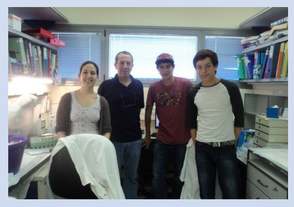
The Heart of Signal Transduction

Technion Israel Institute of Technology
SciTech 2013 Summer Science Camp

THE BRUCE RAPPAPORT FACULTY OF MEDICINE

Ari Mandler (USA), Charlie Hillman (UK)
Supervisors: Lilach Koren, Prof. Ami Aronheim



Abstract

Pathological cardiac hypertrophy is a hallmark of cardiac remodeling. Various biological stressors such as pressure load, volume overload, and neurohormonal activation can induce a hypertrophic response characterized by increased myocardial cell size, death and collagen accumulation. In some cases, this extracellular growth can result in myocardial stiffness and ultimately ventricular dysfunction. Prolonged, hypertrophy can also lead to dilated cardiomyopathy, heart failure, and death. Conclusively, cardiac remodeling is an essential adaptive response to the surrounding environmental changes and consequently may cause heart failure. Cardiomyopathy is one of the leading causes of death in developing countries.

Background

Activating transcription factor 3 (ATF3), a member of the basic leucine zipper family[1], is a protein that regulates gene transcription through binding to specific DNA sequences[2]. It is an immediate early gene[3] expressed at low levels in cells at normal conditions, but is increased in response to various mechanophysical, metabolic and genetic stresses[4]. Phenylephrine (PE), an Alpha 1-adrenergic agonist, is used as a stressor to induce an increase in blood pressure. It has already been shown that PE induces the expression of Egr1, a transcription factor that co-regulates ATF3, which has been associated with cardiac hypertrophy for many years. In this experiment, we compared ATF3 expression in mRNA with similar transcription factors (Fos, c-Jun) that constitute the AP1 family. Recently, the widespread notion that Egr1 can promote ATF3 mRNA expression implies the positive regulation of ATF3 transcription through a common signal transduction pathway[5]. Our lab has shown that ATF3 over-expression in whole animal models leads to ventricular hypertrophy[1], and is now moving onto in- vitro tissue culture models to examine the molecular processes in cardiomyocyte hypertrophy in the absence of blood pressure. Cardiomyocytes are fully differentiated cells and the isolation and maintenance of adult cardiomyocytes is a difficult task.

Method

Cell Isolation

Perfused heart was digested with collagenase, DNase, and Protease solution to disrupt extracellular networks.

Next cardiac tissue was triturated to separate cardiomyocyte cells.

Calcium was added gradually in order to preserve cell contractility.

Cells were plated on laminin [an extracellular protein] coated either cover slips or dishes to provide cell support.

PE (1 μM) was added to culture media to examine ATF3 expression for 0.5 and 2 hours.

Quantitative Real time PCR

Total RNA was extracted from culture cell lysate. mRNA was used as a template for cDNA synthesis prepared with reverse transcriptase.

Real time PCR was performed using sybergreen solution bound to double stranded cDNA for quantitative measurement.

Immunofluorescent staining

Nuclear membrane was permeabilized to allow nuclear staining.

Epitope blocking was performed with 10% fetal calf serum to avoid non-specific staining.

Primary antibody was applied for 1h followed by incubation with secondary antibody conjugated with specific fluorophore.

Images were taken with confocal microscope at X40 magnification.

Results

Isolated cardiomyocytes were either left untreated or treated with PE for 0.5h and 2h. While untreated cells displayed no ATF3 expression, PE treated cells showed significant ATF3 nuclear staining (red fluorescence). ATF3 expression in the PE treated cells was reduced following two hours of PE exposure, compared with the 0.5 hour induced cells. The staining for α-actinin (green fluorescence) and DAPI (blue fluorescence) was observed in both treated and untreated cells.

