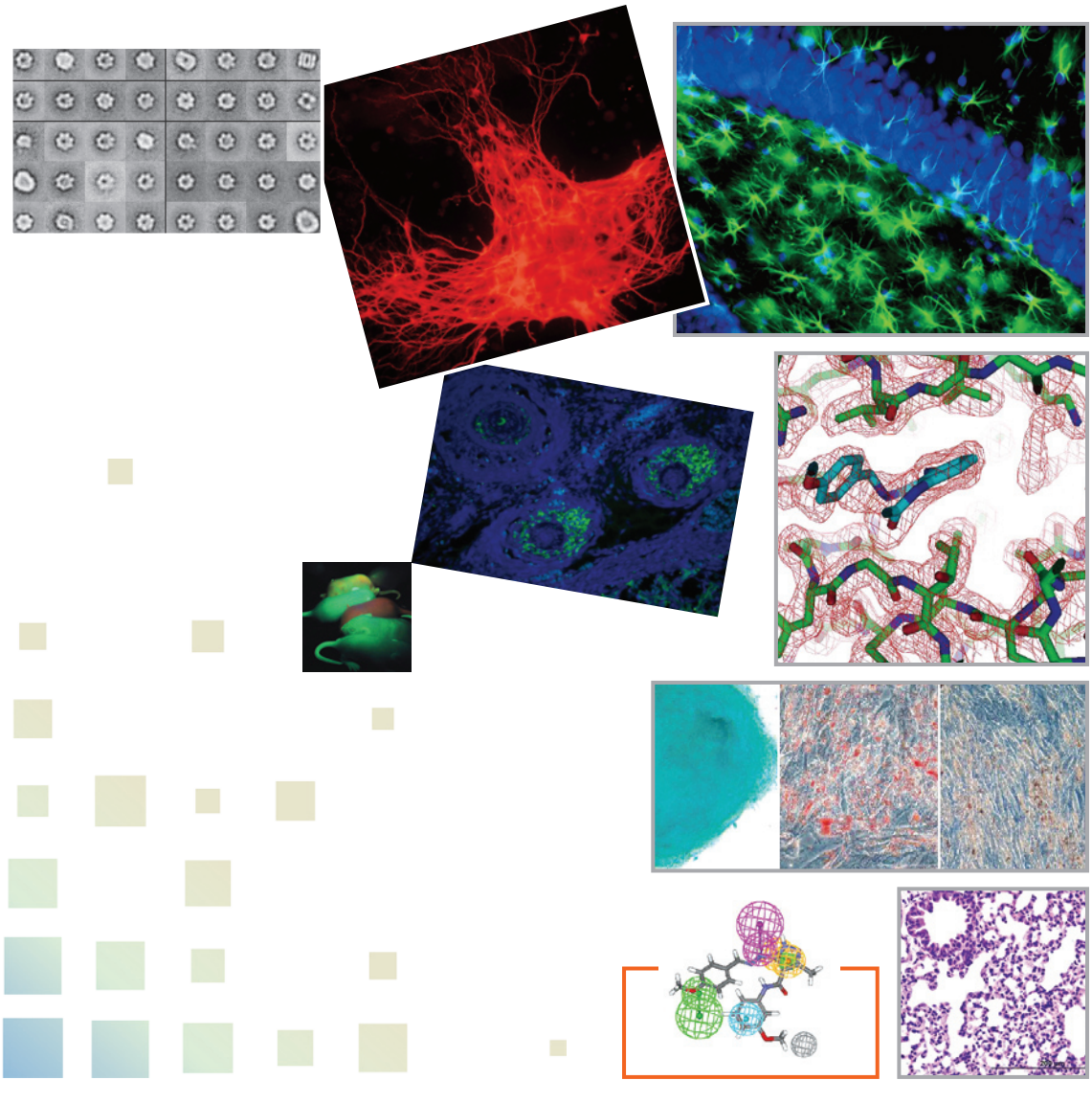
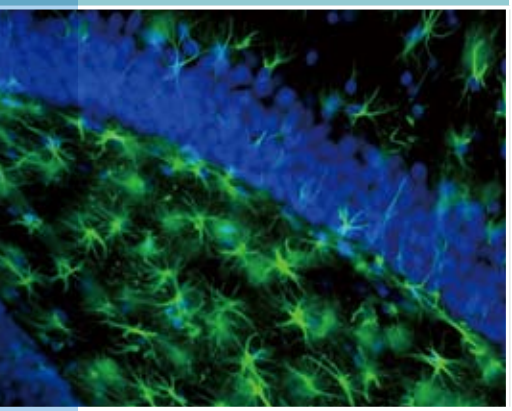
National Health Research Institutes

Annual Report 2008





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Photograph by Dr. Sabrina Wang

Foreword

Anniversaries are a time to reflect on past achievements and ponder future goals.

On the occasion of the National Health Research Institutes' tenth anniversary in 2006 we recognized NHRI's potential to guide the next ten years of biomedical research in Taiwan. Now, as NHRI approaches its fourteenth year and continues to fulfill its role as a leader in that field, we present the NHRI Annual Report 2008. This English-language edition of our annual report focuses mainly on accounts of intramural research programs over the past year. More importantly, however, it provides an invaluable record, allowing us better to dream how far we can advance by reminding us how far we've come.

Since its establishment in 1996, NHRI has done more than keep abreast with the many new materials and techniques used in biomedical and health research; it has even helped advance these through NHRI's state-of-the-art intramural research programs, conducted on the NHRI campus. In addition, NHRI provides extramural research funding based on high-standard peer review. NHRI also builds core facilities and makes research resources accessible to biomedical researchers nationwide. And NHRI's collaboration with other institutions and medical centers is helping create a research network that is enhancing health standards around the country.

This annual report outlines NHRI's recent research highlights and achievements, which have value not just in the advancement of human knowledge and health care but also in their potential applications in the biotechnology industry. Some of these research findings have already become well known and guided policy makers in formulating national health strategies. Moreover, certain drug discovery and vaccine developments are important for national preparedness for emerging infectious diseases and may save human life in times of pandemic.

I hope that the NHRI Annual Report 2008 will reflect well the dedication of NHRI's scientists and staff to contribute to the scientific community and to improve health for people not just in Taiwan but all over the world.

Kenneth K. Wu, M.D., Ph.D.
President of NHRI

National Institute of Cancer Research

Mission

The National Institute of Cancer Research is NHRI's first institute. Its task is to prevent, diagnose, and treat locally prevalent cancers by integrating and coordinating measures to improve the health of citizenry through basic scientific and clinical research. These missions are consolidated through studies at laboratories, clinical wards, outpatient clinics, and clinical research laboratories, as well as at the Taiwan Cooperative Oncology Group (TCOG) and the Subspecialty Training Program for medical staff.

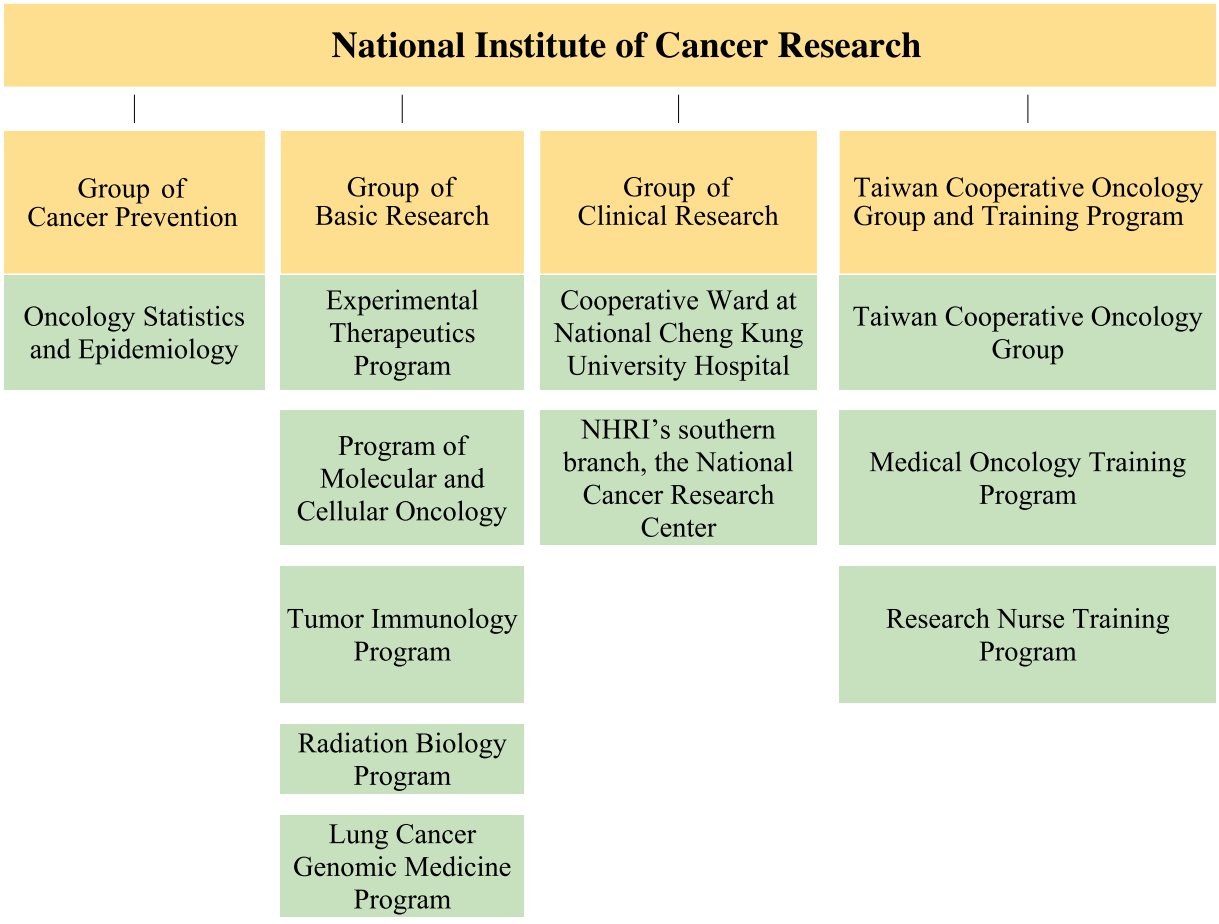
The institute focuses on translational medicine. Clinicians and basic scientists work together to develop novel technology to improve cancer treatment.

Major Progress

A. Clinical Research

Cancer has been Taiwan's number one cause of death since 1982. In 2004, cancer was responsible for more than one-fourth (27.2%) of all deaths in the nation. Such epidemiological data make clear why cancer has long been recognized as an important disease and significant public health issue. To help end Taiwan's deficit in well-trained, competent oncologists, a previous organization that evolved into the National Institute of Cancer Research began an oncology-training program in February 1987. The program, which continues, covers areas such as medical, surgical, gynecological, and radiation oncology. Because cancer types and tumor biology sometimes differ between Taiwan and the West, Western medicines may not always be optimal for cancer patients in Taiwan; thus, the institute recognizes the need to conduct its own investigations of tumor biology and to develop novel therapies for cancer.

To fulfill its missions, the institute cooperates with outside research wards and laboratories, including those previously at Veterans General Hospital and National Taiwan University Hospital. Although the cooperative ward at National Taiwan University Hospital closed in 2005 because of a scheduled move to National Defense Medical College, the outpatient clinics and cooperative studies are continuing. In the spring of 2006, the institute established affiliation



with a new site at Tri-Service General Hospital. During the same period, the Division of Cancer Research was upgraded to NHRI's first institute: the National Institute of Cancer Research. In the spring of 2007, the clinical part of the institute moved to Tainan, where it is collaborating with National Cheng Kung University Hospital in the establishment of the National Cancer Research Center, Southern Branch. The research center was mandated by the Cancer Act, a law initially proposed by National Institute of Cancer Research Director Jacqueline Whang-Peng and passed by Taiwan's legislature. This landmark act which facilitates clinical cancer research and the care of cancer patients in Taiwan, covers expenses for clinical trial drugs, special investigations, and other expenses incurred by patients on clinical trials.

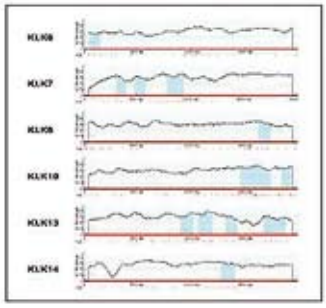


Figure 1. Potential DNA methylation sites of the KLK genes. Unlike other KLK members, most of the invasion-related KLK genes (i.e. KLK7, KLK10 and KLK13) contain multiple CpG islands.

From January to December 2007 the institute gave the first final report of phase II study of triplet combination chemotherapy (GOFL) in advanced pancreatic cancer (L.T. Chen and H.J. Ch'ang, P.I.s), in 2008 ASCO GI Cancer Symposium, January 2008 in Orlando, Florida, USA. The team has completed patient accrual in two multi-center trials: a phase II and pharmacokinetic study of S-1 in advanced gastric cancer (J. Whang-Peng, P.I.) and a randomized phase II trial of ADI-PEG20 in advanced hepatocellular carcinoma (L.T. Chen, P.I.). The team also opened two new phase I trials of liposomal-irinotecan plus weekly 24-hour infusions of high-dose 5-FU/LV in refractory advanced solid tumors (L.T. Chen, P.I.) and a randomized, phase I trial of RAD001 (an mTOR inhibitor) in advanced hepatocellular carcinoma (L.T. Chen and H.S. Shiah, P.I.s). In addition, the team has successfully negotiated with international industrials to sponsor several new trials over the next few years, such as a phase I and pharmacokinetic study of sorafenib plus S-1 in refractory solid tumors (L.T. Chen and H.S. Shiah, P.I.s), a phase I trial of biweekly PEP02 in oxaliplatin-refractory colorectal cancer (J.Y. Chang and J.C. Lee, P.I.s), a phase II trial of cetuximab-based chemoradio-therapy for locally advanced squamous cell carcinoma of the esophagus (C.H. Hsu and H.S. Shiah, P.I.s), a phase III trial of adjuvant gemcitabine with/without concurrent chemoradiotherapy in post-operative pancreatic cancer (TCOG3207, L.T. Chen and H.J. Ch'ang, P.I.s), and an international phase III trial of gemcitabine versus S-1 versus gemcitabine/S-1 in advanced pancreatic cancer.

