The Elongated First Fibronectin Type III Domain of Collagen XIV Is an Inducer of Quiescence and Differentiation in Fibroblasts and Preadipocytes*

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Collagen XIV (CXIV) is a fibril-associated collagen that is mainly expressed in well-differentiated tissues and in late embryonic development. Because CXIV is almost absent in proliferating and/or differentiated tissues, a functional role in maintaining cell differentiation is suspected. We demonstrate antiproliferative, quiescence- and differentiation-inducing effects of human CXIV and its recombinant fragments on mesenchymal cells. In primary human fibroblasts, in mouse 3T3 fibroblasts and in 3T3-L1 preadipocytes, CXIV reduced de novo DNA synthesis by 75%, whereas cell numbers and viability remained unaltered. Cells showed no signs of apoptosis, and maximal proliferation was restored when serum was supplemented, thus indicating that CXIV induced reversible cell quiescence. Exposure of fibroblasts to CXIV in vitro led to cellular bundles and clusters. CXIV also triggered trans-differentiation of 3T3-L1 preadipocytes into adipocytes, as could be shown by lipid accumulation and by expression of the glucose transporter Glut4. These effects were also observed with the amino-terminal recombinant fragment Gln29-Pro154 that harbors the first fibronectin type III domain and a 39-amino-acid extension, whereas no activity was found for all other recombinant CXIV fragments. Based on these finding the development of small molecular analogs that modulate fibroblast cell growth and differentiation, e.g. in wound healing and fibrosis, seems feasible.

The extracellular matrix forms a complex molecular network that determines the specific histarchitecture of tissues, providing cells with a scaffold and with signals that direct polarization, migration, proliferation, survival, and differentiation. Most of the structural extracellular matrix molecules, such as collagens, non-collagenous glycoproteins, and proteoglycans are multidomain proteins exerting different biological functions. CXIV belongs to the group of fibril-associated collagens with interrupted triple helices. It is mainly found in well differentiated, collagen I (CI)-rich tissues, which suggests a role of CXIV in collagen fibril organization (1, 2). CXIV is produced by fibroblasts, myofibroblasts, and hepatic stellate cells and is expressed late in embryonic development, whereas it is virtually absent in proliferating tissues like tumor stroma, indicating a possible function in induction of cellular quiescence and differentiation (3–6). CXIV is expressed as a homotrimeric protein of ~650 kDa and contains a variety of structural and functional domains. Each chain consists of the two short collagenous helices COL1 (Gly1469-Leu1620) and COL2 (Gly1664-Pro1786) and of three non-collagenous domains (see Fig. 1, 4, 7–13)), whereas NC3 contains 7.25 fibronectin type III (FN-III) domains typically consisting of ~90 amino acid residues. Beside its specific interactions with other extracellular matrix molecules such as collagens type I and VI, CXIV displays numerous cell binding properties. The cell adhesion site for fibroblasts was identified within a stretch of 154 amino acids (Gln29-Pro154) including the first FN-III repeat (14). Furthermore, a not fully characterized amino-terminal 16-kDa fragment of CXIV isolated from rat granulation tissue was shown to be chemoattractive (15), underlining the functional potential of the amino-terminal domain. Because CXIV is almost exclusively found in differentiated tissues, we investigated its effects on proliferation and differentiation in different fibroblast populations, including preadipocytes, the latter being a well established model to study cell differentiation (16). We could show that human CXIV and in particular its elongated first FN-III domain comprising amino acids 29–154 are potent inducers of cellular quiescence and differentiation.

**MATERIALS AND METHODS**

**Cell Lines and Reagents**—Mouse embryonic fibroblasts (CCL-92) and 3T3-L1 preadipocytes (CL-173) were purchased from the American Type Culture Collection (Manassas, VA). Human fibroblasts (HF) were isolated from human foreskin tissue and used up to the eighth passage. If not noted otherwise, all reagents were obtained from Merck or Sigma and were of the highest purity available.

