Laminin-1 Promotes Angiogenesis in Synergy with Fibroblast Growth Factor by Distinct Regulation of the Gene and Protein Expression Profile in Endothelial Cells

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Laminins are widely distributed extracellular matrix proteins. Certain laminin isoforms are predominant in vascular basement membranes and may be critical in maintaining the stability of the mature vessel. On the other hand, formation of new vessels during angiogenesis requires degradation of the basement membrane, exposing the endothelial cells to other laminins in the surrounding extracellular matrix. We studied the effects of laminin-1 (LN-1) in different in vitro and in vivo models for angiogenesis. LN-1 induced angiogenesis in the chicken chorioallantoic membrane to the same extent as fibroblast growth factor-2 (FGF-2), and vascular development in embryoid bodies was stimulated in a synergistic manner by FGF-2 and LN-1. LN-1 promoted differentiation of endothelial cells in three-dimensional collagen gels, both in the absence and presence of FGF-2. Formation of tubular structures induced by LN-1 was accompanied by increased expression of Jagged-1, a marker of endothelial differentiation, and increased levels of FGF-2 and FGFR-1 transcripts. LN-1 did not regulate signal transduction pathways known to operate down stream of FGF-2. Thus, phosphorylation of ERK was detected in FGF-2- but not in LN-1-treated cells. Taken together, this suggests that laminins may play a fundamental role in angiogenesis by directly affecting gene and protein expression profiles in endothelial cells.

Angiogenesis, the formation of new vessels from preexisting capillaries, is a prerequisite for embryonic development. In the healthy adult, physiological, regulated angiogenesis is an integral part of tissue growth, wound healing, and the female menstrual cycle. A number of diseases such as cancer and inflammatory diseases including rheumatoid arthritis, diabetic retinopathy, and ischemic heart and limb disease (for reviews, see Refs. 1–3) are accompanied by deregulated angiogenesis.

In the angiogenic process (for a review see Ref. 4), the quiescent endothelial cells (ECs) of the mature vessel become activated by exposure, e.g. to angiogenic growth factors, which leads to degradation of the surrounding matrix and to proliferation and migration of ECs, which eventually form new vessels. These vessels mature by deposition of a new matrix and recruitment of pericytes. Many soluble factors are implicated in regulation of angiogenesis, including vascular endothelial growth factor A (VEGF-A) and fibroblast growth factor 2 (FGF-2) (for a review, see Ref. 5). It has also been recognized that extracellular matrix interactions are important in angiogenesis, and accordingly, interference with αv integrin function leads to inhibition of angiogenesis (6, 7).

Laminins are trimeric molecules composed of α-, β-, and γ-chains. Five distinct α-, three β-, and three γ-chains have been identified thus far, and these combine to form at least 14 different laminins (8, 9). Of these, LN-8 (α4β1γ1) and LN-10 (α5β1γ1) are localized in vascular basement membranes (10). LN-α4 mice are viable and fertile but display certain vascular abnormalities, leading to mild transient hemorrhages in the skin of newborn mice (11). Moreover, blood vessel walls in glioblastoma multiforme show increased expression of LN-8 as compared with normal brain (12). The murine laminin α5 chain is detected only in larger vessels in the embryo, whereas 3–4 weeks after birth, there is prominent expression also in capillaries (13, 14). Targeted inactivation of the gene encoding the laminin α5 chain results in disturbed placental vessel formation and a severe effect on brain and limb development, leading to embryonal death at day 17 (15).

LN-1 (α1β1γ1) is expressed during early embryogenesis (16) and has been implicated in regulation of epithelial as well as EC function. The effect of gene inactivation of the α1 chain has not yet been reported. Targeted inactivation of the γ1 chain gene leads to failure of basement membrane formation and death at embryonic day 5.5 (17) prior to the onset of vasculogenesis. However, because this chain is found in several laminins, the results cannot be used as evidence for a role of laminin-1 in early embryogenesis. LN-1-derived peptides have been shown by Kleinman and co-workers (18–20) to promote or inhibit EC function by modulating EC adhesion. In human colon adenocarcinoma, overexpression of the laminin α1 chain increases vascularization of the tumor, leading to enhanced...
growth (21). We now report that LN-1 promotes differentiation of ECs in vitro and angiogenesis in the chicken chorioallantoic membrane assay. Together with FGF-2, LN-1 synergistically promotes vessel development in embryoid bodies. The molecular mechanisms underlying the effect of LN-1 appears to involve direct gene-regulatory effects, distinct from those induced by angiogenic growth factors.

