Comparing with the control group. Immunohistochemistry showed that normal
significantly the ST-segments decreased (P<0.01) and markedly decreased (P<0.01) ; the damage of myocardial tissue was mild, comparing with the control group. Immunohistochemistry showed that normal distribution and content of Cx43 (P<0.05). And myocardial Ca\textsuperscript{2+} ATPase were decreased (P<0.05) ; the concentration of MDA reduced (P<0.05). And myocardial Ca\textsuperscript{2+} ATPase had no statistical difference (P=0.05).

Conclusions: The myocardial ischemia-reperfusion injury could lead to Cx43 decrease quickly, and maldistribution; Ca\textsuperscript{2+} ATPase were significantly decreased. These were closely related to the severity of I/R. W1XG1 can greatly improve the expression of Cx43 in myocardial ischemia-reperfusion, reduced damage of myocardial tissue. The mechanism of the action might be related to the increase of Ca\textsuperscript{2+} ATPase and alleviate IR1 induced by calcium overload, and have protective effects on myocardium.

**GW25-e0796**

Attenuation of CLOCK-BMAL1 transcriptionally decreases the expression of β1-AR to anticipate the occurrence of ventricular arrhythmia after chronic heart failure

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**Objectives:** Circadian rhythms influence the incidence of sudden cardiac death but the underlying mechanisms are not well defined. We sought to investigate the role of the β-adrenoceptor (β-AR) in cardiac circadian disorders and ventricular arrhythmia (VA) after chronic heart failure (CHF).

**Methods:** CHF was created by transverse aorta constriction. Circadian variations of the myocardial expressions of β1-AR, β2-AR and cardiac genes CLOCK and BMAL1 were examined by real time reverse transcription polymerase chain reaction. Western blot and immunohistochemistry. Chromatin immunoprecipitation assay (ChIP) and luciferase (LUC) were applied to determine whether CLOCK and BMAL1 regulate β1-AR expression. Electrocardiograms were recorded in vivo and/or ex vivo. Ventricular tachyarrhythmias were induced by isoproterenol and programmed electrical stimulation (PES).

**Results:** Normal guinea pigs showed circadian oscillations in both the myocardial expression of β1-AR and CLOCK-BMAL1, but not for β2-AR. However, these circadian rhythms were significantly blunted or even abolished in guinea pigs with CHF. And the expression of β1-AR and CLOCK-BMAL1 were attenuated in guinea pigs with CHF. The decrease of β1-AR and CLOCK-BMAL1 induced the expression of β1-AR. The arrhythmia severity after CHF.

**Conclusions:** Circadian rhythms of myocardial β1-AR and CLOCK-BMAL1 activities are disturbed after CHF. Attenuation of CLOCK-BMAL1 transcriptionally decreases the expression of β1-AR to anticipate the occurrence of ventricular arrhythmia after CHF.

**GW25-e1556**

Differenitiatng Human Induced Pluripotent Stem Cell into Functional Cardiomyocytes by Modulating Wnt/β-catenin Signaling

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**Objectives:** Functional cardiomyocytes generated from human induced stem (h-iPS) cells can potentially offer an unlimited cell source for therapies in cardiac disease, cardiovascular regeneration medicine, and the study of cardiac development. In this study, we aimed to establish a method for differentiating h-iPS cells into beating cardiomyocytes through sequentially modulating Wnt/β-catenin signaling using small molecule inhibitors.

**Methods:** 2011B, h-iPS cell line was chosen for the experiment. 4 days after seeding single cells with different cell number (0.5, 0.75, 1.0, and 1.5 million cells per well) in each well of Matrigel-coated 12-well plate with mTeSR1 + 5 μM T27632 (ROCK inhibitor), the medium was changed into RPMI/B-27 (without insulin) with different concentration of GSK3 inhibitor CHIR99021 (6, 8, 10 and 12 μM) 1 day. After that the cells were then subjected to RPMI/B-27 medium (without insulin) alone for 2 days, then followed by 2 μM IWP2 (Wnt inhibitor) in RPMI/B-27 (without insulin) for 2 days. Then the medium was changed with RPMI/B-27. To identify the differentiated cardiomyocytes, quantitative PCR and immunofluorescence were conducted to detect the specific markers of cardiomyocytes.

**Results:** The first beating cluster of cells could be observed on day 8, and Robust spontaneous contraction occurs by day 12. Especially, the well with 0.75 million cells and 6 μM CHIR99021 were showed highest percentage of beating cardiomyocytes (about 35%). Quantitative PCR results showed that the mRNA of myosin light chain 2 (MLC-2), cardiac troponin T (cTNT) and β-actin were all highly expressed. Immunofluorescent results were further showed that both specific and intensive signals of CTNT and β-actin could identify the sarcomeric structures indicating that generated cardiomyocytes has relatively mature sarcomere assembling similar as adult’s.

