Elongated Nuclear Sheet and Intranuclear Myelin Figure of Human Medulloblastoma

Eiichi Tani, Juji Takeuchi, Yutaka Ishijima, Noboru Higashi, Eiichi Fujihara, Toshio Ametani, and Kyozo Ando

Department of Neurosurgery, Kyoto University Medical School [E. T., J. T., Y. I.,] and Institute for Virus Research, Kyoto University [N. H., E. F., Shogoin, Kyoto, Japan, and Department of Neurosurgery, Osaka Red Cross Hospital, Osaka, Japan [T. A., K. A.]]

SUMMARY

Nuclei of medulloblastoma cells revealed two types of structural changes: formations of cistern-limited nuclear sheets and intranuclear vacuolar profiles or myelin figures. The cistern-limited nuclear sheets were extremely elongated and were contained on either side by the perinuclear cisterns. They were usually composed of a central granular or beaded heterochromatin layer between less dense layers, were further limited on either side by the inner nuclear membrane, and showed an approximately constant width of between 350 and 430 Å. The central granular heterochromatin layer in the cistern-limited nuclear sheet was continuous with the peripheral beaded layer of the heterochromatin granules in the nuclei. In addition, in the nuclear periphery a less dense layer was seen between the inner nuclear membrane and the peripheral heterochromatin layer. The structural association of the inner nuclear membrane and its underlying less dense layer, as well as the peripheral heterochromatin layer, was similar in the cistern-limited nuclear sheet and in the nuclear periphery and seemed to form a structural and functional unit. The intranuclear vacuolar profiles and myelin figures were irregular in form and might be derived from the nuclear membranes by a complicated, irregular invagination. The membranes of the vacuolar profiles and myelin figures demonstrated increased density and thickness and surrounded flocculent substances or islands of cytoplasm and were not covered by the heterochromatin layers.

INTRODUCTION

Medulloblastomas are derived from the most primitive form of glial cells and commonly arise in the posterior midline or vermis of the cerebellum. Electron microscopy of medulloblastomas showed 2 types of cells (41, 44). The 1st type was characterized by the presence of a nucleus with a heavy chromatin net, as well as by the presence of fine filaments and glycogen particles in the cytoplasmic processes. In addition, cilia, microtubules, and intercellular attachment devices were occasionally observed. The 2nd type exhibited evenly distributed chromatin in the nuclei and microtubules in the cytoplasmic processes. However, little attention has been drawn to the fine nuclear structure of these cells. This report is concerned with several characteristics found in the nuclei of human medulloblastomas.

MATERIALS AND METHODS

The materials used in this study were taken from 2 patients with tumors in the posterior midline of the cerebellum. Hematoxylin and eosin staining of paraffin-embedded specimens revealed medulloblastomas with round or slightly oval nuclei and scant cytoplasmic bodies of pyriform shape. There was no newly formed supporting meshwork of reticulin fibers. The reticulin which was present was part of the already existing perivascular regions. Specimens for electron microscopy were carefully removed, without interrupting the vascular supply to the tumors, and placed immediately into a cold, 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4). The specimens were rapidly diced into small pieces which were then fixed in phosphate-buffered 2.5% glutaraldehyde solution (pH 7.4) at 4° for 2 hr. After being washed briefly in the phosphate buffer solution, the specimens were postfixed in 1% OsO₄ in 0.1 M phosphate buffer solution (pH 7.4) at 4° for 2 hr, dehydrated in an ascending concentration of alcohol, and embedded in Epon. All embedded blocks were cut with glass knives on a LKB ultramicrotome. Thin sections were stained with uranyl acetate for 5 min and with lead citrate for 3 min on the grid and were examined with a HU-11 A electron microscope.

