Ultrastructural Characteristics of Human Neurilemoma Cell Nuclei

Satish Chandra, Michael J. Jerva, and Jack D. Clemis

Departments of Research [S. C.] and Surgery [M. J. J., J. D. C.], Mercy Hospital and Medical Center, Chicago, Illinois 60616

SUMMARY

Nuclei of human neurilemoma cells exhibit deep and extensive invaginations of part of their surface. Such invaginations contain cytoplasmic matter. However, in areas of the nucleoplasm distant from the invaginations, small membrane-bound bodies, some of which contain a “nucleoid,” occur either singly or grouped together and enclosed within a large membrane body. These small bodies are not considered virus-like. Degenerated nuclei from cultured tumor tissue contain spherical bodies, 130 to 230 nm in diameter, with spikes on their surface similar to those seen on envelopes of herpes-type viruses. Significance of these bodies in vivo and in vitro tumor tissues is not known.

INTRODUCTION

Variations in ultrastructural nuclear morphology are known to occur in pathological conditions. Most common among these are certain inclusions generally referred to as nuclear bodies and alterations in the nuclear surface configuration. Whereas many different forms of nuclear bodies have been described in various neoplastic diseases of man (4–6, 16, 23, 25, 26), indentations and projections of nuclear envelope are mostly found in lymphoproliferative neoplasms (1, 12, 18), nasopharyngeal carcinoma (14), and in cells infected with herpes-type virus (8, 9, 13, 22). This study describes both these ultrastructural characteristics in human neurilemoma cell nuclei and some unusual spherical structures in degenerated nuclei in cultured tumor tissue.

MATERIALS AND METHODS

**Tumor Tissue.** Samples of neurilemoma tissue from 3 patients were received from surgery in sterile 0.01 M phosphate-buffered saline containing penicillin and streptomycin. The tumor was very soft and a piece of it was processed for electron microscopy while the rest was used for in vitro cultivation. Growth and morphological characteristics of cultured neurilemoma cells will be described elsewhere (manuscript in preparation).

**Electron Microscopy.** Tumor tissue was fixed overnight in 2.5% glutaraldehyde in phosphate-buffered saline. It was sliced into approximately 1-cm pieces and washed twice in phosphate-buffered saline. It was postfixed for 1 hr in chromosmeium and after dehydration in graded concentrations of alcohol it was embedded in Epon-Araldite mixture. Sections 1 to 1.5 μm thick were cut on glass knives, stained with toluidine blue (7), and examined under a light microscope. Thin sections were cut on an LKB Ultrotome with a diamond knife, stained with lead and uranyl acetate, and examined under a Siemens-Elmiskop I electron microscope at an accelerating voltage of 80 kV.

RESULTS

Neurilemoma cells have oval nuclei in which the chromatin material is slightly marginated and also distributed throughout the nucleoplasm in small clumps (Figs. 1 to 4). The nucleolus is large and has the characteristic granular appearance. The cells were characterized as tumor cells because of the presence of (a) basement membrane around most of them, a fact which has supported the hypothesis that neurilemoma is derived from Schwann cells, and (b) specific granules not present in fibroblast cells of the stroma.

**Nuclear Invaginations.** Tumor cell nuclei generally have smooth surfaces; however, in some areas the nuclear surface is invaginated. Nuclear invaginations vary both in depth and in number (Figs. 1 to 6). Approximately 10% of nuclei in any field exhibit such invaginations. Since a part of a nucleus appears to be indented (Figs. 1 and 2) only certain planes of sectioning will contain indented nuclei, while in other planes the same nucleus will appear round or oval; a much greater percentage of tumor cell nuclei could therefore be invaginated.

Nuclear invaginations have bizarre shapes (Figs. 2 and 3) within the nucleoplasm, suggesting either a number of deep invaginations over a certain area of nuclear surface or extensive branching of an invagination within the bulk of a nucleus. Since the invaginations are lined with nuclear membrane with attached chromatin material, cytoplasmic matter can sometimes be seen within such invaginations (Figs. 2 to 5, C). In some areas, on the other hand, the double nuclear membrane with its attached chromatin is not seen and it becomes difficult to attribute precisely the partially enclosed matter as cytoplasmic (Figs. 3 and 4, arrows). Curved arrows (Fig. 3) point to material that appears similar to that within nuclear invaginations in other areas but is not enclosed by a nuclear envelope. The electron micrographs (Figs. 2 to 5) also exhibit the presence of a more conspicuous and abundant amount of perichromatin material along the nuclear invagination membranes than...
along the non-nuclear invagination membranes of tumor cells.

