Investigation of the role of β-catenin at different mechanisms (hypertrophy, apoptosis, fibrosis, inflammation) in cardiomyocytes.

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Abstract

Background:

β-catenin is known to have dual functions. It is part of adherens junctions in the catenin/ cadherin complex, necessary for cell structure and adhesion, and it serves as a transcription factor. Cytoplasmic β-catenin levels are normaly kept low through continuous preteasome-mediated degradation, which is controlled by a complex containing GSK-3/APC/Axin. When cells receive Wnt signals, the degradation pathway is inhibited, and consequently β-catenin accumulates in the cytoplasm and nucleus. Nuclear β-catenin interacts with transcription factors such as lymphoid enhancer-binding factor 1/ T cell-specific transcription factor (LEF/TCF) to activate transcription. Although the critical roles of β-catenin during development and in neoplasic disease have been well described previously, little is known about the role of β-catenin in cardiomyocytes.

Materials and Methods:

To overexpress the β-catenin, neonatal rat cardiomyocytes and H9c2 cardiomyoblast cells were transfected with pCMV β-catenin plasmid or treated with AngII and β-catenin inhibitor for a dose/time-dependent course.To dectect the role of β-catenin in the hypertrophy, apoptosis, fibrosis, and inflammation in cardiomyocytes, the western blotting, actin staining, cytoplasmic and nuclear fractionation, and Flow cytometry were analyzed separately.

Results:

IHC analysis found β-catenin was increased in the acute infraction tissue and condensed with the partial area in the granulation tissue from the human cardiovascular tissue with myocardial infarction (MI) disease. Therefore, we checked that the protein expression of β-catenin in the heart of pathological condition rat models.We found β-catenin was increased and translocated to the nucleus in the Diabetes,obesity, and hypertension heart tissues.

β-catenin overexpression caused the protein markers increased in the hypertrophy, apoptosis, fibrosis and inflammation measured by Western blot. In addition, endogenous β-catenin overexpression by a dose-dependent AngII treatment induced myocardial hypertrophy through the IGF2R signaling pathway previously published by our Lab.When neonatal rat cardiomyocytes were treated with AngII and β-catenin inhibitor, the IGF2R signaling pathway was blocked. Taken together, our data showed that β-catenin expressed and affected the hypertrophy, apoptosis, fibrosis, and inflammation in cardiomyocytes under the pathological condition.

Results

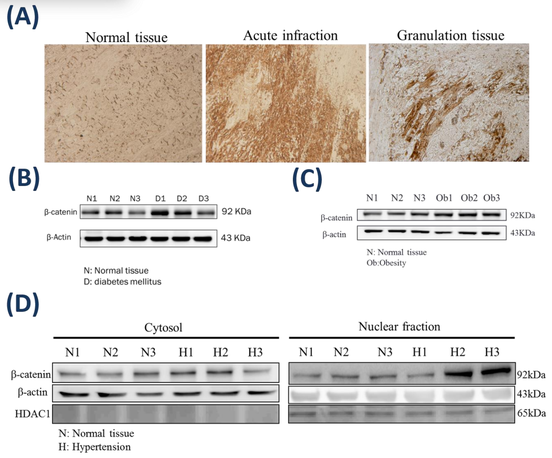

Fig. 1. Immunohistochemical analysis for β-catenin in sections from the human cardiovascular tissue with myocardial infarction(MI) diseases and hearts of animal metabolic syndrome. (A) Immunohistochemical analysis, the β-catenin protein levels were increased in the acute infraction tissue and condensed with the partial area in the granulation tissue visualized brown color. Panel : a normal tissue (n=10), an acute infraction (n=10), and a granulation tissue(n=10). Final magnifications: ×200 (bar, 200μm). (B and C) The protein expression of β-catenin in the hearts of Diabetes and Obesity rat models which measured by Western blot were increased compared to normal tissues. (D)Western blot for β-catenin in the nuclear fractions of the hearts of hypertension rat models. β-catenin nuclear translocation significantly increased in the hypertension heart tissues.

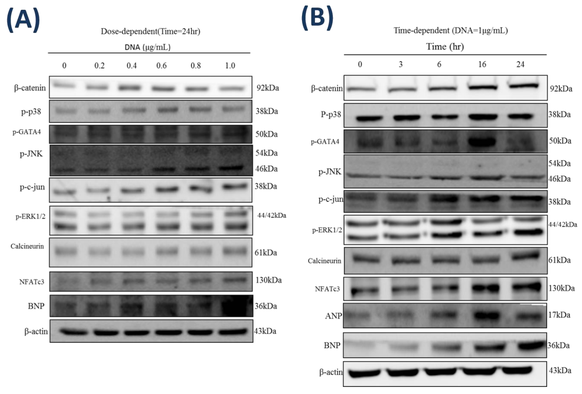

Fig. 2. . β-catenin overexpression cause the concentric hypertrophy protein markers increased. (A) Neonatal Rat Cardiomyocytes were transfected with a dose-dependent pCMV β-catenin plasmid for 24 hrs. (B) H9c2 cells were transfected with a 1μg/ml pCMV β-catenin plasmid for a time-dependent course. After the treatment, the concentric hypertrophy proteins were measured by Western blot.

