PS-1-I

Keynote: Heart-Derived Progenitor Cells for Cardiac Repair

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Potential repair by cell grafting or mobilizing endogenous cells holds particular attraction in heart disease, where the meager capacity for cardiomyocyte proliferation — mediated in part by down-regulation of telomerase — likely contributes to the irreversibility of heart failure. Whether cardiac progenitors exist in adult myocardium itself is unanswered, as is the question whether undifferentiated cardiac precursor cells merely fuse with pre-existing myocytes. Here we report the existence of adult heart-derived cardiac progenitor cells expressing stem cell antigen-1. Cardiac Sca-1+ cells are Lin-, Kit-, CD45-, CD34-, excluding hematopoietic cells in blood or bone marrow as the source, contain virtually all the residual telomerase activity in adult myocardium, and encompass the smaller set of so-called "side population" (SP) cells, which are specifically associated with long-term self-renewal in bone marrow and other organs. Initially, the cells express neither cardiac structural genes nor Nkx2.5 but differentiate in vitro in response to 5' - azacytidine, in part depending on Bmpr1a, a receptor for bone morphogenetic proteins. Given intravenously after ischemia/reperfusion, cardiac Sca-1+ cells home to injured myocardium. Using a Cre/Lox donor/recipient pair (α MHC-Cre/R26R), differentiation was shown to occur roughly equally, with and without fusion to host cells. Cardiac Sca-1+ cells offer auspicious properties for cardiac repair, including high levels of telomerase, homing to injured myocardium, and dependence on the well-defined BMP pathway. We emphasize the incompleteness of myocardial repair as executed by all endogenous mechanisms collectively, including whatever progenitors exist intrinsic and extrinsic to the heart. Thus, there exists both need and opportunity to augment cardiac Sca-1+ cell number or function.

PS-1-II

Combination With Cardiotrophin-1 Gene Transfection Enhanced the Protective Effect of Myoblast Transplantation on the Transition to Heart Failure in Dahl Rats

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Skeletal myoblast transplantation to the myocardium can be a promising candidate for the treatment of heart failure. This strategy is also useful as a tool for cell-mediated gene therapy. Cardiotrophin-1 (CT-1), a member of the interleukin-6 superfamily, has hypertrophic and cardioprotective properties. We reported previously that myoblast transplantation (MB) alleviated the transition from compensatory left ventricular hypertrophy (LVH) to congestive heart failure (CHF) in Dahl salt-sensitive hypertensive (DS) rats. Furthermore, transplantation of myoblast transfected with CT-1 gene using retrovirus (MB+CT) enhanced the effect of myoblast transplantation. In this study, we investigated the mechanisms underlying this salutary effect. Histological examination revealed that the cardiac myocyte-size at the CHF stage was 20% larger in MB+CT group than MB group, indicating that CT-1 secreted from the grafted cells prevented the LV remodeling by inducing hypertrophy of the adjacent myocardial cells. In this DS rat model, activation of local renin-angiotensin (AT) and endothelin (ET) systems in the heart contributes to the transition to CHF. The AT system was markedly up-regulated during the transition to CHF in sham group(injected with PBS). However these changes were attenuated in both MB and MB+CT groups, and the degree of attenuation was greater in MB+CT group than MB group. The ET system remained also unchanged in both MB and MB+CT groups. These results suggest that a decrease in wall stress due to the preservation of LV wall thickness attenuated the expression of such neurohumoral factors. In addition, we revealed that CT-1 has a survival effect on myoblasts in vitro. Thus, it is possible that CT-1 augmented the graft survival ratio in the myocardium. By these mechanisms, transplantation of skeletal myoblasts combined with CT-1 gene transfection could be effective for the treatment of heart failure.

PS-1-III

Regeneration Therapy Against Dilated Cardiomyopathy

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Colony stimulating factors including granulocyte colony-stimulating factor (G-CSF) have multi-potent actions such as acceleration of wound healing, in addition to proliferating and differentiating effects on granulocytes and releasing bone marrow stem cells. We report here that the long-term treatment with G-CSF resulted in a marked improvement of survival of cardiomyopathic hamsters of UM-X7.1 strain compared with the untreated animals (100% vs 53%). Cardiac function was significantly improved and the heart was less affected by fibrosis and dilatation in the G-CSF treated group. These beneficial effects against congestive heart failure (CHF) were accompanied by a lower rate of autophagy-like cardiomyocyte degeneration, a lower level of tumor necrosis factor-alpha (a cardiotoxic cytokine), an increased activity of tissue matrix metaloproteinases (anti-fibrotic action), and an augmented regeneration of cardiomyocytes. The present findings imply a novel therapeutic efficacy of G-CSF to heart failure due to non-ischemic cardiomyopathy.

PS-1-IV

Endogenous Bone Marrow-Derived Stem Cells Reconstituted Myocardium Only in the Small Proportion After Acute Myocardial Infarction

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Objective Our recent study showed granulocyte-colony stimulating factor (G-CSF) promoted bone marrow cells to migrate into the infarcted heart and they differentiated into cardiomyocytes. However, we still do not know in what degree bone marrow-derived cardiomyocytes reconstitute myocardium at the injured area. In this study, we verify the proportional contribution between bone marrow (BM) and non-bone marrow (n-BM) for regenerating neomyocardium after myocardial infarction. Methods Eight C57BL/6 mice were irradiated (900 cGy), and GFP-mouse-derived-bone marrow cells (GFP-BMC: $1x10^6$ cells) were injected. Four weeks later, the left descending coronary artery was ligated. Recombinant human G-CSF (200 μg/kg/day, 8 days) was injected. At four weeks after ligation, hearts were fixed for histology. The proportions of cardiomyocytes derived from BM and n-BM were calculated after taking the chimeric rate into consideration. Results The chimeric rate was 54.6 ± 5.9 %. At the infarcted border area, the total cell number was 1000.3 ± 56.5 / mm^2 and mobilized bone marrow-derived GFP-BMC was at 103.3 ± 13.1 / mm^2. After compensation with the chimeric rate, there were the BM-derived TnI-positive cells at 23.9 ± 4.1 / mm^2, nestin-positive cells at 12.9 ± 2.6 / mm^2, and Ki67-positive cells at 18.3 ± 2.6 / mm^2, respectively. There were significant differences in the contribution of TnI- (6.7 ± 1.7 % vs. 93.3 ± 1.7 %), nestin- (2.4 ± 0.5 vs. 97.6 ± 0.5), and Ki67- (3.9 ± 1.0 vs. 96.1 ± 1.0) positive cells derived from BM and n-BM. Conclusions Bone marrow was one of the origins of regenerated cardiomyocytes, however the contribution of bone marrow-origin was very small compared with non-bone marrow-origin in the infarction model.

PS-1-V

Myocardial Tissue Reconstruction by Cell Sheet Engineering

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Recently, cell transplantation has been proposed as a new therapy and holds enormous potential to repair damaged heart. In direct transplantation of dissociated cells, it is difficult to control shape, size and location of the transplanted grafts. To solve these problems, further advanced therapies to transplant bioengineered 3-dimensional (3-D) myocardial tissue have been investigated. In contrast to popular approach seeding cells onto 3-D biodegradable scaffolds, we have developed novel technology "cell sheet engineering" that layers cell sheets to reconstruct 3-D tissues. Cell sheets can be obtained by culturing isolated cells on intelligent cell culture surfaces grafted with temperature-responsive polymer, poly(*N*-isopropylacrylamide)(PIPAAm), from which confluent cells detach as a cell sheet simply by reducing temperature. By using this technology, neonatal rat cardiomyocyte sheets were successfully layered and electrically communicative pulsatile 3-D myocardial tissues have been engineered. Engineered myocardial tissues grew and survived up to one year *in vivo*. To overcome the limitation of engineered tissue thickness caused by primary insufficient oxygen permeation, implantation of layered cell sheets was repeated at enough interval for neovascularization. The overlaid grafts revealed synchronized beating and thicker vascularlized myocardial tissues without necrosis. When 10-time transplantations of 3-layer grafts were performed, the beating was visible and touchable via skin and the graft thickness was about 1mm. These results indicate that the limitation of engineered-tissue thickness due to primary hypoxia can be dissolved by cell sheet manipulation and multi-transplantation technique. This new strategy might contribute to tissue engineering research field as well as myocardial tissue repair.

