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SCARA5 and Suprabasin are Hub Genes of Co-expression Network Modules Associated with Peripheral Vein Graft Patency

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Abstract

Objective—About 30% of autogenous vein grafts develop luminal narrowing and fail because of intimal hyperplasia or negative remodeling. We previously found that vein graft cells from patients that later develop stenosis proliferate more in vitro in response to growth factors than cells from patients that maintain patent grafts. To discover novel determinants of vein graft outcome we have analyzed gene expression profiles of these cells using a systems biology approach to cluster the genes into modules based on their co-expression patterns and to correlate the results with growth data from our prior study and with new studies of migration and matrix remodeling.

Methods—RNA from 4 hour serum- or PDGF-BB-stimulated human saphenous vein cells obtained from the outer vein wall (20 cell lines), was used for microarray analysis of gene expression followed by weighted gene co-expression network analysis. Cell migration in microchemotaxis chambers in response to PDGF-BB and cell-mediated collagen gel contraction in response to serum were also determined. Gene function was determined using siRNA to inhibit gene expression before subjecting cells to growth or collagen gel contraction assays. These cells were derived from samples of the vein grafts obtained at surgery, and the long term fate of these bypass grafts was known.

Results—Neither migration nor cell-mediated collagen gel contraction showed a correlation with graft outcome. While 1,188 and 1,340 genes were differentially expressed in response to

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treatment with serum and PDGF, respectively, no single gene was differentially expressed in cells isolated from patients whose grafts stenosed compared to those that remained patent. Network analysis revealed 4 unique groups of genes, which we term modules, associated with PDGF responses, and 20 unique modules associated with serum responses. The "Yellow" and "Skyblue" modules, from PDGF and serum analyses respectively, both correlated with later graft stenosis (P=.005 and .02, respectively). In response to PDGF, Yellow was also associated with increased cell growth. For serum, Skyblue was also associated with inhibition of collagen gel contraction. The hub genes for Yellow and Skyblue (i.e. the gene most connected to other genes in the module), SCARA5 and SBSN, respectively, were tested for effects on proliferation and collagen contraction. Knockdown of SCARA5 increased proliferation by 29.9 ± 7.8% (P<.01), while knockdown of SBSN had no effect. Knockdown of SBSN increased collagen gel contraction by 24.2 ± 8.6% (P<.05), while knockdown of SCARA5 had no effect.

Conclusion—Using weighted gene co-expression network analysis of cultured vein graft cell gene expression, we have discovered two small gene modules, which comprise 47 genes, that are associated with vein graft failure. Further experiments are needed to delineate the venous cells that express these genes in vivo and the roles these genes play in vein graft healing starting with the module hub genes SCARA5 and SBSN, which have been shown to have modest effects on cell proliferation or collagen gel contraction.

Introduction

In spite of the growing number of less invasive alternatives, autogenous vein grafts continue to be used to bypass diseased segments of coronary and peripheral arteries. Greater than 300,000 vein bypass grafts are placed in the United States per year and about 30% develop luminal narrowing and faileither because of intimal hyperplasia or negative remodeling. The biological underpinnings of these derangements in vascular healing are still poorly understood, and current treatment for failed reconstructions is limited to surgical or endovascular intervention at considerable cost and morbidity. Thus, further research into potential pharmacological targets to prevent failure remains a priority.

In a previous study we found that vein graft cells from patients that develop stenosis proliferate more in vitro in response to growth factors than do cells from patients whose grafts remain patent 12 months after surgery. In an attempt to discover novel determinants of vein graft outcome we have analyzed whole genome gene expression profiles of these cells. We stimulated the cells with PDGF, since our previous study indicated a differential response to this growth factor. In addition, we also used serum which signals primarily through G protein coupled receptors, as an alternative to PDGF which signals via tyrosine kinase receptors. Because complex diseases such as vein graft failure most likely involve many interacting genes, we then used a systems biology approach to cluster the genes into groups (i.e. modules) by performing all pair-wise comparisons of gene expression among the >20,000 genes. The variability associated with treatment and individual patient differences allowed clustering into modules based on the similarity of changes in gene co-expression patterns. The aggregate behavior of genes of each module was represented by an "eigengene" value, which was then correlated with growth data from the prior study and with newly acquired rates of migration and of collagen gel contraction, the latter as an in

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vitro model of negative remodeling. In this study, we have identified two gene modules, one after PDGF-BB stimulation and the other after serum stimulation, that are correlated with vein graft failure and with either cell proliferation or collagen gel contraction.

