Human Cervical Intraepithelial Neoplasia: Fine Structure of Dysplasia and Carcinoma In Situ

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SUMMARY

Cervical biopsies were taken from women with abnormal Papanicolaou smears and from normal women serving as controls, using the colpomicroscope as an aid in localizing lesions on the exposed portion of the cervix. Half of each biopsy was processed for light microscopy, and the other half was processed for both phase contrast and electron microscopy. The fine structure of dysplasia and carcinoma in situ was compared with normal control epithelium. The dysplastic lesions differed markedly from the normal; there were changes in nuclear size, contour, and chromatin distribution. Cytoplasmic constituents varied in amount and distribution. Cell surfaces and intercellular contacts were altered and were progressively more abnormal as the severity of the dysplastic lesion increased. It was often difficult to distinguish between dysplasia and carcinoma in situ at the ultrastructural level, since the lesions had similar fine structural alterations and differed from one another only in degree.

INTRODUCTION

Carcinoma in situ (CIS) of the human uterine cervix is generally accepted as being a precursor of invasive squamous cell carcinoma; the fine structure of this lesion, as well as that of invasive carcinoma, has been studied by a number of investigators (2, 8, 9, 11, 16, 28). Several alterations were shared by the two lesions. Nuclear changes included enlargement, irregular contours, polylobation, multinucleation, and condensation of nuclear chromatin. Increased numbers of non-membrane-associated ribosomes, often occurring as aggregates, as well as increased amounts of rough-surfaced endoplasmic reticulum were present in the cytoplasm of the epithelial cells. These cells also exhibited increased numbers of surface microvilli. In CIS, the epidermal basement membrane was relatively straight, and decreased numbers of desmosomes and tonofilaments were observed. The number of desmosomes and tonofilaments in invasive carcinoma varied with the degree of differentiation; although these structures were sparse in the poorly differentiated neoplasms, they were numerous in the well-differentiated lesions.

Cervical dysplasia has recently been under investigation by various techniques, and almost all workers agree that the lesion can progress in certain instances to CIS (13, 17, 22, 27), the major disagreement being in the percentage of dysplastic lesions which regress or progress. This spectrum of changes beginning with dysplasia and ending with invasive carcinoma allows study of the earliest epithelial neoplastic change known in the human; the early part of the spectrum may be analogous to the "minimal deviation" hepatomas produced experimentally in animals (18). Since no systematic electron microscopic study of dysplasia of the human cervix has been published, this project was undertaken to examine the fine structural changes occurring in early cervical neoplasia.

MATERIALS AND METHODS

The colpomicroscope was utilized as an aid in accurately determining the distribution of neoplastic epithelium on the exposed portion of the cervix (21) in women who were known to have abnormal Papanicolaou smears. Cervical biopsies were then taken from these regions in 29 patients as well as from 10 patients whose cervical epithelium served as normal controls. The biopsy specimens were then bisected. One portion was fixed in Bouin's solution, stained with hematoxylin and eosin, and examined under the light microscope. The other portion was fixed in phosphate-buffered (pH 7.6) 6.25% glutaraldehyde, postfixed in 2% veronal acetate-buffered (pH 7.4) osmium tetroxide with added sucrose, dehydrated in graded acetone solutions, and embedded in Araldite. Solutions of glutaraldehyde of lesser tonicity were used to fix some biopsies, but when compared with the sections fixed in 6.25% solution, such sections showed no differences in cell contacts and cell contours. Thick (2-3 microns) sections of the plastic-embedded material were stained with Paragon multiple stain (26) and examined under the light microscope for confirmation of the diagnosis and orientation. Thin sections were stained with lead citrate and uranyl acetate and examined under RCA EMU-3F and Siemens Elmiskop I microscopes.
Ten dysplastic lesions, three of CIS, and six control biopsies were found to be suitable for electron microscopic examination and provide the basis for this report.

