Fibroblast Growth Factor-2 Induces Lef/Tcf-dependent Transcription in Human Endothelial Cells* 

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Lef/Tcf proteins belong to a family of architectural transcription factors that control developmental processes and play an important role in oncogenesis. Classical activators of Lef/Tcf-dependent transcription comprise the Wnt family of proteins, which translocate β-catenin into the nucleus and allow the formation of transactivation-competent Lef/Tcf-β-catenin complexes. Here we show that in human endothelial cells fibroblast growth factor-2 (FGF-2) reduces GSK-3 activity and augments nuclear levels of β-catenin. FGF-2 induced Lef/Tcf-dependent transcription of a cyclin D1-luciferase construct. Gel shift assays revealed binding of Tcf-4 as the only Lef/Tcf family member and of β-catenin to the Lef/Tcf site in the cyclin D1 promoter. Cotransfection with a dominant negative Tcf-4 construct inhibited the FGF-2-induced cyclin D1 promoter activity. Overexpression of an uninhibitable GSK-3β mutant resulted in partial inhibition of FGF-2-mediated cyclin D1 induction. The importance for cyclin D1 in FGF-2-induced angiogenesis in vivo is shown in cyclin D1−/− mice, where FGF-2-induced new vessel formation was significantly reduced compared with FGF-2-induced angiogenesis in cyclin D1+/+ mice. In conclusion, FGF-2 is a novel modulator of Lef/Tcf-β-catenin signaling in endothelial cells, suggesting that angiogenic properties of FGF-2 are at least in part mediated by Lef/Tcf-β-catenin activation.

Lef/Tcf (lymphoid enhancer factor/T-cell factor) proteins belong to a family of architectural transcription factors, which are typically activated by the so-called Wnt signaling pathway. Members of the Wnt family bind to a seven-transmembrane receptor called frizzled, which results in the translocation of β-catenin from the cytosol into the nucleus (1). As a functional consequence, β-catenin associates with Lef/Tcf proteins and induces transcription of target genes, such as c-myc, cyclin D1, connexin 43, c-jun, fra-1, or the urokinase receptor (2–6). This Wnt signaling pathway has been shown to play an important role in developmental processes as well as in oncogenesis. It is evolutionarily highly conserved, and proteins are homologous in vertebrates, Drosophila, Xenopus, Caenorhabditis elegans, and even Hydra and nonmetazoan organisms (7–11).

In the cytosol, β-catenin is bound to a large regulatory protein complex including axin/conductin, β-TrCP, the adenomatous polyposis coli protein, protein phosphatase 2A, casein kinase 1, and glycogen synthase kinase-3 (GSK-3) (12–20). After “priming” phosphorylation of β-catenin by casein kinase 1, GSK-3 constitutively phosphorylates β-catenin and thereby prevents its nuclear translocation (12). Phosphorylated β-catenin is ubiquitinylated and subsequently proteosomally degraded (21). Wnt proteins down-regulate GSK-3 activity and hence translocate β-catenin into the nucleus, thereby increasing Lef/Tcf-dependent transcription. Besides Wnt proteins, an increasing number of molecules have been shown to modulate members of the Wnt pathway. GSK-3 activity is reduced by growth factors (22, 23), B cell antigen receptor ligation (24), phorbol esters (25), and lithium (26). Finally, the transcriptional activity of the Lef/Tcf-β-catenin complex itself can be modulated by direct binding of co-activators and co-repressors like groucho (27), Brg-1 (28), PEA-3 (29), p300 (30), and histone deacetylases (31).

It has been recently shown that human endothelial cells have a functional Wnt signaling pathway (32). Following Wnt-1 overexpression, Lef/Tcf-dependent transcription is induced. Based on two observations that (i) FGF-2 (also called basic FGF) alters subcellular distribution of β-catenin in endothelial cells (33) and that (ii) FGF-2 down-regulates GSK-3 activity in Xenopus embryos (34), we explored FGF-2 effects on Lef/Tcf-dependent transcription in endothelial cells. Here we report that in human umbilical vein endothelial cells FGF-2 reduces GSK-3 activity and enhances β-catenin translocation into the nucleus. This is followed by the up-regulation of a cyclin D1 promoter construct in an Lef/Tcf-dependent fashion.

