Fibroblast Growth Factor 10 Induces Proliferation and Differentiation of Human Primary Cultured Keratinocytes

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Fibroblast growth factor 10 is a novel member of the fibroblast growth factor family, which is involved in morphogenesis and epithelial proliferation. It is highly homologous to the keratinocyte growth factor (or fibroblast growth factor 7), a key mediator of keratinocyte growth and differentiation. Both fibroblast growth factor 10 and keratinocyte growth factor bind with high affinity to the tyrosine kinase keratinocyte growth factor receptor. Here we analyzed the effect of fibroblast growth factor 10 on primary cultures of human keratinocytes, grown in chemically defined medium, and we compared the proliferative and differentiative cell responses to fibroblast growth factor 10 with those induced by keratinocyte growth factor and epidermal growth factor. Cell counting, 5-bromo-2′-deoxyuridine incorporation, and western blot analysis showed that fibroblast growth factor 10, similarly to keratinocyte growth factor, not only is a potent mitogen for human keratinocytes, but also promotes the expression of both early differentiation markers K1 and K10 and late differentiation marker filaggrin in response to the Ca^{2+} signal, and seems to sustain the proliferative activity in suprabasal stratified cells. Immunoprecipitation/western blot analysis revealed that fibroblast growth factor 10, similarly to keratinocyte growth factor, is able to induce tyrosine phosphorylation of keratinocyte growth factor receptor and of cellular substrates such as PLCγ. ***Key words: fibroblast growth factor 10/human keratinocytes/keratinocyte growth factor. J Invest Dermatol 116:623–628, 2001***

The fibroblast growth factors (FGF) constitute a family of at least 19 members and are responsible for different activities on cell proliferation and differentiation, embryonic development, angiogenesis, and wound healing (for a review, see Mason, 1994). FGF-10 is a novel member of the FGF family, whose biologic activities remain to be elucidated (Yamasaki *et al*, 1996; Emoto *et al*, 1997). It has recently been reported that FGF-10 plays a key role in controlling directional outgrowth and inducing epithelial buds in lung development (Bellusci *et al*, 1997; Park *et al*, 1998). Moreover, FGF-10, together with other members of the FGF family, appears to be essential for signaling induction during limb development (Xu *et al*, 1998). Finally, generation of FGF-10 deficient mice leads to lack of both limb and lung formation (Min *et al*, 1998; Sekine *et al*, 1999).

When compared to the other members of the FGF family, both the amino acid sequence and the tissue expression of FGF-10 show high similarity to those of the keratinocyte growth factor (KGF/FGF-7). Unlike the other known FGF, KGF acts specifically on epithelial cells (Finch *et al*, 1989), modulating their growth and differentiation. In fact, KGF not only is a mitogen for murine (Rubin *et al*, 1989; Duglosz *et al*, 1994) and human cultured keratinocytes (Marchese *et al*, 1990), but also promotes their early differentiation program (Marchese *et al*, 1990; 1997) and inhibits their terminal differentiation and apoptosis (Hines and Allen-Hoffman, 1996). In addition, KGF seems to play a key role in reepithelialization during normal human and experimental wound healing (Werner *et al*, 1992, 1994; Staiano-Coico *et al*, 1993; Marchese *et al*, 1995). Similarly to KGF, FGF-10 seems to exert mitogenic activity on mouse and rat keratinocyte cell lines (Emoto *et al*, 1997; Igarashi *et al*, 1998). Expression of FGF-10 does not seem to be modulated, however, during experimental wound healing (Beer *et al*, 1997), although the topical addition of the growth factor promotes wound repair in the same model (Jimenez and Rampy, 1999).

Both FGF-10 and KGF bind only and with high affinity (Igarashi *et al*, 1998) to the KGF receptor (KGFR), a splicing variant of FGF receptor 2 (FGFR2), expressed exclusively on epithelial cells (Miki *et al*, 1991, 1992). The two growth factors show a different sensitivity to heparin in their mitogenic activity, however, implying that the extracellular matrix may play a role in regulating the cellular response to FGF-10 and KGF (Igarashi *et al*, 1998).

We have recently shown that KGFR is up-modulated *in vitro* during differentiation from basal to suprabasal keratinocytes (Marchese *et al*, 1997), suggesting that receptor expression may control the proliferative–differentiative cell program, and consistent with the ability of KGF, unlike the epidermal growth factor (EGF), to induce both cell proliferation and expression of differentiation markers in response to the Ca^{2+} signal (Marchese *et al*, 1990, 1997). To ascertain if FGF-10 is able to exert activities similar to KGF on

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Abbreviations: FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; KGF, keratinocyte growth factor; KGFR, keratinocyte growth factor receptor.

