BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Miano, Joseph Michael

eRA COMMONS USER NAME (credential, e.g., agency login): jmiano

POSITION TITLE: J. Harold Harrison, MD, Distinguished University Chair in Vascular Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
SUNY College at Cortland, New York	B.S.	1986	Biology and Physical Education/Exercise Physiology
New York Medical College, Valhalla, NY	M.S.	1988	Experimental Pathology
New York Medical College, Valhalla, NY	Ph.D.	1992	Experimental Pathology
University Texas MD Anderson Cancer Center	Postdoc	1995	Molecular and Developmental Biology

A. Personal Statement

Throughout my 37 years of basic research, I have thrived on flexibility in thought and action to make innovative discoveries in VSMC biology with the most cutting-edge tools. As a graduate student in the lab of Michael Stemerman, I conceived and designed studies examining mRNA expression of scores of genes - a forerunner of microarray analyses – in the vessel wall following balloon injury. I soon discovered many of the vascular injury-induced genes were SRF-CArG target genes. Next, I introduced my post-doc advisor, Eric Olson, and his lab to VSMC biology and the need to discover a SMC version of MyoD. There, I cloned and elucidated expression or promoter activity of several VSMC-restricted genes including *Cnn1*, *Myh11*, and *Tagln* (aka *Sm22*α), all of which are SRF-CArG dependent. We patented the *Sm22*α promoter (US Patent No. 5,837,534) and the IP was later licensed to Genentech. In my independent lab, I initiated experiments focused on identifying all functional CArG boxes in the human and mouse genome (CArGome) and those with functional variants (CArG Variome). Concurrent work resulted in my lab first reporting on Myocardin (MYOCD) acting as a molecular trigger switch for VSMC differentiation; we have since published 38 papers on various aspects of MYOCD biology. My lab engineered one of the first SMC Cre driver mice (*Sm22*α*-Cre*) and we used it to generate one of the first tissue-specific knockouts of *Srf*. Recently, my lab generated and characterized the first VSMC-restricted Cre driver mouse (*Itga8-CreER*^{T2}), thereby circumventing lethal visceral myopathies encountered with the current gold standard, *Myh11-CreER*^{T2}. We conducted the very first RNA-seq study in human VSMC to define a novel human vascular cell-restricted long non-coding RNA (*SENCR*).[1] My lab was also among the first cardiovascular research labs to utilize the revolutionary CRISPR-Cas9 system of genome editing in mice, and we reported the very first regulatory element edit in vivo.[2] We have since generated >50 strains of 'CRISPRized' mice,[3] including one of the first applications of prime editing in the mouse.[4] I frequently speak at local, national, and international meetings and workshops related to CRISPR genome editing, including the inaugural World CRISPR Day in October 2020, where Nobel Prize winner, Jennifer Doudna, delivered the keynote. We collaborate and assist many labs on CRISPR-related projects and have developed improved methods for successful genome editing in mice. My collective training and experience have served me well in the generation of novel hypotheses of translational relevance and the ability to motivate, instruct, and direct lab personnel in the day-day operations of the lab. I have trained 50 PhD candidates and graduated 3 who have gone on to successful careers in research or clinical practice. I have also trained 21 post-doctoral fellows, most of whom have either started up their own externally-funded laboratories or work in pharmaceutical or biotechnology companies. I am well-equipped, both technically and intellectually, to advance knowledge in gene regulation, VSMC differentiation control, mouse genetics, functional variants of clinical import, and potential treatment strategies using CRISPR genome editing tools, all of which are critical for the next generation of scientists.