**Cell Culture**—Cells were cultured in a humidified atmosphere at 37 °C and 5% CO2. Standard culture medium consisted of Dulbecco’s modified Eagle’s medium with 862 mg/liter L-alanyl-L-glutamine, 4.5 g/liter glucose (Invitrogen), 50 μg/ml streptomycin, 50 units/ml penicillin, 50 μg/ml l-ascorbic acid (Biochrom, Berlin, Germany), supplemented with 10% heat-inactivated fetal calf serum (FCS, Invitrogen). Cell layers were detached with 0.05% trypsin, 0.02% EDTA solution (Biochrom). Experiments were performed in 96- and 24-well tissue culture plates with flat bottom (BD Biosciences). To ensure homogenous adherence, cells were spun down to the bottom of the plates for 10 min at 1,000 × g 6-well tissue
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FIGURE 1. Domain structure of human CXIV and recombinant CXIV fragments. CXIV consists of three identical chains of 210–220 kDa. The short collagen domains COL1 and COL2 are interrupted by two short non-collagenous sequences NC7 and NC2. The large NC3 domain comprises two von Willebrand factor A (VWF)-repeats, 7.25 fibronectin type III modules and the unrelated NC4 region (9, 18). Overlapping CXIV fragments were produced as GST fusion proteins and are specified by their amino- and carboxyl-terminal amino acid residues.

Secretion of CXIV from Human Placenta—Collagens were purified as described previously with modifications (14, 17, 18). Briefly, all procedures were carried out at 4 °C, and all centrifugation steps were conducted at 11,000 × g for 30 min. Freshly collected human placentas were washed, homogenized, and preextracted in buffer A (50 mM Tris, 1% Triton X-100, 0.05% sodium azide, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 2 mM N-ethylmaleimide, 4.5 mM NaCl, pH 7.4). After the first centrifugation, insolubles were harvested in the same buffer containing 0.5 mM NaCl. For isolation of CXIV, NaCl was added to the cleared extract to a final concentration of 4.5 mM. After 24 h, the resulting precipitate was dissolved in 50 mM Tris, 2 mM urea, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 2 mM N-ethylmaleimide, and 90 mM NaCl, pH 7.4 (buffer B) and incubated with DEAE-cellulose (Whatman, Maidstone, UK) for 10 h. Unbound material was diluted with buffer B containing no NaCl to a final concentration of 70 mM NaCl and again treated with DEAE-cellulose for 10 h. Bound CXIV was eluted with buffer B containing 300 mM NaCl. Low molecular weight contaminants were removed by ultrafiltration with a molecular size exclusion of 300 kDa (Amicon, Witten, Germany). The final protein solution with a concentration of 200–300 μg/ml was stored in aliquots at −20 °C. CI was isolated by pepsin digestion, fractional salt precipitation in acidic and neutral buffers, followed by ion exchange and molecular sieve chromatography as described (19–21). Purified CI was lyophilized and was redissolved in 150 mM acetic acid at a concentration of 2 mg/ml. Purity >90% of all collagen preparations was confirmed by non-reducing and reducing SDS-PAGE.

Recombinant CXIV Fragments—Sequences spanning the coding regions of the following non-collagenous domains of CXIV were amplified from human placental cDNA by polymerase chain reaction (18): Gln29–Phe115, Gln29–Pro154, Pro67–Val380, Ser336–Val395, Ile627–Lys1010, Ala1008–Lys1257, and Phe1120–Pro1462. All PCR products were cloned into pGEX-2T (Pharmacia Biotech, Freiburg, Germany), which introduces the DNA coding for a 26-kDa fragment of glutathione S-transferase (GST). Fusion proteins were expressed in Escherichia coli MN522 (Invitrogen), purified by affinity chromatography using glutathione-Sepharose 4B (Pharmacia Biotech), characterized as to their correct size and purity >90%, and stored in 50 mM Tris-HCl, pH 8.0, with 5 mM glutathione.

Immobilization of Collagens and Recombinant CXIV Fragments—CXIV and CI at different concentrations were immobilized to tissue culture plates for 1 h at 37 °C or overnight at 4 °C. Remaining binding sites were blocked with 0.1% bovine serum albumin in phosphate-buffered saline (PBS) for 1 h at 37 °C. Coating efficiencies were determined as described before (22, 23). Before adding cells, wells were washed three times with PBS.

