The Academy of Cardiovascular Research Excellence

International Society for Heart Research
North American Section

ACRE@ISHR-NAS Pre-Conference Symposium
Final Program Agenda

6/26/2023 (1:00 pm-8:00 pm)
Meat Science and Animal Biologics Building
Lecture Hall 1111, UW at Madison, 1933 Observatory Dr.
Madison, WI 53706

Registration is free but required via answering the ACRE Symposium participation question @ the 42nd ISHR-NAS Meeting Madison 2023 online registration (https://ishrnorthamerican.org/meetings/).

1:00 -1:15 pm **Opening remarks**
President of ACRE, Dr. Xuejun (XJ) Wang
2023 ISHR-NAS meeting Co-Chair, Dr. Ying Ge
President of ISHR-NAS, Dr. David Lefer

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1:15-3:10 pm **Session I: Featured Lectures** (23 mins (18+5) each, total 105min)

Modulators: Guo-Chang Fan, Ph.D.; Long-Sheng Song, Ph.D.

Cardiac Sarcomere Protein Quality Control and BAG3: Repairing the Engine without Stopping the Car
Jonathan Kirk, Ph.D.
Associate Professor and Vice Chair, Department of Cell and Molecular Physiology
Director, Loyola Proteomics Core
Loyola University Chicago Stritch School of Medicine
Maywood, IL

Long Non-coding RNAs: Dark Materials Behind the Human Heart

Lei Yang, Ph.D., FAHA
Professor
Department of Pediatrics, Anatomy, Cell Biology & Physiology
Herman B Wells Center For Pediatric Research
Indiana University School of Medicine
Indianapolis, IN, 46202

Translational Control of Cardiac Pathophysiology: When mRNA Meets the Heart
Peng Yao, Ph.D.
Associate Professor
Department of Medicine
Aab Cardiovascular Research Institute
Department of Biochemistry & Biophysics
The Center for RNA Biology
University of Rochester School of Medicine and Dentistry
Rochester, New York

Targeting Post-Translational Protein Modification against Ischemic Heart Failure
Yajing Wang, M.D., Ph.D.
Professor and Director of basic and translational research
The University of Alabama at Birmingham (UAB)
Birmingham, AL

PKA and heart disease
Xiongwen Chen, Ph.D.
Professor and Dean
Pharmacy School of Tianjin Medical University
Tianjin, China

3:10-3:25 pm Break

3:25 – 4:25pm Session II: Oral Presentations of Selected Abstracts #1 (12 mins (10+2) each)

Modulators: Liya Yin, M.D., Ph.D.; Kevin Y Xiang, Ph.D.

Michael Zhang, University of Minnesota, Minneapolis, USA
Loss of free fatty acid receptor 4 impairs left ventricular functional recovery after ischemia-reperfusion: a novel role for phosphodiesterase 6c (ISHR-NAS Abstract # 122)

Samiksha Giri, University of South Dakota, Vermillion, USA
The COP9 Signalosome Promotes Neointimal Hyperplasia through Cullin Deneddylation Dependent and Independent Mechanisms (ISHR-NAS Abstract # 19)

Zachery Gregorich, University of Wisconsin-Madison, Madison, USA
Proximity labeling proteomics uncovers the putative mechanism of disrupted RBM20 nuclear import in RBM20 cardiomyopathy (ISHR-NAS Abstract # 45)

Vivian Si Chen, University of Rochester, Rochester, USA
Phosphodiesterase 10A inactivation protects against doxorubicin-induced cardiotoxicity and concomitantly inhibits tumor growth (ISHR-NAS Abstract # 123)

Haofei Wang, The University of North Carolina at Chapel Hill, Chapel Hill, USA
Delineate the postnatal cardiomyocyte maturation using single-nucleus RNA-seq (ISHR-NAS Abstract # 119)

4:25 -4:40 pm Break

4:40 – 5:40 pm Session III: Oral Presentations of Selected Abstracts #2 (12 mins (10+2) each)

Modulators: Rongxue "Rosie" Wu, M.D., Ph.D.; Huabo Su, Ph.D.

