Multilaminar Endoplasmic Reticulum and Abnormal Mitosis in Hodgkin Tumor Cells

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SUMMARY

A multilaminar alteration of endoplasmic reticulum (ER) has been observed in tumor cells of eight patients with Hodgkin's disease and a patient with histiocytic lymphoma. These multilaminar structures are more numerous in dividing cells and thus appear to arise primarily during mitosis. The stacked membranes in the multilaminar structures possibly result from abnormal sticking of organelle membranes, as evidenced in this study by adherence of ER to other elements of ER, nuclear envelope, mitochondria, or lipid droplets. Multilaminar ER was identified in all mitotic tumor cells, a rare mitotic plasma cell, and numerous interphase Hodgkin cells. The paucity of multilaminar ER in normal mitotic cells and its virtual absence for normal interphase cells suggest that this structure represents a pathological alteration in tumor cells from patients with Hodgkin's disease and histiocytic lymphoma. The multilaminar defect of ER is associated with other atypical features of ER in Hodgkin tumor cells, including the excessive length and curving of ER profiles, the collapse of the ER cisternae, and the overall sparsity of this organelle.

Other abnormalities observed in mitotic Hodgkin tumor cells include the presence of disorganized microtubules, large cytoplasmic vacuoles, and abnormally clumped chromosomal material and the persistence throughout mitosis of bodies suggestive of nucleoli and of the nuclear bodies of interphase cells.

INTRODUCTION

A multilaminar structure composed of 4 stacked membranes of ER or of ER with nuclear envelope has been described previously in various conditions. This multilaminar ER was initially observed in dividing sarcoma cells as a double tubular structure which was thought eventually to transform into ER (29). This structure has more recently been interpreted as aggregates of granular reticulum. The multilaminar ER has been observed in mitotic rat thymocytes (25) and mitotic and interphase leukemic cells exhibiting viruses in vivo (34) and has been reported in phytohemagglutinin-stimulated lymphocytes (30), mitotic and rare interphase tumor cells (6, 8, 9, 14), and virally induced mitotic cells in vitro (21). In other studies, the stacked membranes were not noted in phytohemagglutinin-stimulated mitotic lymphocytes from either normal individuals (22, 37) or Hodgkin patients (10) and were not observed in malignant or normal mitotic plasma cells (11, 12, 16) or cultured tumor cells (19, 32). This structure has not been encountered in normal interphase cells and, although observed on one occasion in normal dividing mammalian cells in vivo (25), is generally thought not to occur in such cells (23, 32, 33). The multilaminar reticulum thus appears primarily confined to some tumor cells or cultured cells in mitosis and occurs rarely in nondividing neoplastic cells (8, 14, 33). Although the presence of multilaminar ER has been briefly noted previously in Hodgkin tumor (26, 28), it has not been observed in several ultrastructural studies of Hodgkin neoplasms (4, 7, 13, 17, 18) and has not been previously investigated. In this study, we demonstrate extensive multilaminar structures involving granular reticulum and nuclear envelope of both mitotic and interphase tumor cells in Hodgkin specimens and mitotic cells in histiocytic lymphoma (reticulum cell sarcoma). Such structures appear abnormal and are thought possibly to be related to the sparsity of ER and great excess of unbound ribosomes in the tumor cells (18).

MATERIALS AND METHODS

Lymph nodes from 8 patients and a spleen from 1 patient with Hodgkin's disease and a lymph node from a patient with histiocytic lymphoma (reticulum cell sarcoma) were removed surgically. The patients ages ranged from 4.5 to 55 years, and none had received prior therapy for their cancer. A portion of each specimen was fixed in buffered 10% formalin and processed routinely for light microscopic diagnosis. All specimens from Hodgkin patients revealed tumor of the mixed cell variety, as diagnosed by surgical pathologists of the University Hospital. Tumor cells replaced normal structure in the node from the patient with histiocytic lymphoma.

Another portion of each specimen was fixed 1 to 2 hr in 3% glutaraldehyde in 0.1 M, pH 7.4, cacodylate buffer at 4°. The glutaraldehyde-fixed specimens were rinsed with 7.5% sucrose, buffered with 0.1 M cacodylate at pH 7.4 and then were postfixed 1 hr in 2% osmium tetroxide, dehydrated, and embedded in Epon. Thin sections were stained with a uranyl acetate and lead citrate sequence and viewed in a...
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Hitachi HS-8 electron microscope at an accelerating voltage of 50 kV.