OBSERVATIONS

Controls

The fine structure of normal cervical squamous epithelium will only be reviewed briefly, since it has been described in detail by others (1, 6). An undulating basement membrane, measuring 400-500 Å in thickness, is separated from the basal layer of epithelial cells by a space measuring up to 450 Å in width. Half desmosomes are seen within the basal cells in relation to this basement membrane. In the lower third of the epithelium, the nuclei are oval or elongated and the chromatin appears coarsely clumped (Fig. 1). A single round nucleolus is usually present. The cytoplasm of these cells contains numerous single non-membrane-associated ribosomes as well as small amounts of granular endoplasmic reticulum. Mitochondria are especially numerous between the nuclei and the basal aspects of these cells. Adjacent epithelial cells are separated by an intercellular space of variable width, into which small microvilli project. Flocculent material and occasional leukocytes are seen within this space. Tonofilbrils course throughout the cytoplasm of the epithelial cells but are most conspicuous in the peripheral portion of these cells. The tonofilbrils, which are composed of tonofilaments measuring approximately 70 Å in diameter, terminate on the inner face of the attachment plaques of desmosomes. Mitotic figures are seen rarely in this region. Higher in this layer, nuclear chromatin is more granular, desmosomes more numerous, non-membrane-associated ribosomes less numerous, and tonofilbrils more prominent. Glycogen particles, occasionally forming small aggregates, are distributed throughout the cytoplasm.

In the middle third of the epithelium the cells are larger and the nucleo-cytoplasmic ratio decreased (Fig. 2). Large aggregates of glycogen encircle the nucleus and occupy the adjacent portion of the cytoplasm. Tonofilbrils are concentrated in the peripheral portions of the cells (Figs. 2, 4, 14). Mitochondria are less numerous and are usually found near the nuclei.

In the upper third of the epithelium the cells are flattened in a plane parallel to the surface; many cells are devoid of nuclei and the small nuclei present have compact chromatin (Fig. 3). The cytoplasm of these cells is virtually filled with glycogen. The intercellular spaces are narrower, mitochondria are extremely sparse, and tonofilbrils are once again concentrated around the periphery of the cells.

Dysplasia

Light Microscopy. The criteria used for the diagnosis of dysplasia and CIS are those outlined by the Committee on Histological Terminology for Lesions of the Uterine Cervix of the International Congress of Exfoliative Cytology (3). Photomicrographs of three of the dysplastic lesions examined in this study, illustrating mild, moderate and severe dysplasia, are shown in Figs. 5-7.

Electron Microscopy. In the cells in dysplasia there are alterations in nuclear size and configuration, in the cell surfaces and intercellular contacts, and in the amount and distribution of the cytoplasmic constituents.

The nuclei of dysplastic cells are markedly enlarged and frequently are either multilobate or multinucleate (Fig. 9). The nuclear envelopes exhibit numerous invaginations and the nuclear chromatin appears to be coarsely clumped, especially in the more severe lesions (Figs. 9, 11, 13). The nucleoli are often multiple and exhibit irregular profiles (Fig. 12). Also, in accord with the light microscopic findings, mitoses are sometimes found in the upper layers of the epithelium (Fig. 16). Rarely, mitotic figures are seen which seem to contain abnormal numbers of chromosomes (Fig. 17). Increased numbers of leukocytes are found in the intercellular spaces throughout all layers of the epithelium (Figs. 9, 11).

The cell surfaces and intercellular contacts are altered in all cases, but these alterations are most pronounced in the advanced lesions. Desmosomes, although decreased in number in the advanced lesions (Figs. 9, 11-13, 15) have their usual substructure (Fig. 10). There is a parallel decrease in the number of tonofilbrils in these lesions (Fig. 10). Increased numbers of microvilli are found in all layers of the dysplastic epithelium (Fig. 15) when compared to the normal epithelium (Fig. 14). The microvilli are elongated and frequently have branching processes that interdigitate with those of adjacent cells. This increase in surface microvilli, present in the earliest lesions even when desmosomes appear normal in number, is perhaps the most startling finding in dysplastic cells. The number of half-desmosomes seen in relation to the basement membrane, and the basement membrane proper, do not differ from that observed in control epithelium.