Yanghai Zhang, University of Wisconsin-Madison, Madison, USA
Disruption of RS domain function in RBM20 is causative in dilated cardiomyopathy (ISHR-NAS Abstract # 12)

Md Salim Ahammed, University of South Dakota, Vermillion, USA
PKA-mediated phosphoregulation and activation of 26S proteasomes protect against cardiac hypertrophy and heart failure induced by systolic overload (ISHR-NAS Abstract # 22)

Weiyue Wang, Mayo Clinic, Rochester, USA
ERK/MAPK inhibition exerts therapeutic effects on TTNtv cardiomyopathy in zebrafish (ISHR-NAS Abstract # 121)

Yu Yan, University of California, Los Angeles, USA
Linking the Genetic Variants and Altered Protein PTM Landscape in Cardiovascular Diseases via Artificial Intelligence (ISHR-NAS Abstract # 125)

Eng Soon Khor, University of Rochester, Rochester, NY, USA
eIF4G2 Is a Regulator of Pro-Fibrotic mRNA Translation in Cardiac Fibroblasts. (ISHR-NAS Abstract # 161)

5:40 – 6:15 pm Acknowledgements, concluding remarks, and social events
The Immediate Past President of ACRE, Dr. Jiang (JC) Chang
The President Elected of ACRE, Dr. Zhao Wang
The Local Symposium Programming Committee Chair, Dr. Wei Guo

6:15 – 8:00 pm Dinner

8:00 pm Conclusion

ACRE@ISHR-NAS Program Committee:
Co-Chairs: Drs. XJ Wang, Wei Guo, Li Qian, Liya Yin
Dr. Huabo Su
Dr. Y. Kevin Xiang

American Journal of Cardiovascular Disease

Abstracts Selected for Oral Presentation

Michael Zhang, University of Minnesota, Minneapolis, USA

Loss of free fatty acid receptor 4 impairs left ventricular functional recovery after ischemia-reperfusion: a novel role for phosphodiesterase 6c

(ISHR-NAS Abstract # 122)

Abstract

Omega-3 polyunsaturated fatty acid treatment can reduce cardiovascular events in heart failure, and free fatty acid receptor 4 (Ffar4) is a GPCR for medium and long chain fatty acids that is activated by omega-3 fatty acids. Using a mouse model of ischemia (60 min)-reperfusion (I/R), we found that in mice with systemic deletion of Ffar4 (Ffar4KO), loss of Ffar4 had no effect on infarct size 24 hr post-I/R, but significantly impaired LV systolic function recovery 28 days post-I/R. To examine the molecular basis for this impaired recovery, we performed bulk RNA-seq comparing sham to the infarcted regions of hearts from wildtype and Ffar4KO mice 3-days post-I/R. We found that phosphodiesterase 6c (PDE6c) transcription was increased 5.6- fold in Ffar4KO hearts compared to WT hearts. Subsequent immunoblotting confirmed that PDE6c protein expression was increased two-fold in Ffar4KO hearts. To measure the functional implications of PDE6c overexpression, ELISA-based cyclic GMP assays were performed. There was no difference in cGMP concentration in whole heart or isolated adult mouse ventricular myocytes under basal conditions between Ffar4KO and WT. However, after stimulation of cGMP production with the soluble guanylyl cyclase stimulator, vericiguat, cGMP concentration failed to increase in Ffar4KO myocytes, suggesting increased phosphodiesterase activity. Finally, in a publicly available single cell RNA-seq database, we found that PDE6c transcription was increased two-fold in cardiomyocytes of humans with ischemic cardiomyopathy. Collectively, our results suggest that loss of Ffar4 worsens ischemic cardiomyopathy and identify, for the first time, a novel cardioactive cGMPhydrolyzing phosphodiesterase.