Methods

Detailed methods can be found online (see Appendix 1. Supplemental Methods). In brief, the same human saphenous vein cell lines used in our previous study were used for the gene expression and network analysis study with additional cell lines added for other experiments. The age of vein donor patients and the prevalence of diabetes, hypertension, dyslipidemia, renal insufficiency, or coronary artery disease were not significantly different in those who developed vein graft stenosis or not (Supplemental Table I). These cells originate from explants of an easily dissected outer layer of the vein that was separated from a visually distinct, white, lumenal layer after removal of endothelium. We have previously described these cells as adventitial fibroblasts, but immuno-histochemical staining for smooth muscle alpha actin demonstrates that the dissection plane goes through the outer circular muscle layer of the media. Thus, we now call these cells "outer wall cells" rather than fibroblasts to more accurately define their derivation. Cells were treated for four hours with either PDGF-BB or serum after serum-starvation for 3 days. Total RNA was isolated and gene expression profiling was performed with Illumina HT-12 v3 Expression BeadChips. Differential gene expression analyses to compare FBS or PDGF-BB treated cells to serum-free medium controls were performed using Patterns of Gene Expression (PaGE v5.1.6). Genes were considered to be differentially expressed if their expression changed at least 2 fold with less than a 1% false discovery rate.

Expression levels of 23,493 genes were used to identify groups of genes (modules) whose expression is highly correlated with each other by performing all pair-wise comparisons of gene expression across all microarray samples using weighted gene co-expression network analysis (see Appendix 1. Supplemental Methods). Modules were named with unique colors. A summary of the network construction procedure is presented in Supplemental Figure 1. Cell migration in response to PDGF-BB with and without heparin was determined using a microchemotaxis chamber (Neuroprobe, Inc.). Collagen gel contraction, which was used as a model of vascular remodeling, was determined using bovine collagen. The function of genes was determined using gene knockdown that was achieved using electroporation of siRNA (Amaxa Basic Nucleofector Kit Primary Fibroblasts, Lonza, Inc.) followed by proliferation or collagen gel contraction assays.

Results

Cell Migration and Collagen Gel Contraction

We have previously demonstrated a correlation between vein graft patency at one year and in vitro proliferation of vein outer wall cells. Therefore, we measured the ability of these cells to migrate and contract collagen gels in vitro to determine whether graft patency was associated with these measures of cellular function. Migration in response to PDGF-BB showed no correlation with graft outcome (Fig 1). In addition, while the inhibitory effect of heparin on serum- and PDGF-mediated proliferation has been shown to be less in cells from

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patients whose grafts failed, we found that 100 μg/ml heparin did not inhibit PDGF-BB-mediated cell migration and there was no difference as a function of graft outcome (data not presented). Finally, cell-mediated collagen gel contraction, which was near maximal by 24 hours (Fig 2A), showed no correlation with graft outcome (Fig 2B).

Global Gene Expression and Network Analysis

1,188 and 1,340 genes were differentially expressed in response to treatment with FBS and PDGF, respectively (supplementary file 1 and 2 available directly from the author: rkenagy@uw.edu). However, no single gene was differentially expressed in cells isolated from patients whose grafts stenosed compared to those that remained patent.

While individual genes may not show association with the vein graft outcome, groups of genes that share a common function or are part of the same biological pathway may be abnormally regulated in stenotic veins. Therefore we constructed co-expression networks from gene expression data and clustered genes into modules of genes that may share similar functions. We analyzed the serum and PDGF-BB data separately because some outcomes were related to treatment with serum (collagen gel contraction) and some to treatment with PDGF (microchemotaxis and proliferation in response to PDGF).

Network Modules after Serum Stimulation – Skyblue Module

Twenty modules were identified by network analysis of gene expression after treatment with serum (16,790 genes with $P_{detection}<0.01$ in at least one sample; Table I and Supplementary File 3 [lists module memberships and is available directly from the author: rkenagy@uw.edu]). The module called Skyblue was positively associated with graft stenosis. Skyblue was also positively associated with collagen gel area meaning it was negatively associated with gel contraction. Several of the other modules were also positively and negatively associated with gel contraction. There was no significant enrichment in gene ontology terms in any of modules after Bonferroni correction.

Network Modules after PDGF Stimulation – Yellow Module

Network analysis of gene expression after treatment with PDGF-BB was limited to those genes with $P_{detection}<0.01$ in at least one sample and having a coefficient of variation of at least 10% (N=2847 genes). Four gene modules were identified (table II and Supplementary File 4; the latter is available directly from the author: rkenagy@uw.edu) and the module called Yellow was positively associated with graft stenosis. In addition, Yellow tended to be positively associated with entry into S phase in vitro using ^{3}H-thymidine incorporation data from our prior study with these same cell lines. Yellow was not significantly associated with PDGF-mediated cell migration. There was no significant enrichment in gene ontology terms in any of modules after Bonferroni correction.

