Department of Organ Pathophysiology and Internal Medicine Health Service Center

Outline and Research Objectives

In 1972, Health Service Center was founded under the auspices of the Ministry of Education and Culture to keep sound physical and mental environment in the University of Tokyo. The director of the Center has been elected from a member of Professors in Faculty of Medicine and dealt with two main services, 1) Primary care for undergraduate, graduate and postgraduate students as well as Faculty Staff and 2) Health Control strategy. At the opening of the Center, the main task was restricted to the Student with infectious disease including tuberculosis and malnutrition and medical check of all students in several aspects. The Center has three branch offices, in Hongo, Komaba and Kashiwa Campuses from 2001.

Recently, the number of students in the University is enormously increasing and reached about 25,000. The University also contains Faculty Staffs about 5,000. Target diseases include a wide variety of sickness, ranging from acute but minor problems like common cold, acute gastroenteritis, several accidents during experiment, injuries in sports, insomnia or neurosis to grave or life-threatening sickness such as malignant neoplasms, AIDS, Hepatitis C, imported infections from foreign countries, DMI and suicide. Graduate and Postgraduate Students are actually main human resources to produce the Research output in Laboratory. Their burdened life in both hard works to do until midnight and competitive stress to reach a top-level scientist would cause several serious problems.

Official duties of the Center contain 1) Routine medical care of several problems, 2) Health Check after entrance exam, 3) Regular Health check of all Faculty Members, 4) Regular Health Check for Students from Abroad, 5) Medical Education Programming for chronic diseases, 6) Regular Health Check for Students and Faculty Members treating hazardous materials or radioisotopes, 7) Issue of Health Check Forms, 8) Health Consultation in general, 9) Health Check for the candidates of new staffs and so on.

Faculties and Students

Professor and Chair	Teruhiko TOYO-OKA, M.D., Ph.D. (1991-)
Associate Professors	Yoshio UEHRA, M.D., Ph.D. (1999-)
	Tsukasa SASAKI, M.D., Ph.D. (2000-)
Lecturers	Shu UWATOKO, M.D., Ph.D. (1997-)
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	Katsuyuki ANDO, M.D., Ph.D. (1997-)
	Miyuki SADAMATSU, M.D., Ph.D. (2000-)
	Hisako HIKIJI, M.D., Ph.D. (2001-)

Associates 8

Past Research and Major Accomplishments

From 1976, the author has addressed to the pathophysiology of the most serious and popular problems in medicine in Japan, heart failure. Precise mechanism of the development is still unknown on advanced heart failure from pressure overload secondary to hypertension, several kinds of valvular stenosis, volume overload due to congenital heart anomaly or valvular insufficiency or from unidentified etiology including idiopathic cardiomyopathy. Based on an exact diagnosis, the treatment should be performed, considering the clinical setting of the patients.

After finishing Ph.D. course in the laboratory of Prof. Setsuro EBASI, who is one of the most outstanding scientists in muscle biology, Dr. Tomoh MASAKI and I have purified calcium- activated neutral protease (CANP or calpain) from cardiac muscle for the first time and characterized both the biochemical and pathological features. Studies on calpain continued when I moved to the Department of Cardiology in San Diego or Physiologisches Institut in Heidelberg where calpain was found to be involved in the loss of myofibrillar proteins in myocardial infarction or platelet aggregation.

Using *in vivo* ^{1}H and ^{31}P-NMR, the author has indicated structure abnormalities of sarcolemma as the cause for dilated cardiomyopathy (DCM) that is still the most grave and incurable heart failure. The hereditary form of DCM due to gene abnormalities of cardiac actin, dystrophin, several sarcoglycans (SG) and desmin has been reported in human cases. We have discovered gene deletion of δ-SG in human and hamster with hereditary DCM and identified its breakpoint (Sakamoto *et al., Proc.Natl.Acad.Sci.USA* 1997; Tsubata *et al., J.Clin.Invest.* 2000). Comparing with normal control, BIO 14.6 strains that develop DCM following a hypertrophy phase, some, but not all, SGs except δ-SG remained. On the other hand, TO-2 strains, which develop DCM from an early stage, did not express any SG at all (Kawada *et. al., FEBS Lett.* 1999). These results indicate the scheme that the integrity of cell membrane is normally preserved when all SG are present, but as demonstrated with BIO 14.6 hamsters, develops compensatory hypertrophy when SG is partially present, and finally reaches advanced heart failure, when all SGs were lost. Results from northern blot analysis confirmed abundant mRNA expression of α–, β–, and γ-SGs, and their promoter structures were preserved. Accordingly, we concluded that the actual cause of DCM is the gene mutation of δ-SG and loss of other SGs were originated from post-translational level.