Samiksha Giri, University of South Dakota, Vermillion, USA

The COP9 Signalosome Promotes Neointimal Hyperplasia through Cullin Deneddylation Dependent and Independent Mechanisms (ISHR-NAS Abstract # 19)

Abstract

Background: Neointimal hyperplasia (NH) is a common pathological response to vascular injury mediated primarily by vascular smooth muscle cell (VSMC) migration and proliferation. The COP9 signalosome (CSN) is formed by 8 canonical subunits (CSN1-CSN8) with its deneddylation activity residing in CSN5. Each or some of the 8 subunits may have deneddylation-independent function. Despite strong evidence linking the CSN to cell cycle regulation, the role of the CSN in vascular biology remains obscure. Methods and Results: Immunohistochemistry revealed increased CSN5 in the neointima VSMCs of the pulmonary arteries of human pulmonary hypertension ($p=0.0019$). Left common carotid artery (LCCA) ligation produced NH and increased the mRNA and protein levels of CSN subunits in the LCCA wall of adult wild-type mice ($p<0.05$). Smooth muscle-restricted CSN5 knockout (CSN5-SMKO) impaired Cullin deneddylation, suppressed nuclear-export of p27 in vessel walls, and markedly inhibited VSMC proliferation (0.102 ± 0.07 vs. 1.00, $p<0.001$) in mice. Further, pharmacological inhibition of CSN deneddylase by CSN5i-3 suppressed the formation of NH in wild-type mice and hyperproliferation of control VSMCs. On the contrary, CSN8 hypomorphism (CSN8-hypo) exacerbated NH (2.36 ± 0.5 vs. 1.00 ± 0.3, $p<0.0001$) and VSMC proliferation in vivo and in cellulo, which is associated with increased cytoplasmic CSN5 mini-complexes and nuclear-export of p27. Nuclearexport inhibition with leptomycin or genetically disabling CSN5 nuclear-export but not disabling CSN5 deneddylase

activity suppressed the PDGF-BB induced increases in PCNA and restored p27 nuclear localization in hypomorphic VSMCs ($p<0.05$). Conclusion: The CSN promotes VSMC proliferation and NH in injured vessels through deneddylation activity and CSN5-mediated nuclear export.

Zachery Gregorich, University of Wisconsin-Madison, Madison, USA

Proximity labeling proteomics uncovers the putative mechanism of disrupted RBM20 nuclear import in RBM20 cardiomyopathy (ISHR-NAS Abstract # 45)

Abstract

RNA binding motif 20 (RBM20) is a muscle-specific splicing factor. Genetic mutations in the nuclear localization signal (NLS) in RBM20 cause an aggressive dilated cardiomyopathy (DCM). It is currently thought that this disease results from aberrant splicing and sarcoplasmic RBM20 biomolecular condensates that form secondary to impaired nuclear localization. Understanding how mutations in the RBM20 NLS disrupt nuclear import will be crucial for developing therapeutic strategies to restore nuclear localization. The goal of this study was to define changes in the RBM20 interactome associated with NLS mutations and identify potential mechanism(s) of disrupted nuclear import. In situ proximity labeling was performed in H9c2 cells transduced with wild-type (Wt) or mutant RBM20 (S640G in rat analogous to S637G in human) fused to the promiscuous biotin ligase TurboID. Biotinylated proteins were purified by streptavidin pulldown, digested on-bead, and identified using proteomics. Proteomics analysis identified 103 and 56 proximity partners in cells transduced with Wt and S640G RBM20, respectively. These proximity partners included both shared and unique putative interactors. Gene ontology analysis revealed that these proteins play important roles in protein trafficking and splicing regulation. In particular, a nucleoporin Agfg1and an importin beta Kpnb1 were identified as putative RBM20 interactors. These two proteins are important for protein nucleocytoplasmic transport and altered or disrupted interactions between these proteins and RBM20 could explain impaired nuclear localization. We are in the process of validating these interactions and determining the role they play in RBM20 nuclear import.