Function of Yellow and Skyblue Hub Genes in Cell Proliferation and Collagen Gel Contraction

Of particular interest Yellow and Skyblue are small gene sets comprising less than 30 genes, but they share five genes (highlighted in table III). In addition, the paralogs, HSPA12A and HSPA12B, are members of Skyblue and Yellow, respectively. Since hub genes are the most

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highly connected genes to other members of each module, we tested the effect of siRNA-mediated knockdown of SCARA5 (Scavenger Receptor Class A, Member 5), the Yellow hub gene, and of SBSN (Suprabasin), the Skyblue hub gene, on cell proliferation and collagen gel contraction. In the collagen gel contraction assay, knockdown of SCARA5 and SBSN by their respective siRNA was 92±2% and 97±1% just before suspending cells in the collagen (mean±SEM, N=5). Knockdown of SCARA5 had no effect on collagen gel contraction, while knockdown of SBSN led to a modest increase in contraction (Fig 3A).

PDGF-BB increased levels of SCARA5 mRNA by 196±37% of 2% FBS alone (N=6) measured at the end of the proliferation assay (3 days), but had no effect on SBSN mRNA (93±14% of 2% FBS alone; N=4). Knockdown of SCARA5 and SBSN by their respective siRNA in the presence of PDGF was 93±5% and 77±10% measured at the end of the proliferation assay. In contrast to collagen gel contraction, knockdown of SBSN had no effect on proliferation, while knockdown of SCARA5 led to a modest increase in proliferation (Fig 3B).

Discussion

Using weighted gene co-expression network analysis, we have found that two modules of genes named Yellow and Skyblue, each of which comprise less than 30 genes, are positively associated with vein graft failure. In contrast, while expression of more than 1000 genes was significantly altered by serum or PDGF, no single gene was associated with graft failure. Yellow was also positively associated with entry into the cell cycle, which is not surprising given that the cell lines used for this network analysis were those in which an association between cell cycle entry and vein graft outcome was previously demonstrated. Furthering the connection between the Yellow module and growth, the hub gene for the Yellow module, SCARA5, was induced by PDGF-BB. Hub genes are the most highly connected (i.e. the highest kWithin in Table III) to other members of the module. SCARA5 was also found to be an inhibitor of PDGF-BB-mediated growth suggesting a negative feedback mechanism on the proliferative response. While the effect of SCARA5 on growth was modest, this might be expected for a clinical effect that takes months to appear and is mechanistically multifactorial. SCARA5 has endocytotic activity, which is similar to other members of the SCARA family, that appears to inhibit inflammatory fibrotic responses. It has also been described as a tumor suppressor, that is associated with epithelial-mesenchymal transition and mesenchymal stem cell differentiation, . The inhibitory effect of SCARA5 on growth has been ascribed to the binding of FAK, which in turn inhibits downstream src-p130Cas activity. In this regard, a study of smooth muscle cells reported that targeted knockout of FAK did not influence cell growth, which appears to preclude a role for FAK in the inhibitory effect of SCARA5. However, Ojala et al reported that expression of SCARA5 in mice is limited to specific populations of fibroblasts in the interstitial space of most organs and is not found in smooth muscle alpha actin positive cells. Thus, in the vein SCARA5 may be limited to non-smooth muscle adventitial cells in which it may play a role in growth regulation.

While we previously described our venous outer wall cells as adventitial fibroblasts, we have determined that the outer layer used to derive these cells contains both adventitia and part of

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the outer circular muscle layer of the media. In addition, the adventitia contains longitudinal cords of smooth muscle alpha actin-positive cells. Thus, both outer and inner wall cells come from tissue containing smooth muscle alpha actin-positive cells. However, we have observed significant differences between outer and inner wall cells. Outer wall cells respond more to growth factors than inner wall cells and the association between cell growth and graft patency was greater with the outer wall cells. In addition, we have found that the in vitro growth of outer wall cells, but not inner wall cells, is associated with the genotype of the −838C>A p27^{Kip1} single nucleotide polymorphism (manuscript in preparation) that is itself associated with peripheral vein graft failure. It is not clear whether the outer wall cells are derived from a smooth muscle cell lineage, but future experiments to determine whether these cells have epigenetic marks indicating smooth muscle lineage, such as H3K4me2 at the MYH11 locus, should be informative. While a role for adventitial cells as a source of arterial intimal cells continues to be controversial−, the little data available suggest that an adventitial role in vein graft intimal hyperplasia is possible, . Adventitial progenitor cells in rat and human arteries and veins can differentiate into smooth muscle alpha actin-positive cells−, which irrespective of any role in intimal hyperplasia may be involved in remodeling.