Alterations in both cytoplasmic organelles and other cytoplasmic constituents are most prominent in the upper layers. Glycogen is markedly decreased in amount in all biopsies examined and is virtually absent in some (Figs. 9, 11-13, 15). The large perinuclear aggregates of glycogen in control epithelium are not found in the dysplastic cells. Mitochondria are seen throughout all layers of the epithelium, but no consistent difference in size and shape of these organelles from normal was noted. There is a striking increase in the number of non-membrane-associated ribosomes (Figs. 9-33); these ribosomes are often arranged in aggregates (Inset Fig. 10). There is a lesser increase in the amount of rough-surfaced endoplasmic reticulum. In addition, epithelial cells with a cytoplasmic ground substance of high electron opacity (Fig. 9) similar to those described previously in squamous cell carcinoma of the cervix (11), are occasionally seen within the dysplastic lesions. Golgi zones and lysosomes are present in cells of dysplasia, but they appear normal in number and configuration.

Carcinoma in Situ

Light Microscopy. The photomicrograph of one of the carcinomas in situ studied is shown in Fig. 8; it is representative of the histologic appearance of the other two.

Electron Microscopy. The fine structural alterations in CIS, as in dysplasia, involve cell nuclei, cell surfaces and their contacts, and cytoplasmic organelles. The nuclei are intermediate.
in size between those found in control and dysplastic epithelia. Nuclear profiles are more regular than those of dysplastic cells; multinucleate forms are rare. Nucleoli are frequently multiple and have irregular outlines (Fig. 18). Mitotic figures are found in all epithelial layers. The microvilli are similar in appearance to those described in the dysplastic cells and are very numerous (Figs. 18, 19). The number of desmosomes, when compared with those from dysplastic lesions, is decreased, and tonofibrils are decreased correspondingly. The basement membrane is less undulating, but the number and substructure of the half-desmosomes do not differ appreciably from that seen in the dysplastic lesions. Virtually no glycogen is found in the cytoplasm of these epithelial cells. Mitochondria are found throughout all levels of the epithelium, and the numbers of non-membrane-associated ribosomes and cisternae of the granular endoplasmic reticulum are similar to those seen in the cells of the dysplastic lesions. Many leukocytes are found in the intercellular spaces.

**DISCUSSION**

It is readily apparent from this study that dysplastic cells from the human cervix differ markedly in morphology from normal cells. Although it is both possible and conventional to subclassify cervical intraepithelial neoplasia into mild, moderate, and severe dysplasia and CIS by light microscopy, such a subclassification is difficult at the cellular level.

The nuclear enlargement and the irregularity in nuclear shape are well-known features of early cervical neoplasia (10, 19). Such alterations are known to occur in neoplasms with abnormal chromosome numbers, a finding which has been reported as being characteristic of dysplasia and CIS (12). The nuclear enlargement may also reflect the increase in DNA synthesis that accompanies the decreased generation time in dysplasia and CIS (20). The coarse chromatin clumping may also be partially related to the decreased generation time since in such a population a higher percentage of cells than normal would be expected to be in prophase. Mitotic figures are rarely observed in normal epithelium but are frequently seen in dysplasia and CIS, in keeping with the more rapid growth rate. The mitotic figures in dysplasia do not generally differ from those of normal cells, but rare mitotic figures are observed in which the number of chromosomes appear to be increased, although quantitation of this observation is difficult in a thin section. Such cells appeared to be degenerating, suggesting that some cells with hyperdiploid chromosome numbers may enter mitosis but fail to complete cell division. This has been previously suggested as a possible factor to account for the discrepancy between the abnormal chromosome numbers found in direct squash preparations and the diploid numbers found in cultured cells (23).

