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Coordination of Keratinocyte Programming in Human SCC-13 Squamous Carcinoma and Normal Epidermal Cells

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Exploiting the sensitivity of neoplastic keratinocytes to physiological effectors, this work analyzes the degree of coordination among differentiation markers in the established human epidermal squamous carcinoma cell line SCC-13 in comparison to normal human epidermal cells. This analysis showed that overall keratin content was modulated substantially and in parallel with particulate transglutaminase activity in response to variation of calcium, retinoic acid, and hydrocortisone concentrations in the medium. The changes in keratin expression were evident primarily in the striking stimulation by hydrocortisone or calcium and the virtual suppression by retinoic acid of species in the 56–58 kd region, which have not previously been reported subject to such physiological modulation. In contrast, involucrin levels were coordinated only to a limited degree with particulate transglutaminase activity and keratin content. The very low involucrin levels observed in low calcium medium were increased 5- to 10-fold in high calcium medium. However, they were also increased 5- to 30-fold in low calcium medium by retinoic acid, a clear example of uncoupling. Activities of the tissue transglutaminase were altered considerably by the various culture conditions but were not obviously coordinated to keratinocyte markers. In normal epidermal cells, the suppressive effect of retinoic acid was much more evident with particulate transglutaminase than involucrin levels. While calcium had a large stimulatory effect on both markers, hydrocortisone had little or no influence. These results emphasize the potential importance of quantitative analysis of differentiation markers for resolving the contribution of physiological elements in coordination of cellular programming.

Normal differentiation of the stratified squamous epithelia involves a coordinated series of changes in morphology and protein expression in maturing keratinocytes. The stages of differentiation are now partially understood at the molecular level as alterations in expression of markers such as the keratin intermediate filaments and the proteins participating in formation of the cross-linked envelope (Green, 1979). Recently, a major envelope precursor protein involucrin (Rice and Green, 1979; Watt and Green, 1981) and a particulate transglutaminase (Lichti et al., 1985; Thacher and Rice, 1985), which cross-links involucrin to certain membrane proteins (Simon and Green, 1984, 1985), have been identified and specific antisera prepared, making these highly useful markers in assessing keratinocyte differentiation in tissues and cultured cells (Banks-Schlegel and Green, 1981; Parenteau et al., 1986). Since regulation of the differentiation program now can be studied in detail in culture as a consequence of improvements in methods for serial propagation of human epidermal keratinocytes (Rheinwald and Green, 1975), finding conditions influencing the expression of such markers will help elucidate physiological control of the differentiation program and its perturbation in pathological situations.

Continuous lines of human keratinocytes have been established from a number of squamous cell carcinomas of the epidermis and oral cavity (Rheinwald and Beckett, 1981) and have been shown to exhibit defective regulation of terminal differentiation (Rheinwald and Beckett, 1980). As distinguished from the mixture of differentiation states in cultures of normal keratinocytes, these cells express substantial differentiated character only after reaching confluence or upon growth arrest (Cline and Rice, 1983). They exhibit marked sensitivity to physiological modulation of their differentiated state, which makes them valuable for study of mechanisms by which physiological agents act. For example, vitamin A and hydrocortisone can modulate envelope forming ability (Cline and Rice, 1983), pattern of keratin proteins expressed (Kim et al., 1984), overall keratin content (Rice and Cline,

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1984), and transglutaminase activity (Thacher et al., 1985). The less stringent growth requirements of neoplastic keratinocytes are especially advantageous in such work. Thus, these cells are amenable to more complete analysis of certain elements of differentiated function than normal human epidermal cells, which are difficult to grow in medium depleted of calcium or supplemented with high retinoid concentrations. We have previously observed in these cells the differential regulation by retinoids and calcium of two distinct transglutaminases (the particulate "keratinocyte-specific" and the ubiquitous "tissue" forms) and have demonstrated the sensitivity of the cells to calcium in expression of the particulate enzyme responsible for envelope formation (Rubin and Rice, 1986, 1988).

Neoplastic cells characteristically exhibit instability in expression of normal function and concomitant manifestation of new properties, including those conferring malignancy (Fidler and Hart, 1982; Rubin, 1985). A major goal of cancer research is to describe this instability at molecular and cellular levels to understand its consequences for tumor progression. A feature critical for progression is the loss of growth control, which neoplastic cells exhibit coincident with altered differentiation. For example, sustained germinative capacity even after suspension in semisolid medium is a distinctive characteristic of the defective terminal differentiation exhibited by malignant keratinocytes (Rheinwald and Beckett, 1980). As in the leukemias and other neoplasias (Greaves, 1986; Marks et al., 1987), suppression of or divergence from the normal differentiation program appears connected to aberrant growth properties (e.g., less stringent growth requirements) of the malignant keratinocytes in some fundamental way that further investigation may help elucidate. To this end, the present investigation characterizes the sensitivity of the highly responsive SCC-13 line and of normal epidermal cells to three agents which have large effects on the state of differentiation and explores the coupling of keratinocyte differentiation markers in these cells in different physiological environments.

