A Comparison of Surface Ultrastructures of Normal, Papillomatous, and Carcinomatous Epidermal Cells*

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The surface ultrastructure of normal rabbit epidermal cells and Vx2 carcinoma cells was investigated by Coman and Anderson (3) by means of electron microscopy, using replica methods. They found differences between the two types of cells and concluded that a change in the ultrastructure of the cell surface accompanies the transformation from a normal to a carcinomatous state. It is possible that this structural change reflects the invasive character of cancer cells. If so, such a change would not be expected in the pre-invasive stage of the neoplastic transformation. This hypothesis can be tested by examination of the Shope papilloma cells, which can be considered a transitional stage between normal rabbit epidermis and the Vx2 carcinoma. The surface ultrastructure of the papilloma, as a non-invasive neoplasm, might be expected to resemble the surface of the normal cell rather than that of the malignant. Experiments were therefore designed to compare the surface ultrastructures of Shope papilloma cells, normal rabbit epidermal cells, and Vx2 carcinoma cells.

MATERIALS AND METHODS

There was great difficulty in collecting adequate numbers of normal epidermal cells for examination. Coman and Anderson (3) scraped cells from the rabbit's back into a drop of balanced salt solution after depilation with barium sulfide paste. Numerous attempts to collect enough undamaged cells by this method were unsuccessful, and therefore other technics were investigated. The method of Billingham and Reynolds (1), slightly modified, was adopted and gave excellent results. The method depends on the tryptic digestion of thin slices of skin to promote the separation of individual cells. Although treatment with trypsin may alter the cell surfaces, differences among various types of cells can still be studied by treating all material in a similar fashion.

Normal epidermal cells were collected from the dorsum of the rabbit's ear while the animal was anesthetized with nembutal. The ear was closely shaved, washed free of soap, and dried by being patted with lint-free lens tissue. The thinnest possible tangential sections were then cut with a new razor blade.

The skin sections were almost transparent, consisting of the full epidermis and superficial dermis. These were stretched on a clean microscope slide, dermis side down. A piece of water-proof electrical tape (Scotch brand) was placed over the epidermal portion and pressed firmly. The tape was removed with the section adhering by its epidermal side. The attached skin was rinsed in Ringers and placed in a small Petri dish containing 0.5 per cent crude trypsin (GBI) in buffered Ringer solution, pH 7.8, (1). This was incubated for 1 hour at 38°C. The tape and attached tissue were then removed and rinsed several times with physiological salt solution.

The excess fluid was blotted with filter paper and the dermis gently teased away with fine forceps, leaving a portion of the epidermis attached to the tape. Since most of the basal layer of the epidermis was still fixed to the dermis, this was stretched out on a watch glass, basal cells up. Both samples were then covered with a few drops of physiological salt solution and their exposed surfaces scraped gently with a small scalpel blade. The loosened cells floated in the saline and were transferred to serological tubes. The scraping procedure was repeated twice with fresh saline. The collections usually contained large clumps which were easily broken up into individual cells by vigorous pipetting.

Papilloma cells were obtained from month-old papillomas raised on the ears of domestic rabbits. Vx2 carcinoma cells were obtained from stock tumors maintained by transplantation. Both types

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were exposed to trypsin simultaneously with the normals.

The tumor-bearing rabbits were anesthetized with nembutal and the tumors removed surgically. All keratinized material in the papillomas and necrotic material in the carcinomas were dissected away exposing the grayish-white tumor tissue, which was then cut into thin slices, rinsed in Ringer solution, and fixed to the tape. These sections were treated in the same manner as the normal tissue except that they were scraped directly, since there was no dermis to be lifted away.

All three types of cells were washed 3 times in physiological salt solution by centrifugation. After the third washing, the cells were resuspended in 1 per cent osmic acid and fixed for 5 minutes. They were then washed 3 times with distilled water. Suspensions of all cells were spread on clean mica slips and allowed to air-dry.

Chromium-carbon replicas of the specimens were made by the technic of Coman and Anderson (3), as modified by Nowell and Berwick (4). The replicas were mounted on copper grids and examined with a Philips electron microscope.

RESULTS

Normal epidermal cells.—These appeared both as individual cells and as small sheets of cells. The sizes of the single cells were variable, ranging from 10 to 25 μ in diameter, and, for the most part, the edges of the cells were clearly outlined and intact. At low magnification, the position of the cell nucleus was seen outlined by the collapsed cell membrane (Fig. 1). The cell surface at this magnification appeared wrinkled throughout. At higher magnification, the wrinkles were seen to consist of many tongue-like structures projecting from the surface (Fig. 2). These have been identified as prickles (3). The surfaces of the prickles consisted of closely packed granules ranging from 60 to 125 A in diameter. These small granules also appeared on the cell surface between the prickles, and, in addition, there were larger aggregates, 250 to 300 A in diameter, evenly dispersed throughout. The total surface effect was one of a fine homogeneous granularity.

Vx2 epidermoid carcinoma cells.—These cells appeared more fragile than the normals. The edges were often poorly delineated and obscured by fine debris. This probably was produced by rupture of the membrane during drying with extrusion of the cellular contents. At low magnification, the cells invariably showed larger spherical surface structures from 0.3 to 1.0 μ in diameter (Fig. 3). Although they sometimes appeared to be quite superficial and easily detachable, only rarely were they seen free. The wrinkling of the cell surface was not as prominent a feature as it was in the normal. This absence of wrinkling became clear at the higher magnifications when it was seen that the prickles did not exist (Fig. 4). The prickles seemed to have been replaced by the large spheres already described. These appeared variously as slight protrusions or bulges in the surface, with progressive development to fully formed spheres. The background structure no longer consisted of packed small granules. Instead, there was a mixture of large nodules loosely thrown together to give a rough, pitted effect. Small particles were distinguished on the nodular surfaces. These varied between 150 and 300 A in diameter. The variation in nodular size and the loose, haphazard arrangement of the aggregates gave the carcinoma cells a rough coarseness easily distinguishable from the normal cells.

