An Electron Microscopic Study of Bowen’s Disease

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SUMMARY

Bowen’s disease was studied by electron microscopy. Conspicuous findings included: discontinuity of the basement membrane; cytoplasmic projections of epidermal cells through these breaks; widened intercellular spaces; a decrease in intact desmosomes some of which are apparently replaced by villous projections; an increased number of cytoplasmic organelles, many with an abnormal appearance; aggregation of tonofilaments and other organelles resulting in dyskeratosis; and increased nucleolar size and the observation of virus-like particles.

INTRODUCTION

The purpose of this report is to detail the fine structure of intraepidermal squamous cell carcinoma (squamous cell carcinoma in situ or Bowen’s disease).

MATERIALS AND METHODS

A. Four clinically and histologically typical cases of Bowen’s disease were studied. After excision the specimens were placed in cold 6% glutaraldehyde fixative in 0.1 M phosphate buffer at pH 7.2 with 5% sucrose for two hours. In order to remove aldehyde from the tissue, specimens were transferred to pH 7.2 phosphate with 5% sucrose for 18 hours. The tissues were then transferred to 1% OsO₄ fixative and 0.1 M phosphate-buffered formalin, dehydrated in graded alcohols, embedded in epoxy resin (Maraglas), and heated in an oven at 60°C for 48 hours to produce polymerization. Sections were cut on a microtome (Porter-Blum) and placed on naked copper grids. The sections were floated on a uranyl acetate-acetic acid staining solution followed by lead plumbite.

B. The acid phosphatase reaction was performed on one specimen. After the 18-hour wash as above, the epidermis was sliced at 10- to 20-μ intervals with an S and F Tissue Sectioner. Preparation in this manner allows relatively even penetration of staining materials, and the tissue is easy to handle since the epidermis is still attached to the unsectioned dermis.

The staining solution for the enzyme reaction was prepared according to Barka and Anderson’s (1, 10) adaptation of the Gomori acid-phosphatase procedure. Specimens prepared as described were incubated at 37°C for 15 minutes in this solution. The tissues were then transferred to 1% OsO₄ fixative and treated as described in Section A. Control sections were prepared by deletion of the substrate. Sections were studied by means of a Hitachi HU-11B electron microscope.

RESULTS

The surface of epidermal cells in Bowen’s disease is irregular (Figs. 1, 2). Villous projections protrude into wide intercellular spaces. Some of these projections are in contact with similar projections from adjacent cells giving the appearance of rudimentary or malformed desmosomes. Normal appearing, intact desmosomes are few in number.

Interruptions in the continuity of the basement membrane are numerous (Fig. 1). Cytoplasmic projections protruding through these breaks are directly in contact with the dermis.

Nuclei vary in size, shape, and even number. Multinucleated giant cells with closely packed nuclei are present. The nuclear outlines are moderately irregular. Distinct bilamellar nuclear membranes are usually present. Nucleolar size is markedly increased. Measured by the method of Nix et al. (9), the average nucleolus was 60% larger than controls. The enlarged nucleolus consists of dense strands which branch to form a reticulate network. This reticulate appearance may be seen in normal epidermal cells but is usually less distinct.

Within the cytoplasm there is an abundance of Golgi apparatus and endoplasmic reticulum. The mitochondria exhibit extreme polymorphism (Fig. 3). Accumulations of glycogen are observed (Fig. 4). The quantity of melanin is markedly reduced within epidermal cells. Most cells contain no melanin at all, although dendrites containing melanin are present between the cells.

Tonofilaments are relatively sparse at the periphery of the cell. Formation of keratin within basal and spinous cells occurs without keratohyaline formation through condensation of tonofilaments which have lost their desmosomal attachments. The resulting dyskeratosis may be focal (Fig. 5) or may involve the entire cell (Fig. 6). Cytoplasmic organelles are frequently entrapped within the aggregating tonofilaments but disappear as keratinization progresses. The acid phosphatase reaction is positive within these incompletely keratinized condensations. Many of these dyskeratotic cells are cannibalized by surrounding keratinocytes (Fig. 6).