MATERIALS AND METHODS

Cell culture

SCC-13 keratinocytes (passages 30–41) were inoculated at a density of 2–5 $\times$ 10^5 cells/6 cm dish and grown with irradiated 3T3 feeder layer support (Rheinwald and Beckett, 1981) in Dulbecco-Vogt Eagle's medium supplemented with 5% fetal bovine serum. One day after inoculation, the cultures were rinsed twice in PBS (137 nM NaCl – 3 mM KCl – 8 mM Na_2HPO_4 – 2mM KH_2PO_4, pH 7.2) and given fresh medium, which was changed twice weekly. The fresh medium contained calcium concentrations of 0.025 mM (determined by atomic absorption spectrophotometry) or higher as specified and fetal bovine serum depleted of steroids with charcoal-dextran (Armelin and Sato, 1974) and of calcium with Chelex-100 (BioRad Laboratories, Richmond, CA) ion-exchange resin (Brennan et al., 1975). In some experiments, the cells were treated with hydrocortisone (0.4 μg/ml) or retinoic acid (1 μg/ml) as indicated or solvents alone (0.1% ethanol or dimethylsulfoxide, respectively) as control. Normal human epidermal cultures (third passage) were inoculated at a density of 3.5 $\times$ 10^5 cells/6 cm dish with feeder layer support and grown in medium additionally supplemented with epidermal growth factor (10 ng/ml), cholera toxin (9 ng/ml), insulin (5 μg/ml), transferrin (5 μg/ml), and triiodothyronine (20 pM). SCC-13 cells were switched to low calcium or retinoic acid-containing medium at low density (usually 1 day after inoculation), whereas the normal cells were switched at 20–30% of confluence to insure that they would achieve a confluent state. All assays were performed on cultures that had been confluent for 7–9 days to minimize the influence of possible variations in growth rate or withdrawal from the cell cycle.

Transglutaminase assay

Cultures were rinsed three times in PBS, scraped into 0.3–0.5 ml of ice-cold buffer (10 mM Tris-HCl (pH 7.4) – 1 mM EDTA – 1% Emulgen 911), and stored frozen at −70°C. Samples were thawed, adjusted to 1 ml in buffer containing 1 mM dithioerythritol (DTE), disrupted by Dounce homogenization (30–40 strokes), and centrifuged at 150,000g for 30 min at 4°C. The supernatant was given a second high-speed centrifugation, and the resulting soluble extract was adjusted to 1% in Emulgen 911 and assayed. The pellet from the initial centrifugation was resuspended and sonicated in 1 ml of the Tris-EDTA buffer containing 1% Emulgen 911 and centrifuged again at high speed to yield the particulate extract. Aliquots (25–50 μl containing 20–150 μg of protein) were incubated for 30 min at 37°C in 0.26 ml final volumes containing 0.5 mg of dimethyl casein (Means and Feeney, 1968), 0.1 M Tris-HCl (pH 8.2), 4 mM $CaCl_2$, 0–0.4 mM EDTA, 5 mM DTE, and 0.5 μCi (4 nmoles) of ^{3}H-putrescine. Trichloracetic acid-precipitable radioactivity was recovered on glass fiber filters, rinsed, and scintillation counted.

Anion-exchange chromatography

Soluble or particulate extracts were applied to 1 $\times$ 4 cm colums of Whatman DE-52 resin equilibrated at 4°C with 50 mM Tris-HCl (pH 7.5) – 1 mM EDTA – 0.1% Emulgen 911 – 0.02% NaN_3 buffer. The columns were developed with a linear gradient of NaCl (0–0.5 M) in this buffer as previously described (Rubin and Rice, 1986). Aliquots (0.1 ml) of each fraction were assayed for transglutaminase activity.

Keratin quantitation

Cultures were rinsed three times in PBS, scraped into 1 ml of 10 mM Tris-HCl (pH 7.5) – 1 mM EDTA – 1 mM DTE buffer, and frozen. The thawed cells were Dounce homogenized and, after an aliquot was removed for determination of total cell protein, centrifuged 30 min at 150,000g. The pellets were washed twice in 1 ml of 10 mM Tris-HCl – 1 mM EDTA – 1% Triton X-100, once in this buffer containing 0.5 M NaCl (Wu and Rheinwald, 1981), and finally in buffer without NaCl. The pellets were recovered by centrifugation for 2 min at 10,000g. After washing, the keratins were suspended in the Tris-EDTA buffer containing 2% sodium dodecyl sulfate and 1 mM DTE and dissolved in a boiling water bath. These fractions were then electrophoresed in 10% polyacrylamide gels (Laemmli, 1970), stained with Coomassie Blue, and the keratin

bands quantitated by laser densitometry in parallel with known amounts of an ovalbumin standard. Inclusion of the protease inhibitors antipain and leupeptin in the extraction had no effect on the keratin pattern.