Future plans:

With the National Cancer Research Center's Southern Branch established in 2007, the institute has turned its focus to:

- collaborating with National Cheng Kung University Hospital to establish the National Cancer Research Center;
- promoting interaction between clinical researchers and basic scientists at NHRI and National Cheng Kung University and the university's hospital, with the interaction focusing on domestically prevalent cancers, i.e. head and neck cancer, and gastrointestinal cancer (including hepatobiliary and pancreatic cancers);
- expanding the National Institute of Cancer Research's collaboration with other major medical centers and teaching hospitals, in order to promote cancer-patient care and clinical and translational cancer research in southern Taiwan;
- facilitating phase I and early phase II clinical trials for new drugs and new indications development at the National Institute of Cancer Research, and assisting operations of National Cheng Kung University Hospital's center for clinical trials and research in oncology;
- facilitating collaboration with other international oncology groups for multi-nation, early-phase clinical trials;
- providing better training programs for our fellows and oncology-research nurses.

Although NHRI is still a young organization, its National Institute of Cancer Research has already established a reputation for designing and conducting important early-phase clinical trials, starting from phase I trials for combination chemotherapy (i.e. weekly docetaxel plus infusional 5-FU/leucovorin and cisplatin in advanced gastric cancer, and biweekly gemcitabine, oxaliplatin, and infusional 5-FU/leucovorin in advanced pancreatic cancer) and continuing to the first-in-human phase I trial for cytotoxic agents (i.e. GL331 and liposomal irinotecan) and still more trials, such as several exploratory phase I/II trials for new indications (i.e. thalidomide, ADI-PEG20, and RAD001 in advanced HCC). The institute is planning collaboration with National Cheng Kung University Hospital to set up a clinical trial platform for new drug and new indication development in oncology.

B. Basic Research

The Group of Basic Research is divided into several study programs, including the programs of experimental therapeutics, molecular and cellular oncology, tumor immunology, radiation biology, and lung cancer genomic medicine. The experimental therapeutics program has discovered that several novel microtubule inhibitors and topoisomerase II inhibitors — such as AHMA, indole, stilbenes, and sulfonamide synthetic compounds — are potential anticancer drugs. Traditional microtubule inhibitors, such as paclitaxel and vincristine, are the substrate of MDR and subsequently lead to drug resistance, while the institute's novel drugs function differently and have great potential in the management of various malignancies, particularly for patients with drug resistance. These promising results have been published in the *Journal of Medicinal Chemistry*, *Cancer Research*, *Biochemical Pharmacology*, and the *Journal of Pharmacology and Experimental Therapeutics*. One of the leading compounds, BPRL075, has completed all preclinical toxicology and is ready to enter early phase clinical trial.

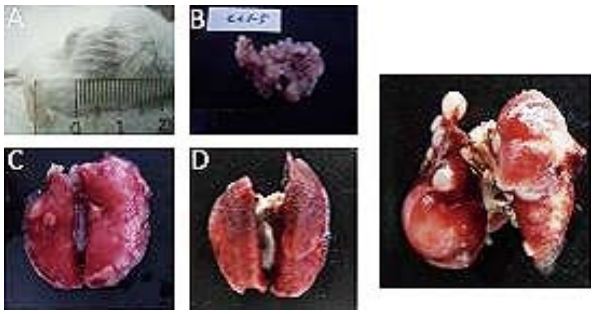


Figure 2. Establishment of the mouse model for investigating lung cancer metastasis. Left panel: SCID mice were subcutaneously inoculated with 5×10^6 CL 1-5 cells in the dorsal region Right panel: SCID mice were injected with CL 1-5 cells through tail vein. Metastatic nodules on lungs were observed after several weeks of injection.

Because drug resistance is a major obstacle in cancer treatment, studying the mechanism of drug resistance and exploring strategies to overcome it are also priorities of the institute. The team found that copper transporter ATP7A plays a role in modulation of platinum resistance in oxaliplatin-resistant cells. In addition, Dr. Liu also found that a transporter is responsible for the cancer cells resistant to gemcitabine. Dr. Chung found that AXL up-deregulated by chemotherapeutic agents increased invasiveness of cancer cells and demonstrated that salfasalazine is able to overcome its resistance. These promising results have been published in *Cancer Research* and the *British Journal of Cancer* and are helpful for the design of future clinical trials.

In the program of molecular and cellular oncology, Dr. Wu has identified several metastasis-related genes, such as Ep-CAM, that are important for lung cancer cell metastasis. He also found the silencing of Ep-CAM is regulated by DNA methylation and histone modification. Dr. Chang demonstrated that kallikrein 13, a newly identified protease gene, enhances malignancy of lung cancer cells through loss of epigenetic control. Dr. Chuang focused on the role of AXL, Carbonic anhydrase 3, and SAF in cancer invasion and metastasis. He found receptor tyrosine kinase AXL is induced by chemotherapy drugs and that overexpression of AXL confers drug resistance in acute myeloid leukemia; this was published in *Cancer Letter 2008*. Dr. Huang's research team has focused on

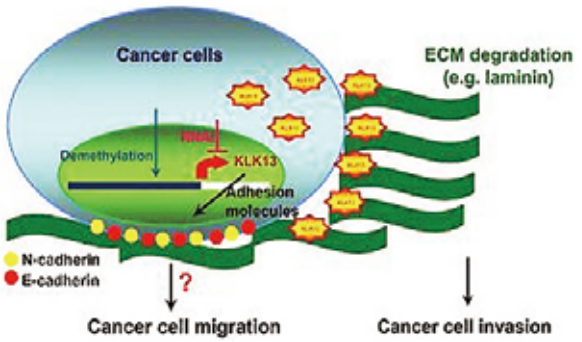


Figure 3. Kallikrein 13 (KLK13), a newly identified prtoease gene, enhances the malignancy of lung concer cells through loss of epigenetic control.

the studies of cancer proteomics and tumor-associated macrophages. They found that macrophage induce MCF-7 cell migration via the integrin-FAK-paxillin pathway. Dr. Yu found that HPV E7 oncoprotein can interact with TIEG1 to prevent infected cells from undergoing suicidal apoptosis. About 90 apoptosis-related genes were found to be altered by TIEG1. Dr. Chen's research team has demonstrated that EBV plays an etiological role in the carcinogenesis of NPC. He found that amplicons on chromosome 3 contain oncogenes induced by recurrent TPA/SB exposure and Epstein-Barr virus reactivation in a nasopharyngeal carcinoma cell line.

In the program of tumor immunology, Dr. Shih has successfully demonstrated that induction of anti-ENO1 immunity was sufficient to prevent and suppress tumor growth in immuno-competent mice. Relevant U.S. and Taiwan patents have been filed for, and the work published in an international journal. Additionally, these findings can provide not only alternative cancer treatment for cancer patients but also a novel potential tumor marker for prognosis of lung cancer.

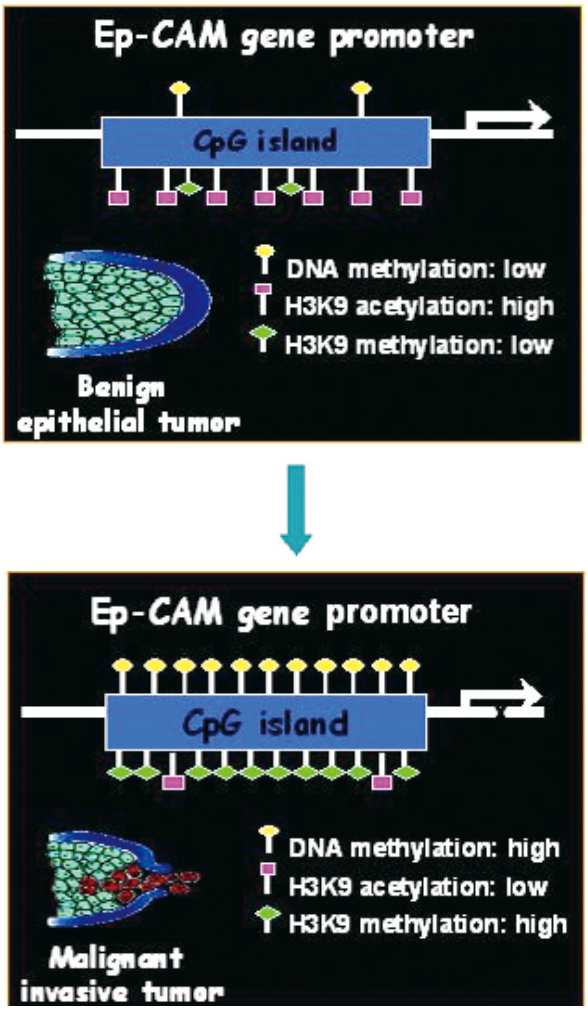


Figure 4. Silence of Ep-CAM expression is regulated by DNA methylation and histone modification.

In the program of radiation biology, Dr. Ch'ang focused her basic research on radiation-related normal tissue toxicity using the intestinal tract of mice as a model and later published a cutting-edge research report in *Nature Medicine* regarding target switching of intestinal tissue to radiation. Her current basic research project, supported by NHRI, is on the role of mesenchymal-epithelial interaction in radiation-induced intestinal mucosa damage repair.

And in the program of lung cancer genomic medicine, Dr. Wu focused TR4 responsive element (TR4RE) in SERPIN family protein and cyclin D1 gene transcription site position determination.

C. Taiwan Cooperative Oncology Group (TCOG)

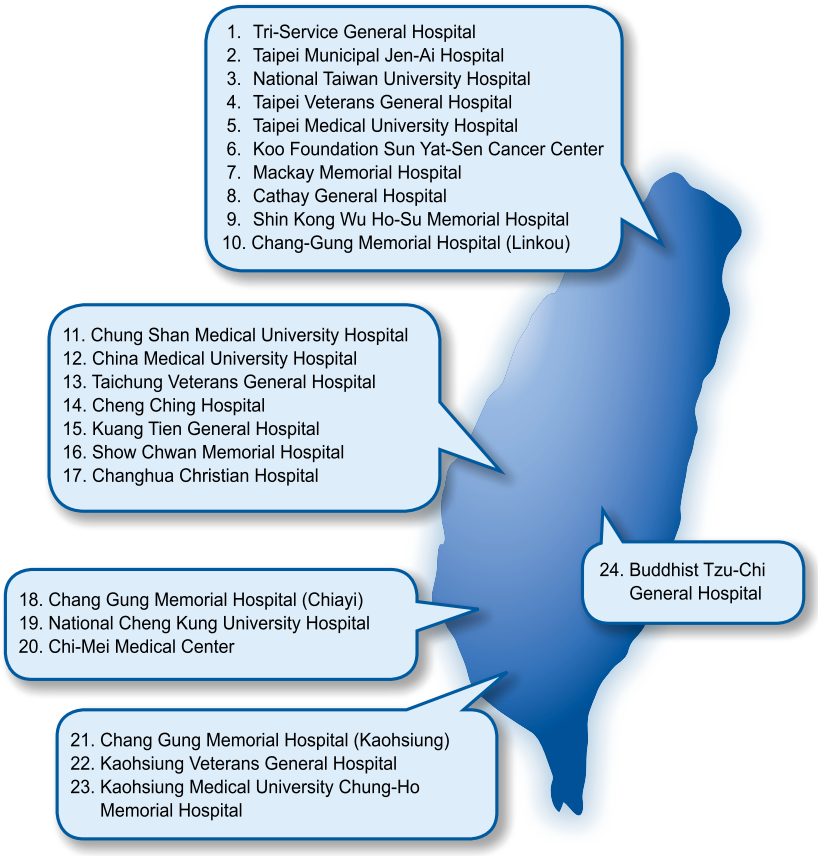
As the first multi-center clinical-trials organization in Taiwan, the Taiwan Cooperative Oncology Group conducts clinical trials for cancer treatment. This network connects 24 teaching hospitals islandwide and serves approximately 75% of all cancer patients in Taiwan.

Each of the group's many specialized committees — including the Scientific Advisory Committee, Board Committee, Executive Committee, four disease committees, four quality-monitoring committees, and four modality committees — is responsible for the formulation of specific cancer diagnosis consensus statements on treatment and prevention, clinical trials' research protocol write-ups, and patient enrollments. The audit committee also conducts yearly audits to assure high-quality data collection and compliance with GCP in TCOG trials.

Based on its evidence-based outcomes, TCOG has produced therapy guidelines and consensus statements on cervical, breast, oral cavity, lung, colorectal, gastric, prostate, and nasopharyngeal cancer, along with patient guidelines for brain tumors and radiotherapy, and cancer-practice guidelines for clinicians.

In August 2007, TCOG revised its "Guidelines for the Practice of Gynecologic Oncology" and released "Guidelines for the Practice of Cancer Pain Management." Audits of case records of clinical trials at nine member hospitals were performed the following month.

In addition, TCOG held its eleventh annual meeting in December 2007 in conjunction with



the Asia-Pacific Congress on Malignancies of the Digestive Tract. This yearly event provides an important opportunity for clinicians, researchers, and medical professionals in the region to exchange information about cancer treatment.

With assistance from NHRI's Division of Biostatistics and Bioinformatics, the Clinical Research Information Management System continues to facilitate the conduct of all clinical trials and to establish standard operating procedures for clinical trial data flow and management.

TCOG has completed 21 trials to date and has 10 ongoing trials.

D. Subspecialty Training Program

For effective utilization of currently available clinical and basic research resources and to better help manage the most important medical problems in Taiwan, training programs are being developed to upgrade clinical laboratory techniques of specialists in medical oncology, surgical oncology, and gynecological oncology.

- **Medical Oncology Training Program**

  One oncologist from Cheng Kung University Hospital graduates from this program every year.
- **Radiation Oncology Training Program**

  The institute's first radiation oncology training program yielded fifteen graduates from several leading hospitals.
- **Research Nurse Training Program**

  This continuing education program invites oncologists, statisticians, and nursing specialists to provide lectures or group seminars for clinical research nurses every three months.
- **Gynecological Oncology Training Program**

  So far ten gynecologists have completed this two-year program.

Institute of Cellular and System Medicine

Mission

The Institute of Cellular and System Medicine aims to provide new information on the mechanisms of important chronic diseases and disorders, and to use the knowledge to develop new diagnostic methods and therapeutic agents for prevention and treatment by using integrated cellular, molecular, and system approaches. The institute was created in March 2008, integrating three research units: the Stem Cell Research Center, the Cardiovascular and Blood Research Center, and the basic research component of the Division of Gerontology Research. The main research activities of these three research units are to understand cellular and molecular aspects of human multi-organ/system chronic diseases and to develop cell-based strategies for treating those diseases. To achieve the goals, the Stem Cell Research Center focuses on novel methods for developing adult stem cells and exploring their therapeutic potentials; the Cardiovascular and Blood Research Center focuses on vascular protection mechanisms and therapeutic strategies; and the basic research component of Division of Gerontology Research focuses on investigating the role of inflammation on musculoskeletal cells and aging-related cellular changes. An interdisciplinary stem cell research program was established to foster basic, translational and clinical research on stem cells. Taiwan's executive branch of government selected the stem cell program to establish the nation's flagship stem cell center. The program will focus on basic and clinical research pertinent to cardiovascular, musculoskeletal, and peripheral nerve diseases.

