Telomere dysfunction contributes to reduced cell viability, altered differentiation, and impaired regenerative/proliferative responses. Recent advances indicate that telomerase activity confers a pro-angiogenic phenotype to endothelial cells and their precursors. We have investigated whether telomerase contributes to tissue regeneration following hind limb ischemia and vascular endothelial growth factor 165 (VEGF$_{165}$) treatment. VEGF delivery induced angiogenesis and increased expression of the telomerase reverse transcriptase (TERT) and telomerase activity in skeletal muscles and satellite and endothelial cells. Adenovirus-mediated transfer of wild type TERT but not of a dominant negative mutant, TERTdn, significantly induced capillary but not arteriolar formation. However, when co-delivered with VEGF, TERTdn abrogated VEGF-dependent angiogenesis, arteriogenesis, and blood flow increase. This effect was paralleled by in vitro evidence that telomerase inhibition by 3'-azido-3'-deoxythymidine in VEGF-treated endothelial cells strongly reduced capillary density and promoted apoptosis in the absence of serum. Similar results were obtained with adenovirus-mediated expression of TERTdn and AKTdn, both reducing endogenous TERT activity and angiogenesis on Matrigel. Mechanistically, neo-angiogenesis in our system involved: (i) VEGF-dependent activation of telomerase through the nitric oxide pathway and (ii) telomerase-dependent activation of endothelial cell differentiation and protection from apoptosis. Furthermore, detection of TERT in activated satellite cells identified them as VEGF targets during muscle regeneration. Because TERT behaves as an angiogenic factor and a downstream effector of VEGF signaling, telomerase activity appears required for VEGF-dependent remodeling of ischemic tissue at the capillaries and arterioles level.

Vascular endothelial growth factor (VEGF)$^1$ is a potent and cell-specific angiogenic factor, which enables the formation of new vascular structures in normal (1) and ischemic tissues (2). Recently, the VEGF range of cellular targets has been potentially extended beyond endothelial cells because its receptors are expressed in other tissues (3, 4). Specifically, the presence of VEGF receptor 2 (VEGFR-2) in regenerating muscle fibers and skeletal muscle satellite cells (3, 5) suggests a specific action of VEGF on these cells during post-ischemic tissue regeneration and gene therapy interventions (4). Beyond evidence that VEGF multiple signaling pathways regulate proliferation, migration, and differentiation of endothelial cells (6), little is known about the mechanism(s) by which VEGF promotes angiogenesis in vivo in normal or ischemic tissues. A growing body of literature assigns to telomerase a potentially relevant role in angiogenesis and cardiovascular disorders (7, 8). To date, however, no evidence has linked VEGF and the regulation of the endogenous catalytic subunit (TERT) of telomerase, which is limiting for enzymatic activity.

Here, we have investigated the role of telomerase in tissue remodeling after VEGF gene transfer in a rat model of hind limb ischemia. We found that telomerase is an important downstream effector of VEGF-mediated vascularization in vivo involved in the regulation of capillarogenesis. In fact, adenovirus-mediated transfer of the wild type hTERT gene in ischemic rats induced development of new capillaries and reduced apoptosis, indicating a direct contribution of TERT to angiogenesis in vivo. Telomerase activity is required for this process, because delivery of a dominant negative mutant of TERT (TERTdn) failed to promote formation of new capillaries. Most strikingly, when co-delivered with VEGF, the TERTdn completely abrogated VEGF-dependent angiogenesis and arteriogenesis, emphasizing the role of the enzyme in the vascular system. Similarly, the inhibition of endogenous telomerase by 3'-azido-3'-deoxythymidine (AZT) in VEGF-treated cells strongly reduced

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$^1$ The abbreviations used are: VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; TERT, human TERT; dn, dominant negative; AZT, 3'-azido-3'-deoxythymidine; NO, nitric oxide; NOS, nitric-oxide synthase; eNOS, endothelial NOS; bFGF, basic fibroblast growth factor; HUVEC, human umbilical vein endothelial cell(s); Ad, adenovirus; PBS, phosphate-buffered saline; TRITC, tetramethylrhodamine isothiocyanate; PI3K, phosphatidylinositol 3-kinase; ANOVA, analysis of variance; RT, reverse transcription; TNF, tumor necrosis factor; ERK, extracellular signal-regulated kinase; aFGF, acidic fibroblast growth factor; TRAP, telomeres repeat amplification protocol; TUNEL, TdT-mediated dUTP nick end labeling; TERT, telomerase reverse transcriptase.
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MATERIALS AND METHODS

Reagents and Antibodies—Human recombinant VEGF<sub>165</sub>, aFGF, and fibroblast growth factor (bFGF) (R&D System), 7-nitroindazole (Santa Cruz Biotechnology), PD98059 (Biomol), SB202190 (Alexis Biochemicals), AdNull and AdhTERT were obtained from Lea Harrington (University of Toronto), anti-Desmin (Dako), goat anti-rabbit-biotinylated secondary antibodies unconjugated or conjugated with fluorescein or Texas Red (Vector Laboratories) were also used.

