

10:15 a.m.

11:15 a.m.

404-5

### Vascular Endothelial Growth Factor (VEGF) Plays a Central Role in the Development of Diabetic Cardiomyopathy and Gene Transfer of VEGF Improves Heart Failure in Long-Standing Streptozotocin-Induced Diabetic Rat

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**Background:** Diabetic cardiomyopathy (DCM), which is characterized by interstitial fibrosis and microvascular pathology, progressively leads to heart failure (HF). Using the streptozotocin-induced diabetic rat model, we evaluated the natural course of DCM over a period of one year and tested whether VEGF gene transfer could improve diabetic HF. **Methods and Results:** Diastolic dysfunction developed in the early phases and progressed in all rats. Systolic dysfunction developed later and became significant in 24% of the rats. Downregulation of myocardial VEGF expression was detected early in the course of DCM, followed by increased apoptosis of endothelial cells (ECs), decreased numbers of circulating endothelial progenitor cells (EPCs), and finally by decreased capillary density and impaired myocardial perfusion. Ultimately, apoptosis and necrosis of cardiomyocytes (CMCs) ensued along with fibrosis. Based on this observed sequence of events, we evaluated a strategy of angiogenic gene therapy (GT) via direct intramyocardial injection of plasmid DNA encoding VEGF(phVEGF<sub>165</sub>). Rats were randomized to receive phVEGF<sub>165</sub>, LacZ plasmid or sham operation (n =14, each group). Only the VEGF group showed increased capillary density and decreased EC and CMC apoptosis. Bone marrow (BM) transplantation studies documented evidence for in situ differentiation of BM derived EPCs into ECs in the VEGF group. These anatomic findings were accompanied by significant improvement in cardiac function in the VEGF group alone. **Conclusion:** Here, we first demonstrate that down-regulation of VEGF plays a central role in the pathogenesis of DCM and that replenishment of VEGF, using a direct myocardial GT approach, can improve HF by augmenting neovascularization through angiogenesis and vasculogenesis and by reestablishing VEGF homeostasis in the myocardium.

406-2

### Stem Cell Factor Plays a Critical Role in Neointimal Formation by Bone Marrow Derived Progenitor Cells

Subodh Verma, Chao-Hung Wang, William Stanford, Richard D. Weisel, University of Toronto, Toronto, Canada

We hypothesized that stem cell factor (SCF) is a critical mediator of restenosis facilitating mobilization, homing & transdifferentiation of progenitors. Femoral injury was induced in wildtype, WBB6F1 (*W/W<sup>h</sup>*) (*c-kit* deficient) & WCB6F1 (*Sf/Sf*, Steel-Dickie) (mSCF deficient) mice & neointimal formation, SCF, *c-kit*, SMA & MMP-9 quantified. Progenitor cell mobilization was evaluated by circulating SCF levels & CD34<sup>+</sup> cells. Vascular injury following BM transplantation using donor cells expressing eYFP (BMT<sup>YFP→Wild</sup>) was performed. The effects of SCF & SMCs on Lin<sup>-</sup> BM cell adhesion to fibronectin was studied in addition to coculture of eYFP<sup>+</sup> Lin<sup>-</sup> BM cells with activated SMC. The effects of PDGF & A-II on SCF expression following vascular injury were evaluated, as were the effects of long-term ACEI. *W/W<sup>h</sup>* & *Steel-Dickie* mice exhibited marked attenuation in neointimal formation at day 14 & 28 (P<0.001) In wild-type mice vascular injury resulted in the elevation of sSCF compared to *Steel-Dickie* mice & induced an increase in circulating CD34<sup>+</sup> progenitors (P<0.01). An increase in SCF, proMMP-9 & MMP-9 expression in the adventitia occurred within the first week following vascular injury. BMT<sup>YFP→Wild</sup> indicated the presence of *c-kit* positive cells within the medial layer expressing SMA. SCF induced a marked increase in adhesion of BM cells to fibronectin suggesting a direct role of SCF in progenitor recruitment (P<0.001). Activated rat SMCs increased the adhesion of BM cells & coculture of eYFP Lin<sup>-</sup> BM cells with SMC showed clusters of cells expressing both eYFP & SMA, suggesting that SMC activation, marked by SCF expression, is required for progenitor differentiation. PDGF & A-II upregulated SMC SCF expression in a dose dependent fashion, & ACEI (BMT<sup>YFP→Wild</sup>) suppressed the number of BM derived neointimal cells(P<0.01). In conclusion, vascular injury upregulates local mSCF expression & activates MMP-9, facilitating the shedding of sSCF with resultant mobilization and homing of BM progenitors to sites of vascular injury. *In-vivo* deficiency of SCF or its receptor, *c-kit* is associated with reduced neointimal formation. Targeting SCF may serve as a novel therapeutic strategy for restenosis.

11:30 a.m.

## YOUNG INVESTIGATOR AWARDS

406

### Young Investigators Awards Competition: Molecular and Cellular Cardiology

Monday, March 08, 2004, 11:00 a.m.-12:15 p.m.  
Morial Convention Center, Room 257

11:00 a.m.

406-1

### Hypoxia Regulatable Aav-2 Vector Protects Against Cardiac Ischemia/Reperfusion Injury

Alok S. Pachori, Luis G. Melo, Lunan Zhang, Richard E. Pratt, Victor J. Dzau, Brigham & Women's Hospital, Boston, MA, University of Saskatoon, Saskatchewan, SK, Canada

**Purpose:** We have previously demonstrated that constitutive overexpression of heme-oxygenase 1 protects against myocardial injury. The purpose of this study is to test the efficacy of a hypoxia inducible vector encoding heme oxygenase 1, for protection against myocardial ischemia and reperfusion injury by determining the inducibility of the vector during ischemia and protection against ischemia and reperfusion injury.