Another consistent and striking alteration occurs at the cell surfaces. In all the biopsies of established (moderate or severe) dysplasia studied, despite the increase in numbers of microvilli, the desmosomes are decreased in number, often markedly so. Such a decrease in desmosomes, as well as an increase in microvilli, has been described in cells of invasive carcinoma of the human cervix (8, 9). The decrease in the number of desmosomes is in accord with the finding that neoplastic cells are more loosely attached to one another than are normal cells (5). The ability to form desmosomes may also explain the close apposition of normal cervical epithelial cells in vitro and the lack of such apposition in cells from dysplasia and CIS (29).

The lack of adhesiveness between the epithelial cells in dysplasia and CIS could also account for the increased number of leukocytes within the altered epithelium, since it would facilitate the penetration of the epithelium by inflammatory cells. No alterations in the substructure of the desmosomes, as described by other workers (16), were observed in the present study.

Cells from any level of a dysplastic epithelium are different from the cells at a comparable level in normal epithelium. This is probably due in large part to the presence in dysplasia of cells of a more undifferentiated type which have an increased rate of division. Surface cells in dysplasia, although they may appear flattened under the light microscope have cytoplasm containing large numbers of ribosomes, numerous mitochondria, and decreased or absent glycogen stores, which is in keeping with their less differentiated state. Increased numbers of ribosomes and polyribosomes have been reported in cervical squamous cell carcinoma (11, 28) and in other neoplasms (4, 7). The increase in ribosomes in all layers of the epithelia in dysplasia and CIS, and their occurrence in aggregates in both lesions, is consistent with a high level of RNA and protein synthesis, whereas disaggregation or disappearance of ribosomes, such as is noted in the upper layers of normal cervical squamous epithelium, denotes a decrease in, or cessation of, cellular synthesis of these products (14, 15).

The intramitochondrial inclusions described in methylcholanthrene-induced cervical carcinoma in the mouse (24, 25) were not observed in this study. It is of interest, however, that the alterations in both cytoplasmic organelles and cell surfaces observed in the human dysplastic epithelium resemble those of experimentally produced cervical neoplasia.

In this electron microscopic examination of human cervical dysplasia and CIS, alterations are found which vary somewhat from one lesion to the other, but which seem to be part of a spectrum of changes that become more prominent with the increasing severity of the lesions and which make the advanced dysplasias virtually indistinguishable from CIS. This study emphasizes the artificial nature of a separation of dysplasia from CIS.

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**REFERENCES**


Figs. 2-4 and 9-19 are electron micrographs. Figs. 5-8 are light micrographs of hematoxylin and eosin-stained paraffin-embedded sections. The insets in Figs. 1, 9, 12, 13, and 18 are phase contrast micrographs of 2- to 3-µ-thick, araldite-embedded sections stained with Paragon multiple stain. × 600.

Fig. 1 inset. Micrograph of biopsied control epithelium. Figs. 1-4 are electron micrographs from this biopsy.

Fig. 1. Epithelial cells from the lower third of control epithelium. The cytoplasm of these cells contains numerous non-membrane-associated ribosomes and small numbers of mitochondria. Numerous desmosomes are present. Small microvilli project into the intercellular spaces. × 11,000.

Fig. 2. A cell from the middle third of control epithelium. A large aggregate of glycogen (G) is present adjacent to the nucleus, while other glycogen particles are dispersed in the cytoplasm. Ribosomes are less prominent than in the cells of the lower third of the epithelium. Tonofilbrils are seen in the periphery of the cells. Desmosomes and microvilli are readily apparent. × 11,000.

Fig. 3. Upper third of control epithelium. The epithelial cells are flattened and the cytoplasm is filled with glycogen particles. The intercellular spaces are narrower than in the other layers. Few mitochondria can be seen in this layer. × 11,000.

