DIFFERENTIATION OF CULTURED HUMAN KERATINOCYTES: EFFECT OF CULTURE CONDITIONS ON LIPID COMPOSITION OF NORMAL VS. MALIGNANT CELLS

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SUMMARY

Differentiation in keratinocytes can be experimentally modulated by changing the culture conditions. When cultured under conventional, submerged conditions, the extent of cellular differentiation is reduced in the presence of low calcium medium and is enhanced in medium containing physiologic calcium concentrations. Moreover, cultures grown at the air-medium interface or on a dermal substrate, or both, differentiate even further. Herein we report the effect of culture conditions on lipid composition in normal human keratinocytes and three squamous carcinoma cell (SCC) lines that vary in their capacity to differentiate as assessed by cornified envelope formation. Under submerged conditions, the total phospholipid content was lower, triglyceride content higher, and phospholipid:neutral lipid ratio lower in direct correlation to the degree of differentiation in these cultures. When grown at the air-medium interface on de-epidermized dermis, evidence of further morphologic differentiation was found only for well-differentiated SCC cells and normal keratinocytes. Similarly, the phospholipid content remained high in poorly differentiated SCC cells and it decreased modestly in well-differentiated SCC cells and markedly in normal keratinocytes. In all cell lines the triglyceride content was increased and cholesterol content decreased when compared to parallel submerged cultures, but these differences were most pronounced in well-differentiated cell lines. Acylceramides and acylglucosylceramides were found only in normal keratinocytes and only under the most differentiation-enhancing conditions. These studies demonstrate differentiation-related changes in the lipid content of both normal and neoplastic keratinocytes.

Key words: lipids; differentiation; keratinocytes; squamous cell carcinomas.

INTRODUCTION

Cultured keratinocytes provide a useful model to study the regulation of epidermal differentiation, because the degree of their differentiation can be experimentally modified. First, changes in extracellular calcium alter differentiation (16,42), but even at physiologic calcium concentrations, the extent of maturation, as determined by a number of protein biochemical and ultrastructural markers, does not achieve that found in vivo (18,40). Second, attachment of keratinocytes to a biological matrix and exposure to an air-medium interface accelerates differentiation (2,13,24,32,34,46). Third, epidermal carcinoma cell (SCC) lines exhibit variable defects in their differentiation capacity (37,38), and this can be modified further by changes in either extracellular Ca" concentration or growth at the air-medium interface (4,5,7,31).

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The lipid composition of normal human keratinocytes also is related to the extent of keratinocyte maturation; however, only when cells are grown at an air-medium interface are substantial quantities of sphingolipids, critical constituents of the epidermal permeability barrier (reviewed in 9), generated (32,46). Moreover, Ponec et al. (30) have shown that rates of lipogenesis are inversely related to differentiation in several SCC cell lines; i.e. highest in poorly differentiating (line SCC-4) cells, intermediate in moderately differentiating (line SCC-15) cells, and lowest in both well-differentiating (line SCC-12F2) cells and normal keratinocytes. However, it is not known whether these differences in rates of lipogenesis also are accompanied by differences in bulk lipid content or composition or both. In the present study we compared the lipid composition of normal keratinocytes and three SCC lines (SCC-12F2, SCC-15, SCC-4) in relation to their capacity to differentiate, as determined by cornified envelope formation and morphologic criteria (37-39). The results presented here show that the lipid composition of the malignant cell lines differs...
from normal keratinocytes, and that some of these
differences are related to their state of differentiation,
changes that become more apparent when these cell lines
are grown at an air-medium interface. Second, acyl sphingolipids were generated only by normal keratinocytes
and only when these cells were grown under the most
differentiation-enhancing conditions.

MATERIALS AND METHODS

Cell Culture

Submerged culture. Three juvenile foreskin keratinocyte cell lines (Passages 1 to 4) derived from donors (ages
1 to 2 yr) and SCC lines (SCC-4, SCC-15, SCC-12F2) (37)
were cultured on irradiated mouse 3T3 fibroblast feeder
layers (35). Media, Dulbecco-Vogt and Ham's F12 (3:1),
were supplemented with 5% fetal bovine serum (FBS) and
0.4 μg/ml hydrocortisone. Normal keratinocytes were
supplemented with 10⁻⁶ M isoproterenol (14) and 10
ng/ml epidermal growth factor (EGF) (36). For low-
calcium conditions, calcium-free Dulbecco-Vogt medium
was mixed with standard Ham's F12 (3:1) and supplement-
ed with 5% chelax-treated FBS (16) and the other
additives listed above. The final calcium concentration
was 0.06 mM, as determined by flame photometry.

Air-exposed culture. The de-epidermized dermis
(DED) for air-exposed cultures was prepared as described
by Regnier et al. (34). Briefly, cadaver skin (stored at 4°C
in 85% glycerol) was carefully washed with phosphate
buffered saline (PBS) and incubated for 3 to 5 d in PBS at
37°C. Subsequently, the epidermis was scraped off and
the remaining dermis irradiated (3000 R) and washed
several times with culture medium. The dermis was then
placed on the stainless steel grid and 0.5 X 10⁶ normal
keratinocytes (Passages 2 or 3) or SCC cells were
inoculated inside a stainless steel ring (diameter 1 cm)
placed on the top of the dermis. After 24 h the ring was
removed, and the level of culture medium (normal
calcium) was adjusted to just reach the height of the grid.
This method ensures that the cells are exposed to air
throughout the remaining period of culture. A parallel set
of normal keratinocytes were seeded onto the dermal
substrate, as described above, but were maintained
submerged in medium of the same composition.

Cell Morphology

Cells cultured to confluence on plastic were detached
with dispase (15). Both epidermal sheets and cells
cultured on DED were fixed in 4% buffered formalde-
hyde, embedded in paraffin, sectioned, and stained with
hematoxylin and eosin.

Lipid Extraction, Fractionation, and Quantitation

Cells grown under submerged conditions were harvest-
ed after 10 d by trypsinization (0.25% trypsin, 0.02%
EDTA in PBS, pH 7.5) and washed several times with
PBS. Cells grown on DED were harvested after 10 d using
thermolysin. The epidermal sheet was then washed
several times with PBS. Lipids were extracted (3), with the
addition of 0.25 M KCl to ensure extraction of polar species. Organic phases were dried under a stream of
nitrogen, redissolved in chloroform:methanol (2:1), and
weighed. Aliquots of cell suspensions and epidermal sheet
residues were lysed in 1 N NaOH for protein determinations
(25).

Lipids were fractionated by thin layer chromatography
(TLC) using several one-dimensional systems, as de-
scribed in detail elsewhere (32). Lipid fractions were
quantitated after charring by photodensitometry in
reference to authentic standards (32). Lathosterol, which
has a TLC mobility very close to that of cholesterol, has
been tentatively identified by the brownish color
produced after charring the plates at 100°C. Data
represent the mean of two to three determinations on two
or more independent experiments.

RESULTS

Cell Morphology

As previously described (4), varying the calcium
concentration in the culture medium resulted in
morphologic changes in normal keratinocytes and in SCC
cell lines. In low-calcium media, both normal and

Fig. 1. Hematoxylin-and-eosin-stained vertical paraffin sec-
tions of confluent cultures of normal keratinocytes (a), SCC-12F2
(b), SCC-15 (c), and SCC-4 (d) cells cultured under submerged
conditions in medium containing normal calcium (1.6 mM)
concentration.