Proposal For Sarnoff Fellowship Application
December 2005

1) describe in 1 page a clinical or scientific problem, review literature, and define the relevance of the problem to cardiovascular medicine:

Heart failure is a disease affecting 1-5% of the US population. A failing heart is the shared end outcome of a number of inciting processes – coronary artery disease, myocardial infarct, valvular abnormalities, hypertension, as well as inherited mutations in sarcomeric and cytoskeletal proteins.[i] Broadly speaking, heart failure occurs when cardiac output is no longer able to sustain the metabolic needs of the body. This results in an overall imbalance in supply and demand, leading to cardiac hypertrophy and subsequently to heart failure. Two general forms of cardiac hypertrophy exist: pathologic (or maladaptive) and physiologic (or adaptive) hypertrophy.[ii] The former is seen in heart failure and situations of pressure overload, while the latter is involved in exercise-induced hypertrophy (the athlete's heart).

Over the past few years, we have come to better understand the intracellular signaling pathways mediating cardiac hypertrophy. Such pathways are important subjects of investigation; they offer targets for intervention with the ultimate goal of preventing heart failure. Of the several pathways mediating cardiac hypertrophy, one has recently received attention for its tendency to induce physiologic more than pathologic hypertrophy – namely, the pathway involving PIP3/PI3K/Akt.

In adaptive hypertrophy, this pathway begins with insulin, IGF or growth hormone activating a receptor tyrosine kinase in the plasma membrane of a cardiac myocyte. PI3K isoform p110alpha phosphorylates kinase Akt, and eventually results in the upregulation of protein synthesis machinery and transcription factors involved in hypertrophy.[iii] I propose to activate the PI3K pathway in a manner so as to favor physiologic hypertrophy over pathologic. As will be discussed, the pathway participates in both physiologic and pathologic hypertrophy, but only the latter seems to result in worsened outcomes and increased mortality.

The PI3K pathway has been shown to be of singular importance in growth and exercise-induced hypertrophy (i.e. adaptive hypertrophy).[iv] Constitutive activation of kinases in the adaptive arm of the PI3K pathway has resulted in cardiac hypertrophy,[v] whereas deletions have resulted in diminished cardiac growth and progression to a cardiomyopathic state.[vi]

The maladaptive hypertrophy PI3K arm is initiated by the binding of endothelin, beta-agonists, or angiotensin II to Gq protein-coupled receptors in the plasma membrane. The PI3K isoform p110gamma (vs. p110alpha in adaptive hypertrophy) associates with Gq subunits to continue signaling.[vii] Recent research has shown that p110gamma is necessary for pressure-induced hypertrophy but not normal growth.[viii] This contrasts to the aforementioned role of p110alpha in normal growth but not pathologic hypertrophy.

I hope to activate the PI3K signaling cascade in such a way to select physiologic over pathological hypertrophy. If I succeed, I would hope to contribute to a better understanding of cardiac hypertrophy, and the distinction between pathologic and physiologic hypertrophy. With continued research, it might one day be therapeutically applicable to modify the PI3K pathway in failing human hearts. This could lead to better outcomes and reduced mortality of heart failure. The current mainstays in heart failure treatment – beta adrenergic receptor antagonists, angiotensin-converting enzyme inhibitors, and diuretics – are attempts to reverse or palliate heart failure. A modifier of the PI3K pathway could conceivably *prevent* heart failure, and would be the first medical therapy to do so. If successful, such a therapeutic development would have important implications in the progression and clinical management of heart failure.

2) describe in 1 page various strategies or experiments to examine the problem:

In order to examine the problem of cardiac hypertrophy in heart failure, I have chosen the PI3K pathway as a focus. I would like to activate the arm of PI3K to effect physiologic hypertrophy without causing pathologic hypertrophy. As an intracellular signaling pathway, the PI3K pathway offers a number of targets for modification.

Perhaps the most obvious place to begin is at the outset of the signaling pathway. Most intracellular signaling pathways involve extracellular ligands binding to receptors on the plasma membrane. These ligands can be growth factors, hormones, or other neuroendocrine signaling molecules. The receptors range from receptor tyrosine kinases (RTKs) to G protein-coupled receptors and cytokine receptors. Pathway modulation at the receptor-ligand level involves either interrupting or facilitating ligand binding and consequent receptor activation. In the case of PI3K, both a G protein coupled receptor and a RTK are involved, the former in maladaptive and the latter in adaptive hypertrophy.[ix] Possible interventions include blocking the interaction between ligand and Gq coupled receptor on the sarcolemma, or inducing the ligand-RTK interaction at the plasma membrane.

A cellular signaling pathway can also be modified immediately downstream from the receptor. With G protein-coupled receptors, the active G subunit can be modified to change pathway activity. With the receptor tyrosine kinase, the kinase domain may be activated or inhibited in order to increase or decrease signaling, respectively. Beyond the receptors are intracellular signaling molecules such as RAS, phosphatases and other kinases such as Jak, Akt, MAPK.[x] These kinases can be experimentally activated by endogenous or constructed activators. Similarly, they can be inhibited by specific synthetic inhibitors, or – if they exist – in vivo inhibitors. In the PI3K pathways, p110alpha and p110 gamma kinases are such targets for modification.

