Cell Differentiation and Tumor-promoting Action in Skin Carcinogenesis

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SUMMARY

The early ultrastructural changes induced by the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate in mouse epidermis previously transformed by treatment with an initiating dose of 7,12-dimethylbenz(a)anthracene are described and compared with those in the basal cells of papillomas and carcinoma that appear later after repeated promoter treatment. Groups of cells with fine structural characteristics distinct from those of the other epidermal cells are observed 48 hr after a single application of the promoter to skin previously treated with an initiating dose of 7,12-dimethylbenz(a)anthracene. With repeated weekly treatment of the promoter, progressively larger groups of the atypical cells are observed, forming small papillomas by the fifth week. The striking similarity between the fine structural features of the atypical basal cells in the focal groups and those of the basal cells in papillomas and carcinomas, which develop later, suggests that these groups of phenotypically altered cells observed early after promotion are precursor neoplastic cells from which papilloma and carcinoma induced by the two-stage technique of skin carcinogenesis are derived.

The absence of these phenotypically altered basal cells after treatment with a single, initiating dose of 7,12-dimethylbenz(a)anthracene, indicates that these phenotypic characteristics are acquired very early after treatment with the promoter and suggests that the phenotypic expression of the neoplastically transformed cells is the essential event of tumor-promoting action. On this basis, it is proposed that the events occurring during promotion can be dissociated into two main components. First, there are the changes induced by the promoter in the epidermal stem cells or in their microenvironment that enable the expression of the neoplastic phenotype. Second, there are the specific phenotypic alterations that occur in the transformed cells in response to promoting stimuli and the subsequent changes in these phenotypic properties during progression of the neoplastic process. The key to understanding tumor-promoting action is the ability to determine which of the many changes induced by promoters are essential for the phenotypic expression of neoplastic transformation.

Two main possibilities are suggested to account for the phenotypic alterations observed in the basal cells of the atypical focal groups, papilloma and carcinoma. First, they are mutant-like cells induced to express their new phenotypic properties by the tumor promoter. Second, these phenotypic alterations in the neoplastic stem cells are due to loss of differentiated traits in the course of carcinogenesis and the acquisition of features found in embryonic cells. Whatever the nature of these phenotypic changes, they occur mainly in the basal cells, and their progeny differentiate normally, indicating that the neoplastic phenotype is reversible and that the relevant and essential changes to epidermal neoplasia are those of the stem cell fraction of the tumor population.

It is proposed that the ability of the promoter to modulate gene activity, perhaps derepressing genes and inducing reversal of the epidermal cell differentiation, may be the alteration that is essential for the activation of the gene loci that code for the neoplastic phenotype.

INTRODUCTION

Relatively few studies have attempted to analyze the alterations that occur at a cellular level during tumor promotion (2, 4, 14, 15, 19, 21, 22). Indeed, the failure of the work so far performed (1, 6, 20, 21, 24) to define which of the cellular alterations induced by promoters are relevant to their promoting action has prevented the formulation of a rational strategy for the study of the molecular biology of promotion that is needed to obtain insight into the nature of the events that occur in this stage of neoplastic evolution.

If we consider that, in the 2-stage technique of skin tumorigenesis, the “initiated” or transformed cells induced by treatment with a carcinogen may remain latent for the life-span of the animal without showing any morphological characteristics to distinguish them from the neighboring nonneoplastic cells in the epidermis, and that a promoting factor is required to allow expression of their neoplastic phenotype and growth to form a clinical neoplasm, 2 questions can be raised. First, which of the cellular changes induced by promoters lead to the phenotypic expression of the neoplastic transformation? Second, what are the phenotypic characteristics that the initiated cells acquire early in promotion?

