathology of mitoses [1]. In each

No. 1 Department of Internal Medicine, I. P. Pavlov First Leningrad Medical Institute. Leningrad City Oncologic Dispensary. Department of Internal Medicine, S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR R. G. Uglov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 12, pp. 763-765, December, 1986. Original article submitted December 19, 1985.

 0007-4888/86/0012-1774$12.50

case 5000 cells were analyzed for this purpose, and the number of cells in mitosis was determined (in promille) and the number of pathological mitosis among them (in %).

EXPERIMENTAL RESULTS

Nucleoli of human large intestinal cells, impregnated with silver, had irregular outlines (Fig. 1). They were small in size, varied from 1 to 4 in number, and did not differ significantly from nucleoli in cells of different individuals. By contrast, nucleoli in tumor cells were enlarged, and their polymorphism was sharply increased.

The mean number of nucleoli in cells of macroscopically unchanged mucosa and of adenomas and adenocarcinomas of the large intestine was about equal (Fig. 2a), although the value of this parameter in adenomas and adenocarcinomas varied within wider limits than in the control.

A study of the number of argentophilic granules in the nucleoli showed it to be significantly higher (34.0 ± 9.7) in the cells of malignant tumors than in the unchanged mucosa (12.3 ± 3.1). In this respect adenomas occupied an intermediate position, and the considerable variability of this parameter evidently reflects the morphologic heterogeneity of this group of tumors. The biological behavior of the different histological types of adenomas of the large intestine is known to be heterogeneous. In particular, doubts have recently been expressed on the ability of tubular adenomas to become malignant [7], whereas villous adenomas are considered to be essentially precancerous or even malignant in character from the start [7].

In the present investigation the mean number of silver granules in nucleoli of villous and tubulo-villous neoplasms was 22.5 ± 9,3, whereas in cells of simple (tubular) adenomas it was 16.2 ± 8.6 (the difference between the parameters is not statistically significant). The mean number of argentophilic granules in the nucleoli in 17 (36%) of the 48 adenomas studied reached the value of the corresponding parameter in cancer cells (more than 25 silver granules per nucleus). Analysis of these adenomas showed that 13 of them had a villous or tubulo-villous structure, with a well-marked villous component. In 7 cases there was gross atypia of the epithelium of the carcinoma in situ type, and in 5 other cases invasion was found through the corresponding muscular layer of the mucosa. As regards the remaining four adenomas of this group, two of them were in the immediate proximity of a malignant tumor, and in one case the adenomas were multiple in character.

Comparison of the results of silver impregnation of NO with mitotic activity of the cells in the group of adenomas (Fig. 3) revealed no strict parallel between the number of silver granules in the nucleoli and the mitotic index of the cells studied. Meanwhile correlation between activity of NO and the presence of pathological mitoses in the cells was quite definite.

The results of silver impregnation of NO of tumor cells in the adenocarcinoma group were sufficiently homogeneous. They indicate higher activity of NO of the tumor cells, with a mean number of silver granules of over 25 per nucleus, than of the control and the adenomas. The exceptions were cases of relatively small (16.6) and relatively large (61.7) numbers of silver granules in the nucleoli (Fig. 3b). The first, in our opinion, can be explained by commencing necrosis of the large tumor which was studied, and death of the cells. The other case, when the mean number of silver granules in the nucleoli (61.6) was about twice the number of corresponding silver granules in cells of the other tumors, suggests the possibility of an increase in the number of actively functioning NO in the cells of that tumor.

The results described above thus confirm previous conclusions [3, 6] regarding increased activity of NO in human tumor cells, which can be largely attributed to disturbance of differentiation of the cells studied, and also to an increase in their proliferative activity. Another important mechanism of the increase in number of silver granules in tumor cell nucleoli may be an increase in the number of acrocentric chromosomes with actively functioning NO in them, for this has been demonstrated several times in other types of tumors [4].

It is difficult as yet to determine what causes lie at the basis of the increased activity of NO and increased mitotic activity of tumor cells. Not the least likely of the possible causes must evidently be amplified and activated oncogenes [8-10].

The data so far obtained show that at least two stages are needed for a malignant process to arise, including in the large intestine. One is linked with activation of oncogenes such as C-myc or C-myb, in the cells, for in the modern view these may endow a changed cell with high proliferative potential. Another stage of malignant transformation leads to disturbance

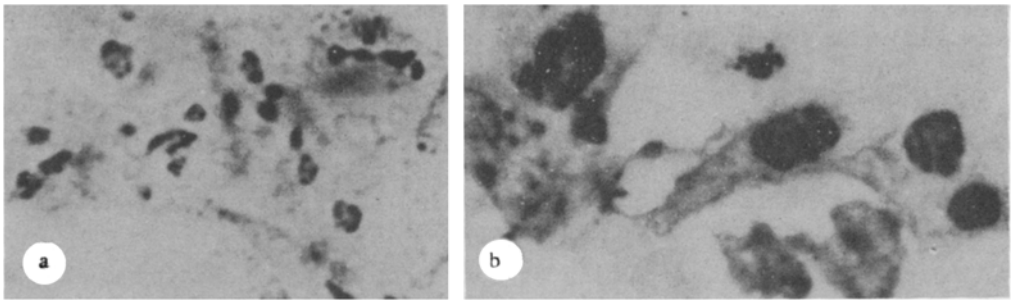

Fig. 1. Cells from biopsy material obtained from pathologically unchanged mucosa (a) and from an adenocarcinoma (b) of the large intestine, stained with silver nitrate. Arrows indicate enlarged nucleoli of tumor cells and increased number of silver granules in them compared with control. 900×.

