Novel Proangiogenic Effect of Factor XIII Associated With Suppression of Thrombospondin 1 Expression

Rima Dardik,* Arieh Solomon,* Joseph Loscalzo, Regina Eskaraev, Ann Bialik, Iris Goldberg, Ginette Schiby, Aida Inbal

Objective—Factor XIII (FXIII), a plasma transglutaminase that stabilizes fibrin clots at the final stages of blood coagulation by crosslinking fibrin monomers, is essential for embryonic implantation and participates in tissue remodeling and wound healing, processes that involve angiogenesis. The aim of our study was to analyze the effect of FXIII on angiogenesis using in vitro and in vivo models and to examine the role of FXIII in the basic steps of angiogenesis, ie, migration, proliferation, and apoptosis/cell survival.

Methods and Results—In the Matrigel tube formation model, only FXIIIa caused a dose-dependent enhancement of array formation. This proangiogenic effect was not associated with alterations in vascular endothelial growth factor (VEGF) protein levels nor VEGF or VEGFR2 mRNA levels. FXIIIa, but not nonactivated or transglutaminase-inactivated FXIII, significantly enhanced endothelial cell migration and proliferation and inhibited apoptosis. After treatment of HUVECs with FXIIIa, almost complete disappearance of mRNA of thrombospondin 1 (TSP-1) and a marked reduction in the secretion of TSP-1 protein were observed. A reduction in TSP-1 protein synthesis, although to a lesser extent, was observed on treatment of microvascular endothelial cells with FXIIIa. In a rabbit cornea model, injection of FXIIIa caused neovascularization associated with almost complete disappearance of TSP-1 in the cornea.

Conclusions—These results show that FXIIIa exhibits a novel proangiogenic activity that is associated with downregulation of TSP-1 and also involves stimulation of endothelial cell proliferation and migration and inhibition of apoptosis. These findings might shed light on the mechanism by which FXIII mediates tissue repair and remodeling. (Arterioscler Thromb Vasc Biol. 2003;23:1113-1120.)

Key Words: PLEASE ■ SUPPLY ■ KEYWORDS

Angiogenesis is the process of formation of new capillaries from preexisting blood vessels and is an essential process in embryonic development, normal physiological growth, wound healing, and tumor expansion. The process of angiogenesis consists of several steps, which include the stimulation of endothelial cells by growth factors, degradation of the extracellular matrix by proteolytic enzymes, migration and proliferation of endothelial cells, and, ultimately, capillary tube formation. Inhibition of endothelial cell apoptosis to promote cell survival is also considered to be essential for angiogenesis. Endothelial cell migration and proliferation are critical steps in the angiogenic process.

Factor XIII (FXIII) is a plasma transglutaminase that stabilizes fibrin clots in the final stages of blood coagulation. Thrombin-activated FXIII catalyzes formation of covalent crosslinks between γ-glutamyl and ε-lysyl residues of adjacent fibrin monomers to yield the mature clot. FXIII circulates in plasma as a heterotrimer composed of 2 A-subunits and 2 B-subunits. The A-subunit contains the active site of the enzyme and is synthesized by hepatocytes, monocytes, and megakaryocytes. The B-subunit serves as a carrier for the catalytic A-subunit in plasma and is synthesized by the liver.

The FXIII A-subunit gene belongs to the transglutaminase family, which comprises at least 8 tissue transglutaminases. These enzymes crosslink various proteins and are involved in many physiological and pathological processes, such as hemostasis, wound healing, tumor growth, skin formation, and apoptosis. Similar to tissue transglutaminases, FXIII participates in tissue remodeling and wound healing, as can be inferred from a defect in wound repair observed in patients with inherited FXIII deficiency. FXIII also participates in implantation of the embryo during normal pregnancy; women homozygous for FXIII deficiency experience recurrent miscarriages.

