RAPID INDUCTION OF MORPHOLOGICAL CHANGES IN HUMAN CARCINOMA CELLS A-431 BY EPIDERMAL GROWTH FACTOR

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ABSTRACT

The morphological effects of epidermal growth factor (EGF) on human carcinoma cells A-431 have been examined by scanning electron microscopy. These flat polygonal cells normally exhibit only small membrane folds, but show extensive ruffling and extension of filopodia within 5 min of exposure to EGF at 37°C. This ruffling activity is transient, subsiding within another 5–15 min, but several other changes in surface morphology follow. Within the first hour of exposure to the hormone, the cell surface becomes exceedingly smooth and the nuclei seem to protrude above the plane of the otherwise thin monolayer, giving the cells a "fried egg" appearance. Cells at the edges of colonies gradually retract from the substrate, leading to reorganization, by 12 h, of the monolayer into multilayered colonies. EGF thus induces both rapid and long-term alterations in the morphology of these epidermoid cells.

KEY WORDS   scanning electron microscopy ·
membrane ruffling · growth factor ·
motility · cell shape change

The regulation of the shape and motility of cultured cells is currently a subject of interest from both morphological and biochemical points of view. In several instances, hormones have been shown to influence the morphology of target cells in culture (e.g., references 27, 21, 16).

In our recent studies concerning the mechanism of action of epidermal growth factor (EGF), we have used the human epidermoid carcinoma cell line A-431, taking advantage of the extraordinarily high number (2–3 × 10^6) of EGF receptors present on these cells (9, 12). This property of A-431 cells has facilitated the visualization of hormone binding and internalization (12, 13) as well as the discoveries that EGF rapidly stimulates fluid pinocytosis in whole cells (14) and protein phosphorylation in isolated membranes (6, 7).

In this report, we describe rapid and striking morphological changes which are induced in A-431 cells by treatment with EGF.

MATERIALS AND METHODS

Cell Culture

A-431 human epithelioid carcinoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS). Semiconfluent cultures were prepared in 35-mm Falcon dishes containing 22-mm square cover glasses.

Scanning Electron Microscopy (SEM)

Samples were processed for SEM by a modification of the OTOTO method (20). Cells were washed rapidly with Dulbecco's phosphate-buffered saline (PBS) and fixed for 1 h with 4% glutaraldehyde in PBS. After three 10-min washes with 0.1 M sodium phosphate buffer (pH 7.1), cultures were postfixed for 2 h with 1% OsO_4 in the same buffer. Six distilled-water washes were followed successively by treatment with 1% thiocarbohydrazide (TCH) (30 min), 1% OsO_4 (2 h), 1% TCH (30 min), and 1% OsO_4 (2 h). Between the TCH and OsO_4 treatments, and after the final incubation with OsO_4, cultures were washed six times for 2.5 min each with distilled water. The cultures on coverslips were then dehydrated through a graded ethanol series and critical-point dried from CO_2. Coverslips were cut into quarters, which were mounted on aluminum stubs with graphite paste. All

fixation, washing, and dehydration steps were performed at room temperature.

Samples were viewed without further coating on a Hitachi S-500 microscope operated at 20 kV. Kodak Min-R X-ray film, developed with a Kodak RP X-Omat processor, was used for micrography.

Materials

Mouse EGF was purified as described previously (22). The A-431 cell line was originally obtained from Dr. J. DeLarco (9).

RESULTS

Striking changes in cell shape occur with remarkable speed when monolayer cultures of A-431 cells, growing in DMEM supplemented with 10% FCS, are treated with EGF (100 ng/ml) at 37°C. These changes are illustrated in Figs. 1–8 with the use of SEM.

Before EGF treatment, control A-431 cells appear polygonal in outline and well flattened (Fig. 1). Cell surface structure is somewhat variable, but the predominant surface extensions are small plicae (folds); microvilli are less frequently observed. These plicae are also the predominant type of surface extension observed in A-431 cells by transmission electron microscopy (TEM) (13).