**MATERIALS AND METHODS**

**Reagents**—LN-1, LN-10/11, and leukemia inhibitory factor (LIF) were from Invitrogen. FGF-2 was purchased from Roche Applied Science. In addition, two noncommercial sources of LN-1 (kindly provided by Dr. Rupert Timpl, Max Planck Institute for Biochemistry, Martinsried Munich, Germany) and by Dr. Kristofer Rubin, Dept. of Medical Biochemistry and Microbiology, Uppsala University, Sweden) were tested. All three LN-1 preparations had the same effects on endothelial cell responses (data not shown). The commercial LN-1 preparation did not contain FGF-2, VEGF-A, or PDGF-BB as detected by enzyme-linked immunosorbent assays. "Quantikine" kits, catalog no. DVE00 (VEGF-A) and DFB 50 (bFGF), R&D Systems, Minneapolis, MN, 5 pg detection limit or by immunoblotting of cells exposed to LN-1 to detect activated growth factor receptors. Moreover, LN-1 did not modulate, either positively or negatively, thymidine incorporation in endothelial cells, indicating that LN-1 preparation did not contain contaminating growth-modulatory factors. The antibody reagents used were: anti-Jagged-1 (sc-11376) and anti-F-actin (sc-1616) (Santa Cruz Biotechnology, Santa Cruz, CA); anti-Akt (No. 9272, AKR mouse T-cell lymphoma, Santa Cruz, CA); anti-phospho-Akt (No. 209, Ser 473), anti-ERK-1/2 (No. 9102) and anti-phospho-ERK-1/2 (No. 9101) (Th2/2 Tyr2/4, Cell Signaling, Beverly, MA); anti-mouse CD31 antibody (No. 01591, Pharmingen, San Diego, CA); horseradish peroxidase (HRP)-conjugated anti-rabbit-IgG (NA 934, Amersham Biosciences); HRP-conjugated anti-goat IgG (A4174, Sigma); anti-rat IgG antibody (BA 9400); and HRP-conjugated streptavidin (SA-5004, Vector Laboratories Inc., Burlingame, CA).

**Tissue Culture and Collagen Gel Assay**—Immortal mouse brain endothelial (IBE) cells (22) were routinely cultured on gelatin-coated tissue culture plastic in Ham’s F12, 10% fetal calf serum (Invitrogen) supplemented with mouse y-interferon, 20 units/ml. The cells were starved overnight in Ham’s F12, 0.1% bovine serum albumin (BSA) prior to overnight in Ham’s F12, 10% fetal calf serum (Invitrogen), 1.2 mm sodium pyruvate (Invitrogen), 1% mouse monoclonal (Sigma), and 1.0 unit/ml LIF. At day 0, 1200 cells in hanging drops were allowed to aggregate in 20 μl of medium without LIF and, from this point, in the presence or absence of 5 or 20 ng/ml FGF-2. At day 4, individual embryoid bodies (EBs) were placed in polystyrene culture-treated glass slides (BD Biosciences). From day 8 the medium was supplemented as indicated with 1 μg/ml LN-1, and the bodies were cultured until day 12. The time points for FGF-and LN-1 addition were chosen for optimal effects. All treatments were performed on four bodies/condition. For quantification, the area of CD31-positive structures was measured using the “Easy Image” software (Tekno Optik, Stockholm, Sweden).

**Immunohistochemical Staining**—EBs were fixed in 0.5% zinc acetate, 0.5% Ca acetate in 0.1 M Tris HCl, pH 7.4, overnight. Endogenous peroxidase activity was blocked in 3% H2O2 in methanol for 30 min, and unspecific binding sites were blocked with blocking buffer (kit NEL 700; PerkinElmer Life Sciences) for 1 h. EBs were incubated with primary rat anti-mouse CD31 antibody diluted 1:800 in blocking buffer for 1 h, and finally with HRP-conjugated streptavidin diluted 1:200 in blocking buffer for 30 min. The AEC substrate kit (Vector Laboratories) was used for staining according to the manufacturer’s instructions. Slides were mounted in aqueous mounting medium (DAKO, Glostrup, Denmark) and analyzed using a Nikon Eclipse IX100 microscope with a Nikon Eclipse DXY 1290 camera.