**Methods:** Eight week old male Sprague-Dawley (SD) rats were divided into two groups (n=15-16/group) and injected with either AAV-4HRE-mSV40-HO-1 OR AAV-4HRE-mSV40-LacZ (4 X 10<sup>11</sup> pfu/ml). Four weeks after injection, animals from both groups were randomized and subjected to either sham surgery or surgery consisting of a 1 hour ischemia followed by reperfusion for 6, 12 and 24 hours. Following reperfusion, tissues were harvested and used for protein and RNA isolation and TTC staining for determination of infarct size. Separate group of animals were used for echocardiographic analysis of cardiac function following I/R injury.

**Results:** Our results indicate that compared to baseline, ischemia/reperfusion injury resulted in a significant increase in HO-1 protein and activity levels in HO-1 transduced animals. More importantly, there was a significant decrease in the infarct size in HO-1 treated animals versus the control group (31.0 ± 5.58 vs. 12.1 ± 2.15, p<.01) as well as complete improvement in cardiac function parameters such as ejection fraction, septal and posterior wall thickness and LV volume and area, one month after injury, as determined by echocardiographic analyses.

**Conclusion:** In this study we have demonstrated that expression of exogenous hHO-1 can be regulated by hypoxia developed during global ischemia. The data indicate that overexpression of hHO-1 during ischemia and early reperfusion has a marked protective effect on the myocardium. This is the first evidence of the therapeutic efficacy of inducible expression and provides proof-of-concept for the feasibility of this approach for protection against ischemia/reperfusion injury.

406-3

### Protecting Ischemic Hearts With a Hypoxia-Sensitive Heme Oxygenase-1 Plasmid System

Yao Liang Tang, Yi Tang, Y. Clare Zhang, Keping Qian, M. Ian Phillips, All Children Hospital, Children Research Institute, St. Petersburg, FL, Stanford University, Stanford, CA

**Background:** Although human heme oxygenase-1(hHO-1) could provide a useful approach for cellular protection in the ischemic heart, constitutive over-expression of hHO-1 may lead to unwanted side effects. We designed a hypoxia-regulated hHO-1 gene therapy system named vigilant hHO-1 plasmid system, which is based on an oxygen dependent degradation domain (ODD) from the hypoxia inducible factor-1-alpha (HIF-1 $\alpha$ ) that can sense ischemia and switch on hHO-1 gene in the heart.

**Methods and Results:** In vitro experiment, HEK293 cells were transfected with vigilant hHO-1 plasmid, it produced a robust hHO-1 induction upon exposure to low oxygen. In a mouse model for myocardial infarction, the vigilant hHO-1 plasmids turned on hHO-1 genes specifically to ischemic myocardium. At 10 days after gene transfer, Masson trichrome staining showed significantly fewer fibrotic areas in vigilant hHO-1 plasmids treated mice compared with saline control (43.0±4.8% vs 62.5±3.3%, P<0.01), the reduction of interstitial fibrosis is accompanied by an increase in myocardial hHO-1 expression in peri-infarct border areas, concomitant with reduced apoptosis in the ischemic myocardium compared to saline control. By use of a cardiac catheter in close-chest preparation, heart from vigilant hHO-1 plasmids treated mice showed improved recovery of contractile and diastolic performance after MI compared with saline control.

**Conclusions:** This study documents the beneficial regulation and therapeutic effect of vigilant plasmid-mediated hHO-1 gene transfer. This novel prophylaxis gene transfer strategy can provide cardiac specific protection from future repeated bouts of ischemic injury.

11:45 a.m.

406-4

### Mice Lacking the Transcription Factor CHF1/Hey2 Show Decreased Arterial Neointimal Formation After Injury and Impaired Vascular Smooth Muscle Cell Responsiveness to Growth Factors

Yasuhiko Sakata, Fan Xiang, Zhiping Chen, Yoriko Kiriyaama, Caramai N. Kamei, Daniel I. Simon, Michael T. Chin, Brigham & Women's Hospital, Cambridge, MA

**Background:** The hairy-related bHLH transcription factor, CHF1/Hey2, has been shown to play an important role in zebrafish vascular development, however, no anatomical defects in the vascular system have been reported in CHF1/Hey2-deficient mice. **Methods:** We investigated the functional vascular phenotype of CHF1/Hey2-deficient mice by measuring vascular remodeling after injury in vivo and primary cultured vascular smooth muscle cell (VSMC) function in vitro. **Results:** Neointima formation after arterial wire injury was markedly decreased in KO compared to WT mice (0.025±0.011 mm<sup>2</sup> in WT (n=13) vs. 0.016±0.008 mm<sup>2</sup> in KO (n=12), p<0.05). Analysis of primary cultured VSMCs revealed that CHF1/Hey2 deficient VSMCs proliferate comparably to WT VSMCs but showed decreased migration in response to platelet derived growth factor (PDGF) (62.6±10.3/CPF vs. 37.2±13.5/CPF, p<0.01) and heparin-binding epidermal growth factor-like growth factor (HB-EGF)(27.4±7.7/CPF vs. 6.4±3.7/CPF, p<0.05) as assessed by modified Boyden chamber assay. Further, PDGF and HB-EGF induced diminished