Wound healing as well as embryo implantation are complex processes that involve cell proliferation and angiogene-
sis. Relatively little is known about the role of FXIII in angiogenesis. Human recombinant tissue transglutaminase was shown to enhance angiogenesis in a rat dorsal skin flap chamber.\(^6\) Dallabrida et al\(^6\) showed that FXIII can support endothelial cell adhesion and modulate tube formation in a fibrin gel. The participation of FXIII in embryo implantation and wound healing implies that FXIII may have a role in angiogenesis. Thus, the aim of our study was to analyze the effect of FXIII on angiogenesis using in vitro and in vivo models and to examine the role of FXIII in the basic steps of angiogenesis, ie, endothelial cell migration, proliferation, and apoptosis/cell survival.

Methods

Cells

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords and maintained in EGM-2 medium (Clonetics) by standard procedures as described previously.\(^10\) Cells from the first and second passages were used for the Matrigel tube formation assays, and cells from the fourth to sixth passages were used for all other experiments.

Human microvascular dermal (neonatal) endothelial cells (HMVEC-d) were purchased from Clonetics and cultured in EGM-MV medium (Clonetics) according to the manufacturer's instructions. Cells from the fourth passage were used for the experiments.

FXIII Activation

The source of FXIII was FXIII concentrate, Fibrogammin-P (Aventis Behring). After reconstitution, 2 mL of 100 U/mL (approximately 1000 \( \mu \)g/mL) FXIII was incubated with thrombin immobilized on Activator \( \times 1000 \). One milliliter of packed buffy volks 200 \( \mu \)L of bovine thrombin, 1 mg/mL of trypsin, and 1 mmol/L of CaCl\(_2\) was added, and the mixture was incubated at 37°C for 2 hours. FXIII activation was monitored by measuring FXIII activity using a chromogenic assay (Berichrome, Dade Behring). FXIIIa was inactivated by treatment with 3 mmol/L iodoacetamide (Sigma) for 30 minutes at 22°C to block transglutaminase activity; free iodoacetamide was then removed by dialysis.

Matrigel Assay

HUVECs (1 \( \times \) 10\(^3\) cells/well) were plated on Matrigel (Becton Dickinson) previously diluted 1:1 with medium M199 and allowed to adhere for 2 hours at 37°C. Either FXIII or FXIIIa was then added at concentrations of 10, 20, or 50 \( \mu \)g/mL. In some experiments, 50 \( \mu \)g/mL FXIIIa was added together with polyclonal goat anti-FXIII antibody (Affinity Biologicals; 50 \( \mu \)g/mL). Goat anti TSP-1 antibody (Santa Cruz; 50 \( \mu \)g/mL) was added either alone or in combination with 50 \( \mu \)g/mL FXIIIa in other experiments. Endothelial array or tube formation in Matrigel was visualized by light microscopic examination.

HUVEC Migration Assays

Migration of ECs was measured using 2 assays, the Boyden migration assay and the wound migration assay. The migration assay was performed by a modification of the procedure described previously using a Boyden chamber (Neuro Probe).\(^11\) HUVECs grown overnight in EBM media (Clonetics) were treated with trypsin, resuspended in EBM media at 100,000 cells/300 \( \mu \)L, and added to the upper chamber. Added to the lower chamber was 50 \( \mu \)g/mL FXIII, FXIIIa, or iodoacetamide-treated FXIIIa. A polycarbonate filter (8-\( \mu \)m pores) was placed between upper and lower chambers. The chamber was incubated for 24 hours at 37°C. After incubation, the filter was removed and the cells migrating through the filter and adhering to the lower chamber were incubated for 1 hour at 37°C with 4 \( \mu \)g/mL Calcein AM (Molecular Probes) in Hanks’ buffered saline and counted in a plate reader (SpectraMax Gemini, Molecular Devices Corp). The wound-migration assay has been previously described and used with slight modification.\(^12\) Briefly, monolayers of HUVECs were grown in Petri dishes. A small incision was made adjacent to the confluent cell-dense region using a small razor blade. Cells were removed from approximately half of the plate by gentle scraping. After wounding, the cells were allowed to migrate for 24 hours. Migration of the cells across the sharp wound edge to the cell-free region was assessed by phase microscopy and counting the cells grown in cell culture medium in the absence or presence of 50 \( \mu \)g/mL FXIII, FXIIIa, or iodoacetamide-treated FXIIIa.