Negative remodeling has been reported to be a large contributor to luminal loss in vein grafts, , and grafts that do not undergo adequate positive remodeling during the first month are more prone to later failure, . Knockdown of SBSN (Suprabasin), the hub gene for the Skyblue module, increased collagen gel contraction, but did not alter cell proliferation despite evidence that SBSN promotes growth of carcinoma cells, . The Skyblue module was positively associated with graft failure, but it was negatively associated with collagen gel contraction. Simplistically, one would expect the contraction of vessel size seen with negative remodeling to be associated with increased collagen gel contraction. However, a counter-intuitive inverse relation between collagen gel contraction in vitro and negative arterial remodeling in vivo has also been reported when Rho-kinase or RHAMM are blocked, . An explanation of these conflicting results may lie with a mechanoregulation model proposed by Grinnell and colleagues. In this model cell migration and collagen translocation can cause tissue movement in a strain-dependent manner. Support for this model comes from observations after vascular injury. Releasing tension by external wrapping of arteries causes tissue regression through apoptosis and loss of extracellular matrix. SMCs in collagen gels that float freely and are not attached to the side walls of the tissue culture dish exhibit higher rates of cell death and lower production of extracellular matrix production than SMCs in gels under tension. Finally, increasing tension by stretching arteries ex vivo causes cell proliferation and increased tissue mass, . Tension experienced by the cell has been shown to regulate which signaling pathways are utilized including FAK, . Thus, it is possible that the lack of tension in the floating gel assay may have altered the function of module genes and masked an association of graft failure with collagen contraction.

While neither the Yellow nor Skyblue modules showed significant gene ontology enrichment, a review of the literature shows many of the members of these modules are associated with cell proliferation and inflammation (Table IV; see Supplementary Table I for references), which is consistent with what is known of the natural history of vein graft healing and pathology. In addition, the venous vasa vasorum, which oxygenates the

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adventitia and most of the media, is disrupted by surgical dissection and distension from elevated pressures that compress the wall. Data from animal models indicates that cell death is prevalent, necessitating cell proliferation. Eleven of 25 and 6 of 19 named genes in the Yellow and Skyblue modules, respectively, are associated with inflammation or cell death. Of interest, one of these is CFD (complement factor D) and two single nucleotide polymorphisms of complement receptor 1 were found to be associated with vein graft failure. Nine Yellow and 7 Skyblue genes are associated with cell proliferation. In addition, of the 5 genes common to Yellow and Skyblue two are associated with proliferation (ANGPTL5 and SBSN), two with progenitor cell function (ANGPTL5 and PRG4 (hemangiopoietin)), and two with inflammation (PRG4 and DMKN) (Supplementary Table I). DMKN and SEPP1 (members of Yellow) both inhibit skin wound healing and SEPP1 also inhibits recovery from hind-limb ischemia, . SBSN and DMKN are co-regulated during embryonic epidermal development and the fifth common gene, RAI-2, may be involved in cell differentiation solely based on known functions of retinoic acid. Finally, FOLR3, which is also linked to development and progenitor cells, is a secreted isoform that was found to be upregulated in monocytes from patients with early onset cardiovascular disease.

In conclusion, weighted gene co-expression network analysis using cultured vein graft cells has led to the discovery of two small gene modules, which comprise 47 genes that are associated with vein graft failure. While basic cardiovascular risk factors were evenly distributed among the samples associated with stenosis or no stenosis (Supplemental Table I), a weakness of the study is the lack of detailed anatomical information about the diameter or other characteristics of the bypass graft. Further experiments are needed to discriminate the venous cells that express these module genes in vivo and the roles these genes play in vein graft healing starting with the module hub genes SCARA5 and SBSN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Few genes have been identified as associated with coronary and peripheral arterial graft failure. Using network analysis to cluster genes based on co-expression patterns in cells cultured from vein grafts with known clinical outcome, we found two gene modules associated with graft failure. The hub gene of one module, SCARA5, is a cell growth inhibitor, while the hub gene of the other module, SBSN, inhibits collagen matrix remodeling. Delineation of the venous cells that express SCARA5 and SBSN in vivo and the roles these genes play in vein graft healing may lead to preventative treatments for vein graft failure.