Ongoing and recently completed projects:

R01 HL138987
Miano (PI)
07/01/18 – 06/30/22
Transcriptional control of myocardin and the MYOCARDome

R01 HL136224
Miano (PI)
12/15/18 – 10/31/22
Role of smooth muscle calponin in vascular pathobiology

R01HL139794
Long (PI), Role: Co-I
01/18/19 – 12/31/22
Function and regulation of TSPAN2 in vascular disease

R01 HL147476
Miano (PI)
03/05/19 – 01/31/23
Regulation and function of SRF in vascular pathobiology

R01 HL132574
Gupte/Miano (MPI)
09/01/17 – 05/31/21
Regulation of vascular SMC phenotype by a novel isoform of glucose-6-phosphate dehydrogenase

Citations:

1. Bell RD, Long X, Lin M, Bergmann JH, Nanda V, Cowan SL, Zhou Q, Han Y, Spector DL, Zheng D, **Miano JM.** Identification and initial functional characterization of a human vascular cell enriched long non-coding RNA. *Arterioscler.Thromb.Vasc.Biol*. 34:1249-59, 2014. PMC4024079, (183 cites).
2. Han Y, Slivano OJ, Christie CK, Cheng AW, **Miano JM**. CRISPR-Cas9 genome editing of a single regulatory element nearly abolishes target gene expression in mice. *Arterioscler.Thromb.Vasc.Biol*. 35:312-315, 2015. PMC4304932, (36 cites, >2,900 reads on Research Gate).
3. **Miano JM**, Zhu QM, Lowenstein CJ. Cutting Edge Review: A CRISPR path to engineering new genetic mouse models for cardiovascular research. *Arterioscler.Thromb.Vasc.Biol*., 36:1058-75, 2016. PMC4882230, (27 cites).
4. Gao P, Lyu Q, Ghanam AR, Lazzarotto CR, Newby GA, Zhang W, Choi M, Holden K, Walker II JA, Kadina AP, Munroe RJ, Abratte CM, Schimenti JC, Liu DR, Tsai SQ, Long X, **Miano JM**. Prime editing in mice reveals the essentiality of a single base in driving tissue-specific gene expression. *Genome Biol*, 16;22(1):83, 2021. PMC7962346, (>4,000 views and picked up by 8 news outlets).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2019-present J. Harold Harrison, MD, Distinguished University Chair in Vascular Biology, Medical College of Georgia at Augusta University, Augusta, Georgia
2019-present Adjunct Professor, Aab Cardiovascular Research Institute, University of Rochester School of Medicine & Dentistry, Rochester, New York
2017-present Synthego Corporation, Redwood City, California
2009-2019 Associate Director, Aab Cardiovascular Research Institute of the University of Rochester School of Medicine & Dentistry, Rochester, New York
2009-2018 Honorary Professor of Xi'an Jiaotong University College of Medicine
2007-2009 Interim Director, Aab Cardiovascular Research Institute of the University of Rochester School

of Medicine & Dentistry, Rochester, New York
2003-2015 Associate Professor of Medicine (tenure in 2007), Aab Cardiovascular Research Institute of the University of Rochester School of Medicine & Dentistry, Rochester, New York
2001-2012 Consultant, Socratech, LLC, Rochester, New York
1999-2003 Assistant Professor of Medicine, Center for Cardiovascular Research, University of Rochester School of Medicine & Dentistry, Rochester, New York
1998-2001 Consultant, Introgen Therapeutics, Inc., Houston, Texas
1995-1999 Assistant Professor of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin

Scientific and Professional Memberships

Member, North American Vascular Biology Organization (1996-present), American Heart Association (1999-present), International Society for Transgenic Technologies (2017-present); Editorial Board Member of *Circulation Research* (1997-2013), *Am Journal of Physiology, Heart & Circulatory Physiology* (2000-2007), *Journal of Molecular & Cellular Cardiology* (2001-2007), *Journal of Biological Chemistry* (2004-2009), *Physiological Genomics* (2003-2010), *Arteriosclerosis, Thrombosis and Vascular Biology* (2012-pesent), *Scientific Reports* (2017-2020), *Cardiovascular Research* (2014-present), *Gene and Genome Editing* (2020-present); Associate Editor of *Arteriosclerosis, Thrombosis and Vascular Biology* (2007-2012), *Vascular Pharmacology* (2017-present); Consulting Editor of *Circulation Research* (2010-2013); Member, American Heart Association Study Section, Natl, Vascular Wall Biology I (1999-2001), American Heart Association Study Section, Northeast 2 Affiliate (1999-2001), External Advisory Committee for NIH COBRE grant, Univ. Nevada, Reno (2006-2015), Standing Member, Vascular Cell and Molecular Biology Study Section (2014-2018), External Scientific Advisory Board, University of Virginia Robert M. Berne Cardiovascular Research Center; Co-Chair, American Heart Association NEA 5 Study Section (2006), AHA Sessions symposium on microRNAs in vascular biology (2011); NIH Workshop: Role of long noncoding RNA in cardiovascular, lung and blood disease (2016)