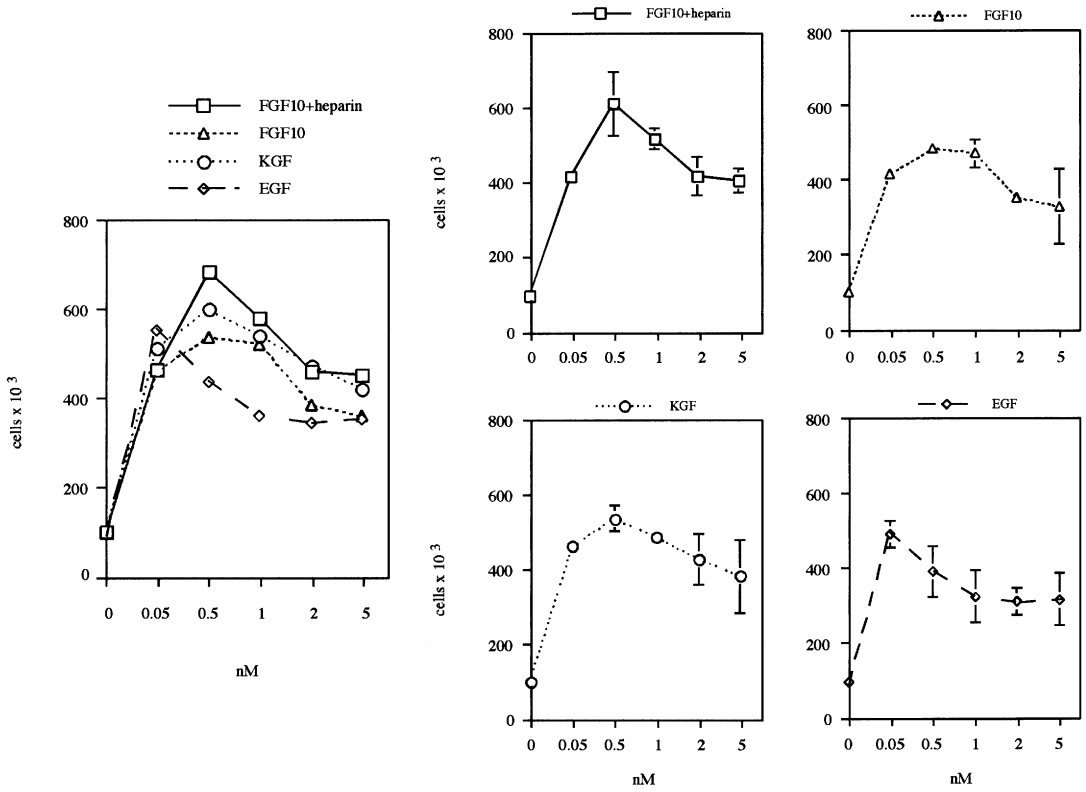

Figure 1. Comparison of the proliferative activities of FGF-10 (in the presence or absence of 0.3 μg per ml heparin), KGF, and EGF on human primary cultured keratinocytes grown in standard low Ca^{2+} medium. Treatments and cell counts were performed as described in *Materials* and *Methods*. The results represent the mean values from three different experiments.

primary cultures of human keratinocytes, we compared here the proliferative and differentiative cell response to FGF-10, KGF, and EGF. Our results show that FGF-10 is a potent mitogen for human keratinocytes. In addition, similarly to KGF and differently from EGF, FGF-10 allows the expression of the early suprabasal differentiation markers K1 and K10 and of the late differentiation marker filaggrin, promoting therefore the initiation and progression of the keratinocyte maturation program.

MATERIALS AND METHODS

Human keratinocyte culture Primary cultures of human keratinocytes derived from neonatal foreskin as previously described (Rheinwald and Green, 1977) were maintained in MCDB 153 medium (Clonetics, San Diego, CA), supplemented with 0.5 μg per ml hydrocortisone, 5 μg per ml insulin, 5 μg per ml gentamycin, and 70 μg per ml whole bovine pituitary extract. Ca concentration in the medium was maintained at 0.15 mM (Boyce and Ham, 1983). Cell adhesion was achieved by precoating the tissue culture 24-well plates used for the proliferation assay with 1 μg per ml of fibronectin (Sigma Chemical, St. Louis, MO). Cultures were supplemented with recombinant human FGF-10 (Igarashi *et al*, 1998) (0.05–5 mM, 1–100 ng per ml), with EGF (0.05–5 mM, 3–300 ng per ml; Collaborative Research, Lexington, MA), or with KGF (0.05–5 mM, 1.4–140 ng per ml; Upstate Biotechnology, Lake Placid, NY). To measure cell number, cultures were harvested by incubation in 0.5% trypsin, 0.2% ethylenediamine tetraacetic acid (EDTA) for 15 min at 37°C, and the cell counts were performed in triplicate using a hemocytometer.