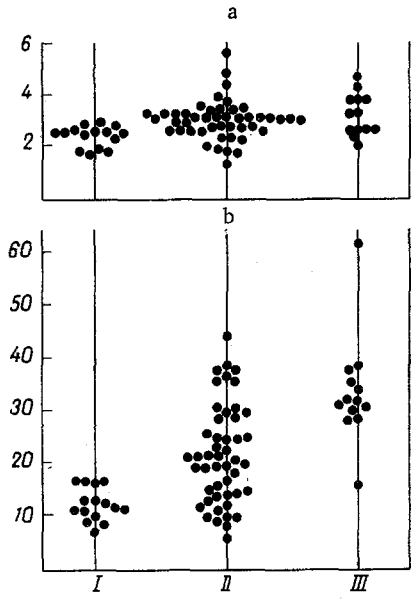

Fig. 2

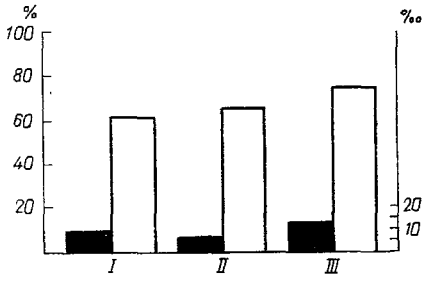

Fig. 3

Fig. 2. Quantitative assessment of number and functional activity of cell nucleoli of pathologically unchanged mucosa (I), and adenomas (II) and adenocarcinomas (III) of the large intestine, stained with silver. a) mean number of nucleoli in nuclei of cells studied; b) mean number of silver granules per nucleus.

Fig. 3. Mean values of frequency of mitosis (in %) and number of pathological mitoses among them (in per cent) in cells of adenomas grouped in accordance with number of silver granules in nucleoli. I) under 20 granules per nucleus; II) from 20 to 29.9 granules per nucleus; III) more than 30 granules per nucleus.

of differentiation of certain types of cells and often coincides in time with activation of C-ras and certain other oncogenes in them [2, 10]. From this aspect some of the adenomas of the large intestine studied may have been tumors in the first stage of malignant transformation. If this is so, the discovery of such a characteristic feature of cancer as high rC activity in cells of adenomas may be an important step in differential diagnosis and evaluation of prognosis of these tumors. This applies in particular, evidently, to villous tumors, in which high NO activity was found more often than in the rest, and the risk of conversion into adenocarcinoma, according to observations by many investigators, is particularly great.

The results of the first cytogenetic investigations of cells of tubular and villous adenomas and of adenocarcinomas of the large intestine, demonstrating significant and nonrandom changes of karyotype even in the stage of "benign" adenomas, and the steady increase

in their complexity, with increasing development of features of malignancy of the tumor, may serve as indirect confirmation of the validity of this concept [15].

LITERATURE CITED

1. I. A. Alov, Cytophysiology and Pathology of Mitosis [in Russian], Moscow (1972), p. 168.
2. M. V. Blagosklonnyi, Vopr. Onkol., No. 7, 8 (1985).
3. N. N. Mamaev, N. V. Begiya, et al., Abstracts of Proceedings of an All-Union Conference [in Russian], Tbilisi (1983), p. 179.
4. N. N. Mamaev, S. E. Mamaeva, et al., Tsitologiya, No. 2, 161 (1980).
5. N. N. Mamaev, S. E. Mamaeva, et al., Tsitologiya, No. 1, 45 (1984).
6. N. N. Mamaev, N. V. Bebiya, S. E. Mamaeva, et al., Byull. Éksp. Biol. Med., No. 4, 477 (1985).
7. K. M. Pozharisski, Arkh. Patol., No. 5, 76 (1978).
8. I. F. Seits, S. N. Fedorov, et al., Vopr. Onkol., No. 8, 52 (1985).
9. K. Alitalo, K. Saksela, et al., Genes and Cancer, New York (1984), p. 383.
10. K. Alitalo, R. Wingvist, et al., Proc. Natl. Acad. Sci. USA, 81, 4534 (1984).
11. H. Busch, Y. Daskal, et al., Cancer Res., 39, 857 (1979).
12. W. M. Howell and D. A. Black, Experientia, 36, 1014 (1980).
13. H. Kacerovska, Z. Likovsky, and W. Smetana, Neoplasma, 28, 513 (1981).
14. O. J. Miller, D. A. Miller et al., Proc. Natl. Acad. Sci. USA, 73, 4531 (1976).
15. A. Reichmann, P. Marin, and B. Levin, Int. J. Cancer, 28, 431 (1981).