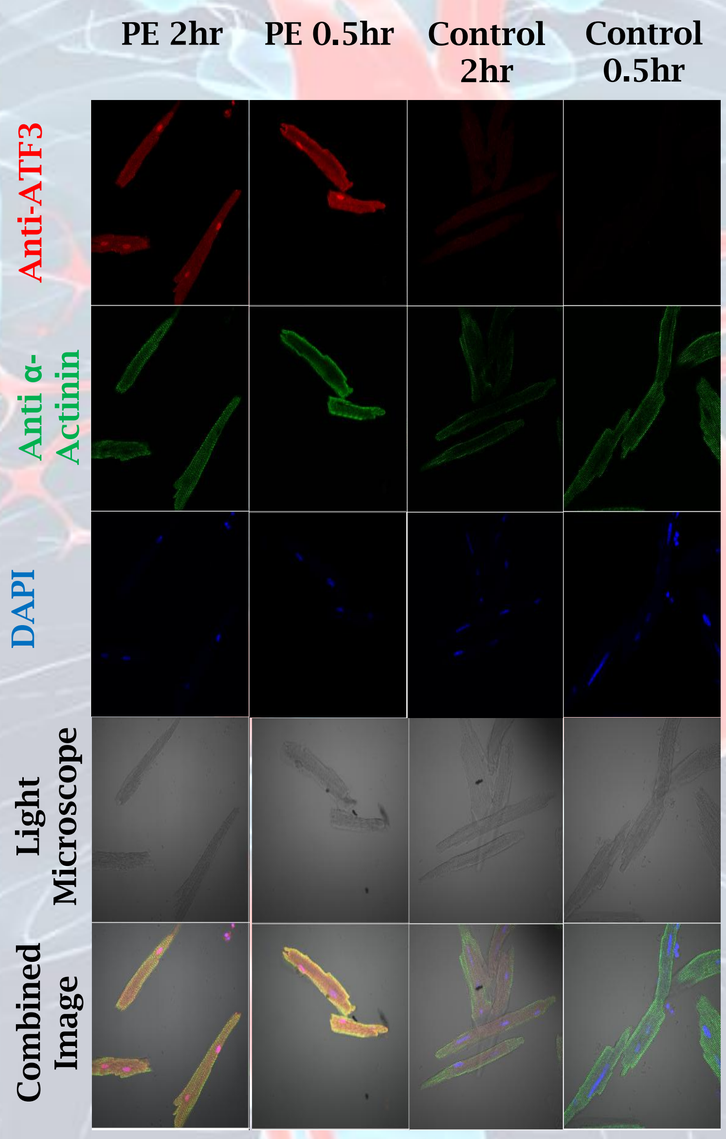


Figure 1: Images from the confocal microscope slides; immunostained cardiomyocytes with anti-ATF3 and anti-α-actinin at the indicated time points

Isolated cardiomyocytes were either treated with PE for 2 hours or left untreated. mRNA was isolated from cardiomyocytes and its levels analyzed by qRTPCR. mRNA levels were then normalized with a housekeeping gene. c-Jun mRNA was induced following PE induction. Conversely, the levels of ATF3, Egr1 and Fos were unchanged.

Figure 2: Quantitative real time PCR examining the mRNA expression of ATF3, c-Jun, Egr1, and Fos following 1 μM of Phenylephrine. The results were normalized with a known housekeeping gene GAPDH.

Discussion

We successfully isolated cardiomyocytes from adult wild type mice. We sought to examine ATF3 expression in response to PE in vitro by immunostaining and qRT-PCR. At the two hour time point, the ATF3 protein is readily observed while no induction of ATF3 mRNA is observed. Interestingly, high levels of c-Jun mRNA is persistent for 2 hours following PE treatment.

Conclusion

PE induction in a tissue culture model of adult cardiomyocyte cells results in increased ATF3 expression at the protein level. Further experiments are required to follow ATF3 transcription in shorter time points. Due to the increase in the qRTPCR measurements, further questioning should go into the relationship and co-regulation between c-jun and ATF3. In addition, a quantitative measurement using computerized programs is needed to follow ATF3 induction. Further experimentation can advance our knowledge on the exact signaling mechanisms that relate ATF3 expression to cardiac hypertrophy (Figure 3).

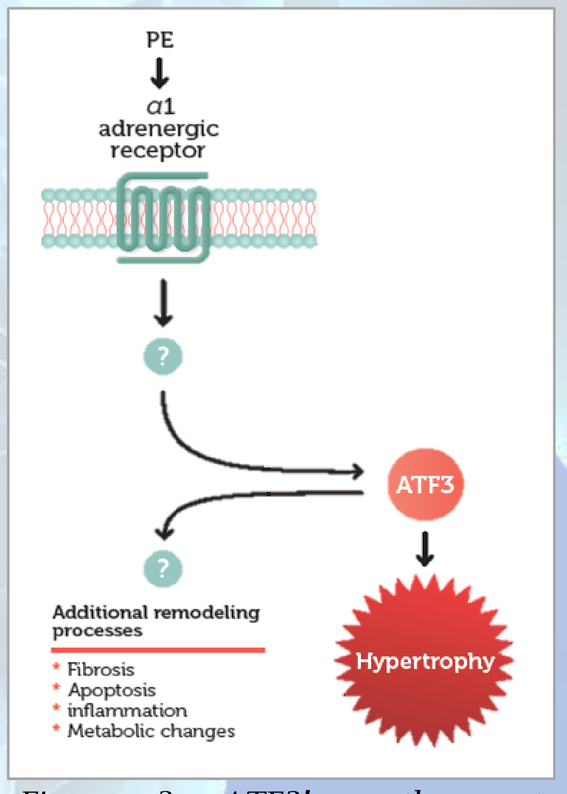


Figure 3: ATF3's pathway to remodeling processes

References

[1] Koren L, Elhanani O, Kehat I, Hai T, Aronheim A; Adult Cardiac Expression of the Activating Transcription Factor 3, ATF3, Promotes Ventricular Hypertrophy; PLoS One, (2013) 8(7):e68396

2 Latchman DS;Transcription factors: an overview: Int J Biochem Cell Biol., (1997) 1305-12:(12)29

[3] Hai T, Wolford CC, Chang YS; ATF3, a hub of the cellular adaptive-response network, in the pathogenesis of diseases: is modulation of inflammation a unifying component?; Gene Expr., (2010) 1-11:(1)15

[4] Hai T, Wolfgang CD, Marsee DK, Allen AE, Sivaprasad U; ATF3 and stress responses; Gene Expr., (1999) 321-35:(4-6)7

[5] GIRALDO, Alejandro; BARRETT, Oliver P. T.; TINDALL, Marcus J.; FULLER, Stephen J.; AMIRAK, Emre; BHATTACHARYA, Bonhi S.; SUGDEN, Peter H.; CLERK, Angela; Feedback regulation by Atf3 in the endothelin-1-responsive transcriptome of cardiomyocytes: Egr1 is a principal Atf3 target; Biochem J., (2012) 444(Pt 2): 343–355

Acknowledgements

We would like to thank Lilach Koren for her never ending support and guidance throughout the project, and to Prof. Ami Aronheim for hosting and guiding us through our research in his lab.

We would also like to thank the Michael and Morven Heller Charitable Foundation, and the Dr. Istvan Madaras Scitech Foundation Program for their generosity and donation