Dense osmiophilic bodies are sometimes seen within the nuclear invaginations. Some of these are membrane bound, while others are irregular, very osmiophilic, and not distinctly membrane bound (Figs. 3 and 4, heavy arrows). Some lie in nucleoplasm with only a single membrane around them, (Fig. 4, arrows).

Projection of Nuclear Envelope. Because of extensive invaginations of neurilemoma nuclei, projections of nuclear envelope are often observed (Figs. 1, 3, 5, and 6, NP). Occasionally, these projections are large and connect 2 lobes of a nucleus (Fig. 1, right).

Small Membrane-bound Bodies. Nuclei of neurilemoma cells also contain small membrane-bound bodies approximately 130 nm in size. Some of these contain a dense "nucleoid." They occur in clusters either free in the nucleoplasm (Figs. 5 and 6, arrows) or enclosed within a large membrane-bound body (Figs. 5 and 6, heavy arrows). Such bodies do not occur within nuclear invaginations and were not observed in the cytoplasm of tumor cells.

Nuclear Bodies. Nuclear bodies similar to those described by other investigators (5, 16) are found in nuclei of neurilemoma cells. These are nearly spherical bodies of fine fibrous material that gives them a whorl-like appearance. They often contain small spherical bodies (Figs. 7 and 8) some of which may be bound by a membrane. In Fig. 7 small dense bodies (arrows) measuring about 45 nm have an electron-lucent substance around them and are embedded in an amorphous matter that does not appear to be chromatin. The latter has a granular matrix (Fig. 7, heavy arrow). Occasionally, nuclear bodies also contain segments of membrane (Fig. 7, M). Similar membrane segments are also found free in the nucleoplasm. Fig. 8 shows a membrane-bound spherical body, measuring approximately 120 nm, within a nuclear body.

Cultured Cell. Nuclei of cultured neurilemoma cells generally exhibit normal ultrastructural morphology. However, in 5 to 10% of cultured cells the nucleus shows margination and condensation of chromatin (Fig. 9). Such cells also contain tubuloreticular structuring or undulating tubules (9) associated with endoplasmic reticulum in the cytoplasm (Figs. 9 and 10, arrows). In 1 culture, certain areas that represented degenerated nuclei contained unusual spherical bodies of various sizes (Fig. 11). Whereas a majority of these were 130 to 230 nm in diameter, few larger oval bodies were occasionally seen within the degenerated nuclear mass. These bodies were devoid of any osmiophilic substance, and at higher magnifications their surface had a bilamellar structure with spikes on the outermost and the innermost surfaces (Figs. 12 and 13). Such bodies were not seen in healthy cells. Fig. 14 shows for comparison spikes (arrows) on the envelopes of HSV* type 1 and on membranes that may be involved in the maturation of HSV type 1. Other membranes (heavy arrows) do not contain such spikes.

DISCUSSION

The most common morphological characteristic of a tumor cell nuclei is the increased size of its nucleolus. In the electron microscope, nuclear bodies have been described with increased frequency in pathological and neoplastic tissues as compared to normal tissues (5, 16). The former category includes viral infection.

Indentation of nuclear membrane and formation of lobules have been described in certain human neoplasms. In lymphoproliferative cancers, these ultrastructural variations are consistently observed both in vivo (1, 12, 14) and in vitro (8, 9, 13, 22). Such variations in nuclear surface were not reported in tumors of the nervous system (21, 25, 26) including a study of 50 cases of human neurilemoma (10, 11). It is possible that they escaped detection because of their localization on only a part of the nuclear surface as described in the present study.

Nuclear bodies have been observed in diseases associated with the human central nervous system, for example, in encephalitis (5) and subacute sclerosing leukoencephalitis (16). Their presence was reported in human brain tumors (5, 16, 25, 26) and in experimental lesions produced in brains of laboratory animals (23). Intranuclear small membrane-bound spherical bodies (Figs. 5 and 6) which may contain some dense material within them and which may either occur free in the nucleoplasm or within a larger membrane-bound body have not been described in the human central nervous system. They are not cytoplasmic bodies since they are not enclosed by nuclear membrane with the chromatin material on the outside as are areas marked Cy in Figs. 2 to 6. The possibility that they represent certain pathological change(s) in an otherwise apparently healthy cell cannot be ruled out. Although these membrane-bound bodies have nucleoid and are of the same size as herpes-type viruses, they are not considered virus-like particles.