Major Progress

Major progress made during 2007–2008 is summarized below, within the following main categories:

- Aging-Related Musculoskeletal Diseases
- Cardiovascular Medicine
- Regenerative Medicine

Aging Research

A. Role of Inflammation in the Pathogenesis and Treatment of Osteoarthritis

Osteoarthritis is characterized by chronic degeneration of the cartilage of the joints, particularly weight-bearing joints, such as knees and hips. Excessive weight and continual "wear and tear" have long been thought to be two of the major factors behind osteoarthritis. Recent clinical studies, however, have indicated that inflammation plays a critical contributing factor in the progression of this disease. As a result, researchers in the division have adopted a new approach to investigate the immune mechanism in inflammation-mediated osteoarthritis and to look for potential therapies for preventing this disorder. Over the past three-and-a-half years, the team has established the preparation and culture of chondrocytes obtained from osteoarthritis patients receiving total hip or total knee replacement and 5-7 chondrocyte cell lines by immortalizing chondrocytes from OA patients. The immunomodulatory effects and mechanisms of all-trans retinoic acid (t-RA) — an active derivative of vitamin A in chondrocytes, activated by ilterleukin-1 (IL-1) or tumor necrosis factor alpha (TNF-α) — have been demonstrated. In addition, retinoic acid inhibits IL-1- and TNF-α-induced chemokine production through COX-2-dependent and COX-2-independent signaling pathways in human chondrocytes.

B. Impaired Atrial Natriuretic Factor Signaling in Aging

Atrial natriuretic factor (ANF), a peptide hormone, exerts potent effects on the regulation of intravascular volume, electrolyte balance, and blood pressure. Its effects are mediated mainly by its receptor, membrane-bound guanylate cyclase GC-A. The team has found that GC-A is subjected to inhibitory constraints exerted by cyclophilin A (CypA). In addition, a novel cDNA encoding guanylate cyclase regulatory protein (GCRP) that associates with GC-A in an ANF-dependent manner and activates GC-A has been cloned. Therefore, it is likely that the binding of ANF induces the association of GCRP with GC-A and the dissociation of CypA from GC-A. These changes in protein–protein interactions cause a conformational change on GC-A, leading to its activation.

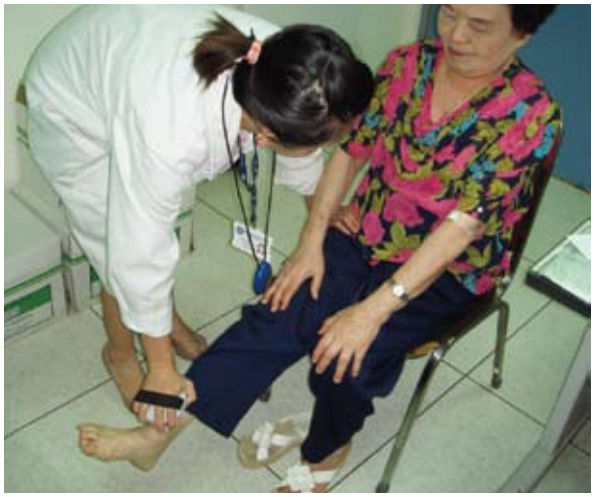
Aging is characterized by the accumulation of advanced glycation end products (AGEs), which have been shown to activate NAD(P)H oxidase and produce superoxide. Preliminary studies from the division found that AGEs activate Nox-4 NAD(P)H oxidase in HEK293 cells, and that expression of Nox-4 or addition of superoxide generators such as menadione and diamide inhibits ANF-stimulated GC-A activity in LLC-PK1 cells. Superoxide interacts with NO and forms peroxynitrite; and the division found that peroxynitrite, menadione, and diamide also inhibit the activity of GC-c (the catalytic domain of GC-A),

indicating that peroxynitrite interacts with the catalytic domain of GC-A.

The team is working to identify the proteins involved in the activation of NAD(P)H oxidase by AGEs in LLC-PK1 and HEK293 cells; inhibition of ANF-simulated GC-A activation by peroxynitirite on specific Tyr and Cys residues; and modification of GC-A, GCRP, and/or CypA in LLC-PK1 cells by AGEs precursors.

C. Skeletal Progenitor Cell Defects and Tissue Repair in Aging

The deficiency of old osteoprogenitor cells and their detrimental effect on bone formation activity has been extensively studied. The team has previously shown that the reduced number of osteoprogenitor cells in aging is attributed to the defeats in mitogenic response of osteoprogenitor cells to growth factors such as IGF-I, PDGF, and bFGF. IGF-I has been identitied as probably the most effective growth factor to modulate mitogenic and osteogenic lineage expression of osteoprogenitor cells. The defects along the IGF-I cascade are multifaceted. The team has demonstrated that local infusion of IGF-I in old rats can enhance bone formation activity and effectively reduces the age defect in bone volume.



Cardiovascular Medicine

Cardiovascular disease is a leading cause of death worldwide, including Taiwan. Understanding the regulatory mechanisms may help provide new mechanistic insight and targets for drug discovery. The main research focus is on protective mechanisms of prostacyclin in endothelial cells, cysteine-rich protein 2 in vascular smooth muscle cells, immune system activated by TLR ligands.

A. Prostacyclin Protects Cell Survival by a Novel Transcriptional Mechanism

1. Prostacyclin (PGI2) protects endothelial cell against apoptosis via PPARδ-mediated 14-3-3ε upregulation

Endothelial cell is the primary type in PGI2 production. PGI2 inhibits platelet aggregation and controls vascular tone. Results of experiments support the hypothesis that PGI2 protects endothelial cell survival. The team's recent work shows that PGI2 prevents H2O2-induced apoptosis via nuclear receptor, PPARδ. Activated PPARδ forms heterodimers with RXR, which activate or suppress an array of genes; but it was not clear which genes were involved in cell survival. The team identifies 14-3-3ε proteins as the PPAR-δ effector. Ligand-activated PPARδ binds PPAR response elements (PPRE) situated at the 5'-flanking region of human 14-3-3ε gene and stimulates 14-3-3ε promoter activity. Human umbilical vein endothelial cells (HUVECs) treated with carbaprostacyclin or L-165041 have a 2–3-fold increase in 14-3-3ε proteins. Increase in 14-3-3ε is accompanied by enhanced sequestration of Bad in cytoplasm and reduced Bad translocation to mitochondria. Suppression of 14-3-3ε with PPAR-δ RNA interference results in reduced HUVEC survival, whereas 14-3-3ε overexpression protects HUVEC from H2O2-induced damage. These results indicate that COX-2 derived PGI2 protects endothelial cell survival by activating PPARδ, which promotes 14-3-3ε expression. Increased 14-3-3ε augments Bad sequestration and thereby reduces Bad-induced apoptosis via the mitochondrial pathway. Thus, PPARδ activation is a target for therapy of cardiovascular diseases. This work was published in 2006 in *Arteriosclerosis, Thrombosis, and Vascular Biology* (26:1481–1487).

2. Nonsteroidal anti-inflammatory drugs (NSAIDs) induce cell death via PPARδ to 14-3-3ε pathway

A number of NSAIDs, including sulindac and indomethacin, induce colorectal cancer cell apoptosis. The research team carried out experiments to determine whether PPAR-δ/14-3-3ε pathway is involved in H-29 or DLD-1 apoptosis by NSAIDs. The results reveal that sulindac and indomethacin induce apoptosis in a PPARδ dependent manner. NSAIDs suppress PPARδ expression and thereby 14-3-3ε expression. 14-3-3ε overexpression prevents NSAID-induced apoptosis. These results indicate that NSAIDs induce colon cancer cell apoptosis by suppressing PPAR-δ expression, which results in reduction of 14-3-3ε and the capacity for Bad binding.

As use of NSAIDs as well as selective COX-2 inhibitors is associated with increased risk of coronary heart disease, the team wondered if NSAIDs induce endothelial apoptosis. Both sulindac and indomethacin induce HUVEC apoptosis. They suppress PPAR-δ proteins and promoter activity as well as 14-3-3ε protein and promoter activity. The 14-3-3ε suppressing effect is abrogated by PPAR-δ siRNA. Furthermore, PPARδ siRNA inhibits HUVEC apoptosis induced by NSAIDs. Sulindac inhibits 14-3-3ε and 14-3-3ε promoter activity. Adenoviral PPAR-δ transduction of HUVEC increases PPAR-δ but fails to rescue 14-3-3ε or apoptosis because of suppression of PPAR-δ by sulindac. Aspirin or sodium salicylate at 1 mM has no effect on PPARδ, 14-3-3ε, or apoptosis. Aspirin at 5 mM, however, induces apoptosis and suppresses PPARδ and 14-3-3ε in a manner similar to sulindac. These results reveal that PPARδ to 14-3-3ε pathway is the target of NSAIDs, which may contribute to cardiovascular complications. Aspirin at therapeutic concentrations (≤1 mM) appears to be safe as it does not interfere with this cell survival pathway.

In summary, the team has discovered a transcriptional signalling pathway that mediates cell survival. NSAIDs induce cancer and endothelial cell apoptosis by suppressing this pathway. This work was published in 2007 in *Cancer Research* (67:3185–3191) and is soon to be published in *Molecular Pharmacology.*

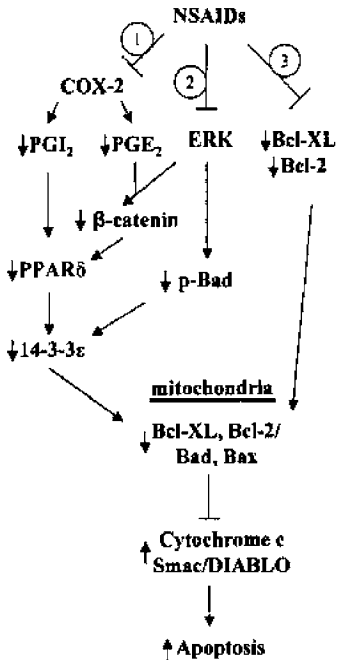


Figure 1. Schematic illustration of multiple biochemical processes which NSAIDs induce apoptosis via suppression of 14-3-3.

B. Cysteine-Rich Protein 2 in Vascular Smooth Muscle Cells

Cysteine-rich protein (CRP) 2 is a vascular smooth muscle cell (VSMC) expressed LIM-only protein. Using Csrp2 (gene symbol of the mouse CRP2 gene)-deficient mice, the team previously demonstrated that an absence of CRP2 enhances VSMC migration and increases neointima formation following arterial injury. Given the inhibitory role of CRP2 in VSMC migration, upregulation of CRP2 by factors present at the injured vessel wall may serve as an adaptive mechanism and reduce neointima formation. TGFβ is one of the factors released at the injured site and has been shown to upregulate many smooth muscle marker genes expression; its role in lesion formation, however, remains controversial. The goal was to investigate whether TGFβ upregulates CRP2 and the mechanisms by which TGFβ induces CRP2 expression.

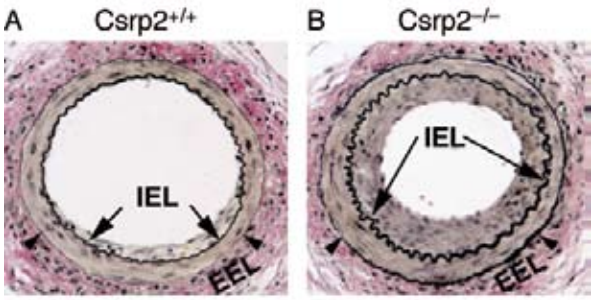


Figure 2. Vascular injury increases intima thickening in Csrp2$^{-/-}$ mice. Femoral arteries were harvested 14 dafter wire injury. Verhoeffs staining for elastin (black) was performed on sections from (A) WT (Csrp2$^{+/+}$) and (B) Csrp2$^{-/-}$ mice. Representative setions are shown. Arrows indicate IEL and arrowheads indicate EEL of the vessels.

1. TGFβ induces CRP2 expression in vascular smooth muscle cells

To examine the possibility that TGFβ might regulate CRP2 expression, primary cultured VSMCs were treated with TGFβ (10 ng/ml), and CRP2 protein levels were evaluated by Western blot analysis. TGFβ induced CRP2 protein expression as early as 2 h; the induction continued at 4 h. A threefold induction was observed at 8 h; and this level of induction was maintained at 24 h when compared with untreated controls (Fig. 2A). Similarly, Northern blot analysis revealed that TGFβ induced CRP2 mRNA expression in a time-dependent manner. A maximal threefold induction was observed at 4 h, which preceded maximal protein induction at 8 h and was maintained at 24 h when compared with untreated controls.

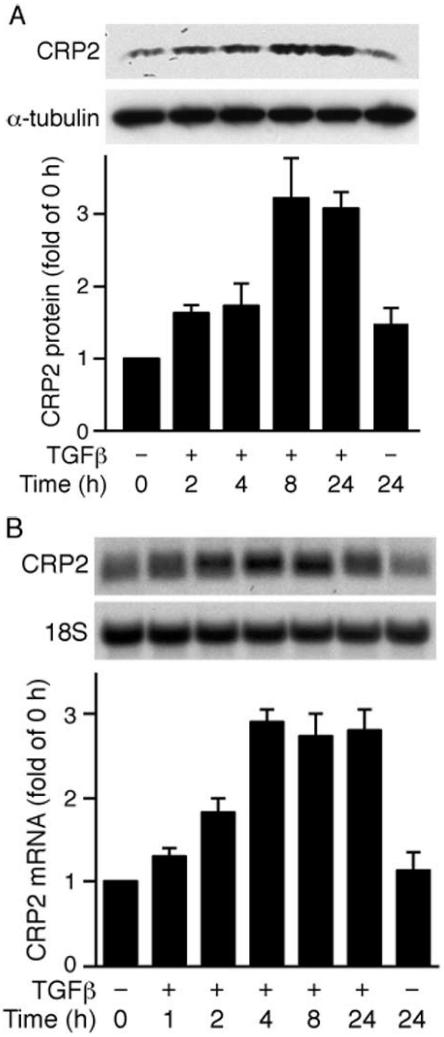


Figure 3. TGFβ increases CRP2 expression levels in VSMCs. VSMCs were exposed to TGFβ (10 ng/ml) for various times. (A) protein extracts were harvested for Western blot analysis. (B) Northern blot analysis was performed from total RNA isolated at the indicated times.

2. TGFβ does not alter the half-life of CRP2 mRNA

To elucidate the mechanisms underlying CRP2 mRNA induction, experiments with the transcriptional inhibitor actinomycin D were performed. VSMCs were pre-incubated with vehicle or actinomycin D for 30 min, then treated for 4 h with or without TGFβ. Northern analysis revealed that in the absence of actinomycin D, TGFβ substantially induced CRP2 mRNA expression, whereas actinomycin D blocked induction of CRP2 mRNA. Given that TGFβ can regulate mRNA levels of many target genes by altering mRNA stability and half-life, CRP2 mRNA half-life in VSMCs stimulated with or without TGFβ was measured. In the absence of TGFβ, the half-life of CRP2 mRNA was approximately 14 h. The half-life of CRP2 mRNA in TGFβ treated cells was also approximately 14 h, indicating that TGFβ has no effect on CRP2 mRNA half-life.

