Developmentally Timed Expression of an Embryonic Growth Phenotype in Vascular Smooth Muscle Cells

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Abstract  Little is known about the phenotypic changes that occur in vascular smooth muscle cells (SMCs) as the developing aorta undergoes the transition from a loosely organized, highly replicative tissue to a morphologically mature, quiescent tissue. In the present study, we have characterized the in vivo pattern of SMC replication during intrauterine and neonatal aortic development in the rat and have cultured and assessed the in vitro growth properties of embryonic, fetal, and neonatal vascular SMCs. Embryonic SMCs, which exhibited a very high in vivo replication rate (75% to 80% per day), demonstrated a significant potential for self-driven replication, as assessed by the ability to proliferate under serum-deprived conditions. Several lines of evidence suggest that the autonomous growth of SMCs in the "embryonic growth phenotype" may be driven by a unique mechanism independent of known fetal SMC mitogens: embryonic SMC replication was not associated with the detectable secretion of mitogenic activity capable of stimulating adult SMCs, and embryonic SMCs were mitogenically unresponsive to a variety of known adult SMC growth factors. The capacity for self-driven growth was lost by embryonic day 20, suggesting that important changes in gene expression and phenotype occur in developing SMCs between embryonic days 18 and 20. Taken together, the data describe a unique embryonic growth phenotype of vascular SMCs and suggest that the replication of aortic SMCs during intrauterine development is self driven, self regulated, and controlled by a developmental timing mechanism. The conversion of SMCs from the embryonic to the late fetal/adult growth phenotype will likely be found to be an important component of a developmental system controlling vascular morphogenesis. (Circ Res. 1994;74:189-196.)

Key Words  • vascular smooth muscle  • aortic development  • smooth muscle cell phenotype  • smooth muscle cell replication

The uninjured blood vessel wall is a remarkably quiescent tissue, with a vascular smooth muscle cell (SMC) replication index of <0.06% per day.1-2 Significant proliferation of SMCs occurs only during development (a rare event), and after vascular injury. In the adult, unscheduled replication of SMCs is an important contributor to atherogenesis, the maintenance of hypertension (through vascular remodeling), and the restenosis of vascular grafts and angioiastenoses (reviewed in Schwartz et al3). Adult SMCs replicating after injury exhibit many features of "fetal" SMCs,4-8 suggesting that the expression of certain developmental phenotypes may contribute to vascular disease processes. Therefore, it seems critical to understand the various "growth phenotypes" expressed by SMCs throughout vascular development.

We have begun to study, in a rat developmental model, the phenotypic changes that occur in vascular SMCs as the developing aorta undergoes the transition from a highly replicative to a fully quiescent tissue. We envision that multiple and probably redundant mechanisms, involving growth suppressor genes (reviewed in References 9 and 10), extracellular matrix molecules,11-13 cell-cell or cell-matrix adhesion molecules,14 growth inhibitory cytokines,15-18 changes in levels of expression of growth factors and their receptors,19,20 and changes in cellular phenotype may contribute to the suppression of SMC replication in the normal adult blood vessel wall. Elucidation of these endogenous, developmentally regulated growth-suppressive mechanisms may provide the basis for the eventual development of therapeutic strategies capable of reducing or preventing SMC replication after vascular injury.

In the present study, we describe the in vivo time course of replication of aortic rat SMCs during embryonic, fetal, and postnatal life. In addition, we describe the isolation, culture, and partial characterization of vascular SMCs from embryonic, fetal, and neonatal aortas. The data collectively define a distinct embryonic growth phenotype of vascular SMCs characterized by a very high in vivo replication rate and by the expression of significant and unique serum-independent growth capabilities in vitro. The capacity for self-driven growth in vitro appears to be lost between gestational days 18 and 20, suggesting an important underlying phenotypic transition of SMCs during fetal life. The data suggest that the replication of embryonic SMCs (eSMCs) is self driven and self regulated in nature and is controlled by a developmental timing mechanism. The factors that regulate the expression and loss of this SMC growth phenotype are likely to be important components of a developmental system controlling vascular morphogenesis and, possibly, SMC replication in vascular disease states.

