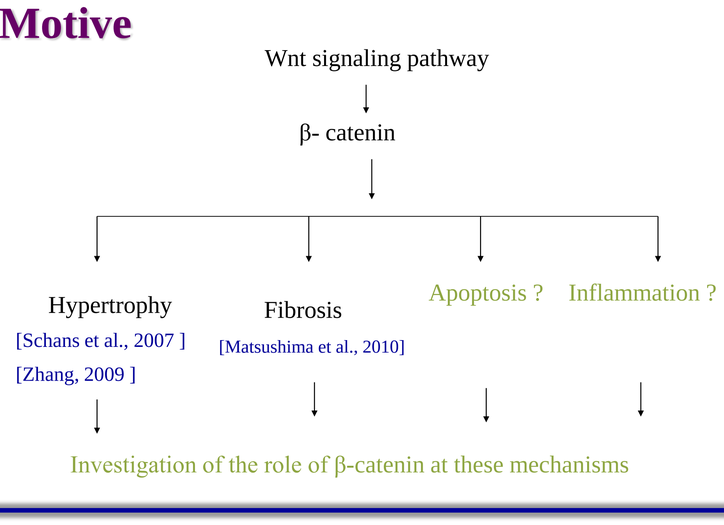
# Investigation of the role of β-catenin at different mechanisms (hypertrophy, apoptosis, fibrosis, inflammation) in cardiomyocyte

Shan-Men Lin[1], Wei-Wen Kuo[2], and Chih Yang Huang[1, 3 *]
*1 Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan*
*2Department of Biological Science and Technology, China Medical College, Taichung, Taiwan*
*3Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan*
E-mail address: cyhuang@mail.cmu.edu.tw

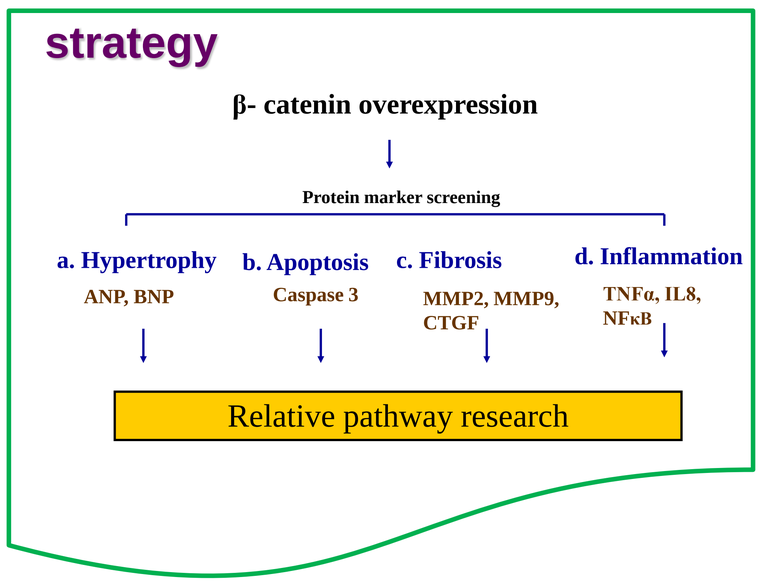
## Abstract

**Cardiac hypertrophy, apoptosis, fibrosis and inflammation are the pathological characteristics of cardiomyocyte and often lead to heart failure. In recent years, several studies have shown the involvement of Wnt/β-catenin signaling in these pathological characteristics, however, the detailed mechanism is still not clear. So, the purpose of this study is to determine whether the β-catenin functions in these pathways which imply heart damage and to find the relative mechanisms. First strategy of this study is to overexpress β-catenin in H9C2 and to screen the protein marker of hypertrophy, apoptosis, fibrosis and inflammation by western blotting. The data of western blotting suggest that, the overexpression of β-catenin in H9C2 enhance the protein level of hypertrophic markers (such as ANP and BNP), the inflammation cytokine (such as TNFα), as well as the NFκB which is the master switch controlling inflammation. The scheme of this study will to screen the other protein such as Caspase 3 (apoptosis marker) or MMP2, MMP9 (fibrosis marker) further. So hope to find the complete influence of β-catenin in H9C2. On the other hand, for the hypertrophic effect of β-catenin, we performed the actin staning to make a double confirm. To summarize, our data had to point out the possible relation between β-catenin and cardiac hypertrophy and we will attempt to find the complete influence of β-catenin at cardiomyocyte in the future.**