EXPERIMENTAL PROCEDURES

Cell Culture—Human umbilical vein endothelial cells (HUVEC) were isolated and cultured according to standard procedures as previously described (35). Cells were used at passages 3–6. Before stimulation with FGF-2 (20 ng/ml; R & D Systems, Indianapolis) and heparin (5 units/ml), cells were serum-starved overnight in Iscove’s modified Dulbecco’s medium (Invitrogen) containing 5% bovine serum albumin (for GSK-3 assays, 1% fetal calf serum was added).

Western Blotting—Cells were incubated in 10 mM NaOH-Hepes, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, and 0.5% Nonidet P-40 on ice followed by centrifugation at 800 × g for 5 min at 4 °C. Supernatants were

* The abbreviations used are: GSK-3, glycogen synthase kinase-3; HUVEC, human umbilical vein endothelial cell(s); VEGF, vascular endothelial growth factor; PMA, phorbol 12-myristate 13-acetate.

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discarded, and pellets containing the intact nuclei were washed in 10 mM NaOH-Hepes, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl. Thereafter, nuclei were lysed in a hypertonic salt buffer containing 20 mM NaOH-Hepes, pH 7.9, 10% glycerol, 420 mM NaCl, 1.5 mM EDTA and incubated for 45 min on ice. After centrifugation at 10,000 × g for 5 min at 4 °C, the supernatants containing the nuclear extracts were collected, and protein concentrations were adjusted to 0.5 mg/ml. Immunoblotting was performed with a monoclonal anti-β-catenin antibody (Sigma) as described elsewhere (33).

Analysis of GSK-3 Activity—GSK-3 activity was measured in crude extracts based on a method described previously (36). Briefly, HUVEC were collected with a cell scraper and homogenized in 30 μl of buffer containing 20 mM NaOH-Hepes (pH 7.4), 200 mM NaCl, 100 mM NaF, 5 mM MgCl₂, 1.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin and 10 μg/ml aprotinin. After 15 min lysis on ice, cells were centrifuged at 13,000 × g for 15 min at 4 °C.

Four μl of the protein extract were incubated in a buffer containing 25 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, and 10 mM MgCl₂, together with a GSK-3-specific peptide derived from the CREB transcription factor (final concentration 140 μM; New England Biolabs, Frankfurt, Germany) and 5 μCi of [γ-32P]ATP at 30 °C for 15 min. Thereafter, aliquots were spotted onto P81 phosphocellulose paper (Merck), washed two times with 75 mM phosphoric acid, and measured by scintillation counting. The difference between the kinase activity in the presence or absence of 40 mM LiCl was considered to represent the specific GSK-3 activity (33). To control for nonspecific phosphorylation, aliquots of the reaction mixtures were boiled with sample buffer and separated on a 10% polyacrylamide gel. Moreover, recombinant GSK-3 (0.5 units; New England Biolabs) was run in parallel. The gels were dried under vacuum at 80 °C and autoradiographed.

Reverse Transcription-Polymerase Chain Reaction—Cells were washed twice with ice-cold phosphate-buffered saline, and cytoplasmic extracts were prepared by lysing the cells in 200 μl of lysis buffer containing 20 mM NaOH-Hepes (pH 7.8), 1.5 mM MgCl₂, 10 mM KCl, 1 mM dithiothreitol, 0.1% Nonidet P-40, and 1 unit of RNase inhibitor (Roche Molecular Biochemicals). Nuclei were pelleted, and the cytoplasmic supernatants were used for mRNA extraction with oligo(dT) beads according to the manufacturer’s instructions. First strand cDNA synthesis was done with a kit from Roche Molecular Biochemicals according to the manufacturer’s protocol. Primers and cycling conditions for Lef/Tcf (37) and Wnt (32) were described elsewhere. For PCR with Wnt primers, different cycle numbers were tested to ensure linearity of amplification.