Shope papilloma cells.—In general, two types of papilloma cells were seen at low magnification. About half the cells appeared as in Figure 5. They were about 12-15 μ in diameter and had moderately prominent surface wrinkles. At higher magnification (Fig. 6), the prickles were less numerous than in the normal cells, but they still lay on a background which closely resembled the normal, i.e., a homogeneous surface with evenly distribut-
ed, fine particles. The rough coarseness characteristic of the surface of the Vx2 carcinoma cell was not present.

The other principal type of papilloma cell appeared at low magnification as in Figure 7. Here the wrinkles were absent, having been replaced by round, regular protrusions on the cell surface. These protuberances were apparently formed by the collapse of the surface membrane over dense loci within the cell. This type of cell somewhat resembled the Vx2 carcinoma at low magnification (Fig. 3). At high magnification, however, the coarseness of the malignant surface was not present. The small particles making up the background surface varied more in size than in Fig. 6, and there was a greater tendency to aggregate into varying sized clumps. In other words, the background surface was obviously altered from the normal in that the regularity of particle size was lost.

DISCUSSION

The cell surfaces of normal epidermis, Shope papilloma, and Vx2 carcinoma differ in two respects. There are relatively gross differences, involving structures of 0.5 μ and greater; and there are finer or ultrastructural differences, involving structures 50-300 A.

By means of the gross changes, one can distinguish between normal epidermal cells on the one hand, and papilloma and carcinoma cells on the other. Normal epidermal cells have many prickles regularly arranged on their surfaces. In the papilloma, these prickles become flattened and are replaced by low-lying spherules. These, in turn, seem to develop in the carcinoma into haphazardly scattered, well-developed spherical bodies. The significance of this phenomenon is unknown, but it may represent focal intracellular condensations which are made visible by the collapse of the cell membrane over these areas of increased density. These grosser changes may also correspond to the existence and development of neoplasia.

By means of the finer or ultrastructural changes, one can distinguish between normal and papillomatous cells on the one hand, and carcinoma cells on the other. These changes involve the fine granular structure which makes up both the principal cell surface and the surface of the protuberances on the cell. Normal cells have a fine and uniform granularity. Papilloma cells, for the most part, resemble normal cells in this respect, although there are small variations in particle size and aggregations which tend to disrupt the homogeneity. In contrast to these, the Vx2 cell surface is completely disorganized, with a wide range of particles and aggregates—all combined in a most haphazard manner.

This organization of fine structure may distinguish mutually adherent non-invasive cells from those with little adhesive strength, i.e., cells with invasive properties. Previous studies of the surfaces of normal and leukemic lymphocytes tend to support this hypothesis (4). Both these cell types are normally invasive, and both have surfaces which show wide ranges in the size of their granules and aggregates. The phenomenon of invasiveness is associated with reduced adhesiveness and loss of calcium (2), and it is tempting to associate these characteristics with the physical changes seen by electron microscopy; but the relationship is still too tenuous to justify more than a suggestion along these lines.

SUMMARY

Experiments were made to compare the surface ultrastructures of normal rabbit epidermal cells, Shope papilloma cells, and Vx2 carcinoma cells by electron microscopy of carbon-chromium replicas.

Differences were found between normal and carcinoma cells, both at low and high magnifications, similar to those previously reported. The surfaces of papilloma cells presented a transitional form between normal and malignant cells. At the ultrastructural level, the papilloma cells resembled the normal cells, i.e., their surfaces consisted of more homogeneous fine granules and fewer coarse aggregates. At a grosser structural level, the papilloma cells resembled the Vx2 carcinoma rather than the normal cells, i.e., there was a disappearance of prickles and their replacement by spherical protuberances.

It was concluded that the transition to neoplasia was not positively demonstrable at the ultrastructural level but that the change to invasiveness might be reflected by the macromolecular structural changes which are present.

REFERENCES

Fig. 3.—Vx2 carcinoma cell with poorly delineated borders and prominent spherules. Prickles are no longer seen.

Fig. 4.—Higher magnification of portion of cell in Figure 3. Cellular contents have been partially extruded. Prickles have been replaced by large spherules whose surfaces are composed of large aggregates and clumps with marked variations in size.
Fig. 5.—Papilloma cell with large nuclear outline and moderately prominent wrinkles. Such cells cannot be distinguished from normal cells.

Fig. 6.—Higher magnification of portion of cell in Figure 5. The prickles are flattened and assume a plaque-like appearance. The fine surface still remains homogeneous, with no more variability of granule size than that seen in the normal.
Fig. 7.—Papilloma cell with prominent low-lying spherules. Wrinkles are no longer present to any great extent.

Fig. 8.—Higher magnification of portion of cell in Figure 7. Some plaque-like prickles still remain, but most have been replaced by the spherules studding the cell surface. The fine structure is more variable in size than in Figure 6 but still does not approach the coarse appearance of the Vx2 carcinoma cell surface seen in Figure 4.
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