## Motive




## strategy




## Method

**Cell culture ：**

H9c2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% glucose, 100 units/ml penicillin and 100 μg/ml streptomycin in 5% CO 2 at 37℃. After β- catenin transfection, the cells were harvested and extracted for analysis.

**Transient transfection ：**

H9c2 cells were seeded into 10cm dishes in DMEM containing 10% FBS and, on the next day, medium was replaced with fresh DMEM medium with penicillin 2 h before transient transfection, and β- catenin was introduced into cells by using PureFection™ Nanotechnology-based Transfection Reagent according to the manufacturer's guidelines.

**Western blot analysis ：**

Proteins were separated in 8-12% SDS-PAGE and transferred to nitrocellulose membranes. Nonspecific protein binding was stopped in blocking buffer [5% milk, 20 mM Tris-HCl (pH 7.6), 150mMNaCl, and 0.1% Tween-20] and blotted with specific antibodies in the blocking buffer at 4℃ overnight. After incubation with secondary antibody for 2 h, Densitometric analysis of immunoblots was performed using Fuji LAS 3000 imaging system.

**Actin staining ：**

H9c2 cells were inoculated into 12-well plate. After treatments, cells were fixed with 4% paraformaldehyde solution for 10 min at room temperature. After a rinse with PBS, cells were treated with permeation solution (0.5% Triton X-100) for 10 min at 4℃. Following wash with PBS, samples were first incubated with actin staining reagent containing Rhodamine-conjugated phalloidin with high affinity for actin. The cells were also stained with 1 μg/ml DAPI for 30 min to detect cell nucleus using UV light microscopic observations (blue). Samples were analyzed in a drop of PBS under a fluorescence and UV light microscope with an excitation wavelength 495 nm and a detection wavelength in 520 nm (red). The increasing cell size and intracellular actin polymerization were measured by Axio Vision LEsoftware.