Materials and Methods

Animals and In Vivo Analysis of SMC Replication

Pregnant female Sprague-Dawley rats were obtained from Harlan Sprague Dawley, Indianapolis, Ind. At gestational days
12 to 20, pregnant females were injected intraintraperitoneally with 100 mg bromodeoxyuridine (Brdu, Sigma Chemical Co, St Louis, Mo) per kilogram body weight. Animals were injected 17, 9, and 1 hour before death; the timing of the initial treatment was identical for all animals. Pregnant females were then killed, embryos and fetuses were removed, and tissues (either whole embryos or dissected aortic segments) were preserved overnight in 10% phosphate-buffered formalin. Aortic tissues from postnatal animals (days 1, 16, 30, and 60) were obtained according to the same protocols. Samples were processed for histology, and sections were stained immunocytochemically with a monoclonal antibody against BrdU (Becton-Dickinson).21 Antigen-antibody complexes were visualized with an avidin-biotin immunoperoxidase system (Pierce, Rockford, Ill). To ensure the proper distinction of the vascular SMC layer from the underlying adventitia, selected samples of embryonic tissues were stained immunocytochemically with a monoclonal antibody specific for the smooth muscle isoform of \( \alpha \)-actin22 (kindly provided by Dr G Gabbiani, University of Geneva) (data not shown). Sections were lightly counterstained with hematoxylin and eosin, and the percentage of BrdU-labeled nuclei was determined from three morphological sites along the aorta: the aortic arch, the thoracic aorta (just proximal to the diaphragm), and the abdominal aorta (just distal to the diaphragm). Data are presented as the mean±SEM of counts from a minimum of three animals.

Cell Culture and In Vitro Analysis of Cell Replication

Aortic tissue was removed from rat embryos, fetuses, and postnatal animals, carefully microdissected free from the surrounding adventitia, and then placed into tissue culture wells, as explants, in DMEM supplemented with 10% calf serum (CS). For the earlier embryonic and fetal time points, cells grew out of the explants within 24 hours (compared with 7 to 14 days for adult tissues), and cultures derived from up to six explants were pooled before subculture. Cells were characterized as SMCs, as described below. Cell replication was analyzed by cell number assays, \(^{[3]H}\)thymidine incorporation as previously described,23 or BrdU histochemistry as described above. Cells cultured in serum-free medium (SFM) were maintained in stock 1× DMEM without any additions. All tissue culture reagents and supplies were obtained from Gibco/BRL, Gaithersburg, Md. All experiments were performed with cells in the second through seventh passages and were performed in triplicate with cells from a minimum of two isolates.

Analytical Techniques

Northern analyses were performed essentially as previously described.23 cDNA probes used include a 500-bp EcoRI/HindIII fragment of a cDNA encoding bovine elastin24 and a 1.3-kb PsI fragment of a cDNA encoding bovine actin (which recognizes \( \alpha \), \( \beta \), and \( \gamma \)-actins).25

Indirect immunofluorescence to detect \( \alpha \)-smooth muscle-specific actin was performed on methanol-permeabilized second-passage SMCs as previously described,26 using a monoclonal antibody specific for the SMC \( \alpha \)-actin isoform.22

Results

Analysis of SMC Replication During Aortic Development In Vivo

BrdU immunocytochemistry was used to assess the replication rates of vascular SMCs throughout development in the rat. The injection protocol used ensured that the nuclei of all cells entering S phase in the 24 hours before death would be labeled with BrdU. Replication indexes were determined for the aortic arch, the thoracic aorta (proximal to the diaphragm), and the abdominal aorta (between the diaphragm and the renal arteries). Because no differences were noted among the data derived from the three sites for any given developmental age, the data were pooled for clarity of presentation (Fig 1). During embryonic life (embryonic [e] days 13 to 17 [e13 to e17]), the SMC replication index was consistently very high, averaging 75% to 80%. At the transition to fetal life, SMC replication slowed dramatically, showing a significant (35% to 40%) drop in the percentage of labeled nuclei between days e17 and e19 (Fig 1). SMC replication remained constant (at ~40%) for the duration of fetal life. During postnatal life, the growth of the aorta slowed gradually, to <0.5% at 60 days. Others have repeatedly shown that, in normal adult animals, the SMC replication index is <0.06% per day.1,2

The statistically significant drop in SMC growth rate observed between days e17 and e19 correlated well with striking morphological and organizational changes that occur over this time period during aortic development (data not shown). The embryonic aorta (eg, at day e17) is morphologically unorganized and contains densely packed cells with little intercellular space. By day e19, when the replication index averaged <40%, intercellular spaces increased, and cells had begun to organize into lamellated units. Proliferating SMCs were not preferentially localized to any area (eg, inner versus outer lamellae) within the blood vessel wall at any developmental stage. The data define two distinct stages of tissue growth during intrauterine aortic development: (1) an embryonic stage (days e13 to e17), characterized primarily by rapid SMC proliferation, and (2) a late fetal stage (days e19 to e21), characterized by moderate SMC proliferation and increasing morphological organization. The data suggested that a change in SMC growth phenotype may occur between days e17 and e19.

