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Detection of Epidermal Growth Factor Receptors and E-Cadherins in the Basolateral Membrane of A431 Cells by Laser Scanning Fluorescence Microscopy

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We have examined the localization of epidermal growth factor (EGF) receptors and E-cadherins in cultured A431 cells, an epidermoid carcinoma cell line, using a laser scanning fluorescence microscope. The fluorescence signals generated by monoclonal antibodies against EGF receptor and E-cadherin were localized mainly in the cell-cell contact sites, about 3 μm in width. When these areas for cell adhesion were scanned perpendicularly, fluorescence was detected from a point 4 μm from the cell base for a distance of 8 μm towards the cell surface (about 16 μm from the cell base). These data suggest the subcellular localization of EGF receptors and E-cadherins in the basolateral membrane of A431 cells.

Key words: EGF receptor — E-cadherin — Cell attachment — Laser scanning microscopy

The polypeptide growth factors such as epidermal growth factor (EGF) are important determinants for the regulation of cell proliferation and differentiation.[1,2] An important aspect in understanding the regulatory role of these growth factors is the identification and localization of their receptors. The EGF receptors have been localized on the "cell surface" at the light microscopy level by using radio-labeled EGF,[3] fluorescently labeled EGF[4] and monoclonal antibodies against EGF receptors.[3,5] By using immuno-electron microscopy, EGF receptors have been localized at cell-cell contact sites of A431 cells.[6] The study of polarized cells *in vivo* and *in vitro* revealed the condensed localization of EGF receptors at the basolateral membrane instead of the apical membrane.[7,8] These data strongly suggest the possible co-localization of EGF receptors with cell adhesion molecules at cell-cell attachment sites. In this short communication, we describe the localization of EGF receptors and E-cadherins, an epithelial cell type adhesion molecule, in A431 cells using a laser scanning fluorescence microscope. The results suggest the co-localization of these molecules in the basolateral membrane of A431 cells.

A431 cells were grown on cover slips under previously described conditions,[9] fixed with cold acetone, air-dried and immersed in phosphate-buffered saline (PBS, pH 7.2). The cover slips were immersed in 3% bovine serum albumin (BSA) in PBS and subsequently reacted with primary antibodies B4G7 for EGF receptor[10] and HECD-1 for E-cadherin[11] (a gift from Dr. M. Takeichi, Kyoto Univ.) after 1:200 dilution. These preparations were reacted with FITC-labeled rabbit anti-IgG as a second antibody (Silenus). Finally, they were mounted with 10% glycerol in PBS. Normal mouse IgG was used as the staining control.

Confocal microscopy[12] was performed using the MRC-600 unit (Bio Rad), which was attached to the phototube of a conventional fluorescence microscope (Optiphot, Nikon). An argon ion laser operating at 488 nm was used as the excitation source. The scan time was 1.0 s per frame. Ten frames 2 nm each in Z-axis from the bottom to the surface of fixed cell were examined for each antibody. Images were photographed by using a MULTI SCAN (Toshiba).

Fluorescence images of A431 cells stained with monoclonal antibodies against EGF receptor and E-cadherin are shown at every 2 μm thickness from the cell base (Fig. 1 for E-cadherin and Fig. 2 for EGF receptor). Fluorescence can be detected mainly at the lateral membrane of A431 cells and the distribution patterns of EGF receptor and E-cadherin are essentially the same. The highest fluorescence intensity is detected in the 10 μm section from the cell base and this high-intensity fluorescence is localized within an area of about 3 μm in width. Fluorescence is detectable in the regions between 4 μm to 12 μm from the cell base. Fluorescence intensity of EGF receptor in the edges of cell surface which are free from cell attachment is very weak and estimated to be 9 times less than that in the cell attachment sites (Fig. 3). Thus, EGF receptors and E-cadherins are localized at cell adhesion areas. These findings are summarized as a scheme with apparent dimensions in Fig. 4.