Plasmids, Transfection, and Luciferase Assays—The cyclin D1 promoter constructs are based on the pA3-luc backbone and contain a 1745-bp fragment of the human cyclin D1 promoter (4). One promoter construct has a wild type Lef/Tcf site (5′-GCTTGTATCTT-3′), and the other has a mutated Lef/Tcf site (5′-GCTTGTGCCCTT-3′). Dominant negative Tcf-4 in the pcDNA3 neo vector was a kind gift from Dr. Hans Clevers, University Medical Center Utrecht, The Netherlands. To control for nonspecific phosphorylation, a kinase-negative control, a hormone-inducible dominant negative Tcf-4 construct in the pEGRE5 vector was used (Invitrogen), which is not expressed without the addition of an edesine analogue. The uninhibitable A9GSK-3β construct in the pCR3.1 vector was a kind gift from Dr. Morris Birnbaum (Howard Hughes Medical Institute, University of Pennsylvania (38)). HUVEC were transfected in 10-cm Petri dishes with 15 μg of plasmid DNA using the calcium phosphate precipitation technique. 24 h later, cells were trypsinized, resuspended, and seeded into 35-mm dishes. HUVEC were either starved in Iscove’s modified Dulbecco’s medium containing 5% bovine serum albumin and 5 units/ml heparin or in Iscove’s modified Dulbecco’s medium containing 5% bovine serum albumin and 20 ng/ml FGF-2 and 5 units/ml heparin (where indicated, vascular endothelial growth factor (VEGF; 10 ng/ml; Strathmann, Hamburg, Germany). 19 h later, cells were lysed, and luciferase activities were measured with a kit from Promega according to the manufacturer’s protocol. Protein concentrations were measured with a Bradford protein assay kit from Bio-Rad, and luciferase values were normalized to the obtained protein concentrations. In transcription experiments using the A9GSK-3β construct, normalization was done by Renilla luciferase under the control of the thymidine kinase promoter, and luciferase assays were performed with the dual luciferase assay system (Promega).

Nuclear Extracts and Electrophoretic Mobility Shift Assays—Nuclear extracts were prepared as described above. Protein concentrations were determined with the Bradford protein assay (Bio-Rad). Radiolabeling of the oligonucleotide containing the cyclin D1 Lef/Tcf site (5′-tggtttagagt-gaagaataacagacgccgag-3′) was performed with [γ-32P]ATP using T4 polynucleotide kinase (Roche Molecular Biochemicals) and purification with Microspin G-25 columns (Amersham Biosciences). Thereafter, the complementary strand was annealed. For the binding reactions, 5 μg of nuclear extract were incubated with 100,000 cpm of radiolabeled oligonucleotide in a 15-μl reaction mix containing 20 mM NaOH-Hepes, pH 7.9, 75 mM NaCl, 1 mM dithiothreitol, 2 mM MgCl₂, 10% (v/v) glycerol, and 1 μg of salmon sperm DNA for 20 min at room temperature. Where indicated, 1 μg of anti-Lef/Tcf antibodies (EΔAlpha, Boston, MA) or anti-β-catenin antibody (BD Transduction Laboratories, Los Angeles, CA) were added to the nuclear extract 10 min prior to the addition of the oligonucleotide. DNA-protein complexes were resolved on 6% nondenaturing polyacrylamide gels in 0.3 × TBE buffer, dried, and visualized by autoradiography.

Matrigel Plug Assay for Angiogenesis—To study blood vessel formation in vivo, we used the matrigel plug assay of angiogenesis (39). For this assay, growth factor-reduced matrigel (Becton Dickinson) was supplemented with FGF-2 (500 ng/plug) and injected subcutaneously into the abdominal midline of 6–8-week-old C57Bl6 mice. Mice were either wild type or null for cyclin D1 expression (40, 41). Plugs were excised from mice after 2 weeks, fixed in 10% neutral buffered formalin, and paraffin-embedded. Sections of embedded plugs were stained with CD31, and vessels were quantitated by calculating the average number of vessels per field for each plug.

RESULTS

FGF-2 Enhances Nuclear Translocation of β-Catenin and Reduces GSK-3 Activity—In resting endothelial cells, β-catenin is predominantly located within the cytosol (33). Fig. 1a shows an immunoblot of nuclear extracts from HUVEC. Compared with the unstimulated controls, β-catenin levels in the nucleus increased by a mean of 2–3-fold after 1 h of stimulation with FGF-2 and decreased thereafter. A Coomasie gel was run in parallel to show equal protein loading. Since down-regulation of GSK-3 activity precedes nuclear translocation of β-catenin (42), we stimulated HUVEC with FGF-2 and performed kinase assays. After 15 min, GSK-3 activity was significantly decreased by FGF-2 and slowly increased after 30 min (Fig. 1b). As reported previously, phorbol 12-myristate 13-acetate (PMA) reduces GSK-3 activity (25, 43). Thus, for control purposes, HUVEC were stimulated with PMA, which led to a reduction of GSK-3 activity comparable with that seen with FGF-2. To confirm specificity of the kinase assay, we analyzed the kinase reactions on SDS-polyacrylamide gels. The GSK-3-specific CREB peptide was phosphorylated and seen as a distinct band on SDS gels. Phosphorylation was inhibited by the noncompetitive GSK-3 inhibitor LiCl. As a control, we also analyzed recombinant GSK-3 on SDS gels (Fig. 1c).