RESULTS

Nuclei of human medulloblastoma cells were variable in shape; some were round or ovoid, and others were irregularly ovoid or lobulated, frequently demonstrating an irregular infolding of the nuclear membrane. One of the most characteristic features in the nuclei was an elongated nuclear sheet (Figs. 1 to 11), up to 4 μ long, limited on either side by an electron-lucent cleft. The electron-lucent cleft was continuous with the perinuclear space at the origin of the nuclear sheet, usually surrounded by the folded outer and inner nuclear membranes, and often irregular in width because of an undulation of the outer nuclear membrane alone. The nuclear sheet usually consisted of a central granular or beaded heterochromatin layer between less dense layers and was...
further limited on either side by a layer of the folded inner nuclear membrane. The total width of the nuclear sheet was remarkably constant and ranged from 350 to 430 Å (Figs. 1 to 11).

The relationship of the nuclear sheets to the main portions of nuclei and the cytoplasm was variable in appearance in sections and classified into 3 main features. The 1st feature (Figs. 2 to 7 and 11) consisted of a long, often tortuous extension of the nuclear sheet reaching out into the cytoplasm. Such long, pedunculated processes of chromatin often showed a terminal loop and sometimes a nuclear loop in its course (Fig. 4). Second, the elongated nuclear sheet connected as a bridge with 2 adjacent lobulated portions of nucleus (Figs. 5, 7, and 8), and a nuclear loop was sometimes formed in the course of the bridging nuclear sheet (Fig. 8).

Last, mostly single and occasionally multiple parallel nuclear sheets were often observed lining a limited or considerable portion of the nuclear perimeter (Figs. 1 and 9). Generally, it appeared likely that as the incidence of cells with nuclear sheets increased the number of nuclear sheets per nucleus also increased, and multiple, separate, or parallel nuclear sheets were observed in the cell profile. The nuclear loop formed in the course of the nuclear sheet usually revealed nuclear pores in its nuclear membrane (Figs. 1 to 11). Often a similar single row of heterochromatin granules was clearly found beneath the inner nuclear membrane of the nuclear sheet (Fig. 13). It might reasonably be assumed that the intranuclear vacuolar profiles or myelin figures were sometimes visible inside the fused nuclear membrane (Fig. 13). They were not correlated structurally with any nuclear inclusions and were different from the nucleoplasm. Flocculent substances were often diffusely scattered in the vacuolar profiles and myelin figures (Figs. 3, 7, and 15), in which -cytoplasm occasionally was evident (Fig. 15). When many sections were carefully examined, it was found that the outer and inner nuclear membranes were invaginated into the nuclei or evaginated into the cytoplasm and were so close to, or fused with, each other so as to show an increased opacity and thickness (Figs. 1, 3, 7, 13, and 15). The heterochromatin granules along the inner nuclear membrane were not seen beneath the fused nuclear membranes (Figs. 1, 3, 13, and 15). Instead, flocculent substances similar in appearance to those in the vacuolar profiles or myelin figures were sometimes visible inside the fused nuclear membrane (Fig. 13). It might reasonably be assumed that the intranuclear vacuolar profiles and myelin figures could be formed by a complicated invagination and an increase in opacity and thickness of the fused inner and outer nuclear membranes. The tendency to form myelin figures was not limited to the nuclear membranes to be continuous with the peripheral heterochromatin layer in the nuclear periphery (Figs. 1, 7, and 8). Consequently, the peripheral heterochromatin granules in the nuclear perimeter appeared to be fused with each other to form the central heterochromatin granules in the nuclear sheet. However, the heterochromatin granules in the nuclear sheet were similar in size and arrangement to those in the nuclear periphery. A less dense layer between the central heterochromatin layer and the inner nuclear membrane of the nuclear sheet was usually irregular in outline on the heterochromatin side, because of the presence of the closely arranged heterochromatin granules, and was approximately 70 to 100 Å wide (Figs. 1 to 11).