**Reverse Transcription PCR**—IBE cells on collagen-coated Petri dishes (800,000 cells/dish) were serum-starved overnight (Ham’s F12, 0.1% BSA). Fresh serum-free medium was added with or without FGF-2, 5 ng/ml. After 24 h, RNA extraction was performed using RNeasy Mini Kit (Qiagen). Alternatively, 800,000 FGF-2-treated cells undergoing tubular morphogenesis in the collagen assay were used. The upper gel layer was removed, and cells were released by collagenase treatment (type IV, 750 μl, 0.1%, Sigma-Aldrich; 20 min, 37 °C), collected, and spotted at 4 °C in 1% RNAase-free dextran (Qiagen) until RNA extraction. Reverse transcription was carried out using Superscript II (Invitrogen), poly(dT) primers, and 2 μg of RNA. The PCR reactions, using primers as indicated in Table I, were performed at 60 °C annealing temperature, except the PCR-reaction for γ3 was carried out using 5 cycles with 66 °C, 5 cycles with 63 °C, and finally 35 cycles with 59 °C.

**Oligonucleotide primers used for PCR (shown in Fig. 4A)**

| Gene | Forward primer (5′-3′) | Reverse primer (5′-3′) | Product length (bp) |
|------|------------------------|------------------------|---------------------|
| α1   | GCGCTAAAGATTCCAGGCC   | TCTGTCAAAGACTCTG      | 626                 |
| α2   | GGTCGCTGACATCAGCTACTG | GGTCGAGGAGAAAGGCTG    | 589                 |
| α3   | TGGAGCTTCTTTGCGTCG    | CGATACGCCCCTTGTG      | 550                 |
| α5   | CCTGTGCTTGGGTCGCAAGAG | AAAAGAACCGGTGCCATGAA  | 437                 |
| β1   | GCTGGATCTGCCGTTGCAGAG | GGCGGAATTCTAAGAGGCTGAA | 702                 |
| β2   | CTGCGCGCTGATGACTCC  | ACGATTGCTGCCAGACAC    | 550                 |
| β3   | CAGCAGCTATGCTGGCAG    | ACTGATTGCTGCCACACAC   | 544                 |
| γ1   | GGGGATTCGCAAGATCAGATGCTTCCGAG | 676                 |
| γ2   | TATAGCGAGATGGGAGGGAGC | TAGTCCTCACGAGTGGAGAGC | 549                 |
| γ3   | AAAGGAGTATGAAGGGGACGC | AGGCAGTCAAGGGAAGGAGAAG | 552                 |
Quantitative Real-time PCR—Primers targeting β-actin, Jagged-1, FGF-2, FGFR-1, VEGF-A, VEGFR-2, the five LN-1 chains, the LN-1 β1- and LN-1 γ1-chains, and the collagen α1 (IV)-chain (Table II) were designed using Primer Express 1.5a (Applied Biosystems, Foster City, CA) and synthesized by Invitrogen.

For RNA preparation and cDNA synthesis, IBE cells cultured on collagen I were lysed in the dish, homogenized through an 0.9-mm syringe, and run through a QiAshredder spin column (Qiagen). Total RNA was extracted according to manufacturer’s protocol using an RNA was extracted according to manufacturer’s protocol using an RNAse-free DNase I-treated (Amersham Biosciences), and RNA levels were determined by spectrophotometry.

One µg of RNA from each condition was used for reverse transcription (USB, Cleveland, OH) to create cDNA. Reverse transcription PCR parameters were 37 °C for 1 h and 95 °C for 5 min. For the real-time PCR, the ABI Prism 7700 (Applied Biosystems) was used with SYBR Green Master mix (Applied Biosystems) and 0.25 µM primer. PCR parameters were 95 °C for 10 min (95 °C, 15 s; 60 °C, 60 s) × 45. All samples were run in triplicate, and the level of target gene expression was calculated in relation to the β-actin level in each sample.