Demonstration of a Distinct Embryonic Growth Phenotype Expressed by SMCs During Aortic Development

SMC replication during vascular development may be driven by factors exogenous to the SMCs (eg, growth
factors or matrix molecules from adjacent cell types or from the plasma), by an autogenous mechanism endogenous to the SMCs, or by a combination of such factors. To assess the contribution that autogenous mechanisms may play in driving the growth of SMCs during development, we isolated SMCs from the aortas of embryos at days e13, e14, e16, and e17, from fetuses at days e18 to e21, and from neonates and adults at days 1, 7, 30, and 60. For comparison purposes, we also cultured embryonic adventitial fibroblasts, which exhibited a “swirling” pattern of growth, a spindle-shaped morphology, and a lack of serum-independent growth potential. All of our SMC cultures (embryonic through adult) exhibited the “hill-and-valley” pattern of organization characteristic of adult SMCs (Fig 2). SMC cultures exhibiting a “cobblestone” pattern of growth, as described for a subset of SMCs derived from neonatal rat aortas,20 were not observed in our studies; however, the explant methodologies used to derive our SMC cultures may not be optimal for the derivation of “epithelioid” SMCs. As a more rigorous test of the SMC nature of our embryonic and fetal cells, we examined the cells with respect to two additional markers known to be expressed in SMCs during early aortic development: elastin and α-smooth muscle actin.28 Total RNA was prepared from second-passage cultures and probed with cDNAs specific for elastin or actin. As shown in Fig 3, the SMC cultures were found to express significant levels of elastin and α-actin mRNAs. To verify that the α-actin message encoded smooth muscle-specific α-actin, we used immunofluorescence techniques to visualize the expression of this protein using a monoclonal antibody specific for the smooth muscle-specific actin isoform.22 As shown in Fig 4, the cultures were uniformly composed of cells that stain positive for the presence of smooth muscle-specific α-actin. The combined presence of the hill-and-valley pattern of organization, a high level of expression of elastin mRNA, and the expression of the smooth muscle α-actin isoform verified that our cultures were of SMC origin.

To assess the capacity of the embryonic cells for self-driven replication, we plated eSMCs (and postnatal SMCs as controls) at sparse density in 10% CS, allowed the cells to attach and spread overnight, and then cultured the cells in serum-free DMEM or in DMEM supplemented with 0.1% CS for up to 12 days. The medium was not changed during the course of the experiment, and cell numbers were determined in triplicate at 72-hour intervals. As shown in the top panel of Fig 5, embryonic (day e17) SMCs replicated freely under low serum (0.1%) or serum-free conditions, whereas neonatal (day e1) and adult (day 60) SMCs became growth-arrested and lost viability when maintained in low-serum or serum-free conditions. As shown below, the capacity for growth in SFM is limited to embryonic (days e13 to e17) and early fetal (day e18) SMCs.

To verify these cell number analyses, we used BrdU histochemistry to determine the number of e17 SMCs replicating after 72 hours under serum-starved conditions. Under these conditions, adult SMCs become largely growth-arrested, exhibiting labeling indexes of <10%.29 Adult and e17 SMCs were plated at sparse

Fig 2. Photomicrograph showing morphological appearance typical of confluent cultures of embryonic and fetal vascular smooth muscle cells (SMCs). Embryonic SMCs at confluence exhibit the “hill-and-valley” pattern of growth that is characteristic of adult SMCs in culture. All embryonic, fetal, and neonatal SMCs exhibited this morphology. Shown is a confluent culture of SMCs from an embryo at day 13. Bar=500 μm.