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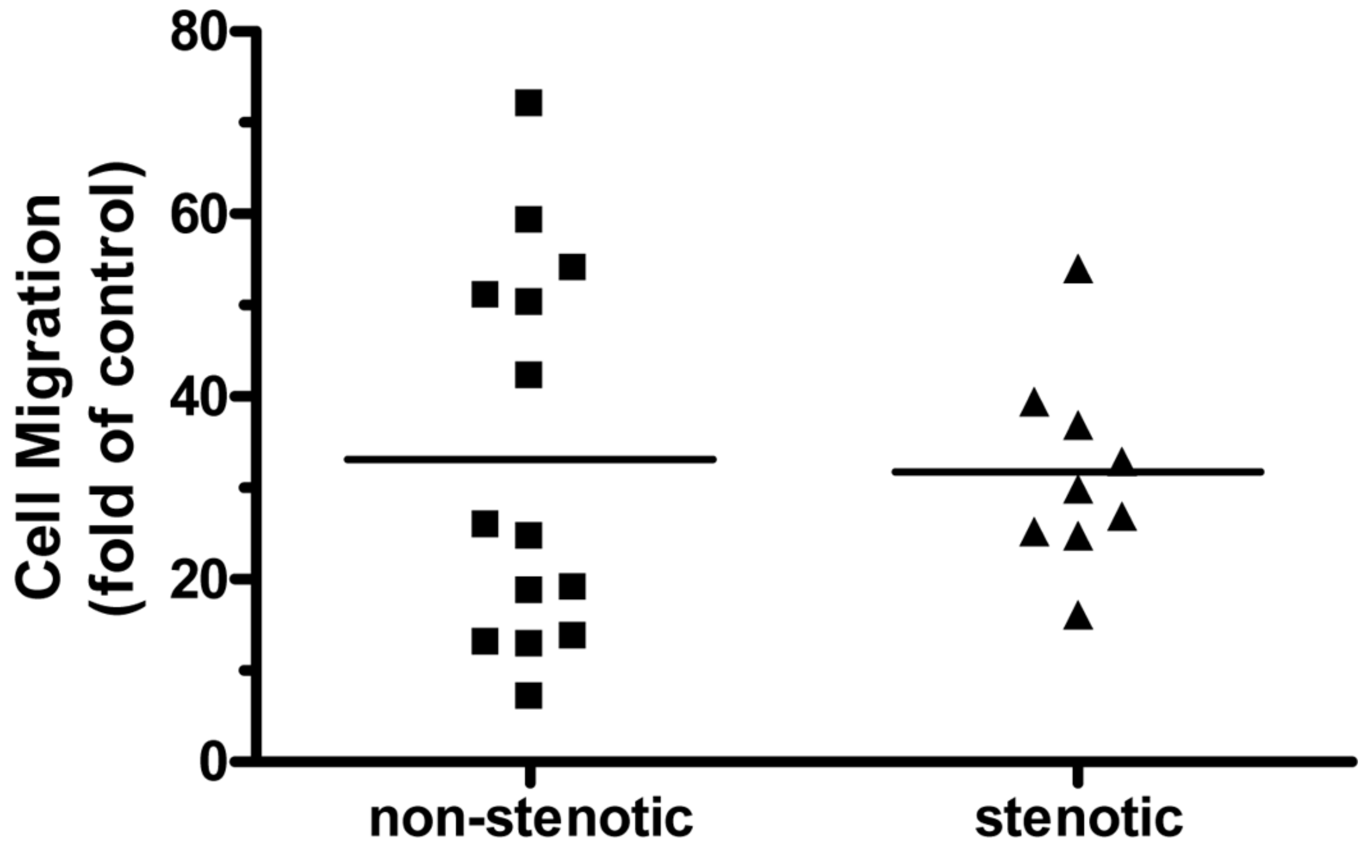

Figure 1.

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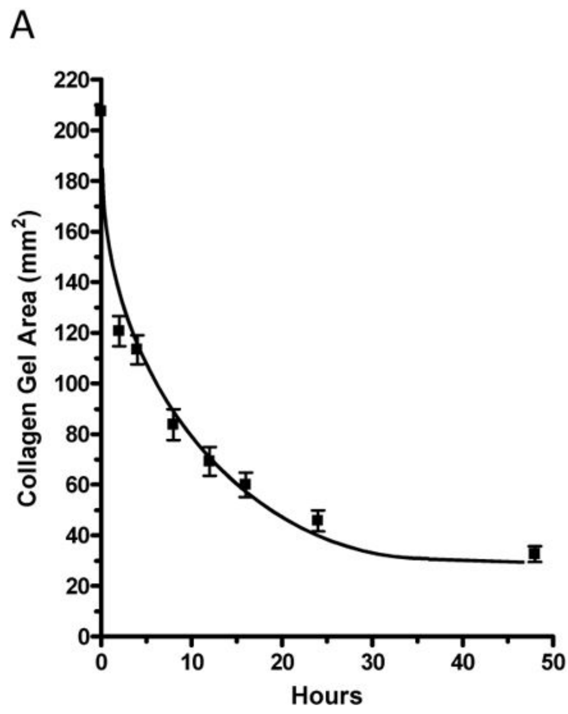