PS-1-VI

G-CSF and GM-CSF Differentially Affect the Regeneration of Infarcted Myocardium by Bone Marrow-derived Cells

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We compared the effects of G-CSF and GM-CSF on the regeneration of infarcted myocardium by mobilized bone marrow cells in a bone marrow transplantation model using transgenic GFP-mice. Unexpectedly, these two cytokines had opposite effects on survival and cardiac function after myocardial infarction. Mice received bone marrow transplantation, underwent myocardial infarction, and then received cytokines by subcutaneous injection for 10 days. GM-CSF worsened subacute survival, elongated and thinned the infarcted area, and deteriorated cardiac function. This was explained by the finding that a number of macrophages were mobilized into the infarcted area by GM-CSF at the acute phase compared with the saline or G-CSF group. In contrast, G-CSF administration significantly improved survival and cardiac function. This phenomenon was explained by the findings that the recruitment of GFP^+CD45^- cells was markedly increased in the infarcted area of G-CSF-treated mice compared to the saline or GM-CSF group at the chronic phase (60 days). Furthermore, approximately 10% of GFP^+ cells were also $actinin^+$, suggesting a cardiomyocyte phenotype. FISH analysis negated the possibility of cell fusion between donor and recipient cells. Appropriate cytokine therapy may therefore augment the repair of damaged myocardium by mobilized bone marrow cells and prevent cardiac remodeling.

PS-1-VII

Gene Therapy to Induce Cardiac Regeneration

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Myocardial infarction (MI) is a leading cause of cardiac morbidity and mortality in many countries, however, the treatment of MI is still limited. We here demonstrate a novel gene therapy to promote regeneration of the heart after MI using leukemia inhibitory factor (LIF) cDNA. We injected LIF plasmid DNA into the thigh muscle of mice immediately after inducing MI. Intramuscular injection of LIF cDNA resulted in a marked increase in circulating LIF protein levels. Two weeks later, left ventricular remodeling such as infarct extent and myocardial fibrosis was markedly attenuated in the LIF cDNA-injected mice compared to vehicle-injected ones. More myocardium was preserved and cardiac function was better in the LIF-treated mice than vehicle-injected ones. Injection of LIF cDNA not only prevented the death of cardiomyocytes in the ischemic area but also induced neovascularization in the myocardium. Furthermore, LIF cDNA injection increased the number of cardiomyocytes in cell cycle and enhanced mobilization of bone marrow cells to the heart and their differentiation into cardiomyocytes.

PS-1-VIII

Effects of Cellular Cardiomyoplasty on Ventricular Remodeling Assessed by Doppler-echocardiography and Topographic Immunohistochemistry

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Background. Myocardial infarction (MI) promotes deleterious remodeling of the myocardium, resulting in ventricular dilation and pump dysfunction. Supplementing infarcted myocardium with neonatal myocyte would attenuate deleterious remodeling. We analyzed cardiac function and histological regeneration of the damaged myocardium after cellular cardiomyoplasty by Doppler-echocardiography and immunohistochemistry.
Methods and Results. Experimental MI was induced by 24-hours coronary ligation followed by reperfusion in adult male Lewis rats and neonatal myocytes were injected directly into the infarct and peri-infarct regions. Three groups of animals were studied at 4 weeks after cellular cardiomyoplasty: noninfarcted control (control), MI plus sham injection (MI), and MI plus cell injection (MI+cell). Ventricular remodeling and cardiac performance were assessed by Doppler-echocardiography or contrast echocardiography. At 4 weeks after cellular cardiomyoplasty, MI+cell hearts exhibited attenuation of global ventricular dilation and cardiac function compared with MI hearts not receiving cellular cardiomyoplasty. Immunohistochemically, connexin-43-positive small cells were often observed in the vicinity of infarction in MI+cell heart. By electron microscopy, these cells contained myofilaments with Z-bands and poorly developed intercalated disks, suggesting neonatal myocardial cells.
Conclusions. Implanted neonatal myocytes form viable grafts after MI, resulting in attenuated ventricular dilation and enhanced contractile function. Cellular cardiomyoplasty may be a beneficial therapy after MI.

PS-1-IX

Regeneration of Cardiac Myocytes by Cell Cycle Regulators

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Since cardiac myocytes withdraw from the cell cycle and never proliferate soon after birth, regeneration of cardiac myocytes is expected to be a novel therapeutic approach for heart failure. We aimed to compel cardiac myocytes to re-enter cell cycle by modulation of cell cycle regulators. Mitogenic stimulations on cardiac myocytes, such as serum exposure, up-regulate expression of cyclin D1, a key regulator of the G1-to-S phase transition. However, we found that cyclin D1 localizes predominantly cytoplasm in post-mitotic neonatal cardiac myocytes, and that ectopically expressed cyclin D1 and cyclin-dependent kinase 4 (CDK4) can not enter to the nucleus. We next produced adenovirus vector containing cyclin D1 directly linked to viral nuclear localization signals (NLSs). The cyclin D1/NLS co-expressed with CDK4 induces cell cycle progression leading to cell division in neonatal cardiac myocytes. Furthermore, the cyclin D1/CDK4 nuclear import promotes the reentry of adult heart cardiac myocytes in the cell cycle in vivo as evaluated by staining with Ki-67 antibody. These results demonstrate that cyclin D1 nuclear import is tightly blocked in post-mitotic cardiac myocytes and that overcoming of this inhibitory mechanism is sufficient to induce the proliferation of cardiac myocytes.

PS-2-I

Long-Term Evaluation of Patients With Hypertrophic Obstructive Cardiomyopathy

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There are few reports about long-term prognosis of hypertrophic obstructive cardiomyopathy (HOCM), especially in patients treated with medical therapy. We evaluated long-term prognosis in patients treated with β-blockers/class I-antiarrhythmic drugs compared with pacing or surgical therapy. Method: Eighty-two patients of HOCM, who had pressure gradient (PG>30mmHg without provocation; mean PG=78 ± 31mmHg) at left ventricular outflow tract, were treated with β-blockers/class I-antiarrhythmic drugs, dual-chamber pacing therapy or surgical therapy (myectomy/mitral valve replacement). We evaluated long-term prognosis (improvement of PG, morbidity and mortality) between patients treated with medical therapy (M-group; 56 patients), with pacing and medical therapy (P&M-group; 14 patients) and with surgical therapy (S-group; 12patients), retrospectively. Mean follow-up duration was 8.4 ± 5.5 years. Result: In M, P&M and S-group, patients with improvement of percent change in PG (>50% reduction) were 45%, 79% and 100%, respectively. However, cardiovascular morbidity was 20%, 43% and 25%, respectively. The major cardiovascular morbid events were tachy-arrhythmia and heart failure. Furthermore, in M, P&M and S-group, cardiovascular mortality was 5.4%, 0% and 8.3%, respectively. The overall mortality in this study was 15%. Conclusion: When considered together, there was no relation between percent changes in pressure gradient, morbidity and mortality. These results suggest that the magnitude of PG is not directly related to the long-term prognosis in patients with HOCM, and HOCM has a benign prognosis in terms of cardiovascular mortality.

