ULTRASTRUCTURAL ASPECTS OF PREGLIOMA

M. S. Abdullakhodzhaeva and T. K. Ibragimov

UDC 616.831-006.484-036.3-091.8

A study of submicroscopic changes in astrocytes after intracerebral injection of the carcinogen 9,10-dimethyl-1,2-benzanthracene suggests that the so-called true period of preglioma begins on the 45th day of the experiment, when astrocytes of a typical ultrastructure begin to appear against the background of degenerative changes. Changes in the submicroscopic organization of the astrocytes during chemical carcinogenesis can be subdivided into three stages: I) intracellular hyperplasia, due to traumatic brain injury; II) degenerative changes, connected with disturbance of the blood supply to the tissue and the direct action of the carcinogen on it; III) atypical ultrastructural reorganization.

KEY WORDS: chemical carcinogenesis; astrocytes; brain tumors; glioma.

It has now been established that the development of tumors in the CNS is preceded by pretumor changes [1, 2, 7, 9, 10, 11, 13, 14]. Meanwhile, much remains to be explained in the interpretation of the morphogenesis of pretumor changes [3, 7, 8, 11].

This paper gives the results of a study of the ultrastructure of astrocytes in the course of chemical carcinogenesis, undertaken in continuation of an investigation of pretumor conditions in the CNS [4, 5].

EXPERIMENTAL METHOD

There were four series of experiments. In series I a pellet of paraffin wax (control) was implanted into the brain of 54 intact animals, in series II a pellet of the pure carcinogen 9,10-dimethyl-1,2-benzanthracene (DMBA) was implanted in 35 rats, and in series III (53 rats) and IV (50 rats) pellets of paraffin wax and DMBA were implanted respectively 45 days after pinealectomy. Forty intact animals served as the control. Material for investigation was taken on the 1st, 5th, 15th, 30th, 45th, 60th, 90th, 120th, and 150th days of the experiment. The pellets were implanted in the right parietal region of the rats' brain. Pinealectomy was performed by the method of Simonnet and Thieblot.

Seven induced brain tumors also were studied. Material for electron microscopy was treated in the usual way. Electron micrographs were obtained with magnifications of 3000 to 90,000, followed by photographic enlargement of 3-3.5 times.

EXPERIMENTAL RESULTS

Changes in the submicroscopic organization of the astrocytes were found to differ in type in different periods of chemical carcinogenesis.

On the 15th-30th days of the experiment astrocytes with oval nuclei and with karyogranules located predominantly beneath the nuclear membrane were more commonly seen. The perinuclear space was widened (Fig. 1). The granular endoplasmic reticulum (GER) consisted of cavities of different shapes and sizes with numerous attached ribosomes. A sharp increase in the number of polysomes, hypertrophy of the lamellar complex, and the presence of lysosomes were observed in the cytoplasm. The mitochondria had well-defined membranes, their cristae were disoriented, and the matrix was clear. By the 45th day the intensity of intracellular hyperplasia in the astrocytes was reduced. On the 60th-90th days the cytoplasm of most astrocytes was more osmiophobic than at the previous time of investigation. Their nuclei were round or oval in shape and the nuclear pores were dilated. The volumes of the lamellar complex and GER were reduced. The mitochondria were mainly round, with a

Department of Pathological Anatomy, Tashkent Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 371-375, April, 1979. Original article submitted May 29, 1978.

 0007-4888/79/8704-0384$07.50

Fig. 1. Ultrastructure of reactive astrocyte on 30th day after intracerebral implantation of DMBA. Widening of perinuclear space, translucency of matrix of mitochondria (M), and hyperplasia of astrocyte organelles; 15,000 ×.

dense matrix and well-marked cristae. The number of ribosomes and polysomes was sharply reduced. Many lysosomes were present in the osmiophobic cytoplasm. The outer nuclear membrane formed cavities of different sizes because of widening of the perinuclear space.

However, careful study of a large number of serial sections through the brain tissue in the neighborhood of the DMBA revealed, as early as on the 45th-90th days, individual cells that differed sharply in their ultrastructure from astrocytes with degenerative and reactive changes. The number of such cells increased considerably later (120-150 days) when, as morphological investigations [3, 4] have shown, microgliomas appear. The nuclei of these cells were irregular in outline because of the many deep invaginations formed by the nuclear membrane. A well-marked focal widening of the perinuclear space with the formation of cavities of different sizes, in close contact with the mitochondria and cisterns of the rough reticulum, were characteristic. The lamellar complex mostly showed hyperplasia and hypertrophy of its vacuolar component. Mitochondria of varied shape and size were extremely swollen, with disorganization and reduction of their cristae and with complete or partial translucency of their matrix. Often close contacts were observed between the mitochondria and dilated vacuoles of the GER. Laminar bodies also were present.