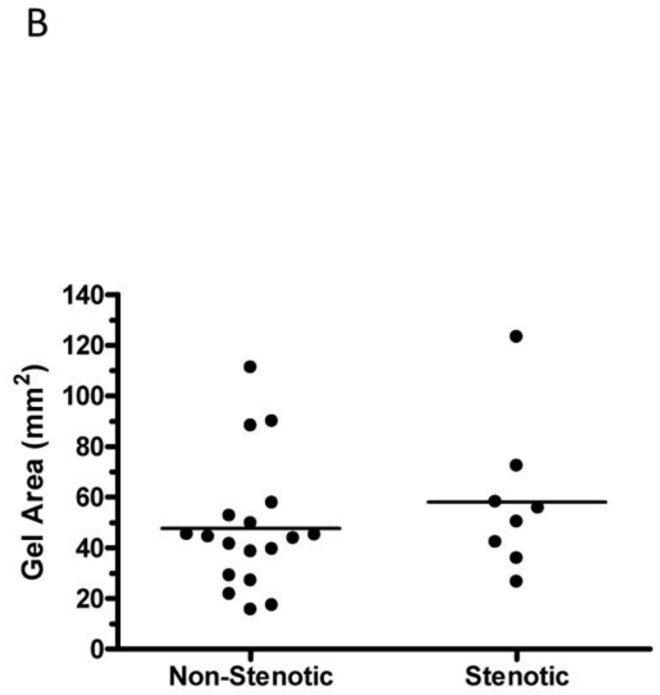

Figure 2.

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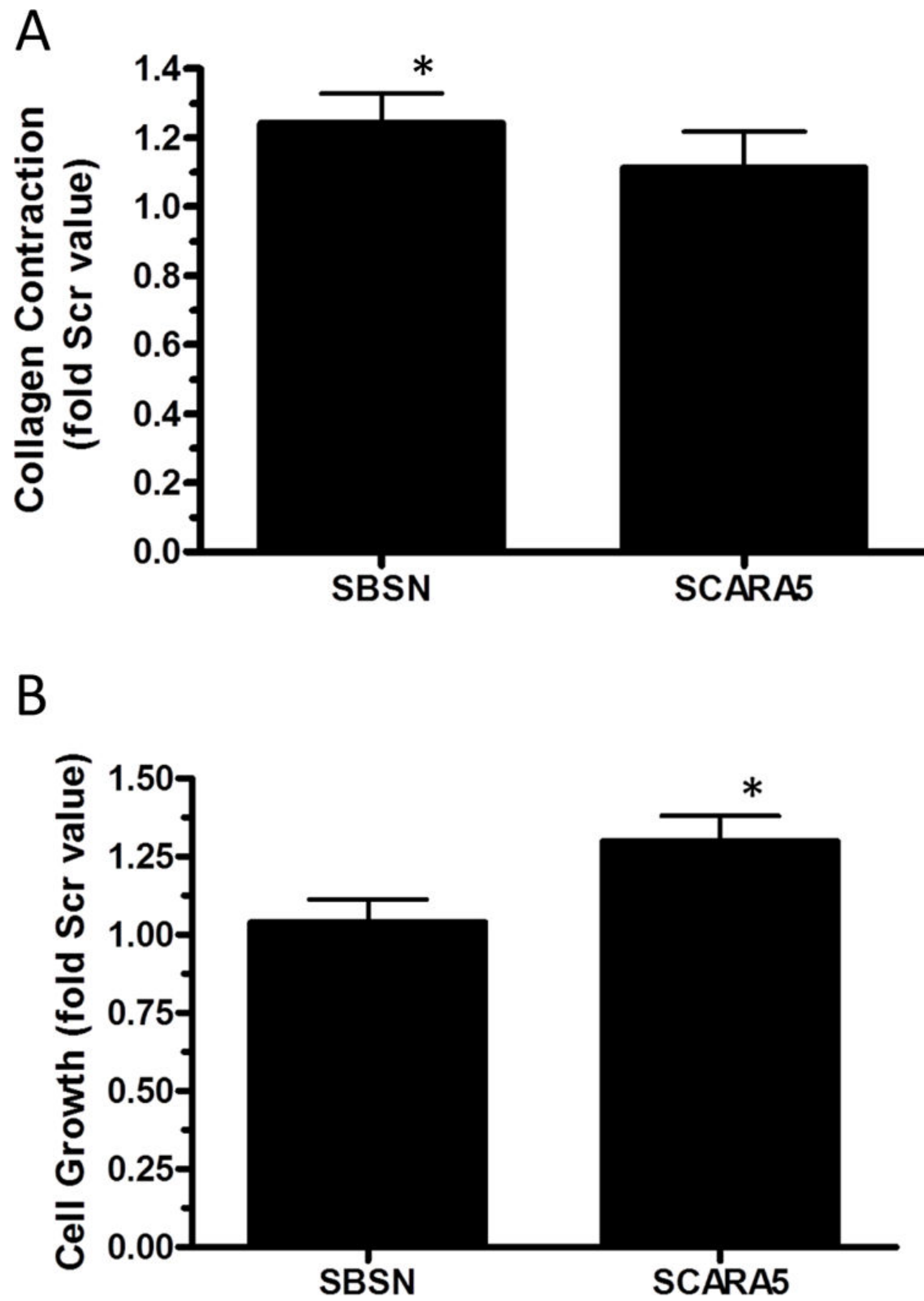