HUVEC Express All Known Lef/Tcf Family Members and Wnt-5a—So far, four human members of the Lef/Tcf family are known: Lef-1, Tcf-1, Tcf-3, and Tcf-4. To explore which of them are expressed in HUVEC, reverse transcription-PCR was performed. As shown in Fig. 2a HUVEC express Lef-1, Tcf-1, Tcf-3, and Tcf-4 mRNA. Except for Tcf-3, all primers yielded one specific band of the expected size. PCR with Tcf-3-specific primers resulted in two bands. According to the expected size, the lower band is specific for Tcf-3, and the identity of the upper band remains to be determined. To exclude the possibility that nuclear translocation of β-catenin is mediated via de novo synthesis of Wnts, we performed semiquantitative reverse transcription-PCR. Neither in unstimulated nor in FGF-2-treated HUVEC (6 h) could we detect Wnt-1 mRNA. Also, degenerate primers, which amplify several Wnt members (44), did not show an increase in mRNA expression by FGF-2. As a control, we analyzed Wnt-5a expression, which so far is the only known Wnt member expressed in endothelial cells but unable to activate the Wnt pathway (32). Also, Wnt-5a mRNA expression was not enhanced by FGF-2 in endothelial cells. Thus, FGF-2 effects on GSK-3 activity and on nuclear translocation of β-catenin were independent from de novo synthesis of Wnt proteins.

FGF-2 Activates Cyclin D1 Transcription in a Lef/Tcf-dependent Manner—To investigate whether the FGF-2-induced nuclear
translocation of β-catenin enhanced Lef/Tcf-dependent transcription, we used luciferase reporter plasmids containing a 1745-bp sequence of the human cyclin D1 promoter, which harbors the wild type or a mutated Lef/Tcf site (4). Following transfection into HUVEC, we found a 4–6-fold induction of the wild type cyclin D1 promoter upon FGF-2 stimulation (Fig. 3). In contrast, the promoter with the mutated Lef/Tcf site responded only insignificantly to FGF-2 (a mean of 1.2-fold). VEGF, used as a positive control, induced expression of both constructs about 3-fold.

**Fig. 1.**

*a*, FGF-2 enhances nuclear translocation of β-catenin. HUVEC were stimulated with FGF-2, and nuclear lysates were prepared at the indicated time points. Lysates were immunoblotted as described using a polyclonal anti-β-catenin antibody. One of three independent experiments is shown. Densitometrical analysis revealed a mean 2–3-fold increase in nuclear β-catenin. To show equal protein loading, aliquots from each time point were separated and stained with Coomassie Blue. 
b, GSK-3 activity is down-regulated by FGF-2 and PMA. HUVEC were stimulated with FGF-2 or PMA, lysed, and analyzed for GSK-3 activity at the indicated time points as described under “Experimental Procedures.” Each bar represents the mean ± S.D. of three independent experiments; each experiment was performed in duplicate. c, GSK-3 specifically phosphorylates the CREB peptide. Aliquots of untreated and LiCl-treated kinase reactions from HUVEC lysates (two left lanes) were loaded on a 17% polyacrylamide gel. An experiment with recombinant GSK-3 is included as a positive control (two right lanes). The gel was dried, and bands were subsequently visualized by autoradiography. Note that the gel contains free [γ-^32P]ATP and CREB peptide only; no other proteins have incorporated radioactivity.

**Fig. 2.**

*a*, expression of members of the Lef/Tcf family in HUVEC. Cytoplasmic RNA was isolated and reverse transcribed, and PCR was conducted with specific primers for Lef-1, Tcf-1, Tcf-3, and Tcf-4. 
b, semiquantitative RT-PCR demonstrating that FGF-2 does not exert significant effects on the expression of Wnt family members. PCR was performed with specific primers for Wnt-1 and Wnt-5a and with degenerate primers amplifying several members of the Wnt family. Glycer-aldehyde-3-phosphate dehydrogenase was included as a positive control for the integrity of the cDNA; PCR on RNA without reverse transcription showed no amplification products excluding contamination with genomic DNA. In order to ensure linearity of amplification, PCR with Wnt primers was done for several cycle lengths and optimized to the linear phase of the PCR (Wnt-1, 35 cycles; Wnt-5a, 25 cycles; Wnt-deg, 40 cycles). The PCR products were separated on a 2% agarose gel and visualized by ethidium bromide staining. The identities of the bands were verified by their expected size. **MWM**, molecular weight marker.

