Mucous Metaplasia and Gap Junctions in the Vitamin A Acid-treated Skin Tumor, Keratoacanthoma

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SUMMARY

Desmosomes are the usual cell junctions found in normal rabbit epithelium as well as in the untreated keratoacanthoma. This study reports the finding of a second cell junction, the gap junction, when epithelium, normal or tumorous, is subjected to topical applications of vitamin A acid. The gap junction forms early in mucous metaplasia (after 2 days of application of vitamin A acid) and appears before the gross appearance of mucus. The presence of the gap junction occurs when there is an increase in the rough-surfaced endoplasmic reticulum and Golgi cisternae and vesicles. It is possible that the early appearance of the gap junction facilitates and mediates the mucous metaplasia. This suggestion is strengthened by the fact that the gap junction, once present in the mucus-producing tumor, is sparse when the tumor reverts back to the dry, keratotic condition upon cessation of vitamin A acid applications.

INTRODUCTION

The keratoacanthoma is a skin tumor which can be produced in experimental animals (5, 6, 21–24) and humans (5, 26). It is characterized by rapid growth, excessive keratinization, and spontaneous regression. In several previous studies, vitamin A acid was topically applied to the tumor in rabbits (22–24). Two significant results were an accelerated regression and the presence of a mucous metaplasia in the tumor. The dry, keratotic keratoacanthoma secreted copious amounts of mucus. Cytologically, both the rough-surfaced endoplasmic reticulum and the Golgi complex, usually sparse in normal epithelium and in the untreated keratoacanthoma, were markedly increased in the vitamin A acid-treated tumor. In addition, mucigen droplets were observed in the tumor keratinocytes after vitamin A acid applications. Cessation of the vitamin applications causes the mucus secreting tumor to revert back to the dry, keratotic condition.

This study reports the finding of a 2nd cell junction, in addition to the desmosome, and correlates its appearance with the above-named cell organelles and mucous metaplasia. These observations yield further information on mucous metaplasia in the vitamin A acid-treated keratoacanthoma.

MATERIALS AND METHODS

Eighteen albino male rabbits (average weight, 2 kg) had the inner surface of their left ear auricles painted twice weekly with 1% 7,12-dimethylbenzanthracene in equal parts of lanolin and mineral oil. After 7 weeks, all the rabbits had developed keratoacanthomas on their ears, with a yield of 6 to 8 tumors/ear. After the 7th week (14 applications), the applications of carcinogen to the ears were stopped. Sixteen of the 18 rabbits received daily applications of 3% vitamin A acid (kindly supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.) in equal parts of lanolin and mineral oil. Each application of vitamin A acid (average, 0.10 g) was applied to a specific tumor by means of a wooden stick spatula, resulting in a thin film of the drug over the surface of the tumor. The vitamin A acid applications were continued daily for 5 days. Two of the vitamin A acid-treated animals were left alone for 2 weeks. Two of the 1% 7,12-dimethylbenzanthracene-treated animals received no vitamin A acid.

Five additional rabbits served as controls; the inner surfaces of their ear auricles were painted only with vitamin A acid in the same amount and schedule as was applied on the ears with tumors. One separate rabbit was painted only with lanolin and mineral oil.

Biopsies were taken daily, beginning 24 hr after the initial application of vitamin A acid and continuing 48 hr after the last application. The biopsies were sliced into 1-cu mm pieces and placed in 4% glutaraldehyde in phosphate buffer for 1.5 hr, followed by fixation in 2% osmium tetroxide buffered to pH 7.4. The tissues were dehydrated in graded strengths of ethanol and embedded in Epon 812. Sections 1 μm thick and ultrathin sections were cut on a Reichert ultramicrotome, stained with uranyl acetate and then with lead citrate, and examined in a Siemens Elmiskop IA electron microscope.

RESULTS

Gross Results. Examination of the keratoacanthomas after 3 days of daily applications of vitamin A acid revealed the keratotic tumor to have a moist appearance. There was no visible change in the tumors prior to Day 3. On the 4th day, the tumors produced a viscous exudate and, by the 5th day, the exudate was copious and yellow-white. On the 7th
day, the exudate was very copious and became crusty. Lifting the crust from the tumor revealed a very soft and a very wet tumor. The 2 vitamin A acid-treated animals that were left alone for 2 weeks now had dry, keratotic tumors which previously were mucous secreting. Biopsies were taken of these tumors.

**Microscopic Results.** The ultrastructural morphology of the keratoacanthoma as well as normal rabbit skin epithelium has been described (21). The normal epidermal cells contain tonofibrils and a sparse, rough-surfaced endoplasmic reticulum and sparse Golgi complex. Desmosomes are quite prominent. The keratinocytes of the tumor contain a sparse, rough-surfaced endoplasmic reticulum, Golgi complex, and many tonofibrils. The only evident cell junction is the desmosome.

Tumors treated with vitamin A acid reveal an increase in the rough-surfaced endoplasmic reticulum as well as an increase in the Golgi complex (Fig. 1) after 2 days of vitamin A acid applications. However, at this time period, beside desmosomes, another cell junction appears. The membranes at this junction are closely apposed, not fused, and are distinguished by a pattern of particles with periodicity of 80 A. The overall thickness of the cell junction is 190 to 200 A. Similar substructure and dimensions have been described for the gap junction (7, 8, 14–16).

