Methods: UA patients who treated with statin (n=6) and without statin (n=6) whole blood was collected. The total miRNAs profile was analyzed by miRNA array. 17 significant differentially upregulated miRNAs were significant. MiRNAs targets were predicted by three bioinformatic tools. Putative targets were clustered into different pathway and cell types by DAVID functional tool. GEO database Stable (n=6) vs rupture plaque macrophage (n=5); Early (n=15) vs Advance carotid atherosclerotic plaque (n=16) provided information were calculated by sam to figure out pathway in the rupture plaque. By using cytoscape to visualize the miRNAs regulated functional network.

Results: From the miRNA array data, 17 differential expressed miRNAs in the statin group compare without statin group in the UA patients. MiRNAs cells type target pathway network appears that in a target plaques macrophage enriched in pathway of hemostasis, signaling in NFκB, opioid signaling; b. Platelet in pathway of hemostasis, signaling by Rho GTPases, signaling by NFκB, integrin signaling, surface T cell receptor, signaling by EGFR; c. Monocyte in pathway of Signaling by NFκB, Apoptosis, hemostasis, signaling by PDGF, Aoxon guidance, signaling by Notch; d. Endothelial cell. Signaling by NFκB, Apoptosis signaling by Rho GTPases, membrane trafficking etc. MiRNAs targeted resident macrophage of rupture plaque are major in signaling for NFκB, signaling by Rho GTPases and hemostasis. The NGF signaling pathway, hemostasis and Rho GTPase signaling also emerged in the carotid plaque. After combine the two miRNA target networks, only the hemostasis, Rho GTPase singnaling pathway and signaling in immune system was targeted.

Conclusions: Our bioinformatic analysis on UA patients’ miRNAs profiles suggested MiRNAs functioning in the plaque macrophage and platelet miRNAs targets are major enrich in hemostasis, while in monocyte NFκB apoptosis and Rho GTPase and in exosomal miRNAs, which was involved in the atherolescerotic lesion, indicate that statin induced miRNAs target signaling pathway of Rho GTPase. Hemostasis in plaque and additional signaling in immune system was targeted by miRNAs in vulnerable plaque.

GW25-e5120
Exosome-mediated transfer of miR-210 from mesenchymal progenitor cells to cardiomyocytes contributes to cardiac function preservation
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Objectives: It has been previously demonstrated that hypoxia preconditioning (HP) potentiated the therapeutic effect of mesenchymal progenitor cells (MPC) on myocardial infarction (MI) injury. Whether exosome secreted from various cells, have been well recognized to play critical roles in cell-to-cell communication, we documented and characterized the exosomal miRNAs secreted from MPC and how it modulated the functions in cardiomyocytes under MI injury. Methods: For HP, MPC was subjected to repeated cycles of hypoxia (30 min) with intermittent reoxygenation (10 min) for three cycles. In vitro, CM was incubated in hypoxia for 24 hours to establish injury model. Concentrated conditioned medium (CM) of MPC was extracted to be applied in the subsequent experiments, and exosome was isolated from CM by differential centrifugation. GW4869, a special inhibitor to exosome, was used to interdict exosome release. The miRNAs transcrition in exosome was analyzed by microarray and verified by quantitative PCR. Alternatively, ago-miR-210 or antago-miR-210 transfection was performed to up- or down-regulate the miR-210 expression in CM, respectively. In vivo, rats were subjected to left anterior descending (LAD) ligation followed by injection of either CM-MPCHP-GW4869 or CM-MPCHP-Ago-miR-210. Results: Our investigation evidenced that exosome-mediated transfer of miR-210 from MPC to CM facilitated the myocardial survival in hypoxia, providing a novel insight into the mechanism of cell-to-cell transfer for cardiovascular diseases.