Cell Proliferation as Determined by [3H]Thymidine Uptake—5 × 103 cells from preconfluent cultures were transferred to extracellular matrix protein-coated or uncoated 96-well tissue culture plates. In experiments with dissolved recombinant CXIV fragments, the standard culture medium was replaced with the appropriate assay medium 2 h after seeding. These cultures were incubated for additional 6 h before 5–10 μg/well of the respective fusion protein were added. After 20 h of incubation all cultures received 0.5 μCi/well [6-3H]thymidine with a specific activity of 5 mCi/mmol (Amersham Biosciences) for 4 h. The cell culture supernatants were discarded, and cells were fixed with 200 μl of 10% trichloroacetic acid for 30 min. Samples were lysed and subjected to partial DNA hydrolysis with 50 μl of 200 mM NaOH, neutralized with 50 μl of 800 mM HCl, and collected onto a glass fiber filter (Skatron, Lier, Norway). [3H]Thymidine incorporated into DNA was determined for 3 min using β-scintillation (LKB Wallac Turku, Finland).

Cell Number Determination by Sulforhodamine B Staining—To determine cell numbers, samples were compared with a standard curve of 6.25 × 102–2 × 105 cells of the respective cell line that were allowed to adhere for 2 h. A colorimetric assay with sulforhodamine B, specifically staining globular proteins was performed as described before (24). In brief, adherent cells were fixed with 10% trichloroacetic acid for 30 min and dried at room temperature before adding 0.4% sulforhodamine B in 1% acetic acid for 30 min. After a final wash with 1% acetic acid and microscopic inspection of the dried cell layer, bound sulforhodamine B was eluted (5 min) with 10 mM Tris base. Extinctions were measured using an ELISA-Reader (Dynatech Laboratories) at a wavelength of 560 nm.

Quantification of Apoptosis by Flow Cytometry—105 3T3 cells were incubated in a 6-well tissue culture plate for 24 or 72 h, detached mechanically and washed with PBS. Cells were fixed with 70% ethanol at −20 °C for 30 min, incubated in 500 μl of PBS containing 250 mg/liter RNase A at 37 °C for 30 min and stained with 50 μg of propidium iodine (ICN, Costa Mesa, CA) in a final volume of 1 ml. Cellular DNA content was analyzed using a FACS Calibur device and the Cell Quest Software (BD Biosciences). Fragmented, S phase and M phase DNA was defined by its average size.

Trans-differentiation of 3T3-L1 Preadipocytes—Trans-differentiation of 3T3-L1 fibroblasts to adipocytes was induced with slight modifications as described before (16). Transwell inserts (polyethylene/ cellulosic acetate, 0.4-μm mesh; Corning, Schiphol-Rijk, The Netherlands) were coated overnight at 4 °C with 3 μg of CXIV or CI/well in 100 μl of PBS. After a wash with 200 μl of PBS, 5 × 103 3T3-L1 cells in 100 μl of culture medium were added to each transwell.

To induce differentiation with soluble recombinant CXIV fragments, 3 μg of the respective GST fusion protein were added and renewed with every medium exchange. For control experiments medium was replaced by standard culture medium containing 1.7 μM insulin, 1 μM dexamethasone, and 500 μM methylxanthine at day 2 after confluence (positive control). Culture medium was completely exchanged every second day.

Lipid Droplet Staining with Oil Red O—An Oil Red O solution was prepared as follows: 530 mg of Oil Red O were dissolved overnight in 150 ml of isopropanol, filtered (mixed cellulose membrane filter unit, 0.45 μm; Millipore, Molsheim, France), and 112.5 ml of bidistilled water
Proteins were separated under reducing conditions with 200 mM dithi-othreitol using 12% SDS-PAGE and blotted (55 min, 10 °C, 300 mA) to a polyvinylidene fluoride membrane using a wet blotting device (Amer-sham Biosciences). Transfer efficiency was checked by reversible protein stain of the membrane (MemcodeTM; Pierce). After blocking unpecific binding sites with 5% skim milk powder in washing buffer (0.1% Tween 20 in PBS, 1 h), membranes were incubated with a polyclonal Glut4-specific antibody overnight at 4 °C (25). Binding of a horseradish peroxidase-coupled goat anti-rabbit secondary antibody was visualized using a non-radioactive detection system (ECLTM; Amer-sham Biosciences).

Statistical Analysis—For statistical analysis Sigma Stat software (Jandel Scientific, San Rafael, CA) was used. One-way analysis of variance was performed with the Tukey test. \( p \leq 0.05 \) was considered to be significant.