Involucrin determination

Involucrin was measured in cytosolic and solubilized particulate extracts using an enzyme-linked immunoassay (Parenteau et al., 1987). The extracts were incubated overnight at 4°C with a 1:4,000 dilution of rabbit anti-involucrin antiserum (Rice and Green, 1979) in assay buffer (Dulbecco's phosphate-buffered saline ($-Ca^{++}$, $-Mg^{++}$) – 0.25% gelatin – 2 mM EDTA – 0.5% Tween 20 – 0.2% NaN_3). Aliquots of each sample were then transferred to a microtiter plate (Nunc) containing adsorbed involucrin (1 ng/well) and incubated at room temperature for 30 min. These plates were then washed with assay buffer and incubated 1 hr with Protein A – alkaline phosphatase conjugate (Parenteau et al., 1987) or goat anti-rabbit IgG-alkaline phosphatase in assay buffer. p-Nitrophenylphosphate (1 mg/ml) in 0.05 M Na_2CO_3 buffer (pH 9.8) containing 1 mM $MgCl_2$ was used as substrate. Absorbance values at 405 nm were recorded using a Titertek Multiskan. The amount of involucrin was calculated from a standard curve generated from a parallel assay using homogenous, chromatographically purified involucrin (Rice and Green, 1979). Assay sensitivity was in the range of 0.025 ng.

Protein concentration

Measurements were made using the bicinchoninic acid (Pierce Chem. Co., Rockford, IL) method of Smith et al., 1985).

RESULTS

Expression of keratinocyte and tissue transglutaminases

In the absence of exogenously added retinoic acid, the transglutaminase activity in SCC-13 cells was associated almost entirely with the particulate fraction. Upon anion exchange chromatography, as shown in Figure 1**B**, the enzyme activity eluted as a single peak at approximately 0.1 M NaCl from DEAE-cellulose columns. However, growth in medium containing retinoic acid resulted in complete suppression of this enzyme, which accompanies a suppression of envelope competence, as observed earlier (Thacher et al., 1985; Rubin and Rice, 1986). We previously found that SCC-13 cells did not express the tissue (type II) transglutaminase when grown in culture medium supplemented with untreated serum, and hence this form is not necessary for envelope cross-linking (Thacher and Rice, 1985). At the rather high concentration of retinoic acid employed presently (3.3 μM), 10-fold higher than in earlier work (Thacher et al., 1985), it is evident that this form, eluting from DEAE-cellulose at 0.2 M NaCl, is expressed (Fig. 1A). That result supports our more recent observation that the high retinoic acid concentration stimulates transglutaminase activity in SCC-13 cells grown in low calcium (0.025 mM) medium, but prevents expression of the particulate form of the enzyme (Rubin and Rice, 1986). Parallel experiments employing DEAE cellulose chromatography have also shown that the similar SCC-12B2 cells

