OPPOSITE EFFECTS OF EGF ON INVOLUCRIN ACCUMULATION OF
A431 KERATINOCYTES AND A VARIANT WHICH IS NOT
GROWTH-ARRESTED BY EGF

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SUMMARY

The A431 cell line is composed of malignant keratinocytes derived from a vulval epidermoid carcinoma. These cells have the peculiarity to stop their proliferation when they are treated with physiological concentrations of EGF, which is a mitogen for normal keratinocytes. We reported earlier that EGF induces involucrin accumulation in A431 cells and proposed that the arrest of proliferation triggers differentiation as shown by the induction of this cornified envelope precursor protein. To test this hypothesis, we compared the A431 subclone 15, which is not growth arrested by EGF-treatment, to the parental A431 cells. We found indeed that EGF reduces the involucrin content of clone 15 cells in a dose dependent manner. These opposite effects of EGF on the expression of terminal differentiation marker involucrin in A431 and A431 clone 15 keratinocytes were observed in defined medium as well as in presence of fetal calf serum. Nevertheless, when growth of parental A431 cells was inhibited by treatment with TGF-β or simply when cultures reached confluency, no involucrin accumulation was observed. Therefore growth arrest per se is not directly correlated with the induction of differentiation.

Key words: A431 cells; involucrin; EGF; hydrocortisone; TGF-β; epidermal differentiation.

INTRODUCTION

In stratified epithelia of different origins involucrin, the major precursor protein of the cornified envelope, is expressed only in the upper layers appearing in the course of the outward migration of the epithelial cells accompanying their terminal differentiation. In skin diseases (psoriasis, keratinization disorders, tumours), involucrin expression is modified or presents abnormal patterns (5,14,15). In cultures of normal human keratinocytes, the expression of involucrin begins in the first cell layer above the proliferative “basal” layer (1,2,22). Watt and Green reported that involucrin expression correlates with growth arrest, cell enlargement, protein content and spatial distribution (29,30,31,32). A431 is an epidermal cell-line derived from a vulval epidermoid carcinoma. These malignant keratinocytes have been extensively studied for their peculiarity to stop proliferation when treated with physiological concentrations of epidermal growth factor (EGF) (3,16), which stimulates the growth of normal keratinocytes (4,12,23). Gordon Gill and colleagues reported (9,18) that A431 clones whose growth is stimulated or unaffected by EGF have both a reduced number of EGF receptors compared to parental growth-inhibited A431 cells. They showed that growth response depends indirectly on the number of EGF receptors present on the cell surface, meanwhile their metabolism, binding and internalization characteristics are unchanged. They also noted that morphological changes induced by EGF are independent of its effects on growth.

We recently showed that differentiation of A431 cells witnessed by the involucrin content was increased after EGF-treatment (24). We interpreted these data by suggesting that proliferation arrest induced by EGF in A431 cells triggered involucrin expression. The existence of EGF-resistant subclones allowed us to test this hypothesis, since EGF does not provoke growth arrest in these cells (8,18). We compared involucrin contents of both A431 wild type and EGF-resistant clones in several culture conditions to test if the inhibition of proliferation by EGF, or EGF by itself, is responsible for the increase of involucrin expression. Two different techniques were employed: first semi-quantitative western blotting was performed by diluting the antigen-containing extracts, second western blots were screened by densitometry.

We observed indeed that, contrary to its effect in wild type A431 cells, EGF reduced involucrin content of clone 15. Since TGF-β inhibits proliferation of normal human keratinocytes (25), we tested its effect on growth and
differentiation of A431 cells: We found that although TGF-β slowed down proliferation of parental A431 cells in defined medium to the same extent as does EGF, it does not provoke significant involucrin accumulation in these cells.

MATERIALS AND METHODS

Materials. Chemically defined medium MCDB 153 was prepared in our laboratory as described by Peehl, Boyce, and Ham (6,7,19), modified (20,21) and supplemented with Calcium (10⁻³), Insulin (5 µg/ml), Ethanolamine (10⁻⁴ M), Phosphoethanolamine (10⁻⁴ M), Hydrocortisone (5 × 10⁻⁵ M) and EGF (10, 50 or 100 ng/ml), whenever used. All chemicals and organic salts were obtained from Sigma, trace elements were from Merck (Darmstadt, FRG), Fluka (Buchs, CH) and Sigma. EGF (culture grade) was obtained from Collaborative Research (Lexington, MA, USA). TGF-β was obtained from R&D Systems Inc. (Minneapolis, MN, USA). All products for electrophoresis and western blotting were purchased from Biorad Laboratories (Richmond, CA, USA). DME and F-12 media were purchased from GIBCO (France) and fetal calf serum and trypsin-EDTA from Boehringer Mannheim (France).

Cell culture. A431 cells were from Dr. P. Vigier (Orsay, France) and clone 15 was kindly provided by Dr. Rodrigo Bravo (EMBL, Heidelberg, FRG) (8). This subclone was obtained by mutagenesis and selective culture (as described in references 9 and 18).

A 1:1 mixture of DME and F-12 Media, supplemented with 10% fetal calf serum was used for stock cultures. All media were changed three times/wk. For growth measurements, the A431 cells were seeded at a density of 2.9 to 6.5 × 10⁵ cells/35 mm dish and cell number in two dishes was counted separately in an isotonic suspension using a Coulter Counter (Coultronics, France) every two days.

Semi-quantitative assay of involucrin in A431 cells. High-salt insoluble protein extracts were prepared as described by Winter et al. (33) in the presence of aprotinin (10 µg/ml). The supernatant obtained after the first homogenization was taken as the high-salt soluble protein fraction. 0.5 µg, 1 µg and 5 µg of high-salt soluble proteins of A431 cells cultured seven days in MDCB 153 with different supplementations were separated in a 8.5% SDS polyacrylamide slab-mini-gel (17) and then transferred to nitrocellulose sheets (27) using the Hoefer mini-electrophoretic and mini-transfer-units (Hoefer, San Francisco, USA).

Involucrin was revealed by the immunoperoxidase staining procedure described by Glass et al. (11). The polyclonal antibody against involucrin was obtained from Biomedical Technologies Inc. (MA, USA) and used at a dilution of 1:20 in TBS 3% Regilait. This antiserum colors the cytoplasm of differentiated epidermal cells and reacts on western blots specifically with one band of MW...