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Redistribution of cytosolic FGFR1 after induced migration of urothelial cells in culture

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Abstract

The distribution of cytosolic fibroblast growth factor receptor 1 (FGFR1) was studied in correlation to cell migration in urothelial cell line g/G. Cell motility was analysed with a new method using consecutive series of photographs of cells relocated on CELLocate coverslips and with image analysis software. The results confirmed that FGF1 stimulated cell motility only when cells were grown on collagen I coating. During the transition from sessile to motile cell phenotype a complete redistribution of cytosolic FGFR1 was revealed. In sessile cells, FGFR1 had a filamentous distribution and its location matched cytokeratin 7. In cells of the migrating phenotype, the distribution of FGFR1 was diffuse, mainly located in cytosol. Our data reveal that the location of cytosolic FGFR1 depends on the motile characteristics of the cell. The results also indicate that attachment of cells to collagen I is crucial for the induction of urothelial cell motility with FGF1.

Keywords: FGF1; FGF receptor 1; Cytokeratins; Collagen; Cell motility

1. Introduction

Fibroblast growth factors exert their effect on cells through their mainly membrane-bound receptors. Receptors for fibroblast growth factors belong to a family of structurally related tyrosine-kinase receptors. The FGF receptors are encoded by four genes (FGFR1–FGFR4), and several splice variants have been identified (Johnson et al., 1990; Dickson et al., 2000). All FGFRs respond to FGF1, which appears to be a universal FGFR ligand (Ornitz et al., 1996). These receptors are glycoproteins composed of two or three extracellular immunoglobulin (Ig)-like domains, a hydrophobic transmembrane domain and a cytoplasmic region that contains the tyrosine-kinase domain. Binding the members of the fibroblast growth factor family induces receptor dimerization, resulting in autophosphorylation of the kinase domain and phosphorylation of the effector signalling proteins (Hughes, 1997). FGFR1 differs from other FGF receptors for their unstable connection to membranes, due to an atypical transmembrane domain (Myers et al., 2003). A high percentage of FGFR1 molecules become released from the endoplasmic reticulum into the cytosol. There they bind the cytosolic FGF1 and the complexes of receptors and FGFs are further translocated to the cell nucleus, where they activate multiple genes (Stachowiak et al., 2003). Those FGFR1 molecules which remain attached to membranes of the endo-membrane system continue their transport to the plasma membrane, where they can be activated by binding extracellular FGF1. FGF1 may thus act in a dual mode, i.e., by interaction with cell–surface receptors and with cytosolic receptors (Wiedlocha et al., 1994).

On binding of extracellular FGFs to receptors on the plasma membrane, signal transduction pathways, including MAPK and phospholipase C, are activated (Mason, 1994). The consequence is a change in cell shape, the distribution of actin filaments and enhanced cell motility. A discoid or polygonal shape is characteristic of sessile epithelial cells. A circular actin bundle is located close to the lateral border of these cells (Vasiliev, 2004). Migrating cells, on the other hand, exhibit a spindle shape with a long, extended tail (Boyer

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et al., 1989; Morton and Tchao, 1994; Bonneton et al., 1999). In the body of migrating cells, actin filaments organize as stress fibres that contribute to adhesion to the cell-substratum and to pulling the cell in a particular direction (Boyer et al., 1989; Mitchison and Cramer, 1996).