Within 5 min of addition of EGF to the cultures, a spectacular burst of ruffling activity begins (Fig. 2). Large lamellipodia, typically several μm in height, rise up around the entire periphery of virtually every cell. Such activity is never observed in untreated cultures of A-431 cells, although smaller lamellipodia are sometimes seen at colony edges in such cultures. Consistent with previous observations that filopodia are often closely associated with lamellipodia (26, 2, 17), a proliferation
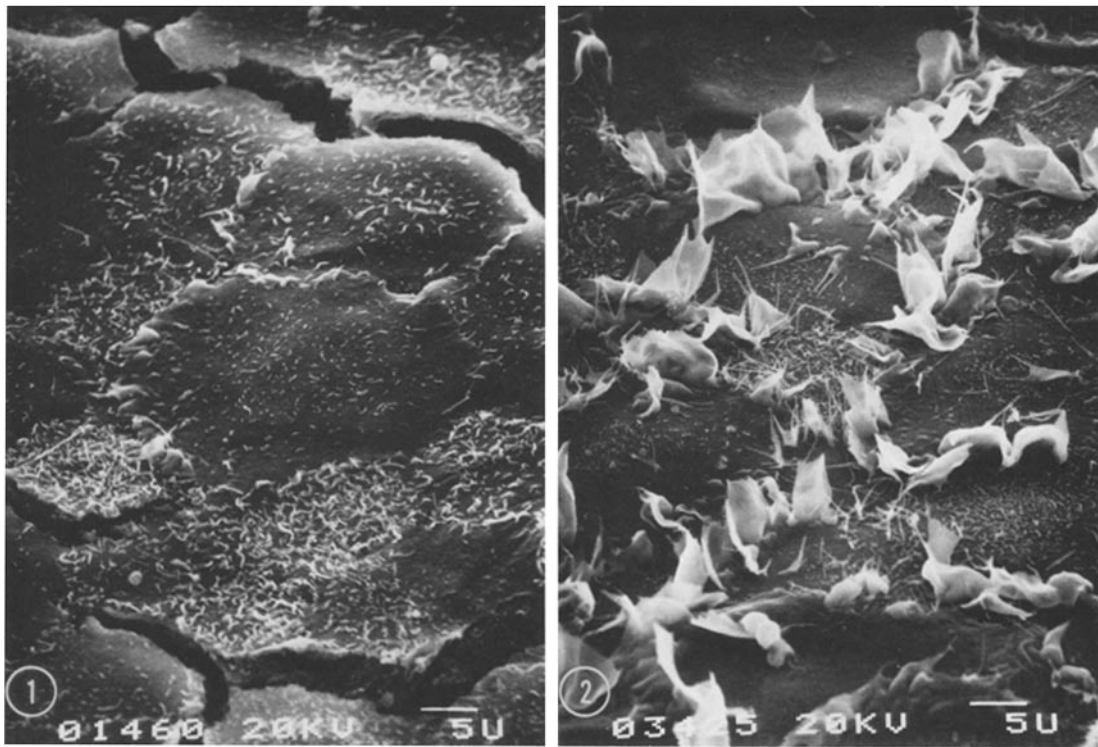
FIGURES 1–8   Human carcinoma cells A-431 in DMEM supplemented with 10% FCS were incubated at 37°C in the presence or absence of EGF (100 ng/ml) for the indicated times, then fixed and processed for SEM. Bars, 5 μm.

FIGURE 1   Untreated control cells. Numerous small plicae are observed on the cell surface. Breaks in the monolayer are artifacts caused by shrinkage during critical-point drying.

FIGURE 2   Cells exposed to EGF for 5 min. Numerous lamellipodia and filopodia have appeared on the cell surface, especially near the periphery.

of filopodia accompanies the induction of ruffling by EGF (Fig. 2).

This unusual ruffling phenomenon comes to an end as quickly as it begins; by 10 min after the addition of EGF, most of the ruffling activity has subsided (Fig. 3). Filopodia projecting several μm into the medium at the cell periphery, as well as an occasional ruffle, are the only reminders of the intense activity observed just 5 min earlier. Additional morphological changes continue to occur, however. At 10 min after addition of EGF, cells at the edges of colonies are beginning to retract from the substrate (Fig. 4). Such cells often display blebs as well as retraction fibers.

By 20 min, most lamellipodia and filopodia have been withdrawn, but the cell morphology has not returned to the untreated control state. As viewed by SEM the cells resemble fried eggs, exceedingly flattened except for a central dome-shaped protrusion, presumably housing the nucleus (Fig. 5). At later time points, this "fried egg" appearance persists, but the cell surface becomes smoother (Fig. 6, 1 h).