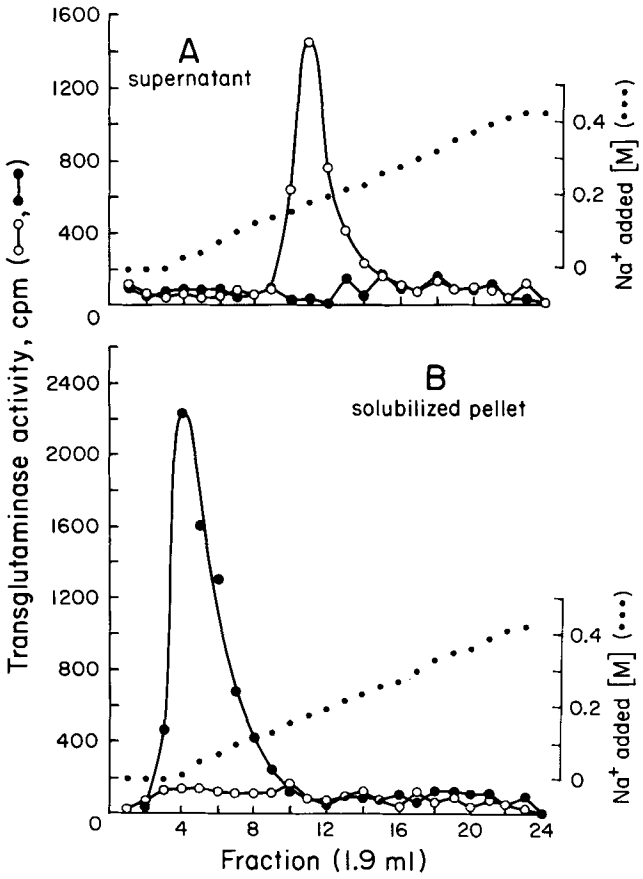

Fig. 1. Anion exchange chromatography of transglutaminases expressed by SCC-13 cells. Cultures were grown in 1.8 mM calcium-containing medium in the presence (○) or absence (●) of 3.3 μM retinoic acid and 8–9 days after confluence were fractionated into soluble (panel **A**) and particulate (panel **B**) extracts. The peaks correspond to designated types I (keratinocyte) and II (tissue) transglutaminase in order of elution as previously described (Thacher and Rice, 1985; Rubin and Rice, 1986).

grown in low calcium medium in the presence of 3.3 μM retinoic acid and 1 μM hydrocortisone express only the tissue transglutaminase (Rubin and Rice, unpublished).

Modulation of keratinocyte programming in SCC-13

We examined the extent to which markers of keratinocyte differentiation were coordinately regulated by calcium, retinoic acid, and hydrocortisone in the growth medium. Particulate and soluble (tissue) transglutaminases, involucrin, and keratin content were measured in each culture in parallel under different growth conditions in the presence of 5% fetal bovine serum treated with charcoal and Chelex resin (Fig. 2, I–IV). In some experiments where the action of calcium was not investigated, the serum was untreated (Fig. 2, V, VI). Cultures maintained in untreated serum displayed a heightened response to hydrocortisone, exhibiting levels of particulate transglutaminase and involucrin several fold higher than in cells maintained in treated serum (Fig. 2, IV, VI). This reflects either a

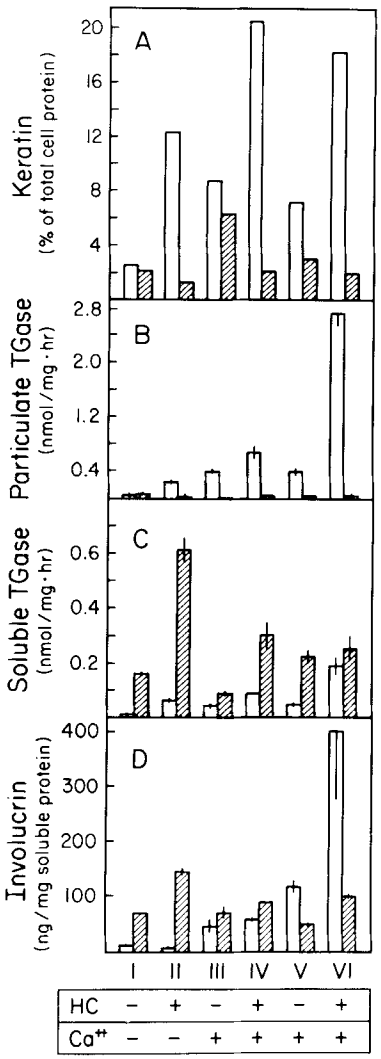

Fig. 2. Modulation of differentiation markers in SCC-13 cells by culture conditions. Levels of keratin (**A**), particulate transglutaminase (panel **B**), soluble transglutaminase (panel **C**), and involucrin (panel **D**) were measured 8 days after confluence in cultures grown with (hatched bars) or without (clear bars) 3.3 μM retinoic acid. As indicated below panel **D**, the concentration of calcium (Ca^{++}) in the medium was 1.8 mM (+) or 0.025 mM (−) and hydrocortisone (HC) was added at 1 μM (+) or omitted (−). I, II, III, IV: cultures grown in medium supplemented with serum treated with charcoal and chelex resin; V, VI: cultures grown in medium supplemented with untreated serum. Error bars give the range of values in duplicate cultures. Panel **A** is representative of several independent experiments.

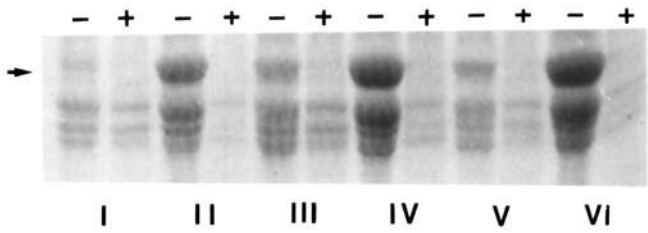

Fig. 3. Modulation of keratin electrophoretic pattern in SCC-13 cells by culture conditions. A 20% (**I–IV**) or 10% (**V, VI**) aliquot of the keratin fraction from one culture was electrophoresed for each condition, labeled I–VI as in Figure 2. The arrow at the left edge of the gels points to the 56–58 kd region keratins, prominent in the absence (−) but almost totally suppressed in the presence (+) of 3.3 μM retinoic acid.

requirement for specific cofactors that are removed by the serum treatment or a more general change in the responsiveness of the cells.