Honors and Awards

2020, invited speaker at inaugural World CRISPR Day; 2014, Vascular Biology Special Recognition Award (AHA); 2003-2007, Established Investigator of the American Heart Association; 2003, Fellow, American Heart Association; 1997-1998, Culpeper Foundation Award; 1997, Manitowoc Heart-A-Rama Research For Life Award, AHA; 1993-1995, NRSA Fellowship under Dr. Eric N. Olson, MD Anderson Cancer Center; 1992 Helen S. Page Memorial Ph.D. Award, New York Medical College.

C. Contributions to Science: 135 publications; >8,900 citations; h = 50; i10 = 99 (Web of Science)

1. Defining the genomic response to acute vascular injury (1988-1992)

My dissertation research was an outgrowth of studies showing that experimental balloon catheter injury elicits neointimal formation in animal models. While much work had focused on the role of platelets and other cell types in the initiation and progression of experimental neointimal formation, essentially nothing was known about changes in VSMC gene expression in the vessel wall. By charting the expression of some 40 genes at early (0-2 hrs) and later (2hrs-14 days) time points post-balloon injury, and relating these findings to the onset of platelet adhesion and subsequent SMC DNA synthesis, I was able to build a timeline of potential 'molecular triggers' for neointimal formation. During this work, I was able to adapt to new innovations (eg, PCR) that facilitated more precision in experimentation. Publications arising from my dissertation were among the very first to define early- and delayed-response genes following balloon injury, and these data inspired clinical studies targeting one such gene (*MYC*) with antisense oligonucleotides for treating restenosis. I conceived all studies below and served as primary investigator (a-c) or co-investigator (d) in these publications:

a. **Miano JM**, Tota RR, Vlasic N, Danishefsky K, Stemerman MB. Early proto-oncogene expression in rat aortic smooth muscle cells following endothelial removal. *Am.J.Pathol.* 137:761-765, 1990. PMC1877546, (116 cites).
b. **Miano JM,** Vlasic N, Tota RR Stemerman MB. Smooth muscle cell immediate-early gene and growth factor activation follows vascular injury: A putative in vivo mechanism for autocrine growth. *Arterioscler.Thromb.* 13:211-219, 1993. PMID: 8427857, (136 cites).
c. **Miano JM,** Vlasic N, Tota RR, Stemerman MB. Localization of Fos and Jun proteins in rat aortic smooth muscle cells after vascular injury. *Am.J.Pathol.* 142:715-724, 1993. PMC1886785, (88 cites).

d. Pritchard KA, O'Banion MK, **Miano JM,** Vlasic N, Bhatia UG, Young DA, Stemerman MB. Induction of cyclooxygenase-2 in rat vascular smooth muscle cells in vitro and in vivo. *J.Biol.Chem.* 269:8504-8509, 1994. PMID: 8132578, (139 cites).

2. Elucidating SMC-restricted gene expression and SMC promoter activities (1992-Present)

Post-doctoral studies were a logical continuation of my PhD work. I initiated a number of cloning projects to define the pre(post)-natal expression profile of candidate SMC-restricted genes and characterize promoter activity of some of these genes both *in vitro* and *in vivo* as first steps toward defining factors controlling SMC differentiation. These foundational studies revealed the mammalian expression profile of smooth muscle isoforms of myosin heavy chain and calponin with the former proving to be most specific. Meanwhile, in collaboration with Li Li, we showed for the first time the expression profile of SM22α in mice and the activity of its promoter. This resulted in the discovery of one of the first SMC-specific promoters (patent #5,837,534 granted in 1998). These studies provided essential information that lead to the development of several mouse reagents for SMC-specific targeting of transgenes, including Cre for cell restricted knockouts. I conceived the SMC-related projects in the Olson lab or recruited/collaborated fellow Olsonites in the following:

