The Surface Ultrastructure of Normal and Metaplastic Cervical Epithelia and of Carcinoma in Situ

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SUMMARY

The surface ultrastructure of normal and abnormal human cervical epithelium was examined by scanning electron microscopy. The characteristic appearance of normal columnar and squamous cells and of cells undergoing physiological metaplasia are described; marked differences between these cells and cells from carcinoma in situ were observed.

INTRODUCTION

CIS of the human cervix uteri is widely accepted as a preinvasive lesion that often progresses to invasive squamous carcinoma. Similarly, CIS is believed to arise by progression from cervical dysplasia, the earliest detectable neoplastic change in cervical epithelium (16). However, there is disagreement as to how often there is progression from dysplasia to CIS, and from CIS to invasive carcinoma, and as to how often lesions remain static or perhaps even regress (6, 14, 15). In addition to occasional neoplastic change, cervical columnar epithelium may undergo physiological transformation to squamous epithelium by a process of metaplasia. This process may occur at any time, but is most common during puberty and during the 2nd and 3rd trimesters of the 1st pregnancy (4). Neoplastic changes most frequently occur at the squamo-columnar junction or within the colposcopic transformation zone (that part of the cervix which has undergone metaplasia), and Coppleson and Reid (4) have suggested that epithelium that is undergoing metaplasia may be more susceptible to carcinogenic agents than mature columnar or squamous epithelium.

The fine structure of normal metaplastic and dysplastic epithelia and of CIS and invasive carcinoma of the cervix has been studied extensively (9, 18). Increasing degrees of abnormality were noted in nuclear size and shape, chromatin distribution, number and arrangement of cytoplasmic organelles, and surface membrane features, during the change from normal to grossly neoplastic tissue. In particular, increased numbers of desmosomes, tight junctions, and tonofilaments and increased numbers of microvilli were seen in more abnormal cells.

Recently, the SEM was used to examine cancer cells both in culture (1) and from tissues in vivo (20), and striking differences between the surfaces of normal and neoplastic cells were seen. The SEM allows examination of surfaces at magnifications from X 20 to X 50,000, producing quasi 3-dimensional images with great depth of focus. Because of the surface differences reported in transmission electron microscopy studies of normal and abnormal cervical epithelia, we undertook this project to examine the surfaces of these tissues by SEM preliminary findings (19) indicated that normal and abnormal cervical cells showed characteristic features that might be of use in the diagnosis of cervical neoplasia (12). The observations reported here extend these studies in relation to normal cervical epithelium and CIS and, in lesser detail, to metaplasia.

MATERIALS AND METHODS

Specimens were obtained from nonpregnant patients who had undergone gynecological surgery. During this study, 20 normal specimens and 40 specimens that showed various types of abnormal epithelium were examined. The cervix was usually rinsed lightly with 0.9% NaCl solution for removal of adherent mucus, although some specimens were fixed unwashed. With the 0.9% NaCl solution technique of colposcopy described by Koller (13) and Johannisson et al. (11), areas of cervix were selected for study and removed by wedge or cone biopsy. The classical method of colposcopy, with acetic acid and Schiller’s iodine, was avoided because of the effect of these reagents on cell surface morphology. The biopsy specimens (epithelium, 3 to 6 x 5 to 10 mm; stroma, 3 to 5 mm) were washed fairly vigorously with ice-cold 0.9% NaCl solution from a Pasteur pipet for removal of blood and any remaining mucus, and were fixed in 2.5% glutaraldehyde in cacodylate-buffered sucrose (17). Care was taken to avoid touching the epithelial surface, and the specimens were transferred between reagents by means of a pin that transfixed the stroma. After fixation, the specimens were washed in cacodylate-buffered sucrose solution, dehydrated through graded aqueous acetones, and dried from absolute acetone at room temperature. Dry specimens were mounted with Araldite [Ciba-Geigy (U.K.) Ltd., Duxford, Cambridge, England] with the epithelial surface up on aluminum specimen stubs, coated in a vacuum with gold-palladium about 20 nm thick, and examined in a Stereoscan Mark IIA SEM (Cambridge Scientific Instruments Co. Ltd., Cambridge, England). A few specimens were fixed in

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2The abbreviations used are: CIS, carcinoma in situ; SEM, scanning electron microscope.

