The Ultrastructure of Preinvasive Cancer of the Corneal Epithelium

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SUMMARY

Preinvasive cancer of the corneal epithelium was studied electron microscopically for the first time. Major findings consisted of numerical reduction in desmosomal junctional complexes with, in many places, a decrease in degree of cell interdigitation; increased numbers of cytoplasmic organelles; perinuclear condensation of tonofilaments with a consequent increase in electron lucency at the cell periphery; and occasional foci of more advanced dyskeratosis. Nuclear changes included increase in size, occasional multilobulation, nucleolar enlargement and reduplication, chromatin granularity, and excessive mitotic activity.

INTRODUCTION

Cancer of the corneal epithelium, although rare, is well recognized and, in common with neoplasia in other squamous epithelia, shows all grades from dysplasia to invasive carcinoma. The stage corresponding to carcinoma in situ in the skin has likewise been generally referred to as Bowen's disease. McGavic (9) in 1942 was the first to describe the lesion in the eye and to apply the eponymous nomenclature but, in that there are some important differences between skin and corneal epithelium, others such as Zimmerman (18) deprecate the use of this term in the ocular situation. Thus, the squamous epithelium of skin normally gives rise to keratinization, whereas this occurrence in the cornea is pathological; and in some respects the corneal lesion is more nearly analogous to carcinoma in situ of cervical epithelium.

Although there have been a number of histological reports of preinvasive neoplasia of the corneal epithelium [Irvine (7)], there has not as yet been any record of its ultrastructure. In presenting our findings in such a case, we do so against the background of carcinoma in situ of cervical epithelium.

MATERIALS AND METHODS

Local removal of an opalescent lesion affecting the corneal epithelium and suspected of being malignant was performed on an 80-year-old man.

The specimen was immediately bisected; one-half was fixed in 10% formal-0.9% NaCl solution and subsequently was embedded in paraffin wax for conventional light microscopy; the other half was fixed in 1% isotonic osmium tetroxide at pH 7.4 and subsequently was embedded in Araldite for electron microscopy. Sections of Araldite-embedded blocks were cut on a LKB Ultratome 111 with glass knives, and thick sections (1 to 2 μ) were stained with toluidine blue for examination and orientation by light microscopy. Thin sections (500 to 800 Å) of the selected areas were cut and stained with saturated alcoholic uranyl acetate and lead citrate, and electron micrographs were taken with an AEI EM6 electron microscope.

RESULTS

Light Microscopy. The epithelium over the central part of the corneal specimen was slightly thickened and abnormal, in that it was hypercellular and individual cells showed an increased nucleocytoplasmic ratio with distinct loss of polarity. The cell nuclei were hyperchromatic, generally were enlarged, and frequently showed 2 or more prominent nucleoli. Mitotic figures were plentiful, while multilobulated nuclei and even multinucleated cells were not uncommon. Occasional instances of individual cell keratinization, characterized by intense cytoplasmic eosinophilia and nuclear pyknosis, were also seen. The glycogen content of much of the abnormal epithelium, as judged by diastase-controlled periodic acid-Schiff staining, was reduced, compared with that of neighboring healthy tissue. In places, the entire thickness of the epithelium was involved, whereas in others the abnormality was most apparent in the basal and middle layers. At the lateral edge of the epithelium, the junction between the dysplastic and normal epithelia was abrupt. Although in some areas there appeared to be abnormally wide intercellular spaces with well-defined intercellular bridges, other parts showed much less obvious extracellular spaces, with increased staining intensity of many of the constituent cells in the basal and midepithelial layers. Such “dark” cells were particularly obvious in toluidine blue-stained sections of the osmium-fixed material (Fig. 1).

A few red blood cells were present on the surface of the cornea, and occasionally they were also seen between the abnormal epithelium and Bowman's membrane. The basement membrane of the epithelium was intact, and no abnormality was observed in the underlying Bowman's membrane and superficial lamellae of the substantia propria.

Ultrastructure. Electron microscopy generally confirmed and amplified the histological appearances.
In the basal zone, there was loss of the normal architecture, with variation in cell size and shape. Reduction in the number of hemidesmosomal attachments to the basement membrane was associated in occasional areas with separation of cells and subepithelial incarceration of red blood cells and granular material presumed to be plasma. Intercellular cytoplasmic borders were often straight or smooth, due to close packing of the cells and reduction in the number and extent of interdigitating processes (Fig. 2). Desmosomes were also reduced in number, but in the main they preserved a normal pattern (Fig. 3), although in places associated with widened intercellular spaces there was some disturbance of the desmosomal-tonofilament attachments. Mitotic figures and scattered foci of dyskeratosis were also present (Fig. 4).

There was similar loss of polarity with cell pleomorphism in the middle layers of the epithelium. Intercellular spaces in some areas were excessively wide, whereas in others they were nonexistent, the individual cell boundaries being indistinct and in places totally unrecognizable. Desmosomes were again fewer than normal, and interdigitations were reduced. Dyskeratosis and mitotic activity were evident to a rather more prominent degree than was seen in the basal zone.

Parallel changes were present in the superficial layers, although loss of polarity was overall less obvious. At this level there was almost complete loss of interdigitation between adjacent cells, and in many places the intervening spaces were widened with the formation of clefts (Fig. 5).