PS-2-II

Keynote: Changing Concepts on Prevalence, Phenotypic Expression and Management of Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is of great clinical significance as the most common cause of sudden cardiac death in the young, as well as cardiovascular disability and death due to heart failure at any age. HCM is the most common of the genetic cardiovascular diseases with an estimated prevalence of about 1:500 in the general population similarly reported from diverse geographic areas including the U.S., Japan and China. The observation that HCM is uncommonly encountered in cardiology practice suggests that the majority of individuals who carry a mutant gene for this disease remain undiagnosed, including many who may be at an unacceptably high risk for sudden cardiac death.

The sine qua non for diagnosis is an otherwise unexplained hypertrophied, but nondilated left ventricle, generally regarded as the HCM phenotype. However, left ventricular hypertrophy may not be present at all ages or phases of life --- including delayed-onset adult morphologic conversions --- which has caused recommendations for family screening to become more complex and the period of morphologic (echocardiographic) surveillance to be greatly extended.

The management of HCM is dependent on identifying the particular clinical course pathway which is most appropriate for each patient. For example, about 25% of patients will develop atrial fibrillation, which will become the major determinant of prognosis...and for which treatment is largely based on control with drugs. A substantial minority of patients may be judged, by virtue of risk factors, to be at unacceptably high-risk for sudden death --- and 2 deserving of consideration for an implantable defibrillator targeting primary (prophylactic) or secondary (after cardiac arrest) prevention of sudden death. Finally, an important subset of patients develop progressive heart failure associated with obstruction to left ventricular outflow, and are refractory to maximum medical management with negative inotropic drugs such as beta-blockers, verapamil or disopyramide. In that event, the gold standard treatment is the ventricular septal myectomy operation. Alternative treatment options to surgery have evolved for selected patients who would otherwise be operative candidates, including dual-chamber pacing and alcohol septal ablation. The latter technique effects reduction in symptoms and outflow obstruction, but leaves a residual intramyocardial scar which may become the nidus for re-entrant ventricular arrhythmias.

PS-3

Keynote: Genetic and Cellular Pathways in Heart Failure: Towards Biologically Targeted Therapy

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As an approach to unraveling the pathways for heart failure, our laboratory has utilized mouse model systems to dissect pathways that lead to dilated cardiomyopathy, and to identify new genetic and cellular principles that can be applied to this disease. A major component of heart failure progression now appears to be chronic increases in wall stress that trigger specific signaling pathways for critical cell responses, which include cell death pathways, survival cues, hypertrophic responses, and associated changes in the downstream pathways. A series of scientific and therapeutic advances have arisen from the initial discovery of a genetic link between defects in cytoskeletal Z disc proteins and dilated cardiomyopathy in MLP deficient mice, including the identification of patients that harbor Z disc genetic defects, the discovery of components of the cardiac muscle stretch sensor, the elucidation of the pivotal role of defects in calcium cycling in heart failure progression, and the validation of phospholamban inhibitory peptides as a new therapeutic strategy in genetic and acquired forms of the disease. More recently, we have utilized the mouse and rat to discover a novel, rare cardiogenic progenitor cell, islet-1 positive cardioblasts, in the post-natal heart. The identification, localization, characterization, renewal, and cardiogenic differentiation of this novel cardiac cell lineage will be discussed, as well as implications of these findings for translational studies of human heart failure. In summary, this talk will highlight a framework for utilizing mouse models to study the physiology and cell biology of heart failure.

PS-4-I

Keynote: From Myocarditis to Cardiomyopathy – Understanding the Gene-Environment Interaction that Leads to the Phenotype of Heart Failure

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Congestive heart failure is the number one rising epidemic in cardiovascular disease. Much of this arises from the aging population and the improved survival of patients with injured hearts. Understanding of the pathogenesis of cardiomyopathies and myocarditis involve the general concept of gene and environment interactions. From various genetic association studies, a number of single gene disorders leading to cardiomyopathies have been identified. Currently, mutations of the contractile proteins promote the development of hypertrophic myopathic phenotype. On the other hand, mutations in cytoskeletal proteins or calcium regulatory proteins tend to produce a dilated phenotype. Recent elegant work from Japanese colleagues suggest that the affinity between the cytoskeletal and the contractile elements determines dilated vs. hypertrophic cardiomyopathy.

The process that leads to the development of the heart failure phenotype generally involves structural remodeling of the heart. This can be simplified into remodeling at the myocyte level, which is exemplified by the hypertrophy and elongation of the myocytes, together with changes in the signaling process, cytoskeletal rearrangement and alterations of contractile function and calcium regulation. This is accompanied by alterations in matrix and matrix-myocyte interactions between the myocytes. The latter includes the changes in integrin isoforms, matrix composition and rates of collagen turnover. Ultimately this can lead to the process of fibrosis and mechanical and electrical isolation of the myocytes, a setting that predisposes to mechanical dysfunction and electrical dyssynchrony.

It is interesting to note that there is a very delicate interplay between the environment and the host in ultimately determining whether there will be progression towards heart failure. I will briefly given two examples of where an environmental disease outcome is determined by the host genetic repertoire, and conversely a genetic condition that can be completely ameliorated by environmental modulation.

For example, viral myocarditis leading to dilated cardiomyopathy is typically caused by an external infective agent – the virus, and that one would presume that the entire outcome should be determined by the viral virulence. However, now we understand that a significant component of the disease is also caused by the host inflammatory response to the viral infection. The elaboration of cytokines following activation of both innate and acquired immunity also contributes to the development of cardiomyopathy. More recently, we have identified the host stress signaling protein p56lck, which is a cell surface tyrosine kinase responsible for T-cell proliferation and host myocyte remodeling through intracellular growth pathways such the MAP kinase ERK1/2. This leads to growth and hypertrophy of the cell, as well as alterations of the contractile characteristic of the heart. Furthermore, the cytokines appear to modify the integrins present on the myocytes, thus alter its interaction with the matrix. Therefore, we see that the outcomes of heart failure in this externally triggered disease is critically dependent on the host response repertoire determining favourable or detrimental remodeling, and in turn host survival.

An opposite scenario in contrast is the development of cardiomyopathy in patients with congenital anemias or hemochromatosis, where excessive iron leads to the oxidative destruction of the myocardium, leading to early heart failure. We have very recently identified that the iron enters the myocardium through L-type calcium channels, and that this iron entry can be blocked by conventional L-type calcium channel blockers. We now have evidence that in genetic forms of anemia and cardiomyopathy, the administration of calcium channel blockers will completely prevent the disease. This is an example of how one could post-natally alter the environment to prevent a genetic disease realizing its natural history in developing heart failure.

For the common type of cardiomyopathy and heart failure, for example following myocardial infarction, the outcomes are predicted on both the genetic repertoire of the host and the environmental variables in the healing period. Therefore, knowing the host genetic repertoire, and the ability to tailor the environment to achieve maximum protection for the host, will be the challenge for the heart failure scientific arena. The promised ability to remove the offending environmental factors, or modifying the gene-gene interactions to obviate the phenotypic expression of a genetic defect will be a challenge for all of us. It is a through the understanding of these processes that we can make a difference for our patients in the future.

