171 NANO-STRUCTURAL AND MOLECULAR REMODELLING EXTENDS TO THE REMOTE REGION IN THE POST MYOCARDIAL INFARCTED PORCINE HEART Â€ " A BASIS FOR HEART FAILURE DEVELOPMENT?

Ashraf Kitmitto*, Mahmoud M Nossier, Hayley J Bennett, Christian Pinali, Bernard Davenport, Rachel Walker, Lucy Murfitt, Nadim Malik, Cathy Holt. *University of Manchester*; **Presenting Author*

10.1136/heartjnl-2016-309890.171

Introduction A common consequence of coronary artery disease is myocardial infarction (MI). Following an MI a sequence of pathological events occur with necrosis and acute inflammation leading to the formation of a stable fibrous scar. However, although many patients now survive an MI many also go on to develop heart failure (HF), with cellular remodelling implicated as a precipitating factor. However, the nano-architectural changes and associated molecular remodelling remains poorly understood. Mitochondria occupying between 30–40% of the cardiomyocyte volume, play a central role in cardiac energetics, with evidence to indicate dysfunction in the post-MI heart.[1] Here we have combined 3-D electron microscopy with biochemical and quantitative mass spectrometry methods, to investigate morphological changes to mitochondria within both the peri-infarct and remote regions. We have interrogated structural changes in the context of protein, molecular, level remodelling.

Methods and results We have employed a translationally relevant porcine model of MI, presenting mild to moderate left ventricular dysfunction (n = 3, control and MI).[2] Animals were studied 1 month post-MI when the scar region has stabilised. Tissue was sampled from the peri-infarct and remote regions and a corresponding region from the control hearts, fixed and prepared for serial block face scanning electron microscopy as previously described.[3] Tissue was also lysed and analysed by quantitative mass spectrometry and western blotting. 3-D reconstruction of the mitochondria within both the peri-infarct region and remote area determined that they were smaller in terms of volume (no. of mitochondria = 389, $P < 0.01$; no. of mitochondria =262 $P < 0.05$ respectively) compared to control. There was also a change to the distribution of the subsarcolemmal and inter-fibrillar mitochondria in both regions post-MI. Quantitative mass spectrometry identified between 1400–2000 proteins within each tissue sample with alterations (both up and down regulation) of proteins associated with Î² oxidation and OXPHOS.

Conclusion These data reveal that mitochondrial structural rearrangements are accompanied by expression level changes to proteins regulating cardiac energetics. Importantly, the data also indicate that while morphological remodelling is more acute within the peri-infarct region the remote areas of the infarcted heart are also undergoing cellular maladaptations. Currently, there are no treatments that specifically target cellular structural remodelling post-MI. Here we show that mitochondrial remodelling is a feature of the post-MI heart; targeting these changes at the structural and molecular level may represent a novel treatment strategy for improved outcomes.

REFERENCES

1 Dodd, M. S., Atherton, H. J., Carr, C. A., Stukey, D. J., West, J. A., Griffin, G. K., Radda, K., Clarke, K., Heather, L. C., Tyler, D. J. Impaired in-vivo mitochondrial Krebs cycle activity after myocardial infarction assessed using hyperpolarized magnetic resonance spectroscopy. *Circ Cardiovasc Imaging*. 2014;**7**(6);895-–904

2 Malik N, Farrell KA, Withers SB, Wright EJ, Holt CM. A novel porcine model of early left ventricular dysfunction for translational resaerch. *Research Reports in Clinical Cardiology*. 2013;**4**:1–7

3 Pinali C, Bennett H, Davenport JB, Trafford AW, Kitmitto A. Three-dimensional reconstruction of cardiac sarcoplasmic reticulum reveals a continuous network linking transverse-tubules: this organisation is perturbed in heart failure. *Circ Res*. 2013;**113**:1219–30

172 A SLOW-RELEASING SYNTHETIC PROSTACYCLIN AGONIST Â€ŒONO 1301-SRÂ€ COMBINED WITH OMENTAL FLAP INCREASES MYOCARDIAL BLOOD FLOW AND REDUCES MICROVASCULAR RESISTANCE, ASSOCIATED WITH FUNCTIONAL RECOVERY, IN A PORCINE CHRONIC MYOCARDIAL INFARCTION MODEL