Study of individual cells at higher magnification showed conspicuous nuclear and cytoplasmic abnormalities.

The nuclei were frequently enlarged and sometimes appeared to be multiple, although serial sections showed that these were more often multilobulated single nuclei. While in general there was a double nuclear membrane, the 2 layers were not always distinct and, as might be expected, the membrane was absent from cells undergoing mitotic division. The nuclear chromatin tended to be granular, ribonucleoprotein granules were prominent, and the nucleoli were often multiple and increased in size (Fig. 6).

Cytoplasmic changes comprised fasciculation of tonofilaments into dense bundles, linked as a rule with a conspicuous perinuclear arrangement (Fig. 7), and an increase in mitochondria, endoplasmic reticulum, ribosomes, primary and secondary lysosomes, and Golgi vesicles. The cytoplasmic organelles were also largely concentrated around the nucleus, with the result that the periphery of the affected cell was often unusually electron lucent. Where the cells were actively dividing, the perinuclear tonofilament bundles showed a curious tendency to be interwoven and randomly oriented (Fig. 8). A bizarre feature was the incorporation of desmosome-like structures within the cytoplasm associated with loss of intercellular contact (Fig. 9). Those cells corresponding to the dark cells seen in light microscopy were characterized by wrinkled, condensed nuclei and electron-dense cytoplasm, related to an increased concentration of tonofilaments, organelles, and glycogen granules.

Some cells were distinguished by condensation of cytoplasmic components, especially tonofilaments, and by either nuclear pycnosis or dispersion of the nuclear content. Occasional cells also contained lipid, and in advanced stages the individual structures of such dyskeratotic cells were barely recognizable, the whole being largely reduced to an aggregate of homogeneous, electron-dense material. These cells, which were present in all layers, were commonly surrounded and sometimes engulfed by nonkeratinized cells, a process that, in the context of Bowen's disease of the skin, has been referred to as cannibalization (10) (Fig. 10). The subepithelial basement membrane was normal and intact. Similarly, no abnormality was observed in Bowman's membrane or the superficial stroma.

**DISCUSSION**

Comparison of the ultrastructural findings in this case of corneal intraepithelial cancer with corresponding lesions in other epithelial tissues, such as skin (10, 14) and cervix uteri (15), reveals many points of similarity. In all 3 situations, there is reduction in intact desmosomes, a predisposition for dyskeratosis, and nuclear abnormality. The only point at which the findings in the corneal lesion differ from those in other comparable situations is the relative absence of elongated intercellular processes and microvilli that are such a noticeable feature in the epidermal and cervical neoplasia (2, 5, 10, 14, 15). Similar processes are described in frank carcinoma of the corneal epithelium (11, 16). The reason for this difference is not immediately apparent, but, since cytoplasmic projections have been considered to be pseudopodia extending to contact neighboring cells (17), it may be relevant that in the present case the presence of such processes was intimately related to the degree of tissue compactness.

Reduction in desmosomal cell junctions is a characteristic of cancer (1, 4) and is thought to be related to the enhanced mobility and decreased adhesiveness shown by malignant cells. Seiji and Mizuno (14) comment on the presence of desmosome-like structures within the cytoplasm of a case of epidermal carcinoma in situ and suggest that these structures have been phagocytosed following the disruption of cell junctions coincident with mitotic cell division. Our own finding within a single cell of all stages, from disruption to invagination and eventual incorporation of desmosomes, strongly supports this suggestion. These desmosomal abnormalities were not, however, usually associated with an obvious deficiency of their tonofilamentous attachments, such as have been implicated in the pathogenesis of dyskeratotic lesions in the skin (3, 10, 14). In this respect, the corneal lesion compares more closely with neoplasia of the cervix uteri, another tissue that does not normally keratinize and in which there is a similar preservation of desmosome-tonofilament attachments (15). The dyskeratosis observed in occasional cells is in all other respects similar to that of epidermal Bowen's disease (10, 14), in that it consists of aggregated tonofilaments concentrated in the middle of the cell, admixed with degenerate nuclear components. As such it falls into the malignant type of dyskeratosis, which is distinguished by a lack of keratohyaline granules seen in normal keratinization and in benign dyskeratosis (8).

Increased mitotic activity, sometimes resulting in bizarre multinucleate and multilobulate forms, is well-recognized in...
preinvasive squamous cell cancer (12), while nucleolar enlargement and multiplicity have also been described (10, 15). The encircling of dividing nuclei by tonofilaments banded together in short, thick fascicles, as remarked upon by Seiji and Mizuno (14), was likewise a feature of the present case. However, the significance of this observation is obscure, although the suggestion has been made that when the filaments surround individual chromosomes they interfere with the normal process of cell division (14).

The increase in free ribosomes, as well as in endoplasmic reticulum, the proliferation of mitochondria, and the reduced amounts of glycogen in all but the dark cells are probably related to a relative lack of differentiation and enhanced cell activity. There is some reason to believe that the increased nuclear and cytoplasmic density of the dark cells is a reflection of cellular compression (13). Moreover, while comparable cells have been described in cervical carcinoma (15), there is no evidence that the increased density is peculiar to malignant states, dark cells having been reported in a number of nonneoplastic corneal disorders (6, 13).

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REFERENCES

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