**Conclusions:** Taken together, we established a method for human cardiomyocytes differentiation from h-iPS cell with relatively high efficiency. This method will provide an unlimited cell source for our future cell replacement therapy research.

**GW25-e1110**

The effect of estrogen on adipocyte triglyceride and its mechanism

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**Objectives:** To investigate the impact of 17β-estradiol (17β-E2) on triglyceride metabolism in adipocyte and its mechanism.

**Methods:** 3T3-L1 adipocytes were cultured and differentiated into mature adipocytes in vitro. The adipocytes were divided into three groups: (1) estrogen groups: the adipocytes were treated with 17β-E2 with various concentrations (0, 10-6, 10-7 and 10-8 mol/L) for 24 hours or 48 hours. (2) selective estrogen receptors (ER) antagonists (PHTPP (selective ERα antagonist) and/or GW25-e1556 (selective ERβ antagonist) for one hour and the adipocytes in the blank control group were maintained in plates containing culture medium alone. Then they were treated with: (1) 10-6 mol/L for 48 hours; (3) lipopolysaccharide (LPS) groups, 100ng/ml 24 hours for 48 hours, the adipocytes were treated with 10-6 mol/L 17β-E2 for 48 hours. The adipocytes in the control group were treated with LPS alone. The levels of triglyceride in mature adipocytes and the concentration of intercellular lipase (IL) in supernatant in LPS groups were measured using enzyme-linked immunoassorbent assay (ELISA). The protein expression of hormone-sensitive lipase (HSL), adipose triacylglycerol lipase (ATGL) and perilipin in adipocytes were detected by Western blot.

**Results:** (1) With the increase of the 17β-E2 concentration, the triglyceride levels in mature adipocytes tend to decrease, especially in the group treated with 10-6 mol/L 17β-E2 for 48 hours. Compared with control, 10-6 mol/L and 10-5 mol/L 17β-E2 groups, the levels of triglyceride in mature adipocytes of the group treated with 10-6 mol/L 17β-E2 were significantly lower (all P<0.05). However, the difference of the triglyceride levels in adipocytes between 10-5 mol/L and 10-4 mol/L 17β-E2 groups and control group had no statistical significance (all P>0.05). (2) Compared with control group, the triglyceride levels in mature adipocytes were significantly lower in 17β-E2 group and 17β-E2+PHTPP group (all P<0.05). The triglyceride levels in the 17β-E2+MPP group were no significant differences comparing with control group (P>0.05). (3) There were no significant differences of the triglyceride levels in mature adipocytes among LPS group, 17β-E2+LPS group and control group (all P>0.05). The IL-6 level in the supernatant of the cultured mature adipocytes in LPS group and 17β-E2+LPS group group were higher than in control group (all P<0.05). However, the IL-6 levels were similar in LPS group and 17β-E2+LPS group (P>0.05). (4) The expression of HSL, ATGL and perilipin of matured adipocytes in 17β-E2 group, 17β-E2+MPP group, 17β-E2+PHTPP group, LPS group, 17β-E2+LPS group and control group were measured by Western blot. Compared with control group and 17β-E2+MPP group, the expression of ATGL was markedly elevated in 17β-E2+PHTPP group (P<0.05). The expression of ATGL was similar among all groups (all P>0.05). The expression of HSL and perilipin in all the groups had no significant differences (all P>0.05).

**Conclusions:** 17β-E2 may up-regulate the expression of ATGL which may be mediated by ER2, then promote triglyceride hydrolysis in mature adipocytes. The effect of estrogen on triglyceride metabolism in adipocytes may be attenuated or disappeared in the presence of inflammation.

**GW25-e1596**

Nonpeptide angiotensin-(1-7) analogue AVE 0991 modulates proliferation of cardiac fibroblast via regulating Smad pathways

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**Objectives:** AVE 0991, the nonpeptide angiotensin-(1-7) (Ang-(1-7)) analogue, is recognized as having beneficial cardiovascular effects. However, the mechanisms have not been fully elucidated. This study was designed to investigate to the effects and possible mechanism of AVE0991 on angiotensin II (AngII)-induced proliferation of cardiac fibroblasts.

**Methods:** Proliferation of cardiac fibroblast was induced by AngII. The cultured cardiac fibroblasts were incubated with AngII (10-6 mol/L) for 24 h after pretreatment with AVE 0991 (10-6 mol/L) and/or Ang-(1-7) receptor antagonist A-779 (10-6 mol/L) for 24 h. The cell proliferation and the DNA synthesis were measured by MTT assay and EdU incorporation assay. Smad2, 3, 4 protein expression was detected using western blot analysis.