**RESULTS**

**LN-1 Induces Angiogenesis in CAM**—The potential effect of LN-1 on angiogenesis in the chicken embryo was investigated. The CAM of day 10 fertilized chicken eggs was exposed by removal of part of the shell. Filter paper discs soaked in LN-1, FGF-2, or both were applied to the CAM. Three days later, the filter discs were cut out and photographed. Representative CAMs are shown in Fig. 1A. The extent of vessel formation and the morphology of the vessels in the LN-1-treated chicken CAM was similar to that in the FGF-2-treated CAM. There was no appreciable additional increase in vessel formation in the CAMs receiving both FGF-2 and LN-1 (see Fig. 1B for quantification). Similar effects were seen with several different sources of laminin, either commercial or prepared in two different laboratories. The effect was independent of whether LN-1 was in complex with nidogen or not (data not shown). The LN-1 preparation did not contain contaminating growth factors such as FGF-2, VEGF-A, or PDGF-BB, as demonstrated using sensitive enzyme-linked immunosorbent assays (data not shown).

Furthermore, incubation of endothelial cells with high concentrations of LN-1 did not affect the incorporation of [3H]thymidine. Thus, the effects of LN-1 on CAM angiogenesis, or in the models shown below, were not due to contaminating growth modulatory factors in the LN-1 preparations.

**Synergistic Stimulation of CD31-positive Vessels in Mouse EBs by LN-1 and FGF-2**—We next examined the effects of LN-1 on the vascular development in EBs. Mouse embryonic stem cells were aggregated in hanging drop cultures in the absence of LIF. On day 4, the EB aggregates were placed in microscopic chamber slides and further cultured until day 12, when the EBs were fixed and stained with antibodies against CD31 (platelet EC adhesion molecule; Fig. 2A). Cells were treated with or without FGF-2 from day 0 and with or without LN-1 from day 8, at which point angiogenesis was evident in the EBs. There was no effect on vessel formation by addition of 5 ng/ml FGF-2, but treatment with 20 ng/ml FGF-2 increased the number of vessels in the EBs. LN-1 alone did not affect vessel formation at either 1 or 5 µg/ml. Vessel formation in the EBs was efficiently stimulated by a combination of the suboptimal concentration of 5 ng/ml FGF-2 and 1 µg/ml LN-1 (Fig. 2A). In contrast, co-stimulation of EBs with 5 ng/ml FGF-2 and 1 µg/ml LN-10/11 did not result in increased vessel formation. The vessels in the FGF-2/LN-1-treated bodies formed a tight capillary plexus-like structure (Fig. 2B).

Quantification of the CD31-positive vessel structures showed a synergistic effect of treatment with 5 ng/ml FGF-2 and 1 µg/ml LN-1 (Fig. 2C).
LN-1 Induces Tubular Morphogenesis of ECs in Three-dimensional Collagen I Gel—To understand the molecular mechanisms underlying the vessel promoting effect of LN-1, we performed tubular morphogenesis assays in the absence and presence of LN-1. IBE cells were seeded on a gel of polymerized type I collagen, treated with LN-1, FGF-2, or both, and embedded in a second layer of collagen I in the continued presence of the different supplements. Formation of tubules was initiated by cells treated with LN-1, FGF-2, or both at 7 h after seeding (Fig. 3). These structures were absent in the cultures devoid of supplement (control). At 24 h, control cells and cells treated with LN-1 only had undergone apoptosis. FGF-2-treated cells showed a network of branching lumen-containing tubules (25). Individual cells presented several protrusions that contributed to the branching and the varying thickness of the tubules. Cells treated with both LN-1 and FGF-2 displayed elongated structures of a more uniform thickness with less branching. Individual cells appeared fully integrated in the tubular structures.

It is possible that endogenous synthesis and deposition of certain laminin isoforms is critical for FGF-2-induced EC tubular morphogenesis, and we therefore analyzed laminin transcript levels. Cells were seeded on collagen gels and, for reference, on gelatin-coated dishes in the absence and presence of 20 ng/ml FGF-2 and incubated for 24 h. Transcript levels of four \( \alpha \)-chains, three \( \beta \)-chains, and three \( \gamma \)-chains were analyzed by PCR (Fig. 4A). Of the \( \alpha \)-chains, \( \alpha 1 \) was not detected and \( \alpha 3 \) and \( \alpha 5 \) were expressed at very low levels. FGF-2 treatment slightly increased \( \beta 2 \) expression, but there was no reproducible difference between the collagen gel and the gelatin-coated cultures in this respect.