In the pretumor period the ultrastructure of the GER in the astrocytes of atypical structure was considerably modified and became very variable. By the study of its submicroscopic organization three types could be distinguished: 1) a poorly developed, disorganized GER, consisting of separate cisterns and vacuoles of different sizes. Many ribosomes and polysomes could be found in the cytoplasm of these cells; 2) a well developed GER, consisting of greatly dilated cisterns and large vacuoles of irregular shape, occupying a large part of the cytoplasm (Fig. 2); 3) a powerfully developed GER in the form of a system of branched tubules with many attached ribosomes. Astrocytes containing this type of GER were very similar in their ultrastructure to glandular cells.

In some cases communication between the ribosomes and membranes of the rough reticulum was disturbed, so that there were more free than membrane-bound ribosomes, a characteristic feature of malignant tumors.

The study of the ultrastructure of cells of the astrocytoma and glioblastoma multiforme revealed their identity with atypical astrocytes in the pretumor period. For instance, astrocytoma cells were characterized by oval nuclei with a winding membrane and a uniform

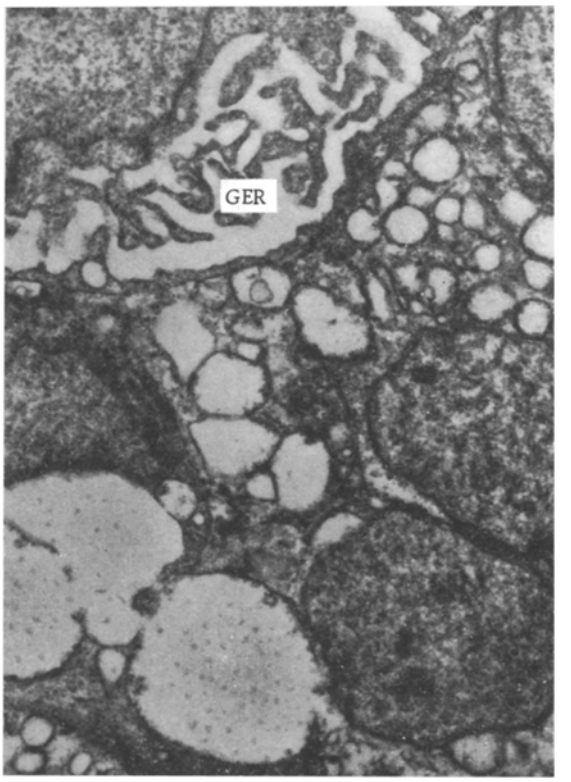

Fig. 2

Fig. 3

Fig. 2. On 90th day after intracerebral implantation of DMBA, GER of atypical structure present in astrocytes in the form of numerous cavities of different sizes, together with a few ribosomes. Mitochondria (M) swollen, with translucent matrix and destroyed cristae; 50,000 ×.

Fig. 3. Variability of ultrastructure of glioblastoma multiforme cells. Cell with branched cisterns of GER, similar in ultrastructure to cells of glandular organs, visible in top part of photograph; 15,000 ×.

distribution of karyogranules. Regions with marked widening of the perinuclear space were closely linked with the cisterns of the rough reticulum. Mitochondria consisted of vacuoles of different sizes. Hyperplasia of the membrane profiles and dilatation of their cavities were observed in the GER, as a result of which the cytoplasm was filled with numerous vesicles with an extremely irregular distribution of ribosomes. The lamellar complex was represented mainly by large vacuoles, uniformly filling the cytoplasm.

Cells of glioblastoma multiforme were characterized by nuclei divided into many lobules by deep invaginations. Widening of the perinuclear space was distinctly localized, with the formation of relatively large lacunae in the cytoplasm. Degranulation and the formation of large cysts were particular features of the rough reticulum in this tumor. Similar cells also were found in the pretumor period. The mitochondria consisted of osmiophobic vacuoles and the integrity of the membrane in one area was disturbed. This is an important fact, for it evidently is related to the provision of energy for the actively functioning GER. The close proximity of the mitochondria to the dilated cisterns of the GER and to the outer nuclear membrane in the zone of widening of the perinuclear space, observed in the tumors, and the direct continuity between the perinuclear space and the dilated cisterns of the GER are evidence of intensive protein synthesis in the tumor-forming astrocytes.