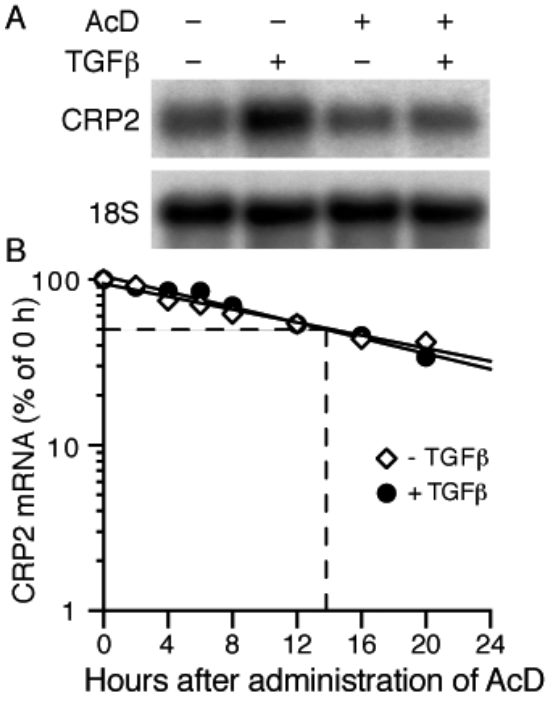
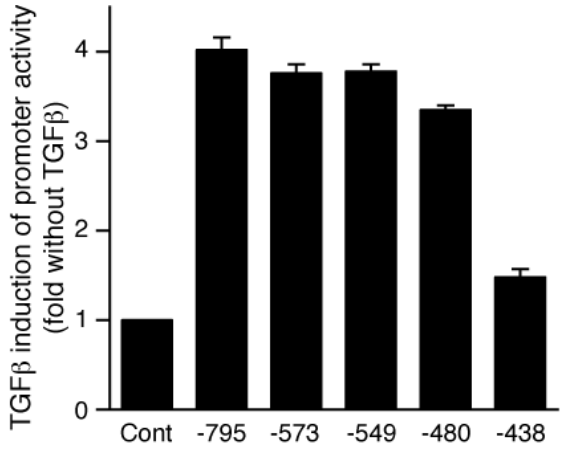


Figure 4. TGFβ does not alter CRP2 mRNA half-life. (A) VSMCs were preterated with vehicle (95% ethanol) or transcriptional inhibitor actinomycin D (AcD) for 30 min and then stimulated without or with TGFβ (10ng/ml) for 4 h and analyzed. TGFβinduced CRP2 mRNA expression in the absence of AcD. In comparison, Acd blocked the CRP2 mRNA induction by TGFβ. (B) TGFβ does not alter CrP2 mRNA half-life. VSMCs were stimulated with or without TGFβ for 4 h and then AcD was administered to the cells. Total RNA was extracted at the indicated times after administration of AcD and Northern blot analyses were performed. The normalized intensity was then plotted as a percentage of the 0 h value (in log scale) against time.

3. Csrp2 promoter bp -480 to -438 confers TGFβ response

To determine whether elements responsible for TGFβ induction were present in the Csrp2 promoter, VSMCs were transiently transfected with luciferase plasmid -795Csrp2-luc, containing –795 bp of the Csrp2 promoter. TGFβ increased -795Csrp2 promoter activity by approximately fourfold, indicating that elements responsible for the TGFβ induction of CRP2 were located in the –795 proximal promoter.



To localize the elements, a series of Csrp2 5'-deletion promoter-luciferase constructs were generated and transiently transfected into VSMCs. Similar to -795 construct, -573 construct showed a 3.8-fold induction by TGFβ. Deletion of the 5' sequences to bp -549 retained TGFβ inducibility. Additional deletion to bp –480 only slightly diminished the responsiveness to TGFβ. Significantly, further deletion to bp -438 abolished the promoter induction by TGFβ, indicating that the region between bp -480 and -438 was required for the TGFβ stimulation of Csrp2 promoter activity.

These data indicate that TGFβ induces CRP2 expression at the transcriptional level via the promoter region between bp –480 and –438. Future studies will define the cis-acting elements and cognate transcription factors that mediate transcriptional activation of Csrp2 gene by TGFβ.

Regenerative Medicine

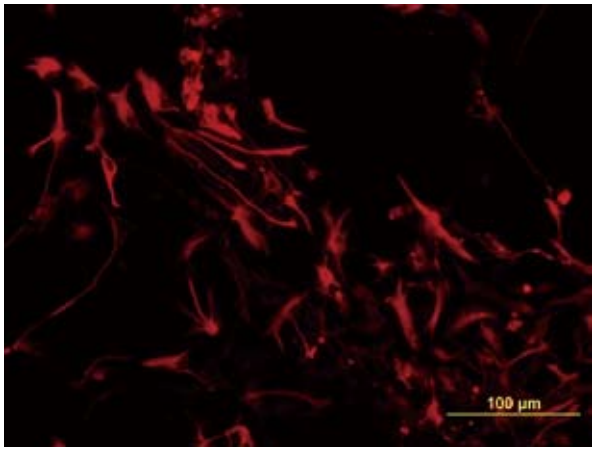
The group of regenerative medicine aims to establish a cGMP-level cell-processing center to serve as a core laboratory for clinical trials of cell therapy and regenerative medicine. The group conducts research in the following areas: neural stem cells in medical application, embryonic stem cells and placenta stem cells, mesenchymal stem cells and immunity, and stem cells hair follicle cycles. The team is also helping guide the creation of a stem cell law.

A. Neural Stem Cells

1. Research and development of adult and embryonic Neural Stem Cells

Fibroblast growth factor (FGF) 1 and 2 regulate neural stem cell growth, differentiation, and self-renewal capacity. FGF1 is unique among FGFs in its binding to all known FGF receptors (FGFRs). In previous research the team found that brain-specific FGF1B promoter was active in mouse neural stem cells; also a fragment of 18-bp of FGF1B promoter, important for the regulation of brain-specific FGF1 expression, was characterized. The team then developed a new approach to isolate neuronal progenitor cells from mouse and human brain tissues with fluorescent protein expression using F1B-GFP or F1B-Red (F1B-FPs). Based on the cell isolation, proliferation, and differentiation condition experiments, the center arrived at two conclusions: (1) FGF1 could replace FGF2 in culture medium to isolate brain stem cells from mouse brain at embryonic day 17.5 (E17.5) and postnatal day 1 (P1) in serum-free condition, and (2) F1B-FP plasmids could be used in selection for neural progenitors.

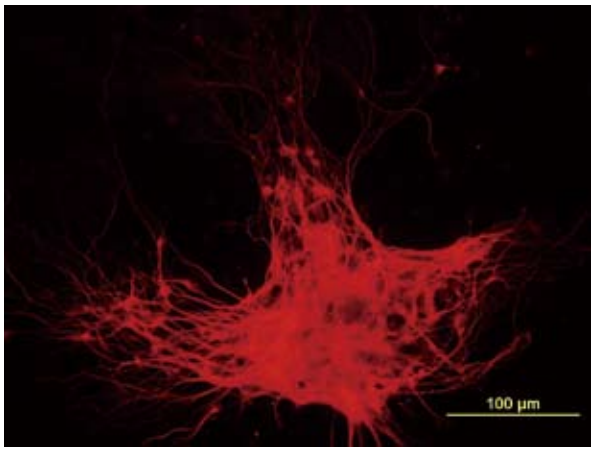
The team further showed that a combination of mouse neural stem cells and novel biomaterial-based nerve conduits could facilitate the repair of rat sciatic nerve injury. Based on this discovery, the team is planning to use human neural stem cells to repair peripheral nerve injuries not only in rats and pigs but also humans.



2. Neurogenic stem cell niches in the brain: crosstalk between neural stem cells and the surrounding cells

Various cell types, their secreted factors, and extracellular components guide neurogenesis process and could lead development of neurodegenerative diseases. The team has set up two sources of neural stem cells (NSC) culture for these studies: the embryonic stem cell (ESC)-derived neural stem cell, and the neurosphere-forming cells from prenatal, postnatal, and adult mouse brain. Primary cultures of other brain cells such as astrocyte and microglia are established. NS cells are co-cultured with brain endothelial cells, microglia, and astrocytes to decipher

the neurogenic components and their mechanisms. One molecule that the group is focused on is the type II transmembrane serine protease that has been shown to play a role in cell differentiation, growth, migration/invasion, and angiogenesis. The team found that this protease is expressed on the surface of ESC, its derived NSC, and adult neurospheres but is absence from astrocytes- the largest cell population in the brain. Current data suggest that this protease has little effect on the proliferation and survival of ESC-derived NSC whereas could be involved in neuron maturation and NSC mobility. For NSC homing, the team was able to maintain a living brain slice culture for three weeks and saw the migration of the implanted NSC.



An embryonic stem cell (ESC)-derived neural stem cell culture has been set-up. This cell line carries a GFP under the control of the promoter of Sox1, while the ESC-derived NSC is used in co-culture with brain endothelial cells, microglia, and astrocyte to decipher the neurogenic components and their mechanisms.

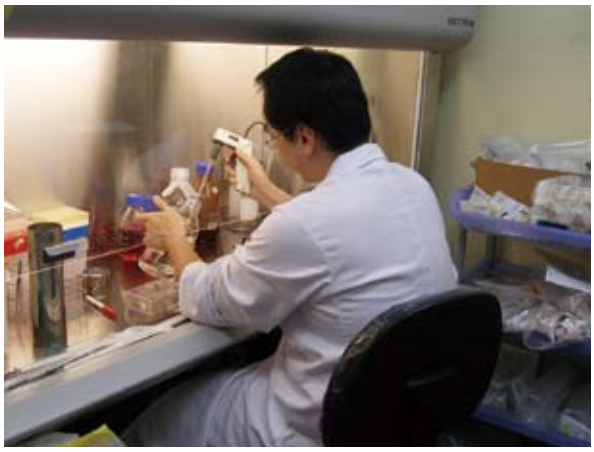
One molecule that the group is focused on is a type II transmembrane serine protease that has been shown to play role in epidermal differentiation and tumor angiogenesis. The team found that this protease is expressed on the surface of their ESC, its derived NSC, and the brain endothelial. The preliminary data suggest that this protease has little effect on the proliferation and survival of ESC-derived NSC whereas promotes its neuronal differentiation. The team will investigate this further.

3. Characterization and medical application of human neural stem cells

The center uses F1B-GFP reporter to transfect cells derived from human tissues, including embryos, fetal brain, cord blood, bone marrow, lipoaspirate, scalp, foreskin, amnion and placenta to identify the rare neural stem cells in these populations. GFP-tagged human adult and fetal neural stem cells would have enormous importance in clinical applications. Since neural stem cells usually reside in the brain and are difficult to access for autologous grafting, the method that the center proposes would allow identifying neural stem cells from sources that are more easily accessible, such as cord blood, bone marrow, and placenta. A facile means to identify and isolate neural stem cells could lead to developing strategies for stem cell-based therapies for neurodegenerative and traumatic diseases and as an adjunctive therapy for brain tumors.

Identification of neural stem cells in human brains and adipose tissues

The group has established F1B-GFP transgenic mouse lines and identified neural stem cells that express green fluorescent protein (GFP) from such transgenic mice. In addition, the mouse neural stem cells thus isolated, based on GFP expression, could repair the severed rat sciatic nerve and allow the rats to use their crippled hind leg again within one week.



Identification of neural stem cells in human umbilical cord blood and bone marrow

The group has previously reported the isolation of multi-potent mesenchymal stem cells from umbilical cord and human bone marrow by negative immuno-selection and limiting dilution. These cells, under appropriate induction, can be differentiated into various progenies, including neuroglial cells. These differentiated neuroglial cells express marker genes of neuroglial lineage including MAP-2 and GFAP. Also, the *in vitro* function assay by calcium imaging demonstrated that, after differentiation induction, these differentiated cells are functional.

Identification of neural stem cells in human tissues

The group found that the KT98 cell line, which is derived from F1B-Tag transgenic mice, is capable of supporting human embryonic stem cell (hESC) *in vitro* cultures after inactivation. This is a technically important finding since hESCs are heavily dependent on mainly primarily cultured feeders, whether it be murine or human sources.

Establishment of a mouse model for Parkinson's disease by prenatal exposure to lipopolysaccharide

The group is developing the mouse model of Parkinson's disease based on a rat model published using lipopolysaccharide stimulation in pregnant female mice. In addition, the group has been working to establish culture systems for DA neuron differentiation to facilitate the studies in vitro. The team showed that after NSC being driven to midbrain phenotype differentiation, the tyrosin hydroxylase expressing, neuron-looking cells could be seen after two weeks' culture in medium containing FGF2.

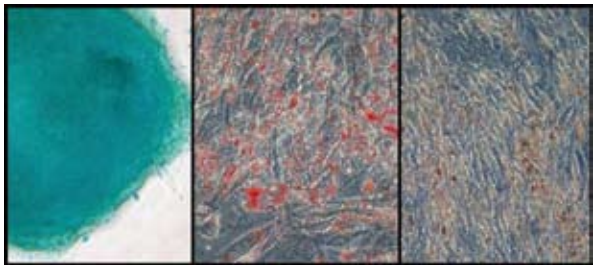
B. Placenta - Derived Multipotent Cells

Human Placenta-Derived Multipotent Cells: further immune profiling and chondrocytic regeneration in animal arthritis models

Current sources of stem cells include embryonic stem cells (ESCs) and adult stem cells (ASCs). Both sources, however, are potentially problematic: ESCs, with significant ethical considerations, tumorigenicity concerns, and paucity of cell lines; and ASCs, which are possibly more limited in potential. Collaboration with Cathay General Hospital has resulted in the discovery of a population of multipotent cells from the human term placenta. These placenta-derived multipotent cells (PDMCs) exhibit many markers common to bone marrow mesenchymal stem cells and embryonic stem cells. PDMCs also possess strong immuno-suppressive properties, which are useful in transplantation.

This year, by investigating the endothelial differentiation of PDMCs, a population of CD34(-)/CD133(-)/Flk-1(-)cells, the group found that the combined use of endothelial growth factors and high shear stress is synergistic for the endothelial differentiation of PDMCs. These findings were published recently in the *Journal of Biomechanics*.

In terms of further immune characterization, the group has found that PDMCs are also resistant to natural killer cell (NK) cytotoxicity. The group's *in vivo* studies of PDMCs have begun to evaluate the effect of transplantation into immune-deficient versus wild-type animals. The team hopes to utilize PDMCs in rodent disease models of cartilage, a tissue with a limited capacity for self-repair.