RESULTS

CXIV Inhibits Fibroblast Proliferation—To test whether native CXIV affects cell proliferation, DNA synthesis of human HF and mouse 3T3 embryonic fibroblasts was determined after 24 h of culture on 3 μg/well immobilized CXIV. In comparison to culture on immobilized CI, CXIV reduced \([\text{H}]\)thymidine incorporation to 30 and 40%, respectively (Fig. 2A). Significant reduction of DNA synthesis was shown for 0.5–3 μg/well immobilized CXIV.

The Elongated First FN-III Domain Harbors the Antiproliferative Site in CXIV—HF and 3T3 cells were incubated in the presence of 1 μg/well of the individual immobilized recombinant CXIV fragments that cover the entire non-collagenous domain NC3 of CXIV. A clear and strong antiproliferative effect was only seen with the elongated first FN-III domain Gln29-Pro154, because \([\text{H}]\)thymidine uptake was dramatically decreased by 75% in HF and by 91% in 3T3 cells when compared with the GST control, which itself had no effect on proliferation (Fig. 2B). All other fragments, including fragment Gln29-Phe115 that lacks the last 39 amino acid residues of the elongated first FN-III domain Gln29-Pro154, caused no or only a modest reduction of DNA synthesis, between 10 and 35% of control, except for fragment Pro478-Val580, which slightly stim-u-lated DNA synthesis in both cell lines.

Soluble Gln29-Pro154 Enhances the Antiproliferative Effect of Immobi-lized Native CXIV—To assess the impact of the spatial presentation of CXIV fragments Gln29-Pro154 and Pro478-Val580 on their anti- or pro-proliferative effects, recombinant fragments were added to HF and 3T3 cells cultured on immobilized native CI or CXIV. As found with the immobilized fragment, soluble Gln29-Pro154 strongly reduced, whereas Pro478-Val580 slightly increased DNA synthesis of both cell types (Fig. 3). In 3T3 cells the combination of immobilized CXIV and soluble Gln29-Pro154 led to an additive reduction of proliferation, whereas this effect was not observed in HF (Fig. 3, black bars).

Effect of Native CXIV and Soluble CXIV Gln29-Pro154 on DNA Synthesis of 3T3-L1 Preadipocytes—3T3-L1 preadipocytes are a well estab-lished model to study mesenchymal cell differentiation. Soluble frag-ment Gln29-Pro154 reduced DNA synthesis of 3T3-L1 cells by 45%,
whereas immobilized Gln\textsuperscript{29}-Pro\textsuperscript{154}, native CXIV, and CI had no significant effect (Fig. 4A). However, 3T3-L1 cell DNA synthesis was reduced significantly on immobilized CXIV compared with the CI control in the presence of 10\% FCS. 

**Antiproliferative Effects of CXIV Are Not due to Toxicity or Apoptosis and Are Fully Reversible**—To eliminate the possibility that CXIV or fragment Gln\textsuperscript{29}-Pro\textsuperscript{154} are toxic to cells, viability, apoptosis, cell cycle, and restimulation experiments with high FCS concentrations were performed (Fig. 5). Compared with the respective controls, cell numbers on immobilized CXIV or the recombinant CXIV fragments Gln\textsuperscript{29}-Phe\textsuperscript{115} and Pro\textsuperscript{78}-Val\textsuperscript{160} were not changed (Fig. 5A), whereas immobilized fragment Gln\textsuperscript{29}-Pro\textsuperscript{154} slightly reduced HF cell numbers. These effects were also demonstrated for the 3T3 fibroblasts, and the results were confirmed by a fluorometric assay measuring the intracellular esterase activity by the enzymatic conversion of 4-methylumbelliferyl heptanoate (data not shown). Thus, the presence of CXIV does not contribute and fragment Gln\textsuperscript{29}-Pro\textsuperscript{154} contributes only minimally to the reduced DNA synthesis observed for HF and 3T3 cells. DNA synthesis of HF that were precultured on immobilized CXIV or recombinant CXIV fragments under conditions of reduced serum (0.5\% FCS) could be rescued by the addition of 10\% FCS (Fig. 5B). This effect was independent of the concentration of CXIV or CXIV fragments used prior to the addition of 10\% FCS, demonstrating that CXIV and fragment Gln\textsuperscript{29}-Pro\textsuperscript{154} induced quiescence but not cell necrosis or apoptosis. In addition, cell cycle analysis using flow cytometry showed no relevant changes in the relative amount of apoptotic 3T3 cells after culture on immobilized CXIV for 24 or 72 h (Fig. 5C).

