CYTOCHEMICAL, LUMINESCENCE, AND ELECTRON MICROSCOPE ANALYSIS OF RIBONUCLEIC ACID AND MITOCHONDRIA IN HUMAN CANCER CELLS CULTIVATED IN VITRO

M. I. Leikina, V. I. Grebenshchikova, and V. S. Karpov

UDC 616-006.6-018.1-008.939.633.2 + 616-006.6-018.11

Cultures of human cancer tissue are now widely used for studying many problems in cytology, virology, and oncology. For this reason the use of various methods to investigate the structure and cytochemistry of human cancer cells when cultivated in vitro is of great interest.

The object of the present investigation was to study the distribution and concentration of ribonucleic acid (RNA), the structure and distribution of the mitochondria, and the activity of certain oxidative enzymes of the mitochondria in the cells of a culture of carcinoma of the human larynx (strain HEp-2) using the luminescence and electron microscopes and histochemical methods of investigation.

EXPERIMENTAL METHOD

Experiments were carried out on 3-4 day cultures of HEp-2 grown on cover slips in bubbles under penicillin in a stationary position on Medium No. 199 with the addition of 10% bovine serum and antibiotics.

The RNA was detected by Brachet's method after fixation of the cultures with 96° ethyl alcohol. RNA was also studied in living cells and cells fixed with alcohol by means of the ML-2 luminescence microscope after staining for 4-5 min with 1:100,000 acridine orange.

The mitochondria were studied by means of the optical microscope in cultures fixed with calcium-formol by Baker's method and stained with acid fuchsin by Altman's method. The activity of certain enzymes concentrated in the mitochondria was also studied: succinate dehydrogenase (the method of Seligman and Rutenberg in Hirono's modification [5]), and malate and isocitrate dehydrogenases (by the method of Hess, Scarpelli, and Pearse [4]).

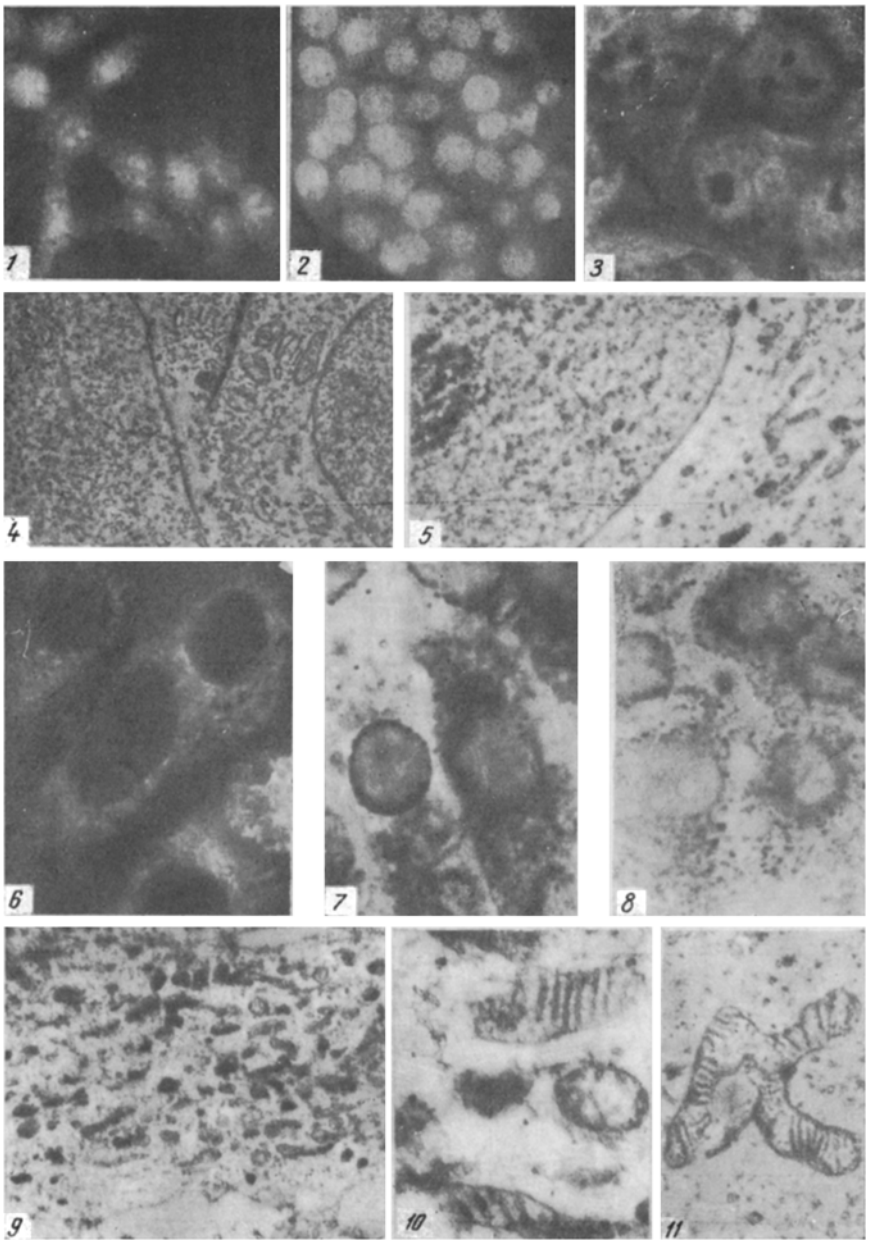
The luminescence microscope was used to detect the mitochondria after fluorochroming with oxytetracycline for 3 min in a concentration of 1:100,000. To study the ultra structure of the cancer cells the cultures were fixed by Palade's method, and embedded in a mixture of methyl and butyl methyacrylates. Blocks were cut on the Dashevskii UMD-5 microtome. The investigation was carried out on the Jem-5g electron microscope.