PS-4-II

The Mammalian Target of Rapamycin (mTOR): a New Molecular Target for Heart Failure

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Background Inflammatory responses play important roles in the pathogenesis of heart failure. Rapamycin, a lipophilic macrolide, inhibits cytokine production and has been used as an immunosuppressant in clinical practice. The mammalian target of rapamycin (mTOR) is an intracellular target of rapamycin. mTOR interacts with the insulin-phosphoinositide 3-kinase pathway that plays a critical role in heart size regulation (Shioi et al. EMBO J 19:2537;2000, Shioi et al. Mol Cell Biol 22:2799;2002, Crackower et al. Cell 110:737;2002). **Methods and Results** To examine the role of mTOR in cardiac hypertrophy and failure, rapamycin was administered to mice with ascending aortic constriction (Shioi et al. Circulation 167;1664;2003). Activity of ribosomal S6 kinase 1 (S6K1), an effector of mTOR, in the aortic constricted heart was increased by 5.6 fold. Pre-treatment of mice with 2 mg/kg/day of rapamycin completely suppressed S6K1 activation and S6 phosphorylation in response to pressure overload. The heart weight/tibial length ratio of vehicle treated aortic banded mice was increased by 34% compared with vehicle treated sham operated mice, and rapamycin suppressed the load induced increase in heart weight by 67%. Rapamycin did not cause body weight loss, lethality, or left ventricular dysfunction. To further examine the role of mTOR in heart failure, rapamycin was administered to rats immunized with cardiac myosin. Rapamycin significantly attenuated the development of heart failure in the rat model of autoimmune myocarditis. **Conclusions** mTOR or its target (s) appears to play an important role in cardiac hypertrophy and failure. Rapamycin may be an important therapeutic modality for heart failure.

PS-4-III

Adenovirus-mediated Gene Transfer of ICOSIg Fusion Protein Ameliorates Ongoing Experimental Autoimmune Myocarditis

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Experimental autoimmune myocarditis (EAM) has been used as a model for human myocarditis. We previously demonstrated that blockade of B7/CD28 or CD40/CD40 ligand (CD40L) had a potential preventive effect on EAM, but less therapeutic effect on ongoing EAM. Thus, we searched for the involvement of other costimulatory molecules in EAM. We demonstrated the expression of inducible costimulator (ICOS)/ICOSL molecules in the lymph nodes, spleen, and heart in the EAM rat. We constructed adenovirus vectors containing ICOSIg (Adex1CAICOSIg) to achieve effective inhibition of ICOS/ICOSL interaction, and examined the effects of Adex1CAICOSIg on EAM. Adex1CAICOSIg treatment shortly after the immunization did not inhibit the onset and severity of EAM as compared with control rats. On the other hand, delayed treatment with Adex1CAICOSIg strikingly inhibited ongoing EAM. The survival rate in rats treated with Adex1CAICOSIg was significantly higher than that of the control group. Furthermore, the affected area ratio of the Adex1CAICOSIg treatment group was significantly lower than that of the control group. This study indicates that ICOS/ICOSL costimulation makes an important contribution to the progression of EAM and that the blockade of this pathway by gene transfer has therapeutic potential for ongoing autoimmune myocarditis.

PS-4-IV

Autoantibodies Against β1-Adrenergic Receptors in Patients With Idiopathic Dilated Cardiomyopathy

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Autoimmunity is suggested as one of the causes of idiopathic cardiomyopathy. We tested the hypothesis that autoimmune mechanism via cardiac β-adrenergic receptors played a role in mediating the pathophysiology in such patients. Male Japanese white rabbits were immunized by the subcutaneous injection of a synthetic peptide corresponding to the second extracellular loop of the β1-adrenergic receptors for 6 months. Concentric left ventricular hypertrophy, as well as diastolic dysfunction as manifested by an increase in left ventricular end-diastolic pressure and a decrease in the first derivative of left ventricular pressure decay was noted. Myofiber disorganization and interstitial fibrosis along with cellular hypertrophy were noted microscopically. β-Adrenergic receptor uncoupling was observed along with increased expression of inhibitory guanine-nucleotide binding (G) protein and G-protein-coupled receptor kinase type 5. These alterations were reversed by adjunctive use of β-adrenergic receptor blocker, bisoprolol. Bisoprolol, an inverse agonist, completely abolished agonist-like effect of the autoantibodies in vitro. Such autoantibodies were found in 40 (38%) out of 104 patients with idiopathic dilated cardiomyopathy. The presence of the autoantibodies was associated with ventricular tachycardias on Holter monitoring. In addition, the presence of the autoantibodies was associated with higher prevalence of sudden cardiac death than those without antibodies. Cox proportional hazard analysis demonstrated that the presence of autoantibodies against β1-adrenergic receptors was an independent predictor of sudden cardiac death as well as poor ejection fraction. ***Conclusions:*** Concentric hypertrophy as well as β-adrenergic receptor uncoupling can be induced by autoimmune mechanisms via β1-adrenergic receptors in rabbits. Autoantibodies against β1-adrenergic receptors predicted sudden cardiac death relating to serious ventricular arrhythmias in patients with idiopathic dilated cardiomyopathy. Autoimmune mechanisms via β1-adrenergic receptors may play a role in mediating persistent myocardial damage and fatal ventricular arrhythmias in such patients.

PS-4-V

Roles of Costimulatory Molecules in the Development of Murine Acute Myocarditis Caused by Coxsackievirus B3

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It has been shown that NK cells and then antigen-specific T-cells infiltrate the heart and play a pivotal role in the myocardial damage involved in acute myocarditis. We have previously reported that costimulatory molecules belonging to immunoglobulin and integrin superfamilies such as ICAM-1/ LFA-1, VCAM-1/ VLA-4, and B7-1/CD28 as well as to TNF receptor/ligand superfamilies such as CD40/CD40L, 4-1BB/4-1BBL, and Fas/FasL play critical roles in the development of myocardial injury involved in murine viral myocarditis. In contrast to the most other costimulatory molecules which deliver positive signals to activated T-cells, CTLA-4, PD-1/PD-1 ligands (PD-L1/PD-L2), which are known as negative regulatory costimulatory molecules, may downregulate T-cell receptor (TCR) and/or CD28-mediated T-cell activation signals. Blockade of PD-1 mediated signaling pathway by in vivo anti-PD-1 mAb administration significantly increased cytokine production and cytotoxicity of infiltrating cells rather than induction of their apoptosis and aggravated the myocardial inflammation. These data raise the possibility of immunomodulating therapy by suppressing the positive costimulatory signals and enhancing negative costimulatory signals to prevent myocardial damage and improve the prognosis of patients with viral myocarditis. For the main signal for T-cell activation, we analyzed the TCR Vb clonality of heart - infiltrating cells as well as peripheral blood lymphocytes (PBLs) of patients with DCM by single strand conformation polymorphism (SSCP) technique. 39.6 % of clonotypes of heart-infiltrating cells were shared among all 3 distinct parts within the same heart, strongly suggesting that these clones (common heart clones) recognized a specific antigen common throughout the heart. In PBLs of the patients, 46.0 % of the expanded clones were identical with the common heart clones. Therefore, it may be possible to screen cardiac antigens and to identify targets for immunotherapeutic intervention by analyzing TCR clonality of PBLs.

PS-4-VI

Hepatitis C Virus and Inflammatory Mediators in Myocarditis and Cardiomyopathies

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Recent studies suggest that hepatitis C virus is involved in development of dilated cardiomyopathy, hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy in addition to myocarditis. Furthermore, left ventricular aneurysm represents the same morbid state not only after myocardial infarction but also after myocarditis.