Examination of the vitamin A acid-treated tumor keratinocytes on the 3rd day depicts a further increase in the number of cisternae of the Golgi complex (Fig. 2). In addition, there is an increase in the amount of rough-surfaced endoplasmic reticulum, and mucigen droplets are now present. The gap junction is found more frequently, although not associated with every keratinocyte (Fig. 3). From the 5th to 7th day, mucigen droplets and condensing vacuoles are found in most of the keratinocytes (Fig. 4). The extensive Golgi complex contains smooth-surfaced membranes arranged in lamellar arrays of cisternae. There are many small vesicles and large vacuoles (Fig. 5). “Gap junction-bounded vesicles” (20) are frequently found (average, 6 of 11 cells) (Fig. 6). In several instances, the gap junctions are associated with desmosomes (Fig. 7).

Those tumors that secreting mucus and reverted back to the dry, keratotic condition have keratinocytes that show a sparse number of mucigen droplets and a sparse number (average, 1 of 19 cells) of gap junctions.

Normal epidermal cells subjected to vitamin A acid produce a slight increase in the Golgi complex and rough-surfaced endoplasmic reticulum and occasional gap junctions and mucigen droplets by the 7th day.

**DISCUSSION**

The biosynthesis of glycoproteins has received considerable investigative attention. Present evidence indicates that the Golgi saccule is the site where carbohydrate is synthesized and is added to protein, the latter synthesized on the ribosomes of the rough-surfaced endoplasmic reticulum (9, 10, 12). Neutra and Leblond (18, 19) reported that the Golgi saccule then becomes the mucigen droplet.

The topical application of vitamin A acid to normal epithelium and to the keratoacanthoma in rabbits results in a viscous exudate (mucus). The mucus is scant in normal epithelium and very marked in the tumor. At the ultrastructural level, mucigen droplets are evident in the keratinocytes after 3 days of vitamin A acid application. The droplets become more numerous on succeeding days. Previous investigations have shown that the droplets stain positive with Mayer’s mucicarmine and positive with periodic acid-Schiff stain (23, 24).

After 2 days of vitamin A acid treatment, there is an increase in the rough-surfaced endoplasmic reticulum and Golgi cisternae and vesicles, although mucigen droplets and mucus are not evident until the 3rd day. These organelles increase in number as the mucous metaplasia continues. Deluca et al. (2) have shown that retinol is required for the formation of mucus-secreting goblet cells in the rat intestine. Levinson and Wolf (13) have studied the effect of vitamin A acid on glycoprotein synthesis in rabbit keratoacanthoma. After vitamin A acid treatment, labeled fucose and glucosamine uptake into glycoprotein was greatly stimulated. They state that the induction of the synthesis of a fucose-containing glycopeptide is particularly significant, since fucose is characteristic of secreted glycoproteins and is generally not metabolized to other sugars (17).

A very interesting finding in the present study is the early presence (after 2 days of vitamin A acid applications) of a cell junction, in addition to the desmosome, the substructure and dimensions of which have been described for the gap junction. The gap junction appears only after the application of vitamin A acid and is not found in normal rabbit epithelium nor in the untreated keratoacanthoma.

The structure of the gap junction in this study is similar to gap junctions reported in a wide variety of tissues, including myocardium (16), liver (7, 8), cervical epithelium (16), tissue culture cells, (4, 11, 25), basal cell cancer (3), and wool follicle cells (20). The gap junction has been implicated as a low-resistance pathway for cell-to-cell coupling. Electrical coupling between cells does not imply that currents are normally passing from one cell to the other, but shows that areas exist for the preferential exchange of small ions between cells. Lowenstein (14) has suggested that the junction may be instrumental in exchanging substances that control cellular growth and differentiation. This hypothesis has been reported by other investigators (11, 16, 25).

The present study has demonstrated that gap junctions form early (before the gross appearance of mucus) and continue to increase in number along with the Golgi complex and rough-surfaced endoplasmic reticulum in mucous metaplasia. It is possible that the early appearance of this cell junction facilitates and mediates the mucous metaplasia and is not a by-product of mucous metaplasia. This suggestion is strengthened by the fact that the gap junction, once present in the mucus-producing tumor, is sparse when the tumor reverts back to the dry, keratotic condition upon cessation of vitamin A acid applications.

We are now studying the gap junction with tracer materials (lanthanum and horseradish peroxidase) and the freeze-fracture technique to confirm the findings obtained with thin-section electron microscopy.
REFERENCES

Mucous Metaplasia and Gap Junctions

Fig. 1. Micrograph depicts Golgi cisternae and vesicles (arrows) in the keratoacanthoma after 2 days of vitamin A acid applications. Tissues in this and succeeding micrographs were stained with uranyl acetate and lead citrate. × 40,000.

Fig. 2. Arrows point to the increase in the number of cisternae of the Golgi complex after 3 days of vitamin A acid applications. × 40,000.
Fig. 3. Gap junction (arrows) in the vitamin A acid-treated keratoacanthoma. × 84,000.

Fig. 4. Mucigen droplet and condensing vacuole in the keratinocyte subjected to vitamin A acid. × 32,000.

Fig. 5. The extensive Golgi complex in mucous metaplasia containing smoothsurfaced cisternae, small vesicles, and large vacuoles. × 36,000.
Fig. 6. Gap junction-bounded vesicle in mucous metaplasia. x 129,000.

Fig. 7. Gap junction (GJ) is associated with a desmosome (D) in several instances in mucous metaplasia. x 136,500.
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