Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Vascular Research Initiatives Conference (VRIC) 2022
Translational Immunology and Cardiovascular Disease

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22-VIRC-455-AHA-VD

The Histone Methyl Transferase (SUV39H1) Promotes Smooth Muscle Cell Dedifferentiation

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Objectives: The phenotypic plasticity of vascular smooth muscle cells (VSMCs) is central to growth and remodeling processes, but also contributes to the pathology of atherosclerosis, restenosis, and other cardiovascular diseases. This ability of VSMCs to reversibly differentiate and dedifferentiate is incompletely understood. SUV39H1, a histone methyltransferase, specifically trimethylates Lys-9 of histone H3 (H3K9me3), resulting in epigenetic transcriptional repression. We hypothesized that SUV39H1 plays a role in VSMC phenotypic switching.

Methods: Using knockdown, quantitative polymerase chain reaction, Western blot, chromatin immunoprecipitation, RNA sequencing, and murine vascular injury to determine the role of SUV39H1 in VSMC plasticity.

Results: A quantitative polymerase chain reaction array screen of epigenetic regulators revealed that SUV39H1 is upregulated with platelet-derived growth factor -induced dedifferentiation, but downregulated with rapamycin-induced differentiation in human coronary artery smooth muscle cells. SUV39H1 knockdown promoted differentiation measured by increased contractile gene, protein expression, enhanced contractility, decreased migration, proliferation, and dedifferentiation-associated gene expression. RNA sequencing transcriptomics confirmed changes in multiple pathways consistent with a role for SUV39H1 in promoting human coronary artery smooth muscle cell dedifferentiation. Mechanistically, SUV39H1 knockdown suppressed expression of KLF4, the master transcriptional regulator of VSMC dedifferentiation, decreasing KLF4 messenger RNA stability and upregulating miRNA143, a known repressor of KLF4. siSUV39H1 also increased expression of KDM4a, a JMJD family lysine demethylase that targets H3K9me3. Chromatin immunoprecipitation assays at contractile gene promoters showed significant decrease in the H3K9me3 mark and increase in H3K27Ac after SUV39H1 knockdown. In vivo, we noted a significant increase in SUV39H1 and H3K9me3 expression in murine carotid artery ligation induced intimal hyperplasia.

Conclusions: We identify SUV39H1 as an epigenetic regulator of VSMC phenotype whose expression and activity increase with dedifferentiation in vitro and in vivo. Platelet-derived growth factor promotes H3K9me3 repressive marks at contractile genes by promoting expression of SUV39H1, which also inhibits the KDM4a. Understanding the role of SUV39H1 in VSMC plasticity may reveal new therapeutic strategies for treating vascular diseases.

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22-VIRC-527-AHA-VD

Single Cell Gene Expression Of Brachial Artery In Response To Increased Shear Stress

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Objective: Blood flow dynamics modulate vascular development, homeostasis, and contribute to vascular pathobiology. However, it is unknown how shear stress influences cellular transcriptomics of the arterial wall. Two-stage hemodialysis access surgeries provide an opportunity to compare native vessels with vessels after exposure to significantly altered hemodynamics.

Methods: Single-cell sequencing was performed on brachial artery samples obtained from three patients at the time of first and second stage surgeries. Arterial cellular composition and temporal changes in gene expression were quantified after sequencing and functional analyses were performed on differentially expressed genes.

Results: Compositional changes and gene expression dynamics were highest in the smooth muscle cell (SMC) populations followed by fibroblasts. SMCs were clustered into contractile (classical) and modified populations, with the latter having lower expression of contractile markers. Downregulation of the transcription factor ARNTL was the most significant controller in contractile SMCs and fibroblasts, and second behind heat-inducible factor 1α in modified SMCs. Macrophages had a significant increase in composition, but few dynamic genes suggest a return to quiescence. Endothelial cells showed minimal changes with dynamic genes responding to mechanical stimulus. An ingenuity pathway analysis revealed activation of EIF2 signaling in both SMC and fibroblast clusters, promoting translation. The top divergent pathway among SMC clusters is PI3/AKT signaling, with increased vascular remodeling, proliferation, and cell death in the contractile group.

Conclusions: Cellular processes are overall downregulated in modified SMCs, which represent the majority of SMCs after vascular adaption. This study is the first single-cell characterization of normal human artery and its cellular response to increased hemodynamic forces, specifically detailing cellular compositional changes and their associated dynamic genes.