\[^{3}H\]-Thymidine Incorporation

HUVECs were seeded on 24-well tissue culture plates at a density of 1 \( \times \) 10\(^3\) cells/well. After 24 hours, the cell culture media was replaced with serum-free media, and 48 hours after seeding, 50 \( \mu \)g/mL FXIII, FXIIIa, iodoacetamide-treated FXIIIa, or 10 \( \mu \)g/mL bFGF was added. Seventy-two hours after seeding, 2% FBS was added to the media along with 1 \( \mu \)Ci/well \[^{3}H\]-thymidine, and the cells were incubated for an additional 16 hours. After this time period, the cells were washed twice with PBS and incubated in 10% TCA overnight at 4°C. The cells were then washed twice with 100% ethanol and dried. Precipitated DNA and protein were solubilized with 1 N NaOH, and the radioactivity was counted in a liquid scintillation counter.

MTT Assay

HUVECs were seeded in a 96-well tissue culture plates at a density of 5 \( \times \) 10\(^3\) cells/well. After 24 hours, the cell culture media was replaced with serum-free media, and 48 hours after seeding, 50 \( \mu \)g/mL FXIII, FXIIIa, iodoacetamide-treated FXIIIa, or 10 \( \mu \)g/mL bFGF was added. Seventy-two hours after seeding, 2% FBS was added to the media and incubated for 16 hours. After this incubation, 1 mg/mL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide; Sigma) was added to the media and the culture incubated for an additional 3 hours. After the incubation, the medium was removed, the cells were solubilized in isopropanol containing 0.4 N HCl and 10% Triton X-100, and the amount of the dye released from the cells was measured at 560 nm by ELISA reader (Molecular Devices). Final quantification of dye release was obtained after subtraction of absorbance at 650 nm.

Apoptosis Assay

Apoptosis in HUVECs was determined by TUNEL using an in situ cell death detection kit (Roche) in which dUTP is labeled with fluorescein to allow the direct detection of the labeled DNA strand breaks. Cells grown overnight in complete EGM-2 medium (Clonetics) were treated with 50 \( \mu \)g/mL FXIII, FXIIIa, or iodoacetamidetreated FXIIIa for 24 hours after which they were fixed, permeabilized, and labeled according to the manufacturer’s instructions. To highlight the nuclei, the cells were also stained with DAPI in mounting media (Vector) and covered with cover slips. The apoptotic cells were counted by direct visualization using fluorescence microscopy.

Gene Array Analysis

Total RNA from HUVECs treated with 50 \( \mu \)g/mL FXIIIa for 16 hours at 37°C or of control, untreated cells was isolated using the Qiagen RNA isolation kit (Qiagen). RNA was then reverse transcribed into cDNA by MMLV reverse transcriptase (Promega) in the presence of 50 \( \mu \)Ci \[^{32}P\]dCTP. Each radiolabeled cDNA sample was hybridized to a gene array membrane containing 100 genes associated with angiogenesis using the hybridization buffer supplied by the manufacturer. Hybridization conditions and washings of the membranes were performed according to the manufacturer’s instructions (Superarray Inc). The membranes were exposed to Kodak X-ray film for 16 hours at \(-70°C\), and the autoradiograms were compared.

