

SYMPOSIUM

The Future is Now: Frontiers on Display at Yale-NAVBO Cardiovascular Inflammation and Remodeling Symposium 2014

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Earlier this year, 200 researchers from across the globe gathered at the Omni New Haven Hotel at Yale University in New Haven, Connecticut, for 3 days of talks from 30 of the leading pioneers in modern cardiovascular medicine. From May 8 to 10, 2014, scientists discussed and dissected topics ranging from the clinical treatment of atherosclerosis to the molecular biology of leukocyte-endothelial cell interactions. With other sessions exploring vascular malformation and aneurysm, hypertension, the endothelial-mesenchymal transition (endo-MT[†]), and the role of metabolism in cardiovascular disease, conference participants gained striking insights into rapid advances and ongoing challenges in the field of cardiovascular inflammation and remodeling.

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†Abbreviations: ALK1, activin receptor-like kinase 1; AMP, adenosine monophosphate; CANTOS, Canakinumab Anti-Inflammatory Thrombosis Outcomes Study; EC, endothelial cell; endo-MT, endothelial-mesenchymal transition; ERR- α , estrogen-related receptor alpha; FGF, fibroblast growth factor; GWAS, genome-wide association study; HDAC, histone deacetylase; HHT, hereditary hemorrhagic telangiectasia; HIF, hypoxia-inducible factor; hsCRP, high-sensitivity C-reactive protein; HuR, Hu protein R; IL, interleukin; KLF, Krüppel-like factor; LBRC, lateral border recycling compartment; MRI, magnetic resonance imaging; MEF, myocyte enhancer factor; MITF, microphthalmia-associated transcription factor; NAVBO, North American Vascular Biology Organization; PECAM, platelet endothelial cell adhesion molecule; PPCM, peripartum cardiomyopathy; PGC-1 α , peroxisome proliferator-activated receptor-gamma coactivator-1 alpha; PH, pulmonary hypertension; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; TEM, transendothelial migration; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; ZAP, zeta chain-associated protein kinase.

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INTRODUCTION

Begun in 2013 as a new collaboration between the North American Vascular Biology Organization (NAVBO) and Yale University, the annual Yale-NAVBO symposium in cardiovascular medicine has rapidly developed into one of the cornerstones of the world of cardiology. This year, the focus of the conference was on cardiovascular inflammation and remodeling, with keynote addresses given by Hal Dietz of Johns Hopkins University and Zoltan Arany of Harvard University. Invited speakers included many Yale faculty, as well as speakers from across North America and as far as Germany and India. Invoking disease models from hypertension to peripartum cardiomyopathy, the conference highlighted the critical role of cardiovascular health in both longevity and quality of life and underlined the fact that despite significant and ongoing advances across the spectrum from bench to bedside, much still remains to be done.

TOPICS

Atherosclerosis

Renu Virmani, from the CVPATH Institute in Maryland, began the atherosclerosis section with insights from histopathology, focusing on the differences between atherosclerotic plaques that are progressive as compared to those that are not, wherein plaque cap composition and the pattern of calcification predict progression. Virmani further contrasted “positive remodeling,” a process associated with acute plaque rupture, with “negative remodeling,” a process leading to chronic total occlusion, and correlated these with distinct patterns of calcification described in plaques [1].

Moving from the pathology to the molecular biology of atherosclerosis, Mukesh Jain of Case Western Reserve University gave an overview of the Krüppel-like factor (KLF) family of transcription factors as they relate to cardiovascular inflammation and vascular function. The KLFs regulate systemic metabolic homeostasis, mediate statin-enhanced nitric oxide production, and by

competing with NF κ B for coactivators, they oppose inflammatory pathways to control the phenotype of the vascular endothelium [2,3]. Later in the session, Laura Shankman presented smooth muscle cell lineage tracing data showing that KLF4 represses smooth muscle cell marker genes — a component of a transition to a macrophage-like state [4].

Carlos Fernandez-Hernando of Yale discussed the vascular phenotypes of Akt1, Akt2, and Akt3 knockout mice. Deletion of Akt1 in mice results in one of the few murine models of spontaneous myocardial infarction, enhancing lesion progression when combined with the well-known ApoE knockout [5].