To further search for transcript regulation of laminin chains during tubular morphogenesis, transcripts levels for the five \( \alpha \)-chains and the \( \beta 1 \)- and \( \gamma 1 \)-chains, as well as the collagen IV transcripts, were determined at several different early time points by real-time PCR (Fig. 4B). Cells were seeded on collagen gels and treated with or without 20 ng/ml FGF-2 for 1, 3, or 7 h. Transcripts for the \( \alpha 1 \)- and \( \alpha 3 \)-chains were not detected. Immunoblotting confirmed that the ECs did not express the \( \alpha 1 \) chain (data not shown). FGF-2 treatment increased \( \alpha 5 \) mRNA levels at 3 h and increased the \( \beta 1 \)-chain mRNA more than 4-fold at 7 h. This transient increase did not appear to result in increased levels of protein, however. By immunoblotting we
were unable to detect increased synthesis or secretion of laminins during tubular morphogenesis (data not shown).

To address the question of whether endogenous production of laminins is critical in tubular morphogenesis, human ECs were treated with neutralizing antibodies against laminin-binding integrin α6/β1 and α6/β3. Only marginal inhibition was detected (data not shown). Thus, analysis of laminin transcript levels, protein expression, and function suggested only a small, if any, contribution of changes in the expression levels of endogenous laminins during tubular morphogenesis.

Induction of Jagged-1 Expression by LN-1—To understand the molecular mechanisms underlying the angiogenic effect of exogenous LN-1, we asked whether LN-1 could modulate expression of angiogenic growth factors FGF-2 and VEGF-A and their receptors and the differentiation marker Jagged-1. Jagged-1 is a ligand for the Notch receptor family, and correct temporal activation of Notch receptors is critical for proper vascular development. Quantitative real-time PCR analyses were performed on RNA isolated from cells treated with FGF-2, LN-1, or a combination of the two for 1, 3, or 7 h (Fig. 5). Treatment with LN-1 led to increased expression levels of Jagged-1 and FGF-2/FGFR-1 at 3 and 7 h, respectively. In contrast, LN-1 did not significantly affect the expression levels of VEGF-A or VEGFR-2.

FGF-2 treatment led to increased transcription levels of Jagged-1 at 3 h, to an extent similar to that seen in the LN-1-treated cells. FGF-2 treatment also led to increased expression of both VEGF-A/VEGFR-2 and FGF-2/FGFR-1. For FGF-2 transcription, the effect was dramatic, with a close to 70-fold higher expression levels in the FGF-2-treated cells, indicating a positive autocrine loop.

Co-treatment of the IBE cells with FGF-2/LN-1 led to a synergistic increase in Jagged-1 transcripts at 1 and 3 h, but at 7 h the transcript levels were severalfold below that in the control cells. The dramatic effect of FGF-2 on FGF-2 transcript levels was attenuated in cells treated with FGF-2/LN-1. The increase in VEGF-A and VEGFR-2 transcript levels was slightly more pronounced in cells receiving combined treatment for 1 and 3 h compared with FGF-2 alone. Combined treatment led to a decrease at 7 h of FGFR-1, VEGF-A, and VEGFR-2 transcript levels, similar to the effect on Jagged-1 transcript levels. These data indicate fine-tuned regulation of the expression levels of angiogenic modulators in this model.

We further analyzed the level of Jagged-1 expression in IBE cells undergoing tubular morphogenesis by immunoblotting. IBE cells on collagen gels were lysed after treatment with FGF-2, LN-1, or both (Fig. 6). Individual treatment with LN-1
and FGF-2 increased expression of Jagged-1 at 3 h after seeding on collagen. Treatment with the combination of LN-1 and FGF-2 further increased the level of Jagged-1 expression. At 7 h, the effect of LN-1 treatment on Jagged-1 expression was essentially lost, whereas the FGF-2 effect remained. Blotting using phosphospecific ERK1/2- or phospho-Akt antibodies showed that LN-1 failed to activate these signal transduction components. Treatment with FGF-2 led to induction of phospho-ERK1/2 but not phospho-Akt (Fig. 6). Thus, both real-time PCR and immunoblotting showed that LN-1 alone could promote increased expression levels of Jagged-1, which may be an important mediator of the LN-1 effect on ECs.