As shown in Figure 2B, retinoic acid (3.3 μM) suppressed the particulate enzyme activity to barely detectable levels under all the medium conditions examined without significantly affecting cell growth. In the absence of added retinoid, the enzyme levels were 3- to 11-fold higher in high (1.8 mM) than in low (0.025 mM) calcium-containing medium. Regardless of the calcium concentration, cultures grown in the presence of hydrocortisone (1 μM) had approximately twice (I–IV) or 7-fold (V, VI) the enzyme specific activity of those grown without added steroid. By contrast, the tissue transglutaminase, an enzyme found in many cell types (Chung, 1972) and hence not a marker of keratinocyte differentiation, was stimulated in the cytosolic fractions of such cultures by treatment with retinoic acid in the growth medium (panel C). In addition, the maximal degree of retinoid stimulation was seen in low calcium medium, where the content of the particulate enzyme, a small percentage of which may be found in a soluble form (Thacher et al., 1985), was minimal.

As a fraction of total cell protein, the keratin content was 2- to 5-fold higher in cells grown with hydrocortisone in the medium regardless of the calcium concentration (panel A). In the presence of hydrocortisone at high or low calcium concentration, retinoic acid in the medium suppressed the keratin content by 90%. As illustrated in the gel patterns (Fig. 3), the suppression was especially dramatic for keratin protein in the region of 56–58 kd, which was virtually eliminated. This action was quite noticeable even at 0.3 μM retinoic acid (Rong, Rubin, and Rice, unpublished). In contrast, these keratins were readily apparent from cells grown in low calcium medium, especially in the presence of hydrocortisone.

The regulation of involucrin in these cultures exhibited 3 distinctive features. First, for cultures grown in medium supplemented with treated serum and without retinoic acid, the involucrin content in high calcium medium was 5- to 10-fold that in low calcium medium (Fig. 2D, I–IV). Second, addition of retinoic acid to low calcium medium stimulated involucrin levels 5- to 30-fold (Fig. 2D, I–II), the higher value being observed in the presence of hydrocortisone. Third, in cultures grown in medium supplemented with untreated serum, hydrocortisone produced a 4-fold higher level of involucrin, a level that was 4- to 8-fold that observed when retinoic acid also was added to the medium (Fig. 2D, V–VI). Previous experiments with SCC-13 cells have shown a similar degree of involucrin suppression by retinyl acetate in high calcium medium (Cline and Rice, 1983).

Regulation in normal human epidermal cells

Expression of the particulate enzyme (little tissue transglutaminase was detected) was clearly sensitive to calcium and retinoic acid (Fig. 4, lower panel). In

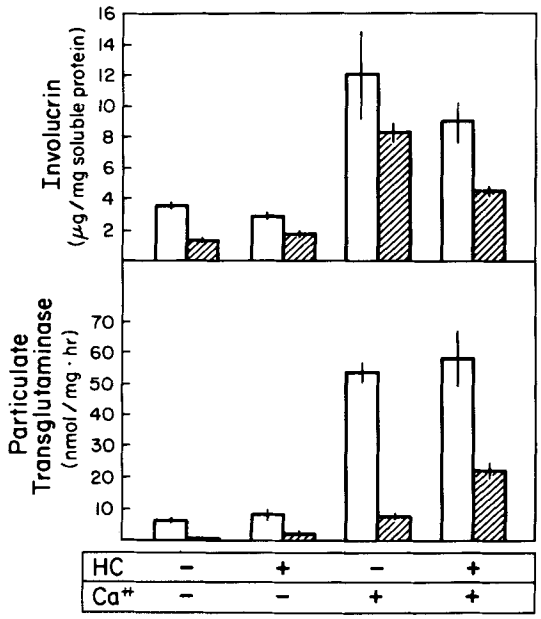

Fig. 4. Effect of culture conditions on involucrin and particulate transglutaminase in normal human epidermal cells. The cells were inoculated at a density of 3.5×10^5/6 cm dish. After 4 days, when the colonies covered 20–30% of the dish, the cultures were rinsed 3 times with the appropriate high or low calcium medium and given high or low calcium medium containing chelex- and charcoal-treated serum with the indicated effectors or solvent controls. One week after reaching confluence the cultures were washed 3 times in serum-free medium and scraped into 1 ml of 10 mM Tris-HCl (pH 7.4) – 1 mM EDTA – 1 mM DTE on ice and immediately frozen until assay of transglutaminase (lower panel) or involucrin (upper panel). Upon thawing, the cultures were disrupted with 10 strokes of a motor driven teflon/glass homogenizer in 6 ml of buffer, and particulate fractions were isolated by centrifugation. Hatched bars: 3.3 μM retinoic acid; clear bars: DMSO solvent controls. ± 1.8 mM $CaCl_2$ and 1 μM hydrocortisone as indicated below the figure. Error bars give the range of values in duplicate cultures.