Vivian Si Chen, University of Rochester, Rochester, USA

Phosphodiesterase 10A inactivation protects against doxorubicin-induced cardiotoxicity and concomitantly inhibits tumor growth (ISHR-NAS Abstract # 123)

Abstract

Cyclic nucleotides play critical roles in cardiovascular biology and disease. PDE10A is able to hydrolyze both cAMP and cGMP. PDE10A expression is induced in various human tumor cell lines and PDE10A inhibition suppresses tumor cell growth. Chemotherapy drug such as doxorubicin (DOX) is widely used in chemotherapy. However, cardiotoxicity of DOX remains to be a serious clinical complication. In the current study, we aim to determine the role of PDE10A and the effect of PDE10A inhibition on cancer growth and cardiotoxicity induced by DOX. We found that PDE10A deficiency or inhibition alleviated DOX-induced myocardial atrophy, apoptosis, and dysfunction in C57Bl/6J mice. RNA-seq study revealed a number of PDE10A-regulated signaling pathways involved in DOX-induced cardiotoxicity. PDE10A inhibition increased the death, decreased the proliferation, and potentiated the effect of DOX on various human cancer cells. Importantly, in nude mice with implanted ovarian cancer xenografts, PDE10A inhibition attenuated tumor growth while protecting DOX-induced cardiotoxicity. In isolated CMs, PDE10A contributed to DOX-induced CM death via increasing Top2β expression, mitochondrial dysfunction, and DNA damage by antagonizing cGMP/PKG signaling. PDE10A contributed to CM atrophy via potentiating FoxO3 signaling via both cAMP/PKA and cGMP/PKG dependent

signaling. Taken together, our study elucidates a novel role for PDE10A in cardiotoxicity induced by DOX and cancer growth. Given that PDE10A has been already proven to be a safe drug target, PDE10A inhibition may represent a novel therapeutic strategy in cancer therapy, with effects preventing DOX-induced cardiotoxicity and simultaneously antagonizing cancer growth.

Haofei Wang, The University of North Carolina at Chapel Hill, Chapel Hill, USA

Delineate the postnatal cardiomyocyte maturation using single-nucleus RNA-seq

(ISHR-NAS Abstract # 119)

Abstract

Throughout postnatal development, cardiomyocytes (CMs) undergo a series of molecular, structural, and functional changes to prepare the heart for efficient and consistent beating throughout an individual's lifespan. Multiple layers of regulation are involved in the maturation of CMs, including the activation of transcriptional regulatory networks, metabolic switches from glucose to fatty acids, and intercellular crosstalk. Defining the key factors and signaling pathways that regulate CM maturation can guide the promotion of CM maturation and the diagnosis and treatment of heart disease patients. However, due to the absence of in vitro models that accurately mimic the microenvironment, particularly the cell-cell interactions that occur during heart development, there have been no systematic studies profiling and integrating these regulatory layers involved in postnatal CM maturation. In this study, we performed single-nucleus RNA sequencing (sn-RNAseq) on hearts harvested from both genders at Postnatal Day 0 (P0), P7, P14, and P21. Our findings revealed extensive transcriptome remodeling throughout the postnatal stage. Through SCENIC analysis, we identified putative transcription regulators involved in postnatal CM maturation. Additionally, we profiled the cell-cellinteractions that actively participate in different stages of CM maturation and identified the crucial ligand-receptor pairs that can stimulate CM maturation.Overall, our sn-RNA-seq approach has established the development trajectory of postnatal CMs. The putative transcription factors and ligand-receptor pairs identified in our study can lead to improved maturation of both differentiated and reprogrammed CMs and novel therapeutic strategies for cardiac disease.

Yanghai Zhang, University of Wisconsin-Madison, Madison, USA

Disruption of RS domain function in RBM20 is causative in dilated cardiomyopathy

(ISHR-NAS Abstract # 12)