RESULTS

The tumor cells in Hodgkin lesions ranged from 20 to 40 μm in diameter and usually enclosed 1 nuclear profile but, occasionally, could be identified as Reed-Sternberg cells from their content of 2 or 3 nuclear profiles. Interphase tumor cells were observed in all of the patients studied, and mitotic cells were seen in 6 of the 8 patients with Hodgkin's disease and the 1 patient studied with histiocytic lymphoma. The nuclei of Hodgkin tumor cells measured 10 to 20 μm in diameter, and in turn, enclosed bizarre nucleoli measuring up to 8μm in greatest dimension (Fig. 1). The nuclear chromatin was finely dispersed and the nuclei often contained several nuclear bodies composed of a moderately dense rim and a less dense core. The nucleoli consisted mainly of abundant granular component with sparse interspersed dense component and a few small foci of par amorpha. Structural variability was evident among both multinucleated Reed-Sternberg cells and uninnuclear Hodgkin cells in the same specimen as described previously (4, 17, 18). Some tumor cells resembled immunoblasts with sparse collapsed ER and abundant free polyribosomes (18) (Fig. 2). whereas other tumor cells often contained abundant microfilaments, a few granules, fairly abundant mitochondria, and variable amounts of ER (Fig. 1). The specimen with histiocytic lymphoma disclosed numerous interphase tumor cells with fine structural features similar to those previously described for these cells (31).

Reed-Sternberg and Hodgkin cells and histiocytic lymphoma cells exhibited a multilaminar membrane alteration (Figs. 1 to 9). This change was observed in mitotic and interphase cells of lymph node and spleen from 6 Hodgkin patients and of the lymph node from the patient with histiocytic lymphoma. In 2 Hodgkin patients, no mitosis was encountered, and the multilaminar ER was not present in the interphase tumor cells observed in this study. The prevalence of multilaminar ER in tumor cells was not related to the age of the patients or the extent of their disease. The multilaminar structure frequently consisted of a collapsed cisterna of rough ER closely approximated or adherent over a variable distance to another cisterna of ER (Figs. 1 and 2). In other profiles, these structures were composed of variably long segments of nuclear envelope of interphase cells or persistent nuclear envelope of mitotic cells in similar approximation to rough ER (Figs. 3 and 4). At the point where adherence of 2 cisternae terminated, the multilaminar reticulum appeared to branch into cisternae of ER (Figs. 5 and 6) or nuclear envelope and ER (Figs. 3, 4, and 7). The multilaminar profiles accounted for a minority of the rough ER and nuclear envelope in approximately 15% of the interphase tumor cells encountered (Fig. 1), but comprised all or most of the granular reticulum in all of the 15 mitotic tumor cells (Fig. 2).

The approximated cisternae lay separated by a narrow distance in some areas and exhibited 4 membrane layers. Less commonly, the 2 central membranes appeared fused throughout most or all of the multilaminar ER so that it was composed of 2 outer layers comparable in thickness to the usual cytoplasmic unit membrane plus an inner, thicker layer (Figs. 1 and 9). Very sparse particles presumed to be ribosomes and amorphous material adhered to the cytoplasmic surface of the outer layers of the multilaminar ER. The multilaminar elements of ER frequently contacted or enwrapped other cell organelles such as lipid droplets (Fig. 9). ER occasionally was approximated closely to mitochondria in such a way that the 2 mitochondrial membranes and 2 ER membranes formed a 4-layered structure (Fig. 9) similar to multilaminar ER. Some irregular projections of collapsed ER appeared folded back upon themselves. Occasionally, profiles of ER or multilamimated structures formed a complete circle (Figs. 1, and 7 to 9).

Two mitotic plasma cells in 1 tumor specimen displayed the multilaminar ER in the 1 or 2 profiles of granular reticulum nearest the nucleus (Fig. 10). These were the only dividing plasma cells encountered.

Cells presumed to be Hodgkin tumor cells were observed in various stages of mitosis (Fig. 2). These dividing cells were interpreted as neoplastic cells in mitosis from the similarity of their cytoplasmic features to those of Hodgkin and Reed-Sternberg cells and the atypical appearance of their chromosomal mass (Fig. 2). The chromosomal material in mitotic cells had an abnormally clumped appearance and irregular contour. Microtubules associated with this chromosomal material often appeared tortuous and disoriented rather than linear and aligned perpendicular to chromosomal material. Remnants of nuclear material resembling nucleoli (5) were seen in the cytoplasm of tumor cells in all stages of mitosis (including late telophase). Also, bodies showing a cortex and less dense center and resembling nuclear bodies of interphase tumor cells persisted in the cytoplasm of occasional mitotic tumor cells.