## Condition test

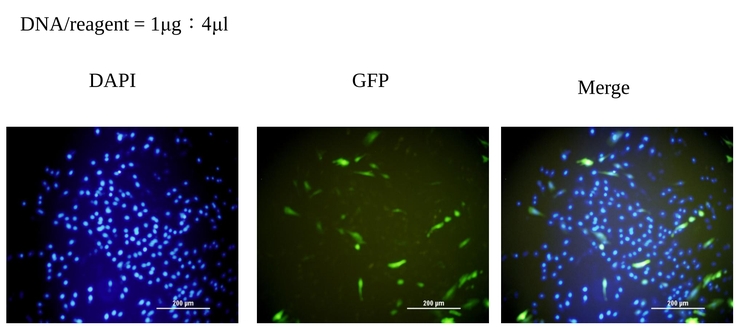



**Figure 1. Transfection of pEGFP-Rubicon by purefection in H9C2**

**Plasmid check**

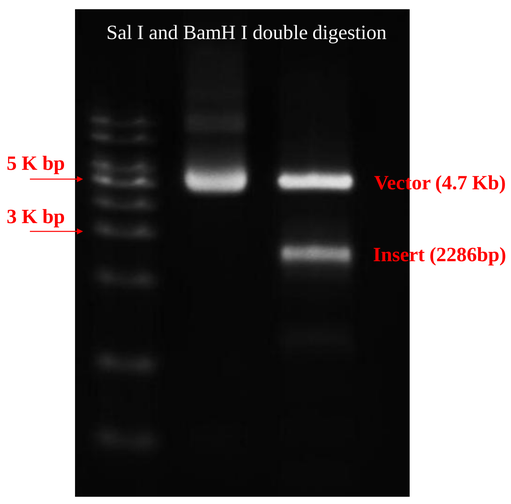



**Figure 2. The Gel electrophoresis of β-catenin plasmid after Sal I and BamH I double digestion**

## Result

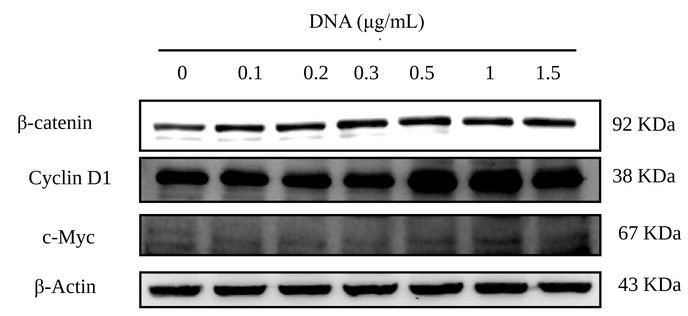



**Figure 3**
**Overexpression of β-catenin induced the level of Wnt pathway arget gene increase in H9c2 cardiomyoblast cells with dose-dependent.**
**The H9C2 was transfected with β-catenin plasmid (indicated concentraction) for 24 hours.**

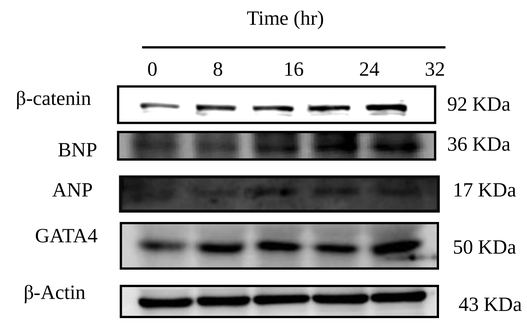



**Figure 4**
**Overexpression of β-catenin induced pathiological hypertrophy marker increase in H9c2 cardiomyoblast cells with time-dependent..**
**The H9C2 was transfected with β-catenin plasmid (1μg/mL) for indicated hours.**

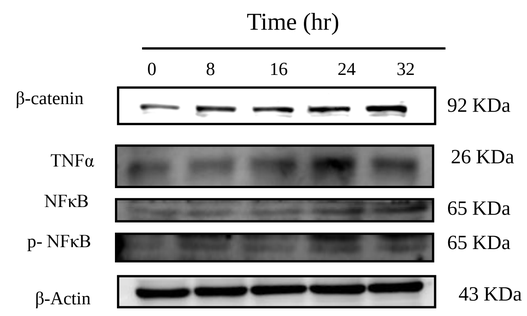



**Figure 5**
**Overexpression of β-catenin induced inflammation factors increase in H9c2 cardiomyoblast cells with time-dependent. .**
**The H9C2 was transfected with β-catenin plasmid (1μg/mL) for indicated hours.**

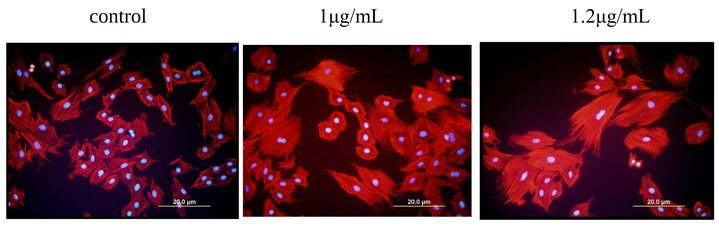



**Figure 6**
**Overexpression of β-catenin induced cell size increase in H9c2 cardiomyoblast cells with dose-dependent.**
**The H9C2 was transfected with β-catenin plasmid (indicated concentraction) for 24 hours.**

## Conclusion

**The data of this study had to point out the possible relation between β-catenin and cardiac hypertrophy and we will attempt to find the complete influence of β-catenin at cardiomyocyte in the future..**