Some of the cellular alterations induced by the tumor promoter TPA in mouse epidermis have been characterized (14, 15, 18) and compared with those induced by hyperplastic and phlogistic agents (16, 17) that have little or no tumor-promoting activity. The differences in the pattern of cellular changes induced by these different kinds of agent indicate that they induce different types of cellular reprogramming (16, 17) and suggest that the ability of the tumor promoter to modify gene activity, causing a reversal of cell

1This work was supported by Grant-in-Aid MA-4340 from the Medical Research Council of Canada.

Received February 19, 1974; accepted July 25, 1974.

2The abbreviations used are: TPA, 12-0-tetradecanoyl-phorbol-13-acetate; DMBA, 7,12-dimethylbenz(a)anthracene; RER, rough endoplasmic reticulum.
This report describes the ultrastructural changes induced in mouse epidermis during the promoting stage of skin tumorigenesis. The fine structural characteristics of groups of atypical cells observed early after promotion, before the macroscopic appearance of tumors, are analyzed and compared with those of the basal cells of the papillomas and carcinomas that developed later. The existence of common ultrastructural features in the cells of these different stages of neoplastic evolution is suggested, and the relevance of these changes to promotion discussed.

MATERIALS AND METHODS

Chemicals. TPA (chromatographically pure by thin-layer chromatography) was kindly supplied to us by Dr. J. B. Jones (Department of Chemistry, University of Toronto, Toronto, Canada). DMBA was purchased from Eastman Kodak Co., Rochester, N. Y. All solvents and chemicals were reagent grade and were used without further purification.

Animals and Treatment. Female Swiss-Webster mice (ICR strain, Blue Spruce Farms, Inc., Altamont, N. Y.) were 6 to 7 weeks old at the beginning of the experiments. Mice were shaved and treated according to the 2-stage technique of skin tumorigenesis described previously (18). A single dose of 0.1 pmole of DMBA was used as initiator. One week later, weekly applications of 0.016 pmole of TPA were started. Groups of 5 mice were killed weekly for 7 weeks, 48 hr after TPA treatment, for a study of the sequential morphological changes that occur in the epidermis in the period that precedes macroscopic tumors and at the time at which the first tumors appear. Groups of mice with papillomas of various sizes or with carcinoma were killed after 40 weekly TPA treatments. In another group, TPA treatment was stopped after 25 weeks and the mice were left without promoting treatment for 1 year. Mice in which the papillomas did not regress were killed at this time, and papillomas of various sizes were excised. All mice were killed by neck fracture between 9 and 11 a.m.

Electron Microscopic Studies. Fragments from the skin of the back or from papillomas and carcinomas were fixed, processed, and embedded for electron microscopy (14). Representative samples were also fixed and processed for optical microscopic examination (18). Epon-Araldite blocks were cut on a Porter-Blum MT-1 microtome with glass knives. Areas suspected of abnormality in the period that preceded macroscopic tumors and representative areas of papillomas and carcinomas were selected for thin sectioning by examining 0.5 μm sections by light microscopy. Thin sections stained with lead hydroxide by Method A of Karnovsky (7) or double stained with uranyl acetate and lead hydroxide (26) were examined in a Philips 200 electron microscope with 60 kV acceleration voltage.

RESULTS

Sequential Fine Structural Changes Observed in Mouse Epidermis During 2-Stage Skin Carcinogenesis

Fine Structural Changes Induced in Mouse Epidermis before Tumors Appear. The ultrastructural changes induced in the epidermis 48 hr after TPA application to mouse skin treated previously with an initiating dose of DMBA are, in most areas, similar to those observed 48 hr after a single application of TPA to normal mouse skin, as previously reported (14). The basal cells are increased in size and the intercellular spaces between them are markedly dilated (Fig. 2). The nuclei are larger, the chromatin is mostly dispersed, and the nucleolus is prominent. The cytoplasm contains numerous polysomes and enlarged mitochondria. Profiles of RER and Golgi complex are prominent. However, groups of epidermal cells with morphological characteristics distinct from the other epidermal cells can be found at this time. The cells in these groups have an irregular shape, are usually smaller, and their matrix is more electron dense than the other epidermal cells (Fig. 1). The nucleus of these dark cells is irregular in shape; the nucleolus is large, and the chromatin, although mostly dispersed, forms many dense aggregates. The cytoplasm is densely packed with ribosomes and polysomes, not attached to membranes. The mitochondria are larger, richer in cristae, and more numerous than in the other epidermal cells. Cisternal or tubular profiles of RER are few and the Golgi complexes, although present, are not prominent. The basal surface of these cells is usually more undulant than that of other epidermal cells, but their attachment to the basal lamina and the distribution of hemidesmosomes are unchanged. The cells of the 2nd or 3rd layer above the basal cells retain some of the characteristics of the dark cells in these foci, but the cells in the more superficial layers differentiate as in the other areas of the epidermis.