Paul Ridker of Brigham and Women’s Hospital highlighted current clinical trials that aim to reduce cardiovascular inflammation. Prefacing an overview of current and recent cardiovascular clinical trials involving inflammatory modulation, Ridker summarized current evidence of the links between inflammation and cardiovascular events. Both high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) are independent predictors of events, and the magnitude of independent risk conferred by higher levels of inflammation is now recognized as being at least as great as that conferred by blood pressure and lipids. The formal test of inflammatory atherothrombosis will be the highly anticipated Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) trial of antibody-mediated IL1 β blockade, with phase II trial data showing significant reduction in inflammation without major changes in lipid profile [6,7].

Endothelial-Mesenchymal Transition (endo-MT)

Raghu Kalluri of Beth Israel Deaconess Medical Center opened the endo-MT session with a look at the role of endothelial to mesenchymal transition (endo-MT) in vascular development and pathology. Kalluri discovered that endo-MT — specifically, the emergence of fibroblasts from endothelial cells — is a key contributor to cardiac fibrosis. The transition, Kalluri found, is induced by TGF- β 1 and requires SMAD3. These insights may one day make it possible to develop specific anti-fibrotic therapies [8].

Michael Simons of Yale began by noting that continuous FGF expression is required for maintenance of the normal vasculature. Fibroblast growth factor (FGF), Simons observed, had previously been shown to inhibit TGF- β signaling in several different contexts. But what was the mechanism of this inhibition and what was its functional role? Using both *in vitro* and *in vivo* systems, Simons demonstrated that FGF suppresses TGF- β signaling by modulating expression of the miRNA *let-7*. The functional significance of this suppression, Simons found, is that if FGF signaling is blocked (e.g., by any of several key inflammatory cytokines), *let-7* levels fall dramatically, TGF- β signaling increases, and endo-MT occurs, contributing to neointima formation [9].

Hypertension and Pulmonary Hypertension

Jens Titze and David Harrison, both of Vanderbilt University, gave conference participants a detailed guide to the role of immune cells in modulating hypertension. Titze began by asking a key question: Does the skin actually have a “kidney” function? As Titze found, it does. Mononuclear phagocyte system cells are attracted by electrolyte accumulation in the skin; in turn, these cells initiate the expression and secretion of vascular endothelial growth factor C (VEGF-C), which triggers an increase in lymphatic capillary density and ultimately enhances electrolyte clearance. If this pathway is blocked, high blood pressure may develop [10]. Titze went on to describe the development of an accurate, noninvasive clinical assay for quantifying tissue sodium content in patients: ^{23}Na magnetic resonance imaging (MRI). The improved interpretability of this assay over previous methods (such as serum or weight measurements) may have significant clinical implications [11,12].

Harrison continued the conversation by turning from macrophages to other immune cells. Harrison discovered that downstream of angiotensin II, superoxide production and isoketal formation in dendritic cells can lead to isoketal-modified proteins being presented as neoantigens. This in turn triggers a chain of events that begins with T cell bind-

ing and activation and ultimately leads to hypertension. Isoketal scavengers, which would block this pathway, may thus represent a promising new class of drug for treating high blood pressure in patients [13].

Evangelos Michelakis, from the University of Alberta, began a session on pulmonary hypertension, beginning with the general metabolic basis of the disease and the history of recognizing the disorder [14]. Focusing on the mitochondria, Michelakis outlined a link between metabolic dysregulation, metabolic syndrome, and the pathologic remodeling of pulmonary hypertension. Interestingly, Michelakis reported that a novel treatment for glioblastoma is in early trials for pulmonary hypertension, as it reverses the pathologic remodeling by promoting mitochondria-induced apoptosis [15].

Hyung Chun of Yale continued with the discussion of emerging targets for the treatment of pulmonary hypertension (PH), noting that while all currently available therapies are aimed at vasodilation, new therapies are needed to address the hyper-proliferative aspect of the disease pathogenesis [16,17]. Chun presented data that a transcriptional regulatory axis involving the transcription factor myocyte enhancer factor-2 (MEF2) and the transcriptionally repressive histone deacetylases govern the expression of specific miRNAs that oppose smooth muscle hyper-proliferation in an animal model. Extending from these findings, Chun showed emerging data using animal models of PH that a chemical inhibitor of class II histone deacetylases (HDACs) can modulate these molecular pathways to rescue pulmonary arterial pressure and right ventricular function.

Continuing on the same topic, Ruben Tuder of the University of Colorado at Denver presented a mouse model utilizing shistosomiasis and used the model to demonstrate a role for signal transducer and activator of transcription-6 (STAT6)-driven TGF- β production in PH pathogenesis [18].