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10% neutral formal-0.9% NaCl solution instead of glutaraldehyde and were processed as described above. After glutaraldehyde fixation and a cacodylate wash, other specimens were washed thoroughly in distilled water, quenched in isopentane, cooled in liquid nitrogen, and freeze dried (3) in an Edwards-Pearse tissue freeze-drier (Edwards High Vacuum Ltd., Crawley, Sussex, England) before they were mounted, coated, and examined in the SEM.

After examination in the SEM, all specimens were processed to provide hematoxylin- and eosin-stained sections (2) for confirmation of identification of the areas observed in the SEM. In order to examine the undersurface of cervical cells, we stripped cells off the cervix, using the membrane filter techniques of Evans et al. (5). Pieces of filter [Millipore (U.K.) Ltd., Wembley, London, England] were applied to the cervix, then were removed, fixed, and processed for the SEM as described above, except that dehydration was done in graded ethanol, because the filter was soluble in acetone.

The dimensions given in “Results” were obtained by measuring cell and tissue features on scanning electron micrographs and are thus approximate dimensions only. Accurate measurements from scanning electron micrographs can only be obtained by stereometric analysis of stereo pairs of micrographs; facilities for this procedure were not available.

RESULTS

With the SEM, large pieces of tissue could be examined and the interrelationship between different types of epithelium (normal and abnormal) could be assessed. A complete specimen of normal tissue (Fig. 1) taken from the region of the cervical external os shows the demarcation line between the relatively smooth squamous epithelium and the greatly dissected columnar epithelium. Fig. 2 shows a section from this specimen taken after SEM examination.

Normal Columnar Epithelium. Seen at low magnification, the most striking features of columnar epithelium (Figs. 1 and 3) were the many long ridges and finger-like villi. Between the ridges were deep clefts lined by columnar epithelium extending into the stroma. The villi and clefts did not lie at right angles to the general plane of the epithelium but were oriented obliquely towards the external os. The squamocolumnar junction was usually sharply defined, unless metaplasia was present (see below).

At higher magnification, the individual columnar cells could be seen (Fig. 4). These covered the entire surfaces of the villi, clefts, and any intervening areas and were the exposed tips of the elongated cells seen in sections. The cells were small (about 4 μm in diameter), irregularly polygonal, of uniform size, and closely packed, presenting a “cobblestone” appearance. The surface of the cells was slightly raised and was covered with many short microvilli (about 2 μm long). These were frequently matted together and partially obscured by mucus that was difficult to wash away completely. No intercellular junctions could be seen between columnar cells. Ciliated cells, seen among columnar cells (Fig. 5), occur infrequently in cervical smears; and their function, if any, is not clear, although it has been assumed that they produce movement of cervical mucus (10). In the SEM, ciliated cells covered large areas of the epithelium of the endocervical canal but occurred rarely on the ectocervix.

In addition to villi and the large ridges and clefts, small invaginations of the epithelium, lined with columnar cells, were seen occasionally (Figs. 6 and 7). These were the clefts or Fluhrmann’s crypts (7), which delved into the stroma of the cervix and which are frequently, but incorrectly, termed glands.

Normal Squamous Epithelium. Squamous epithelium differed markedly from columnar epithelium, appearing smooth in contrast to the dissected surface of the latter. The epithelial surface of washed specimens was covered with large (30 to 40 μm in diameter), flat polygonal cells with well-defined nuclei and raised terminal bars between the adjacent cells. The cells were arranged in an irregular, pavement-like pattern with some cells partially overlying those beneath (Fig. 8). At higher magnification, the surfaces of these cells showed a typical pattern of microridges (Fig. 9). These microridges were approximately 0.15 μm wide with spaces of about 0.25 μm between them. The length of these microridges varied considerably (up to 40 μm), and there was much branching and anastomosis. However, on normal squamous cells, microridges less than 1.0 μm long rarely occurred (Fig. 10). In the center of the cells, the microridges showed no particular orientation (Fig. 10) but, peripherally, some orientation parallel to cell boundaries was seen (Fig. 11). The terminal bars (about 0.5 μm high) appeared to be formed by the folding over and interdigitation of the edges of adjacent cells [cf. transmission electron microscopy studies (9)]. To determine whether these surface features were artifacts attributable to the method of specimen preparation, we examined squamous epithelia from formalin-fixed and freeze-dried specimens. No significant difference could be seen between the specimens prepared by the 3 procedures. In addition, a very similar pattern of microridges was seen on the surfaces of squamous cells from the cervix and buccal mucosa of rats and ferrets.