Shin Yajima*, Shigeru Miyagawa, Satsuki Fukushima, Yoshiki Sakai, Akima Harada, Kayako Isohashi, Tadashi Watabe, Hayato Ikeda, Genki Horitsugi, Jun Hatazawa, Yoshiki Sawa. *University Graduate School of Medicine*; **Presenting Author*

10.1136/heartjnl-2016-309890.172

Introduction Coronary microvascular dysfunction (MVD) has been shown to be the major cause of persistent myocardial ischaemia and progressive left ventricular (LV) remodelling in the chronic myocardial infarction (MI) heart. ONO-1301SR is a slow-releasing synthetic prostacyclin agonist, being developed to induce angiogenesis by upregulating a variety of proangiogenic cytokines in the targeted region. In addition, pedicle omental flap covering over the LV surface reportedly yields proangiogenic effects on chronic MI heart. We herein hypothesised pedicle omental flap covering, combined with ONO-1301SR placement. over the LV surface may be effective on the MVD and related functional deterioration in a chronic MI heart. To test this hypothesis, clinically latest imaging studies were applied in a porcine chronic MI model.

Methods A mini-pig chronic MI model was generated by placing an ameroid constrictor around the left anterior descending artery for 4 weeks. The mini-pigs were then randomly assigned into the following 4 groups; sham group, omental flap alone (OM group), ONO-1301SR alone (ONO group), ONO-1301SR plus omental flap (ONO+OM group, n = 6 each).

Results At 4 weeks after the treatment, ^{13}N-ammonia PET study showed that the ONO+OM group, but not the other groups, displayed a significantly increase in myocardial blood flow (189 ± 89% increase, $P < 0.05$) and coronary flow reserve (179 ± 121% increase, $P < 0.05$) under the stress compared to those under the rest. This trend was predominated in the left circumflex territory. In addition, coronary angiogram and pressure-temperature sensor wire study showed that the ONO+OM and the ONO groups, but not the OM group, showed a significantly greater number of collateral arteries and smaller indexed of microvascular resistance, in particular, in the left circumflex territory, compared to the sham group. Moreover, echocardiographical EF was significantly greater in the ONO+OM group (50 ± 7%) and the ONO group (48 ± 3%), compared to the OM group (41 ± 3%) and the sham group (38 ± 5%) at 4 weeks. Furthermore, histological density of CD31-positive capillaries and CD31SMA-double positive arterioles were significantly greater in the ONO+OM group than the sham group or the OM group, in association with a significantly smaller myocyte size and interstitial fibrous area in the ONO+OM and the ONO group compared to those in the other groups. However, expression of proangiogenic cytokines, such as *vascular*

endothelial growth factor, hepatocyte growth factor or *stem cell-derived factor-1*, were not significantly different among the groups, indicating that angiogenic process might have been completed at the 4 weeks after the treatment.

Conclusions ONO-1301SR combined with omental flap therapy promoted myocardial angiogenesis with collateral artery growth, leading to recovery of the cardiac function in a porcine ICM model. This combined therapy may be useful in regenerating myocardial microvasculature.

173 FUNCTIONAL ROLE OF MICRORNA-214 IN MODULATING VASCULAR SMOOTH MUSCLE CELL FUNCTIONS AND NEOINTIMA FORMATION

Tayyab Adeel Afzal*, Qingzhong Xiao. *Queen Mary University of London*; **Presenting Author*

10.1136/heartjnl-2016-309890.173

Background/Objective Vascular smooth muscle cell (VSMC) proliferation and migration play a pronounced role in the pathophysiology of atherosclerosis and neointima formation. microRNAs are short, single stranded, non-coding 22 nucleotide RNAs involved in post transcriptional gene silencing. Increasing evidence has suggested an important role of miRNAs in regulating VSMC functions. However, little is known about the functional involvements of miR-214 in VSMC proliferation/migration and vessel injury-induced neointima formation. In the current study, we aimed to study the functional importance of miR-214 and its target genes in VSMC proliferation, migration and neointima SMC hyperplasia.