5-Bromo-2′-deoxyuridine (BrdU) incorporation and immunofluorescence Cells, grown on tissue culture chamber slides (Nunc, Naperville, IL), were treated for 3 d with each growth factor at a concentration of 20 ng per ml. At the end of treatment, 100 μM BrdU (Sigma) was added to the medium and the cultures were incubated for 24 h at 37°C to allow BrdU incorporation. Cells were then fixed in 4% formaldehyde in phosphate-buffered saline (PBS) for 30 min at 25°C, followed by treatment with 0.1 M glycin for 20 min at 25°C and with 0.5% HCl, 0.1% Triton X-100 for an additional 45 min at 25°C to allow permeabilization. After extensive washing in PBS, cells were buffered with 0.1 M $Na_2B_4O_7$ and incubated with anti-BrdU monoclonal antibody (Sigma) (1:50 in blocking buffer: PBS 0.5% bovine serum albumin, 0.5% Tween 20) for 30 min at 25°C in a humidity chamber, followed by goat antimouse IgG-Texas Red (1:50 in blocking buffer) (Jackson Immuno Research Laboratories, PA). For double immunofluorescence, cells were incubated with anti-BrdU as above and with anti-K1 polyclonal antibodies (Berkeley Antibody, Richmond, CA) (1:100 in PBS) visualized with goat antimouse IgG-fluorescein isothiocyanate (1:10 in PBS) (Cappel Research Products, Durham, NC) and goat antirabbit IgG-Texas Red (1:50 in PBS) (Jackson Immuno Research Laboratories) after appropriate washing with PBS. Colocalization of the two fluorescence signals was analyzed by recording and merging single stained images using a cooled CCD color digital camera SPOT-2 (Diagnostic Instruments, MI) and FISH 2000/H1 software (Delta Sistemi, Roma, Italy).

Western blot analysis Cells cultured for 6 d in medium containing 1 mM Ca^{2+} concentration and supplemented with 20 ng per ml FGF-10, KGF, or EGF were lyzed in RIPA buffer [10 mM Tris, pH 7.4; 50 mM NaCl; 1 mM EDTA; 10 mM KCl; 1% Nonidet P-40; 0.1% sodium dodecyl sulfate (SDS); 0.05% Tween 20] supplemented with protease inhibitors [10 μg per ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg per ml leupeptin]. Twenty-five micrograms of total proteins were resolved under reducing conditions by 10% SDS polyacrylamide gel electrophoresis (PAGE) and transferred to reinforced nitrocellulose (PROTRAN, Schleider & Schuell, Keene, NH). The membrane was blocked with 3% nonfat dry milk in PBS with 0.05% Tween 20 for 4 h at room temperature. After washing for 20 min in PBS 0.05% Tween 20, the membrane was incubated with anti-K1 polyclonal antibody diluted 1:500 (Berkeley Antibody), with anti-K10/13 mouse monoclonal antibody diluted 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA), and with antifilaggrin monoclonal antibody diluted 1:500 (Biomedical Technologies, Stoughton, MA) for 1 h at 25°C, followed by enhanced chemiluminescence (ECL) detection (Amersham, Arlington Heights, IL).