The ultrastructural alterations observed in the epidermal cells after repeated weekly TPA treatment of initiated skin are, in most areas, similar to those observed at 48 hr, although the opening of the intercellular spaces is not as marked. The main difference is in the groups of dark cells. These groups grow progressively larger and, although their main morphological characteristics are maintained, develop more irregular and bizarre shapes. The intercellular spaces between them open more widely, and numerous papillary projections appear in their surface (Figs. 3 and 4). Cisternal profiles of the RER are more conspicuous after repeated treatments. The large groups of these cells observed after 4 or 5 weekly TPA treatments resemble the small tumors that start to appear macroscopically 5 weeks after treatment (Fig. 5).

Papillomas. The fine structural characteristics of the basal cells of the small papillomas observed in the 5th or 6th week after promoter treatment are different from those of the basal cells in the neighboring hyperplastic epidermis. The basal cells in these papillomas are predominantly of 1 cell type, forming a fairly homogeneous population. Their ultrastructural characteristics resemble those of the dark cells observed at earlier times, (Fig. 5). Like the atypical cells seen earlier, the cells of the papillomas have irregular contours, the matrix is markedly electron dense, and the intercellular spaces are widely open. The cytoplasm is densely packed with polysomes and ribosomes not attached to membranes, although the mitochondria are smaller and have less cristae than those in the dark cells 48 hr after TPA treatment (Fig. 1). Contact between the cells is reduced, and they remain attached to each other only by desmosomes or where papillary projections from their surface meet. No alteration is observed in their attachment to the basal lamina. Mastocytes are frequently seen close to the epidermodermal junction in the areas of tumor growth. The
cells in the uppermost layers of the papillomas differentiate, as do the cells in corresponding layers of the hyperplastic epidermis.

The cells in the basal layer of the large papillomas, from mice killed after 40 weekly TPA treatments, form a heterogeneous population composed of 3 main cell types. One of the types is similar to the dark cells of the small papillomas (Fig. 6) and a 2nd resembles the dark cells of atypical clumps of basal cells observed earlier (Fig. 7). The 3rd cell type observed in the large tumors is similar to that of the basal cells in the neighboring nontumoral hyperplastic epidermis (Fig. 8). Although the 2 former cell types predominate, in some areas of these papillomas, groups of cells of each of the 3 types are frequently observed side by side. In the peripheral areas of these tumors, the cells have a more orderly distribution, and the 3rd type of cell predominates. In all these areas, the cells in the upper layers are well differentiated and indistinguishable from cells in the corresponding layers of the neighboring hyperplastic epidermis. In occasional areas at the epidermodermal junction, the basal lamina has disappeared, forming gaps through which cytoplasmic projections from the basal cells extend into the upper dermis and balloon out (Fig. 9). The fine structure of papillomas present in mice 1 year after promoter treatment was stopped resembles that of the large papillomas described above.