Fig. 4. Middle third of normal epithelium. The typical substructure of several desmosomes is shown. Note the larger size of the glycogen particles as compared with the free ribosomes. Dense networks of tonofilaments are present in the cytoplasm of these cells. × 56,000.

Fig. 5. Mild dysplasia. The full thickness of the epithelium is composed of cytologically atypical cells with irregular nuclei arranged in a disorganized pattern. Multinucleated cells are common and several mitotic figures are present, occurring in the lower and middle thirds of the epithelium. Only the lower third of the lesion is occupied by undifferentiated cells. × 200.

Fig. 6. Moderate dysplasia. This lesion is similar to that illustrated in Fig. 5, except that more mitoses are noted, and a greater portion of the epithelium is composed of undifferentiated cells. × 200.

Fig. 7. Severe dysplasia. The majority of the cells in this lesion are undifferentiated, and mitotic figures are common. It is distinguished from carcinoma in situ by the layer of differentiated cells occupying the surface region of the biopsy. × 200.

Fig. 8. Carcinoma in situ. The full thickness of the epithelium is composed of undifferentiated neoplastic cells; mitoses are abundant and occur in all cellular layers; multinucleated cells are rare. × 200.

Figs. 9-11 are taken from the same biopsy of cervical dysplasia illustrated in inset Fig. 9. Figs. 9 and 10 are from the middle third of the epithelium; Fig. 11 is from the upper third.

Fig. 9. The nuclei of the cells of this early lesion are enlarged and have irregular contours. Note the electron opacity of the cytoplasm of the cell in the central portion of the figure. Glycogen is virtually absent and large numbers of ribosomes are scattered throughout the cytoplasm. Many mitochondria are present, but desmosomes and tonofilbrils appear to be somewhat decreased in number. A leukocyte (L) is seen in an intercellular space. × 11,000.

Fig. 10. The substructure of the desmosomes in the central portion of the figure is similar to those in Fig. 4. Large microvilli are seen in the intercellular space. Numerous ribosomes, but virtually no glycogen, are present in the cytoplasm of these cells. × 56,000.

Fig. 10 inset. Numerous aggregates of non-membrane-associated ribosomes in the cytoplasm of a dysplastic cell. × 56,000.

Fig. 11. Numerous microvilli but only a few desmosomes are seen between these epithelial cells. Note the paucity of glycogen, the large numbers of ribosomes, and numerous mitochondria in the cytoplasm of the cells. A portion of two enlarged nuclei, with relatively loose chromatin, are shown. Two leukocytes (L) are present in the extracellular spaces. × 11,000.

Fig. 12. Portions of several cells from the middle third of the dysplastic epithelium in the inset. These cells contain small amounts of glycogen, large numbers of non-membrane-associated ribosomes, and numerous mitochondria. Tonofilbrils are not prominent. Flocculent electron-opaque material is seen in the intercellular spaces. × 11,000.

Fig. 13. Upper third of dysplastic epithelium from biopsy illustrated in this inset. The nuclei are enlarged, have irregular contours and display coarse clumping of their chromatin. Desmosomes are markedly decreased in number. Increased numbers of elongated microvilli project into the intercellular spaces. The cytoplasmic constituents of these cells are similar to those shown in Figs. 9-12.

Figs. 14, 15. Micrographs from the middle thirds of control and severe dysplasia respectively. Note the marked decrease in desmosomes, the increased numbers and complexity of the microvilli, and the virtual absence of glycogen in the dysplastic cells. × 7,500.

Figs. 16, 17. These illustrate mitotic figures within dysplastic lesions. Fig. 16 exhibits the usual number of chromosomes while the chromosomes in Fig. 17 seem to be increased in number. × 3,500.

Figs. 18, 19. These are from the middle and upper third respectively of the carcinoma in situ shown in the inset of Fig. 18. The changes in the nuclei, cytoplasm, and cell surfaces are very similar to those described in dysplastic lesions (Figs. 9-13). × 11,000.
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