Western Blot Analysis of TSP-1 in Conditioned Medium

HUVECs or HMVEC-d were incubated with FXIIIa at concentrations of 10, 20, or 50 \( \mu \)g/mL for 16 hours at 37°C. Conditioned
medium of FXIIIa-treated and untreated cells was collected, centrifuged for 5 minutes at 700g, and incubated with heparin-Sepharose (5 mL medium/0.5 mL of packed beads). The beads were then washed with 10 mL TBS (0.15 mol/L NaCl and 20 mmol/L Tris-HCl, pH 7.5) followed by washing with 10 mL TBS, 0.25 mol/L NaCl, and 20 mmol/L Tris-HCl, pH 7.5. The beads were boiled in 0.2 mL nonreducing electrophoresis sample buffer, and the eluted protein was subjected to 3% to 8% SDS-PAGE gel (Novex). The SDS gel was electrophoretically transferred to an Immobilon membrane (Millipore). The blot was blocked in a 3% BSA solution in TBS (TBS/BSA) for 16 hour at 4°C and incubated with a monoclonal anti-TSP-1 antibody (Sigma) diluted 1:500 in TBS/BSA for 2 hours at 22°C. The blot was washed with TBS containing 0.1% Tween-20 (TBS/Tween) and incubated with peroxidase-conjugated goat anti-mouse immunoglobulins (Jackson ImmunoResearch Laboratories) at 1:2000 in TBS/Tween for 1 hour at 22°C. After washing in TBS/Tween, the blot was developed using an enhanced chemiluminescence kit (ECL; Amersham Pharmacia Biotech).

Analysis of VEGF Level in Conditioned Medium

Supernatants of HUVECs grown either in the absence or presence of FXIIIa (50 μg/mL) were analyzed for VEGF levels using the VEGF DuoSet ELISA kit (R&D Systems): 1A, 1C, and 1D). No effect was observed on addition of FXIII or FXIIIa, and array formation was analyzed 16 hours after plating. Addition of 20 to 100 μg/mL of FXIII caused a marked, dose-dependent increase in endothelial cell content, resulting in a significant increase in tube thickness compared with that observed in untreated samples (Figures 1A, 1C, and 1D). The effect of FXIIIa was abolished by a polyclonal anti-FXIII antibody (50 μg/mL; Figure 1F).

Immunohistochemical Analysis of TSP

Sections from FXIIIa- or PBS-treated rabbit corneas were incubated with polyclonal goat anti-human TSP-1 (1:100 in TBS/BSA; Santa Cruz Biotechnology) for 2 hours at 22°C. The slides were then washed with TBS and incubated with biotinylated anti-goat IgG for 1 hour at 22°C, followed by washing in TBS and incubation with avidin-conjugated peroxidase (ABC kit; Vector) for 1 hour at 22°C and stained with 3-amino-9-ethyl carbazole substrate (Zymed). The slides were counterstained with hematoxylin.

Statistical Analysis

The data were analyzed by one-way ANOVA with post hoc comparisons. P<0.05 was considered significant.

Results

Effect of FXIIIa on Endothelial Tube Formation

HUVECs were plated on Matrigel in the presence or absence of FXIII or FXIIIa, and array formation was analyzed 16 hours after plating. Addition of 20 to 100 μg/mL of FXIIIa caused a marked, dose-dependent increase in endothelial cell content, resulting in a significant increase in tube thickness compared with that observed in untreated samples (Figures 1A, 1C, and 1D). No effect was observed on addition of nonactivated (Figure 1B) or acetamide-treated (Figure 1E) FXIIIa. Iodoacetamide-treated FXIIIa decreased migration by 30%. Similar results were obtained using the Boyden-migration assay (Figure 2); 50 μg/mL FXIIIa increased migration ≈2.5-fold (258±72% of control) (P<0.001 by ANOVA), whereas no significant

In Vivo Model of Rabbit Cornea Vascularization

New Zealand albino male rabbits (3.0 kg in weight) were used in this study. Surgery and care were performed according to ARVO guidelines for animal research. General anesthesia was achieved by intramuscular injection of xylazine 2% and ketamine HCl. Using a guidelines for animal research. General anesthesia was achieved by intramuscular injection of xylazine 2% and ketamine HCl. Using a

Effect of FXIII on Endothelial Cell Migration

In the wound migration assay, migration of the cells across the sharp wound edge to the cell-free region was assessed. As shown in Figure 2, 50 μg/mL FXIIIa increased the migration of the cells greater than 7-fold (747±328% of control) (P<0.001 by ANOVA). Treatment of the cells with nonactivated FXIII had no effect on migration. Iodoacetamide-treated FXIIIa decreased migration by 30%. Similar results were obtained using the Boyden-migration assay (Figure 2); 50 μg/mL FXIIIa increased migration ≈2.5-fold (258±72% of control) (P<0.001 by ANOVA), whereas no significant
change in migration was observed with treatment of the cells with similar concentrations of FXIII or iodoacetamide-treated FXIIIa.