Carcinomas. The striking differences observed in the ultrastructural characteristics of the cells in the carcinomas are evident in the nodules of an invasive epidermal carcinoma (Fig. 10). The cells in the basal layer of the nodules are usually smaller than in the layers above them and have very irregular and bizarre shapes. The electron density of their matrix is much higher than that of the cells in the other layers of the nodule. The intercellular space around them is open, and they are attached to neighboring cells by a few desmosomes only (Fig. 12). The cells are separated from the connective tissue by a thin basal lamina (Fig. 12). Profiles of the RER and Golgi complexes are inconspicuous; numerous, densely packed polysomes are the dominant feature of the cytoplasm. Tonoofilaments are very few or, in some cells, absent. The overall ultrastructural features of these cells are those of undifferentiated embryonic cells. The cells in the layers above the basal have progressively more differentiated characteristics (Figs. 10 and 11) and are indistinguishable from the cells in normal epidermis. The isolated carcinoma cells observed in the dermis, or groups of a few such cells, usually lack a basal lamina, and their ultrastructural features are similar to those of the basal cells in the carcinomatous nodule.

DISCUSSION

Groups of atypical cells with fine structural characteristics distinct from those of the other epidermal cells appear after a single application of the tumor promoter TPA to mouse skin previously treated with an initiating dose of DMBA. With repeated weekly treatments with the promoter, progressively larger groups of these atypical cells are observed, preceding the macroscopic appearance of tumors. The focal distribution of these atypical cells, their absence after a single treatment with an initiating dose of DMBA (A. N. Raick, unpublished data), the proliferation they undergo with repeated treatment with promoter and, more particularly, the similarity between their fine structural characteristics and those of the basal cells of the papillomas and carcinomas, suggest that these phenotypically altered cells observed early in promotion are the precursor neoplastic cells from which the papillomas and carcinomas induced by the 2-stage technique of skin tumorigenesis are derived.

However, cells with morphological features that resemble those of the atypical cells described in this report appear when TPA is applied to normal mouse skin (14, 15) and in epidermal wounds (17), although TPA has little or no tumorigenic activity alone (5, 18), and wounding has only a weak promoting activity (1, 6). The features of the atypical cells are thus not peculiar to neoplastic cells but occur also in other variants induced in the epidermal population. It remains to be determined whether specific cellular differences exist that could be used as markers to enable us to distinguish between the atypical cells that appear after treatment of initiated skin with the promoter and other variants that have some morphological features in common. Moreover, the neoplastic nature of these atypical cells seen soon after promotion needs to be established in a more clear-cut way.

If we consider that the groups of atypical cells observed soon after promotion are the cells transformed by the initial treatment with carcinogen, their absence after a single application of an initiating dose of DMBA (A. N. Raick, unpublished data) indicates that the expression of their phenotypic characteristics is induced by the tumor promoter. Thus, it may be concluded that the phenotypic expression of the cells transformed by the carcinogen is one of the critical cellular events of tumor-promoting action. If this view is accepted, the events occurring during the promoting process can be dissociated into 2 main components. First, there are the changes induced by the promoter in the epidermal cells or in their microenvironment that enable the phenotypic expression of the transformed cells, and perhaps also of other variants present in the epidermal cell population, that are normally repressed. Second, there are the specific phenotypic alterations that occur in the transformed cells in response to the promoting stimuli. Identification of the changes, among many induced by promoters in mouse skin, that are essential for the phenotypic expression of the transformed cells is the key for an understanding of tumor-promoting action. The analysis of the phenotypic characteristics of the atypical cells might enable us to define the differences that exist between the neoplastic cells and their normal counterparts, and might disclose those features that are peculiar to the neoplastic cells. The isolation of pure clones of these atypical cells is a basic step in building a model system to analyze at a molecular level the changes in their biological properties.