A similar less dense layer was also frequently visible between the inner nuclear membrane and the peripheral heterochromatin layer (Figs. 1, 2, 7, 8, and 10) and was continuous with the less dense layer in the nuclear sheet (Figs. 1, 7, and 8). When sectioned tangentially, ill-defined microvesicular profiles and striations were revealed in the nuclear sheet (Fig. 12). When the central heterochromatin layer in the nuclear sheet partly disappeared, the inner nuclear membranes limiting either side of the nuclear sheet were close to each other and surrounded the less dense layer alone.

On occasion, the chromatin granules which are usually situated beneath the peripheral heterochromatin granules that form a beaded dense layer along the inner nuclear membrane were not found (Figs. 1, 2, 7, 8, and 10). When margination of the chromatin granules was evident, closely packed chromatin granules beneath the peripheral heterochromatin granules were often randomly oriented and very occasionally arranged parallel to the peripheral heterochromatin layer in a regular periodicity of approximately 70 to 100 Å (Fig. 5). A less dense layer, similar in appearance to that between the inner nuclear membrane and the peripheral heterochromatin layer, was evident between parallel heterochromatin layers near the nuclear periphery, and there was no evidence of an electron-lucent cleft there.

Another characteristic feature in the nuclei of medulloblastoma cells was the presence of vacuolar profiles and myelin figures of varying sizes and shapes (Figs. 3, 7, and 13 to 15). They were not correlated structurally with any nuclear inclusions and were different from the nucleoplasm. Flocculent substances were often diffusely scattered in the vacuolar profiles and myelin figures (Figs. 3, 7, and 15), in which -cytoplasm occasionally was evident (Fig. 15). When many sections were carefully examined, it was found that the outer and inner nuclear membranes were invaginated into the nuclei or evaginated into the cytoplasm and were so close to, or fused with, each other so as to show an increased opacity and thickness (Figs. 1, 3, 7, 13, and 15). The heterochromatin granules along the inner nuclear membrane were not seen beneath the fused nuclear membranes (Figs. 1, 3, 13, and 15). Instead, flocculent substances similar in appearance to those in the vacuolar profiles or myelin figures were sometimes visible inside the fused nuclear membrane (Fig. 13).
but was also seen in the cytoplasmic membranes of the endoplasmic reticulum, mitochondria, and cell membranes.

**DISCUSSION**

The cistern-limited nuclear sheets observed in the present study are not unique to medulloblastoma and are found in other tissues in terms of nuclear sheets, nuclear blebs, nuclear pockets, nuclear projections, or triple-membered structures. In the central nervous system, they were seen in an intracranial cancer induced by a simian adenovirus (30), in retinoblastoma (3, 7, 36), and in fetal retina (36). In cancers, similar structures also were reported in various types of leukemia and lymphomas (1, 2, 4, 16–18, 20, 29, 31–34, 37, 43, 45), lung carcinoma (31), pleomorphic adenoma of the parotid gland (31), and dermatofibrosarcoma (31), as well as in different cultured cell lines infected with virus (5, 8). In the nonneoplastic pathological conditions, similar cistern-limited chromatin was shown in the neutrophils and eosinophils of a congenital chromosomal abnormality (trisomy syndrome) (23, 28). In addition, many examples of the cistern-limited nuclear sheets were reported in a variety of presumably normal tissues e.g., 6-week-old guinea pig thymus (42), fetal human thymus (38), rat pancreatic acinar cells (10), and human leukocytes (24, 39, 40, 45).

The central dense layers of the cistern-limited nuclear sheets were considered as closely packed heterochromatin granules. In this study, similar arrangements of the heterochromatin granules were found beneath the inner nuclear membranes of the main portions of nuclei but were absent in the nuclear pores. Davies and Tooze (14, 15) regarded the membrane-limited chromatin sheets as indicative of the presence of a specific structural unit of the heterochromatin. Later, Davies (12, 13) confirmed this finding when he found regularly oriented layers of chromatin parallel to the nuclear membranes in erythrocytes from chicken and lamprey. The parallel layers of chromatin granules may be seen, therefore, either as layers bounded by infolding of the nuclear membrane or simply as layers of chromatin granules in the nucleoplasm.