Animal Studies—Wistar male rats (Harlan) (3–4-months-old, 300–350 g) were used. Unilateral hind limb ischemia was induced as described previously (1) following a protocol approved by the Institutional Animal Care and Use Committee.

Adenovirus Vectors—Replication-deficient recombinant adenovectors were prepared, stored, and administered as described previously (1, 9).

In all of the vectors, E1 was replaced by the cytomegalovirus-early promoter region gene promoter/enhancer driving the cDNA for human VEGF<sub>165</sub> or LacZ gene (9), the hTERT or hTERTdn cDNA (10), or not (AdNull).

Gene Therapy Protocol—Sham-operated and ischemic rats, immediately after femoral artery removal, were randomly assigned to receive or no gene (AdNull) (1).

Arteriole Length Density—Arteriole profiles included major and minor luminal diameter length. Thereafter, sections were de-paraffinized, rinsed in xylene, and embedded in paraffin. For each arteriole profile, the length density (Ld) according to the formula: Ld = n/L, was averaged to obtain the arteriole length density.

Animals (4–8/time point) were anesthetized and perfused via the left ventricle with 10% formaldehyde for 48 h. The arteriole profile included major and minor luminal diameter length. Thereafter, sections were de-paraffinized, rinsed in xylene, and embedded in paraffin. For each arteriole profile, the length density (Ld) according to the formula: Ld = n/L, was averaged to obtain the arteriole length density.

Experiments were performed in 24-well plates coated with 400 μl/well Matrigel as described previously (17). 5 × 10<sup>4</sup> cells/well in triplicate. TUNEL assay—All of the procedures were performed as previously described (5). Cells or muscle sections were incubated with 5 units of terminal deoxynucleotidyltransferase, 2.5% M<sub>coco</sub>, 0.2 μ/m potassium cacodylate, 25 μM Tris-HCl, 0.25% bovine serum albumin, and 0.5 μM biotinylated 2′-dUTP (biodin-16-dUTP). Samples were incubated with avidine-biotin complex-horseradish peroxidase complex and revealed with 3,3′ diaminobenzidine. HUVEC were plated in the absence of growth factors, and VEGF in combination or not with AZT was added to the medium. After 36–48 h, cells were harvested and cytospun onto glass slides. A total of 20 randomly selected fields were chosen for each slide, and total counts were averaged to obtain the apoptotic index.

Blood flow was measured by ultrasonic transit time flowmetry using a Transonic flowmeter (T 106, Transonic Systems Inc.) coupled to a 100-Hz 0.5-V probe calibrated according to the manufacturer’s instructions. Flowmetry was performed on the residual part of the femoral artery underlying the inguinal ligament in the ischemic limb and on an equivalent portion of the controlateral normoperfused limb. The probe was directly applied on the artery, which was not deflected from its natural course. Space between the vessel and the brackets of the probe was filled with an ultrasonic couplant (HR Lubricating Jelly). Blood flow (ml/min) was measured in both controlateral and ischemic limbs and was expressed as ischemic/controlateral ratio. Data are expressed as the mean ± S.D. Four animals were used for each experimental condition.

Results

VEGF<sub>165</sub> Induces Angiogenesis and Up-regulates TERT in Ischemic Rat—Unilateral hind limb ischemia was induced by removal of the femoral artery in male Wistar rats. Consequences of hind limb ischemia in this model have been well characterized (1). Gene therapy treatment was administered at the time of surgery by intramuscular injection of an adenovector encoding the human isoform of VEGF<sub>165</sub> (AdVEGF<sub>165</sub>) into the right thigh along the projection of the femoral artery. AdhTERT and AdhTERTdn or saline were injected at 10 sites. For all of the experiments, the virus dose was 5 × 10<sup>7</sup> pfu/animal in 0.5 ml except as indicated when a combination of 5 × 10<sup>7</sup> pfu/virus for a total of 1 × 10<sup>8</sup> pfu/animal was used.