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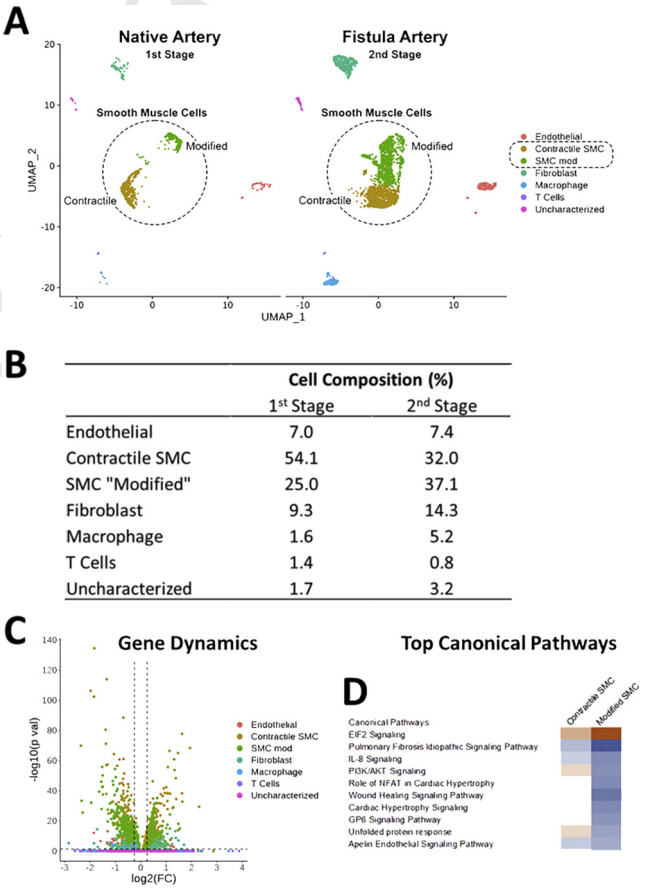

B

	Cell Composition (%)	
	1st Stage	2nd Stage
Endothelial	7.0	7.4
Contractile SMC	54.1	32.0
SMC "Modified"	25.0	37.1
Fibroblast	9.3	14.3
Macrophage	1.6	5.2
T Cells	1.4	0.8
Uncharacterized	1.7	3.2

Fig. ■■■.

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22-VIRC-518-AHA-VD

The Epigenetic Enzyme KMT2A/MLL1 Is a Driver of Coronavirus-associated Coagulopathy

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Objectives: Coronavirus-associated coagulopathy is postulated to be driven by systemic macrophage activation after severe acute respiratory syndrome coronavirus 2 infection and presents with an increased risk of thrombogenesis and hyperfibrinolysis. Previous work shows that the histone methyltransferase KMT2A/MLL1 is a key mediator of inflammatory signaling in monocytes and macrophages (Mo/Mφs). In this study, we sought to identify the regulation of factors important in coronavirus-associated coagulopathy by MLL1.

Methods: Mice with myeloid specific knockout of MLL1 (Cre^+) and littermate controls (Cre^-) underwent intranasal inoculation of 2×10^5 pfu of the murine coronavirus MHVA59, an established model which phenocopies severe acute respiratory syndrome coronavirus 2 infection. Splenic Mφs (surrogate for circulating Mo/Mφs) were isolated and RNA and protein levels of urokinase (Plau; profibrinolytic), urokinase receptor (Plaur; profibrinolytic), and tissue factor (F3/TF; procoagulant) were analyzed using quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. Thromboelastography on whole blood and urokinase activity assays from mouse plasma were performed. Urokinase and tissue factor activity assays were performed on plasma from human samples.

Results: RNA (Figure, *top panel*) and protein (Figure, *bottom*) levels of Plau, Plaur, and F3 were suppressed in the splenic Mφs harvested from sham (intranasal phosphate-buffered saline) and infected Cre^+ animals (*white bars*) compared with splenic Mφs harvested from Cre^- animals (*blue bars*; Figure, *A*). Cre^- mice displayed a shortened reaction time as measured by thromboelastography (Figure, *B*) and elevated plasma urokinase activity levels (not shown). Hospitalized coronavirus disease 2019-positive patients displayed elevated plasma urokinase and tissue factor activity levels (Figure, *C*).

Conclusions: We identify a role for MLL1 for basal expression and for coronavirus-mediated induction of factors important for fibrinolysis and coagulation in murine Mo/Mφs and in driving coagulopathy. Our results suggest that MLL1 blockade may be an attractive strategy to combat coronavirus associated coagulopathy.

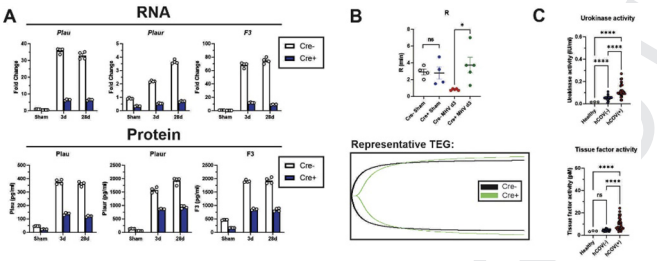

Fig. ■■■.