Methods and Results miR-214 expression was closely associated with VSMC phenotypic modulation in response to different pathological stimuli. miR-214 over-expression in serum-starved VSMCs significantly decreased VSMC proliferation and migration, while knockdown of miR-214 dramatically increased VSMC proliferation and migration, respectively. Proteomic analyses pinpointed that NCK-associated protein 1 (NCKAP1), a major component of the WAVE complex that regulates lamellipodia formation and cell motility, was negatively regulated by miR-214 in VSMCs. NCKAP1 has also been predicted as one of the top targets of miR-214 by using several computational miRNA target prediction tools, and was indeed negatively regulated by miR-214 in VSMCs. Luciferase assay showed miR-214 substantially repressed wild type, but not the miR-214 binding site mutated version of NCKAP1–3â€ ™-UTR-luciferase activity in VSMCs, confirming the NCKAP1 is the functional target of miR-214 in VSMCs. Data from co-transfection experiments also revealed that inhibition of NCKAP1 is required for miR-214-mediated lamellipodia formation and cell motility. Importantly, locally enforced expression of miR-214 in the injured vessels significantly reduced NCKAP1 expression levels, inhibited VSMC proliferation, and prevented neointima SMC hyperplasia after injury.

Conclusions/implications We have uncovered an important role of miR-214 in modulating VSMC functions and neointima hyperplasia. Our findings have suggested that miR-214 represents a potential therapeutic target for vascular diseases.

174 HNRNPA1 IS A CRITICAL REGULATOR IN VASCULAR SMOOTH MUSCLE CELL FUNCTIONS AND NEOINTIMA HYPERPLASIA

Weiwei AN*, Qingzhong Xiao. *Queen Mary University of London*; **Presenting Author*

10.1136/heartjnl-2016-309890.174

Background RNA binding protein heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) plays various roles in transcriptional and posttranscriptional modulation of gene expression. Our previous study has demonstrated for the first time that hnRNPA1 plays an important role in vascular smooth muscle cell (VSMC) differentiation from stem cells *in vitro* and *in vivo*. However, little is known about the functional involvements of hnRNPA1 in VSMC functions and neointima hyperplasia.

Purpose In this study, we aimed to investigate the functional roles of hnRNPA1 in the contexts of VSMC functions, injury-induced vessel remodelling and human atherosclerotic lesions, and the molecular mechanisms involved.

Methods and results Studies used mouse aorta VSMCs showed that hnRNPA1 expression levels were consistently modulated during VSMC phenotype switching. Moreover, VSMCs with hnRNPA1 knockdown had an increased migratory and proliferative ability, but a reduced VSMC-specific gene expression level. Consistently, over-expression of hnRNPA1 significantly reduced VSMC migration and proliferation. Furthermore, our data shows that hnRNPA1 exerts its effects on VSMC functions through modulating IQ motif containing GTPase activating protein 1 (IQGAP1) gene, a well-known important regulator of VSMC migration and proliferation. Mechanistically, hnRNPA1 regulates IQGAP1 mRNA degradation through two mechanisms: upregulating miR-124 and AU-rich element (ARE). Further evidence suggests that hnRNPA1 up-regulates miR-124 through regulating miR-124 biogenesis, and that IQGAP1 is the authentic target gene of miR-124. Importantly, the expression levels of hnRNPA1 were significantly down-regulated during neointimal lesion formation induced by wire injury, suggesting a role for hnRNPA1 in vessel injury-induced neointimal development and progression. In accordance, perivascular ectopic overexpression of hnRNPA1 greatly reduced VSMC proliferation, and inhibited neointima formation in wire-injured carotid arteries. Translationally and consistently, lower expression levels of hnRNPA1 and miR-124, while higher expression levels of IQGAP1, were observed in human atherosclerotic lesions.

Conclusions We have identified hnRNPA1 as a critical regulator in VSMC functions and neointima hyperplasia. Our data provide new insight into the roles of hnRNPA1/miR-124/IQGAP1 regulatory axis in VSMC functions and the pathogenesis of neointima formation and/or angiographic restenosis, and aid the development of novel therapeutic agents for the prevention of these diseases.