To modulate translation of specific mRNA to protein, we demonstrated that transgene expression is most effectively inhibited with CMV promoter-activated antisense DNA plasmid, rather than general antisense DNA (Chen *et al., J.Biol.Chem.* 2000; Wang *et al., Circ.Res.,* 2001). In addition, with approval of ethical committee, we conducted genetic diagnosis of mitochondrial cardiomyopathy and identified a novel DCM-linked D-loop (Shin *et al., Am.J.Hum.Genet.,* 2000).

Since the discovery of dystrophin, heart failure due to dystrophin-related proteins (DRP) abnormalities have been regarded as accompanying symptoms of congenital muscular dystrophy. Although there was a report on proteolysis of dystrophin following viral myocarditis, we have reported most cases of acquired DRP disruption observed in chronic heart failure models were caused by the direct degradation of DRP complex including catecholamine toxicity and heart failure of the old myocardial infarction.

To develop gene therapy of advanced heart failure, we employed several vectors. Using proteoliposome, we have transfected eNOS gene to cardiac muscles *in vivo,* and succeeded initiating apoptosis (Kawaguchi *et al., Circulation* 1997). We have also indicated the necessity to employ other vectors for the efficient, long-term, and biologically inert transfection (Kawada *et al., BBRC,* 1999). Our attempt to transduce the causative gene when it is identified has shown that responsible gene does improve pathophysiology and rescue the hereditary DCM (Kawada *et al., PNAS* 2002).

Current Research

Although the prognosis of advanced heart failure typified by DCM is now gradually improving through various pharmaceutical agents, the final choice is heart transplantation. However, there are still many ethical, social and medical obstacles such as keeping the number of donor hearts, and continuing immunosuppressant therapy after the transplantation during whole life. The development of gene therapy is anticipated as an ideal method to overcome these problems. As a solution for these problems, we proposed the following 3-year-plan to develop new strategy for the prevention and treatment of advanced heart failure (2002 to 2004).

- **Research and Survey Projects in the Ministry of Welfare to investigate the patient population of the mutated gene previously cloned** (2002).
- **Development of new potential gene-delivery system.** Using safe and long-lasting adeno-associated virus (rAAV) as a vector, we previously transferred responsible gene with normal sequence, *via* thoracotomy to above animal model, which improved the prognosis. To examine if this method is clinically applicable to larger subhuman animals, as a step towards clinical use of the basic research, we will conduct preclinical studies on monkeys (2002-2004). Specifically, after initiating large-scale production of rAAV (2002), and simultaneously develop administration methods with high transfection efficiency. (2003)
- **Search for more realistic gene-delivery route.** Clinical situation of the patients with advanced heart failure, however, requires less aggressive route not under open chest surgery. Another administration route via transcoronary route might be preferable to attain the efficient and safe delivery (2004).
- **Strict evaluation of safety in regards to general pathology, immunology, and germ-line cell infection.** Trail of the rAAV administration to skeletal muscle cells is similar but more easily transfectable than myocardial cells (2002-2003).
- **Creation of advanced heart failure presenting human-type DCM in larger animals.** Small animal model does not always represent human diseases. Rhesus monkeys will be employed, because the Ministry of Welfare in Japan has accepted this species in a preclinical study (2003). Animal models of human-like DCM will be highly innovative for the development of various disease treatments.

- **Cell transplantation combined with the gene therapy**. Patients seek treatment only when symptoms become severe. Gene therapy was effective to prevent the disease progression but could not regenerate the normal cells. Transduction of normal sequence transgene to cultured muscle cells and create normalized cells *in vitro* might be promising for the patients after the onset of the symptoms. Fortunately, skeletal myoblasts can proliferate and be transplantable to cardiac tissue and show new development of cardiac muscle cells. Transplantation of genetically normalized myoblasts will be promising for the next generation gene therapy.

Future Prospects

Present project consists of two sections, as follows.