**DISCUSSION**

In this paper, we show that LN-1 stimulates angiogenesis in the chicken CAM and in EBs. Remarkably, LN-1 promoted angiogenesis in the CAM assay to the same extent as FGF-2. We were not able to detect morphological or qualitative differences in the vessels formed by the two stimuli in the CAM or in EBs. This may be due to the presence of complementary endogenous angiogenic factors in these models. Tubular morphogenesis of ECs was promoted by LN-1 alone, accompanied by elevated expression of Jagged-1. We examined whether FGF-2-induced tubular morphogenesis involved endogenous production of laminin by the ECs. We did not detect endogenous production of LN-1 protein or laminin α1 mRNA. We also did not detect FGF-2- or matrix-induced changes in transcript levels at 24 h even though there were transient effects at earlier time points of α5- and β1-chains mRNAs. By immunoblotting for laminin β1- and γ1-chains, we were unable to detect increased secretion of laminins (data not shown). Moreover, blocking antibodies against laminin-binding integrins α3 and α4 did not prevent tubular morphogenesis of human ECs (data not shown). Combined, these data show that LN-1 is not synthesized by the EC model used in this study. Moreover, endogenously produced laminins are not critical in the induction of angiogenesis. We cannot rule out a contribution from endogenous α5-containing laminins or potential novel laminins for which we lack reagents. However, exogenous α5-chain-containing laminin (LN10/11) did not stimulate angiogenesis in the EB model (Fig. 2), which emphasizes the strong influence of LN-1 on *in vitro* angiogenesis.

Jagged-1 is a transmembrane ligand for the Notch family of receptors of which Notch4 is expressed on ECs (26), and both Notch ligands and receptors appear to be restricted to arterial endothelium (27). Upon activation, the Notch intracellular domain is proteolytically released and translocated to the nucleus to serve as a transcriptional regulator (see Ref. 28 for a review of Notch signaling). A number of data indicate that appropriate temporal activation of Notch receptors is required for proper vascular development and angiogenesis. Thus, both targeted *notch* gene inactivation and endothelial overexpression of constitutively activated Notch4 leads to severe vascular defects in mice (29, 30). *In vitro* studies show that Jagged-1 transcripts are increased during tubular morphogenesis of human umbilical cord ECs (31). Moreover, overexpression of Notch4 and Jagged-1 promote tubular morphogenesis by rat brain EC (32). On the other hand, activated Notch4 inhibits *in vitro* angiogenesis in fibrin gels and in the chicken chorioallantoic membrane assay (33). Thus, maintaining the proper expression levels of Jagged-1 and Notch receptors appears to be vital for EC function. This is in agreement with the transient increase in Jagged-1 transcript and protein levels we observed in the LN-1-treated EC.

The level of ERK and Akt phosphorylation was unaffected by LN-1 treatment, indicating that signaling induced by LN-1 does not converge with classical growth factor-induced signaling. Nevertheless, LN-1 treatment up-regulated mRNA levels of FGF-2 and FGFR-1, which may have contributed to the LN-1 activity. Interestingly, cotreatment with LN-1 and FGF-2 changed the mRNA levels of FGF-2, VEGF-A, FGFR-1, and VEGFR-2 in a temporal pattern similar to that observed for Jagged-1, with an early increase and a subsequent drop at 7 h. These data suggest that FGF-2 and VEGF-A signaling may be down-regulated as ECs become more differentiated. Accordingly, FGF-2 and genes regulating FGF-2 signaling are down-regulated in human umbilical cord ECs during differentiation (31). Moreover, VEGFR-2 appears to be important primarily in the early phase in angiogenesis and to a lesser extent in the mature vessel (4, 34).

In the EB model (Fig. 2), LN-1 treatment alone was not sufficient to induce angiogenesis. However, treatment with a suboptimal level of FGF-2 and a low level of LN-1 together resulted in massive angiogenesis, in a synergistic manner, as strong as treatment with high concentrations of FGF-2. The extent to which exogenous LN-1 was incorporated in vascular basement membranes in the embryoid bodies has not been studied in detail. However, exogenous administration of LN-1 has previously been shown to rescue epithelial development in EBs derived from embryonic stem cells expressing dominant-negative FGFR-2 (35) or lacking expression of laminin γ1 (36) or integrin β1 (37), all of which are unable to secrete or produce LN-1. Whether these LN-1-deficient EBs still allow vascular development has not been examined.