Several approaches have been made to detect EGF receptors in cultured cells and polarized epithelial cells *in vivo*. Boonstra[6] made the first report on the subcellular

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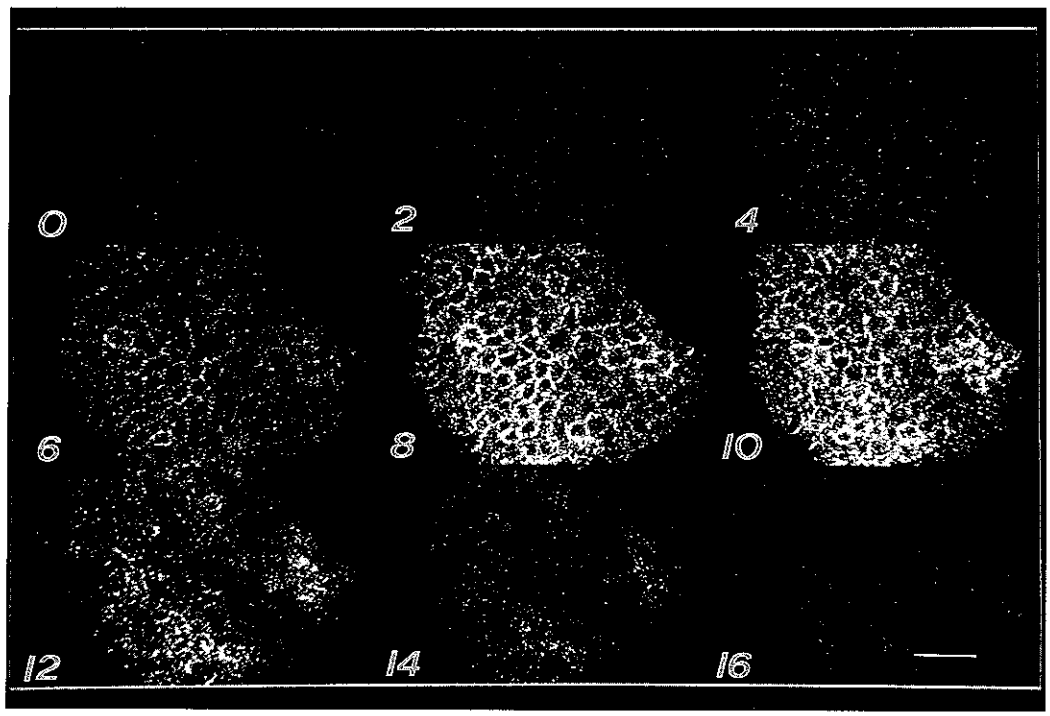

Fig. 1. Fluorescence images of A431 cells stained with antibody to E-cadherin. Fluorescence images at every 2 μm from the cell base (0) to the surface (16) are presented. Bar: 50 μm.

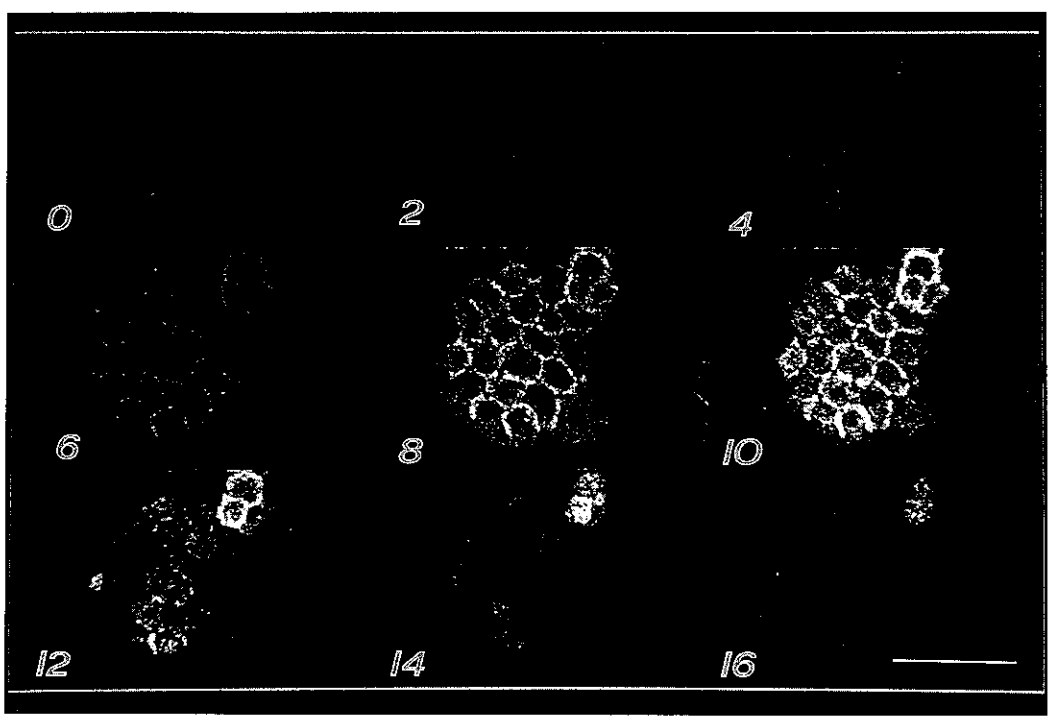

Fig. 2. Fluorescence images of A431 cells stained with antibody to EGF receptor. Fluorescence images at every 2 μm from the cell base (0) to the surface (16) are presented. Bar: 50 μm.