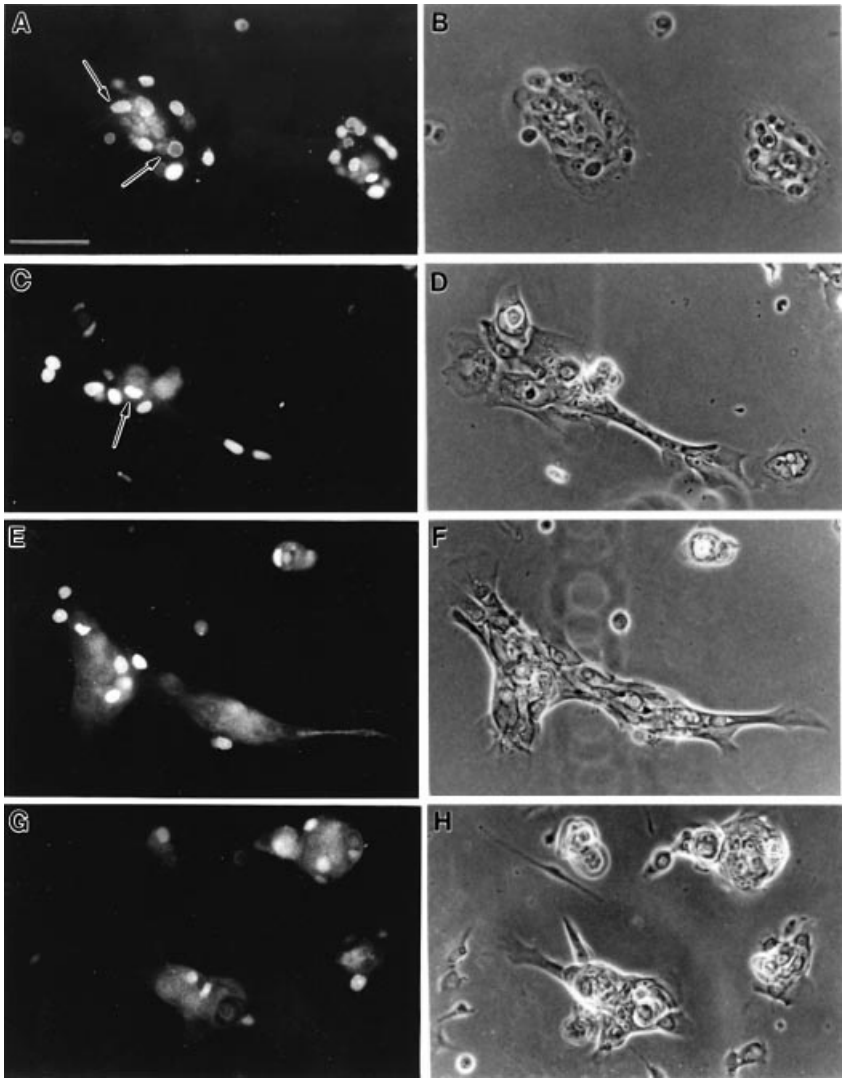

Figure 2. BrdU incorporation in keratinocyte cultures grown in standard low Ca^{2+} medium and supplemented with FGF-10, KGF, EGF, or kept in medium alone. Immunofluorescence with anti-BrdU antibodies reveals that in FGF-10-treated and in KGF-treated cultures most of the cell nuclei were positively stained, compared with a lower number in EGF-treated cells and the minority in control untreated cells. In both FGF-10- (*A*, *B*) and KGF-supplemented (*C*, *D*) cultures, but not in EGF-treated (*E*, *F*) or untreated (*G*, *H*) cultures, BrdU staining was not confined to basal cell nuclei but appeared also on suprabasal cells (*A*, *C*, *arrows*), as shown in parallel phase contrast images (*B*, *D*). *Scale bar*: 10 μm.

Immunoprecipitation and western blot analysis Subconfluent cultures of NIH3T3 KGFR transfectants were lyzed in RIPA buffer (10 mM Tris pH 7.4; 50 mM NaCl; 1 mM EDTA; 10 mM KCl; 1% Nonidet P-40; 0.1% SDS; 0.05% Tween 20) supplemented with protease inhibitors (10 μg per ml aprotinin, 1 mM PMSF, 10 μg per ml leupeptin) and phosphatase inhibitors (25 mM sodium orthovanadate, 20 mM sodium pyrophosphate, 0.5 M NaF). Fifteen micrograms of total proteins were resolved under reducing conditions by 7% SDS-PAGE and transferred to reinforced nitrocellulose (BA-S 83, Schleider & Schuell). The membrane was blocked with 5% nonfat dry milk in PBS with 0.1% Tween 20 for 4 h at room temperature. After washing for 20 min in PBS 0.1% Tween 20 the membrane was incubated alternatively with rabbit anti-Bek polyclonal antibodies, diluted 1:200 (C-17, Santa Cruz Biotechnology), or with mouse anti-Bek monoclonal antibody, diluted 1:200 (C-8, Santa Cruz Biotechnology), both antibodies raised against the carboxy terminal region of the intracellular domain of the FGFR2/KGFR, for 1 h at 25°C, followed by ECL detection (Amersham). For the detection of KGFR tyrosine phosphorylation, NIH3T3 KGFR cells were serum starved for 12 h, treated with 100 ng per ml KGF 0.3 M NaCl or with 100 ng per ml FGF-10 for 10 min at 37°C, lyzed, and processed as above for immunoblotting with antiphosphotyrosine monoclonal antibody diluted 1:1000 (Upstate Biotechnology). For immunoprecipitation/immunoblotting experiments, cell were lyzed in 1% Triton X100, 50 mM HEPES buffer containing 150 mM NaCl, 1% glycerol, 1.5 mM $MgCl_2$, 5 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, and protease and phosphatase inhibitors as above. A hundred micrograms of total protein were immunoprecipitated with 4 μg per ml anti-Bek antibodies. Immunocomplexes aggregated with 50 μl of γ bind Protein-G Sepharose (Pharmacia, Uppsala, Sweden) were washed four times with 0.6 ml of buffer. The pellets were boiled in Laemmli buffer for 5 min and proteins were resolved under reducing conditions by 10% SDS-PAGE and transferred to reinforced nitrocellulose (PROTRAN, Schleider & Schuell). The membrane was blocked with 3% bovine serum albumin in PBS (0.05% Tween 20) overnight and probed for 1 h with antiphosphotyrosine antibody, followed by ECL detection.

For the detection of phospholipase Cγ (PLCγ), the nitrocellulose used for the experiment of immunoprecipitation/immunoblotting described above was stripped for 30 min at 55°C with 100 mM mercaptoethanol, 2% SDS, and 62.5 mM Tris pH 6.7, washed for 15 min in PBS 0.1% Tween 20 at room temperature, and blocked with 5% nonfat dry milk in PBS/Tween for 1 h. The membrane was then incubated with monoclonal antibody anti-PLCγ 2 μg per ml (Upstate Biotechnology) for 1 h at room temperature followed by ECL detection.