Leukocyte-Endothelial Cell (EC) Interactions

Jeffrey Bender of Yale kicked off the leukocyte-EC session with a talk addressing

the role of leukocyte integrin-dependent modulation of inflammatory gene expression in vascular pathology. Downstream of leukocyte integrin LFA-1 engagement, Bender found, an induced Rac2-myosin IIA complex triggers the activation of Hu protein R (HuR), which in turn leads to significant stabilization of labile mRNAs. As Bender noted, this process is relevant *in vivo*: flow recovery is significantly impaired during hind limb ischemia in mice with HuR or myosin IIA deleted in macrophages [19].

Jordan Pober, also of Yale, continued the session, noting that both endothelial cells (which can also function as antigen-presenting cells) and T cells play a critical role in graft rejection. Focusing on T cell transendothelial migration (TEM) downstream of T cell-endothelial cell binding, Pober found that TCR-driven TEM, but not chemokine-driven TEM, requires Vav, Rac, and myosin IIA activation downstream of zeta chain-associated protein kinase-70 (ZAP-70). Given the role of TCR-driven TEM in initiating inflammatory responses, it may be possible to exploit the differences between TCR-driven and chemokine-driven TEM to prevent graft rejection without indiscriminately suppressing the entire immune system [20].

William Muller of Northwestern University further examined leukocyte transmigration, observing that both paracellular and transcellular migration require targeted trafficking of endothelial lateral border recycling compartment (LBRC) membrane. Muller identified both platelet endothelial cell adhesion molecule (PECAM) and CD99 as critical components of the LBRC, helping to stabilize leukocyte-endothelial cell binding prior to transmigration [21]. Although the intracellular tail of CD99 contains no known signaling motifs, Muller found that it is able to function by activating PKA via soluble adenylyl cyclase [22].

Metabolism and Cardiovascular Disease

Lawrence Young of Yale began the metabolism session by discussing inflammation and metabolic disease in the ischemic heart, directing attention toward the ability of adeno-

sine monophosphate (AMP)-activated protein kinase in cardiac myocytes to protect against ischemia-induced necrosis, apoptosis, and contractile dysfunction. The topic then turned to the metabolism of the adipocyte, with Kenneth Walsh of Boston University describing adipose tissue as an endocrine organ. Adipocytes secrete pro-inflammatory factors that contribute to cardiovascular disease, but more rarely, also produce cardiovascular-protective factors such as SFRP5 and adiponectin [23]. The seemingly paradoxical decline in adiponectin in obesity is due to a loss of healthy brown fat — a shift characterized by decreased adipose vascularity, decreased mitochondria, and increased lipid content. Conversely, Walsh showed that induction of brown adipose angiogenesis improves the systemic metabolic defects associated with obesity.

Robert Gerszten presented his group's expansive Massachusetts General Hospital study of metabolic profiles in cardiometabolic disease that combines genome-wide association studies (GWAS) with mass spectrometry of metabolites in human tissue and plasma [24]. Using these data together with existing GWAS, such as that of metabolites within the Framingham study, Gerszten and his associates have identified new and novel genes associated with particular disease-associated metabolites, or the metabolites associated with a specified gene of interest.

Vascular Malformation and Aneurysm

Three Yale professors, with a fourth (Martin Schwartz) moderating, presented an in-depth look at vascular malformation. George Tellides began the session by examining the role of TGF- β in arterial homeostasis and inflammation. Noting that while TGF- β is essential for normal vascular development, it can also promote aneurysm and dissection. Tellides underlined the difficulty of drawing a clear line between “helpful” and “harmful” TGF- β . Using *in vivo* mouse models, Tellides specifically explored whether, as suggested by pathology, TGF- β signaling in smooth muscle cells promotes aortic disease. Surprisingly, Tellides found that the opposite was true: basal TGF-

β signaling in smooth muscle actually impedes disease progression [25].

Turning from TGF- β to one of its receptors, Anne Eichmann described the autosomal dominant genetic disorder hereditary hemorrhagic telangiectasia (HHT), a vascular disease characterized by frequent and severe (sometimes even life-threatening) bleeding. Noting that activin receptor-like kinase 1 (ALK1) is inactivated in HHT patients, Eichmann meticulously uncovered and characterized a direct link between ALK1 and Notch signaling in vascular morphogenesis, providing a potential model for the pathogenesis of HHT [26].