Ultrasonic Transit Time Flowmetry—Blood flow was measured by ultrasonic transit time flowmetry using a Transonic flowmeter (T 106, Transonic Systems Inc.) coupled to a 100-Hz 0.5-V probe calibrated according to the manufacturer’s instructions. Flowmetry was performed on the residual part of the femoral artery underlying the inguinal ligament in the ischemic limb and on an equivalent portion of the controlateral normoperfused limb. The probe was directly applied on the artery, which was not deflected from its natural course. Space between the vessel and the brackets of the probe was filled with an ultrasonic couplant (HR Lubricating Jelly). Blood flow (ml/min) was measured in both controlateral and ischemic limbs and was expressed as ischemic/controlateral ratio. Data are expressed as the mean ± S.D. Four animals were used for each experimental condition.
the adductors and quadriceps of normoperfused (sham-operated) or ischemic rats. Control animals received saline, AdNull, or AdLacZ. As shown in Fig. 1a, VEGF165 gene delivery enhanced capillary density. In treated rats, values were significantly higher than in controls (AdNull) at each time point (3 days, p < 0.0001; 8 days, p < 0.045; and 14 days, p < 0.032).

A large body of literature indicates that VEGF regulates the function of mature endothelial cells and mobilizes vascular precursor cells (18). VEGF also protects vascular cells from apoptosis (19) and stimulates angiogenesis in normal (1) and ischemic skeletal muscles (20). However, the mechanisms underlying this vascularization remain elusive (21). Recent evidence shows that a sustained telomerase activity improves cell survival (22) and enhances the angiogenic properties of endothelial cells and their precursors (23–25). No cooperative angiogenic effect between VEGF and endogenous TERT has been reported to date. We sought to evaluate whether VEGF165 modulates TERT expression and telomerase activity during a time course of 3, 8, and 14 days in rat skeletal muscles. In the untreated animals, rTERT mRNA expression was low (26, 27) and decreased further after ischemia (Fig. 1b) but was markedly enhanced upon VEGF165 gene delivery at all of the time points (p < 0.01). rTERT mRNA did not increase upon injection of control virus (AdLacZ). Telomerase activity paralleled changes in mRNA level (Fig. 1c). It was low but detectable in the skeletal muscles of normoperfused animals and virtually absent following ischemia but substantially rescued by 3 days of treatment with VEGF165.

Expression of the TERT protein was detected (Fig. 1d) in muscle fibers and vascular structures of normoperfused animals but was down-regulated upon ischemia and again substantially rescued by VEGF165 treatment. VEGFRs have been recently detected in non-vascular tissues including regenerating skeletal muscle fibers and satellite cells (5, 16). To investigate a potential correlation between VEGF activation of satellite muscle cells and of TERT expression, serial muscle sections were immunostained with antibodies for TERT or desmin, a specific marker of activated satellite cells (see Ref. 5 and references therein). Satellite cells of ischemic rats at day 3 after VEGF treatment did co-express TERT (Merge). Nuclei were stained with Hoechst 33258 (blue).
establishing a correlation between telomerase activation and muscle regeneration. Satellite cells in normoperfused or ischemic animals receiving saline or AdNull were negative for both markers (data not shown). These results indicate that VEGF165 gene therapy of hind limb ischemia induces TERT expression and function and that this effect is concomitant with the onset of an angiogenic process.

Telomerase Induces Angiogenesis in Ischemic Tissues and Protects Them from Apoptosis—We next queried whether the activation of telomerase is essential for VEGF165-dependent angiogenesis in acute hind limb ischemia or is an epiphenomenon devoid of biological significance. To address this point, we examined in vivo the effect of adenovirus-mediated transfer of the hTERT gene on the formation of blood vessels and on blood flow in rat ischemic muscles at 3, 8, and 14 days after injection (Fig. 2). The expression of the transduced gene was monitored by RT-PCR using primers specific to hTERT. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as control. The results shown are from two randomly chosen rats after 3, 8, and 14 days of treatment. b, capillary density evaluated on hematoxylin-eosin-stained sections is expressed as counts per mm². The effect of TERTdn was evaluated at the time (8 days) when the response to VEGF165 treatment was maximal compared with controls (see Fig. 1a). c, arteriole formation was evaluated on ischemic rats 14 days after gene therapy and expressed as length density per mm². d, blood flow of normoperfused and ischemic tissues was evaluated by ultrasonic transit time flowmetry 14 days after gene therapy treatment. e, TUNEL assays were performed on paraffin sections of ischemic rats 3 days after treatment. Values are expressed as TUNEL-positive nuclei per mm². Statistical significance is indicated as follows: asterisk, AdVEGF165 or AdhTERT versus AdNull p < 0.02; §, AdVEGF165 + AdTERTdn versus AdVEGF165 p < 0.0006; #, 1 × 10⁶ pfu.
The induction of telomerase activity is necessary for VEGF-dependent angiogenesis in vitro. 