The concept of an angiogenesis-promoting function of LN-1 may be difficult to reconcile with its restricted, preferentially epithelial expression pattern (38). However, by using sensitive detection methods, a low but rather general tissue expression of LN-1 in the adult mouse was recently reported, in addition to high epithelially derived expression in some tissues (16). Interestingly, LN-1 was found in sinusoids in liver, indicating a restricted perivascular expression *in vivo*. Furthermore, during active angiogenesis, the invading angiogenic sprout could become exposed to extravascular LN-1. Lastly, we cannot exclude that LN-1 is mimicking a thus far unidentified pro-angiogenic laminin (containing a putative laminin-α6), which is produced by ECs but not detected by the available reagents. In accordance with a role for LN-1 in promoting angiogenesis, Kleinman and colleague (39) have identified structurally related proangiogenic peptides derived from the amino termini of the α1- and γ1-chains (denoted A13 and Y16, respectively). Administration
of LN-1 and A13 increases tumor growth in an ovarian cancer model in nude mice (40). Furthermore, a scrambled and modified version of peptide C16 (C16Y), a potent inhibitor of EC adhesion to LN-1, blocks angiogenesis and tumor growth (41).

In conclusion, based on data from three angiogenesis models we suggest that: (a) LN-1 may play a more active role in angiogenesis than previously recognized possibly by stabilizing the differentiating vessel; (b) LN-1 induces expression of the differentiation marker Jagged-1 through direct effects on ECs; and (c) LN-1 signaling may converge with FGF-2-signaling to promote angiogenesis.