Fig 3. Northern blot analysis demonstrating the expression of high levels of expression of α-actin and elastin mRNAs in embryonic smooth muscle cell (SMC) cultures. Total RNA was prepared from second-passage SMC cultures at embryonic day 13. Northern blots were prepared (using 15 μg total RNA per lane) as described and probed with 32P-labeled cDNAs specific for actin (lane 1) and tropoelastin (lane 2). The positions of migration of the 18S and 28S ribosomal RNAs are shown to the left. Note that the embryonic SMC cultures express high levels of transcripts for α-actin (lower 1.6-kb transcript in lane 1) and tropoelastin, two phenotypic markers for vascular SMCs.
density, switched to SFM for 72 hours, and then labeled with 10 mmol/L BrdU for an additional 24 hours. As shown in the middle and bottom panels of Fig 5, e17 SMCs maintained a high nuclear labeling index (in the experiment shown, >40%) under serum-deprived conditions, whereas the adult cells became growth-arrested, with only a few cells (5% to 6%) staining positive for BrdU.

The serum-independent growth phenotype of embryonic SMCs was stable in culture up to at least 15 passages, without any detectable loss (or gain) of autonomous growth potential (data not shown). Similarly, the serum-dependent growth phenotype of adult SMC cultures was stable for over 30 passages. The distinct growth phenotypes described herein therefore appear to be unrelated to the number of population doublings of the cultures tested.

Evidence That the Replication of eSMCs Is Driven by a Unique Molecular Mechanism Independent of Known Adult SMC Mitogens

eSMCs Do Not Secrete Detectable Mitogenic Activity for Adult SMCs

A variety of conditioned medium experiments were performed to determine whether embryonic SMCs secreted mitogenic activity into the culture supernatant; no such activity was detected using growth-arrested adult SMCs as target cells (data not shown). Because labile growth-promoting factors would not necessarily be detected in conditioned media assays, we also performed coculture experiments as a more rigorous test for the secretion of growth-promoting factors by eSMCs. For these experiments, adult SMCs were plated within 35-mm rings in the center of 100-mm tissue culture plates, allowed to attach and spread overnight, and then maintained in SFM for 72 hours. “Source” cells (either adult or e17 SMCs) were plated in SFM around the periphery of the central ring containing the growth-arrested target cells. The ring was removed after 24 hours, the target and source cells were cocultured in SFM for 48 hours, and the cells were exposed to BrdU for an additional 24 hours. The percentage of BrdU-labeled nuclei was determined by immunocytochemistry. In adult-e17 cell cocultures, as shown in Fig 6, the source e17 SMCs replicated freely with a high (33.6%) labeling index, whereas adult “target” SMCs remained growth arrested (labeling index, <7%). Treatment of the adult cultures with 10% CS increased the labeling index to 74.2%. The replication of adult or e17 target cells was not influenced by coculture with adult source cells. Additional controls (as shown below) indicated that our adult target SMCs respond mitogenically to platelet-derived growth factor (PDGF) isoforms, epidermal growth factor (EGF), insulin-like growth factors (IGFs), and acidic and basic fibroblast growth factors (FGFs). The data suggest that the autonomous replication of e17 SMCs is not associated with the secretion of known mitogens capable of stimulating adult SMCs. However, our data cannot rule out the possibility of “intracrine” stimulation of cell growth by intracellular mitogens, the trapping of secreted mitogens by the extracellular matrix, or the secretion of embryo-specific mitogens whose receptors are not expressed on adult target cells.

eSMCs Are Refractory to Mitogenic Stimulation by Known SMC Peptide Mitogens

The in vitro responses of autonomously replicating eSMCs to various growth-regulatory molecules were determined using BrdU or [3H]thymidine uptake assays. Such an analysis might indicate which factors may contribute to autonomous replication in vitro as well as to the high rate of SMC replication seen in embryonic aortas (Fig 1). Shown in Fig 7 are results comparing the mitogenic effects of the BB homodimer of PDGF (chosen because it can interact with all forms of the PDGF receptor),30 EGF, basic FGF, and 10% CS on e13, e17, and adult SMCs. Sparse cultures were maintained in low (0.1%) CS for 72 hours and then treated with EGF (10 ng/mL), PDGF-BB (10 ng/mL), basic FGF (50 ng/mL), or 10% CS for 24 hours in the presence of 1 μCi/mL [3H]thymidine. Surprisingly, although adult SMCs responded to each of these factors (Fig 7), eSMCs appeared refractory to stimulation. In addition, the stimulatory effects of serum on eSMCs were markedly less than the effects observed for the adult cells. In additional experiments not shown, it was also determined that eSMCs did not respond mitogenically to IGFs, PDGF-AA, or acidic FGF. The [3H]thymidine results shown in Fig 7 were verified using BrdU mitogenesis assays (data not shown); these experiments also ruled out the possibility that the lack of response of eSMCs to the indicated growth factors was due to an already maximal growth rate. The data suggest that eSMC replication cannot be markedly influenced by a
variety of exogenous growth factors known to stimulate
adult SMC mitogenesis.