Recently, we have shown that mast cells play an important role in the pathogenesis of viral myocarditis, and mast cell mediators may be important factors in initiation and development of heart failure. Therefore, antiallergic drugs that stabilize mast cells may be useful for the treatment of myocarditis.

In our murine model of heart failure due to viral myocarditis, phosphodiesterase III inhibitor, pimobendan improved survival, attenuated inflammatory lesions, and decreased production of intracardiac IL-1β, IL-6 and TNF-α and nitric oxide. In our study of pimobendan, but not the other phosphodiesterase III inhibitors, inhibited the activation of NF-kB. Thus, the inhibition of proinflammatory cytokines and NO production by pimobendan is mediated by its inhibitory effect on the activation of NF-kB. More recently, we found that a new NF-kB inhibitor suppressed cytokine production, and prevented development of viral myocarditis.

It has been shown that angiotensin II-induced activation of NF-kB results in increased expression of proinflammatory cytokines, nitric oxide, chemokines and cell adhesion molecules. Recently, we have shown that injection of angiotensin II in mice activated NF-kB, and induced the expression of proinflammatory cytokines in the heart. Moreover, inflammatory responses and NF-kB activation were attenuated in angiotensin II type I receptor (AT1) knock-out mice and in mice treated with an AT1 antagonist, in an animal model of heart failure induced by EMC virus. These results indicate that angiotensin II is a proinflammatory mediator acting through NF-kB/cytokine pathway.

PS-4-VII

The Pivotal Link Between Hepatitis C Virus Infection and Myocardial Damage in Patients With End Stage Renal Failure

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It was recently reported that cardiac troponin T (cTnT), a marker of myocardial injury, was a major predictor of mortality in end stage renal failure (ESRF) patients. The high prevalence of hepatitis C virus (HCV) infection in patients with ESRF has been well-known. **Purpose** Our purpose of this study is to evaluate the predictors of myocardial damage, including HCV infection. **Subject and Method** Ninety-one patients (mean age: 64 years, 59 male, mean duration of dialysis treatment: 3517 days) undergoing chronic dialysis were studied. Pre- and post-dialysis levels of serum cTnT were measured by Elecsys (Roche) and those of > 0.01 ng/ml was defined as abnormal value. We also measured serum calcium, phosphorus, intact parathyroid hormone, creatinine, total cholesterol, hematocrit, plasma brain natriuretic peptide by RIA and serum HCV-RNA by RT-PCR. We assessed their blood pressure, left ventricular diastolic diameter, mass index and fractional shortening by echocardiography. Determinants of elevated cTnT were assessed by logistic regression analysis. **Results** Elevated cTnT levels were detected in forty-nine patients (54%) before dialysis and in thirty-one patients (34%) after dialysis. Twenty-seven patients (30%) were seropositive for HCV, and seventeen patients (19%) had HCV viremia. Patients with elevated cTnT had higher SV1+RV5 in electrocardiogram (3.5 vs. 2.9 mV, $p < 0.05$), higher LVMI (129 vs. 112 g/m2, $p < 0.05$), higher plasma BNP levels (523 vs.308 pg/ml, $p < 0.05$), serum phosphorus (6.2 vs. 5.6 mg/dl, $p < 0.05$) and a higher frequency of cardiovascular diseases (CAD)(49 vs. 12%, $p < 0.001$). Prevalence of HCV viremia tended to be higher in patients with elevated cTnT than those without it (24 vs. 12%). Multiple logistic regression analysis revealed that LVMI (OR 1.05, 95% CI 1.005-1.103), P (OR 2.25, 95% CI 1.139-4.458), HCV viremia (OR 15.94, 95% CI 1.155-211.782) and presence of CAD (OR 15.92, 95% CI 1.442-175.725) were independent predictors of elevated cTnT. **Conclusion** These data show for the first time that myocardial damage is frequently observed in patients with ESRF and associated with left ventricular hypertrophy, ectopic calcification and overwhelmingly with HCV infection.

PS-5-I

Keynote: Molecular Mechanisms of Hypertrophic Cardiomyopathy

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The heart responds to diverse stimuli by morphological and functional adaptation. The cardiac adaptive repertoire is stimulus-specific. For example, endurance exercise yields eccentric hypertrophy, while pressure overload or certain mutant sarcomeric proteins can provoke concentric hypertrophy. We have begun to explore the pathways leading to these physiologic and pathologic adaptations and are probing the roles of gender and diet in modifying them. We recently found that substituting a casein-based diet for the traditional soy-based diet in a mouse model of hypertrophic cardiomyopathy (HCM) has profound and unexpected effects on pathogenesis. It therefore seems plausible that phytoestrogens in standard rodent chow dramatically affect disease and may also affect exercise adaptation. Genetic mouse models have been employed to address the following questions: which known signaling pathways play a role in hypertrophic cardiomyopathy; what role do those same pathways play in exercise adaptation; how do gender and diet modify these adaptations? Mouse models that can potentially answer such questions are: models of HCM with mutations in myosin heavy chain (MyHC) or cardiac troponin T (cTnT), and mice with alterations in cardiac signaling molecules. Genetic crosses among them have begun to yield intriguing answers to these questions.

PS-5-II

The Role of Mitogen-Activated Protein Kinase Cascade in the Transition to Heart Failure

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The mitogen-activated protein (MAP) kinase signaling pathways are implicated as an important contributor to the pathogenesis of cardiac hypertrophy and heart failure. The MAP kinase family consists of the ERK (extracellular signal-regulated protein kinase), JNK (c-Jun NH_2-terminal protein kinase) and p38 MAP kinase. In this study, we attempted to explore the in vivo physiological functions of p38α and Raf-1 in hearts. First, we generated p38α floxed allele mice and cross-bred them with mice expressing the Cre recombinase under the control of the α-myosin heavy chain promoter to obtain cardiac-specific knockout mice of p38α. These conditional mutant mice were born normally, developed to adulthood, were fertile and exhibited normal life span. These mice displayed normal global cardiac structure and function. Upon pressure overload to the left ventricle, they developed significant levels of cardiac hypertrophy as seen in controls. These mice, however, developed cardiac dysfunction and heart dilatation following pressure overload. This abnormal response to pressure overload was accompanied by cardiac massive fibrosis and the appearance of apoptotic cardiomyocytes. The cardiac-specific Raf-1 deficient mice demonstrated left ventricular systolic dysfunction and heart dilatation without cardiac hypertrophy or lethality. The cardiac-specific Raf-1 deficient mice showed a significant increase in the number of apoptotic cardiomyocytes. The expression level and activation of ERK showed no difference. These results demonstrate that both p38α and Raf-1 plays a critical role for cardiomyocyte survival pathway.