It should be pointed out that, since protruding nuclei are not obvious in sections of EGF-treated cells processed for TEM (unpublished observations), it seems possible that the peripheral cytoplasm collapses as an artifact of critical-point drying. Nevertheless, this apparent artifact is specific to EGF-treated cells.

As the process of retraction from the substrate at the edges of colonies continues, cells in these areas begin to pile up on top of one another. This phenomenon is readily observed by 1 h after addition of EGF to the cultures (Fig. 7). By 12 h, the original monolayer has changed to form groups of cells several layers thick (Fig. 8). If the cells are maintained in a continuing presence of EGF, a multilayered morphology persists for at least 5 d.

DISCUSSION

We report here the rapid induction of membrane ruffling in A-431 cells by treatment with EGF. This phenomenon may be a consequence of the
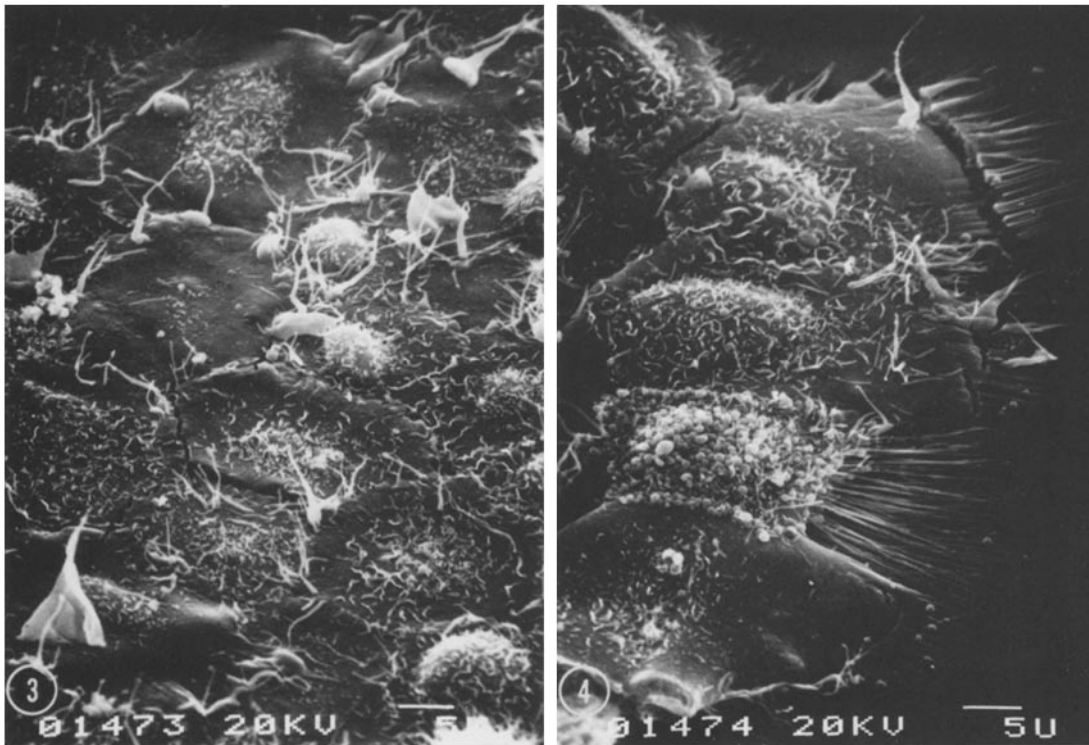

FIGURE 3   Cells exposed to EGF for 10 min. Most lamellipodia have been withdrawn, but a number of filopodia remain around the cell periphery.

