Intranuclear Paracrystalline Fibrillar Arrays in Human Glioma Cells

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SUMMARY

Characteristic paracrystalline fibrillar arrays, some of which displayed interfilamentous septa, were found in the nuclei of human astrocytoma and glioblastoma multiforme cells. In astrocytoma, they were composed of bundles of linear filaments, each about 70 Å thick, oriented in a regular spacing of about 300 Å in periodicity. Others exhibited periodicity of approximately 170 Å. These figures had a diameter of about 40 Å. Some filaments appeared to separate into two parallel components that sandwiched a clear layer, and others consisted of a linear arrangement of short filaments at regular intervals. In addition, a few of them showed small particulates, approximately 170 Å. These figures had a diameter of about 300 Å in periodicity. Others exhibited periodicity of possibly a cross-sections of filaments, which were regularly oriented in a linear array. In glioblastoma multiforme, filaments about 60 Å thick were regularly arranged with a periodicity of around 200 Å.

The paracrystalline fibrillar arrays were closely associated with the fibrillar components of the nuclear bodies in glioblastoma multiforme and usually were associated with free lipid droplets in astrocytoma. On the other hand, lipid droplets as well as nematosomes, tubular structures, and flocculent materials were found in the widened perinuclear gap in the nuclei of astrocytoma cells. In the latter sites, they were surrounded by the inner nuclear membrane alone. On the basis of electron microscopic observations of the nuclei of human glioma cells, two possible origins of the intranuclear paracrystalline arrays might be considered.

RESULTS

Intranuclear fibrillar rodlets in malignant gliomas as reported by Robertson and Maclean (35) were occasionally found in the nuclei of the glioma cells in the present study and were composed of bundles of filaments running parallel with each other (Fig. 1), but the center-to-center distances between the individual filaments did not seem as regular.

On the contrary, intranuclear paracrystalline fibrillar arrays (Figs. 2 to 7 and 11) revealed characteristically a regular spacing of filaments and were variable in size, up to about 1.6 X 1.0 μ. They displayed usually 1 and sometimes 2 axes of the constituent filaments. The chromatin substances were absent in the paracrystalline arrays.

In astrocytoma, the intranuclear paracrystalline arrays were usually associated with lipid droplets that showed no surrounding membrane and varied in number, from 1 to 9 (Figs. 2 to 4 and 7). The paracrystalline arrays showed various features mainly due to the axis of sectioning and were divided into 2 types of spacings, depending upon thickness and center-to-center distance of the filaments. In the 1st type of
spacing, the individual filaments were about 70 Å thick and were arranged in a regular periodicity of about 300 Å (Figs. 2 to 7). The 2nd type of spacing consisted of bundles of filaments about 40 Å thick possessing a periodicity of approximately 170 Å (Fig. 4). Each filament was usually a dense linear structure, not wavy in appearance, and sometimes was separated into 2 dense linear components sandwiching a clear linear layer (Figs. 3 and 4). Some filaments revealed a beaded feature (Fig. 6) and appeared to consist of a linear arrangement of short filaments, the average 200 Å long, at comparatively regular intervals (Figs. 2 and 3). Some paracrystalline arrays consisted of short particulates, each around 70 to 80 Å in diameter, which were linearly oriented at regular intervals of 200 Å (Figs. 6 and 7).

Dense particulates were seen at comparatively regular intervals at the middle between adjacent filaments, and small septa were seen crossing the interfilarmentous space from one filament to an adjacent one (Figs. 3 to 7). In the latter case, the septa crossed the interfilarmentous space perpendicularly or obliquely to the axes of the filaments, and some paracrystalline arrays seemed to be composed of a beaded arrangement of microvesicular profiles. Although the paracrystalline arrays with the interfilarmentous septa appeared to form a lattice, they were different in structure from a regularly oriented filaments. In addition, the linear orientation of dense particulates was about 300 Å, which was similar to the spacing between adjacent filaments described above. Consequently, the linear arrangement of dense particulates could possibly be a cross-section of the regularly oriented filaments. In addition, the linear orientation of short filaments at relatively regular intervals might be an oblique section of the paracrystalline fibrillar array.

In connection with the presence of free lipid droplets closely associated with the intranuclear paracrystalline arrays, similar lipid droplets with a surrounding membrane were seen in the margin (Figs. 9 and 10) and the center (Fig. 8) of nuclei. In the margin, the nuclear membrane was complexly invaginated, and isolated cytoplasm appeared in the nuclei. The cytoplasmic islands in the nuclei were usually surrounded by 2 layers of nuclear membrane. In contrast, the lipid droplets in the vicinity of the nuclear membrane were surrounded by the inner nuclear membrane alone. The inner nuclear membrane also was invaginated to form an intranuclear canaliculus (Fig. 9). The space containing the lipid droplets was therefore a widened perinuclear space in which delicate, flocculent materials also were diffusely scattered (Figs. 8 and 10). In addition, nucleolus-like structures, as well as tubular structures oriented in a parallel way, were present in the widened perinuclear space (Fig. 10). The nucleolus-like structures consisted of a convoluted network of electron-opaque strands, which appeared to be made of an entanglement of tightly packed small filaments and particles. The tubular structures were about 260 Å in diameter and appeared as a microvesicular profile in cross-sections (Fig. 8); the longitudinal sections demonstrated 2 parallel linear structures sandwiching a space of an intermediate density (Fig. 10). Although several lipid droplets were present in the nucleus, the cytoplasm did not show numerous lipid droplets, nor were there degenerative changes in the cytoplasmic organelles.