Abstract

Genetic mutations in RNA binding motif 20 (RBM20) are associated with an aggressive dilated cardiomyopathy (DCM). Three mutations in the arginine/serinerich (RS) domain and one in the RNA recognition motif (RRM) have been introduced in mice. Intriguingly, only mutations in the RS domain cause severe DCM, which indicates that loss of RS domain function is crucial for disease. To test this hypothesis, we generated an RBM20 RS domain deletion mouse model (Rbm20ΔRS). We demonstrate that these mice develop DCM secondary to RBM20 re-localization and sarcoplasmic granule formation. In contrast, mice lacking the RRM (Rbm20ΔRRM) show no RBM20 re-localization or granule formation, and do not manifest DCM. In vitro experiments were used to identify the core NLS in RBM20, which consists of the first nine amino acids in the RS domain. The identification of this sequence as the core NLS in RBM20 is supported by the fact that all three validated DCM-causing mutations are located in this sequence. Further analysis of plasmids containing DCM-associated mutations in other regions of RBM20, as well as in the non-NLS RS domain sequence, confirmed that only mutations in the NLS facilitate re-localization and granule formation. The effect of RS domain phosphorylation on RBM20 nuclear localization was investigated by replacing all phosphorylatable serine residues in the RS domain with either alanine or aspartate. The results suggest that RS domain phosphorylation is dispensable for

RBM20 nuclear localization. Collectively, our findings revealed that disruption of RS domain-mediated nuclear localization is crucial for DCM caused by NLS mutations.

Md Salim Ahammed, University of South Dakota, Vermillion, USA

PKA-mediated phosphoregulation and activation of 26S proteasomes protect against cardiac hypertrophy and heart failure induced by systolic overload

(ISHR-NAS Abstract # 22)

Abstract

The ubiquitin-proteasome system is the main proteolytic pathway for the quality control (PQC) and regulatory degradation of proteins in living cells. The demand for PQC is greater in cells with higher metabolic activity such as cardiomyocytes. Previous cell culture studies unveiled that phosphorylation on Ser14 of RPN6 (pS14-RPN6) mediates the activation of 26S proteasomes by protein kinase A (PKA) but the pathophysiological significance of this phosphoregulation remains to be established. Systolic overload as seen in hypertension and aortic stenosis is a leading cause of heart failure. Here we report that myocardial pS14-RPN6 and ubiquitin conjugates are significantly increased in human heart failure of ischemic or non-ischemic etiologies ($p<0.01$, 0.005) and, similarly, in wild type (WT) mice after transverse aortic constriction (TAC). To investigate the role of pS14-RPN6 upregulation, we then compared the responses to TAC between WT and the newly created Rpn6S14A knock-in mice. TAC-induced increases in myocardial proteasome peptidase activities were significantly attenuated and accumulation of ubiquitin conjugates exacerbated in Rpn6S14A mice ($p<0.0005$). Serial echocardiography revealed that Rpn6S14A mice displayed significantly greater left ventricular (LV) end-diastolic posterior wall thickness and LV mass to body weight (BW) ratios 1- and 12-week post-TAC ($p=0.024$, 0.0003; $p=0.005$, 0.01), and lower ejection fraction at 12-week post-TAC than WT mice. Rpn6S14A mice also showed greater heart weight to tibial length (TL) and ventricular weight to TL ratios at the terminal experiment (12-week; $p=0.035$, 0.023). These novel findings demonstrate compellingly that myocardial pS14-RPN6 curtails cardiac hypertrophy and delays heart failure occurrence during systolic overload.

Weiyue Wang, Mayo Clinic, Rochester, USA

ERK/MAPK inhibition exerts therapeutic effects on TTNtv cardiomyopathy in zebrafish

(ISHR-NAS Abstract # 121)

Abstract

Truncating mutations in TITIN (TTN) gene, encoding the largest human protein, are the major genetic factors for dilated cardiomyopathy (DCM). However, the altered signaling pathways remain poorly understood, preventing the developing of mechanism-based therapy. Here, we utilized a zebrafish ttntv-A mutant, which carries a truncating mutation in an exon encoding the A-band domain of titin, as an in vivo animal model for discovering altered signaling pathways. Using microhomology-mediated end joining (MMEJ)-based CRISPR-Cas9 genome editing technology, we assessed five genes from four candidate cardiomyopathy-related pathways in F0 animals. We identified potential cardioprotective effects of mapk3, map2k1, pde1a inhibition, which were later validated after generating F1 stable mutants. We elected to focus on ERK/MAPK pathway and noted its dynamic activity along the pathogenesis of TTNtv-A DCM. Phosphorylated ERK1/2 level is upregulated at an early stage and then downregulated at a later stage of the disease. The aberrant ERK signaling can be rescued by either mapk3 or map2k1 inhibition. While map2k1 inhibition reduces the phosphorylated ERK, mapk3 inhibition removes the total ERK protein. Transcriptomic studies of the ttntv-A model supported the aberrant activity of ERK/MAPK pathway and uncovered several differentially expressed genes from downstream. Together, our study

identified ERK/MAPK as a potential therapeutic pathway for TTNtv DCM, underscoring zebrafish as a rapid in vivo vertebrate model for discovering new therapeutic target genes for TTNtv DCM.