EXPERIMENTAL RESULTS

On the 3rd-4th day of growth the HEp-2 culture formed a monolayer consisting of polygonal and spindle-shaped cells. RNA was found in the cytoplasm and nucleoli of the cells. The intensity of staining of the nucleoli with pyronine was much higher than that of the cytoplasm. The results of control treatment with ribonuclease showed that all the pyroninophilia of the cell was due to the presence of RNA. In the cytoplasm RNA was revealed as tiny granules scattered diffusely throughout the cell. In some cases a higher concentration of RNA was seen in the perinuclear part of the cytoplasm (see Fig. 3).

In living cells of the HEp-2 culture stained with acridine organe, the bright red luminescence of the RNA could be seen in the form of granules of various sizes scattered throughout the cytoplasm (see Fig. 1). In some cells the granules were rather more concentrated around the nucleus. The nucleoli gave a bright green luminescence. In alcohol-fixed cells stained with acridine orange the pattern of luminescence was different. The RNA showed a reddish-orange luminescence scattered as tiny granules throughout the cytoplasm. The nucleoli gave a yellow luminescence (see Fig. 2).

Department of Cytology and Histology, Moscow University (Presented by Active Member of the Academy of Medical Sciences of the USSR S. E. Severin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 12, pp. 87-90, December, 1966. Original article submitted April 2, 1965.



Cells of a 4-day culture of HEp-2. 1) RNA (unfixed cultures; acridine orange; ML-2 luminescence microscope, objective 40x); 2) RNA (fixation in 96° ethyl alcohol; acridine orange; ML-2 luminescence microscope, objective 40x); 3) RNA (fixation in 96° ethyl alcohol; stained by Brachet's method; optical microscope, objective 60x); 4 and 5) endoplasmic reticulum and ribosomes [electron microscope, magnification 5,000 (4) and 15,000 x (5)]; 6) mitochondria (unfixed cultures; oxytetracycline; ML-2 luminescence microscope, objective 90x); 7) succinate dehydrogenase (reaction with nitro BT; optical microscope, objective 60x); 8) isocitrate dehydrogenase (reaction with nitro BT; optical microscope, objective 60x); 9-11) mitochondria [electron microscope, magnification 2,000 (9), 30,000 (10), and 5,000x (11)].

In the electron microscope the endoplasmic reticulum in the cells of the HEp-2 culture was poorly developed and consisted of a few tubules and vesicles. The ribosomes lay on the membranes of the reticulum, but the greater part of them lay actually in the hyaloplasm, and they were sometimes collected into small groups or rosettes (see Figs. 4 and 5).

In the electron microscope the nucleolus was seen to contain granules (ribosomes) closely packed against each other. They were more tightly packed than in the cytoplasm. No fibrillary structures were seen in the nucleolus (see Fig. 5). The nucleoli were usually round or oval.

Under the optical microscope the mitochondria appeared as granules and short rods, scattered irregularly in the cytoplasm. In some cells, the mitochondria were concentrated in the perinuclear regions. After vital staining with tetracycline, the mitochondria gave a green luminescence and they varied in size. They appeared as short rods and granules. Often the mitochondria were more concentrated on one side of the nucleus or around it (see Fig. 6).