C. Mesenchymal Stem Cells

Role of transforming growth factor beta-1 in immunodulation of human mesenchymal stem cells

The team worked on the mechanisms of the immunomodulatory activities of MSCs and found that MSCs from both umbilical cord blood (uMSCs) and bone marrow (BM-MSCs) showed the cell number-dependent ability to inhibit allogenic T cell proliferation in co-culture. uMSCs demonstrated greater inhibitory responses than BM-MSCs.

It was also found that the production of interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) of T cells significantly increased after co-culturing with uMSCs. Neutralizing antibodies to IL-10 and TGF-β abolished the inhibitory effects. Importantly, $CD4^{+}CD25^{+}$ and $CD8^{+}CD25^{+}$ T cells significantly increased after the co-culturing with uMSCs. Taken together, the results showed that uMSCs induced $CD4^{+}CD25^{+}$ regulatory T cells and modulated the immune system by secreting TGF-β1 and activating the downstream signaling pathways. The experiment's results have been promising.

D. Adult Hair Follicle Stem Cells

Role of TGF-b and Notch signaling in postnatal hair follicle cycling

The hair cycle provides a model system to study hair follicle morphogenesis and stem cell biology. Hair follicle stem cells, residing in a specialized niche called the bulge region, alternate between quiescent and activating state to maintain the hair growth during the hair cycle and re-epithelialize the denuded dermis after wounding. The group applied molecular biology, cell biology, and mouse conditional knockout studies to understand the role of TGF-b3 and Notch signaling in the hair follicle cycling. TGF-b signal has been

shown to generate cytostatic effect and Notch signal has been implicated in the cell fate determination. During recent years it has developed a transgenic mouse that allows the group to manipulate gene expression in TGF-b3 expressing cells, including the developing hair follicle. Moreover, a floxed OFUT1 mouse has been developed by Dr. Stanley's lab to study loss of function of Notch signaling. The groups is currently analyzing the hair follicle morphogenesis resulted from inactivating the Notch signal specifically in the TGF-b3 expressing region, including the hair follicle. The proposed research is likely to be important for understanding wound healing and hair loss disorder in humans.

Division of Biostatistics and Bioinformatics

Mission

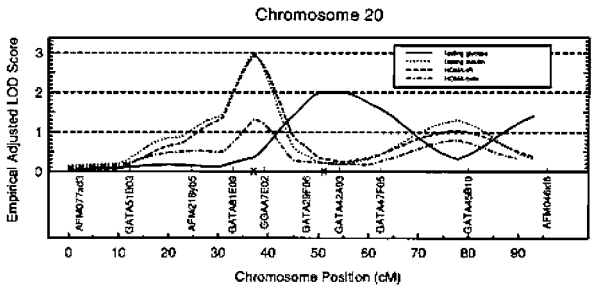
The Division of Biostatistics and Bioinformatics uses statistical knowledge and informatics technology to promote and improve health and welfare in Taiwan. This mission is accomplished by applying statistical principles to advance biomedical research and raise the standard of clinical trials, execute revolutionary and high-quality research on bioinformatics and statistical genetics to advance biotechnology and genomic medicine, administer high-quality theoretical and methodological research on biomedical statistics and bioinformatics, and provide training for researchers and dissemination of biomedical information. The division conducts both biostatistics and bioinformatics methodological research and collaborative research in the areas of clinical trials, pharmaceutical research, genetic studies, genomic studies, and epidemiological studies.

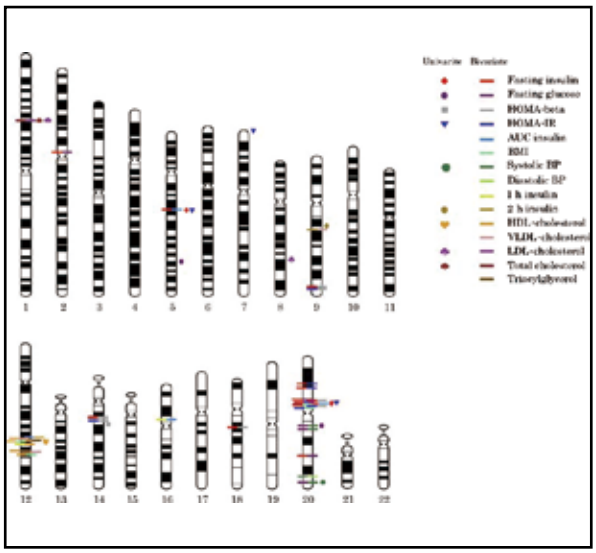
Major Progress

A. SAPPHIRe — a Genetic Study

The division has participated in an international genetic study, SAPPHIRe (the Stanford Asian Pacific Program in Hypertension and Insulin Resistance), which analyzes candidate genes and is performing a genome-wide search to identify major gene loci for hypertension. The study has already discovered insulin resistance syndrome (IRS) is familial in nature and heritable in Chinese and Japanese hypertensive families and that Sagittal abdominal diameter (SAD) as well as waist circumference predicts insulin sensitivity better than waist-to-hip ratio in non-diabetic Chinese hypertensive families. ACE (I/D) and aldosterone synthase (C-344T) are associated with insulin resistance in Chinese and Japanese hypertensive families.

The division had several important achievements in genetics in 2007. The pleiotropic effects of the locus at 37 cM on chromosome 20 are for the following pairs: (1) fasting insulin and insulin AUC (empirical p= 0.0006); (2) fasting insulin and homeostasis model assessment of beta cell function (HOMA-beta) (empirical p= 0.0051); and (3) HOMA of insulin resistance (IR) and HOMA-beta (empirical p= 0.0044). In addition, the peak logarithm of the odds (LOD) scores of linkage between a chromosomal locus and a trait for the pair fasting insulin and HOMA-IR rose to 5.10 (equivalent LOD score in univariate analysis, LOD[1]= 4.01, empirical p= 8.0×10^{-5}) from 3.67 and 3.42, respectively, for these two traits in univariate analysis. Additional significant linkage evidence was identified at 45 cM on chromosome 16 for the pair 1 h insulin and the AUC for insulin, with a LOD score of 4.29 (or $LOD_{[1]}$= 3.27, empirical p= 2.0×10^{-4}). This new locus is also likely to harbor the common genes regulating these two traits (p= 1.73×10^{-6}).





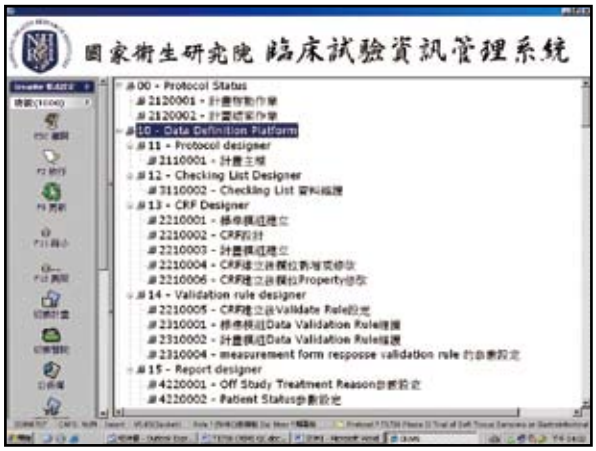
B. Clinical Trials

The division has developed a Clinical Research Information Management System. This customized software provides applications for clinical data management, statistical analysis, clinical operations, and the generation of summary reports on clinical studies. This system has been helping the Taiwan Cooperative Oncology Group (TCOG) with data management, statistical design, and the analysis of its multi-institutional clinical trials, including 12 ongoing trials, 12 terminated trials, and 3 protocols under review. In 2006, this system received FDA Part 11 Compliance accreditation.

The Division of Biostatistics and Bioinformatics established the Audit Committee for TCOG to ensure data quality and monitor the progress of multi-

center trials. In 2007 it audited TCOG clinical trials conducted at selected hospitals.

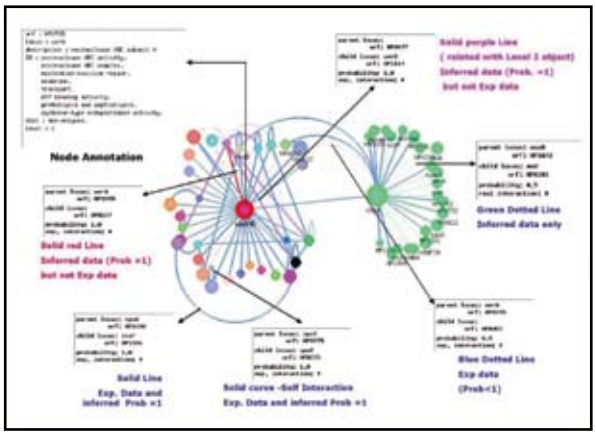
In the area of drug development, the Department of Health invited the division to provide consultation on infrastructure-building for clinical trials in Taiwan and also to participate in the National Clinical Trial Network to help statistical support for non-oncology clinical trials.

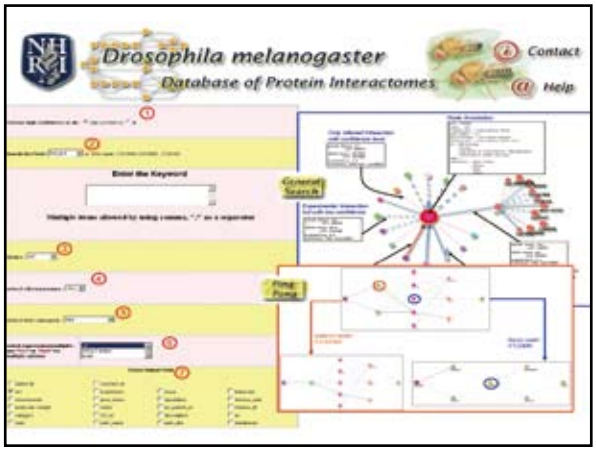


C. Bioinformatics

The Division of Biostatistics and Bioinformatics established the Microarray Analysis Laboratory to provide analytic and statistical support for genomic research projects. The Coding Sequence Database serves as a valuable source for comparative genomics research on more than 50 model organisms. For microarray analysis, the division established an experiment tracking system with normalization procedures. A Bayesian regression approach has also been proposed to analyze array-CGH (comparative genomic hybridization) data.

Achievements in protein-protein interaction database were particularly notable last year. The statistical model for protein interaction database for validation of two-hybrid assays of *Helicobacter pylori*, and prediction of putative protein interactions have been implemented. The *H. pylori* database of protein interactomes (*hp*-DPI) was developed with a succinct yet comprehensive visualization tool integrated with annotation from Genbank, GO, and KEGG. A database of protein interactomes of Drosophila (Fly-DPI) has also been developed.





D. Public Health and Epidemiology

The division has coordinated sampling design and data management for the National Health Interview Survey (NHIS), a large collaborative project with the Bureau of Health Promotion and the Bureau of Controlled Drugs of the Department of Health of Taiwan. The survey has been conducted twice so far: in 2001 and 2005. The next time will be in 2009. The division has developed an information system to help improve the survey and its quality control. The survey's web site at nhis.nhri.org.tw provides useful information. A separate computer-assisted personal interview will be integrated into the survey. The division has also incorporated ArcGIS software for geographic information system analysis of the survey outcomes.

Using the survey outcomes and other related datasets, the division is conducting several epidemiological studies on the relationship between obesity and metabolic disorder diseases, such as hypertension, diabetes, and high cholesterol. In addition, some methodologies on environmental health problems have been established or are close

to completion, including exposure assessment using a physiologically based toxicokinetic (PBTK) model with repeated biomarker measurements, spatiotemporal analysis of dioxin exposure and incinerator, and statistical methodology on dioxin fingerprint analysis.

Genetic epidemiological study of lung cancer

Through the end of 2007, 2850 lung cancer cases (1270 males and 1580 females) were collected, along with 2600 healthy controls (1381 males and 1219 females), 432 sibling-controls (170 males and 262 females), and 138 case-trios. For female cases and controls, after adjusting for age, ethnicity, smoking status, education, BMI, cooking habit, incense burning, regular motorcycle riding, and family female cancer history, the team found that use of hormone replacement therapy (HRT) could reduce relative risk of lung cancer by 30% (OR=0.70; 95% CI, 0.53-0.94; p=0.019). Frequent usage of a fume extractor also has a significant protective effect against lung cancer after appropriate adjustment.

E. Statistical Methodology Research

The division has made good progress in genetics statistics and survival analysis. Incorporating endophenotypes into a family-based allelic association study has been proposed. It is shown that the gain in statistical power of the proposed method is influenced not only by the correlation between the endophenotype and the phenotype of main interest, but also by the endophenotype value.

To improve the precision and efficiency of estimation location, a multipoint linkage disequilibrium mapping approach for case-control studies has been extended to incorporate information on factors influencing the effect of causal genes.

Division staff published several important papers in this area in 2007, including "Non-parametric maximum-likelihood estimation in a semiparametric mixture model for competing-risks data," which applied survival analysis and analysis of family data to good effect, and statistical methodology research papers in the area of evaluation of pharmaceutical products and microarray data analysis.

F. Biostatistics and Bioinformatics Workshops

The division organized two international symposia in 2007 to promote exchange among biostatisticians and biomedical researchers: the Symposium on Recent Developments of Statistics in Biological Sciences (with the National Research Program for Genomic Medicine) and Current Advances in the Evaluation of Research & Development of Translational Medicine (with NHRI's National Institute of Cancer Research).

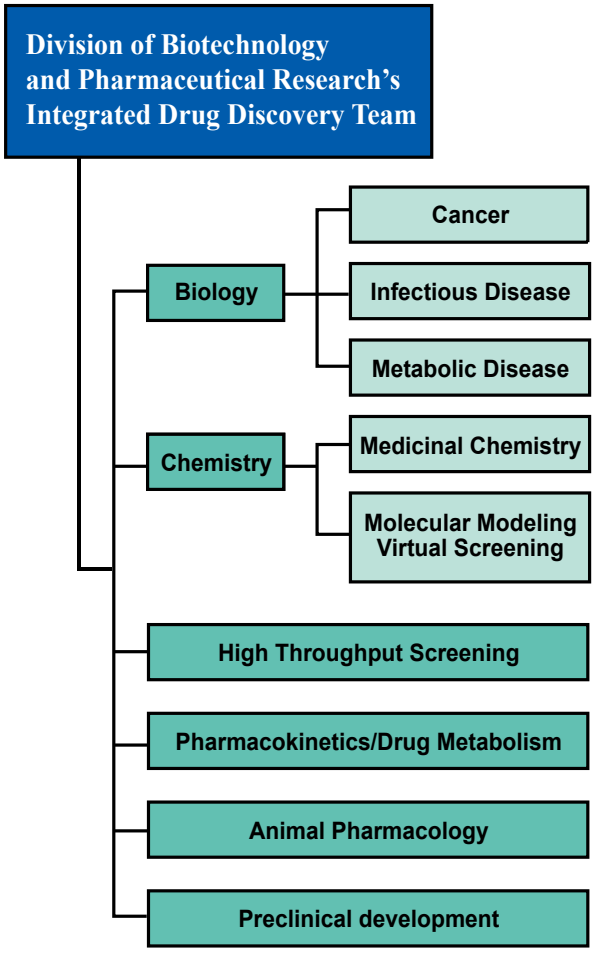
Division of Biotechnology and Pharmaceutical Research

Mission

Established in January 1998, the Division of Biotechnology and Pharmaceutical Research aims to establish, conduct, and support research in new medicines and biotechnology that leads to better health and quality of life. This significant assignment is executed via:

- establishment and maintenance of an integrated multi-disciplinary drug discovery team
- discovery and development of novel therapies in cancers and in infectious and metabolic syndrome diseases
- establishment of collaborative relationships with academic and industrial partners
- core-facility support for the National Science and Technology Program in Biotechnology and Pharmaceuticals.