Figure 3.

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Table I

Association of serum-stimulated gene co-expression modules with graft failure and collagen gel contraction. For gene membership of modules see Supplemental File 3.

Module	# of Genes	Graft Failure	Collagen Gel Area 24 h
MEturquoise	6737	−0.025 p=(0.9)	−0.035 p=(0.8)
MEmidnightblue	123	−0.18 p=(0.3)	−0.31 p=(0.005)
MEdarkorange	27	−0.044 p=(0.8)	−0.3 p=(0.05)
MEroyalblue	74	−0.11 p=(0.5)	−0.084 p=(0.08)
MEdarkturquoise	506	−0.073 p=(0.7)	−0.42 p=(0.03)
MEpurple	255	−0.13 p=(0.4)	−0.53 p=(0.005)
MEblue	3548	−0.022 p=(0.9)	0.00018 p=(0.8)
MEcyan	282	0.045 p=(0.8)	0.32 p=(0.7)
MEgreen	1071	−0.051 p=(0.8)	0.17 p=(0.9)
MEskyblue	21	0.36 p=(0.02)	0.57 p=(6e−09)
MEdarkgreen	54	0.14 p=(0.4)	0.28 p=(0.04)
MElightcyan	101	0.22 p=(0.2)	0.51 p=(0.001)
MElightyellow	1217	0.15 p=(0.4)	0.31 p=(0.03)
MEyellow	909	0.016 p=(0.9)	−0.3 p=(0.9)
MEdarkred	337	0.076 p=(0.6)	−0.33 p=(0.6)
MElightgreen	83	−0.035 p=(0.8)	−0.55 p=(0.05)
MEblack	437	−0.095 p=(0.6)	−0.37 p=(0.5)
MEorange	27	−0.081 p=(0.6)	−0.35 p=(0.1)
MEdarkgrey	708	0.11 p=(0.5)	−0.11 p=(0.3)
MEgreenyellow	250	0.095 p=(0.6)	−0.028 p=(0.3)

P value is enclosed in parentheses.

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Table II

Association of PDGF-stimulated gene co-expression modules with graft failure and PDGF-mediated cell growth and migration. For gene membership of modules see Supplemental File 4.

	Gene #	Graft Failure	Basal-Growth	PDGF-Growth	PDGF & Thrombin-Growth	PDGF & S1P-Growth	PDGF-Migration
MEbrown	387	0.0065 (1)	−0.31 (0.05)	−0.18 (0.3)	−0.22 (0.2)	−0.28 (0.08)	0.13 p=(0.4)
MEturquoise	1237	0.048 (0.8)	−0.37 (0.02)	−0.28 (0.09)	−0.32 (0.05)	−0.36 (0.02)	0.09 p=(0.6)
MEblue	926	−0.028 (0.9)	0.35 (0.03)	0.25 (0.1)	0.28 (0.08)	0.34 (0.03)	−0.12 p=(0.5)
MEyellow	27	0.43 (0.005)	0.45 (0.004)	0.28 (0.08)	0.33 (0.04)	0.29 (0.07)	0.25 p=(0.1)

P value is enclosed in parentheses.

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Table III

Genes of co-expression modules Skyblue and Yellow. kWithin describes the connectivity between genes in each module relative to 1 as the highest connectivity value. Genes common to both modules are highlighted.