**Immobilized CXIV Induces Markers of Differentiation in 3T3 Embryonic Fibroblasts and 3T3-L1 Preadipocytes**—To address the question of whether the reduced proliferation was due to the onset of cellular differentiation processes, growth patterns, lipid accumulation, and Glut4-expression were investigated after treatment with immobilized CXIV and recombinant fragment Gln\textsuperscript{29}-Pro\textsuperscript{154}. When grown on CI, 3T3 embryonic fibroblasts were homogenously distributed during a culture period of up to 44 h (not shown). In contrast, cells cultured on immobilized CXIV for 20–44 h progressively formed ring-shaped complexes (Fig. 6).

In 3T3-L1 adipocytes, immobilized CXIV and soluble fragment Gln\textsuperscript{29}-Pro\textsuperscript{154} were strong inducers of lipid droplet synthesis in 23 and 11\% of cells, respectively, when compared with 45\% observed with the classical inducer mixture containing insulin, dexamethasone, and isobutylmethylxanthine (Fig. 7). Cells grown on non-treated surfaces, on CI, or in presence of GST alone showed no lipid droplet synthesis.

During differentiation of 3T3-L1 cells, lipid droplet synthesis is accompanied by the up-regulation of the glucose transporter Glut4. Therefore, Glut4 protein and Glut4-mediated glucose transport were investigated after the culture of 3T3-L1 preadipocytes in the presence of CXIV (Fig. 8). To directly determine Glut4 protein expression after exposure to immobilized CXIV, soluble fragment Gln\textsuperscript{29}-Pro\textsuperscript{154}, or to the hormone mixture, 3T3-L1 cytosolic fractions were subjected to Western blot analysis (Fig. 8A). Untreated cells showed low base-line expression of Glut4, which was slightly up-regulated by immobilized CXIV alone and almost comparable to the hormone mixture positive control after the addition of the soluble fragment Gln\textsuperscript{29}-Pro\textsuperscript{154} to CXIV-treated cells. Glucose transport was similar in control cells incubated with insulin or cytochalasin B (data not shown) confirming the absence of Glut4 expression. In cells grown on CXIV prior to the addition of 1.7 \mu M insulin, a distinct glucose uptake was induced that reached \textasciitilde65\% of the cells pretreated with the hormone mixture serving as positive control, indicating the induction of Glut4 (Fig. 8B). The specificity of this observation was confirmed because, irrespective of pretreatment, the trans-
port rate in the presence of insulin was reduced to 50% by 15-4/H9262M cytochalasin B, which inhibits Glut4-mediated glucose transport.

**DISCUSSION**

In this study we investigated in vitro effects of human CXIV, an extracellular matrix molecule that is expressed in differentiated mesenchymal tissue, and recombinant CXIV fragments on fibroblast and preadipocyte proliferation, apoptosis, and differentiation. We showed that immobilized native CXIV, and in particular its elongated FN-III domain Gln29-Pro154, induced quiescence and differentiation in human skin fibroblasts and mouse 3T3 cells, which was not associated with apoptosis, because proliferation could be reinduced upon the addition of 10% FCS. Although the CXIV fragment Pro478-Val580 reproducibly had a minor growth-promoting effect, all other recombinant CXIV fragments, covering the remaining 85% of the non-collagenous NC3 sequence of CXIV showed no effect. The results of these noteworthy biological effects of CXIV are in accord with published data on CXIV as a cell adhesion molecule. Thus we and others showed that the first FN-III domain and its immediate carboxyl-terminal extension (equivalent to fragment Gln29-Pro154) mediates attachment of hematopoietic and mesenchymal cells (14, 26, 27). In other reports, a 16-kDa amino-terminal fragment of rat CXIV similar to the recombinant human CXIV fragment Gln29-Pro154 used in our study was identified as a potent neu-
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![Figure 8: Effect of CXIV on Glut4 expression and [3H]glucose transport in 3T3-L1 cells. 3T3-L1 preadipocytes were cultured in the presence of immobilized CXIV, soluble Glu29-Pro134, or the hormone mixture as described for Fig. 7. A, after 5 days, cytosolic fractions of 1 x 10^6 detached 3T3-L1 cells were separated by SDS-PAGE. Glut4 protein expression was detected by Western blot using a Glut4-specific antibody. B, after 9 days in culture cells were treated either with 1.7 μM insulin or with 15 μM cytochalasin B for 20–30 min, and [3H]glucose uptake was measured. One representative of three experiments is shown. Each bar represents the mean of four determinations ± S.D.](Image)