Yu Yan, University of California, Los Angeles, USA

Linking the Genetic Variants and Altered Protein PTM Landscape in Cardiovascular Diseases via Artificial Intelligence (ISHR-NAS Abstract # 125)

Abstract

The growing number of cardiovascular Genome-Wide Association Studies (GWAS)serves as a strong foundation to study the role of variants in cardiovascular diseases. To investigate whether post-translational modifications (PTM) of proteins impact the mechanistic insight underlying the pathogenesis introduced by genetic variants, we explored the link between genetic variants, their mutant products, and their PTMs landscape. In this study, we examined 5,712 Single Nucleotide Polymorphisms (SNPs) from cardiovascular-relevant GWAS, using information extracted from GWAS Catalog (https://www.ebi.ac.uk/gwas/). We constructed a new application using the Artificial Intelligence (AI) model, MIND-S, to define and analyze the altered landscape of 13 frequently occurred PTMs affected by SNPs. Among the cardiovascular SNPs studied, 10 genetic variants were found to alter PTM occurrence with high confidence; 6 SNPs displayed disrupted PTMs, whereas in 4 SNPs, PTMs were promoted. Further analyses revealed that several disrupted PTMs are involved in distinct biological processes, indicating PTMs play important roles linking variants and diseases. Notably, PTMs promoted by genetic variants have not been previously documented, suggesting new targets and functions of PTMs. Taken together, understanding the altered landscape of PTM offers a novel layer of mechanistic insight underlying the pathogenesis introduced by genetic variants.

Eng Soon Khor, University of Rochester, Rochester, NY, USA

eIF4G2 Is a Regulator of Pro-Fibrotic mRNA Translation in Cardiac Fibroblasts (ISHR-NAS Abstract # 161)

Abstract

RNA-binding proteins play a crucial role in regulating gene expression during TGFβ- induced fibrotic response in cardiac fibroblasts. Among them, eukaryotic translation initiation factor 4G2 (eIF4G2) facilitates an alternate form of mRNA translation in response to various stresses. However, the role and mechanism of eIF4G2- mediated translation in cardiac fibroblasts and its contribution to cardiac fibrosis remains poorly understood. In this study, we reported an increase in eIF4G2 protein levels in ischemic failing human and mouse hearts, myocardial infarcted (MI)-derived primary mouse cardiac fibroblasts, as well as in TGFβ-treated human and mouse cardiac fibroblasts. Knockdown of eIF4G2 not only inhibits the proliferation and migration in TGFβ-activated cardiac fibroblasts, but also reduces total collagen secretion. Deep sequencing analysis of RNA associated with polysomes of eIF4G2- depleted human cardiac fibroblasts identified translationally-downregulated genes, primarily enriched in focal adhesion and extracellular matrix (ECM) pathways. RNAbinding protein immunoprecipitation (RIP)-sequencing revealed insulin growth factor binding protein 7 (IGFBP7) mRNA as a major binding target transcript of eIF4G2. Moreover, co-IP- and BioID2-mass spectrometry demonstrated that DDX24 (DEADbox RNA helicase 24) is a vital interacting partner of eIF4G2 in mediating IGFBP7 mRNA translation in cardiac fibroblasts. Notably, we observed that in the MI mouse model, Eif4g2 conditional knockout in POSTN-positive myofibroblast attenuated ECM production, resulting in decelerated cardiac dysfunction and myocardial fibrosis. Overall, this study highlights the role of eIF4G2 as a mediator of TGFβ-induced cardiac fibroblast activation by regulating the translation of IGFBP7 via its complex with DDX24.