As co-activator to induce transcription. To verify binding of β-catenin to the Tcf-4-DNA complex in FGF-2-stimulated HUVEC, we added a monoclonal anti-β-catenin antibody into the
binding reaction. Fig. 4b shows the presence of β-catenin in this complex. As a positive control, we stimulated HUVEC with LiCl, which inhibits GSK-3 activity leading to nuclear accumulation of β-catenin. Nuclear extracts of LiCl-stimulated (3 h, 20 mM) HUVEC supershifted with the anti-β-catenin antibody (Fig. 4b).

**Fig. 5.** a, luciferase analysis showing the influence of ΔN-Tcf-4 on FGF-2-and VEGF-mediated induction of the cyclin D1 promoter. The wild type cyclin D1 promoter was cotransfected with either a control vector carrying a hormone-inducible ΔN-Tcf-4 or a constitutively active ΔN-Tcf-4 construct. Both FGF-2 and VEGF activate the cyclin D1 promoter in mock-cotransfected cells. Constitutively active ΔN-Tcf-4 abolished the FGF-2-mediated induction of the cyclin D1 promoter. In contrast, VEGF effects on luciferase expression were only insignificantly affected by the constitutively active ΔN-Tcf-4. Results are the mean ± S.D. of four independent experiments. Each experiment was done in duplicate. b, GSK-3β is partially involved in the FGF-2-mediated up-regulation of cyclin D1. HUVEC were transfected with the wild type cyclin D1 promoter and an uninhibitable GSK-3β construct (A9GSK-3β) or with the empty vector (pCR3.1), incubated for 19 h in the presence of FGF-2, and assayed for luciferase activity. Firefly luciferase activity was normalized with a cotransfected Renilla luciferase under the control of the thymidine kinase promoter. Shown are the mean ± S.D. of three experiments performed in duplicate.
The FGF-2-induced cyclin D1 promoter activity was abolished by cotransfection with constitutively active ΔN-Tcf-4 but not by cotransfection with the control vector. In contrast, VEGF induction was only minimally affected by ΔN-Tcf-4. To investigate the role of GSK-3 in FGF-2 stimulation of Lef/Tcf-dependent transcription, HUVEC were transfected with the wild type cyclin D1 promoter and a plasmid coding for a constitutively active GSK-3β mutant, where the serine 9 phosphorylation site is mutated to an alanine. After transfection, cells were stimulated with FGF-2 for 19 h. Overexpression of the GSK-3β mutant reduced the effect of FGF-2 on the cyclin D1 promoter by a mean of 33%.

Cyclin D1 Is Required for FGF-2-mediated Angiogenesis—We substantiated the role of cyclin D1 in FGF-2-induced angiogenesis by using the matrigel plug model (39). Growth factor-reduced matrigel supplemented with FGF-2 was injected subcutaneously into cyclin D1+/− mice or into wild type litter mates as previously described (40, 41). New vessel formation was quantified after 2 weeks. As seen in Fig. 6, in cyclin D1−/− mice, FGF-2 induced a robust new vessel formation within the gels (mean 42 ± 1.8 vessels per high power field). In contrast, in mice where the cyclin D1 gene has been deleted, angiogenesis in response to FGF-2 was significantly reduced (mean 16.8 ± 3.1 vessels). In the absence of FGF-2, angiogenesis was virtually absent. Thus, the up-regulation of cyclin D1 is an important component of the angiogenic process in response to FGF-2.

DISCUSSION

Transcription factors of the Lef/Tcf family require the interaction with other proteins to be activated and to modulate gene transcription. β-Catenin is the best characterized protein that activates Lef/Tcf-mediated transcription. In unstimulated cells, β-catenin is mainly located in the cytoplasm. The nuclear translocation of β-catenin is thought to be mainly under the control of the Wnt signaling pathway. The initial observation that human endothelial cells have a functional Wnt signaling pathway came from Wright et al. (32), who transfected endothelial cells with a Wnt-1 construct and found Lef/Tcf-dependent transcription and cell proliferation. However, endothelial cells in culture express Wnt-5a only, which is unable to activate Lef/Tcf-dependent genes. We have confirmed this observation and extended it by showing that even after FGF-2 stimulation, Wnt expression was not induced (iRT-PCR with degenerated primers used by Wright et al. (32)).