Intracytoplasmic virus-like particles were observed in all four specimens (Fig. 7). These particles were target-like spheres of uniform size between 80 and 110 mµ and possessed a uniformly dense nucleoid between 55 and 75 mµ in size. These particles were noted in highest concentration in cells which had

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a marked alteration in structural integrity. Such cells were relatively electron lucent. Structures resembling budding virus particles were observed within the cytoplasm. We have never observed similar particles in normal skin or other pathologic skin specimens. The significance, if any, of this observation is dependent upon additional investigations.

DISCUSSION

The discontinuity of the basement membrane observed in these cases of Bowen's disease is in contrast to the intact basement membrane found in basal cell carcinoma (12). Luibel et al. (6) found that the basement membrane was intact in cervical cancer in situ but was often lacking in invasive cancer of the cervix. In addition, Luse (7) stated that the basement membrane remains intact in cancer in situ but not in invasive cancer, while Dobson (4) found basement membrane dissolution prior to invasion of experimental epidermal tumors. Since Bowen's disease is ordinarily not invasive—at any rate not until after a period of years—this study casts doubt upon the hypothesis that the basement membrane is important in restraining invasion of cancer into connective tissue.

The findings of organelle entrapment and acid phosphatase activity observed prior to dyskeratotic keratinization are exactly analogous to the observed acid hydrolase activity in normal superficial epidermis prior to normal keratinization (11), suggesting a role by hydrolytic enzymes in the degradation of cellular organelles prior to keratinization. The importance of a disturbed desmosomal-tonofilament relationship in abnormal keratinization has been emphasized by Caulfield and Wilgram (3) in Darier's disease and Hailey-Hailey disease. They reported that condensation of tonofilaments following their separation from desmosomes results in dyskeratosis. Braun-Falco and Vogell (2), however, have shown that apparently normal keratinization may occur in areas with abnormal desmosome-tonofilament complexes.

Other investigators (5, 8, 12) have also reported decreased contact between cancer cells. Such lessened adhesion between epidermal carcinoma cells may explain the efficacy of treatment by curettage since such tumor cells are easily separated from surrounding healthy tissue.

The abundance of endoplasmic reticulum and abnormal subcellular organelles suggests very active production of abnormal cell-constituents. The distorted morphology of mitochondria is particularly striking. The Golgi apparatus was also much more prominent than in normal epidermis.

Increased nucleolar size has been observed in a variety of tumors. This finding is not specific for cancer, however, as Nix et al. (9) found nucleolar enlargement following ultraviolet light exposure.

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REFERENCES

Fig. 1. Low power electron micrograph of Bowen's disease at the dermal-epidermal junction. Note cytoplasmic projections (C) extending through the basement membrane (Bm) into the dermis. Villous projections protrude into dilated intercellular spaces. Some of these meet similar projections from other cells to form rudimentary desmosomes (d). Relatively few normal appearing desmosomes (D) are seen. × 10,800.

Fig. 2. Typical Bowen's disease keratinocyte with few intact desmosomes. Papillary projections into the intercellular spaces are numerous. The nucleoli are prominent. Few tonofilaments insert into the desmosomes. Endoplasmic reticulum (er), Golgi apparatus (G). × 17,400.

Fig. 3. Bizarre, but still recognizable, mitochondria (M) are prevalent. Tonofilaments (T). × 54,800.

Fig. 4. Glycogen accumulations are present. Note surrounding membranes (m). × 77,700.

Fig. 5a. Acid phosphatase reaction. Lysosomes (L) are scattered throughout the cytoplasm. × 63,200.

Fig. 5b. Acid phosphatase reaction. Dyskeratotic foci with reaction product. × 37,100.

Fig. 6. Dyskeratotic cell which has been completely engulfed by an adjacent keratinocyte. × 30,000.

Fig. 7. Basal cell with cytoplasm filled with virus-like particles. Inset a shows high magnification of the particles within the square. Inset b shows intracellular virus-like particles. Budding structures (†) as well as mature particles (⊗) are seen. Desmosome (D).

Ultrastructure of Bowen's Disease

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