RESULTS

Proliferation of human keratinocytes in response to FGF-10, KGF, and EGF To analyze the possible mitogenic effect of FGF-10 on primary cultures of human keratinocytes and to compare the proliferative response induced by FGF-10 with that of KGF and EGF, we plated the cells at a density of 2×10^5 per well and grew them for 6 d in standard medium supplemented with varying concentrations of the growth factors. As it has recently been shown that the biologic activity of FGF-10 on a mouse keratinocyte cell line is highly dependent on heparin and that the optimal concentration of heparin required for maximal DNA synthesis is 0.3 μg per ml (Igarashi *et al*, 1998), we used this heparin concentration in our dose–response curve of FGF-10 proliferation

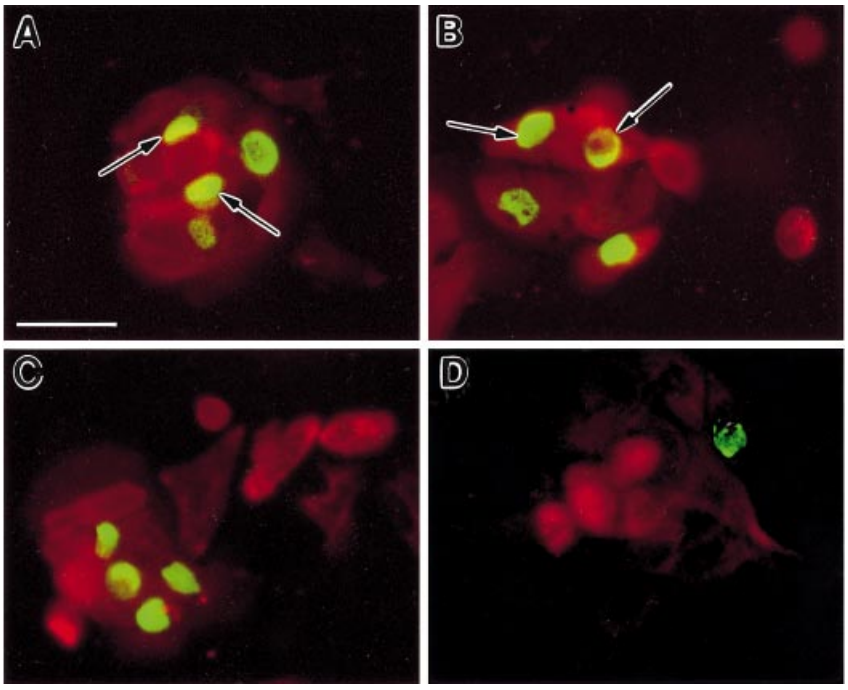

Figure 3. BrdU incorporation and K1 expression in human keratinocytes cultured in standard low Ca^{2+} medium and supplemented with FGF-10, KGF, or EGF, or kept in medium alone. Double immunofluorescence with anti-BrdU (green) and anti-K1 (red) antibodies show codistribution of the nuclear BrdU staining and the cytosolic K1 signal by overlapping single images in FGF-10- (*A*) or KGF-supplemented (*B*) cultures, but not in EGF-stimulated (*C*) or untreated control (*D*) cultures. *Arrows* in (*A*) and (*B*) point to suprabasal cells positive for BrdU and expressing K1. *Scale bar*: 40 μm.

(**Fig 1**). Addition of FGF-10 was also performed in the absence of heparin. A significant increase in cell number (approximately 5-fold) was already detectable at low concentration of the growth factor (0.05 nM) and the maximal mitogenic activity (7-fold increase) was observed at a concentration of 0.5 nM FGF-10. Comparison of the increases in cell number obtained in response to the growth factors revealed that FGF-10 induced the greatest proliferation in our culture conditions and confirmed the heparin requirement for optimal mitogenic activity (**Fig 1**). Thus FGF-10 appears to represent a novel potent mitogen for primary cultures of human keratinocytes.