Cellular signaling can also be modified by changing the activity of downstream steps, such as those mediated by protein kinases and other enzymes. P110, when recruited to the sarcolemma by beta/gamma subunits of activated Gq/11, in turn recruits kinase Akt and eventually causes pathologic hypertrophy. It is possible to inhibit p110 gamma in order to decrease pathologic hypertrophy. PI3Kgamma -/- mice have been found to be protected from hypertrophy, fibrosis, and cardiac dysfunction induced by long-term beta adrenergic receptor activation.[xi] Such knock-out mice could be evaluated for their hypertrophic responses to exercise and to pressure overload. Constitutive activation of p110alpha leads to adaptive hypertrophy in cardiac myocytes, as first demonstrated by Shioi et al. in 2000. Importantly, this p110alpha activation did not translate to premature death or increased heart failure at one year. Dominant negative mutations of p110alpha prevented both normal trophic heart growth and the exercise hypertrophy without increased mortality or failure rates at 1 year.[xii] One could pursue further exploration of constitutively active p110alpha models to determine if maladaptive hypertrophy is prevented or attenuated.

Farther downstream in the PI3K pathway, the serine-threonine kinase Akt stands out as the branch point in both forms of hypertrophy. Akt is activated by both p110gamma and p110alpha kinases, mediating pathologic and physiologic hypertrophy, respectively. Precisely what distinguishes the pathologic hypertrophic Akt signal from the physiologic has not yet been determined. However, it is conceivable that Akt activity could be modified so as to select for physiologic hypertrophy.

At the end of intracellular signaling pathways are transcription factors and upstream modulators of transcription factors such as calcineurin, nuclear factor of activated T cells (NFAT), histone deacetylases and mammalian target of rapamycin (mTOR). These molecules may be upregulated or inhibited to modify transcription and protein synthesis of hypertrophic mediators.

3) explain in 1 page which approach to this problem you find most interesting and why:

In my opinion, the Akt/PDK1 complex stands out as the most interesting target for intervention. Akt marks the beginning of the final common pathway for hypertrophy; it is activated by mediators of both physiologic and pathologic hypertrophy. In this sense, it is a fascinating signaling step – how can a single kinase be a convergence point for such markedly different signals?

It has been suggested that signal intensity or duration – which likely differs in the p110 gamma and alpha pathways – is what may determine adaptive versus maladaptive hypertrophy.[xiii] Exercise and the resultant growth factors would presumably activate Akt/PDK1 for shorter periods than would the prolonged conditions of pressure overload seen with hypertension. If stimulus duration were the distinguishing factor between the two pathways, the question remains whether it would be possible to compare prolonged activation of Akt to shorter term Akt activation. If the time hypothesis were indeed valid, the model with prolonged activation of Akt should show evidence of pathologic hypertrophy, whereas brief activation would result in physiologic hypertrophy.

I propose to study Akt activation in *in vitro* cell cultures of adult cardiac myocytes. I plan to set up two cultures of cells with differing Akt activity: one culture will have constitutively active Akt while the other will have transient, regular periods of Akt activation. I plan to use cardiac cell constructs which fuse active Akt to the estrogen receptor (ER). This ER is folded into an inactive conformation at baseline. Upon cell treatment with estrogen or a partial estrogen receptor agonist such as Tamoxifen, the ER unfolds and transient Akt activation is induced. By using this Akt-ER construct, the effects of turning on Akt will be immediate; alternative methods of regulating Akt at the gene level would have delays associated with gene transcription and translation. I plan to have one cardiac cell culture continually exposed to estrogen, and the other cell culture with daily hour-long burst exposures to estrogen. I will follow these cultures over the course of several weeks to months and measure markers of hypertrophy (see below).

The direct effects of estrogen on the cardiac cells could be a confounding factor in this experiment. As a control I plan also to have normal cardiac cell cultures without the Akt-ER construct, which I would expose to estrogen in a parallel fashion to the construct cells. This would allow me to distill any trophic effects of estrogen alone from the anticipated hypertrophic effects of Akt.

As an experimental endpoint, I plan to measure markers of cardiac hypertrophy in each of the cell culture models proposed. Myosin heavy chain (MHC) isoforms have long been accepted as quantitative indicators of heart failure, and of the reversion to fetal phenotype which occurs with failure. With maladaptive hypertrophy, MHC beta chain expression (low ATPase activity, seen in fetal hearts) increases and MHC alpha chain (high ATPase activity, seen in healthy adult hearts) decreases.[xiv] As such, the MHC beta/alpha ratio and will be an important indicator of pathologic hypertrophy in the cell cultures. I will also measure SERCA2a levels, which are decreased in pathological hypertrophy and heart failure with consequent decrease in Ca^{2+} cycling efficiency.[xv] Other markers I may measure include thyroid hormone receptor expression, ANP/BNP, and collagen isoforms, all of which have been shown to be affected by maladaptive hypertrophy. With these results I would hope to validate the hypothesis that continual Akt activation leads to expression of cardiac markers associated with failure, whereas burst Akt activation Akt does not.

Research results thus far with Akt have been inconclusive. Overexpression of the cardiac specific Akt (Akt1/2) in constitutively active murine Akt mutants has resulted – as one might expect – in cardiac hypertrophy. However, the extent of hypertrophy – and whether or not left ventricular dysfunction ensues – has been inconsistent in these constitutively active mutants: an investigation by Shioi et al in 2002 confirmed LV decompensation over time,[xvi] whereas two other studies in the same year reported no such evidence of cardiac decompensation (all in murine

models). [xvii,xviii] In completing my project, I would hope to help elucidate what distinguishes Akt's adaptive effects from maladaptive effects in cardiac hypertrophy.

My primary goal in this project is to induce physiologic and pathologic hypertrophy in cardiac myocytes by burst and continual Akt activation, respectively. If my hypothesis is correct, I would be simulating in vivo Akt signaling by physiologic and pathologic arms of the PI3K pathway. Understanding what differentiates physiologic from pathologic signaling in cardiac myocytes is paramount; such understanding may eventually be translatable to therapeutic interventions for failing human hearts.

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