The changes observed in the fine structure of the groups of atypical cells and in the basal cells of papillomas and carcinomas clearly suggest that differences exist between their phenotype and that of the basal cells in the other areas of the epidermal hyperplasia induced by the promoter. The atypical cells are more irregular in shape and size, and their nuclear and cytoplasmic matrices are more electron dense. Their surface is more irregular, with microvilli that are finer and more
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numerous than those of the surface of the epidermal cells in the other areas of the hyperplastic epidermis. The cytoplasm of the atypical cells is densely packed with free ribosomes, contrasting sharply with the basal cells in the other areas, in which the free ribosomes are more dispersed in distribution. The basal cells in the small papillomas and 2 of the basal cell types in the large papillomas resemble those of the atypical cells of the focal groups, but the basal cells of the carcinoma deviate further. The very irregular, pleomorphic shape of carcinoma basal cells, the fine and numerous microvilli in their surface, their electron-dense matrix, the densely packed ribosomes not attached to membranes, and the absence or paucity of tonofilaments make them resemble embryonic cells. However, it remains to be determined which cellular changes account for these phenotypic differences and what their significance is to the expression of neoplastic transformation.

The 1st possible interpretation is that these phenotypic changes observed in the neoplastic cells are related to the development of the machinery for cell growth and proliferation. Indeed, cell proliferation and epidermal hyperplasia are among the more conspicuous effects of promoters in mouse skin (14, 18), and many of the changes observed in the epidermal cells after promoter treatment are related to cell growth (14). However, the failure of ethylphenylpropionate to induce groups of atypical cells similar to those seen after promoter treatment of initiated mouse skin, although the increase in the rate of cell proliferation it induces in the epidermis is comparable to that induced by treatment with the promoter (16, 17), argues against this hypothesis. Moreover, if these morphological changes observed in the foci of atypical basal cells, papillomas, and carcinomas are related to the development of the machinery for cell proliferation only, they should be observed also in normal adult mouse epidermis, as it has a relatively high mitotic rate. The absence of these atypical cells in normal mouse epidermis, in hyperplastic epidermis induced by ethylphenylpropionate, and their scattered focal distribution after promoter treatment indicate that, in all probability, their phenotypic characteristics are not exclusively related to cell growth and proliferation.

Another possible interpretation of the phenotypic alterations observed in the basal cells of the focal groups and papillomas is that they are mutant-like cells induced to express their phenotype by the tumor promoter. If we consider these to be cells transformed by the initial treatment with the carcinogen, the changes observed in their morphology would suggest that new phenotypic properties not present in normal adult mouse epidermal cells are acquired by the transformed cells very early after treatment with the tumor promoter, well before the macroscopic appearance of papillomas. The appearance of new antigens in skin papillomas (9, 10), in hyperplastic mammary nodules (23), and in hyperplastic liver nodules (3, 8, 25) indicate that new phenotypic properties are acquired early in the evolution of the neoplastic process and corroborate this hypothesis. However, comparative studies of their biological characteristics and especially of their antigenic properties are needed to determine whether these cells indeed gain new features or whether their phenotypic changes are due to loss of differentiated traits in the course of carcinogenesis, as suggested by the morphological features of the basal cells in the carcinomatous nodule that resemble embryonic cells more than the normal epidermal cells from which they originate. The disappearance of organ-specific antigens early after the administration of carcinogenic agents and in chemically induced cancer of the liver and kidney (27) also suggests the loss of differentiated character in the course of carcinogenesis.

A peculiar feature in the groups of atypical cells, in the papilloma and in the carcinoma, is that the phenotypically altered cells are observed mainly in the basal layer, while the cells in the upper layers have well-differentiated morphological characteristics that are indistinguishable from that of cells in the corresponding upper layers of hyperplastic or normal epidermis. This pattern is similar to that observed by Pierce (11, 12) in teratocarcinoma, by Pierce and Wallace (13) in squamous cell carcinoma, and by Wylie et al. (28) in mammary carcinoma, and it indicates that the neoplastic stem cells are still able to respond to differentiating stimuli and that they differentiate along the pathway followed by the cell type from which they originate. As shown by Pierce (11) and by Pierce and Wallace (13), this normally differentiated progeny of the neoplastic stem cells is incapable of forming tumors when transplanted, although the transplantation of the neoplastic stem cells did give rise to tumors, clearly suggesting that the differentiation of the neoplastic stem cells leads to loss of their proliferative and neoplastic properties. Thus, whatever the nature of the phenotypic alterations in the neoplastic stem cells, whether due simply to loss of differentiated traits and the acquisition of morphology and properties found in embryonic cells or to the acquisition of new phenotypic properties, the neoplastic phenotype is reversible, and at least some neoplastic cells are able to differentiate normally. Second, it can be implied that the cellular alterations critical and essential to epidermal neoplasia are those that occur in the stem-cell fraction of the tumor population. The characterization of these phenotypic alterations in the neoplastic stem cells and the determination of the changes in the stem cells or in their microenvironment that enable the expression of these phenotypic characteristics are key steps for the understanding of the biology of neoplasia.