In other experiments (not shown), we determined
that the inability of embryonic SMCs to respond to
exogenous mitogens was independent of the expression
of autonomous growth capabilities: late fetal, neonatal,
and certain neointimal SMCs, which do not exhibit
autonomous growth potential in culture, show markedly
reduced responses to mitogenic stimulation by a variety
of growth factors (R.A. Majack, C.L. Cook, P.E.
Schwartz, M.C.M. Weiser, R.C. McFall, C.F. Reilly,
manuscript submitted). Autonomous replication and
mitogenic unresponsiveness therefore appear to be sepa-
rate but overlapping developmental phenotypes.

Developmental Timing of Expression of the
Embryonic Growth Phenotype in Aortic SMCs

The BrdU assay described previously (for Fig 5,
middle and bottom panels) was used to determine the
gestational age at which serum-independent growth
capacity is lost during SMC development. The nuclear-
labelling indexes of SMCs derived from animals of
increasing developmental ages were determined after
72 hours in 0.1% fetal bovine serum. As presented in
Fig 8, embryonic (days e13 to e17) and early fetal (day
e18) SMCs maintained replication indexes of 28% to
33% in low serum; SMCs from animals fetal day e20 and
older became largely growth arrested, with labeling
indexes of 7% to 12%. Cell number experiments similar
to those illustrated in the Fig 5 graph were repeated
using SMCs derived from aortas at days e13, e14, e16,
e17, e18, e20, and e21 and postnatal days 1 and 60. The
results were consistent with those shown in Fig 8: cells
derived from the aortas of animals at days e13 to e18 showed significant serum-independent replication,
whereas SMCs from fetal day e20 and older did not
significantly replicate in culture under low-serum
conditions. The data establish that the transition of aortic

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![Graph and images](http://circres.ahajournals.org/)

**Fig 5.** Replication of embryonic and adult vascular smooth muscle cell (SMC) cultures under serum-deprived conditions. Left, embryonic (day 17), postnatal (day 1), and adult SMCs were plated at sparse density in 10% calf serum (CS), allowed to attach and spread overnight, and then cultured in low (0.1%) CS or serum-free medium (SFM) for 9 days. The media were not changed throughout

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SMCs from an embryonic to an adult growth phenotype occurs between gestational days e18 and e20.

Discussion

Little is known about the factors that control vascular SMC replication, phenotype, and organizational behavior during development; even less is known about the factors that establish and maintain SMC quiescence once morphogenesis is complete. In the present study, we describe an SMC embryonic growth phenotype that is characterized by a very high in vivo replication index and a significant capacity for serum-independent replication in vitro. This embryonic growth phenotype was found to be uniquely expressed by embryonic (days e13 to e17) and early fetal (day e18) SMCs in culture. The expression of this serum-independent phenotype does not appear to be associated with the secretion of a mitogen capable of stimulating adult SMCs. In experiments (not shown) using e17 SMCs, we were unable to detect significant autoactivation of PDGF, EGF, IGF, or FGF receptors, as assessed by the tyrosine phosphorylation of immunosolated receptor proteins. The majority of SMC proliferation during aortic morphogenesis therefore appears to occur via a unique, as yet undefined, molecular mechanism. The data suggest that one factor contributing to the acquisition of quiescence in the adult blood vessel wall is the developmentally timed loss of this embryonic self-driven growth phenotype. Elucidation of the factors controlling the expression and loss of this phenotype should significantly advance our understanding of the mechanisms regulating vascular morphogenesis and SMC function during development and after vascular injury.

SMC Replication During Intrauterine Development In Vivo

A number of previous studies have addressed various aspects of aortic development in vivo. Morphological studies have shown that the basic structure of the rat aorta is largely developed by birth31 and that slow growth of the aorta continues postnatally.32-35 Owens and Thompson36 reported that the labeling index of aortic SMCs in newborn rats (after a single 1-hour labeling period) is ~2% and that replication slowly decreases postnatally until adult replication levels are reached by 90 days. Because the available evidence suggested that the bulk of vascular SMC replication during development must occur before birth, we focused our studies on embryonic and fetal tissues and cells. The data reveal an extremely high (75% to 80% per day) replication index during embryonic life (through day e17) and a rapid transition to a significantly lower rate of proliferation by day e19. Our in
vitro data suggest that the high SMC replication rate observed in the embryonic aorta is driven by mechanisms endogenous to eSMCs and that the reduction in aortic SMC replication rate at days e17 to e19 may result, at least in part, from the developmentally timed loss of this phenotype.