**Effect of FXIII on Endothelial Cell Proliferation**

[3H]-thymidine incorporation and MTT uptake were used to determine HUVEC proliferation in the presence or absence of FXIII. As shown in Figure 3A, 50 μg/mL FXIIIa increased the proliferation of HUVECs by approximately 64% (164±5% of control) (P<0.001 by ANOVA). In comparison, addition of 10 ng/mL bFGF (positive control) enhanced cell proliferation by approximately 93% (193±25 of control). Similarly, in MTT uptake experiments, an increase in cell proliferation by 70% on exposure to 50 μg/mL FXIIIa was observed (170±17%; Figure 3B). By contrast, neither FXIII nor iodoacetamide-treated FXIIIa significantly affected proliferation.

**Effect of FXIII on Endothelial Cell Apoptosis**

The effect of FXIII on apoptosis of HUVECs was studied by the TUNEL assay. Figure 4 shows that only in cells exposed to FXIIIa (50 μg/mL) did the number of apoptotic cells decrease significantly compared with untreated cells (0.21±0.09% versus 1.08±0.16%, respectively; P<0.001 by ANOVA). The apoptotic response to the same concentration of FXIII or iodoacetamide-treated FXIIIa-induced apoptosis is similar to that of the untreated cells.

**Effect of FXIIIa on VEGF Production and Secretion**

To explore the mechanism underlying the proangiogenic effect of FXIIIa, we measured VEGF in the supernatant of cultured HUVECs. In addition, the mRNA levels of either VEGF or VEGF receptor-2 (VEGFR2) from HUVECs were assessed by reverse transcriptase–polymerase chain reaction. Neither VEGF protein level nor steady-state VEGF or VEGFR2 mRNA levels were affected by FXIIIa (data not shown).

![Figure 2](image2.png)

**Figure 2.** Effect of FXIII on HUVEC migration using Boyden chamber (black bars) and wound-migration (white bars) assays. In the Boyden assay, each data point is presented as a percent of control from the average±SD of 15 samples in each group. *P<0.001 by ANOVA. In the wound-migration assay, 5 to 10 random fields were examined. Each data point is presented as a percent of control from the average±SD of 12 samples in each group. **P<0.001 by ANOVA.

![Figure 3](image3.png)

**Figure 3.** Effect of FXIII on HUVEC proliferation. HUVECs were seeded and processed as described in Methods. A, [3H]-thymidine incorporation; B, MTT assay. Each data point is presented as a percent of control from the average±SD of 6 samples in each treatment group. *P<0.001 by ANOVA.
Effect of FXIIIa on Endothelial Cell Gene Expression
After treatment of HUVECs with FXIIIa, a modest reduction in the mRNA of the following genes was observed: endoglin, bFGF, integrin α5 subunit, PLGF, and SPARC (osteonectin); however, the most striking finding was the almost complete disappearance of thrombospondin 1 mRNA (Figure I, please see http://atvb.ahajournals.org).

Effect of FXIIIa on TSP-1 Synthesis
As shown by Figure 5A, TSP-1 protein was markedly reduced in a dose-dependent manner in the conditioned medium obtained from each HUVEC and HMVEC-d cultures grown in the presence of FXIIIa compared with controls. The basal amount of TSP1 secreted from HMVEC-d was much lower than that of HUVEC (Figure 5, lanes 0). Blockade of the transglutaminase activity of FXIIIa by iodoacetamide reversed the attenuating effect of FXIIIa on TSP-1 secretion in both cell types (Figure 5B).

To confirm the role of TSP-1 in the effects observed in Matrigel assays, HUVECs were plated on Matrigel in the presence or absence of either anti-TSP-1 antibody or FXIIIa, and tube formation was analyzed 16 hours after plating. Addition of 50 μg/mL of anti-TSP-1 antibody caused enhancement of endothelial cell proliferation similar to that observed with 50 μg/mL FXIIIa (Figures IIA through IIC, please see http://atvb.ahajournals.org).