high calcium medium the activity was 5- to 10-fold that in low calcium medium, and in either case was considerably higher in the absence than in the presence of the retinoid. Hydrocortisone in the growth medium had no observable effect on the calcium stimulation of expression. In the absence of the steroid, transglutaminase activity was 6- to 8-fold higher without than with retinoic acid in the medium, while in the presence of hydrocortisone the activity was only 2- to 3-fold higher without than with the retinoid. Involucrin levels in cultures grown in high calcium medium were approximately 3-fold higher than those from cultures grown in low calcium medium. Retinoic acid in the medium reduced the levels only slightly (≤ 50%), similar to the observations of Green and Watt (1982), while hydrocortisone had, if anything, only a barely detectable suppressive effect.

DISCUSSION

The present measurements of specific protein markers show a limited degree of coordination of the differentiation program in SCC-13 under the influence of retinoic acid, hydrocortisone, and calcium. These findings are summarized in Table 1. In low calcium medium, analogous to the basal state proposed for mouse epidermal cells under this condition (Hennings et al., 1980), involucrin, keratin, and particulate transglutaminase are suppressed. Levels of the latter two markers are stimulated by hydrocortisone and reduced by retinoic acid. In striking contrast, however, the effect of retinoic acid on involucrin content was critically dependent on other medium supplements. The retinoid stimulation (and lack of hydrocortisone action) evident under low calcium conditions, for instance, provides a clear example of uncoupling of keratinocyte programming. The 3-fold difference in involucrin levels in normal cells grown in low calcium versus 1.8 mM calcium-containing medium contrasts slightly with an earlier observation that the proportion of involucrin-positive cells is essentially the same under the two growth conditions (Watt and Green, 1982). It is likely that the present quantitative measurements detected a change in content that was not obvious earlier by immunofluorescence, or that holding the present cultures at confluence for a week or more increased the proportion of involucrin-positive cells.

TABLE 1. Modulation of keratinocyte differentiation in SCC-13[1]

Marker	Agent		
	Calcium (1.8 mM)	Hydrocortisone (1 μM)	Retinoic acid (3.3 μM)
Keratin	↑	↑	↓
Particulate transglutaminase	↑	↑	↓
Involucrin	↑	–	↑[2,3]
		–	–[2,4]
		↓	↓[5]

[1]Overall summary of given agents (↑, positive; –, no effect; ↓, negative).
[2]Charcoal- and chelex-treated serum.
[3]In low calcium medium.
[4]In 1.8 mM calcium-containing medium.
[5]Untreated serum.

By biochemical criteria, especially particulate transglutaminase expression, the normal epidermal cells were clearly sensitive to the culture conditions examined. This sensitivity, however, was quantitatively less than in the SCC-13 cells. To some extent, the difference could be due to their shorter time of exposure to low calcium or high retinoid concentrations. In addition, the normal cells express involucrin and transglutaminase to considerably higher levels than the SCC-13 cells. Thus, their lesser response could also reflect a greater commitment to keratinocyte programming (including the terminal stage) which is less readily perturbed. The present results highlight the utility of the malignant cells beyond their ability to grow under conditions where normal cells grow poorly. On the one hand, they provide a sensitive model for elucidating the actions of physiological effectors of differentiation. On the other hand, to the extent their response differs from that in the normal cells, they provide natural variants useful in resolving critical elements of keratinocyte programming. Our previous finding that the tissue transglutaminase is retinoid-stimulable in the neoplastic but not the normal epidermal cells (Rubin and Rice, 1986) suggested that differences in the keratinocyte program were likely to be observed.

In vivo, involucrin and particulate transglutaminase levels increase at the same time among the maturing cells of the spinous layer of human skin (Thacher and Rice, 1985). The intuitively reasonable suggestion that they are under identical control, however, appears too simplistic in light of present findings in SCC-13 cells. Indeed, more recent experiments have shown that expression of involucrin and transglutaminase in malignant keratinocytes is temporally distinct and can be uncoupled by treatment of the cells with certain carcinogens (Rice et al., 1988). In the normal cells, the greater suppression by retinoic acid of particulate transglutaminase than involucrin is suggestive of the uncoupling that becomes more evident in the neoplastic keratinocyte lines. Further investigation of this uncoupling in SCC-13 cells may not only resolve elements in regulation of keratinocyte markers but also help clarify normal coordination mechanisms. In any case, retinoic acid suppression of envelope competence consistently observed in SCC-13 appears attributable to suppression of the particulate transglutaminase and/or substrates other than involucrin (Thacher et al., 1985; Rubin and Rice, 1986, 1988).