Considering these morphological changes observed in the neoplastic stem cells, what might be the nature of the changes induced by tumor promoter that lead to these phenotypic alterations and the expression of neoplastic transformation? Although we do not yet know the nature of the phenotypic alterations observed in the neoplastic stem cells, the findings in this study and those of Weiler (27) suggest that the expression of the neoplastic phenotype possibly is coupled with the loss of some differentiated traits of the normal stem cells. Indeed, if one considers that neoplastic stem cells are the true neoplastic cells, the observations made in this study suggest that this loss of some differentiated traits may be critical for the expression and maintenance of the neoplastic phenotype. If one views the cellular changes induced in mouse epidermis by the tumor promoter (14, 15, 18) in the context of the above-mentioned considerations, an attractive working hypothesis emerges. The ability of the promoter to modulate gene activity, perhaps derepressing genes and inducing a reversal of the epidermal cell differentiation (14), may be the essential alteration required for the expression of the neoplastic phenotype. As postulated before (14), it is possible that the expression of the neoplastic phenotype can take place
only in cells that are in a relatively more primitive stage of differentiation. It is also possible that the modulation of gene activity induced by the promoter leads to loss of some of the differentiated traits of the epidermal basal cells and to their reversal to a more primitive or undifferentiated stage such as required to activate the gene loci that code for the neoplastic phenotype. Another alternative could be that the gene loci responsible for both these phenotypic properties are inter-related and that the derepression of one of these gene loci would lead to activation of the other. The failure of other hyperplastic agents (that have little or no tumor-promoting activity) to induce reversal of the epidermal cell differentiation (16, 17), and its induction by other promoters and by tumorigenic doses of carcinogens (A. N. Raick, unpublished data), corroborate the hypothesis proposed above. The elucidation of the molecular alterations and the mechanism involved in the cellular reprogramming induced by tumor promoters might lead to new insight into the nature of the events in this early and critical stage of neoplastic evolution.

ACKNOWLEDGMENTS

The author wishes to thank Dr. A. C. Ritchie for his review of the manuscript. Thanks are due to H. Christensen for her technical assistance, and to J. Cetkovski for typing the manuscript.

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Fig. 1. Epidermis 48 hr after application of TPA to mouse skin treated previously with an initiating dose of DMBA. A focal group of atypical dark cells (DKC) is shown. The intercellular space (ICS) between the dark cells is dilated, and many papillary projections can be seen in their surface. The cells have irregular shapes, a markedly electron-dense matrix, and their cytoplasm is packed with polysomes and ribosomes not attached to membrane. The mitochondria (M) are large and rich in cristae. BL, basal lamina; TF, tonofilament; N, nucleus; NO, nucleolus. x 8,400.

Fig. 2. Epidermis, 3rd week of promotion. Shown is a basal cell type seen in most areas of the hyperplastic epidermis. They have a regular shape, and the electron density of their matrix is lower than that of the atypical cells seen in the focal group in Fig. 1. M, mitochondria; MI, mitochondrial inclusion; N, nucleus; NO, nucleolus; TF, tonofilaments; ICS, intercellular space; BL, basal lamina. x 8,736.