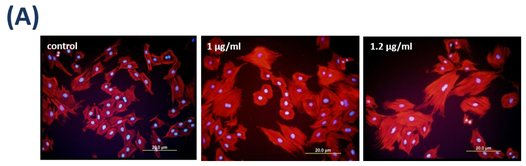

Fig. 3. Effect of β-catenin overexpression on hypertrophy in H9c2 cells by actin staining. (A) Actin staining was used to stain their actin filament with phalloidin-rhodamine. DAPI staining was used to mark the nucleus. Acting staining showed β-catenin increased the cell size.

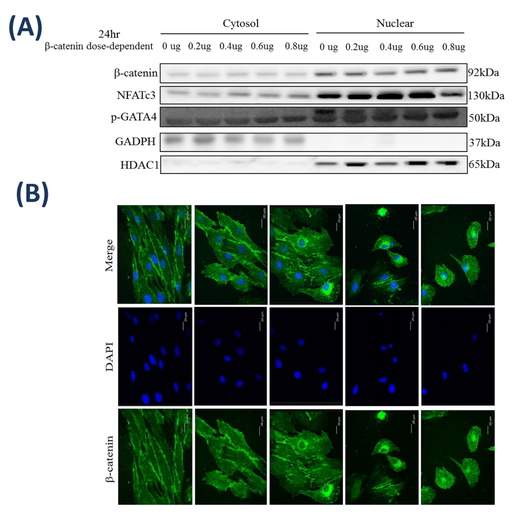

Fig. 4. β-catenin overexpression induced β-catenin , p-GATA4, and NFATc3 nuclear translocation. (A) H9c2 cells were transfected with a dose-dependent pCMV β-catenin plasmid for 24hrs.Total cell lysates from H9c2 cells was extracted by cytoplasmic and nuclear fractionation kit and measured by Western blot.(B) The nuclear translocation of β-catenin was measured by confocal microscopy. Green:β-catenin ,blue: nucleus.

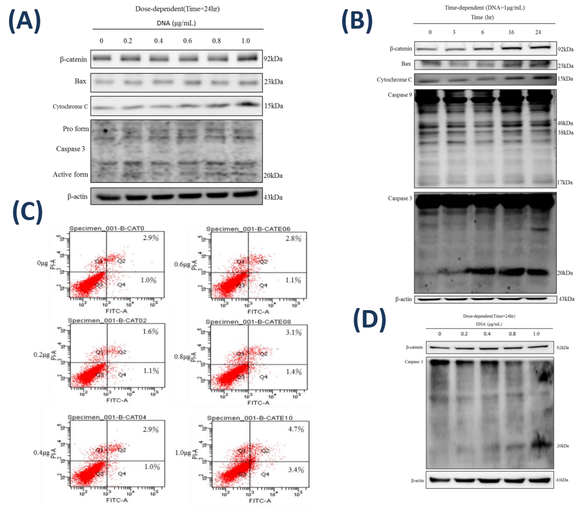

Fig. 5. β-catenin overexpression cause apoptosis. (A) Neonatal Rat Cardiomyocytes were transfected with a dose-dependent pCMV β-catenin plasmid for 24 hrs. (B) H9c2 cells were transfected with a 1μg/ml pCMV β-catenin plasmid for a time-dependent course. After the treatment, the apoptosis protein markers were measured by Western blot. (C) Flow cytometry for apoptosis stained by Annexin V-FITC in χ axis and PI in Y axis. H9c2 cells were treated with a dose-dependent pCMV β-catenin plasmid for 24hrs. The percentages of Q4 represent the change at earlier apoptosis,and the percentages of Q2 represent the change at late apoptosis and/or necrosis. Representative apoptosis percentage of Q2 plus Q4 is shown. The percentage of apoptosis enhanced in the treatment of 0.8μg and 1.0μg pCMV β-catenin plasmid compared to the control cell.(D) H9c2 cells were transfected with a dose-dependent pCMV β-catenin plasmid for 24 hrs. The caspase 3 of apoptosis protein markers was enhanced in the 1.0μg dosage of β-catenin. The result is the same with the Flow cytometry data.

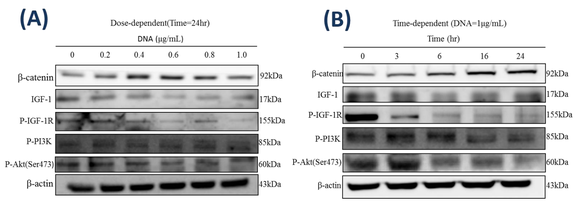

Fig. 6. β-catenin overexpression cause the survival pathway proteins decreased. (A) Neonatal Rat Cardiomyocytes were transfected with a dose-dependent pCMV β-catenin plasmid for 24 hrs. (B) H9c2 cells were transfected with a 1μg/ml pCMV β-catenin plasmid for a time-dependent course. After the treatment , the survival protein markers were measured by Western blot.