Framework of Drug Discovery Programs



Introduction of Core Facilities and Services

- **High-Throughput Screening Core:** The core operates by integrating technologies of automated operation platform, assays for rapid detection, and database management. In the past year, several active compounds have been obtained, including anti-HCV compounds, DPP-IV inhibitors, and anti-cancer and anti-obesity compounds. Acting as a core facility for the National Science and Technology Program for Biotechnology and Pharmaceuticals (NSTP-BP), the high-throughput screening core continues providing services for *in vitro* testing of synthetic compounds and natural products for anti-cancer and anti-diabetes agents in various NSTP-BP projects.

Figure 1. High Throughput Screening System.

- **Recombinant Protein Core:** The core offers a unified technological infrastructure for efficient protein expression and purification to provide sufficient enzymes for large-scale compound screening as well as structural biology initiatives. The core is working to express and purify recombinant proteins, such as epidermal growth factor receptor (EGFR) kinase, Aurora A and B kinases, and influenza neuraminidases.
- **Medicinal Chemistry for Lead Optimization Core:** This supports mission-oriented projects within the division or cross divisions and synthesizes series of analogs for each identified pharmacophore in order to optimize the drug properties.

Figure 2. Compound library.

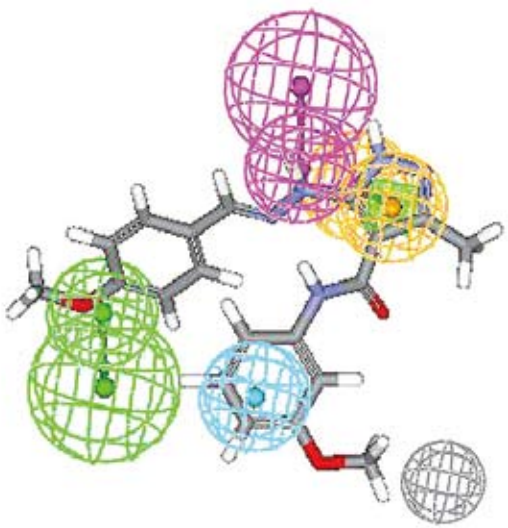


Figure 3. Pharmacophore hypothesis model (Molecular Modeling/Virtual Screening).

- ***In Vivo / Ex Vivo* Pharmacology Core:** The core has established several *in vivo / ex vivo* models and identified several drug leads/candidates against cancer, diabetes, and obesity. It also provides models for toxicological dose range finding in support of drug discovery and preclinical development.

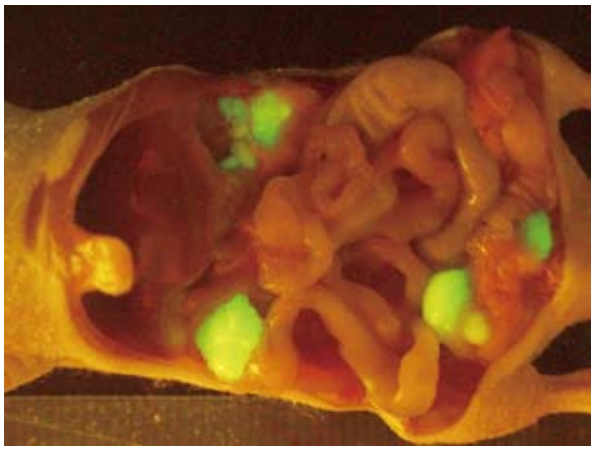


Figure 4. Diffusion of intraperitoneal gastric tumors (fluorescent parts are tumor cells).

- **Pharmacokinetics and Drug Metabolism (PKDM) Core:** This core provides pharmacokinetic (PK), pharmacodynamic, drug metabolism (DM), and toxicokinetic support in characterizing drug disposition and elimination in animals for lead optimization and identification of preclinical development candidates.

Figure 5. Liquid Chromatography Mass Spectrometer.

Major Progress

A. Discovery and Development of Lead Compounds and Drug candidates

Several drug candidates and lead compounds have been identified; and their patent rights have been filed. From January 2007 to June 2008, 44 papers were published and 8 patents were granted.

1. Anti-cancer drug discovery and development

Researchers with the division have discovered two classes of compounds with anti-tubulin activity: DBPR104 and DBPR204. DBPR104, which underwent acute and sub-acute toxicology studies during 2005 and 2006, demonstrates anti-mitotic activity and potentiality in treatment of various malignancies, particularly in patients with drug resistance. DBPR204, an orally active drug candidate, has been in preclinical development since 2007. Meanwhile, both drug candidates have been approved by the Center for Drug Evaluation as critical path program index cases. (Both anti-cancer candidates have been transferred to SynCore Biotechnology Co., Ltd., a subsidiary of Sinphar Group, for further development and clinical studies in August 2008.)

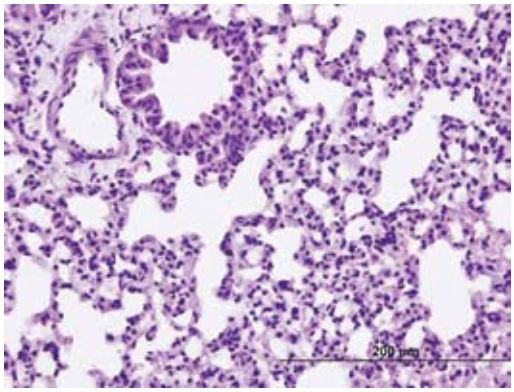


Figure 6. Biopsy of normal lung cell.

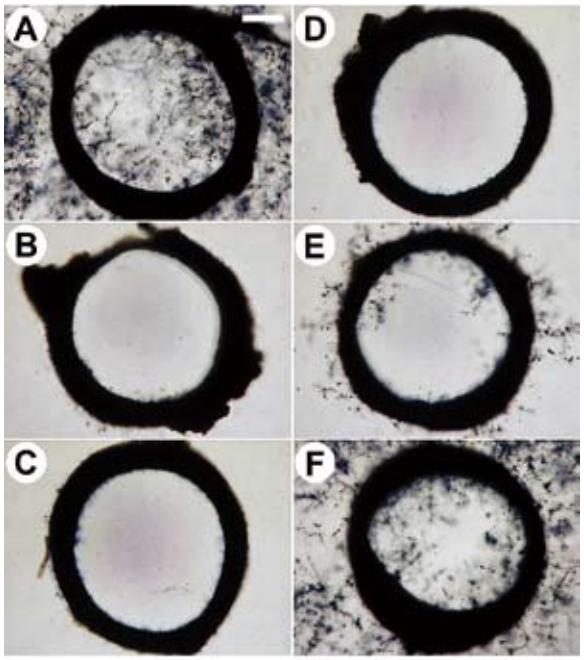


Figure 7. Endothelial growth in rat aorta tube formation assay.

Besides searching for tubulin inhibitors, the division initiated two new target-based cancer projects against Aurora and epidermal growth factor receptor kinase in 2007. The assay development and lead optimization are ongoing with the division's in-house platform technologies of virtual screening and knowledge-based rational design.

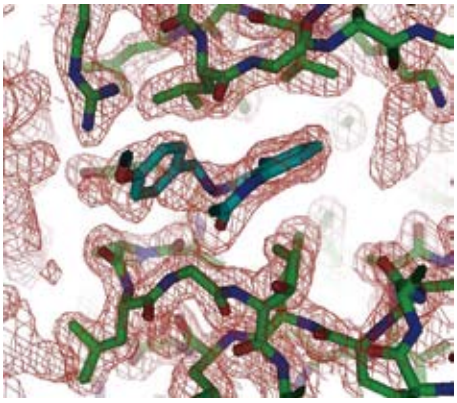


Figure 8. Protein X-ray crystallography density map.

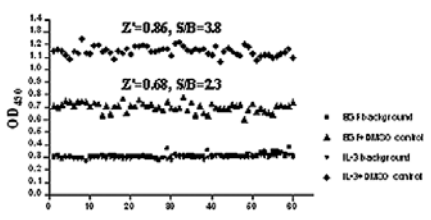


Figure 9. MTS assay optimization and performance.

2. Anti-diabetes drug discovery and development

Dipeptidyl peptidase IV (DPP-IV) inhibition is a new and promising approach for the treatment of type-II diabetes. The drug discovery team has developed four different classes of DPP-IV inhibitors. More than 500 analogues have been synthesized. One drug candidate, DBPR108, had been identified and under preclinical development in 2008.

3. Anti-obesity drug discovery

With the Division of Biotechnology and Pharmaceutical Research's well-developed assay screening, knowledge-based chemical design, and animal models, four series of compounds were discovered and several compounds among hundreds of synthesized analogues were found to exhibit significant weight-loss efficacy. These compounds have great potential therapeutic application in many metabolism-related indications in addition to the treatment of obesity, such as type II diabetes and atherosclerosis.

Figure 10. Breeding the obese mouse.

The Division of Biotechnology and Pharmaceutical Research is collaborating with a local pharmaceutical company on a two-year National Science Council research project aimed at discovering potential lead compounds and drug candidates for further preclinical development.

4. Anti-HCV drug discovery

More than 500 compounds have been synthesized; and recently several compounds were identified as orally bioavailable with high concentration in liver. Its derivatives have been synthesized and assayed in an HCV replicon system, showing similar antiviral activity against HCV. Lead optimization with desirable PK/DM properties for further development is underway.

5. Anti-dengue drug discovery

After four years of effort, the division has developed several crucial technologies to aid drug research. An effective dengue replicon containing reporter gene Luciferase was established. The dengue replicon can be transiently or stably expressed in BHK21 cells to determine the effect of small molecules on dengue virus replication. Dengue replicon is a useful tool for screening small molecules that block dengue virus replication. Most importantly, a patent application has been filed for a novel method to construct stable dengue infectious cDNA (RNA based). Recently, a DNA-based infectious cDNA was also successfully constructed. The knowledge gained from this work will greatly facilitate the study of anti-dengue therapies, such as in the development of drugs and vaccines.

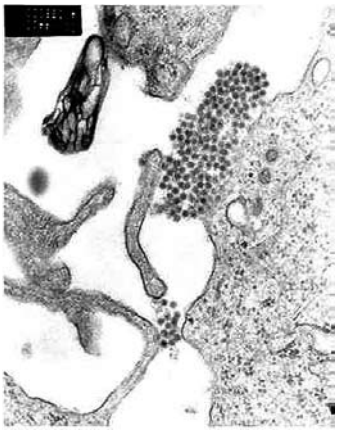


Figure 11. EM of mature dengue 2 virus (×123,000).

B. Preclinical Development of Drug Candidates

After a drug candidate is selected for further development, regulatory agencies require detailed information on the formulation, pharmacokinetics, drug metabolism, safety pharmacology, and animal toxicology of the new candidate for the investigational new drug submission. So far one anti-diabetes and two anti-cancer drug candidates have entered the preclinical development stage.

The division will continue to identify potential lead compounds and drug candidates for further preclinical development.

C. Public Service: meeting the national health challenges

Influenza remains a critical health issue throughout the world. To deal with this pressing health concern, the division has formed a research team with the scientists from Chang Gung University, National Tsing Hua University, National Chiao Tung University, and Academia Sinica to execute a project from the National Science Council on drug discovery against the influenza virus. Since August 2006 the team has been searching for novel compounds to inhibit the reproduction of the influenza virus. By means of well-established assay screening, some different types of molecular influenza virus inhibitors have been identified. More analogs will be synthesized according to structure-activity relationship to identify lead compounds for better anti-virus activity and further PK/DM analysis.

Another aspect of the division's work against influenza was working with Chang Gung University and National Taiwan University's College of Medicine to host the 2007 International Symposium on the Influenza Virus.

Figure 12. 2007 International Symposium on the Influenza Virus.

D. International Collaboration with Genelabs

NHRI, Genovate Biotechnology Co. (Genovate), and Genelabs Technologies Inc. entered into a collaborative research agreement on July 22, 2008, to jointly conduct further research to discover and develop compounds that target the hepatitis C virus.

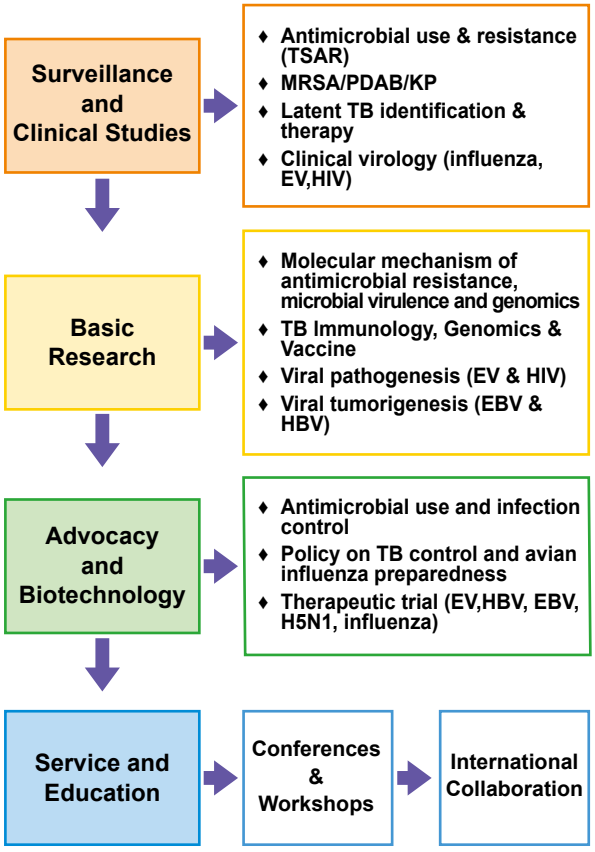
NHRI and Genelabs scientists will jointly work to discover and develop new HCV drug candidates by utilizing Genelabs' know-how and lead compounds on an existing target and NHRI's chemistry and drug discovery expertise.