On the basis of these results it was postulated that the so-called microcysts in the cytoplasm of the tumor astrocytes are formed from mitochondria, dilatation of the cisterns of the GER, and widening of the perinuclear space. Cyst formation in the cytoplasm of the tumor cells is evidently based on intensive protein synthesis in the GER system, confirming Tushevskii's views [12] on the mechanism of cyst formation in human cystic astrocytomas.

The changes described above were found when the carcinogen was implanted into the brain of pinealectomized animals. In the experiments in which DMBA was implanted in the brain of intact animals only the phase of intracellular hyperplasia was observed (on the 15th-30th days), but by contrast with the experiment with the paraffin wax pellet, this phase developed later and lasted longer because of the ability of the carcinogen to inhibit repair processes. Not until the 120th-150th days was this reactive gliosis replaced in some cases by degenerative changes in the astrocytes. This indicates that the degenerative changes developing after pinealectomy constituted the background process against which the neoplastic transformation of the glial cells took place more rapidly. The electron-microscopic data confirmed the writers' previous opinion [3, 4] that the period characterized by the development of degenerative changes in the brain tissues, against the background of which atypical cells appear, must be regarded as a period of preglioma. Proliferation of these cells leads to the appearance of proliferative foci and later of microblastomas.

The results suggest that malignant transformation in neuroglial cells under the influence of DMBA begins much earlier than microscopically demonstrable proliferation, in confirmation of Avtsyn's views [8] regarding the presence of a preproliferative period of tumor development. The following conclusions can be drawn from these results.

1. The period preceding tumor development in the brain is characterized by the development of degenerative changes in the brain cells and the appearance, against this background, of cells of atypical ultrastructure.

2. The basic changes during neoplastic transformation of astrocytes consist of hyperplasia of the organelles determining the degree of protein synthesis in the cell, disturbance of connections between ribosomes and membranes of the GER, hypertrophy of the vacuolar component of the lamellar complex, marked swelling of the mitochondria, and increased activity of the nuclear and plasma membranes.

3. Changes in the submicroscopic organization of the astrocytes developing after intracerebral implantation of a chemical carcinogen can be subdivided into three principal stages: I) intracellular hyperplasia of the organelles, due to brain trauma in the course of intracerebral implantation of DMBA; II) degenerative changes connected both with disturbance of the vascularization of the tissue and with the direct action of the carcinogen on the cells; III) disturbance of differentiation of atypical ultrastructural reorganization.

LITERATURE CITED

1. M. S. Abdullakhodzhaeva, in: Proceedings of a Scientific Conference of Morphologists of Kazakhstan on the Occasion of the 50th Anniversary of Soviet Power [in Russian], Karaganda (1967), p. 3.
2. M. S. Abdullakhodzhaeva, "Histopathology of experimental carcinogenesis in the rat central nervous system," Author's Abstract of Doctoral Dissertation, Moscow (1968).
3. M. S. Abdullakhodzhaeva, Carcinogenesis in the Central Nervous System [in Russian], Tashkent (1971), p. 175.
4. M. S. Abdullakhodzhaeva, T. K. Ibragimov, Ya. Zh. Otepov, et al., in: Abstracts of Proceedings of the 8th All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Tashkent (1974), p. 3.
5. A. P. Avtsyn, in: Proceedings of the 8th International Cancer Congress [in Russian], Vol. 4, Moscow—Leningrad (1963), pp. 373-374.
6. A. P. Avtsyn, Arkh. Patol., No. 11, 3 (1972).
7. A. P. Avtsyn, in: Problems in the Pathological Anatomy of Pretumor Processes [in Russian], Krasnodar (1973), pp. 11-12.
8. A. P. Avtsyn and Ya. P. Yablonovskaya, Byull. Éksp. Biol. Med., No. 8, 46 (1971).
9. V. V. Arkhangel'skii, Arkh. Patol., No. 12, 52 (1961).
10. I. N. Dimant, Experimental Tumors of the Central Nervous System [in Russian], Tashkent (1966), p. 205.
11. V. F. Tushevskii, "Ultrastructural organization of macroglial tumors of the human brain," Author's Abstract of Doctoral Dissertation, Kiev (1976).
12. B. S. Khominskii, Uchen. Zapiski Kiev. Nauch.-Issled. Rentgenoradiol. Onkol. Inst., 5, 150 (1955).
13. H. Kroh, Neuropatol. Pol., 7, 55 (1969).
14. H. Simonnet et al., Acta Endocrinol. (Copenhagen), 7, 306 (1951).
15. A. Zageman and W. Dietz, Experientia, 28, 1344 (1972).