- **Double vector strategy for the development of knockout heart and novel therapy with monkeys.** Previous report that knockout of δ-SG gene in mice caused not only DCM but also coronary spasm (Coral-Vasquez *et al., Cell* 1999). The contribution of myocardial ischemia is of great significance for the pathogenesis of DCM, although they did not show clear evidence to support the scheme. Because of the limitation of handling in small heart, no group has succeeded in the verification of the microvessel spasm. To clarify the contribution of coronary spasm, we are trying to prepare not transgenic mice but knockout heart in large animals like Rhesus, to make it possible to visualize coronary spasm by coronary angiogrrraphy and anaerobic metabolism as follows. AAV consists of 5 subtypes, Serotype I to V. Preliminary experiment showed that type V was the most efficiently transfected both skeletal and cardiac muscle cells *in vivo,* followed by type III and II. We have been verifying *in vivo* since last year and succeed in modifying the rAAVs for gene vector. The rAAV type II as gene therapy vector, known to be safe and to possess a long expression period. In addition to type II, we have already prepared type V and are now making sure the safety. (2002).

Since the serotype of these rAAV vectors completely differ, mutual interference does not take place. This difference of serotypes has crucial meaning to our study. By applying different rAAV vectors at the initial and secondary administration, different antigenicity does not prevent double transfection. In our present study, we will initially administer rAAV type V that is the most efficient with δ-SG antisense, extensively to monkey cardiac muscle *in vivo*, and create a cardiospecific δ-SG deficient heart. Following validation of transfection *via* myocardial biopsy using specific antibody to δ-SG, the pathogenesis will be precisely examined whether they develop human-like DCM (2003).

Subsequently, expression of δ-SG containing normal or abnormal sequence can be examined, using rAAV type II. With the former gene, the rescue will be expected, as demonstrated previously with hamsters. With the latter animals, we will confirm the presence of transgene, and examine the persistence of clinical symptoms of dilated cardiomyopathy by physiological examinations by hemodynamics, echocardiography and assay of BNP (2004).

- **Combination therapy of gene and cell therapy at the advanced heart failure.**

Cell therapy will be promising for the treatment of advanced heart failure after progression to the degenerative process of cardiomyocytes. Simple cell transplantation tried in humans is not applicable to the case with gene-deletion or mutation, because intrinsic gene mutation universally occurs in all tissues including cardiac muscle and skeletal muscle. After skeletal myoblasts in culture were proliferated and transfected by normal gene, the treated and normalized muscle cells can be transplanted to DCM heart. For ethical considerations, genetic diagnosis a priori is essential for identifying responsible gene or at least, actually aggravating factor and for developing gene therapy. In accordance with recommendations by Helsinki Declaration, Science Council, Ministry of Education, Ministry of Health and Welfare and related 8 Academic Societies, we requested for a strict inquisition by ethical committee. As stated above, we have already demonstrated serious consideration to privacy protection in our previous study on nation-wide genetic diagnosis survey for the prevention of sudden death and obtained very meaningful results (Shin *et al., Am.J.Hum.Genet.* 2000).

Research Grants from 1998 to 2002.

1998-2000 The Ministry of Education, Culture, Science and Sports; Section A (1), "Novel gene therapy of dilated cardiomyopathy secondary to disruption of dystrophin-related proteins".

1998-2000 The Ministry of Education, Culture, Science and Sports; Section A (2), "Prevention trial of sudden death with using gene-based presymptomatic diagnosis".

2001 Uehara Memorial Foundation, "Development of gene-based therapy for dilated cardiomyopathy".

2002-4 The Ministry of Education, Science, Culture and Sports; Section A (1), "Gene or cell-engineered therapy of advanced heart failure".

2002-4 The Ministry of Health, Labor and Welfare; Translational Research Project from Basic Medicine to

Clinical Therapy, "Clinical application of gene therapy with cell engineering for the treatment of advanced heart failure".

Select Publications

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12. Toyo-oka T, Arisaka H, Sanma H, Shin WS, Dan Y. and Sugimoto T. Synergistic deleterious effect of micromolar Ca ions and free radicals on respiratory function of heart mitochondria at cytochrome C and its salvage trial. *Biochem.Biophys.Res.Commun.* 163, 1397-1403, 1989.
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