a. **Miano JM,** Cserjesi P, Ligon KL, Periasamy M, Olson EN. Smooth muscle myosin heavy chain marks the smooth muscle lineage during mouse embryogenesis. *Circ.Res.* 75:803-812, 1994. PMID: 8427857, (309 cites).
b. Li L, **Miano JM,** Cserjesi P, Olson EN. SM22α, a marker of adult smooth muscle, is expressed in multiple myogenic lineages during embryogenesis. *Circ.Res.* 78:188-195, 1996. PMID: 8575061, (310 cites).
c. Li L, **Miano JM,** Mercer B, Olson EN. Expression of the SM22α promoter in transgenic mice provides evidence for distinct transcriptional regulatory programs in vascular and visceral smooth muscle cells. *J. Cell Biol.* 132:849-859, 1996. PMC2120743, (278 cites).
d. **Miano JM** and Olson EN. Expression of the smooth muscle cell calponin gene marks early cardiac and smooth muscle cell lineages during mouse embryogenesis. *J.Biol.Chem.*271:7095-7103, 1996. PMID: 8636144, (106 cites).

3. Serum response factor and the CArGome (1994-Present)

Through analysis of numerous SMC-restricted promoters, my lab and others recognized a common DNA-binding element called a CArG box. Serum response factor (SRF) binds the CArG box, and my lab was among a few leading labs that functionally characterized SRF-binding CArG boxes both *in vitro* and *in vivo*. Further, we have been directly or indirectly involved with the genetic inactivation of *Srf* in various tissue types. Inspired by the human genome project and the initial drafts of mouse and human genomes, I turned attention to computational methods for the detection of CArG boxes in the genome. This effort resulted in the discovery of several new SRF target genes, including microRNAs, that contain CArG boxes. Since most variation in DNA sequence occurs in non-coding sequence space, our recent efforts have turned to defining variations of CArG boxes (CArG-SNPs), a novel concept that has exciting opportunities with the advent of state-of-the-art methods in defining genome architectures and genome editing. These studies are important since regulatory SNPs are pervasive in our genome, yet little effort has been expended to understand the nature of these sequence variants. The following publications were conceived and carried out in my independent lab:

a. **Miano JM**. Serum response factor: toggling between disparate programs of gene expression. *J.Mol. Cell.Cardiol.* 235(6):577-93, 2003. PMID: 12788374, (445 cites).
b. **Miano JM**, Ramanan N, Georger MA, de Mesy Bentley KL, Emerson RL, et al. Restricted inactivation of serum response factor to the cardiovascular system. *Proc.Natl.Acad.Sci., USA*. 101:17132-7, 2004. PMC535359, (185 cites).
c. Sun Q, Chen G, Streb JW, Long X, Yang Y, Stoeckert CJ, **Miano JM**. Defining the mammalian CArGome. *Genome Res*. 16:197-207, 2006. PMC1361715, (210 cites).
d. Long X, **Miano JM**. Transforming growth factor-beta1 (TGF-beta1) utilizes distinct pathways for the transcriptional activation of microRNA 143/145 in human coronary artery smooth muscle cells. *J. Biol.Chem*. 286:30119-29, 2011. PMC3191051, (97 cites).

4. Myocardin as a molecular switch for SMC differentiation (2002-Present)

My lab first reported the abundant expression of MYOCD in SMC in vivo and its sharp decrease in expression upon phenotypic modulation of cultured SMC. Importantly, we performed the classic “MyoD

experiment" and showed, for the first time, that ectopic MYOCD expression was sufficient to turn on SMC-restricted genes in a cell that otherwise did not express such genes, a finding that was subsequently replicated by many labs. We went on to show both ultrastructural and physiological evidence for a SMC-like phenotype following ectopic MYOCD expression. These findings were highly significant as they helped resolve a decades-old conundrum over how VSMC de-differentiate in vitro and under pathological conditions of the vessel wall as seen in atherosclerosis and restenosis. The fact that ectopic MYOCD expression reconstitutes the VSMC differentiation program and inhibits their growth suggests a new therapeutic intervention may exist for an array of vascular disorders highlighted by VSMC de-differentiation. We assisted in defining the role of MYOCD in the expression and activity of miR145 and made the discovery of MYOCD overexpression in cerebral vessels of some Alzheimer's disease patients. My lab conceived and drove (a,b) or co-conceived and assisted (c,d) in:

a. Chen J, Kitchen CM, Streb JW, **Miano JM**. Myocardin: a component of a molecular switch for smooth muscle differentiation. *J.Mol.Cell.Cardiol*. 34:1345-56, 2002. PMID: 12392995, (305 cites). Editorialized in Firulli AB, *J.Mol.Cell.Cardiol*. 34:1293-1296, 2002.
b. Long X, Bell RD, Gerthoffer WT, Zlokovic BV, **Miano JM**. Myocardin is sufficient for a smooth muscle-like contractile phenotype. *Arterioscler.Thromb.Vasc.Biol*. 28:1505-10, 2008. PMC2574857, (83 cites). *Editorialized in Parmacek MS, Arterioscler.Thromb.Vasc.Biol.,* 28:1416-17, 2008.
c. Bell RD, Deane R, Chow N, Long X, Sagare A, Streb JW, Guo H, Rubio A, Van Nostrand W, **Miano JM,** Zlokovic BV. SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells. *Nat. Cell Biol*. 11:143-53, 2009. PMC2654279, (191 cites). *Editorialized in Dotti and Strooder, Nat. Cell Biol.* 11:114-16, 2009.
d. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, Lee T-H, **Miano JM**, Ivey KN, Srivastava D. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature*. 460:705-10, 2009. PMC2769203, (1088 cites). *Editorialized in Cell and J.Clin.Invest.*

5. CRISPR-Cas9 genome editing in the mouse (2013-Present)

I was first introduced to CRISPR by Nobel Laureate, Jennifer Doudna, in the summer of 2012. I witnessed the explosive rise of the technology first hand and helped adapt methods to rapidly generate new mouse models. Since 2013, I have been a frequent contributor to the CRISPR online forum and we are the "go to" lab for everything CRISPR at the Medical College of Georgia. Our initial CRISPR-Cas9 experiment, the first of its kind, showed how subtle edits in a regulatory element result in massive reduction of a target gene in the mouse. We have since generated several additional regulatory element edits as well as enhancer deletions, protein-coding point mutations (including SNPs) to model human diseases, *loxP* insertions, and epitope tag insertions for facile tracking of recalcitrant proteins. We are also interested in modeling rare, genetically-isolated human diseases and this is demonstrated by a paper (a) showing a human visceral smooth muscle myopathy with loss of the SRF/MYOCD dependent target gene, *Lmod1*. My lab conceived and drove (a-d) the following studies:

a. Han Y, Slivano OJ, Christie CK, Cheng AW, **Miano JM**. CRISPR-Cas9 genome editing of a single regulatory element nearly abolishes target gene expression in mice. *Arterioscler.Thromb.Vasc.Biol*. 35:312-315, 2015. PMC4304932, (36 cites, >2,900 reads on Research Gate). Editorialized in Musunuru K, *Arterioscler.Thromb. Vasc.Biol.,* 35:496-97, 2015 and cover image.
b. **Miano JM**, Zhu QM, Lowenstein CJ. Cutting Edge Review: A CRISPR path to engineering new genetic mouse models for cardiovascular research. *Arterioscler.Thromb.Vasc.Biol*., 36:1058-75, 2016. PMC4882230, (27 cites).
c. Halim D, Wilson MP..22 authors..Hofstra, RMW, **Miano JM**. Loss of LMOD1 impairs cyto-contractile coupling and causes megacystis microcolon intestinal hypoperistalsis syndrome in humans and mice. *Proc.Natl.Acad.Sci., USA*, 114:E2739-E2747, 2017. PMC5380076, (41 cites). Recommended for F1000Prime.
d. Gao P, Lyu Q, Ghanam A…11 authors…Liu DR, Tsai SQ, Long X, **Miano JM**. Prime editing in mice reveals the essentiality of a single base in driving tissue-specific gene expression. *Genome Biol*, 22(1):83, 2021.

View Miano publications on Pubmed