Retinoids are known to influence the type of keratin subunit proteins expressed in normal human epidermal cells (Fuchs and Green, 1981). Under the present conditions in SCC-13, retinoic acid suppresses a number of keratins to a moderate extent, similar to the coordinated response reported in normal epidermal cells (Gilfix and Eckert, 1985). However, it almost completely prevents accumulation of keratins in the range of 56–58 kd, effectively antagonizing the hydrocortisone-dependent stimulation observed in its absence. More discriminating analysis by 2-dimensional electrophoresis may reveal whether other "epidermal" keratins present in lower amounts, such as the 46 and 50 kd species expressed in SCC-13 cells (Rheinwald et al., 1983), respond coordinately. Although malignant human epidermal cells (SCC-12B and -15) have been shown to be considerably more sensitive than the normal cells in regulation of the 67 kd (K1) and 40 kd (K19) keratins (Kim et al., 1984), which were not expressed by SCC-13 in these experiments, substantial effects using retinyl acetate have not been reported previously on those of 58 and 56 kd (K5 and 6), which are expressed in high yield by all the SCC lines derived by Rheinwald and Beckett (Rheinwald et al., 1983). Transcriptional and translational control mechanisms, the latter recently reported (Tyner and Fuchs, 1986) for the 56 kd "hyperproliferative" keratin (Weiss et al., 1984) in cultured human keratinocytes, appear more plausible than protein degradation. Although mediation of the retinoid effect by a specific protease cannot be completely discounted, general proteolysis appears unlikely due to the concomitant stimulation of involucrin (highly susceptible to protease action [Rice and Green, 1979]) and tissue transglutaminase. In any event, our findings emphasize that marked changes in observed expression of keratins and other markers, perhaps even as large as those observed in some virally transformed keratinocytes (Hronis et al., 1984), could have an underlying basis in alteration of cellular response to physiological effectors.

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LITERATURE CITED

Armelin, H.A., and Sato, G. (1974) Cell cultures as model systems for the study of growth control. In: Chemical Carcinogenesis. Part B. P.O. Ts'o, J.A. Dipaolo, eds. Marcel Dekker, New York, pp. 483–502.

Banks-Schlegel, S., and Green, H. (1981) Involucrin synthesis and tissue assembly by keratinocytes in natural and cultured human epithelia. J. Cell Biol., *90*:732–737.

Brennan, J.K., Mansky, J., Roberts, G. and Lichtman, M.A. (1975) Improved methods for reducing calcium and magnesium concentrations in tissue culture medium: Application to studies of lymphoblast proliferation in vitro. In Vitro Cell. Dev. Biol., *11*:354–360.

Chung, S.I. (1972) Comparative studies on tissue transglutaminase and factor XIII. Ann. NY. Acad. Sci., *202*:240–255.

Cline, P.R., and Rice, R.H. (1983) Modulation of involucrin and envelope competence in human keratinocytes by hydrocortisone, retinyl acetate and growth arrest. Cancer Res., *43*:3203–3207.

Fidler, I.J., and Hart, I.R. (1982) Biological diversity in metastatic neoplasms: Origins and implications. Science, *217*:998–1003.

Fuchs, E., and Green, H. (1981) Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell, *25*:617–625.

Gilfix, B.M., and Eckert, R.L. (1985) Coordinate control by vitamin A of keratin gene expression in human keratinocytes. J. Biol. Chem., *260*:14026–14029.

Greaves, M.F. (1986) Differentiation-linked leukemogenesis in lymphocytes. Science, *234*:697–704.

Green, H. (1979) The keratinocyte as differentiated cell type. Harvey Lect., *74*:101–139.

Green, H., and Watt, F.M. (1982) Regulation by vitamin A of envelope cross-linking in cultured keratinocytes derived from different human epithelia. Mol. Cell. Biol., *2*:1115–1117.

Hennings, H., Michaels, D., Cheng, C., Steinert, P., Holbrook, K., and Yuspa, S.H. (1980) Calcium regulation of growth and differentiation of mouse epidermal cells in culture. Cell, *19*:245–254.

Hronis, T.S., Steinberg, M.L., Defendi, A., and Sun, T.-T. (1984) Simple epithelial nature of some simial virus-40-transformed human epidermal keratinocytes. Cancer Res., *44*:5797–5804.

Kim, K.H., Schwartz, F., and Fuchs, E. (1984) Differences in keratin synthesis between normal epithelial cells and squamous cell carcinomas are mediated by vitamin A. Proc. Natl. Acad. Sci. U.S.A. *81*:4280–4282.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, *227*:680–685.