PS-5-III

Role of Early Inflammatory Changes in Cardiac Remodeling and Diastolic Dysfunction in Hypertensive Rat Heart

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Objectives; Inflammatory process is implicated in cardiac remodeling in various heart diseases. Recently, we reported that TGF-beta-mediated myocardial fibrosis is the major determinant of diastolic dysfunction in hypertensive (HT) rats with suprarenal aortic constriction. Thus, we studied the causal relation of inflammatory changes in myocardial remodeling and cardiac function. Method; HT was induced by constricting supra-renal abdominal aorta of Wistar rats. Results; HT transiently activated myocardial ACE after day 1, peaking at day 3 and returning to basal levels by day 14. Immunoreactive ICAM-1 was transiently expressed on the coronary endothelial cells at days 1-3. Also, MCP-1 mRNA was expressed after day 1 with a peak at day 3, decreasing to lower levels by day 7. Immunoreactive MCP-1 was found in the intramyocardial vessel wall. Macrophages accumulated in perivascular space after day 1, peaking at day 7. Thereafter, reactive myocardial fibrosis and myocyte hypertrophy developed. Non-suppressor dose of candesartan, an AT1 receptor blocker, eliminated early inflammatory changes (MCP-1 expression, macrophage accumulation, and TGF-beta induction) and subsequent myocardial fibrosis without affecting myocyte hypertrophy in the later phase. Furthermore, chronic blockade of MCP-1 or ICAM-1 function by a neutralizing antibody not only prevented macrophage accumulation but also ameliorated the TGF-beta-mediated myocardial fibrosis and diastolic dysfunction, in HT hearts, having no effects on blood pressure and myocyte hypertrophy. Conclusion; macrophage-related inflammation, partially induced by tissue angiotensin II, may play an initial role in myocardial fibrosis and subsequent diastolic dysfunction in HT hearts.

PS-5-IV

Selective Translocation and Cleavage of Dystrophin in Cardiomyocytes and Increased Sarcolemmal Fragility as a Final Common Pathway to Progress Heart Failure in Both the Hereditary and Acquired Origins

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To improve the prognosis of patients with heart failure, the progression mechanism leading to the advanced stage should be precisely clarified. Duchenne/Becker type muscular dystrophy is caused by the mutation in dystrophin (Dys) gene and most of the cases progress to heart failure and poor outcome. Dys makes a complex with the related proteins including δ-sarcoglycan (δ-SG). The deletion or mutation of δ-SG gene has been identified in both TO-2 strain hamsters (Sakamoto *et al., PNAS* 1997) and human families with dilated cardiomyopathy (DCM, Tsubata *et al., JCI* 2000), showing also the grave prognosis. Based on the results from two animal models, TO- 2 strain hamsters as the hereditary heart failure with or without gene therapy (Kawada *et al., PNAS* 2002) and normal rats after the cardiotoxic action with isoproterenol as the acquired origin (Xie *et al., J.Cardiovasc. Pharmacol.*, 2000), we report a novel paradigm that the cardioselective disruption of Dys, the translocation from sarcolemma to myoplasm and increased sarcolemmal fragility would commonly cause advanced heart failure, as is the case of contractility loss in muscular dystrophy of skeletal muscle, irrespective of the hereditary or acquired origins. The mechanism to the advanced stage will be discussed.

PS-5-V

Activation of the Laminin Gamma 1 Chain Gene Promoter Through the BCN-1 Element

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Laminin is a major component of the extracellular matrix. Laminin increases in the myocytes and vascular cells of the diabetic heart and dilted cardiomyopathy. The laminin gamma 1 chain gene contains a highly conserved transcriptional element denoted bcn-1 that was originally shown to be transcriptionally active (Suzuki H, Bomsztyk K. J Biol Chem 271:18981-9). Yeast one hybrid screen of mamalian cDNA libraries was used to identify transcriptional factors that bind to the bcn-1 motief. Using this strategy we identified that transcriptional factor E3 (TFE3) and related transcriptional factor-1 (RTEF) bind to bcn-1 element. The affinity of the in vitro interaction between TFE3 and bcn-1 motif was higher than that seen between RTFE-1 and bcn-1. Transient transfection assays showed that TFE-3 activated the bcn-1 element in either the context of the native laminin gamma 1 chain gene promoter or when the bcn-1 motief was placed upstream of a heterologous promoter. The stimulation of the bcn-1 element by RTEF-1 was not as strong as that seen with TEF3. These results suggest that TEF3 might be involved in the transcriptional regulation of laminin gamma 1 chain gene promoter.

PS-5-VI

Comparison of the Prevalence of Viral Infection in the Myocardium of U.S. and Japanese Patients With End-Stage Idiopathic Dilated Cardiomyopathy

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Objectives: Enteroviruses have been implicated in the pathogenesis of idiopathic dilated cardiomyopathy (IDC). Recently, the association of adenovirus or parvovirus with IDC has been reported. We evaluated the prevalence of infection with a variety of viruses in the myocardium of American and Japanese patients with end-stage IDC. The character and activity of detected viruses were also investigated. Methods: Myocardial specimens from 30 American IDC patients, obtained during heart transplantation, and from 34 Japanese IDC patients, obtained during partial left ventriculectomy, were analyzed for the presence of cardiotropic viruses. Strand-specific detection of enteroviral RNA was performed to determine viral activity in hearts with IDC. Established RT-PCR or PCR techniques were used to detect genomic sequences of influenza viruses, mumps virus, adenovirus, parvovirus, herpes simplex viruses, varicellazoster virus, and Epstein-Barr virus. Results: Enteroviral RNA was detected in 7 (23%) of 30 American patients and in 12 (35%) of 34 Japanese patients. Minus-strand enteroviral RNA, an indicator of active viral RNA replication, was detected in 5 (71%) of 7 plus-strand-positive American patients and in 9 (75%) of 12 plus-strand-positive Japanese patients. Sequence analysis revealed that the viruses detected were coxsackie B viruses such as coxsackievirus B3 and B4. No genomic sequences of other viruses were detected in the myocardium. Conclusions: Active group B coxsackievirus RNA replication in the myocardium was demonstrated in a significant proportion of both American and Japanese patients with end-stage IDC. There was no evidence of persistent infection by other viruses in IDC hearts. Specific therapy should be designed for coxsackievirus-positive patients with IDC.

PS-5-VII

The Role of Extracellular Matrix in the Development of Cardiac Remodeling

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Extracellular matrix (ECM) provides not only mechanical support but also important biological signaling during tissue remodeling. Among various ECM molecules, tenascin-C (TNC) is well known as a regulator of multiple cellular functions in tissue remodeling during embryogenesis, cancer invasion, wound healing and regeneration. In the heart, TNC is expressed during early embryonic development but not detected in the normal adult tissue. However, under pathological conditions such as myocardial infarction or myocarditis, various factors including proinflammatory cytokines, growth factors, hypoxia, acidosis or mechanical stress induce re-expression of TNC. One of the features of TNC is its transient and site specific expression in myocardium closely associated with injury and active inflammation, which suggests important roles during myocardial repair. Our in vitro data and animal experiments using TNC knockout mice have provided several evidences that TNC may regulate cell behavior during cardiac remodeling by modulation of attachment of cardiomyocytes to connective tissue, induction of matrix metalloproteinases, and enhancement migration and differentiation of myofibroblasts that are main players in tissue repair. On the other hand, the restricted expression also suggests that TNC can be a marker for myocardial disease activity. We evaluated the diagnostic value of TNC expression in biopsy specimens from patients with myocarditis, and confirmed that immunoreactivity reflects clinical disease activity. We also succeeded imaging the inflammatory lesion of in rat models using anti-TNC antibody labeled with 111Indium. Furthermore, we measured serum TNC of patients with acute myocardial infarction and found serum TNC level could be co-related with progression of left ventricular remodeling. These data suggest that TNC can be a key molecule to explore a new noninvasive and accurate approach for the diagnosis of myocardial disease activity and cardiac remodeling, and might be a potential therapeutic target.