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22-VIRC-526-AHA-VD

Thrombospondin-5 Is Necessary for Males But Not Females in Hindlimb Ischemia Recovery

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Background: Ameliorating critical limb ischemia (CLI), in part, relies on arteriogenesis and angiogenesis. We have shown that thrombospondin-5 (TSP5) is proangiogenic in vitro. The role of TSP5 in CLI and the differences between males and females are unknown. Blood flow recovery, arteriogenesis, and angiogenesis after limb ischemia is diminished (1) in TSP5-null mice compared with controls, and (2) in female compared with male TSP5 null-mice.

Methods: Fourteen- to 16-week-old wild-type (WT; C57Bl6) or TSP5-null male and female mice underwent left femoral artery ligation and transection (n = 6-9/group). Laser Doppler data were collected preoperatively, after ligation, and at humane killing (day 14). Blood flow recovery was expressed as a ratio of ischemic/nonischemic limb. Immunohistochemistry was performed using anti-α-smooth muscle actin on the vastus lateralis to quantify arteriogenesis and anti-CD31 on the gastrocnemius to quantify angiogenesis. Arteriole and capillary density were determined by vessel counts/5 high-power field. Data were analyzed by analysis of variance with post hoc testing, with a *P* values of less than .05 being significant.

Results: TSP5-null mice had decreased blood flow recovery in males (WT 0.85 ± 0.04 vs TSP5 0.48 ± 0.06; $P < .001$) and females (WT 0.63 ± 0.07 vs TSP5 0.36 ± 0.04; $P < .01$). No difference in flow recovery was seen between male and female TSP5 nulls (male 0.48 ± 0.06 vs female 0.36 ± 0.04; $P < .14$). Arteriogenesis was impaired in male TSP5 null mice (WT 3.91 ± 0.24 vs TSP5 2.6 ± 0.42; $P < .02$), but increased in females (WT 2.5 ± 0.26 vs TSP5 4.35 ± 0.73; $P < .01$). Angiogenesis was decreased in TSP5-null males (WT 10.9 ± 0.93 vs TSP5 7.03 ± 0.84; $P < .01$), but not females (WT 6.1 ± 0.50 vs TSP5 5.6 ± 0.80; $P < .9$). In contrast, flow recovery, angiogenesis, and arteriogenesis were decreased in WT females compared with WT males ($P < .05$).

Conclusions: Our findings suggest that TSP5 is relevant to vascular remodeling following hindlimb ischemia with a divergence in mechanism between males and females. TSP5 is necessary in males for arteriogenesis and angiogenesis. TSP5 in females may be suppressive of arteriogenesis and has no effect on angiogenesis. Further investigation into these sexually dimorphic adaptive processes may lead to specific targeted CLI treatments.

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22-VIRC-457-AHA-VD

Microvessel Oxidative Stress Predicts Changes in Leg Function of Patients with Peripheral Arterial Disease after Supervised Exercise Therapy

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Objective: To evaluate baseline oxidative stress in the microvessels of patients with peripheral arterial disease (PAD) (1) in association with heme oxygenase-1 (HO-1), a critical cytoprotective molecule for oxidative stress (2) as a predictor of the functional and pathophysiologic changes that occur in response to 6 months of supervised exercise therapy (SET). We hypothesized that accumulated oxidative stress in the microvessels of PAD at baseline would predict changes in walking performance, myofiber pathology, and limb function at the end of a 6-month program of SET.

Methods: Twelve claudicating patients received six months of SET per the American College of Cardiology/American Heart Association guidelines. Before and after SET, patients were evaluated for leg biomechanics, overground walking capacity (6-minute walking distance [SMWD]), and maximum walking time on a treadmill. Subsequently, their more affected calf muscle was biopsied for quantification of HO-1 and oxidative stress (carbonyl content) in both myofibers and microvessels. We evaluated the association between HO-1 expression and carbonyl content with correlation and multiple regression.

Results: HO-1 expression and Carbonyl content were strongly associated in the microvessels (n = 1400; r = 0.98; $P < .001$). Increasing the carbonyl content in the microvessels of each patient was associated with increasing HO-1 expression. In a subset of patients, HO-1 expression increased more slowly with increasing carbonyl content. Pre-SET oxidative stress in microvessels was a significant predictor of SET mediated change in SMWD (r = −0.75; $P = .012$) and plantarflexion torque (r = 0.8571; $P = .0065$).

Conclusions: Carbonyl content (oxidative stress) in microvessels of each patient was positively associated with HO-1 expression. However, in a subset of patients, HO-1 expression increased more slowly with increasing carbonyl content. Baseline oxidative stress in the microvessels, which may be a function of the quality of HO-1 expression, was a significant predictor of SET-mediated change in the SMWD and plantarflexion torque. The data suggest that microvessel oxidative damage may contribute uniquely to calf muscle pathology and leg dysfunction in patients with PAD.