The nuclei of human glioblastoma multiforme cells have already been described in detail by Robertson and Maclean (35). In addition to various nuclear structures, including the nuclear bodies and the fibrillar rodlets, paracrystalline fibrillar arrays similar in structure to those in human astrocytoma were evident in the nuclei of human glioblastoma multiforme cells (Fig. 11). Each constituent filament, about 60 Å thick, ran parallel to the others at regular intervals of about 200 Å. In addition, small septa crossed the interfilarmentous space obliquely or perpendicularly at relatively regular intervals of 150 Å. The intranuclear paracrystalline fibrillar arrays here were not associated with lipid droplets. Delicate fibrillar structures surrounding the chromatin substances suggested fibrillar components of nuclear body and appeared to be closely associated with the paracrystalline arrays.

**DISCUSSION**

Siegesmund et al. (36) provided the first definitive ultrastructural description of intranuclear fibrillar rodlets in the central nervous system in a correlative light and electron microscopic study of some neurons. They noted that the rodlets consisted of oriented fibrils or filaments about 50 to 70 Å in diameter, which appeared to be distinctly different from the chromatin in the surrounding neoplasm. Very similar filament formations have subsequently been described in electron microscopic studies of a variety of cells comprising the vertebrate and invertebrate nervous system (9, 11, 14, 17, 23—25, 28, 33—35, 37, 39, 40), pineal chief cells (1), epithelial cells of anterior pituitary of female rabbit and dog epididymis (7), and pancreatic islet β-cells (4). In other tissues, these filament formations have also been encountered occasionally in connection with some viral states (8, 12, 26, 27, 30, 31).

The filaments in most intranuclear fibrillar rodlets reported thus far were closely packed and roughly parallel to each other, usually indicating no regular spacing as observed in the nuclei of human glioma cells in this study. In this regard, the intranuclear lattices reported in a variety of neurons (5, 9, 11, 23, 25, 28, 32) demonstrated a double lattice with arrays of regularly spaced filaments that crossed each other at an angle. However, since the intranuclear lattices appeared not infrequently in the same neuronal nuclei in which the fibrillar rodlets were found and merged or were continuous with a bundle of fibrils (9, 11, 23, 25), the 2 structures might represent variations in the orientation of similar macromolecules. The intranuclear inclusions of reactive cells...
associated with vaccinia virus-infected human skin (30) revealed a paracrystalline array of regularly oriented fibrils that were most similar in structure to the paracrystalline arrays present in astrocytoma cells. Kubai and Ris (18) observed a unique crystalline body, closely associated with the nucleus of the dinoflagellate nucleus, which had longitudinal sections similar in appearance to the present paracrystalline arrays and a honeycomb structure revealed in its cross-sections.

As to the possible origin of the filaments comprising the paracrystalline array, the present observation suggested 2 possibilities. Fibrillar materials of the nuclear body in glioblastoma multiforme shown in Fig. 11 were closely associated with lipid droplets in the perinuclear space of the astrocytoma cells could not be confirmed in this study. Similar layers of the nuclear membranes around the lipid droplets. The morphology of the lipid droplets in the nuclei is paracrystalline array, the present observation suggested 2 possibilities. Fibrillar materials of the nuclear body in glioblastoma multiforme suggested, on the basis of light and electron microscopic observations of fixed sympathetic ganglion preparations, that the nucleolus and its presumed spheroidal granulofibrillary body derivatives were involved in the assemblage and functional dynamics of the fibrillar rodlets. Lane (19), as well as Dutta et al. (10), considered this possibility likely, based principally upon the close proximity of the rodlet formations to the nucleolar complex or its environs, as well as the similarity in morphology, fine structure, and staining reactions between certain well-delineated, largely fibrillar regions within some neuronal nucleoli and the granulofibrillary bodies.

Another possibility was suggested by the fact that the intranuclear paracrystalline arrays were, for the most part, present in association with lipid droplets in the nuclei of the astrocytoma cells in our study. On the other hand, membranes surrounding similar lipid droplets in the nuclei might have arisen from cross-sections of invaginations of the inner nuclear membrane alone. The presence of lipid droplets in the perinuclear gap and the invagination of the inner nuclear membrane were strongly suggestive of this hypothesis. The invagination of the inner nuclear membrane as an intranuclear canaliculus was observed in a variety of cells (6, 15, 16, 21, 41). Our observations of the astrocytoma cells strongly suggest that the free lipid droplets in the nuclei might have arisen from the inner nuclear membranes primarily surrounding the lipid droplets.