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REFERENCES

1. Griffioen, A. W., and Molema, G. (2000) Pharmacol. Rev. 52, 237–268
2. Bodoly, E., Koch, A. E., Kim, J., Szegedi, G., and Szekanecz, Z. (2002) J. Cell Mol. Med. 6, 357–376
3. Carmeliet, P., and Jain, R. K. (2000) Nature 407, 249–257
4. Papetti, M., and Herman, I. M. (2002) Am. J. Physiol. 282, C947–C970
5. Cross, M. J., and Claesson-Welsh, L. (2001) Trends Pharmacol. Sci. 22, 201–207
6. Brooks, P. C., Montgomery, A. M., Rosenfeld, M., Reisfeld, R. A., Hu, T., Klier, G., and Cheresh, D. A. (1994) Cell 79, 1157–1164
7. Brooks, P. C., Clark, R. A., and Cheresh, D. A. (1994) Science 264, 569–571
8. Colognato, H., and Yurchenco, P. D. (2000) Dev. Dyn. 218, 213–234
9. Libby, R. T., Champlaud, M. F., Claudepierre, T., Xu, Y., Gibbons, E. P., Koch, M., Burgess, R. E., Hunter, D. D., and Brunken, W. J. (2000) J. Neurosci. 20, 6517–6528
10. Dai, M., Thyboll, J., Kortesmaa, J., Janson, K., Ivanainen, A., Parivard, M., Timpl, R., Hedin, U., Swedenborg, J., and Tryggvason, K. (2002) J. Biol. Chem. 277, 12741–12748
11. Thyboll, J., Kortesmaa, J., Cao, R., Soininen, R., Wang, L., Ivanainen, A., Sorokin, L., Riesing, M., Cao, Y., and Tryggvason, K. (2002) Mol. Cell. Biol. 22, 1194–1202
12. Ljubimova, J. Y., Lakhter, A. J., Loksh, A., Yong, W. H., Riedinger, M. S., Miner, J. H., Sorokin, L. M., Ljubimov, A. V., and Black, K. L. (2001) Cancer Res. 61, 5601–5610
13. Sorokin, L. M., Pausch, F., Durbeej, M., and Ekblom, P. (1997) Dev. Dyn. 209, 446–462
14. Patton, B. L., Miner, J. H., Chiu, A. Y., and Sanes, J. R. (1997) J. Cell Biol. 139, 1507–1521
15. Miner, J. H., Cunningham, J., and Sanes, J. R. (1998) J. Cell Biol. 143, 1713–1723
16. Sasaki, T., Gitay, R., Talts, U., Timpl, R., and Talts, J. F. (2002) Exp. Cell Res. 275, 185–199
17. Smyth, N., Vatansever, H. S., Murray, P., Meyer, M., Frie, C., Paulsson, M., and Edgar, D. (1999) J. Cell Biol. 144, 151–160
18. Malinda, K. M., Nomizu, M., Chung, M., Delgado, M., Kuratomi, Y., Yamada, Y., Kleinman, H. K., and Ponec, M. L. (1999) FASEB J. 13, 53–62
19. Schnaper, H. W., Kleinman, H. K., and Grant, D. S. (1993) Kidney Int. 43, 20–25
20. Ponec, M. L., Nomizu, M., and Kleinman, H. K. (2001) FASEB J. 15, 1389–1397
21. De Arcangelis, A., Lefebvre, O., Mechine-Neuville, A., Arnold, C., Klein, A., Remy, L., Riedinger, M., and Simon-Assmann, P. (2001) Int. J. Cancer 94, 44–53
22. Kanda, S., Landgren, E., Ljungstrom, M., and Claesson-Welsh, L. (1996) Cell Growth & Diff. 7, 383–395
23. Sasaki, T., Larsson, H., Kreuger, J., Salminivirta, M., Claesson-Welsh, L., Lindahl, U., Hohenester, E., and Timpl, R. (1999) EMBO J. 18, 6240–6248
24. Friedlander, M., Brooks, P. C., Shaffer, R. W., Kincaid, C. M., Varner, J. A., and Cheresh, D. A. (1995) Science 270, 1500–1502
25. Rahamanian, M., Pertofi, H., Kanda, S., Christofferson, R., Claesson-Welsh, L., and Heldin, P. (1997) Exp. Cell Res. 237, 223–230
26. Uyttendaele, H., Marazzi, G., Wu, G., Yan, Q., Sassone, D., and Kitajewski, J. (1996) Development 122, 2251–2259
27. Villa, N., Walker, L., Lindsell, C. E., Gasson, J., Iruela-Arispe, M. L., and Weinmaster, G. (2001) Mech. Dev. 108, 161–164
28. Kadesch, T. (2000) Exp. Cell Res. 260, 1–8
29. Krebs, L. T., Xue, Y., Norton, C. R., Shutter, J. R., Maguire, M., Sundberg, J. P., Gallahan, D., Clouston, V., Kitajewski, J., Callahan, R., Smith, G. H., Stark, K. L., and Gribely, T. (2000) Genes Dev. 14, 1245–1252
30. Uyttendaele, H., Ho, J., Rossant, J., and Kitajewski, J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5643–5648
31. Bell, S. E., Mavila, A., Salazar, R., Bayless, K. J., Kanagula, S., Maxwell, S. A., and Davis, G. E. (2001) J. Cell Biol. 154, 2755–2773
32. Uyttendaele, H., Clouston, V., Wu, G., Roux, F., Weinmaster, G., and Kitajewski, J. (2000) Microvasc. Res. 69, 91–103
33. Leong, K. G., Hu, X., Li, L., Noseda, M., Larrivee, B., Hull, C., Hood, L., Wong, F., and Karsan, A. (2002) Mol. Cell. Biol. 22, 2830–2841
34. Meduri, G., Bausero, P., and Perrot-Plantaz, M. (2000) Biol. Reprod. 62, 439–447
35. Li, X., Chen, Y., Scheele, S., Arman, E., Hoffner-Krausz, K., Ekblom, P., and Lonai, P. (2001) J. Cell Biol. 153, 811–822
36. Aumailley, M., Pesch, M., Tunggal, L., Gaill, F., and Fassler, R. (2000) J. Cell Sci. 113, 259–268
37. Li, S., Harrison, D., Carbonetto, S., Fassler, R., Smyth, N., Edgar, D., and Yurchenco, P. D. (2002) J. Cell Biol. 157, 1279–1290
38. Ekblom, P., Lonai, P., and Talts, J. F. (2003) Matrix Biol. 22, 35–47
39. Ponec, M. L., and Kleinman, H. K. (2003) Exp. Cell Res. 285, 189–195
40. Yoshida, Y., Hosokawa, K., Dantes, A., Kotsui, F., Kleinman, H. K., and Amsterdam, A. (2001) Int. J. Oncol. 18, 913–921
41. Ponec, M. L., Hihino, S., Lebioda, A. M., Mochizuki, M., Nomizu, M., and Kleinman, H. K. (2003) Cancer Res. 63, 5060–5064
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