FIGURE 4   Cells exposed to EGF for 10 min. Cells at edges of colonies have begun to retract from the substrate.
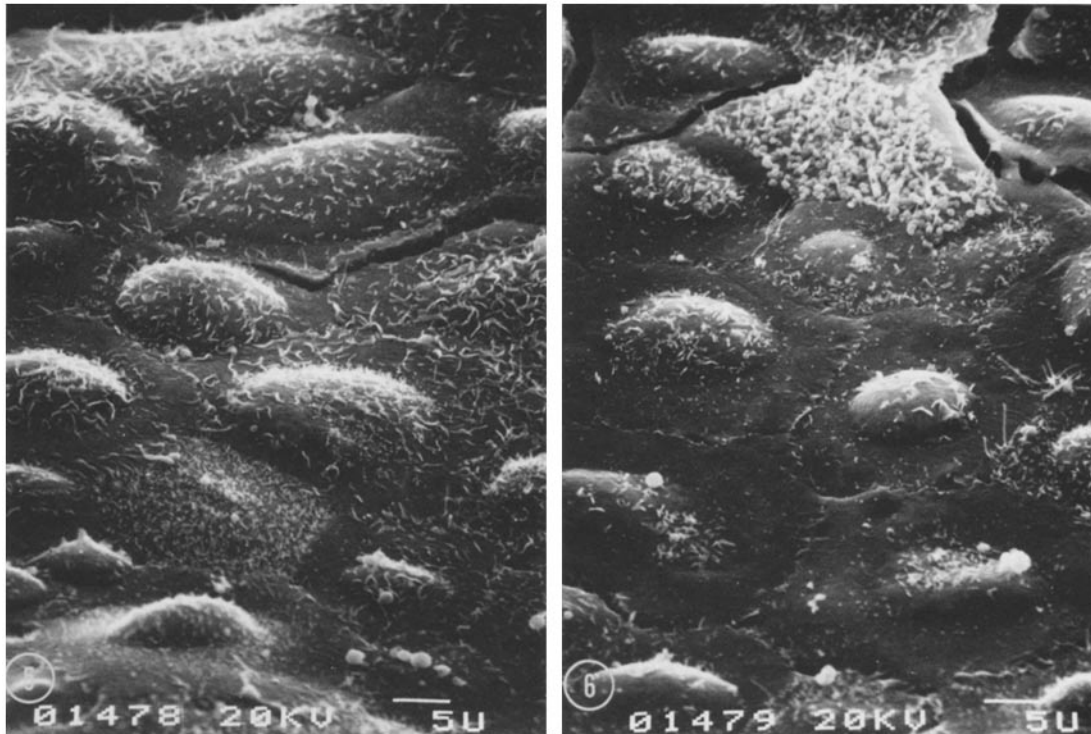
FIGURE 5   Cells exposed to EGF for 20 min. Remaining lamellipodia and filopodia have been withdrawn and cells have taken on a "fried egg" shape.

FIGURE 6   Cells exposed to EGF for 1 h. "Fried egg" appearance has persisted and the cell surface has become very smooth. Blebbed cell at top of micrograph is atypical.

very high number of EGF receptors present in A-431 cells, some 30-fold more than are present on fibroblasts (9, 12).

Stimulation by EGF of ruffling activity in cultured human glial cells has been described (5) but differs from the response in A-431 cells in two important respects. The experiment with glial cells demonstrated the ability of EGF to restore basal levels of ruffling activity to serum-starved cells, while we have described a marked increase in such activity after the addition of EGF to serum-containing cultures of A-431 cells. Also, the time course of stimulation of ruffling is different in the two systems. EGF stimulates a rapid and transient increase in ruffling in A-431 cells, observed within a few minutes of exposure to the hormone. In glial cells, EGF stimulates a gradual and continuing increase in the percentage of cells exhibiting ruffling, first detectable 4 h after the addition of hormone to the cultures. This time course suggests that ruffling in the glial cell system is a secondary

response to EGF and is not directly related to the initial binding of the hormone.

It is noteworthy that in both A-431 cells (14) and glial cells (5) EGF stimulates an increase in fluid pinocytosis, the time course of which parallels that of the increase in ruffling activity. That is, EGF stimulates a gradual increase in fluid pinocytosis in glial cells, while in A-431 cells the pinocytic rate increases very rapidly (within 30 s) but transiently, returning to control levels within 15 min. A close association between macropinocytosis and membrane ruffling was originally described by Lewis (18), and has often been noted (e.g., references 10, 11, 1).