A Distinct Embryonic Growth Phenotype Expressed in Developing Vascular SMCs

The growth phenotype expressed by cultured embryonic and early fetal SMCs is primarily characterized by a significant ability for self-driven replication in serum-free conditions. This characteristic is not significantly expressed by cells derived from adult aortas; our data suggest that the loss of this phenotype occurs during early fetal life, between days e18 and e20. Because the self-driven replication of eSMCs does not appear to be associated with the secretion of known mitogens for adult SMCs, the most likely explanation for our data is that eSMCs replicate in response to a novel embryo-specific mitogen whose receptor is not expressed in adult SMCs. Alternately, a completely intracellular, growth factor-independent growth stimulus might drive e17 proliferation. Studies are ongoing to determine the molecular mechanisms responsible for the self-driven growth of eSMCs and the changes in gene expression that underlie the loss of serum-independent growth potential. Of particular interest will be the elucidation of the timing mechanism that controls the conversion of SMCs from the embryonic to the adult growth phenotype.

On the basis of our in vitro mitogenesis assays, the replication of eSMCs does not appear to be markedly affected by a variety of exogenously added growth factors (Fig 7). These observations are similar to those reported by Hultgardh-Nilsson et al.27 and Querol-Ferrer et al.,28 who found that neonatal rat SMCs exhibited only weak responses to exogenously added PDGF and EGF, relative to adult control SMCs. Our data extend these findings to embryonic SMCs and demonstrate that this lack of responsiveness may involve an intracellular pathway common to a variety of mitogens. Exogenous growth factors may therefore play an unexpectedly minor role in the regulation of SMC replication during vascular development. A variety of data suggest that autonomous growth and mitogenic unresponsiveness are separate, but overlapping, independently regulated developmental phenotypes (R.A. Majack, C.L. Cook, P.E. Schwartz, M.C.M. Weiser, R.C. McFall, C.F. Reilly, manuscript submitted).

Evidence exists to suggest the presence of a unique subpopulation of SMCs that may be preferentially involved in arterial repair and neointimal formation.20,21-24 This "pup/neointimal" SMC subtype is characterized, in part, by an epitheliod morphology, significant expression of PDGF-B chain mRNA, and a lack of expression of PDGF α-receptor mRNA. The eSMC phenotype described herein is characterized by the typical hill-and-valley morphology of SMCs cultured in serum,16 by a lack of expression of PDGF-B mRNA, and by easily detectable levels of PDGF α-receptor mRNA (data not shown). The eSMC phenotype therefore appears distinct from the pup/neointimal subtype. More intriguing is the possible relation between the eSMC phenotype described herein and a "platelet-derived growth factor-independent" subset of adult SMCs described by Schwartz et al.42 This SMC subtype, isolated by selection in plasma-derived serum, proliferates in the absence of platelet-derived mitogens. Similar to eSMCs, the serum-independent replication of these cells was not associated with the detectable secretion of SMC mitogens into the conditioned medium. Our data therefore reinforce and extend the concept that significant serum-independent replication can occur in SMCs expressing specific developmentally regulated or injury-regulated phenotypes.

Potential Relevance of the Embryonic Growth Phenotype to Vascular Disease States

Vascular SMC replication is an important causative factor in the pathogenesis of atherosclerosis, hypertension, and restenosis after angioplasty and vascular reconstructions. A variety of clinical and experimental studies have repeatedly shown that SMCs proliferating after injury exhibit phenotypic changes characteristic of a reversion to a more "fetal" state.3-6 suggesting that developmentally regulated growth phenotypes may also be reiterated in SMCs after injury. Although SMCs cultured from the neointima of rat and human vessels have been shown to produce increased amounts of PDGF-like mitogens,41,43,44 the functional significance (in terms of autocrine growth potential) of these observations has not been determined, and the molecular mechanism underlying the continued replication of neointimal SMCs has not been determined. It will be interesting to determine if neointimal SMCs, replicating after injury, reiterate the embryonic growth phenotype described in the present study. Experiments to elucidate this possibility are in progress.

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