The electron micrographs showed considerable variation in the size and shape of the mitochondria. They were round or oval, or shaped like very long rods. Some mitochondria of a more complex, branching form were seen, especially trilobate. The internal matrix of the mitochondria was electron-permeable. The cristae usually ran perpendicularly to the long axis of the mitochondria and divided them into separate compartments. In some mitochondria, the cristae ran obliquely, and sometimes they were very short and situated at the periphery of the organoid (see Figs. 9-11).

Succinate dehydrogenase activity was observed in the mitochondria. Formazan granules were distributed throughout the cytoplasm. In many cells the activity of the enzyme was higher in the perinuclear zone (see Fig. 7). Malate dehydrogenase activity was also found in the cells of the culture in the form of tiny granules scattered irregularly throughout the cytoplasm. On the whole, the intensity of the reaction was weaker than in the case of the determination of succinate dehydrogenase. The activity of the isocitrate dehydrogenases coupled with di- and triphosphopyridinenucleotide was slightly higher than the malate dehydrogenase activity, but weaker than the succinate dehydrogenase activity. Both these enzymes were found not only in the granules (mitochondria), but also diffusely throughout the cytoplasm. The formazan granules varied in size from tiny particles to relatively large clumps (see Fig. 8).

The number and size of the formazan granules differed during the determination of different dehydrogenases.

In culture in vitro, HEp-2 cells are characterized by a high concentration of RNA, localized in the nucleolus and cytoplasm. The difference in the distribution of RNA in the cytoplasm of living and fixed cells is noteworthy. Whereas in the latter, the RNA was distributed diffusely throughout the cytoplasm, after vital staining with acridine orange, the RNA was concentrated into granules of different sizes. This was evidently due to the fact that acridine orange, like neutral red and certain other substances, is a granular stain, and when it is used for staining cells a reaction of granule formation takes place and the RNA is aggregated into granules. A similar aggregation of RNA in granules was observed in a culture of fibroblasts by B. V. Kedrovskii [1], using neutral red. According to the theory of D. N. Nasonov and V. Ya. Aleksandrov [2], granule formation demonstrates a normal state of the cell and absence of injury to it. After fixation (irreversible changes in the cell) no granule formation takes place and the RBA is distributed diffusely throughout the cytoplasm after staining with acridine orange and pyronine.

The nuclei of the cells of the HEp-2 culture were rich in RNA. This was clear from their cytochemical and electron-microscopic study. Whereas, the RNA of the cytoplasm gave a bright red luminescence, the nucleoli gave a green luminescence; it may be supposed that this was due to differences in the properties of the RNA in the cytoplasm and nucleolus: either a difference in the degree of polymerization of the RNA in the cytoplasm and nucleolus or a difference in the distribution of the ribosomes, because the ribosomes were much more tightly packed in the nucleoli than in the cytoplasm.

The mitochondria in the cells of the HEp-2 culture were very heterogeneous and differed in shape, size, and activity of the enzymes located in them. Differences in the properties of the mitochondria have also been described in the literature as a result of cytological and biochemical investigation [3, 5, 6, 7].

This cytochemical and biochemical heterogeneity of the mitochondria was due primarily to differences in their submicroscopic structure—the degree of development, the size, and the direction of the internal cristae.

The study of the RNA, the mitochondria, and the activity of certain oxidative enzymes showed that in many cells these components are concentrated in the perinuclear region of the cytoplasm, often in the form of a cluster

on one side of the nucleus. This is probably associated with the higher metabolic activity of the perinuclear zone of the cytoplasm. The presence of such a zone in tissue culture cells has been concluded from the results of a number of investigations [8].

The study of the cytochemistry and fine structure of cancer cells using microscopic and submicroscopic methods yields results which are both confirmatory and supplementary.

LITERATURE CITED

1. B. V. Kedrovskii, Byull. Éksp. Biol., 25, No. 6, 450 (1948).
2. D. N. Nasonov and V. Ya. Aleksandrov, The Reaction of Living Matter to External Agents [in Russian], Moscow-Leningrad (1940).
3. R. N. Étingof, I. I. Gumina, and A. A. Krichevskaya, Biokhimiya, No. 2, 354 (1961).
4. R. Hess, D. G. Scarpelli, and G. E. Pease, J. Biophys. Biochem. Cytol., 4 (1958), p. 753.
5. Y. Hirono, Stain Technol., 32 (1956), p. 39.
6. E. Kuff and V. Schneider, in the book: Problems in Cytophysiology [Russian translation], Moscow (1957), p. 226.
7. V. Mutolo and T. Abrignani, Brit. J. Cancer, 11 (1957), p. 590.
8. W. Sandritter and H. G. Schimer, Verh. Dtsch. Ges. Path., 42, Tag (1959), p. 449.
9. N. Schummelfeder, Naturwissenschaften, 44 (1957), p. 467.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