To confirm the ability of FGF-10 to stimulate DNA synthesis in primary cultures of human keratinocytes and to identify the proliferating cells in the growing colonies kept at a standard low concentration of Ca^{2+} (0.15 mM), we performed a BrdU incorporation assay and observed the labeled nuclei at immunofluorescence. Cells, treated with each growth factor for 3 d, were incubated with BrdU for 24 h at 37°C and fixed in formaldehyde; incorporated BrdU was then visualized with anti-BrdU antibody followed by Texas Red-conjugated secondary antibodies. In untreated control keratinocytes, grown in standard medium and plated at low cell density, only a minority of the cells (approximately 20%) showed nuclei positively stained for BrdU (**Fig 2*G***). Parallel phase contrast microscopy revealed colonies small in size and in cell number and composed mostly of basal cells (**Fig 2*H***). When keratinocyte cultures were exposed to FGF-10 (**Fig 2*A*, *B***), KGF (**Fig 2*C*, *D***), or EGF (**Fig 2*E*, *F***) before BrdU incorporation, most of the cells (approximately 80% of FGF-10-treated cells, 70% of KGF-treated cells, and 50% of EGF-treated cells) showed intense BrdU staining in the nuclei. Parallel phase contrast observation of cultures treated with FGF-10 and KGF showed large colonies characterized by an evident increase in size and in cell number compared to the untreated cultures; in addition, in FGF-10- and KGF-treated cultures, the colonies were composed not only of basal but also of suprabasal cells (**Fig 2*B*, *D***). Moreover, in FGF-10-treated and KGF-treated cultures, BrdU staining was not confined to basal cell nuclei but appeared also on suprabasal stratified cells, superimposed on the basal ones (**Fig 2*A*, *C***, *arrows*). The differentiated nature of the suprabasal cells positive for BrdU was unequivocally demonstrated by double immunofluorescence with anti-K1 polyclonal antibodies (**Fig 3**). In control experiments, performed by omission of BrdU from the culture medium during the 24 h incubation, the cell nuclei were all negative. Thus, FGF-10 and KGF, but not EGF, appear to promote the initial stratification of keratinocytes *in vitro* and to sustain their proliferative activity also in cells located suprabasally.

Effect of FGF-10 on the expression of K1, K10, and filaggrin differentiation markers Basal keratinocytes committed to the differentiation program are known to become suprabasal and to express the early differentiation markers K1 and K10 and then the late differentiation marker filaggrin (for a review see Fuchs, 1990). EGF blocks the *in vitro* expression of K1 and filaggrin induced by high concentrations of Ca^{2+}, whereas KGF seems to promote such expression (Marchese *et al*, 1990, 1997). To analyze the effect of FGF-10, compared with KGF and EGF, on keratinocyte differentiation, we exposed keratinocyte cultures to the Ca^{2+} differentiation signal (1.0 mM Ca^{2+}) in the presence of the growth factors. Western blot analysis with anti-K1, anti-K10, and antifilaggrin antibodies showed that both the K1 and K10 early proteins and the late differentiation protein filaggrin were expressed in FGF-10 supplemented cultures as well as in KGF-treated and in control untreated cultures; in contrast, the expression of the two keratins and of filaggrin was highly inhibited by treatment with EGF, as expected (**Fig 4**) (Marchese *et al*, 1990, 1997). Thus, FGF-10, similarly to KGF, promotes the expression of both early and late differentiation markers in response to the Ca^{2+} signal.

Tyrosine phosphorylation and activation of KGFR induced by FGF-10 binding To analyze the effects of FGF-10, compared to KGF, on the phosphorylation of KGFRs, we treated KGFR transfected NIH3T3 cells with FGF-10 (100 ng per ml) or with KGF (100 ng per ml) for 10 min at 37°C. Cell lysates were immunoprecipitated with anti-Bek polyclonal antibodies, which specifically recognize the intracellular domain shared by the two splicing isoforms, FGFR2 and KGFR, followed by immunoblotting with antiphosphotyrosine monoclonal antibody. FGF-10 binding to KGFR induced phosphorylation of a protein species reacting with anti-Bek antibodies and corresponding to the molecular weight of the KGFR (**Fig 5**), as expected (Marchese *et al*, 1998), and no reactivity was detected in control nontransfected NIH3T3 cells (data not shown). Similar tyrosine phosphorylation of KGFR was induced by treatment with KGF; a weak tyrosine phosphorylation of KGFR was detected also in untreated cells (**Fig 5**), most likely as a consequence of autocrine

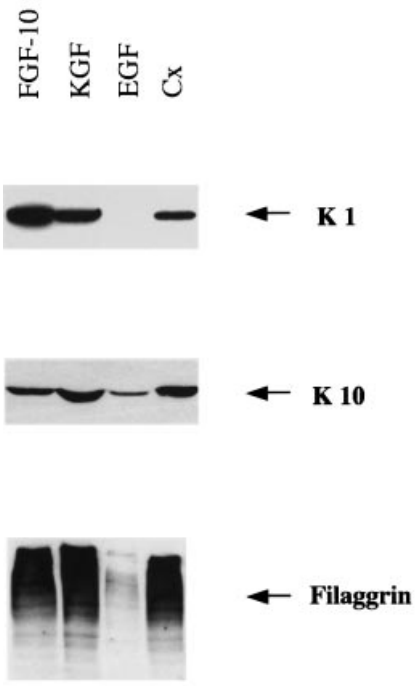

Figure 4. Western blot analysis of the expression of the keratinocyte differentiation markers K1, K10, and filaggrin in keratinocyte cultures exposed to high Ca^{2+} (1.0 mM) concentration and supplemented with FGF-10 (in the presence of heparin), KGF, and EGF. Expression of the markers induced by the Ca^{2+} differentiation signal is not blocked by FGF-10 or KGF treatment, whereas EGF contrasts such expression.