PS-6-I

Keynote: Advances in the Genetics of Hypertrophic Cardiomyopathy: Implications for Understanding Pathogenesis and Impact on Clinical Management

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The clinical diagnosis of hypertrophic cardiomyopathy is based on the demonstration of unexplained left ventricular hypertrophy. Approximately 50% of patients fulfilling diagnostic criteria have disease caused by mutations in sarcomeric contractile protein genes. Mutations in beta myosin heavy chain, myosin-binding protein C, and cardiac troponin T and I are the most common abnormalities detected. Preliminary genotype/phenotype information reveals marked heterogeneity of clinical expression, even within families with the same mutation, indicating an important role for other genetic or environmental factors. To date the clinical impact of the recent advances in understanding of the molecular genetics of HCM has been to provide a molecular gold standard for disease risk, and to provide a framework for prognostic evaluation, particularly in relation to sudden death. Familial evaluation, which has been performed for the genetic studies, reveals a much greater breadth of disease expression than had previously been recognised. Individuals from families with HCM who have electrocardiographic abnormalities in the absence of echocardiographic LVH are common. The presence of mild disease, however, does not preclude serious disease-related complications. It is clear that patients with mutations in cardiac troponin T may die suddenly in the absence of significant morphological abnormalities on echo. It is unclear whether mutations in other genes can also result in sudden death with mild or absent disease expression on echo, but if this occurs it must be rare. The clinical indications and feasibility of DNA diagnostic testing is under evaluation. Preliminary observations suggest a role for mutation analysis in the management of patients suspected of having troponin T disease i.e. when there has been a cardiac arrest or sudden death in a first-degree relative, with mild LVH or near-normal heart weight at autopsy. For the majority of the disease-causing genes, disease expression takes place during childhood, adolescence or in the early adult years. Myosin-binding protein C, however, may cause disease in the later decades. Mutation analysis may also be helpful in patients suspected of having a mutation in myosin-binding protein C, in such patients the ECG and echo may remain normal until the later decades. At present, mutation analysis is not routinely performed outside of research laboratories. Preliminary observations suggest a role for DNA diagnostic testing in HCM but the full potential will not be fully realised until DNA diagnosis is available to the clinician on a routine basis.

PS-6-II

Keynote: Genetics of Dilated Cardiomyopathy

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Dilated cardiomyopathy (DCM) is the most common form of cardiomyopathy, causing approximately 60% of the total cardiomyopathic phenotypes. Approximately 35-40% of cases of DCM are genetically inherited, with autosomal dominant inheritance most typical. However, X-linked, autosomal recessive and mitochondrial inheritance also occurs. We have pursued a hypothetical pathway approach to gene identification and have utilized this approach in an attempt to decipher the mechanisms underlying the disease. In this "final common pathway" hypothesis, we have suggested that the links between sarcolemma and sarcomere via the cytoskeleton are critical to the normal function of the heart and, when disrupted, leads to the development of the phenotype. We have identified multiple cytoskeletal protein-encoding genes causing DCM (dystrophin, δ-sarcoglycan, α-dystrobrevin) using this approach. Recently, we have focused our attention on the actin cytoskeleton and Z disk, identifying mutations in genes encoding MLP, α-actinin2, ZASP and others. The purpose of this talk will be to discuss these causative genes for DCM.

PS-6-III

Novel Molecular Etiology of Hypertrophic Cardiomyopathy

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Idiopathic cardiomyopathy (ICM) is by definition of unknown etiology, but recent molecular genetic approaches have revealed that mutations in genes encoding components of sarcomere or Z-disc elements cause ICM. There are two clinically different ICM; hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), which were thought to be etiologically different. However, we and others reported different mutations of the same component such as titin (TTN) in HCM or DCM. To reveal the mutations in telethonin (TCAP) gene and functional differences between the HCM-related mutations and DCM-related mutations, we have searched for mutations in 260 HCM patients and 130 DCM patients. In addition to a TCAP mutation reported in DCM, another TCAP mutation and two other TCAP mutations were found in DCM and HCM, respectively. We then tested the functional alterations due to the mutations in binding affinity to titin, MLP and another component of Z-disc by using a yeast-two-hybrid assay and a competition pull-down assay. The analyses revealed that the DCM-related mutations decreased the affinity to these Z-disc components, whereas the HCM-related mutations increased the affinity except for MLP. These results demonstrated that the HCM-related TCAP mutations and DCM-related TCAP mutations caused opposite functional changes in the binding affinity among the Z-disc elements as was observed for TTN mutations. These observations clearly indicated that the TCAP is a novel disease gene for HCM and have suggested that HCM is the disease of stiff sarcomere (increasing stretch response), while DCM is the disease of loose sarcomere (deteriorating stretch response).

PS-6-IV

Novel Strategies to Find the New Pathophysiology or Treatment of Chronic Heart Failure: The Molecular Analysis Using DNA Array and SNP or the Data Mining Method Using Medical Records

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There are 2 different methods to find the ways to conquer the cardiovascular diseases. One is to apply the genomic analyses and another is to use the large clinical database. First of all, for chronic heart failure (CHF), we planed the strategy to identify responsible genes expressed in human, canine and murine failing hearts. In human failing hearts, DNA array revealed that about 200 genes including BNP or ANP are up-regulated and about 400 genes including cytokines are down-regulated among 10,000 genes. We linked the DNA array database to the clinical parameters of CHF such as the plasma BNP levels and ejection fraction. Furthermore, we compared DNA microarray data of failing myocardium of these 3 species, where we found several common up-regulated genes including HB-EGF or down-regulated genes including adenosine A2 receptors. Either inhibition of HB-EGF or stimulation of adenosine receptors attenuated both hypertrophic signals and cardiac hypertrophy in murine aorta banding models. Furthermore, we performed SNP analysis in patients with CHF with dilated cardiomyopathy. Among them, we found the SNP differences of adenosine A2 receptors in the control subjects and CHF patients. We are now seeking SNP differences of HB-EGF. DNA microarray or SNP analysis can identify the candidates for the pathophysiology or the novel drugs. Secondly, we evaluated the rationale of the current treatments of CHF using data mining methods (DMM) in 1100 patients with CHF. DMM revealed that beta-blockers, ACEI or ARB independently improved both BNP and EF, and inotropic agents for oral intake worsened both BNP and EF. Interestingly, dipyridamole attenuated the severity of CHF, enforcing us to pursue the possibility of adenosine therapy for CHF. In conclusion, we postulate the two different methods to find the novel strategies to find the pathophysiology or drugs in the cardiovascular diseases in the clinical or molecular database.

PS-7-I

Stabilization of Ryanodine Receptor as a New Therapeutic Strategy Against Heart Failure

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In heart failure, abnormal function of sarcoplasmic reticulum (SR) is one of the major pathogenic mechanisms in heart failure. Here, we assessed the effects of 1,4-benzothiazepine derivative JTV519 (intracellular Ca2+ modulator) and propranolol on cardiac and SR functions.

Methods and Results. SR vesicles were isolated from dog LV muscles {normal (N), n=11; 4-weeks rapid RV pacing with or without JTV519 (JT:14.4 mg/Kg/day) or propranolol (PL:0.05mg/kg/day) [untreated: n=10, JT(+): n=10, PL(+): n=10, respectively]}. 1) In either JT(+) or PL(+), cardiac function was improved [peak +dP/dt of LV pressure (+26%, +12%), time constant of LV pressure decay (-40%, -33%), LV end-diastolic (-11%, -11%) and end-systolic (-16%, -17%) diameters] compared with untreated group. 2) Abnormal SR Ca2+ leak was found in untreated failing SR. A benzothiazepine Ca2+ antagonist diltiazem as well as JTV519 acutely inhibited this Ca2+ leak in failing SR (IC50= 0.1 μM, 0.03 μM, respectively), and neither nifedipine nor verapamil affected the Ca2+ leak. There was no abnormal SR Ca2+ leak either in JT(+) and PL(+). 3) Both JTV519 and propranolol prevented the decrease in the stoichiometry of RyR vs FKBP12.6 assessed by [3H]ryanodine and [3H]FK 506-bindng assays [1:3.6 in normal, 1:1.3 in untreated, 1:3.6 in JT(+), 1:2.4 in PL(+)]. 4) In untreated group, RyR was PKA- hyperphosphorylated, whereas it was reversed both in JT(+) and in PL(+). 5) The amount of RyR-bound FKBP12.6 was tremendously less in untreated group than normal RyR, whereas it was reversed both in JT(+) and PL(+).