Division of Clinical Research

Mission

The Division of Clinical Research conducts research on important infectious diseases in Taiwan and advises the government on policies to solve medical/health problems. The major research focuses of the division are surveillance of antimicrobial resistance and usage, clinical virology (influenza and enteroviruses), and tumor virology, complemented by studies on molecular epidemiology, mechanisms of antimicrobial resistance, and the pathogenesis and virulence of important infectious agents. Since 2004 the division has also conducted research on tuberculosis to assist in controlling this public health threat. An HIV/AIDS study group was formed in 2006. In addition to basic and clinical research, symposia and educational workshops are held regularly to promote interaction among researchers and policy makers to seek consensus on important national policies. Since infectious diseases are a global problem, international collaboration is also encouraged.

Organization Chart



Major Progress

A. Advocacy on Judicious Antimicrobial Use and Research on Antimicrobial Resistance

1. Taiwan Surveillance of Antimicrobial Resistance and advocacy on antimicrobial control policy

Taiwan Surveillance Antimicrobial Resistance is a national surveillance program of antimicrobial resistance, executed mainly by Dr. Yang Lauderdale's team. The program's advocacy to the government in 2001 resulted in an improvement in outpatient clinic's usage of antibiotics. For example, resistance to erythromycin in Gr. A streptococci, which was greater than 50% before 2001, decreased to 17% in 2004, and dropped to 15% in 2006. Significant increases in multidrug-resistant organisms, however, remain to be overcome. Observed increases include methicillin-resistant *S. aureus* (MRSA), which increased from 36% in 2004 to 48% in 2006 in *S. aureus* isolated from outpatients , and the proportion of carbapenem-resistant *Acinetobacter baumannii* (CRAB), which increased from less than 3% in 2002 to 32% in 2006 ($p<0.001$).

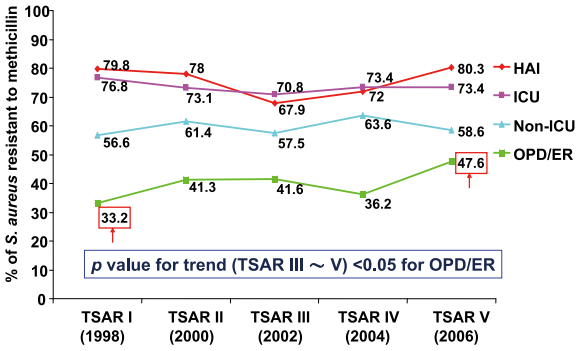


Figure 1. MRSA prevalence in different patient groups.

In 2007 the Division of Clinical Research advised the government to strengthen enforcement of infection control in intensive care units. The division has also been involved in the surveillance of antimicrobial resistance in zoonotic pathogens. The high rate of fluoroquinolone-reduced susceptibility in *Salmonella* (30%) and resistance in *Campylobacter* (>90%) raises an important issue for future control policy in Taiwan.

In fungal infections, Dr. Lo's team conducted the third round of Taiwan Surveillance Antimicrobial Resistance of Yeasts in 2006. *Candida albicans* was the most frequently isolated species, followed by *Candida tropicalis* and *Candida glabrata*. Importantly, the susceptibility to fluconazole changed

significantly. Of the isolates collected in 1999 and 2006, 8.4% and 17.1%, respectively, had minimum inhibitory concentrations to fluconazole ≥ 64 μg/ml, considered as resistant (*Diagn Microbiol Infect Dis*. 2008). In addition, two related diploid sequence types (DSTs, 140 and 98) of *C. tropicalis* isolates with high minimum inhibitory concentrations of fluconazole circulated in Taiwan from 1999 to 2006 (*Diagn Microbiol Infect Dis*. 2007, *J Med Microbiol*. 2007).

Table 1. The susceptibility to fluconazole of *Candida* species collected in TSARY 2006.

MICs (μg/ml)	*C. albicans*	*C. trpicalis*	*C. glabrata*	*C. parapsilosis*	*C. krusei*	Others	Total
≤ 8	407 (97.1)[a]	108 (43.9)	169 (80.1)	61 (98.4)	0	11 (91.7)	756 (78.4)
16-32	2 (0.5)	6 (2.4)	33 (15.6)	1 (1.6)	0	1 (8.3)	43 (4.5)
≥ 64	10 (2.4)	132 (53.7)	9 (4.3)	0	14 (100)	0	165 (17.1)
Total	419	246	211	62	14	12	964

MIC stands for minimum inhibitory concentration. [a]Number of isolates (percentage).

2. Molecular mechanism of drug resistance and bacterial virulence

In genomic studies on *Klebsiella pneumoniae*, the division's Dr. Yang Lauderdale, in collaboration with the Division of Molecular and Genomic Medicine, identified a novel plasmid containing *qnrS* quinolone resistance determinant and SHV-2 β-lactamase. The complete sequence of another plasmid carrying both CTX-M-type ESBL and CMY-type AmpC β-lactamase genes was also reported (*Antimicrob Agents Chemother*. 2006 & 2007).

K. pneumoniae was found to be closely associated with liver abscess in diabetes in Taiwan. Dr. Siu's team was the first group to identify that the serotype K1and K2 *K. pneumoniae* were the major types of *K. pneumoniae* responsible for the development of liver abscess as well as complicated endophthalmitis. They further demonstrated K1/K2 *K. pneumoniae* isolates were more virulent than non-K1/K2 *K. pneumoniae* isolates in neutrophils phagocytosis and intracellular killing model, as well as in the animal model (*Gut*. 2002, *Microbes Infect*. 2004, *J Clin Microbiol*. 2007). In diabetes, they identified that the risk factor of poor glycemic control may contribute to development of liver abscess and distant metastasis (*J Clin Endocr Metab*. 2006).

In drug resistance of fungus, Dr. Lo's team has identified that Rep1p is involved in drug resistance by negatively regulating efflux pump *MDR1* in *C. albicans*. Recently, the team has shown non-lethal *cph1/cph1 efg1/efg1* mutant cells partially protect mice from systemic infections by lethal wild-type *C. albicans* cells (submitted, 2008). The team has also shown that unlike other known peptides (P113 and Pac525) of which antifungal activities were blocked by salts, the antifungal activities of D-Nal-Pac525 are independent from salts.

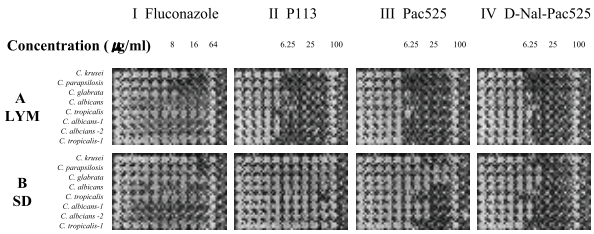


Figure 2. The antifungal activities of D-NAL-Pac525 peptides are independent from salt.

The accessory gene regulator (*agr*) locus controls many of the virulence toxins involved in *Staphylococcus aureus* pathogenesis. AgrC is a two-component signaling receptor for sensing AIP signal molecules. Dr. Chen's lab has successfully identified the key residues, T101A, V107S, and I116S responsible for AgrC activation. This finding provides information for designing AgrC receptor-ligand inhibitors as therapeutic alternatives to control *S. aureus* infections.

B. Research and Advocacy on Tuberculosis Control

Tuberculosis continues to be a major cause of disease and death in Taiwan. Dr. Dou and Dr. Su coordinated a study of molecular epidemiology and genomics of TB strains from different ethnic populations (aborigines and elderly veterans) in Taiwan. They found the Beijing strain predominates among TB patients in the general population (52%) and among veterans (72%), but that the rare Haarlem strain is prevalent (47%) among Taiwan's aborigines (*Infect Genet Evol*. 2008), which is probably related to Taiwan's colonization by the Dutch in the seventeenth century. For Beijing strains, the aborigines have an ancient genotype, distinct from the Han population. The association of TB strains with ethnic and population migration is interesting. In another population-based study in Taipei, the Beijing strain was prevalent, with up to 85% of TB patients below 25 years old; moreover, the Beijing family is associated with a high drug resistance. Both findings suggest a serious issue for TB control in Taiwan (*BMC Infectious Diseases*, revised).

Table 2. Distribution of RD genotype - defined subgroup of *M. tuberculosis* Beijing genotype in the general population, veterans, and aborigine patients.

Population	% of isolates in each RD genotype-defined subgroup strains[a]					
	group 1	group 2	group 3	group 4	group 5	unclassified
General population	0	0	12%	12%	76%	0
Veterans	0	5%	79%	14%	0	2%*
Aborigines	0	31%	44%	25%	0	0

[a] Subgroup 1. RD105 deletion; Subgroup 2. Concurrent deletion of RD105, RD207; Subgroup 3. Concurrent deletion of RD105, RD207, and RD181; Subgroup 4. Concurrent deletion of RD105, RD207, RD181, and RD150; Subgroup 5. Concurrent deletion of RD105, RD207, RD181, RD142 .

*Contain RD105, RD207, RD181, RD150, and RD142.

C. Surveillance of Viruses (Influenza and Enteroviruses) and Preparedness for a Flu Pandemic

1. Surveillance of Influenza Virus

In collaboration with Taiwan's Centers for Disease Control and Professor Shih of Chang Gung Memorial University, Dr. Su and Dr. Wang demonstrated that the influenza virus strain circulating in Taiwan is consistently two years ahead of the vaccine strain recommended by WHO (*J Clin Microbiol*. 2005 & 2006). Dr. Derek Smith, a member of the WHO vaccine committee, invited NHRI's Tainan Virology Laboratory to join the WHO influenza surveillance network as part of the influenza vaccine strain selection process. Dr. Wang and Dr. Chi further demonstrated reassortment of the influenza B virus circulating in Taiwan in 2004/2005, and that the circulating strain was distinct from the vaccine strain (*J Clin Microbiol*. 2006). There is considerable similarity of clinical features between influenza A and B in children. The B Yamagata-like strains were associated with more invasive infections. (*Pediatr Infect Dis J*. 2008). These data together raised important issues on vaccine policy in Taiwan.

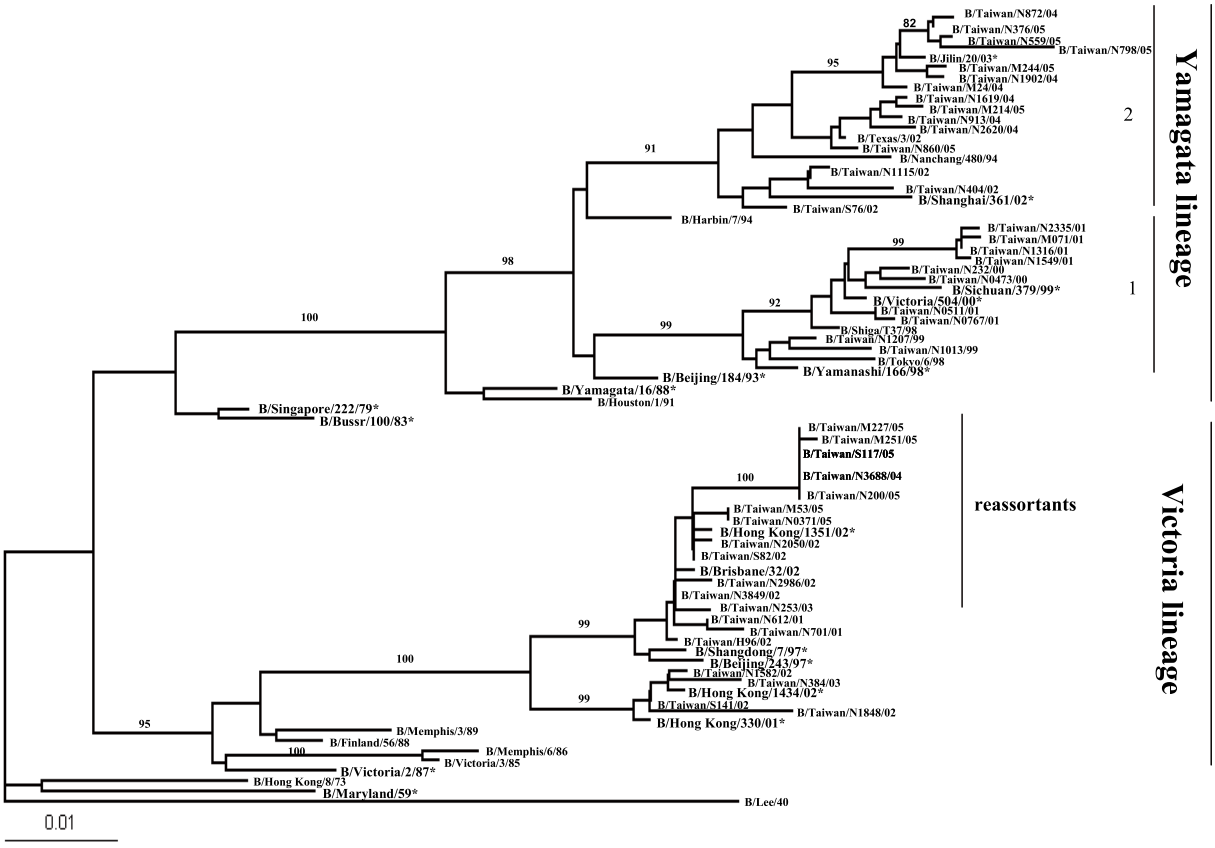


Figure 3. Phylogenetic analysis of HA genes of influenza B virus isolates obtained during the 1999 - 2005 influenza season.

2. Surveillance of Enteroviruses

In enterovirus research, Dr. Wang's team and Dr. Tseng demonstrated the recombination and co-circulation of EV71 and CA16, a finding with important implications for EV71 vaccine development. The epidemiological survey on enteroviral infections that occurred in Taiwan between 2000 and 2005 also provides important data for controlling this disease (*J Med Virol*. 2007).

3. National preparedness for H5N1 Avian Influenza

The Division of Clinical Research has continued to coordinate national policy on H5N1 preparedness, such as through the March 2008 publication of the third edition of a book on H5N1 that provides guidelines to educate the medical community and the public. Collaboration with Professor Osterhaus of Erasmus University in the Netherlands on the prevention of H5N1 infection using a natural product (algae) has recently been completed (June 2008).

Figure 4. Reference book of H5N1.

D. Tumor Virology

1. Pathogenesis and therapy of HBV-related hepatocarcinogenesis

Dr. Su's laboratory demonstrated HBV pre-S mutants play a potential role in the pathogenesis of hepatocellular carcinoma (HCC) and that ground glass hepatocytes represent a preneoplastic lesion of HCC. The pre-S2 mutant, as a viral oncoprotein, can selectively upregulate cyclin A and induce nodular proliferation and the transformation of hepatocytes in transgenic mice. Signal pathways such as VEGF/Akt/mTOR and JAB1/p27/RB/Cyclin A were identified to be associated with tumor development and are potential candidates for prevention or target therapy (*Hepatology*. 2001 & 2005, *Am J Pathol*. 2003, *J Biol Chem*. 2004, *Carcinogenesis* 2004, *Mol Cancer Res*. 2007, *J Gastroenterol Hepatol*. 2008).