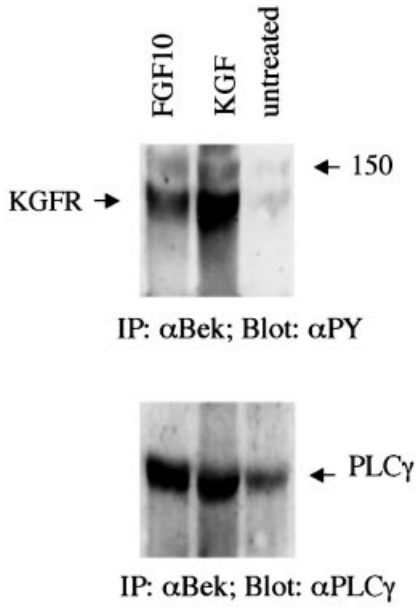

Figure 5. Tyrosine phosphorylation of KGFR and of PLCγ substrate induced by FGF-10 and KGF. Serum-starved KGFR transfected NIH3T3 cells were treated with FGF-10 (100 ng per ml) or KGF (100 ng per ml), immunoprecipitated with anti-Bek antibodies, and immunoblotted with antiphosphotyrosine antibody or with anti-PLCγ antibody after stripping and reprobing the same nitrocellulose.

receptor activation, as NIH3T3 cells are known to produce mouse KGF (Miki *et al*, 1991). The addition of heparin (0.3 μg per ml) to FGF-10 when used at a concentration of 100 ng per ml did not increase the KGFR phosphorylation (data not shown), in agreement with the similar mitogenic activity of FGF-10 at 100 ng per ml in the presence or absence of heparin, as reported by Igarashi *et al* (1998). Moreover, both FGF-10 and KGF stimulated phosphorylation of a 150 kDa protein, which coimmunoprecipitated with the activated KGFR (**Fig 5**) and which was identified, by stripping and reprobing the same nitrocellulose with anti-PLCγ antibody, as the PLCγ protein (**Fig 5**). This result confirms that PLCγ is a major substrate of the KGFR (Shaoul *et al*, 1995) and demonstrates that FGF-10, similarly to KGF, induces not only phosphorylation but also signaling by the KGFR.

DISCUSSION

The process of growth and differentiation of epidermis appears to be finely regulated by the action of several autocrine and paracrine factors with overlapping specificities and functions (Fuchs, 1990). This regulation results in both the proliferation of basal cells and the triggering of terminal differentiation. The use of human keratinocytes, which can be cultured in chemically defined medium, permits analysis in a controlled model system of the activity of single growth factors selectively added to the culture. In this study, we analyzed the effect of the newly identified FGF-10 on cultured human keratinocytes, comparing its activities with those exerted by the homolog KGF and by EGF. As KGF elicits a peculiar response on cultured keratinocytes, positively modulating both their proliferation and early differentiation, and as KGFR appears to be upmodulated during differentiation, we were interested to analyze if also FGF-10, being highly homologous to KGF and having similar receptor specificity, might induce such a response. Our results indicate that FGF-10 exerts both proliferative and differentiative activity on human keratinocytes similar to that of KGF and suggest that FGF-10 may play an important role as a positive regulator of epidermal growth and differentiation *in vivo* and *in vitro*.

The BrdU incorporation assay applied in this study allowed us to identify the proliferating cells in cultures treated with the growth factors. Interestingly, in FGF-10-treated cultures, as well as in KGF-treated cultures, keratinocytes appear to be not only triggered to become suprabasal, consistent with the expression of K1 and K10 differentiation markers, but also induced to maintain a DNA synthesis capacity when located suprabasally. This finding parallels the observation that KGFR are expressed on suprabasal differentiated cells (Marchese *et al*, 1997, 1998) and that KGF, unlike EGF, exerts a mitogenic effect on confluent cells, which are therefore committed to differentiate (Hines and Allen-Hoffman, 1996). Work is in progress to define the different functions and signaling properties of the receptors for both ligands KGF and FGF-10, when present on basal or suprabasal cells, and to elucidate the mechanism of action of the two growth factors in normal epidermal tissues as well as in hyperplastic skin diseases and during human wound healing.

