

Overall research theme:

Molecular mechanisms underlying heart failure, cardiac hypertrophy and arrhythmia.

Latest update:

April 29, 2003

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Characteristics of the research group:

The research group integrates studies involving molecular biology, cell biology and animal physiology with the aim of improving clinical diagnosis and treatment of heart disease. The group combines knowledge in many areas from clinical medicine to protein purification. However, a unique feature is the focus on applying molecular biology methods *in vivo*. We use animal models of congestive heart failure and cardiac hypertrophy and cellular and molecular models of cardiac hypertrophy. The laboratory is well-equipped and we have instruments for measuring rat ECG, real-time PCR, protein analysis, laser scanning microscopy and protein-protein interaction *in vivo*.

Running projects: Titles and abstracts:

Cell-Cycle Proteins as Regulators of Cardiac Hypertrophy.

Approximately hundred thousand people in Denmark are affected by congestive heart failure, which is a condition associated with high morbidity and mortality. A predominant feature of heart failure is cellular hypertrophy, which over time leads to myocyte necrosis and apoptosis. At the molecular level, the involvement of mitotic stimuli and cell-cycle regulatory proteins in hypertrophy shows that there is a relation between hypertrophy and mitosis. Three recent publications of which one is from this project, demonstrate that inhibition of certain cell-cycle proteins can impair cardiac hypertrophy *in vitro*.

This project aims to find the key control molecules for hypertrophic response. We have identified the cell-cycle proteins that are expressed in the heart and shown that the cell-cycle regulators are necessary for development of hypertrophy *in vitro*.

We manipulate the expression of these genes in the heart to study the effect in hypertrophy. This is done with pharmacological and genetic inhibition in rat hearts. Our second approach is to find new targets for therapy by elucidating the signalling mechanism in primary cardiomyocyte cultures.

The project will lead to an enhanced understanding of the mechanism behind the hypertrophic response and possibly allow the design of treatments of malignant hypertrophy in humans.

The Cell-Cycle as a Therapeutic Target in Cardiac Disease.

The hypothesis of this project is that cardiac hypertrophy and proliferation are highly related processes that are controlled by the cell-cycle regulatory mechanism. By mapping this mechanism in cardiac myocytes we will be able to gain an understanding of how to stimulate cell-division in the heart. This knowledge will be important for the development of future strategies for the treatment of cardiac disease. The project is an approach to the possibility of regenerating damaged areas in the heart after an infarct or other cardiac diseases that lead to cell loss. Until recently, the notion of *de novo* cardiomyogenic induction and subsequent cardiomyocyte proliferation in the adult heart was not viewed as a likely prospect. However, the finding that bone marrow-derived stem cells have the ability to differentiate into cardiac myocytes has changed this opinion. In addition, in the adult heart there is a limited population of stem cells that could be stimulated to divide in patients with myocardial damage. We do not know which

of these possibilities that will eventually develop into therapy. However, it is clear that the biological role of the cell-cycle regulatory mechanism in the heart is a highly relevant field in the coming years.

Gene expression patterns in cardiac hypertrophy

We hypothesized that a comparative analysis of hypertrophic gene expression using microarray technology (with 8799 genes) in four rat models of cardiac hypertrophy including aortic banding, myocardial infarction, arterio-venous shunt operation and hormone treatment (angiotensin II and the thyroxin analogue detap) would identify novel genes involved in hypertrophic signalling. From multiple expression analyses of left ventricular RNA, we identified a core set of 135 genes with differential expression in hypertrophic hearts including 49 genes or pathways not previously associated with hypertrophy and 86 genes whose expression had previously been shown to be altered by hypertrophy. This experiment and subsequent RT-PCR and western blotting demonstrated increased expression of a novel cardiogenic factor. This Ca²⁺ binding and angiogenic protein is considered critical for metastatic progression of breast cancer. Immunohistochemistry revealed the novel protein is localized in endothelial cells and vascular smooth muscle cells in the rat and human heart. Purified recombinant protein potently induced morphological hypertrophy and ANP secretion in cardiomyocyte cultures. Conclusions-Our data identified a core set of 135 genes that may signify changes in gene regulation underlying hypertrophic remodelling across multiple experimental forms of cardiac hypertrophy in the rat. One of these genes is produced in blood vessel- and inflammatory-cells and promotes cardiomyocyte hypertrophy suggesting paracrine-signalling plays a significant role in the pathophysiology of heart failure.