Jay Humphrey rounded out the session by highlighting the potential of mathematical modeling in studying the role of thrombus in aortic aneurysms, showing a close correlation between numerical predictions and experimental results in several different contexts.

KEYNOTE LECTURE: FOUND IN TRANSLATION: NEW INSIGHTS INTO THE PATHOGENESIS AND TREATMENT OF MARFAN SYNDROME AND RELATED DISORDERS

The keynote address given by Hal Dietz of Johns Hopkins presented new avenues for therapy of Marfan Syndrome and the related Loeys-Dietz Syndrome. Highlighting nearly 3 decades of work on the pathogenesis of these disorders, Dietz began with the 1991 identification of mutations in the gene *FBN1*, then turned attention to the more recent developments in the molecular signaling defects that arise from these mutations. Central to his recent work is the observation that the cardiovascular manifestations of Marfan Syndrome come from defects in acute signaling mediated by TGF- β , rather than from a structural protein defect, opening the possibility of pharmacologic treatment of this and other related diseases. In a mouse model with a Marfan-like phenotype in the aorta, TGF- β blocking antibodies or chemical inhibitors blocking the phosphorylation of the downstream effector ERK each resulted in phenotypic rescue. Drawing upon genetic databases of Marfan Syndrome patients and databases tracking other genetic determinates

of aortic aneurisms, calcium channel blocker use was positively associated with aortic aneurysm rates, with subsequent work demonstrating a mechanism whereby TGF- β acting through protein kinase C mediates this susceptibility. In the case of Loeys-Dietz Syndrome, Dietz showed that angiotensin II-dependent TGF- β signaling contributes to the aortic aneurysm and dissection pathogenesis, with the clinically well-known angiotensin-II receptor blocker losartan rescuing the phenotype [27]. Mutations in *TGF β 2*, *TGFBR*, and *SMAD3* have been identified as causing Loeys-Dietz [28], and Dietz further presented newer data about loss-of-function mutations identified through genetic linkage — one in a miRNA and one in a protein that inhibits TGF- β — each resulting in aortic phenotypes in mouse models. Thus, the coupling of clinical observations and genetic linkage with experimental models has led to insights both into the molecular pathogenesis of these syndromes, as well as to guidance in their pharmacological management.

KEYNOTE CALABRESI AWARD LECTURE: TRAVELS WITH PGC-1 α

“Follow the data.” That was the message emphasized throughout Zoltan Arany’s energetic inaugural mid-career Calabresi Award lecture describing a decade of work on peroxisome proliferator-activated receptor- γ coactivator-1 alpha (PGC-1 α). A potent transcriptional coactivator often described as a “master regulator” of cellular energy metabolism in general and mitochondrial biogenesis in particular [29,30], PGC-1 α is actually a fairly recent evolutionary addition, thought to function by coordinating pre-existing biological programs. One of these many programs is angiogenesis; as Arany noted, PGC-1 α has been found to regulate blood vessels independently of hypoxia-inducible factor (HIF). Acting through estrogen-related receptor alpha (ERR- α), PGC-1 α can powerfully upregulate the expression of VEGF, PDGF-B, and angiopoietin 2, leading to robust induction of angiogenesis in skeletal muscle *in vivo* [31].

Underlining the long reach of PGC-1 α , Arany went on to observe that certain melanomas express elevated levels of PGC-1 α (up to 1,000 times baseline); these melanomas display increased mitochondrial metabolism and increased reactive oxygen species (ROS) detoxification capacity [32]. Aside from increased metabolism and ROS tolerance, however, PGC-1 α -overexpressing melanoma cells also turn black due to augmented melanin production. Investigating further, Arany found that this effect was directly caused by PGC-1 α , which activates melanin formation by inducing the microphthalmia-associated transcription factor (MITF). In normal skin cells, PGC-1 α functions as a key regulator of human tanning, serving as a link between the UV exposure-induced secretion of α -MSH by keratinocytes and pigment formation in melanocytes [33].

Finally, Arany turned to peripartum cardiomyopathy (PPCM), a life-threatening condition of unknown cause that occurs in \sim 1 in 2000 live births in previously healthy women. A leading cause of modern maternal mortality, PPCM is characterized by heart failure due to left ventricular systolic dysfunction [34,35]. Why, Arany asked, does this particular form of dilated cardiomyopathy only manifest in women, and then only during or shortly after late pregnancy?