Squamous cells that had been stripped off the cervix on membrane filters also showed microridges on their surfaces (Fig. 12), i.e., on the undersurface of the cells as it formed part of the epithelium.

Some specimens that had not been washed prior to fixation were examined. Surface detail was largely obscured by dried mucus but, where the epithelium was visible, numbers of exfoliating cells that were partially detached from the underlying cells could be seen (Fig. 13). The surfaces of many of these exfoliating cells lacked the convoluted microridges seen on squamous cells from washed specimens, and were sparsely covered with small microvilli.

Apart from mucus, which sometimes obscured parts of the epithelium, some nonepithelial cells were occasionally observed lying on the surface of the epithelium (Fig. 14). Some of these nonepithelial cells were readily identified as erythrocytes because of their characteristic shape; the remainder, however, could not be identified with certainty but were assumed to be mostly polymorphonuclear leukocytes because of their size (5 μm) and shape and because exfoliative cytology studies of the cervix show that polymorphonuclear leukocytes are frequently present on the normal ectocervix. Bacteria were also seen lying singly or in clusters on the surface of the squamous cells (Fig. 15). They were usually rod shaped and...
were probably Doderlein's bacillus, *Lactobacillus acidophilus*, which is a commensal of the normal cervix and vagina.

**Squamous Metaplasia.** In this study, no attempt was made to examine a series of specimens, such as could be obtained from pregnant women (4), that would show the development of squamous metaplasia. Nevertheless, the appearance of areas of metaplasia that were seen in specimens from nonpregnant individuals could be correlated with some of the stages recognized by Coppleson and Reid (4). The earliest stage was observed at the tips of columnar villi (Fig. 16) where the even cobblestone pattern was sometimes distorted by the presence of larger cells (10 µm in diameter) scattered among the normal columnar cells. The surfaces of these cells were slightly rounded and were covered with short, close-packed microvilli. No terminal bars between the cells were observed. Coppleson and Reid found that, in the 2nd stage of metaplasia, fusion of columnar villi occurs, and the superficial layers of the metaplastic epithelium consist of cuboidal cells. In the SEM, these cells are seen as islands of flattened, irregularly polygonal cells merging with the surrounding smaller cells (Fig. 17). The surfaces have small, closely packed microvilli, and there is some tendency to formation of microridges (Fig. 18). In specimens with more advanced metaplasia, terminal bars similar to those between mature squamous cells were observed, although the surfaces of the cells were still covered in microvilli rather than microridges (Fig. 18). Since we were able to examine large areas of epithelium by SEM, the multifocal nature of physiological squamous metaplasia was clearly revealed (Fig. 19).

**CIS.** Areas of CIS differed markedly from normal columnar and squamous epithelia, and from squamous metaplasia. At low magnification (Fig. 20), the most obvious characteristic was the disorganized appearance of the epithelium compared with normal areas. The cells were rounded, of diverse sizes and shapes, and did not have interdigitating boundaries (Fig. 21). At higher magnification (Fig. 22), the surfaces of individual cells appeared granular due to the presence of numerous microvilli (about 0.15 µm in diameter). They appeared to be short but were so densely packed that the intervening surface of the cells could not be seen, preventing assessment of their length. Some microvilli formed short anastomoses with other microvilli, but no true microridges as seen on normal squamous cells were observed. A feature of cells from CIS was the presence of "holes" in the surface of the cells (Fig. 23). These bizarre structures may have been artifacts, but were observed only in CIS. Similar holes have been seen in rat tumor cells (A. E. Williams, unpublished results). The junction between CIS and columnar epithelium was frequently very sharply defined (Fig. 24). In 1 of the specimens (Fig. 25), a number of unusual cells were seen between the normal columnar and CIS areas. The exposed surfaces were fairly large (7 x 4 µm) and were elongated parallel to the columnar CIS junction. The surfaces were covered with groups of short cilia or microvilli (about 0.5 µm long), the tips of which were fused together, probably by adherent mucus. This cell type, which has been seen from only this 1 specimen, was designated a "hedgehog cell" on a purely descriptive basis. Unfortunately, the method for cutting sections from SEM specimens had not yet been devised when the specimen was examined, and correlation with histological appearance was not possible.