Skyblue Module		Yellow Module	
Gene	kWithin	Gene	kWithin
SBSN	1	SCARA5	1
GPR177	0.93322	METTL7A	0.86873
PRG4	0.89402	ABCC2	0.86382
ANGPTL5	0.88057	SEPP1	0.86359
TPR	0.8747	LOC644222	0.78888
DMKN	0.77106	DNM1	0.77801
RAI2	0.76135	ASPA	0.75205
C16ORF45	0.70057	SBSN	0.75036
FGD1	0.6904	GSN	0.74069
PROCR	0.68561	CFD	0.72897
GNRH1	0.6623	LAMB3	0.7155
TRH	0.6083	AIF1L	0.70752
HOMER3	0.59784	F10	0.68841
SAMD9L	0.57969	FOLR3	0.67669
LOC652082	0.57307	PRG4	0.65364
TPMT	0.56117	DENND2A	0.6286
HSPA12A	0.52037	CILP	0.61032
CBR1	0.5104	RAI2	0.60388
RASA4P	0.4526	G0S2	0.58433
ABI1	0.41369	TPPP3	0.56034
LOC728081	0.38511	ANGPTL5	0.46327
		AGTR1	0.44939
		DMKN	0.40289
		ADH1B	0.37079
		RASL12	0.35846
		HSPA12B	0.30395

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Table IV

Functional classification of members of the gene modules associated with vein graft failure. References for these classifications are listed in Supplementary Table I.

Function	Yellow genes	Skyblue genes
Cell proliferation	SCARA5 (Scavenger Receptor Class **A** Member 5) SEPP1 (Selenoprotein P Plasma, 1) SBSN (Suprabasin) F10 (Coagulation Factor X) CILP (Cartilage Intermediate Layer Protein) G0S2 (G0/G1switch 2) TPPP3 (Tubulin Polymerization-Promoting Protein Family Member 3) ANGPTL5 (Angiopoietin-like 5) AGTR1 (Angiotensin II Receptor, type 1)	SBSN (Suprabasin) TPR (Translocated Promoter Region, Nuclear Basket Protein) PROCR (Protein C Receptor, Endothelial) TRH (Thyrotropin-Releasing Hormone) SAMD9L (Sterile Alpha Motif Domain Containing 9-like) ANGPTL5 (Angiopoietin-like 5) ABI1 (Abl-Interactor 1)
Migration/Actin Dynamics	SCARA5 (Scavenger Receptor Class **A** Member 5) SEPP1 (Selenoprotein P Plasma, 1) F10 (Coagulation Factor X) TPPP3 (Tubulin Polymerization-Promoting Protein Family Member 3) HSPA12B (heat shock 70kD protein 12B) AGTR1 (Angiotensin II Receptor, type 1)	FGD1 (FYVE, RhoGEF and PH domain containing 1) TRH (Thyrotropin-Releasing Hormone) HOMER3 SAMD9L (Sterile Alpha Motif Domain Containing 9-like) ABI1 (Abl-Interactor 1)
Development	FOLR3 (folate receptor 3 [gamma]) PRG4 (Proteoglycan 4, lubricin) HSPA12B (heat shock 70kD protein 12B)	GPR177 (WNTLESS) PRG4 (Proteoglycan 4, lubricin)
Stem/Progenitor Cells	SCARA5 (Scavenger Receptor Class **A** Member 5) FOLR3 (folate receptor 3 [gamma]) PRG4 (Proteoglycan 4, lubricin) G0S2 (G0/G1switch 2) ANGPTL5 (Angiopoietin-like 5) AGTR1 (Angiotensin II Receptor, type 1)	PRG4 (Proteoglycan 4, lubricin) ANGPTL5 (Angiopoietin-like 5)
Inflammation/Stress/Cell Death related	SCARA5 (Scavenger Receptor Class **A** Member 5) SEPP1 (Selenoprotein P Plasma, 1) GSN (Gelsolin) CFD (complement factor D, adipsin) F10 (Coagulation Factor X) PRG4 (Proteoglycan 4, lubricin) CLIP CILP (Cartilage Intermediate Layer Protein) G0S2 (G0/G1switch 2) AGTR1 (Angiotensin II Receptor, type 1) DMKN (Dermokine) HSPA12B (heat shock 70kD protein 12B)	PRG4 (Proteoglycan 4, lubricin) TPR (Translocated Promoter Region, Nuclear Basket Protein) DMKN (Dermokine) PROCR (Protein C Receptor, Endothelial) HOMER3 SAMD9L (Sterile Alpha Motif Domain Containing 9-like)
Wound Healing	SEPP1 (Selenoprotein P Plasma, 1) DMKN (Dermokine)	DMKN (Dermokine)
Vesicular trafficking/Transmembrane transport/GTPases	SCARA5 (Scavenger Receptor Class **A** Member 5) ABCC2 (ATP-binding cassette, subfamily C (CFTR/MRP), member 2) DNM1 (Dynamin 1) GSN (Gelsolin) DENND2A (DENN/MADD domain containing 2A) DMKN (Dermokine)	TPR (Translocated Promoter Region, Nuclear Basket Protein) DMKN (Dermokine) SAMD9L (Sterile Alpha Motif Domain Containing 9-like) ABI1 (Abl-Interactor 1)