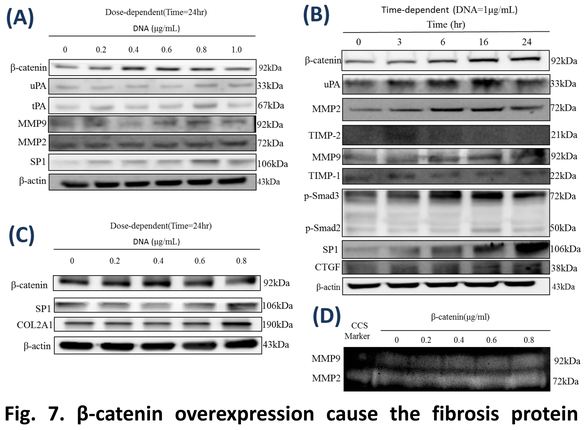

Fig. 7. β-catenin overexpression cause the fibrosis protein markers increased and TIMP-2/-1 decreased. (A) Neonatal Rat Cardiomyocytes were transfected with a dose-dependent pCMV β-catenin plasmid for 24 hrs. (B) H9c2 cells were transfected with a 1μg/ml pCMV β-catenin plasmid for a time-dependent course. After the treatment , the fibrosis protein markers were measured by Western blot.(C) Neonatal cardiac fibroblast cells were transfected with a dose-dependent pCMV β-catenin plasmid for 24 hrs. After the treatment, the conditioned medium was harvested for Zymography assay (D).

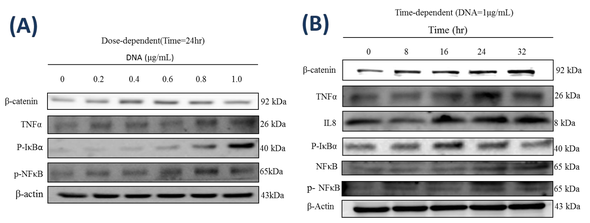

Fig. 8. β-catenin overexpression cause the inflammtory cytokines increased. (A) Neonatal Rat Cardiomyocytes were transfected with a dose-dependent pCMV β-catenin plasmid for 24 hrs. (B) H9c2 cells were transfected with a 1μg/ml pCMV β-catenin plasmid for a time-dependent course. After the treatment , the inflammatory cytokines were measured by Western blot.

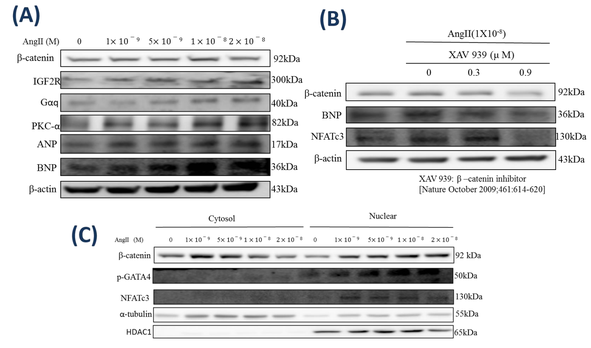

Fig.9. Endogenous β-catenin overexpression by a dose-dependent AngII treatment induced myocardial hypertrophy through the IGF2R signaling pathway. (A) H9c2 cells were treated with a dose-dependent AngII. (B) H9c2 cells were treated with a $1X10^{-8}$M AngII and β-catenin inhibitor. The hypertrophy protein markers decreased when we added the β-catenin inhibitor. (C) H9c2 cells were treated with a dose-dependent AngII. Total cell lysates from H9c2 cells was extracted by cytoplasmic and nuclear fractionation kit . The endogeous β-catenin, and the hypertrophy protein markers (p-GATA4, NFATc3) overexpressed and translocated to the nucleus.

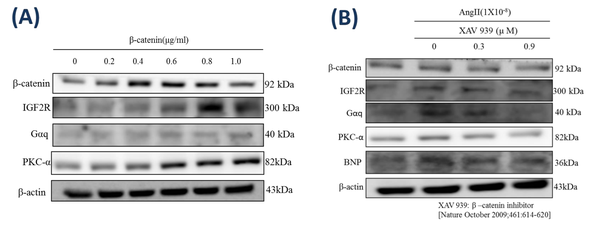

Fig.10. Directed β-catenin overexpression by a dose-dependent pCMV β-catenin plasmid treatment induced myocardial hypertrophy through the IGF2R signaling pathway. (A) Neonatal Rat Cardiomyocytes were treated with a dose-dependent β-catenin pCMV plasmid. (B) Neonatal Rat Cardiomyocytes were treated with a $1X10^{-8}$M AngII and β-catenin inhibitor. After the treatment , the IGF2R, the downstream proteins of the IGF2R signaling pathway , and the hypertrophy protein markers decreased when we added the β-catenin inhibitor.