The morphological alterations induced by EGF in A-431 cells are somewhat similar to those induced by avian sarcoma virus (ASV) in chick fibroblasts (3, 25). Cells infected with a temperature-sensitive transformation-defective mutant of ASV show a dramatic increase in ruffling activity 1–3 h after being exposed to a lowered permissive
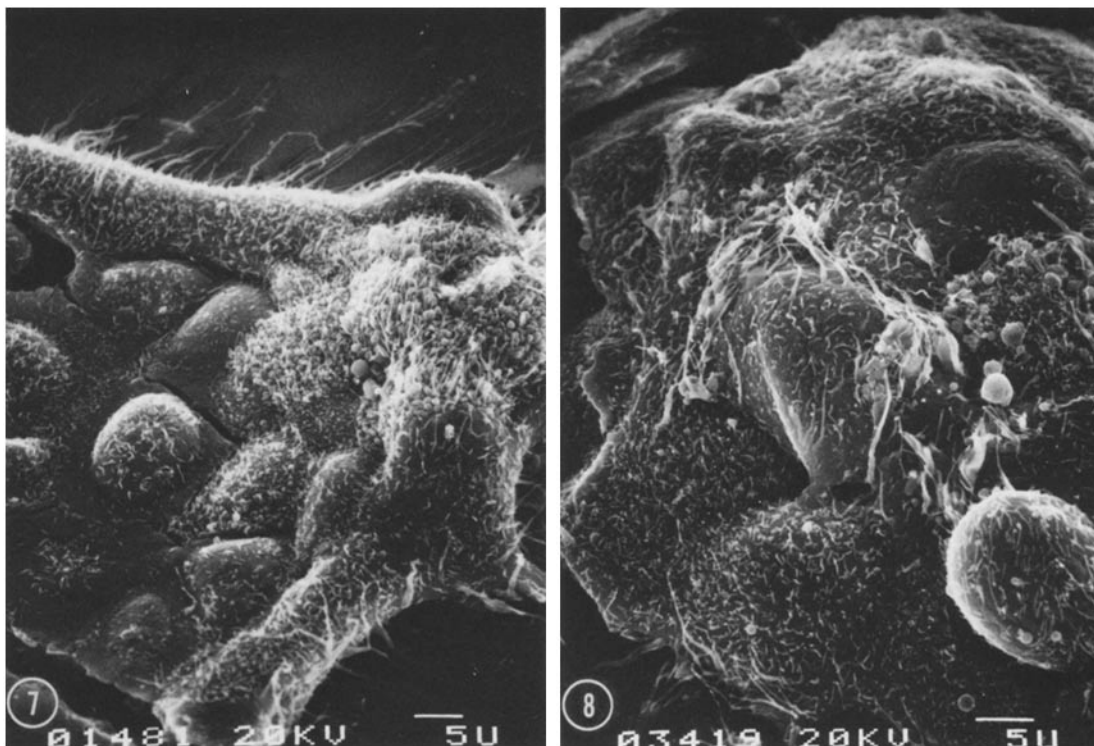
FIGURE 7   Cells exposed to EGF for 1 h. Cells retracting from the edges of colonies have begun to overlap (right). Cells in colony interior remain monolayered and flattened (left).

FIGURE 8   Cells exposed to EGF for 12 h. The retraction process has led to the formation of colonies of overlapping cells.

temperature (3, 25). This is followed by retraction from the substrate at the edges of cells, which leads, by 12-24 h, to cell rounding (25). It is interesting to note that transformation by ASV is apparently mediated by a virally coded protein kinase which specifically phosphorylates threonine residues (8); EGF also stimulates a threonine-specific protein kinase when the hormone is added to isolated A-431 cell membranes (6, 7). This raises the possibility that a phosphorylation event may be responsible for the morphological changes induced by EGF. Certainly the rapidity of the phosphorylation response in A-431 membranes is consistent with the rapidity of the induction of ruffling. The regulation of cell shape by protein phosphorylation/dephosphorylation reactions is suggested by the observations that different states of phosphorylation of spectrin relate to changes in erythrocyte shape (4), that full activity of nonmuscle myosin requires phosphorylation of the myosin light chain (15), and that several other cytoskeletal proteins may be phosphorylated (23, 24, 19).

From preliminary observations of earlier stages in the EGF response, it appears that the large ruffles begin to form in <60 s by direct growth and excrescence of the small plicae present on the surface of control cells. We predict that the rapidity and amplitude of the ruffling response may make the EGF/A-431 cell system a useful model for the study of the mechanisms involved in membrane ruffling.

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