Fig. 3. Epidermis, 3rd week of promotion; basal cell of a focal group of atypical cells. The overall fine structural characteristics resemble those of the atypical dark cells in Fig. 1, although they are more irregular in shape, the intercellular spaces between them are more widely open, and cisternal profiles (CP) of the RER are more conspicuous. BL, basal lamina; N, nucleus; TF, tonofilaments; ICS, intercellular space. x 11,180.

Fig. 4. Epidermis, 4th week of promotion; basal cell of a focal group of atypical cells. The electron density and the other fine structural characteristics of these cells resemble those of the cells in Figs. 1 and 3. CP, cisternal profile of RER; BL, basal lamina; R, ribosomes; TF, tonofilaments; N, nucleus; ICS, intercellular space. x 8,736.

Fig. 5. Epidermis, 6th week after TPA treatment; basal cells of a small papilloma. Their ultrastructural features resemble those of the atypical dark cells of the focal groups (Figs. 1, 3, and 4) and are distinct from those of the basal cells in the neighboring hyperplastic epidermis, which are similar to those of the basal cell shown in Fig. 2. BL, basal lamina; TF, tonofilaments; R, ribosomes; M, mitochondria; N, nucleus; NO, nucleolus; ICS, intercellular space. x 11,180.

Fig. 6. Basal cell of a large papilloma. The fine structural characteristics of this basal cell type resemble those of the atypical dark cells observed in the focal groups and in small papillomas. BL, basal lamina; PC, papilloma cell; N, nucleus; NO, nucleolus; CP, cisternal profile of RER; R, ribosome; M, mitochondria; ICS, intercellular space. x 11,180.

Fig. 7. Basal cell of a large papilloma. This basal cell type has a very high electron density, the cytoplasm is densely packed with polysomes and ribosomes (R), not attached to membrane. CP, cisternal profile of RER; M, mitochondria; GC, Golgi complex; N, nucleus; NO, nucleolus; ICS, intercellular space; BL, basal lamina; PC, papilloma cell. x 8,736.

Fig. 8. Basal cell of a large papilloma. This basal cell type has a more regular shape and its electron density and other ultrastructural characteristics are similar to those of the basal cells in the neighboring hyperplastic epidermis. BL, basal lamina; ICS, intercellular space; M, mitochondria; N, nucleus; NO, nucleolus; PC, papilloma cell. x 11,180.

Fig. 9. Large papilloma. Focal areas of complete disappearance of the basal lamina (BL), forming gaps through which cytoplasmic projections (CYP) of basal cells (BC) extend into the dermis. x 18,800.

Fig. 10. Carcinoma. Partial view of a nodular mass formed in the lower dermis by the cells of an invasive epidermal carcinoma. The cell in the basal layer (BC) has a very irregular shape and a highly electron-dense matrix. The overall ultrastructural features are those of an undifferentiated epidermal cell and they resemble, to some extent, those of the atypical dark cells of the focal groups and papillomas. The cells (DC) in the layer above the basal are more differentiated and have morphological characteristics similar to those of the epidermal cells in normal or hyperplastic mouse epidermis. BL, basal lamina; ICS, intercellular space; CP, cisternal profile of RER; TF, tonofilaments; D, desmosomes; N, nucleus; NO, nucleolus. x 13,800.

Fig. 11. Epidermal cell of the layer closest to the central area of the carcinomatous nodule. These cells (DC) are well differentiated, and their characteristics are indistinguishable from those of the cells in the corresponding layers of the normal epidermis. N, nucleus; TF, tonofilament; ICS, intercellular space; GM, granular material; KH, keratohyaline granule. x 10,750.

Fig. 12. Epidermal cell of the basal layer (BC) of the carcinomatous nodule. These cells have a highly electron-dense matrix and undifferentiated characteristics. N, nucleus; NO, nucleolus; R, ribosomes; GC, Golgi complex; ICS, intercellular space; D, desmosomes; BL, basal lamina. x 14,352.
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_Cancer Res_ 1974;34:2915-2925.

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