DISCUSSION

Since the multilaminar ER has been observed only rarely in normal dividing mammalian cells in vivo (25), the presence of the multilaminar structures in all dividing tumor cells in Hodgkin specimens and histiocytic lymphoma represents, at the least, a pathological increase in this event. The occurrence of this structure in interphase Hodgkin cells would appear to be abnormal, since insofar as we are aware, it has not been previously described in interphase cells in vivo. The presence of multilaminar ER in Hodgkin tumor cells further supports speculation that this structure is more prevalent in tumor cells in general (6, 8, 9, 29). The increased prevalence of multilaminar ER in some dividing cells, as previously recognized (8, 14, 25), suggests that this process occurs primarily, or is accentuated, during cell division. When observed in normal cells, the multilaminar ER does not appear to persist beyond late telophase. Multilaminar ER encountered here in interphase cells argues then against the genesis of the lesion transpiring only during mitosis, unless it has persisted since cell division. For the multilaminar ER in interphase cells to be a residuum of structures formed during mitosis, however, does seem consistent with the invariable presence of this change in mitotic Hodgkin tumor cells and its lesser prevalence in
interphase cells. The lesion thus appears to occur with increased frequency in mitotic cells and to persist abnormally after mitosis in Hodgkin tumors.

Knowledge of the ER in dividing plasma cells in vivo is too limited to determine whether multilaminar ER in mitotic plasma cells is abnormal. However, the structure is absent from illustrated mitotic plasma cells (12, 16) and plasma-cytoma cells (11) and, to our knowledge, has not been previously encountered in such cells. Possibly, therefore, the presence of multilaminar ER in plasma cells in vivo is abnormal and arises from an influence of the neoplastic environment on the cell or as an early manifestation of transformation in the cell line from which the Reed-Sternberg cells derive (18).

Previous investigators (29) have suggested that these stacked profiles of ER develop as a consequence of mitosis-induced proliferation of new nuclear envelope or ER. The formation of the structures may also entail folding of nuclear envelope on itself as it is pulled toward the spindle poles in late prophase (6). Consequently, the term spindle lamellae has been used (6) to describe the multilaminar structures. However, normal dividing cells generally lack multilaminar ER (1, 2, 16, 23, 32, 33, 35) and are not known to replicate ER by a mechanism entailing formation of such structures.

The multilaminar structure, on the other hand, conceivably arises through segments of ER paralleling, contacting, and fusing with nuclear envelope or other ER segments. The structure might develop from ER or nuclear envelope folding back and adhering to itself. The intimate approximation or contact of the ER with mitochondria and lipid droplets, as well as nuclear envelope or other profiles of ER observed in interphase Hodgkin cells, suggests an abnormal tendency of the ER membrane to approach other organelle surfaces in these cells. Perhaps the membrane is altered in such a way as to favor approximation and adhesion to other structures. If the adherence occurs mainly during mitosis, the structure might subsequently pull apart and reform ER or nuclear envelope or simply degenerate, as has been suggested previously (8).

The multilaminar ER is a distinctive structure and clearly differs from the projections of nuclear envelope containing nuclear material as seen in cultured Burkitt lymphoma cells (15) or guinea pig thymus (35). The structure has been interpreted as a form of annulate lamellae, but from the present and previous observations, appears distinct from the latter entity (20).

A number of the cells in which multilaminar ER has been observed heretofore were infected with virus (3, 21, 24, 34). Viral particles and other microorganisms have been observed ultrastructurally in patients with Hodgkin's disease (27). Recently, tubular arrays identified in the ER of cultured Hodgkin cells have been attributed to a possible viral lesion (36). Although cells with multilaminar reticulum disclosed no intracellular microorganisms, prior infection or continued infection in an occult form possibly could play a part in pathogenesis of multilaminar ER in tumor cells and plasma cells of the same specimen.

The increased prevalence of multilaminar ER in Hodgkin tumor cells suggested by the present results conceivably entails impaired genesis of granular reticulum. Defective formation of ER could comprise a basis for the failure of the tumor cells to mature into plasma cells. The lack of ER in these tumor cells apparently results in production of immunoglobulin on unbound ribosomes (18) and failure to secrete the globulins (15). The observation here of small amounts of multilaminar ER in dividing plasma cells infiltrating tumor of presumed B-cell origin suggests that the propensity for development of multilaminar ER exists throughout the B-cell series.

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