Conclusions. Both JTV519 and proplanolol improved cardiac function and attenuated LV remodeling partly by ameliorating the defective interaction of FKBP12.6 with RyR through restoration of PKA-hyperphosphorylation of RyR.

PS-7-II

NF-kB as a New Therapeutic Target for Cardiac Remodeling

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Background Myocardial expression of proinflammatory cytokines may play an important role in the pathogenesis of cardiac dysfunction and remodeling. Since NF-kB is a key transcription factor that regulates inflammatory processes, in the present study, we assessed the hypothesis that cardiotoxic effects of proinflammatory cytokines are mediated by the activation of NF-kB.

Methods and Results Transgenic mice with cardiac-specific overexpression of TNF-α were used as a model of dilated cardiomyopathy. To block the activation of NF-kB, TNF-α transgenic mice were crossed with knockout mice in which the p50 subunit of NF-kB was disrupted. The electrophoretic mobility shift assay demonstrated that NF-kB was activated in the myocardium of TNF-α transgenic mice, while it was completely abolished by the targeted disruption of p50. However, infiltration of inflammatory cells or expression of proinflammatory cytokines in the myocardium of TNF-α transgenic mice was not ameliorated by the disruption of p50. At the age of six weeks, the left ventricle was significantly dilated and the fractional shortening was significantly reduced in male TNF-α transgenic mice, where the disruption of p50 significantly ameliorated ventricular dilatation and improved the fractional shortening. By the end of 12 weeks, 6 of 27 male TNF/ ++ mice and 12 of 69 male TNF/ +- mice died of congestive heart failure. However, none of 29 male TNF/ -- mice died by that time, indicating that the disruption of p50 significantly improved the survival of male TNF-α transgenic mice.

Conclusions - Blockade of NF-kB activation did not ameliorate myocardial inflammation but improved cardiac function and survival in TNF-α transgenic mice. Activation of NF-kB may play an important role in the pathogenesis of myocardial dysfunction and remodeling besides promoting myocardial inflammation.

PS-7-III

A New Challenge of Gene and Enzyme Replacement Therapy for Cardiac Fabry Disease

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Cardiac Fabry disease, an atypical form of Fabry disease, is due to a deficiency of the lysosomal hydrolase α-galactosidase A (α-gal A), and accumulation of ceramide trihexoside (CTH) is observed only in heart. In order to establish gene therapy for Fabry disease including cardiac Fabry disease, we performed an experimental study using α-gal A-deficient mice and retroviral-mediated gene transfer. Bone marrow mononuclear cells were harvested from α-gal A-deficient mice, transduced with the retroviral vector pUMFG/ α-gal A/FLAG and transplanted into lethally-irradiated recipient α-gal A-deficient mice. We analyzed α-gal A activities and CTH levels in organs including heart, liver, spleen, lung, and kidney at 12 or 26 weeks after transplantation. In all mice, α-gal A activities increased in all organs, and decreased levels of CTH were confirmed in heart, liver, spleen, and lung. Thus we have shown long-term systemic enzyme increases and CTH reduction using transduced and transplanted bone marrow cells, providing a possibility of gene therapy for Fabry disease, including cardiac Fabry disease.
We have also evaluated the effectiveness of enzyme replacement therapy using recombinant human α-gal A in a male patient with Fabry disease who showed marked left ventricular hypertrophy. He has received recombinant human α-gal A at a dose of 1 mg per kilogram of body weight every other week for over 2 years. His left ventricular mass was decreased from 752 g to 510 g after the treatment. We speculate that this data might be suggestive of efficacy of enzyme replacement therapy.

PS-7-IV

Reduction in Exercise Capacity, Myocardial Energy Metabolism, and Survival in Rats With CHF Are Greatly Improved by Endothelin-A Receptor Antagonist

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It was reported that endothelin-A receptor antagonist improves survival of CHF, however, it is unclear whether this therapy improves the decreased exercise capacity. We studied the chronic effects of the endothelin-A receptor antagonist YM598 on exercise capacity, impaired myocardial energy metabolism, and poor survival rate of rats with CHF. Rats received coronary ligation or sham-operation. YM598 (1 mg/kg/day) or vehicle (distilled water) was orally administered for 6 months; the survival rate of CHF rats+vehicle was 52.4%, whereas that of CHF rats+YM598 was 92.9% (P=0.013). In survived rats, exercise capacity was assessed by treadmill running (25 m/min). The running time of CHF+vehicle was significantly shorter than that of the sham-rats (25.8 ± 3.7 min vs 52.5 ± 4.4 min, n=12, $p < 0.01$), and that of CHF+YM598 was significantly longer (37.8 ± 3.6 min, n=12, $p < 0.05$) than CHF+vehicle. In compared with the heart of sham-rats, CHF+vehicle rats fell in impairment of myocardial energy metabolism; decrease in mRNA expression and activity of 3-hydroxyacyl CoA dehydrogenase (enzyme for fatty acid β-oxidization) and isocitrate dehydrogenase (enzyme for TCA cycle). Chronic YM598 treatment normalized these changes. These findings suggest that the improvement of the impaired myocardial energy metabolism by endothelin-A receptor antagonist is one of major factors for improvement of the exercise capacity and poor survival rate in rats with CHF.

PS-7-V

Contribution of Mitochondrial Genome Polymorphisms to Idiopathic Cardiomyopathy

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Polymorphisms in the human mitochondrial genome have been used for elucidation of the phylogenetic relationships among various ethnic groups. Because analysis by mitochondrial genetics has detected pathogenic mutations causing mitochondrial encephalomyopathy or cardiomyopathy, most of the mitochondrial single nucleotide polymorphisms (mtSNPs) found in control subjects have been regarded as merely normal variants. We cannot exclude, however, the possibility that the mitochondrial functional differences among individuals are ascribable at least in part to the mtSNPs of each individual. Human life span in ancient history was much shorter than that at the present time. Therefore it is reasonable to speculate that certain mtSNPs that predispose one toward susceptibility to adult- or elderly-onset diseases, such as Parkinson's disease and Alzheimer's disease, have never been a target for natural selection in the past. Similarly, thrifty mtSNPs that had been advantageous for survival under severe famine or cold climate conditions might turn out to be related to satiation-related diseases, such as diabetes mellitus and obesity. To examine these hypotheses, we have constructed the mtSNP database (http://www.giib.or.jp/mtsnp/index_e.html) by sequencing the entire mitochondrial genomes from 672 subjects: 96 each of 7 groups, i.e., centenarians, young obese or non-obese subjects, diabetic patients with or without major vascular involvements, patients with Parkinson's disease, and those with Alzheimer's disease. We have identified mtSNPs associated with age-related conditions such as longevity, Parkinson's disease, and Alzheimer's disease, as well as those related to energy metabolism such as obesity, thinness, and type-2 diabetes, or to atherosclerosis. Given that each mitochondrial haplogroup has different functional characteristics, further studies are necessary to confirm the hypothesis that clinical courses of idiopathic cardiomyopathies are influenced by mtSNPs.