The amino-terminal domain harbors the CXIV receptor binding site(s), and it interacts with heparin and the small dermatan sulfate proteoglycan decorin (17, 26). The identified CXIV receptors are either membrane-bound heparan sulfate proteoglycans (26) and a chondroitin-dermatan sulfate variant of CD44. This CD44 variant is almost exclusively expressed on fibroblasts and hematopoietic cells (17).

It may be possible that cell attachment and the induction of quiescence are mediated by the same receptors (either the CD44 variant or heparan sulfate proteoglycans), or at least receptors that bind to the same region of Glu29-Pro134 (17, 18), thus making experimental investigations difficult because attachment is a main prerequisite for fibroblast survival in vitro. Our results, using immobilized CXIV and soluble Glu29-Pro134, point to a synergistic action in reconstituting a threedimensional CXIV signal, as occurs in vivo, and result in an optimized activation of CXIV receptor(s) for induction of quiescence and differentiation. This is supported by the finding that the two-dimensional culture conditions of in vitro monolayers of human skin fibroblasts that induce tensile stress result in the down-regulation or complete absence of cellular CXIV expression, which can be restored by transfer to a three-dimensional culture system (8, 28).

Growth pattern analysis revealed the formation of characteristic, ring-shaped cell complexes, which formed cell bundles after 44 h. Similar structures were described for cells in contact with quiescence-inducing basement membrane-like substrates, which are a mixture of poorly defined matrix and growth factors, including e.g., laminin-1, type IV collagen, and heparan sulfate proteoglycans (29, 30). The effects seen in our experiments were solely induced by CXIV, and by its elongated FN-III domain Glu29-Pro134, and contamination of our highly purified CXIV, and in particular Glu29-Pro134 by type IV collagen or other matrix components or growth factors/inhibitors could be excluded. In addition, collagen type IV has to be immobilized to induce quiescence (29, 30), unlike to fragment Glu29-Pro134, which is active in soluble form (31, 32).

Induction of quiescence is a hallmark and precondition for the onset of cellular differentiation (33), being of high biological relevance, e.g., in wound healing or in liver fibrosis (34). We therefore looked for further evidence of CXIV-induced differentiation in fibroblasts. The differentiation of 3T3-L1 preadipocytes to adipocytes is a well established cell culture model for a differentiation process (16), that resembles the reversion of an activated myofibroblast to a quiescent hepatic stellate cell or fibroblast (34). Thus, the finding that exposure of cells to native CXIV and fragment Glu29-Pro134 resulted in all features considered characteristic for adipocyte-differentiation in this cell line, e.g., lipid droplet synthesis, increased glucose transport, and Glut4 expression, provides strong evidence for the unique potential of CXIV to induce fibroblast differentiation and quiescence.

The exact signal transduction pathways that emanate from CXIV and its amino-terminal domain Glu29-Pro134 to results in fibroblast quiescence and differentiation-induction remain to be defined. So far, the only CXIV receptor identified on fibroblasts is a chondroitin sulfate variant of CD44. Its signal transduction is linked to cytoskeletal rearrangement and survival (35), involving phosphoinositol-3 kinase and protein kinase B (c-Akt) signaling. This pathway is also active in 3T3-L1 cells when exposed to the insulin-containing hormone mixture used in our experiments, resulting in lipid droplet formation, Glut4 expression, and glucose uptake (36, 37). Recently, peroxisome proliferator-activated receptor-γ up-regulation has been demonstrated to induce adipogenic transcription factors and quiescence in hepatic stellate cells, which makes it likely that peroxisome proliferator-activated receptor-γ and the CXIV-induced cellular alterations share common signal transduction pathways (34).

Taken together our results demonstrate that human CXIV and in particular its elongated first FN-III domain Glu29-Pro134 induce quiescence and differentiation in fibroblasts and preadipocytes. This may open the way for the development of CXIV-based low molecular weight analogs to induce fibroblast quiescence in biological processes as diverse as wound healing and liver fibrosis.

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