Similarly, the boundary between cells of CIS and normal squamous cells was usually well defined. Often the abnormal area was lower than normal squamous epithelium and a lip was observed at the junction (Fig. 26). The squamous cells on the edge of the lip adjacent to and slightly overlying the neoplastic tissue were not completely normal; the surface was covered with short, very closely packed microridges interspersed with microvilli (Fig. 27); yet, these cells seen in section (Fig. 28) after examination in the SEM appeared perfectly normal by the standard criteria of light microscopy.

**DISCUSSION**

This study by SEM of normal and abnormal cervical epithelia has revealed details of the surface structure that had been almost unobtainable by previous methods. It has been possible to map large areas of epithelium and to examine the interrelationship of the various cell types. The ability to confirm the identity of the cell by post-SEM histology was essential, as the identification of cell types in unfamiliar material on evidence of the SEM alone is fraught with uncertainty.

The results indicate that previous studies by transmission electron microscopy have lead to a misinterpretation of the surface structure of squamous cells. Previously described differences between the surfaces of superficial squamous cells and CIS, derived from transmission electron microscopy studies, have been limited to notation of an increase in the density of microvilli on CIS cells relative to normal squamous cells. Without tedious serial ultrathin sections, it would have been difficult to appreciate that the finger-like projections on the surfaces of squamous cells seen in section were, in fact, sections through microridges. The SEM showed that normal squamous cells and CIS cells have entirely different surfaces. Hackeman et al. (9) showed interdigitation of the surface projections on the upper and lower surfaces of superficial squamous cells. The fact that these surface features are recognized as microridges suggests that they may help to maintain the integrity of the epithelium by offering resistance to sideways movement between layers of cells and by increasing the area of cell contact. Conversely, the presence of extremely tightly packed microvilli on the surfaces of CIS cells and the rounded shape of the cells may inhibit interdigitation of adjacent surfaces and may contribute to the well-recognized friability of CIS tissue. It seems probable that microridge structure may be typical of squamous epithelia in general, in view of the similarities observed between specimens from different anatomical sites and species. The microridge structure was lost from squamous cells that were exfoliating and that were presumably dead and keratinized. The structure of columnar cells, as seen by SEM, correlated well with that known from transmission electron microscopy. In addition, the SEM provided a new appreciation of the overall structure of this type of epithelium, particularly with relation to the columnar clefts and ciliated cells. Clearly the surface conformation of normal squamous cells and CIS cells differs. CIS cells do not arise directly from mature, differentiated squamous cells but, rather, as a result of the failure of
immature cells to differentiate fully. Thus the microvilli of the CIS cells have not replaced the microridges on individual squamous cells—the microridges have simply not developed. As yet, the stage in the progression from normality through dysplasia to CIS, at which cells lose the ability to form microridges and form microvilli instead, is unknown. However, it appears that a tendency toward an abnormal surface may be present in cells that are normal by standard histological criteria. Examinations of biopsies of dysplastic epithelium and of other cervical abnormalities such as Trichomonas vaginalis infections, inflammation, and trauma are in process. It is hoped that dysplasia may be differentiated into subgroups—those that will change to cancer and those that will not—on the basis of surface features of the tissue or cells.

Superficially, there was some similarity between cells undergoing metaplasia and those of CIS, perhaps lending support to the hypothesis that cervical neoplasia arises from metaplastic cells. Nevertheless, the 2 types of cells differed, particularly in that squamous metaplasia was an organized process, contrasting markedly with the disorganization of CIS. The presence of numerous microvilli on metaplastic and CIS cells suggests that they may be characteristic of active cells in general rather than of neoplastic cells in particular. These studies are being extended, with the use of specimens from pregnant women and laboratory animals, for a detailed investigation of sequential changes during metaplasia.