The significance of nuclear bodies and of small membrane-bound spherical bodies in human neurilemoma tissue is not clear; however, the former has consistently been observed in viral infections both in vivo and in vitro, concomitant with the presence of an increased amount of perichromatin material as in this study. In human epidermal tissue infected with vaccinia virus, nuclear bodies were present in the infected cells whereas cells at the periphery of the biopsy specimen did not contain these bodies (19). Patrizi and Middelkamp (19) infected cultured WI-26 fibroblast cells with vaccinia virus and observed the presence of nuclear bodies in infected cells only. Other viruses that have been associated with such bodies are adenovirus type 12 (17), simian virus 40 (15), polyoma (3), and herpes simplex and human cytomegaloviruses (20). It is of significance that all these viruses are DNA viruses. It would therefore appear that infection by a DNA virus could be considered as a factor in the morphological manifestation of nuclear bodies in cells. Although no virus has as yet been implicated in the genesis of tumors of the human nervous system, 2 factors should be considered in this regard: (a) HSV is known to lie dormant in man (2) and (b) HSV can transform cultured cells to a state of malignancy (24).

* The abbreviation used is: HSV, herpes simplex virus.
Furthermore, the presence of tubuloreticular structure in cultured cells from the neurilemoma tissues suggests infection by a virus of the herpes group (9) and/or some neoplastic disease process (27). Attempts to isolate a herpes-type virus from cultured neurilemoma cells with cells susceptible to HSV have so far been unsuccessful. The significance of spherical bodies exhibiting spikes (Figs. 12 and 13) in a few degenerated nuclei in neurilemoma cell culture is not known, yet the similarity between these spikes and those on the envelopes of HSV is striking.

Oncogenic viruses generally produce malignant neoplasms. Of the various viruses of the papova group, the papilloma viruses produce benign lesions in their natural hosts. On the basis of the present morphological observations, a role, if any, of a DNA virus in the genesis of human neurilemomas, which are benign in nature, is purely speculative.

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REFERENCES

Human Neurilemoma Cell Nuclei

Fig. 1. A low-power electron micrograph of human neurilemoma showing 2 tumor cell nuclei with deep indentation (arrow) and projections of nuclear envelope (NP). × 3,200.

Fig. 2. Extensive cytoplasmic invagination within a tumor cell nucleus which, in this plane, exhibits a smooth surface. Cytoplasmic matter (Cy) within the nucleus is demarcated by nuclear envelope with chromatin material attached to it. × 12,000.

Fig. 3. Similar to Fig. 2; however, in certain areas, the cytoplasmic matter (Cy) within the invaginations is not completely enclosed by nuclear membrane (arrow). Curved arrows, material that appears similar to that within the nuclear invagination in other areas in this field, but is not enclosed by nuclear envelope. Dense osmiophilic bodies are seen within nuclear invaginations (heavy arrows). NP, nuclear envelope projection. × 21,000.

Fig. 4. Dense osmiophilic bodies, some of which are enclosed by nuclear envelope (heavy arrow), while others have a single membrane around them (arrows). × 21,000.

Figs. 5 and 6. Small membrane-bound spherical bodies, measuring about 130 nm, some of which are free in the nucleoplasm (arrow), while others are enclosed by a single membrane (heavy arrows). NP, nuclear envelope projection; Cy, cytoplasmic matter within nuclear invagination. × 21,000.

Fig. 7. A nuclear body with characteristic whorl-like formation by fibrillar material. This body has osmiophilic spherical bodies, membranous segments (M), and some dense amorphous mass. Within the latter are seen small spherical bodies, 45 nm in diameter, with an electron-lucent substance around them (arrows). Heavy arrow, granular chromatin matter. One membranous segment appears free in the nucleoplasm. × 42,000.

Fig. 8. A membrane-bound spherical body measuring approximately 120 nm within a nuclear body. × 42,000.

Fig. 9. Portion of a cell from a human neurilemoma cell culture exhibiting margination of chromatin. Arrows, tubuloreticular structures in the cytoplasm. N, nucleus. × 7,500.

Fig. 10. Tubuloreticular structures in Fig. 9 at a higher magnification (arrows). N, nucleus. × 30,000.

Fig. 11. An area that appeared to be a remnant of a degenerated nucleus in a culture of human neurilemoma contains spherical bodies, 130 to 230 nm in diameter. × 9,000.

Figs. 12 and 13. Spherical bodies similar to those in Fig. 11 exhibit bilamellar profiles and spikes on their surfaces at higher magnifications. × 78,000.

Fig. 14. For comparison, spikes (arrows) on envelopes of HSV type 1 and on membranes which may be involved in virus maturation. Other cytoplasmic membranes do not have spikes (heavy arrows). × 78,000.
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