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REFERENCES

Beer HD, Florence C, Dammeier J, McGuire L, Werner S, Duan DR: Mouse fibroblast growth factor 10: cDNA cloning, protein characterization, and regulation of mRNA expression. *Oncogene* 15:2211–2218, 1997

Bellusci S, Grindley J, Emoto H, Itoh N, Hogan BL: Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. *Development* 124:4867–4878, 1997

Boyce ST, Ham RG: Calcium-regulated differentiation of normal human epidermal keratinocytes in chemically defined clonal cultures and serum-free serial cultures. *J Invest Dermatol* 81:33–40, 1983

Duglosz AA, Cheng C, Denning MF, Dempsey PJ, Coffey RG, Yuspa SH:

Keratinocyte growth factor receptor ligands induce transforming growth factor α expression and activate the epidermal growth factor receptor signaling pathway in cultured epidermal keratinocytes. *Cell Growth Differ* 5:1283–1292, 1994

Emoto H, Tagashira S, Mattei MG, *et al:* Structure and expression of human fibroblast growth factor 10. *J Biol Chem* 272:23191–23194, 1997

Finch PW, Rubin JS, Miki T, Ron D, Aaronson SA: Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science (Wash DC)* 245:752–755, 1989

Fuchs E: Epidermal differentiation: the bare essential. *J Cell Biol* 111:2807–2814, 1990

Hines MD, Allen-Hoffman BL: Keratinocyte growth factor inhibits cross-linked envelope formation and nucleosomal fragmentation in cultured human keratinocytes. *J Biol Chem* 271:6245–6251, 1996

Igarashi M, Finch PW, Aaronson SA: Characterization of recombinant human fibroblast growth factor (FGF)-10 reveals functional similarities with keratinocyte growth factor (FGF)-7. *J Biol Chem* 273:13230–13235, 1998

Jimenez PA, Rampy MA: Keratinocyte growth factor-2 accelerates wound healing in incisional wounds. *J Surg Res* 81:238–242, 1999

Marchese C, Rubin JS, Ron D, *et al:* Human keratinocyte growth factor activity on proliferation and differentiation of human keratinocytes: differentiation response distinguishes KGF from EGF family. *J Cell Physiol* 144:326–333, 1990

Marchese C, Chedid M, Dirsch OR, *et al:* Modulation of keratinocyte growth factor and its receptor in reepithelializing human skin. *J Exp Med* 182:1369–1376, 1995

Marchese C, Sorice M, De Stefano C, Frati L, Torrisi MR: Modulation of keratinocyte growth factor receptor expression in human cultured keratinocytes. *Cell Growth Differ* 8:989–997, 1997

Marchese C, Mancini P, Belleudi F, *et al:* Receptor-mediated endocytosis of keratinocyte growth factor. *J Cell Sci* 111:3517–3527, 1998

Mason IJ: The ins and outs of fibroblast growth factor. *Cell* 78:547–552, 1994

Miki T, Fleming TP, Bottaro DP, Rubin JS, Ron D, Aaronson SA: Expression DNA cloning of the KGF receptor by creation of a transforming autocrine loop. *Science (Wash DC)* 251:72–75, 1991

Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AML, Aaronson SA: Determination of ligand binding specificity by alternative splicing: two distinct growth factor receptors encoded by single gene. *Proc Natl Acad Sci USA* 89:246–250, 1992

Min H, Danilenko DM, Scully SA, *et al:* FGF-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. *Genes Dev* 12:3156–3161, 1998

Park WY, Miranda B, Lebeche D, Hashimoto G, Cardoso WV: FGF-10 is a chemotactic factor for distal epithelial buds during lung development. *Dev Biol* 201:125–134, 1998

Rheinwald JG, Green H: Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. *Nature* 265:421–424, 1977

Rubin JS, Osada H, Finch PW, Taylor WG, Rudikoff S, Aaronson SA: Purification and characterization of newly identified growth factor specific for epithelial cells. *Proc Natl Acad Sci USA* 86:802–806, 1989

Sekine K, Ohuchi H, Fujiwara M, *et al:* FGF10 is essential for limb and lung formation. *Nature Gen* 21:138–141, 1999

Shaoul E, Reich-Slotky R, Berman B, Ron D: Fibroblast growth factor receptors display both common and distinct signaling pathways. *Oncogene* 10:1553–1561, 1995

Staiano-Coico L, Krueger GJ, Rubin JS, *et al:* Human keratinocyte growth factor effects in a porcine model of epidermal wound healing. *J Exp Med* 178:865–878, 1993

Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT: Large induction of keratinocyte growth factor expression in the dermis during wound healing. *Proc Natl Acad Sci USA* 89:6896–6900, 1992

Werner S, Smola HM, Xiang L, Longaker MT, Krieg T, Hofschneider PH, Williams LT: The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science* 266:819–822, 1994

Xu X, Weinstein M, Cuiling Li, *et al*: Fibroblast growth factor receptor 2 (FGFR2)-mediated reciprocal regulation loop between FGF8 and FGF10 is essential for limb induction. *Development* 125:753–765, 1998

Yamasaki M, Miyake A, Tagashira S, Itoh N: Structure and expression of the rat mRNA encoding a novel member of the fibroblast growth factor family. *J Biol Chem* 271:15918–15921, 1996