The most frequently used technique leading to a diagnosis of CIS is the examination of stained smears of exfoliated cells. Exfoliated cells may also be examined by SEM. Friedlander (8) published scanning electron micrographs of exfoliated normal and abnormal cervical cells. Although he compared these cells with parallel preparations stained by the Papanicolaou stain and examined by light microscopy, he made no attempt to confirm the identification of the individual cells that were examined in the SEM. Preliminary results (19) indicated that it was possible to examine an individual cell by light microscopy and by SEM. A detailed account of the method and its application to the examination of exfoliated cells will be the subject of a later publication.

REFERENCES

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Fig. 1. Complete biopsy specimens of cervical tissue showing epithelial surface. c, columnar epithelium; s, squamous epithelium. X 13.

Fig. 2. Section from specimen shown in Fig. 1 after examination by SEM; c, columnar epithelium. s, squamous epithelium. H & E, X 13.

Fig. 3. Columnar epithelium with villi. v, columnar villi. X 230.

Fig. 4. Detail of surface of columnar cells. Each cell is covered with closely packed microvilli and adherent mucus. X 2,300.

Fig. 5. Ciliated cells in a field of columnar epithelium from the endocervical canal. ci, cilia. X 5,600.

Fig. 6. Entrances to clefts in columnar epithelium. X 135.

Fig. 7. Detail from Fig. 6 showing columnar cells (col) lining entrance to epithelial cleft. e, erythrocyte. X 1,350.

Fig. 8. Pavement-like appearance of squamous epithelium. tb, terminal bars between cells; n, nucleus. X 550.

Fig. 9. Cell from squamous epithelium showing surface microridges (mr). b, cell boundary; n, nucleus. X 1,680.

Fig. 10. Detail of cell surface overlying nuclear region of cell in Fig. 9. No orientation of the microridges is present. X 7,000.

Fig. 11. Detail of cell surface at periphery of cell shown in Fig. 9. Orientation of microridges (mr) parallel to the cell boundary (b) is seen. X 3,900.

Fig. 12. Surface of squamous cells removed by membrane filter technique. The characteristic ridges, similar to that seen on the surface of biopsy specimens, are on the undersurface of these cells as they form part of the epithelium. b, cell boundary; mr, microridges. X 5,200.

Fig. 13. Unwashed biopsy specimens exfoliating squamous cells (e). X 550.

Fig. 14. Erythrocytes, fibrin, and polymorphonuclear cells adherent to the surface of cervical epithelium. f, fibrin; p, polymorphonuclear cell; rbc, erythrocyte. X 3,800.

Fig. 15. Colony of rod-shaped bacteria, probably Doderlein's bacillus, on the surface of squamous epithelium. X 7,000.

Fig. 16. Early stage of metaplasia at tip of columnar villus; formation of giant cell. m, metaplastic cell. X 1,150.

Fig. 17. Later stage of metaplasia showing island of cuboidal cells merging with normal columnar cells; c, columnar cells; cs, cuboidal metaplastic cells. X 630.

Fig. 18. Metaplastic cells showing formations of well-defined terminal bars (tb). c, columnar cells. X 1,330.

Fig. 19. Low-power view of metaplastic area showing multifocal nature of process. mf, metaplastic focus. X 250.

Fig. 20. CIS showing disorganized appearance of epithelium. Note rounded cells; irregular shape, size, and arrangement; and lack of interdigitating boundaries. X 250.

Fig. 21. Detail from Fig. 20 showing granularity of cell surface due to presence of fine microvilli. X 1,250.

Fig. 22. Surface of a cell from CIS (cis) showing closely packed microvilli. X 15,080.

Fig. 23. CIS cells showing surface holes. X 2,400.

Fig. 24. Junctions (j) of CIS and columnar epithelium (c). X 775.

Fig. 25. Hedgehog cells (h) at junctions of CIS and columnar epithelium. X 3,100.

Fig. 26. Squamous epithelial lip overlying area of CIS (cis). s, squamous epithelium. X 235.

Fig. 27. Surface of cells from squamous area in Fig. 26 showing abnormality of cells compared with normal squamous cells. The microridges are closely packed and intermingled with short microvilli. X 5,800.

Fig. 28. Post-SEM histology of specimen shown in Figs. 26 and 27, indicating the apparent normality of squamous cells adjacent to and overlying the area of CIS (cis). ns, normal squamous epithelium. X 258.
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