**a**. Differentiated C2C12 or proliferating HUVEC were grown with or without recombinant VEGF165 (50 ng/ml) and stained with an anti-TERT antibody (α-TERT) or with Hoechst 33258. Magnification is ×10 for C2C12 and ×40 for HUVEC.

**b**. Telomerase activity was assayed in extracts from proliferating (GM) and differentiated (DM) C2C12 cells and in HUVEC in the absence (–) or presence (+) of recombinant VEGF165. Positive and negative controls and internal standard are as shown in Fig. 1.

**c**. Viability of HUVEC cultured in the presence or absence of VEGF and with or without AZT (10 and 100 μM) or heat-inactivated AZT (10 μM and 100 μM) at the indicated time.

**d**. HUVEC cultured on Matrigel in the presence or absence of VEGF and with or without AZT. 

**e**. Sub-G1 percentage of HUVEC cultured in the absence of growth factors for 48 h after adenoviral infection obtained by cytofluorimetric analysis after propidium iodide staining.

**f** and **g**. Quantification of capillary-like structures in each condition is represented as the mean ± S.E. of 10 randomly chosen fields as described under “Material and Methods.” Crtl, control.

**i**. Telomerase activity in VEGF-treated HUVEC was evaluated in each condition by TRAP. Positive and negative controls and internal standard are as described in Fig. 1.
FIG. 3—continued

VEGF was co-delivered with a dominant-negative mutant hTERT (AdhTERTdn), which on its own has no effect on capillary density, VEGF-dependent effects on angiogenesis (Fig. 2a), arteriogenesis (Fig. 2b), and blood flow (Fig. 2c) were abrogated. On the other hand, wild type hTERT was as efficient as or even more so than VEGF in protecting cells from apoptosis (Fig. 2c). All of these observations demonstrate a direct contribution of TERT to the onset of new vessels promoted by VEGF treatment.

Telomerase Activity Is Required for the Onset of VEGF-dependent Angiogenesis in Vitro—To further examine the role of TERT in angiogenesis, we measured TERT expression and telomerase activity in differentiated murine myotubes (C2C12) and HUVEC grown in the absence or presence of 50 ng/ml human recombinant VEGF165 (Fig. 3). As expected (24, 28), endogenous TERT was expressed at negligible levels in differentiated C2C12 or proliferating HUVEC, as assessed by indirect immunofluorescence with a TERT antibody (Fig. 3a). VEGF165 strongly induced TERT mRNA expression in both cell types with peak expression at 20–24 h (data not shown). This effect was paralleled by a significant increase in telomerase activity (Fig. 3b). To analyze functionally the role of hTERT during VEGF-dependent endothelial cell survival and in vitro differentiation, a series of experiments were performed in which hTERT activity was inhibited by AZT (29) or TERTdn expression (Fig. 3, c–i). Fig. 3c shows that, in the presence of serum, AZT does not significantly compromise endothelial cells viability upon treatment for 2–24 h. However, in the absence of other growth factors, VEGF165 pro-survival effects were markedly reduced in cells exposed to AZT (Fig. 3d) or infected with TERTdn (Fig. 3e).

Remarkably, AZT-treated or TERTdn-infected HUVEC cultured in the presence or absence of VEGF failed to form VEGF-dependent capillary-like structures on Matrigel (Fig. 3, f–h). Under the same conditions, both AZT and TERTdn severely compromised telomerase activity (Fig. 3i), thus indicating that a functional enzyme may be required for VEGF165 differentiation and pro-survival effects.