In Vivo Effect of FXIII on Rabbit Cornea Neovascularization
FXIIIa was injected into the right eye of each of the 4 rabbits as outlined in Methods. The effect of FXIIIa on corneal vascularization was compared with that observed after injection of PBS into the left eye. In contrast to the left eyes treated with PBS, blood vessel formation was obvious in the right eyes of all 4 rabbits 48 hours after injection of FXIIIa (Figure III, please see http://atvb.ahajournals.org).

Histological section of the cornea shown in Figure 6B demonstrates the rich network of capillaries grown in the cornea of eyes treated with FXIII compared with those treated with PBS (Figure 6A). Staining of the FXIIIa–treated sections of the cornea with endothelial cell marker isolecitin B4 showed positive stain in the membranes of endothelial cells lining well-formed but also abortive blood vessels (Figure 6C).

Figure 4. Effect of FXIII on HUVEC apoptosis (TUNEL assay). The apoptotic cells were counted by direct visualization using fluorescence microscopy. To calculate the percentage of apoptotic cells in each case, 5 to 10 random fields were examined. Each bar represents average±SD of 8 samples in each treatment group. *P<0.001 by ANOVA.

Figure 5. Effect of FXIIIa on TSP-1 protein level in HUVECs and HMVECs in the absence (A) or presence (B) of the transglutaminase inhibitor iodoacetamide. TSP-1 purified by heparin-affinity chromatography from conditioned media of HUVEC or HMVEC-d grown either in the absence of FXIIIa (line 0) or in the presence of FXIIIa (A) or iodoacetamide-treated FXIIIa (ido-FXIII) (B) at the concentrations (μg/mL) indicated above each lane was subjected to SDS-PAGE and Western blot analysis by a monoclonal anti-TSP-1 antibody B7.
Immunohistochemical staining of the PBS-injected (control) corneas (Figure 6D) for TSP-1 disclosed positive staining in the stromal fibers (collagen fibers) and a few stromal cells (young fibroblasts). In contrast, in FXIIIa-treated corneas, the stromal elements were negative for TSP-1 (Figure 6E).

Discussion

The results of our study show for the first time that FXIIIa has proangiogenic activity. Endothelial cell migration and proliferation are essential steps in the angiogenic process. To understand the role of FXIII in the complex pathways of angiogenesis, we first examined its effect on endothelial cell migration, proliferation, and apoptosis in vitro. At a concentration that significantly enhances HUVEC proliferation in tubes formed in Matrigel, only the activated form of FXIII increased the migration of HUVECs, augmented the rate of thymidine incorporation, and decreased the number of apoptotic cells. Because endothelial cell migration, proliferation, and apoptosis are crucial steps in blood vessel formation, our results are consistent with the proangiogenic nature of FXIIIa. It was previously reported that endothelial cell proliferation and migration are regulated by the binding of VEGF family members to the VEGF receptors Flt-1, Flk-1/KDR, and Flt-4. In our study, neither VEGF protein level nor VEGF or VEGFR2 mRNA levels were affected by FXIIIa.

The mechanism by which FXIIIa affects cell proliferation, migration, and apoptosis is not known. It may be related to the nuclear localization of the enzyme, where it can complex directly with DNA or with other nuclear factors, thereby affecting DNA transcription. A second possibility involves FXIII-inducible signaling initiating downregulation of TSP-1, which is a known inhibitor of migration and proliferation and inducer of apoptosis.

Our observations that FXIIIa exhibits proangiogenic activity in the Matrigel model of angiogenesis contradict the data reported by Dallabrida et al, who showed that FXIIIa inhibits capillary tube formation by human microvascular endothelial cells in a fibrin gel. The reason for this discrepancy is not known. It may stem from the differences in the experimental microenvironment (fibrin gel versus Matrigel), or it may indicate that FXIIIa may have variable effects on different types or sources of endothelial cells. The cells used by this group were HMEC-1, ie, SV40-transformed dermal microvascular endothelial cells that generate much less TSP-1 compared with HUVECs. Thus, if the proangiogenic effect of FXIIIa is mediated at least in part by TSP-1, it is possible that cells with lower TSP-1 synthesis will be less responsive to the effect of FXIIIa.