In spite of the large number of studies on the mechanism of actions of FGFRs after binding their ligands, knowledge of the location of the receptors in a cell is very obscure. The biochemical studies of Citores et al. (1999) first revealed that the majority of FGFR4 is anchored to a detergent-insoluble fraction in cells and they predicted the cytoskeletal attachment of the receptor. Until now, no data have shown whether FGFR1, for which the main location for binding its ligand is in the cytoplasm, has a similar distribution. The cytoskeletal attachment of the receptors would be especially intriguing in epithelial–mesenchymal transition after epithelial injury, during which a dramatic redistribution of the cytoskeleton takes place (Hay, 1995). In the present study, we examined the distribution of FGFR1 in an *in vitro* model of an injury to an urinary bladder epithelium. After mechanical disruption of the basement membrane, which commonly happens after surgical removal of bladder tumours, the epithelial cells come into contact with collagen I as a major component of the extracellular matrix, and also with FGF1, the concentration of which is increased at the area of epithelial injury (de Boer et al., 1994). Thus, in an injured urothelium, cells are simultaneously exposed to collagen I and FGF1 as potential stimulators of epithelial–mesenchymal transition, which is one of the crucial steps in epithelial regeneration. We examined the effect of cell migration on localisation of FGFR1. In our studies, the non-invasive mouse urothelial cell line g/G was used, which resembles normal mouse urothelial cells of the basal or intermediate layer both in terms of morphology and response to growth factors (de Boer et al., 1993). The results indicate that in sessile cells FGFR1 is located close to cytokeratins while in cells with migrating phenotype an apparent redistribution of cytoplasmic FGFR1 takes place. We additionally found that only simultaneous application of FGF1 and collagen I promotes migration of urothelial cells, while the independent use of collagen I or FGF1 did not change the velocity of migration.

2. Materials and methods

2.1. Cell culture

A routine cell culture medium consisting of a 1:1 mixture of Dulbecco's Modified Eagle's medium (DMEM) and Ham's F-12, supplemented with 10% foetal calf serum, 5 μg/ml insulin, 5 μg/ml transferrin, 100 mg/ml hydrocortisone and 5 ng/ml selenite. g/G cells (kindly donated by Dr Vermeij from the Department of Pathology, Erasmus University, NL) were incubated at 37 °C in an atmosphere of 5% CO_2. The experiments were performed in serum-free medium prepared as previously described (de Boer et al., 1993) The serum-free medium was similar to the routine medium, but without foetal calf serum (FCS), and supplemented with 0.1% bovine serum albumin, 4 μM spermine, 4 μM spermidine, 0.1 mM ethanolamine and 1 μM putrescine.

2.2. Preparation of collagen coatings

Vitrogen 100® Collagen is a sterile solution of bovine dermal collagen I (98%) and collagen III (2%), dissolved in 0.012 N HCl. The collagen concentration of Vitrogen 100 Collagen is approximately 3 mg/ml. A 10% solution of Vitrogen 100 Collagen was placed in each petri dish. After 45 min, the petri dishes were rinsed with PBS and the g/G cells were added.

2.3. Treatment with FGF1

The g/G cells were grown on CELLocate® coverslips (Eppendorf) in petri dishes (2.8 cm^2), with or without collagen coating. The cells were plated at a low density (10^3 cells/dish) in a routine medium and maintained for 3 h. The routine medium was then replaced with serum-free medium in order to minimize the effect of the serum, which contains growth factors and molecules of extracellular matrix. After 24 h, the medium was replaced again with a new serum-free medium or serum-free medium with FGF1 (20 ng/ml), in which the cells were maintained for 24 h. Analysis of cell motility and immunofluorescence were then performed.

2.4. Analysis of cell motility

2.4.1. Consecutive recording of cell positions

View areas of CELLocate coverslips with 3–6 cells were chosen and photographed with a Nikon Coolpix 950 digital camera attached to an inverted microscope. The same view area was photographed every 30 min over a period of 6 h. Exact relocation of the area was enabled by CELLocate coverslips with engraved alphanumerically labelled grids. Every 30 min, three view areas of each petri dish in the experiment were recorded. After recording, cells were fixed and prepared for immunofluorescence labelling. Each experiment was done three times and in each experiment three view areas were analysed. Thus, in each experiment the velocity of migration was measured for 36–54 cells.

2.4.2. Analysis of photographs with CorelDRAW computer software

The size of a page was set at 210 × 297 mm (A4). After the first page, 12 more pages were inserted. The net was drawn on the first page with a line drawing tool. The margins were set at 175 μm long, as in CELLocate. The net was later used as the background to measure the travelling distances. Thirteen images of each cell were placed consecutively on each of the 13 pages. A net of CELLocate coverslips on each image enabled exact overlapping with the net on the pages. The cell nuclei were chosen as the centre of gravity and were drawn as a spot on each cell on an image. The spots were marked with different colours to distinguish the cell's actual