The answer, Arany found, is that there are profound hormone changes in late pregnancy. At that stage, even in normal pregnancies, the placenta secretes a potent systemic anti-angiogenic cocktail, with factors like soluble FLT1 sopping up free VEGF in circulation. In normal pregnancies, however, there are sufficient local pro-angiogenic defenses to guard against cardiac dysfunction in the maternal heart. Using a combination of cardiac-specific knockout mice and human clinical samples, Arany demonstrated that PGC-1 α may play a critical role in defending against pregnancy-induced anti-angiogenic factors and that repressed expression of PGC-1 α may be a key cause underlying PPCM [36].

While many important questions about PGC-1 α remain open, as Arany admitted (for

example, why do PGC-1 knockout mice have a mostly normal phenotype, including normal numbers of mitochondria? Does PGC-1 α play a functional role in exercise-induced mitochondrial biogenesis in skeletal muscle [37] or is it actually dispensable [38]?), all in all, Arany's impressively productive decade "travel[ing] with PGC-1 α " strongly supports his advice to just "follow the data" when it comes to research.

REFERENCES

1. Otsuka F, Sakakura K, Yahagi K, Joner M, Virmani R. Has our understanding of calcification in human coronary atherosclerosis progressed? *Arterioscler Thromb Vasc Biol.* 2014;34(4):724-36.
2. SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, et al. KLF2 Is a novel transcriptional regulator of endothelial proinflammatory activation. *J Exp Med.* 2004;199(10):1305-15.
3. Feinberg MW, Cao Z, Wara AK, Lebedeva MA, Senbanerjee S, Jain MK. Kruppel-like factor 4 is a mediator of proinflammatory signaling in macrophages. *J Biol Chem.* 2005;280(46):38247-58.
4. Salmon M, Gomez D, Greene E, Shankman L, Owens GK. Cooperative binding of KLF4, pELK-1, and HDAC2 to a G/C repressor element in the SM22 α promoter mediates transcriptional silencing during SMC phenotypic switching in vivo. *Circ Res.* 2012;111(6):685-96.
5. Fernández-Hernando C, Ackah E, Yu J, Suárez Y, Murata T, Iwakiri Y, et al. Loss of Akt1 leads to severe atherosclerosis and occlusive coronary artery disease. *Cell Metab.* 2007;6(6):446-57.
6. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1 β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CAN-TOS). *Am Heart J.* 2011;162(4):597-605.
7. Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, et al. Effects of interleukin-1 β inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. *Circulation.* 2012;126(23):2739-48.
8. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med.* 2007;13(8):952-61.
9. Chen PY, Qin L, Barnes C, Charisse K, Yi T, Zhang X, et al. FGF regulates TGF- β signaling and endothelial-to-mesenchymal transition via control of let-7 miRNA expression. *Cell Rep.* 2012;2(6):1684-96.