TSP-1 is one of the best-characterized antiangiogenic factors. The regulation of angiogenesis by TSP-1 is complex and involves direct and indirect effects on endothelial cells. In 2 reports, the indirect effects seem to outweigh the direct inhibition of endothelial cell function by TSP-1. In a substantial number of studies, TSP-1 has been shown to inhibit angiogenesis in vitro by inhibiting endothelial cell proliferation, migration, and induction of apoptosis. The antiangiogenic activity of TSP-1 is exerted via binding to the CD36 receptor on the endothelial cell membrane, leading to the recruitment of signaling pathway kinases that ultimately trigger apoptosis.

Previous reports suggested that TSP-1 might be involved in the process of wound healing. Streit et al have shown that overexpression of TSP-1 delays cutaneous wound healing and granulation tissue formation in transgenic mice. In addition, the authors showed that overexpression of TSP-1 significantly inhibited endothelial cell mitogenesis and wound angiogenesis. In accordance with this report and based on our data, overexpression of TSP-1 may occur in the wound area of patients with FXIII deficiency, accounting for insufficient angiogenesis required for wound healing.

Our findings demonstrate that neovascularization of rabbit cornea is associated with almost complete loss of TSP-1. In accordance with our observations, TSP-1 was previously found to inhibit neovascularization in a mouse cornea model; however, it was reported that stimulatory and inhibitory domains exist in TSP-1.

The crosslinking activity of FXIIIa is very similar to that of tissue transglutaminase. Our data show that the transglutaminase activity of FXIIIa is required for suppression of TSP-1 expression, suggesting that crosslinking of TSP-1 may play a role in the proangiogenic effect of FXIIIa.

The FXIIIa-mediated proangiogenic effect may finally clarify its role in vascular remodeling and tissue repair. Based on our findings, we suggest the following hypothesis: during tissue repair processes, plasma FXIII as well as FXIII exposed on the surface of platelets or macrophages are activated by thrombin or other serine proteases and subsequently induce endothelial cell migration and proliferation at the site of injury. The associated downregulation of TSP-1 may be responsible, at least in part, for the complex multi-

**Figure 6.** Representative histological section of rabbit cornea excised 96 hours after injection of PBS (A and D) or FXIIIa (B, C, and E). A and B, H&E stain, magnification $\times 400$. Blood vessels are denoted by arrows. C, GSLI-isoelectin B$_2$ staining. Immunohistochemical staining for TSP-1 and counterstaining with hematoxylin of cornea sections injected with PBS (D) or FXIIIa (E).
factorial process of new vessel formation required for remodeling and wound repair in this paradigm.

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References

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Figure I. Effect of FXIIIa on HUVEC gene expression

Radiolabeled cDNA of HUVEC incubated with either control medium or medium containing 50 µg/ml FXIIIa for 16 hr at 37°C was hybridized to membranes containing 96 genes relevant to angiogenesis. Following hybridization and washes, the membranes were subjected to autoradiography. The modestly reduced genes are shown by thin arrows. The almost complete disappearance of TSP-1 is delineated with a thick arrow.
ENG - endoglin; bFGF – basic FGF; PlGF – Placental Growth Factor; α5 – the α subunit of the fibronectin receptor integrin; SPARC = osteonectin.

Figure II. Effect of anti TSP1 antibody on array formation in Matrigel

1x10^5 HUVEC were plated on Matrigel and incubated with either control medium (A), medium containing 50 µg/ml of FXIIIa (B), medium containing 50 µg/ml of goat anti- TSP1 antibody (C) for 16 h at 37°C and processed as described in Materials and Methods.

Figure III. Effect of FXIIIa on rabbit cornea neovascularization

A-Normal, non injected rabbit eye. B and C- Rabbit eye 48 hr and 72 hr, respectively, following injection of 20 µg FXIIIa. Arrows point to the new vessels formed in response to FXIIIa injection. C presents a wider, denser and deeper vascular network as compared to B.
Online Figure I