Lichti, U., Ben, T., and Yuspa, S.H. (1985) Retinoic acid induced transglutaminase in mouse epidermal cells is distinct from epidermal transglutaminase. J. Biol. Chem., *260*:1422–1426.

Marks, P.A., Sheffery, M., and Rifkind, R.A. (1987) Induction of transformed cells to terminal differentiation and the modulation of gene expression. Cancer Res., *47*:659–666.

Means, G.E., and Feeney, R.E. (1968) Reductive alkylation of amino groups in proteins. Biochemistry, *7*:2192–2201.

Parenteau, N.L., Eckert, R.L., and Rice, R.H. (1987) Primate involucrins: Antigenic relatedness and detection of multiple forms. Proc. Natl. Acad. Sci. U.S.A., *84*:7571–7575.

Parenteau, N.L., Pilato, A., and Rice, R.H. (1986) Induction of keratinocyte type I transglutaminase in epithelial cells of the rat. Differentiation, *33*:130–141.

Rheinwald, J.G., and Beckett, M.A. (1981) Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultured from human squamous cell carcinomas. Cancer Res., *41*:1657–1663.

Rheinwald, J.G., and Beckett, M.A. (1980) Defective terminal differentiation in culture as a consistent and selectable character of malignant human keratinocytes. Cell, *22*:629–632.

Rheinwald, J.G., Germain, E., and Beckett, M.A. (1983) Expression of keratins and envelope proteins in normal and malignant keratinocytes and mesothelial cells. In: Human Carcinogenesis. C. Harris and H. Autrup, eds. Academic Press, New York, pp. 85–96.

Rheinwald, J.G., and Green, H. (1975) Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. Cell, *6*:331–344.

Rice, R.H., and Cline, P.R. (1984) Opposing effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and hydrocortisone on growth and differentiation of cultured malignant human keratinocytes. Carcinogenesis, *5*:367–371.

Rice, R.H., and Green, H. (1979) Presence in human epidermal cells of a soluble protein precursor of the cross-linked envelope: Activation of the cross-linking by calcium ions. Cell, *18*:681–694.

Rice, R.H., Rong, X., and Chakravarty, R. (1988) Suppression of keratinocyte differentiation in SCC-9 human squamous carcinoma cells by benzo(a)pyrene, 12-0-tetradecanoylphorbol-13-acetate and hydroxyurea. Carcinogenesis, *9*:1885–1890.

Rubin, A.L., and Rice, R.H. (1986) Differential regulation by retinoic acid and calcium of transglutaminases in cultured neoplastic and normal human keratinocytes. Cancer Res., *46*:2356–2361.

Rubin, A.L., and Rice, R.H. (1988) Characterization of the calcium sensitivity of differentiation in SCC-13 human squamous carcinoma cells. In Vitro Cell. Devel. Biol., *24*:857–861.

Rubin, H. (1985) Cancer as a dynamic developmental disorder. Cancer Res., *45*:2935–2942.

Simon, M., and Green, H. (1984) Participation of the membrane-associated proteins in the formation of the cross-linked envelope of the keratinocyte. Cell, *37*:827–834.

Simon, M., and Green, H. (1985) Enzymatic cross-linking of involucrin and other proteins by keratinocyte particulates in vitro. Cell, *40*:677–683.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. (1985) Measurement of protein using bicinchoninic acid. Anal. Biochem., *150*:76–85.

Thacher, S.M., Coe, E.L., and Rice, R.H. (1985) Retinoid suppression of transglutaminase activity and envelope competence in cultured human epidermal carcinoma cells: Hydrocortisone is a potent antagonist of retinyl acetate but not retinoic acid. Differentiation, *29*:82–87.

Thacher, S.M., and Rice, R.H. (1985) Keratinocyte-specific transglutaminase of cultured human epidermal cells: Relation to cross-linked envelope formation and terminal differentiation. Cell, *40*:685–695.

Tyner, A.L., and Fuchs, E. (1986) Evidence for posttranslational regulation of the keratins expressed during hyperproliferation and malignant transformation in human epidermis. J. Cell Biol., *103*:1945–1955.

Watt, F.M., and Green, H. (1981) Involucrin synthesis is correlated with cell size in human epidermal cultures. J. Cell Biol., *90*:738–742.

Watt, F.M., and Green, H. (1982) Stratification and terminal differentiation of cultured epidermal cells. Nature, *295*:434–436.

Weiss, R.A., Eichner, R., and Sun, T.-T. (1984) Monoclonal antibody analysis of keratin expression in epidermal diseases: A 48- and 56-kdalton keratin as molecular markers for hyperproliferative keratinocytes. J. Cell Biol., *98*:1397–1406.

Wu, Y.J., and Rheinwald, J.G. (1981) A new small (40 kd) keratin filament protein made by some cultured human squamous cell carcinomas. Cell, *25*:627–635.