Recent studies have concentrated on a layer existing immediately beneath the nuclear membrane in cells from certain vertebrates, termed the “fibrous lamina” by Fawcett (19) and the “zonula nucleum limitans” or “nuclear limiting zone” by Patrizi and Poger (35). These findings are of great interest in the manner in which they may relate to the less dense layer found between the inner nuclear membrane and the central heterochromatin layer of the cistern-limited nuclear sheet in this study. The zonula nucleum limitans is similar to the fibrous layer, 150 to 200 Å thick, found in certain vertebrates (19). The present, less dense layers between the inner nuclear membrane and the peripheral or central heterochromatin layer of the cistern-limited nuclear sheet were much narrower (approximately 70 to 100 Å) and less electron dense than were the fibrous layers. Upon tilting the section in the microscope, Davies contended that the less electron-dense layer corresponded to a triple-structured band of half-width (212 Å) demonstrated in the nuclei of erythrocytes from chickens and lampreys. Although the triple-structured band, consisting of a less dense band, a dense band (130 to 170 Å), and a 2nd less dense band, might be present in the nuclei of medulloblastoma cells, the presence of a heterochromatin layer in the cistern-limited nuclear sheet could be of great significance.

On careful examination of the peripheries of the nuclei in the medulloblastoma cells, the inner nuclear membrane and the underlying less electron-dense layer, as well as the peripheral heterochromatin layer, were clearly visible when the inner nuclear membrane was sharpest, and they seemed to form a structural unit. In addition, the 2nd chromatin layer, parallel and subjacent to the peripheral heterochromatin layer, was not always visible, and the peripheral heterochromatin layer alone was sometimes present in the nuclear periphery of the present medulloblastoma cells, although Davies indicated the presence of parallel heterochromatin layers near the nuclear envelope. The continuity of the 3 structures, the inner nuclear membrane, the less electron-dense layer, and the heterochromatin layer, between the nuclear periphery and the cistern-limited nuclear sheet might suggest that these 3 structures could constitute a significant structural and functional unit. In isolated nuclei of muscle cells, Franke and Schinko (21) observed a frequent indentation of the inner nuclear membrane alone, and they suggested a relatively tight connection of the inner nuclear membrane to the framework of the peripheral nuclear layer. Flattened canaliculi formed by an invagination of the inner nuclear membrane alone were observed in a variety of cells (9, 22, 25–27, 46). The intranuclear canaliculi were also covered by thin layers of heterochromatin, except in the nucleoli. It might be reasonable to assume, therefore, that the cistern-limited nuclear sheet could possibly be related to some widely diffuse type of organization of heterochromatin and to unknown behavior of actively proliferating nuclei.

It was suggested that the intranuclear vacuolar profiles and myelin figures were derived from the nuclear membranes. The myelin figures might be artifactual, since they were seen in hepatocytes of embryo chicks and in brown adipose tissue of perinatal rats only after glutaraldehyde fixation or glutaraldehyde-OsO₄ double fixation (6, 11). However, they have scarcely been observed in nuclei of other types of human gliomas similarly fixed with glutaraldehyde and OsO₄. It might be suggested that the myelin figures in medulloblastoma represent an abundance of available phospholipid or lipoprotein which is disorganized or transferred by glutaraldehyde. Their increased opacity and thickness might suggest a macromolecular alteration of the membrane structure itself, and the heterochromatin layers were usually not present beneath the membranes of the vacuolar profiles and the myelin figures. Consequently, qualitatively different changes might occur in the nuclear membrane of the medulloblastoma cell in the formation of the cistern-limited nuclear sheet and the intranuclear vacuolar profiles or myelin figures.

