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Discussion

Dr Robert C. Robbins *(Stanford, Calif).* You went through it very quickly, but this is a tour de force; technically it is a very difficult model and you are to be congratulated for getting the animals through this. Also impressive is the sophisticated use of molecular genetics that you used here to help answer important questions. Certainly heart failure is an important public health issue. Finally, the experimental therapeutic approach to try to identify potential targets for developing small molecules or other strategies to treat heart failure is impressive.

I have just a couple of comments and questions for you.

GATA-4 and NKx2.5 are things that we are looking at as embryonic stem cells differentiate into cardiac myocytes. I thought it was interesting that you targeted these particular transcription factors. You mentioned the isoform shift of cardiac myosin in your manuscript, and a lot of work has been done by Jeff Robbins in this area. I would like to note that you mentioned cardiac myosin. Because it is so important in allograft rejection and autoimmune disease, can you comment on what you found with cardiac myosin specifically?

Dr Azakie. We used the MF20 antibody, which selects for myosins. The purpose of looking at changes in sarcomeric gene expression was to show that, in fact, there was biochemical hypertrophy. We did not look specifically for different myosin isoforms. What we wanted to show is that despite no change in DNA content, there was an upregulation in myosin proteins. The purpose in evaluating cTnT expression was to show that, in fact, an embryonic gene expression pattern induced a marker of the development of pathologic hypertrophy. We also looked at serum brain natriuretic peptide and found that it was significantly upregulated in shunted animals. We did not look at isoform-specific expression of different myosins.

Dr Robbins. You hit on other things that I was going to ask you about. Have you thought about using microarrays? I've been impressed that often this is a fishing expedition, but it can often direct you to other things. You have done it the old-fashioned way. You have picked a couple of things and gone after them in the classic sense. Have you thought about using microarrays to look for other targets? We have recently discovered a gene termed APLN in heart failure patients who had ventricular assist devices. I wonder whether you could get more targets if you would considered using microarray techniques.

Dr Azakie. We thought about using microarrays, but there are no good ovine arrays available now. They are in preparation. The main issue that my colleagues and collaborators have had with that approach is that it does not always necessarily provide a mechanistic answer toward the switch to a hypertrophic program.

I think the main message here resided in the posttranslational modifications and the functional assays that we performed. Neonatal ovine cardiomyocytes were cultured and cotransfected with cardiac promoters and expression constructs that encoded different transcription factors. We found that TEF-1 and Sp1 were transcriptional activators, whereas Sp3 was a repressor. GATA-4 and NKX2.5 were selected because they are very well-known transcriptional activators that are important for development and really set a standard.

The next level was to look at the posttranslational modifications that occurred, and specifically acetylation and ADP-ribosylation. Cardiac histone acetylation has been implicated in pathologic cardiac growth, and currently there are HDAC inhibitors that are being developed for use in cancer therapy in ongoing clinical trials.

Class I HDAC inhibitors have recently been shown to suppress the development of hypertrophy in a rodent model where the aorta was banded or angiotensin was infused.

The answer to the question, in short, is that we were interested in trying to determine the mechanisms by which the hypertrophic transcriptional program was initiated and that is why we did not do microarrays.

Dr Robbins. You mentioned in your manuscript about models of pressure overload to the left ventricle that are much easier to do, such as constriction of the ascending aorta in transgenic mice. This is very specific to the previous point. You are to be congratulated for focusing in and testing the specific hypothesis, but I wonder whether this is too narrow an area. I understand that your interest is in surgery for congenital heart disease, but how do you think that these data would translate into more generalizable models of ischemic cardiomyopathy or rapid pacing, other models of heart failure?

Finally, how do you choose the next step? Do you try to block Sp1 or do you try to increase Sp3 in pharmacologic methods?

Dr Azakie. This model is interesting because it mimics certain congenital heart defects, but the model can be tailored to produce pressure loading of the left ventricle.

With regard to other more common causes of congestive heart failure, I think that our hypothesis, and the hypotheses of others, is that although there are different types of biomechanical stressors, like pressure overload, volume overload, and ischemia-reperfusion injury, which stimulate different intracellular signaling pathways, we believe that ultimately there is a transcriptional reprogramming that develops within the nucleus of the cardiac myocyte and so really represents a final common pathway.

Finally, with regard to the next step for this line of research, I think our plan will be to use selective class I HDAC inhibitors as well as selective HAT inhibitors in determining whether or not those agents can either reverse the (volume-loaded) hypertrophic process or prevent the development of heart failure.

Dr Ludwig K. von Segesser *(Lausanne, Switzerland).* Did you consider going the other way around and closing the shunt to see how your molecular patterns precede or follow the morphology?

Dr Azakie. That's a very good question. We did not do that. Maybe doing that would be an interesting way to support the mechanisms that we are trying to infer here. It's a very good question and good suggestion. Thank you.