10. Wiig H, Schröder A, Neuhofer W, Jantsch J, Kopp C, Karlsen TV, et al. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. *J Clin Invest.* 2013;123(7):2803-15.
11. Kopp C, Linz P, Wachsmuth L, Dahlmann A, Horbach T, Schöfl C, et al. ²³Na Magnetic Resonance Imaging of Tissue Sodium. *Hypertension.* 2012;59(1):167-72.
12. Kopp C, Linz P, Dahlmann A, Hammon M, Jantsch J, Müller DN, et al. ²³Na magnetic resonance imaging-determined tissue sodium in healthy subjects and hypertensive patients. *Hypertension.* 2013;61(3):635-40.
13. Kirabo A, Wu J, Chen W, Thabet SR, Bikineyeva AT, Dikalov S, et al. Activation of T cells by dendritic cells in hypertension: a potential role of isoketal-modified proteins. *Hypertension.* 2012;60(3):A166.
14. Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circ Res.* 2014;115(1):148-64.
15. Sutendra G, Michelakis ED. Pyruvate dehydrogenase kinase as a novel therapeutic target in oncology. *Front Oncol.* 2013;3:38.
16. Kang Y, Kim J, Anderson JP, Wu J, Gleim SR, Kundu RK, et al. Apelin-APJ signaling is a critical regulator of endothelial MEF2 activation in cardiovascular development. *Circ Res.* 2013;113(1):22-31.
17. Kim J, Kang Y, Kojima Y, Lighthouse JK, Hu X, Aldred MA, et al. An endothelial apelin-FGF link mediated by miR-424 and miR-503 is disrupted in pulmonary arterial hypertension. *Nat Med.* 2013;19(1):74-82.
18. Graham BB, Chabon J, Gebreab L, Poole J, Debella E, Davis L, et al. Transforming growth factor- β signaling promotes pulmonary hypertension caused by *Schistosoma mansoni*. *Circulation.* 2013;128(12):1354-64.
19. Morrison AR, Yarovinsky TO, Young BD, Moraes F, Ross TD, Ceneri N, et al. Chemokine-coupled β 2 Integrin-induced Macrophage Rac2-Myosin IIA Interaction Regulates VEGF-A mRNA Stability and Arteriogenesis. *J Exp Med.* In press.
20. Manes TD, Pober JS. T cell receptor-driven transendothelial migration of human effector memory CD4 T cells involves Vav, Rac and myosin IIA. *J Immunol.* 2013;190(7):3079-88.
21. Mamdouh Z, Mikhailov A, Muller WA. Transcellular migration of leukocytes is mediated by the endothelial lateral border recycling compartment. *J Exp Med.* 2009;206(12):2795-808.
22. Watson R, Muller W. CD99 signals through soluble adenylyl cyclase to regulate leukocyte transendothelial migration. *FASEB J.* 2014;28(1 Supplement):146.6.
23. Ouchi N, Higuchi A, Ohashi K, Oshima Y, Gokce N, Shibata R, et al. Sfrp5 is an anti-inflammatory adipokine that modulates metabolic dysfunction in obesity. *Science.* 2010;329(5990):454-7.
24. Rhee EP, Ho JE, Chen MH, Shen D, Cheng S, Larson MG, et al. A genome-wide association study of the human metabolome in a community-based cohort. *Cell Metab.* 2013;18(1):130-43.
25. Li W, Li Q, Jiao Y, Qin L, Ali R, Zhou J, et al. *Tgfb2* disruption in postnatal smooth muscle impairs aortic wall homeostasis. *J Clin Invest.* 2014;124(2):755-67.
26. Larrivée B, Prahst C, Gordon E, del Toro R, Mathivet T, Duarte A, et al. ALK1 signaling inhibits angiogenesis by cooperating with the Notch pathway. *Dev Cell.* 2012;22(3):489-500.
27. Gallo EM, Loch DC, Habashi JP, Calderon JF, Chen Y, Bedja D, et al. Angiotensin II-dependent TGF- β signaling contributes to Loeys-Dietz syndrome vascular pathogenesis. *J Clin Invest.* 2014;124(1):448-60.
28. Lindsay ME, Schepers D, Bolar NA, Doyle JJ, Gallo E, Fert-Bober J, et al. Loss-of-function mutations in *TGFB2* cause a syndromic presentation of thoracic aortic aneurysm. *Nat Genet.* 2012;44(8):922-7.
29. Cantó C, Auwerx J. PGC-1 α , SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol.* 2009;20(2):98-105.
30. Liang H, Ward WF. PGC-1 α : a key regulator of energy metabolism. *Adv Physiol Educ.* 2006;30(4):145-51.
31. Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girmun G, et al. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1 α . *Nature.* 2008;451(7181):1008-12.
32. Vazquez F, Lim JH, Chim H, Bhalla K, Girmun G, Pierce K, et al. PGC-1 α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. *Cancer Cell.* 2013;23(3):287-301.
33. Shoag J, Haq R, Zhang M, Liu L, Rowe GC, Jiang A, et al. PGC-1 coactivators regulate MITF and the tanning response. *Mol Cell.* 2013;49(1):145-57.
34. Ramaraj R, Sorrell VL. Peripartum cardiomyopathy: Causes, diagnosis, and treatment. *Cleve Clin J Med.* 2009;76(5):289-96.
35. Martin S, Short D, Wong CM, McLellan D. A change of heart: case series of peripartum cardiomyopathy. *Case Rep Obstet Gynecol.* 2013;2013:563158.
36. Patten IS, Rana S, Shahul S, Rowe GC, Jang C, Liu L, et al. Cardiac angiogenic imbalance leads to peripartum cardiomyopathy. *Nature.* 2012;485(7398):333-8.
37. Geng T, Li P, Okutsu M, Yin X, Kwek J, Zhang M, et al. PGC-1 α plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle. *Am J Physiol Cell Physiol.* 2010;298(3):C572-9.
38. Rowe GC, El-Khoury R, Patten IS, Rustin P, Arany Z. PGC-1 α is dispensable for exercise-induced mitochondrial biogenesis in skeletal muscle. *PLoS One.* 2012;7(7):e41817.