VEGF<sub>165</sub> Treatment Induces TERT Expression and Telomerase Activity via PI3K/AKT and Nitric Oxide—It has been reported that VEGF regulates NO production via PI3K/AKT signaling (30), which may account for some of the VEGF-dependent vascular protective effects (19). Fig. 4a shows that PI3K signaling is important for hTERT expression, which is significantly reduced by the PI3K inhibitor LY294002. Consistently, in VEGF-stimulated endothelial cells, the inhibition of PI3K dramatically reduced VEGF-dependent telomerase activity (Fig. 4b) and transcription from the human TERT promoter (Fig. 4c). The regulation of telomerase expression and function via PI3K signaling prompted us to evaluate whether this pathway could be relevant for endothelial cell differentiation. Fig. 4d shows that endothelial cells in which a dominant negative AKT molecule has been overexpressed by means of adenovirus vector exhibit significant reduction in TERT activity, which is paralleled by a reduction in capillary-like structure formation (Fig. 4e). Therefore, we investigated the role of nitric-oxide synthases (NOS) on TERT mRNA expression (Fig. 5a), telomerase activity (Fig. 5b), and TERT promoter transcriptional activity (Fig. 5c). Differentiated C2C12 and proliferating HUVEC were treated with the NOS inhibitor, 7-nitroindazol (7N), in the presence or absence of different angiogenic factors. Recombinant VEGF, αFGF, or βFGF reproducibly induced TERT mRNA (1.5–3-fold the unstimulated level) and telomerase activity (Fig. 5, a and b), revealing a commonality of action of these factors and underscoring the correlation between promotion of angiogenesis and telomerase regulation. Of note, preincubation of cells with 7N abolished only VEGF<sub>165</sub>-dependent effects, indicating that acidic and basic FGF operate via mechanisms that do not involve NO. Preincubation with an inhibitor of p38 mitogen-activated protein kinase pathway (SB203580) had no effect on hTERT mRNA levels (Fig. 5a). Furthermore, VEGF<sub>165</sub> activated the hTERT promoter in transient transfection assays (Fig. 5c), an effect again abrogated by 7N but not by PD098059 (PD), an antagonist of ERK mitogen-activated protein kinase signaling pathway. The role of NO in the VEGF-dependent transcriptional regulation of TERT is further supported by the observation that transfection of a phosphomimetic constitutively activated eNOS mutant (16) in the absence of VEGF up-regulates the hTERT promoter to levels similar to those obtained with VEGF alone (Fig. 5c).

All of the above findings are consistent with our in vivo data indicating that VEGF is capable of inducing TERT expression and telomerase activity in differentiated skeletal muscles and vessels. Furthermore, they reveal that PI3K signaling pathway, which regulates NO synthesis and NO itself, is involved in this process.

**DISCUSSION**

VEGF induces angiogenesis in a variety of tissues by a mechanism involving activation of a signaling cascade in cells expressing the two VEGF receptors. Recently, the expression of VEGFR-1 and VEGFR-2 has been detected also in non-vascular cells such as regenerating muscle fibers and satellite cells (3, 5), suggesting that some non-endothelial cells may be targeted by VEGF during physiologic angiogenesis and angiogenesis accompanying tumor growth or VEGF<sub>165</sub> treatment of ischemia. Skeletal muscle tissue frequently undergoes degeneration after abrupt interruption of blood flow (31) but can regenerate.
rapidly upon activation of satellite cells (32). Interestingly, the production of VEGF increases during muscle regeneration following ischemia (33), although the molecular mechanisms underlying this phenomenon are largely undefined. Here, we have shown that treatment with adenovirus-expressing VEGF_{165} induces revascularization of rat ischemic adductor muscles.

**Fig. 4.** PI3K/AKT pathway mediates VEGF-dependent telomerase induction in endothelial cells. *a*, RT-PCR analysis of hTERT expression in HUVEC cultured in the presence or absence of recombinant VEGF, and the PI3K inhibitor LY-294002 (LY). DMSO, MeSO. *b*, telomerase activity in the same experimental condition of panel a. H.I., heat-inactivated. *c*, transient transfection of the hTERT promoter reporter construct p3996 containing a 3996-bp fragment of the hTERT promoter linked to the luciferase reporter gene. The experiment was performed in bovine endothelial cells (BAEC) in the presence or absence of recombinant VEGF. Results are representative of three independent experiments each performed in duplicate. *d*, telomerase activity detected by TRAP assay in cells infected with AdAKTdn. *e*, quantification of capillary-like structures longer than three cells. The graph is represented as the mean ± S.D. of 10 randomly chosen fields as described under “Material and Methods.”
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This process is paralleled by activation of TERT expression and telomerase activity, which is particularly evident in vascular structures, muscle fibers, and satellite cells. This finding prompted us to query whether expression of telomerase per se would promote angiogenesis. In agreement with the results of others showing that hTERT-expressing cells have increased angiogenic properties, (25) we found that adenoviral transduc-