Here, we report that FGF-2 reduces GSK-3β activity and augments nuclear translocation of β-catenin in human endothelial cells. To analyze gene expression, we first used wild type and mutated Lef/Tcf reporter plasmids (TOPflash and FOPflash; Upstate Biotechnology, Inc., Lake Placid, NY), but both constructs were inducible by FGF-2 (data not shown). This effect has thus to be interpreted as nonspecific and is probably due to the minimal thymidine kinase promoter in front of the luciferase gene. Instead, we used luciferase reporter constructs of cyclin D1, a known target gene of the Wnt pathway came from Wright et al. (32), who transfected endothelial cells with a Wnt-1 construct and found Lef/Tcf-dependent transcription and cell proliferation. However, endothelial cells in culture express Wnt-5a only, which is unable to activate Lef/Tcf-dependent genes. We have confirmed this observation and extended it by showing that even after FGF-2 stimulation, Wnt expression was not induced (iRT-PCR with degenerated primers used by Wright et al. (32)).

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2 W. Holnthoner and P. Petzelbauer, unpublished observations.
β-catenin-Tcf-4 to the cyclin D1 Lef/Tcf site in response to FGF-2. In addition, despite the presence of Lef-1, Tcf-1, and Tcf-4 (at least on the mRNA level), only Tcf-4 bound to the cyclin D1 Lef/Tcf site.

A crucial switchpoint in the signal transduction to Lef/Tcf is GSK-3. Here we show that FGF-2 down-regulates GSK-3 activity. However, the consequences of altered GSK-3 activity on Lef/Tcf-dependent transcription seem to be dependent on the cell type. In HepG2 hepatoma cells, insulin and insulin-like growth factor-1 reduce GSK-3 activity and induce nuclear translocation of β-catenin and Lef/Tcf-dependent transcription in mammary epithelial cells (48). Epidermal growth factor reduces GSK-3 activity but does not induce β-catenin accumulation and transcriptional activity of Lef/Tcf in mammary epithelial cells (49). FGF-1 reduces in GSK-3 activity in neuronal cells, but consequences on β-catenin signaling are unknown (50).

We defined the role of GSK-3 in endothelial cells by cotransfecting a mutant GSK-3β. We show that this constitutively active GSK-3β binds to and regulates FGF-2-induced cyclin D1 promoter activity. This confirms that GSK-3 is involved in the transduction of signals from FGF-2 to Lef/Tcf. However, inhibition by this dominant positive GSK-3 construct was only partial. A possible explanation is based on the finding that in addition to translocating β-catenin from the cytosolic and GSK-3-bound pool, FGF-2 also liberates junctional β-catenin (33). One might speculate that the junctional-derived β-catenin can “bypass” the cytosolic GSK-3β-complex and directly translocate into the nucleus. It should be noted that in contrast to endothelium, in epithelial cells FGF-2 appears to have different effects; it does not liberate catenins from the membrane (51, 52). An alternative explanation is that a portion of β-catenin is linked to GSK-α and thus still accessible to FGF-2 signals, which is supported by the finding that GSK-3β−/− knockout mice show normal early embryonic development, suggesting that GSK-3α can at least partially compensate for GSK-3β (53). However, a direct interaction of GSK-3α with β-catenin has not been shown yet (48). Finally, it should be noted that β-catenin stability can be influenced independently from GSK-3 (e.g. by the IκCas and IκKβ kinases (54)), but FGF-2 does not appear to regulate IκK.

In conclusion, we describe a novel function for FGF-2 in endothelial cells. FGF-2 down-regulates GSK-3 activity, enhances nuclear translocation of β-catenin, and transactivates a cyclin D1 reporter construct in a Lef/Tcf-dependent manner. Moreover, we provide the first physiological evidence for the requirement for cyclin D1 in FGF-2-induced angiogenesis. Further evidence for the importance of Lef/Tcf in angiogenesis comes from recent publications that show roles for β-catenin/Tcf signaling in vascular remodeling (55) and for Tcf-4 in the proliferation of human dermal microvascular endothelial cells (56). Thus, in analogy to Wnt proteins, which control developmental processes like patterning and organ shaping, FGF-2 may interfere with vascular- or angiogenesis by controlling nuclear translocation and transcriptional activation of β-catenin.

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