Extracellular Signal-regulated Kinases control expression of G protein-coupled Receptor Kinase 2 (GRK2)

G protein-coupled receptor kinase 2 (GRK2) phosphorylates G protein coupled receptors resulting in uncoupling from G proteins. These molecules function as the cells internal 'receptor blockers' and have the unique feature of binding only to activated receptors. Furthermore, unlike man made receptor blockers, the GRKs work on many different G protein coupled receptors. In turn, receptors modulate GRK2 expression, however the mechanistic basis for this effect is largely unknown. We have reported a novel mechanism by which receptors use the ERK cascade to regulate GRK2 cellular levels. ERK activation by receptor stimulation elevated endogenous GRK2 while antagonist treatment decreased cellular GRK2. Activating ERK by overexpressing constitutive active MEK-1 or Ras elevated GRK2 protein levels while blocking ERK using PD98059 or dominant negative Ras abolished this effect. These data suggest ERK is a critical regulator of GRK2 levels. Further work suggests this effect is due reduced GRK2 degradation by the proteasome rather than increased GRK2 synthesis. We have observed interesting cardiac hypertrophy phenotypes with several adenovirus containing GRK2 mutated in the aminoacids phosphorylated by Erk.

Recent publications related to the projects described above:

- Busk P.K. and Hinrichsen, R. (2003). Cyclin D in left ventricle hypertrophy. *Cell Cycle* **2**, 91-95.
- Busk P.K., Wulf-Andersen L., Strøm, C.C., Enevoldsen M., Thirstrup K., Warthoe P., Haunsø S. and Sheikh SP. (2003). Multi-protein Bridging Factor 1 is necessary for cardiac hypertrophy and promotes hypertrophic gene expression together with the transcription factor c-Jun. *Exp Cell Res.* **286**, 102-114.
- Theilade J., Strøm C., Christiansen T., Haunsø S. and Sheikh S.P. (2003). Differential G protein receptor kinase 2 expression in compensated hypertrophy and heart failure after myocardial infarction in the rat. *Basic Res Cardiol.* **98**(2):97-103.
- Theilade J, Lerche Hansen J, Haunsø S, & Sheikh SP. (2002). Extracellular Signal-regulated kinases control expression of G protein coupled receptor kinase 2 (GRK2). *FEBS Letters* **528**:195-199.
- Busk P.K., Bartkova, J. Strøm C.C., Wulf-Andersen L., Hinrichsen R., Christoffersen, T.E.H., Latella, L., Bartek J., Haunsø S. and Sheikh SP. (2002). Involvement of Cyclin D activity in left ventricle hypertrophy in vivo and in vitro. *Cardiovascular Research.* **56**, 64-75.
- Jensen AA, Lerche-Hansen J, Sheikh SP, & Bräuner-Osborne H. (2002). Probing intermolecular protein-protein interactions in the calcium-sensing receptor homodimer using bioluminescence resonance energy transfer (BRET). *Eur. J. Biochem.* **269**(20):5076-87.
- Theilade J, Haunsø S, & Sheikh SP. (2001). G protein coupled receptor kinase 2 – a negative feed back molecule *Current Drug Targets, Immune, Endocrine & Metabolic Disorders* **1**, 139-151.
- Lerche Hansen, J, Servant G, Baranski TJ, Fujita T, Iiri T, Haunsø S, & Sheikh SP. (2000). Functional reconstitution of type 2 Angiotensin II receptors. *Circulation Research* **87**, 753-9.
- Baranski TJ, Herzmark P, Lichtarge O, Gerber BO, Trueheart J, Meng EC, Iiri T, Sheikh SP; & Bourne HR. (1999). C5a receptor activation. Genetic identification of critical residues in four transmembrane helices. *J. Biol. Chem.* **274**(22):

15757-15765.

Sheikh SP, Villard J-P, Baranski TJ, Lichtarge O, Iiri T, Meng E, Nissenson RA, & Bourne HR. (1999). Similar structures and shared switch mechanisms of the b2 adrenoceptor and the parathyroid hormone receptor - Zn(II) bridges between helices III and VI block activation. *J. Biol. Chem.* **274**(24): 17033-17041.