GW25-e5168
Impaired Post-Transcriptional Regulation of RyR2 by microRNA-106b-25 Cluster Promotes Atrial Fibrillation
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Objectives: Atrial fibrillation (AF) is the most common sustained arrhythmia yet current pharmacological treatments are only moderately effective. Enhanced sarcomeric plasmic reticulum (SR) Ca2+ leak via rymodine receptor type-2 (RyR2) contributes to AF pathogenesis and preliminary data in atrial samples from paroxysmal AF (pAF) patients showed an upregulation of RyR2 proteins with no change in mRNA levels. This suggests that post-transcriptional regulation of RyR2 by microRNAs (miRNAs) may play a role in RyR2 dysregulation in pAF. Since bioinformatic analysis suggests that miR-106b and miR-93, members of the miR-106b-25 cluster, could target RyR2-3′UTR, we hypothesized that loss of the miR-106b-25 cluster may promote AF via enhanced RyR2-mediated SR Ca2+ leak.

Methods: In vivo, a novel miR-106b-25 luciferase reporter assay was performed in HEK293 cells. Telemetry ECG recordings, intracardiac electrophysiology (EP) studies, Ca2+ imaging and immunocytotoxicity (ICC) using isolated atrial myocytes, and Western blotting (WB) were performed in miR-106b-25 homozygous knockout (miR-106b-25 −/−) mice and wildtype (WT) littermates. Levels of miRNAs and mature miRNAs were measured using quantitative real-time (qRT)-PCR in both mice and atrial samples from pAF patients.

Results: Luciferase reporter assay confirmed that miR-106b targets RyR2-3′UTR. WT and miR-106b-25 −/− mice showed a larger area under the contractile curve (AUC) in hypoxia, as indicated in a remarkable decrease in apoptosis rate of CM, which was significantly lower in CM-MPCHP compared to CM-MPCcontrol, miR-210 was internalized by CM via exosome-intake, resulting in a prominent rise in CM survival in a similar manner to CM-MPCHP incubation. Further, we observed that miR-210 was significantly increased in miR-106b-25 −/− CM, which was lower than WT (P<0.05). In vivo, we performed in mice in miR-106b-25 −/− and WT after 4 weeks of LAD ligation, Telemetry and EP studies showed that miR-106b-25 −/− mice showed a significantly more atrial tachycardia than WT. Telemetry and EP studies showed that levels of miR-106b-25 −/− mice showed a significantly more atrial tachycardia than WT (P<0.05). In vivo, we performed in mice in miR-106b-25 −/− and WT after 4 weeks of LAD ligation, Telemetry and EP studies showed that miR-106b-25 −/− mice showed a significantly more atrial tachycardia than WT (P<0.05). In vivo, we performed in mice in miR-106b-25 −/− and WT after 4 weeks of LAD ligation, Telemetry and EP studies showed that miR-106b-25 −/− mice showed a significantly more atrial tachycardia than WT (P<0.05). In vivo, we performed in mice in miR-106b-25 −/− and WT after 4 weeks of LAD ligation, Telemetry and EP studies showed that miR-106b-25 −/− mice showed a significantly more atrial tachycardia than WT (P<0.05).

Conclusions: These results suggest that abnormal miR-106b-25 regulation of RyR2 expression is an important molecular mechanism involved in the proarrhythmic RyR2 dysregulation in pAF patients. miR-93 mimic may constitute a novel therapeutic strategy for pAF patients associated with RyR2 hyperactivity.

GW25-e0298
Mutational spectrum of the NKX2-5 gene in patients with atrial fibrillation
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Objectives: The functional characteristics of the mutant NKX2-5 proteins were analyzed using a dual-luciferase reporter assay. Methods: As a result, two heterozygous NKX2-5 mutations, including a previously reported P.E21Q and a novel P.T180A mutation, were identified in two families with pAF patients transmitted in an autosomal dominant pattern. The mutations co-segregated with AF in the families with complete penetrance. The detected substitutions, which altered the amino acids highly conserved evolutionarily across species, were absent in 700 control individuals and were both predicted to be causative. Functional analyses demonstrated