The morphology of the lipid droplets in the nuclei is generally classified into 2 forms. The 1st form is a derivative of cytoplasmic invagination into the nuclei, demonstrating 2 layers of the nuclear membranes around the lipid droplets. The other type is free in the nucleoplasm without any association of the nuclear membrane and is usually considered to be formed as a result of destruction of the invaginated nuclear membrane (3, 20, 38). Palay (29) observed fat droplets in the perinuclear spaces of intestinal epithelial cells actively absorbing fat droplets and demonstrated that they arrived in the perinuclear gap by way of the endoplasmic reticulum from the cell surface. In the astrocytoma cells in this study, no marked accumulation of lipid droplets in the cytoplasm was evident. It is unknown at present how the lipid droplets appeared in the perinuclear space of astrocytoma cells.

The origin of tubular or nucleolus-like structures associated with lipid droplets in the perinuclear space of the astrocytoma cells could not be confirmed in this study. Similar nucleolus-like inclusions, although more dense in appearance than the present structures, were designated “nematosomes” by Grillo (13). They were evident in the cytoplasm of sympathetic neurons of adult rat and often were associated with smooth-surfaed and coated vesicles, suggesting that the vesicles might function in transporting bits of this material either to or from other regions of the cell.

If the free intranuclear lipid droplets in the astrocytoma might be derived from the lipid droplets by the invagination and final disappearance of the inner nuclear membrane, the close association of the free lipid droplets and the paracrystalline arrays could suggest that the latter structures might have arisen from either tubular, nematosome, or flocculent materials present in a close association with the lipid droplets in the perinuclear gap. The initial alignment and the ultimate ordering of filaments within an array might be determined by those conformational states of the filament macromolecules that satisfy their intrinsic physicochemical behavior within the array. Local ionic, pH, and related microenvironmental factors would also influence the physicochemical processes involved in filament alignment.

REFERENCES


Fig. 1. An intranuclear fibrillar rodlet (arrow) revealing no exact regularity of spacing is seen near the nucleolus of an astrocytoma cell. x 41,000.

Fig. 2. An intranuclear paracrystalline array with 2 axes of filaments is closely associated with 7 lipid droplets in an astrocytoma cell. The filaments demonstrate a regular spacing of about 300 Å in periodicity, and some of them are composed of a linear arrangement at comparatively regular intervals of short filaments. x 74,000.

Fig. 3. Four lipid droplets are seen in close association with a paracrystalline array with 2 axes of filaments. Some constituent filaments consist of a linear orientation at relatively regular intervals of short filaments (Arrow 1), and others appear to separate into 2 dense linear components sandwiching a less dense layer (Arrow 2). A series of interfilamentous septa also is visible (Arrow 3). x 113,000.
Fig. 4. Two paracrystalline arrays are present near a lipid droplet. The upper fibrillar array exhibits a regular spacing of about 300A in periodicity, and the lower one shows regular spacing around 170 A in periodicity. The individual filaments in the upper array appear to form 2 dense linear components in places (arrow), possibly by separation of each filament. X 103,000.

Fig. 5. A series of septa are seen obliquely or perpendicularly crossing the interfilamentous space between adjacent filaments (Arrow 1). Some filaments are located in the middle of the interfilamentous spaces of a paracrystalline array and appear to interlock with the constituent filaments of adjacent paracrystalline arrays (Arrow 2). Lipid droplets that are present near the paracrystalline array are not shown in this picture. X 68,000.
Fig. 6. Particulates or septa, although obscure, are seen in the interfilamentous spaces. An interlocking of filaments also is evident (Arrow 1). Regularly oriented particulates (Arrow 2) form a portion of paracrystalline array. Lipid droplets, although not shown in this picture, are present in the upper vicinity of the paracrystalline array. $\times 82,000$. 

Fig. 7. An intranuclear paracrystalline array is composed of regularly oriented filaments and particulates and is associated with 5 lipid droplets. $\times 69,000$.

Fig. 8. A lipid droplet surrounded by an inner nuclear membrane alone is present in the center of the nucleus of an astrocytoma cell. Four microvesicular profiles (arrow) are seen between the lipid droplet and the inner nuclear membrane. $\times 39,000$. 

Fig. 9. Two layers of the nuclear membrane of an astrocytoma cell are irregularly invaginated. The inner nuclear membrane is invaginated to form an intranuclear canaliculus on the one hand (Arrow 1) and to surround a lipid droplet on the other (Arrow 2). X 47,000.

Fig. 10. A lipid droplet, as well as a nematosome, 6 tubules, and flocculent materials, is evident in a widened perinuclear space formed by an irregular invagination of the inner nuclear membrane. X 45,000.

Fig. 11. A paracrystalline fibrillar array with interfilamentous septa is seen in the vicinity of the chromatin substance of a glioblastoma multiforme. Delicate fibrillar structures (arrow) surrounding the chromatin appear to be closely associated with the paracrystalline array. X 71,000.
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