**REFERENCES**

Nuclear Sheet and Intranuclear Myelin Figure


Fig. 1. A nucleus of a medulloblastoma cell is irregularly lobulated, and 5 nuclear sheets (Arrow 1) are seen along the nuclear periphery. The outer and inner nuclear membranes appear to be fused with each other, and the chromatin granules are not found beneath the fused nuclear membrane (Arrow 2). A heterochromatin layer is linearly arranged along the inner nuclear membrane in places (Arrow 3). X 30,000.

Fig. 2. A nuclear sheet with a terminal nuclear loop is limited by the perinuclear space. A linear arrangement of heterochromatin granules is seen in the center of the nuclear sheet as well as beneath the inner nuclear membrane of the terminal loop and the main portion of the nucleus. X 67,000.

Fig. 3. In addition to a nuclear sheet with a terminal nuclear loop, the outer and inner nuclear membranes appear to be fused with each other and exhibit increased density (arrow). Four irregular vacuolar profiles limited with the membranes of the increased density are visible in the nucleus. X 58,000.

Fig. 4. A nuclear sheet with a terminal loop forms a nuclear loop in its course. Two nuclear pores (arrows) are visible in the nuclear loop where the heterochromatin layer is absent. X 51,000.

Fig. 5. Four nuclear sheets are seen in a nucleus. Three show terminal nuclear loops, and 1 (Arrow 1) spans 2 lobulated parts of the nucleus. Linearly arranged heterochromatin layers are seen parallel to the peripheral heterochromatin layers in places (Arrow 2). X 58,000.

Fig. 6. Two parallel nuclear sheets are limited with a common perinuclear space, and a space between them (arrow) is limited with the inner nuclear membrane alone. X 84,000.

Fig. 7. A linearly oriented heterochromatin layer is visible beneath the inner nuclear membrane (Arrow 1) and in the centers of 2 nuclear sheets. Continuity is evident in the heterochromatin granules between the nuclear sheet and the periphery of the nucleus (Arrow 2). In addition, 4 myelin figures and vacuolar profiles are found in an irregularly lobulated nucleus and contain flocculent substances in their centers. A portion of cytoplasm is continuous with a myelin figure (Arrow 3). X 41,000.

Fig. 8. A nuclear loop is visible in the course of a nuclear sheet spanned between 2 lobulated portions of a nucleus, and 2 nuclear pores are seen in the loop (Arrow 1). The heterochromatin granules in the nuclear sheet seem to be continuous with the peripheral heterochromatin granules arranged in a layer (Arrow 2). X 71,000.

Fig. 9. A nuclear sheet is seen along the nuclear periphery. A space between the nuclear sheet and the main portion of the nucleus is limited with the inner nuclear membrane alone. X 55,000.

Fig. 10. A portion of cytoplasm is interposed between a nuclear sheet and its main nucleus. In this case, the nuclear sheet is limited by the perinuclear space lined with the inner and the outer nuclear membranes. A linearly oriented heterochromatin layer is seen beneath the inner nuclear membrane. X 57,000.

Fig. 11. Each nuclear sheet is composed of a central, beaded heterochromatin layer between less dense layers and further limited on either side by a layer of the inner nuclear membrane. The outer nuclear membrane along the nuclear sheet is wavy. X 88,000.

Fig. 12. Tangential sections of nuclear sheets exhibit ill-defined striations and microvesicular profiles. X 59,000.

Fig. 13. The outer and inner nuclear membranes appear to be fused with each other and protrude into the cytoplasm. There is no evidence of heterochromatin granules beneath the fused nuclear membranes and, instead, flocculent substances are visible here. X 57,000.

Fig. 14. A myelin figure seems to be continuous with the outer and inner nuclear membranes. There is no evidence of the nuclear membrane between the myelin figure and the nucleoplasm. X 71,000.

Fig. 15. A portion of cytoplasm (C) is invaginated into a nucleus, and a surrounding membrane seems to be formed by fusion of the outer and inner nuclear membranes. The fused nuclear membrane is continuous with a myelin figure (arrow). Flocculent materials are seen in the myelin figures and vacuolar profiles. X 19,000.
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