tion of the hTERT gene in ischemic muscles induced angiogenesis at the level of capillaries. Notably, the arteriolar bed formation was not stimulated by TERT expression, suggesting that telomerase may be a necessary but not sufficient downstream effector of VEGF-dependent angiogenesis and/or that different mechanisms underlie the formation of the two vascular districts (34). This evidence is further supported by the observation that inhibition of telomerase by a dominant negative mutant co-delivered with VEGF abrogates this response and, moreover, negates the effect of the growth factor on capillaries, arterioles, and blood flow increase. Further, our in vitro experiments show that VEGF treatment induces TERT expression and telomerase activity in human endothelial cells and murine differentiated myotubes. Telomerase has been associated with the proliferative capacity, survival, and functionality of endothelial cardiomyocytes (23–26, 35–37) and, more recently, cardiac stem cells (38). Our data are consistent with a similar role of the enzyme during capillary formation in our in vivo and in vitro systems. Of note, the inhibition of the PI3K signaling pathway or nitric oxide production abrogates VEGF-dependent induction of telomerase. Conversely, the expression of a constitutively active phosphomimetic NOS mutant induces TERT promoter activity at levels similar to those of VEGF. These results, confirming that PI3K and NO synthesis play an important role in the VEGF-mediated angiogenic effect (39), provide a link between the angiogenic factor and telomerase expression and function. The observations that induction of telomerase is brought about also by aFGF or bFGF, but not in either case inhibited by 7N, support a strict correlation between the enzyme and promotion of VEGF-dependent angiogenesis while at the same time suggesting that the other angiogenic factors utilize pathways other than those regulating NO synthesis. Remarkably, recent observations indicate that TERT may induce basic bFGF expression in microvascular endothelial cells (40) or epithelial growth factor may increase TERT levels directly by up-regulating its transcription (35). Therefore, the biological function of TERT, at least in vascular cells, may be controlled by a molecular network of autoregulatory loops in which growth factor production plays an important role. Interestingly, recent evidence indicates that, in near senescence endothelial cells, TERT response to VEGF induction is inhibited (41, 42). Remarkably, in this condition, NO production in HUVEC is also deficient (36), thus suggesting that the TERT-dependent VEGF function may be compromised in aging.

We have shown that VEGF treatment of C2C12 cells activates the hTERT promoter, indicating that, also in our system, the increase in TERT mRNA and enzymatic activity could be accounted for by a mechanism operating, at least in part, at the transcriptional level. Further studies are required to better elucidate this regulatory process. The rapid onset of the angiogenic process after expression of telomerase, detectable by day 3 in vivo and within 12–24 h in vitro, would seem to exclude a telomerase effect mediated by substantial lengthening of telomeres. Rather, we favor a model in which telomerase acts as survival factor, capping chromosome ends and thereby protecting cells from apoptosis (43). Recently, a novel extranuclear function of TERT, which protects cells modulating mitochondria calcium influx, has also been reported (44). Telomere dysfunction has also been implicated in myocardocyte apoptosis after heart failure (26, 37, 45). Thus, it is tempting to speculate that the anti-apoptotic effects of VEGF may be, at least in part, imputable to telomerase.

In conclusion, here we document for the first time a positive regulation by VEGF on telomerase activity in vitro and in vivo. Given that telomerase per se can induce angiogenesis, it is

![Figure 5. VEGF treatment induces mouse TERT RNA expression, telomerase activity, and promoter transcription via nitric-oxide synthases.](Image)

**Fig. 5.** VEGF treatment induces mouse TERT RNA expression, telomerase activity, and promoter transcription via nitric-oxide synthases. Differentiated C2C12 and HUVEC, grown in the presence or absence of angiogenic factors (VEGF, aFGF, and bFGF) or solvent alone (MeSO, DMSO), were preincubated with (+) or without (−) the nitric-oxide synthases inhibitor, 7N, or a p38 inhibitor SB 203580 (SB). a, TERT mRNA levels were measured by densitometry, and values were normalized to those of the control housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. The average ratios (TERT/H11002) of three independent experiments are expressed as fold induction (± S.E.) of three independent experiments, each performed in duplicate.

The results represent the average (± S.E.) of three independent experiments.
reasonable to assume that activation of telomerase is an essential requisite for the VEGF-dependent remodeling of ischemic tissue. Moreover, the detection of TERT in the activated skeletal muscle satellite cells provides evidence that this cell population is a target of VEGF during in vivo regeneration of damaged muscle fibers after acute ischemia. Our findings may lead to the design of novel gene therapy interventions based on the combined delivery of VEGF and TERT.

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