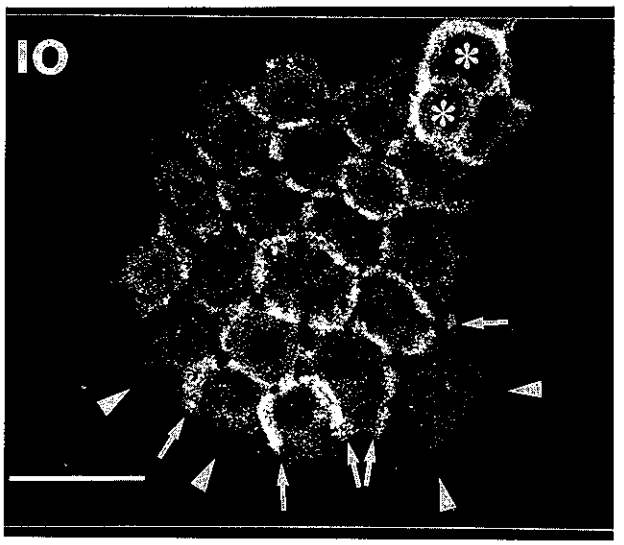

Fig. 3. Higher magnification of the fluorescence images of EGF receptor distribution. The 6th frame (10 μm) of Fig. 2 is shown at higher magnification. Arrowheads indicate edges of cell surface which are free from cell attachment. Arrows indicate the distinct fluorescence signals for EGF receptors at cell-cell attachment sites. These marks are shown only for representative cells. Asterisks indicate the mitotic cells. Bar: 50 μm.

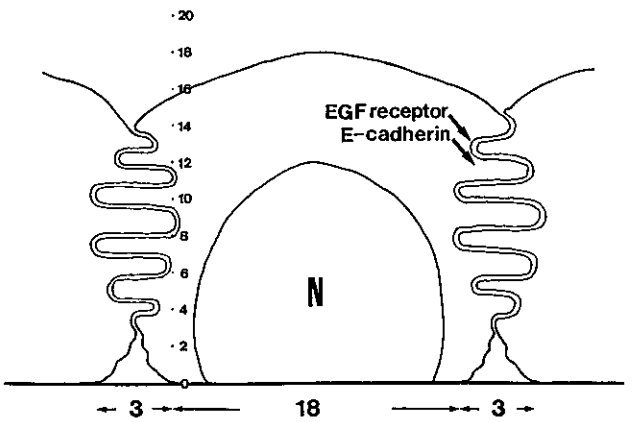

Fig. 4. A schematic view of the localization of EGF receptors and E-cadherins. Numbers indicate apparent distances in μm. Wavy lines indicate the basolateral membrane. N: nucleus.

localization of EGF receptors in A431 cells using immunoelectron microscopy. However, he only noted the receptor localization as "a high number of gold particles on the cell surface, especially on membrane folds."[6] This initial observation was confirmed by the later findings that EGF receptors are condensed in the basolateral membrane of a renal epithelial cell line LLC-PK_1[7] and small intestinal enterocytes.[8] In these cells, EGF stimulated cell proliferation only when it was provided through the basolateral membrane. These studies suggested that EGF receptors may be co-localized with cell adhesion molecules. We thus used the laser scanning fluorescence microscope to examine the localization of EGF receptors and E-cadherins perpendicularly from the cell base to the surface. The distribution of fluorescence signals at every 2 μm was very similar for these two molecules. The addition of 50 m*M* EGTA into A431 cell culture resulted in a diffuse fluorescence signal over the entire cell surface for both (data not shown). These results support the previous reports on the subcellular localization of EGF receptors.[6-8] A possible scheme is shown in Fig. 4 by reconstructing the fluorescence images based on the tomographical images obtained by electron microscopic observation.[6]

Insulin receptors are localized in the basolateral region of canine intestinal entrocytes.[13] Our preliminary data indicated that the c-*erb*B-2 gene products and transferrin receptors are also localized on the basolateral membrane (data not shown). The precursor proteins of TGF-α modulate cell-cell communication.[14] Thus, the localization of receptors to cell-cell attachment sites may take a significant role in the physiological functions of polypeptide hormones and growth factors.

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