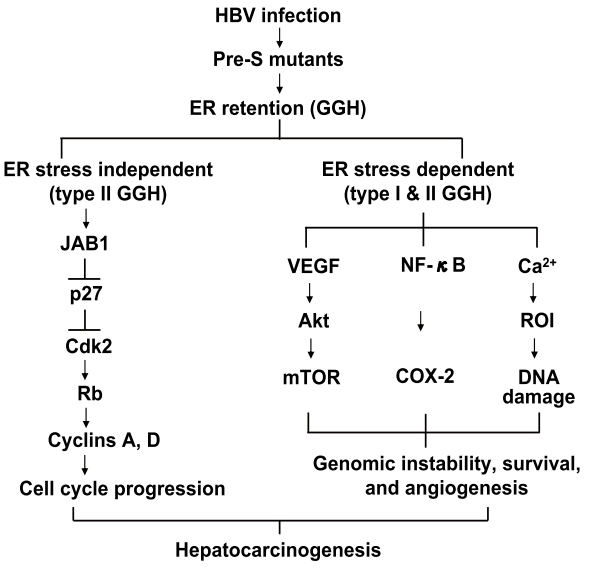


Figure 5. Schematic depiction of the potential signals induced by pre-S mutants and in ground glass hepatocyte (GGH).

2. Pathogenesis and therapy of EBV-associated hemophagocytic syndrome and malignancies (T cell lymphoma and nasopharyngeal carcinoma)

Dr. Su's team demonstrated that the EBV LMP-1 protein can inhibit the XLP-SAP expression and activate the TRAF2,5/NFkB/ERK signaling pathway via ATF-5, leading to enhanced cytokine production, providing a common pathway or pathogenesis for hemophagocytic syndrome (*Blood*. 2005, *Am J Pathol*.

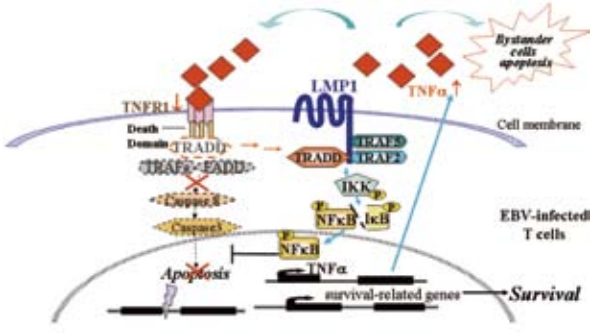


Figure 6. A diagrammatic depiction of the pathogenesis and molecular mechanism for the progression from HLH to chronic active disease or T-cell lymphoma in EBV-infected T cells.

2007 & 2008). Activation of NFkB represents a key pathway for the progression from EBV infection of T cells to T cell lymphoma (*Am J Pathol*. 2007). Animal studies using a PPAR agonist, rosiglitazone, revealed improved control for virus-associated hemophagocytic syndrome (*Blood*. 2005, *Am J Path*. 2007, *J Formos Med Assoc*. 2008, *Blood* submitted).

In NPC studies, Dr. Chang's team found that EBV lytic transactivator Zta enhances chemotactic activity through induction of interleukin-8 in NPC cells, providing a potential link between EBV lytic infection and pathogenesis of NPC (*J Virol*. 2008). In 2008, Dr. Chang also demonstrated a role of endoplasmic reticulum stress in upregulation of an EBV oncoprotein LMP-1, providing a novel mechanism for development and progression of NPC (2008, in submission).

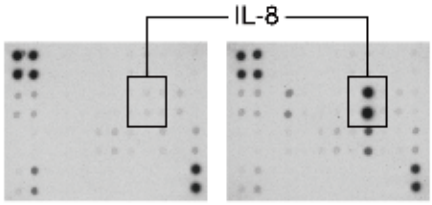


Figure 7. Interleukine-8 (IL-8) is significantly induced upon EBV reactivation in a NPC cell line.

E. International Cooperation

In 2006 the division initiated the Vietnam Virology Laboratory project in cooperation with Taiwan's Centers for Disease Control and National Cheng Kung University Hospital. The project studies enterovirus epidemiology, Milrinone trials for EV71 lung edema, and EBV-hemophagocytic syndrome in Vietnam. In 2008, the division tested serum and throat swab samples from more than 300 Vietnamese patients and found that EV71 and EBV-associated hemophagocytic syndrome are prevalent in Vietnam. Once the project is completed in 2009, the data will be analyzed for publication.

F. Symposia and Education

Since 2007 the division has held 15 academic symposia, workshops, and scientific meetings. The first and second NHRI TB symposia were held on March 3, 2007, and March 8, 2008, respectively, to provide a regular discussion platform among TB researchers and government officers. The Symposium of Medical Mycology was held on June 8, 2007. A MIRL symposium was held on June 9, 2007, to summarize the findings of TSAR and other research projects and to form a consensus to advocate for government actions on controlling antimicrobial misuse and resistance. The 2008 Antimicrobial Susceptibility Testing International Workshop will be held on September 25 and 26.

Figure 8. International cooperation — Vietnam virology laboratory.

Division of Environmental Health and Occupational Medicine

Mission

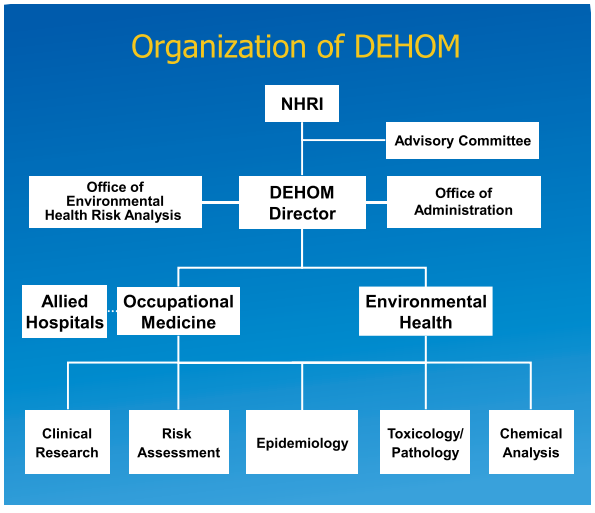
The Division of Environmental Health and Occupational Medicine sets the following as its mission:

- establish world-class research laboratories and conduct vigorous research to address various environmentally and occupationally related health issues of importance in Taiwan;
- establish research teamwork and networks with scientists in other governmental agencies, academic institutes, and research organizations, in efforts to create critical mass and coordinated research in Taiwan;
- promote education and training of future health scientists for the nation;
- provide scientific consultations, assistance, and research expertise to the government in Taiwan on critical health issues confronted by the nation.

Organization Chart

The division now in its ninth year, has 9 Principal investigators, 13 postdoctoral fellows, 17 research assistants, and 3 administrative staff.

In addition, the division operates the mission-oriented Office of Environmental Health Risk Analysis.



Major research themes and activities

The division focuses on three main research-development areas of special importance in Taiwan: metal toxicology, with a special emphasis on arsenic carcinogenesis and cardiovascular diseases; persistent organic pollutants (POPs), with special attention on endocrine disruptors; and issues related to occupational medicine. The division also helps ensure safety in Taiwan by answering the government's call to investigate environmental health concerns in Taiwan, such as dioxin found in ducks and the health risks of importing beef from countries with cases of BSE.

The division has also participated in two NHRI-initiated projects: "Toxicogenomic Studies on Genes and Environmental Interaction of Female Lung Adenocarcinoma in Taiwan" and the Department of Health's priority action project, "Evidence-Based Health Policy Research and Development."

1. Exposure to arsenic was found to enhance the carcinogenicity of tobacco smoke due to alteration of metabolism of pro-carcinogens and p53-related tumor-suppression mechanisms. The enhanced carcinogenicity of tobacco smoke was also increased by excretion of DNA adducts in the urine, including 8-hydroxy-2'-deoxyguanosine and N7-methylguanine. Arsenic was found to potentiate the TNF-α-induced VCAM-1 expression in a redox-sensitive manner. The mechanistic basis of vascular effects of arsenic was elucidated as related to this TNF-α-induction and associated inductions of adhesion molecules and inflammatory cytokines, fibrin deposition, modulation of nitric oxide production, increase in vascular permeability, and apoptosis. The genetic basis of variability in human metabolism of arsenic was initiated in an established cohort from an arseniasis-endemic area to examine polymorphisms of the genes that encode enzymes responsible for arsenic metabolism, such as GSTO1, GSTO2, and AS3MT.

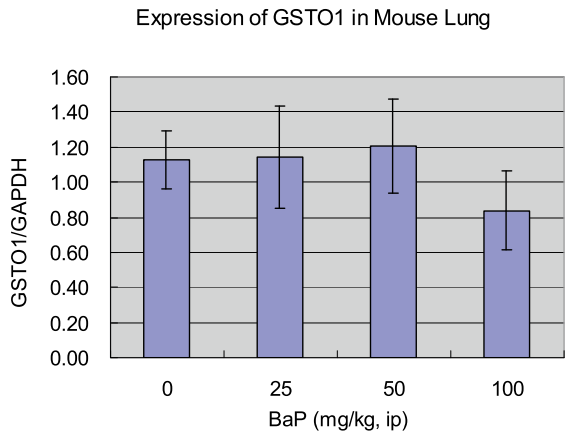


Figure 1. Expression of GSTO1 in mouse liver and lung after treatment of BaP. Male ICR mice (n = 3) received 0, 25, 50, and 100 mg/kg/day of BaP via intra-peritoneal injection for 3 days. At day 4, mice were sacrificed and their liver and lung tissues collected for RNA extraction. Expression of the GSTO1 gene was determined by real-time PCR and standardized by the housekeeping gene GAPDH.

2. For persistent organic pollutants (POPs) in the environment — including dioxins, polychlorinated biphenyls (PCB), and phthalate (PVC stabilizer) — human studies have been set up to examine the epidemiological associations between exposure to dioxins and PCB and endometriosis in women at fertile age, and between maternal exposure to dioxins and intelligence development in children. The endocrine disruption effects of phthalate and their interactions with genes are being especially examined.

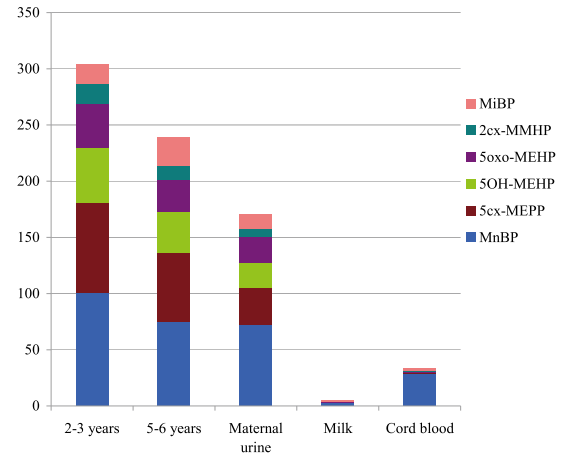
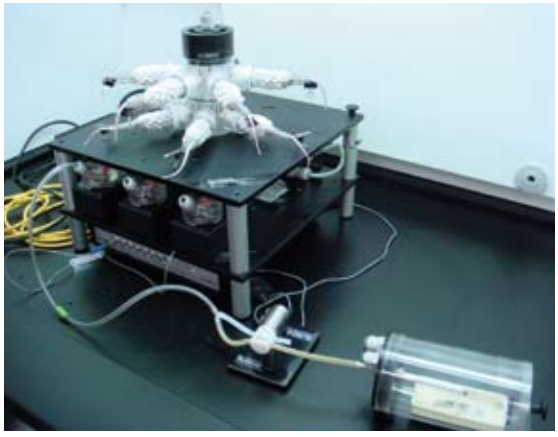


Figure 2. Concentrations (ug/L) of major phthalate metabolites in different specimens and ages from mothers, and their breast milk and children.

3. In addition to its occupational medicine physician - training program, the team is engaged in occupational medicine research focused on (1) occupational lung diseases (including asthma, COPD), (2) allergic skin diseases, (3) occupational cancers, and (4) herniation of intervertebral disc (HIVD).

4. Female lung adenocarcinoma is a disease of particular concern in Taiwan. The team is investigating gene and environment interactions related to this disease to aid prevention and disease-intervention efforts. The team has found that all AhR agonists may be involved in the development of lung adenocarcinoma, and interruptions of AhR related signal pathways may result in disease intervention. A rapid mutagenesis test and PAH analytical method were developed to characterize environmental mixtures as risk factors of female lung adenocarcinoma. Results indicate that $PM_{2.5}$ fractions of motor scooter emissions and cigarette smoke may be potent causative agents of female lung adenocarcinoma. Reduction of exposure to these agents may help prevent female lung adenocarcinoma. Animal models were established to demonstrate that exposure to benzo[a]pyrene stimulated tumor growth of hormone receptor specific lung adenocarcinoma cells in a dose-dependent manner in female nude mice but not in male nude mice.



5. In 2007 the Department of Health initiated the **National Risk Center Initiative Pilot Project** to investigate a host of environmental hazards of national concern, including high levels of heavy metals (Ni, Cd, Cr, As) in farm soils in Changhua County, acrylamide in high-temperature-processed foods, and airborne $PM_{2.5}$ in the ambient environment. The division continues to play an important role in these investigations by using the Geography Information System.

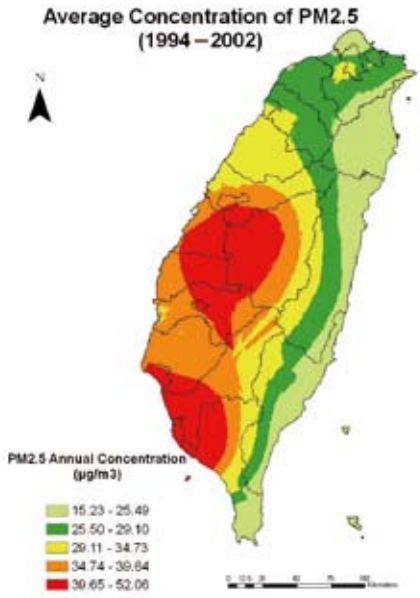


Figure 3. Spatial distribution of $PM_{2.5}$ converted from PM_{10}, 1994–2002.

Major Progress

A. Arsenic Research Program

The division has explored this issue with both *in vitro* and *in vivo* approaches. Epidemiological observations need validation with animal-model studies. With an animal model the division validated the epidemiological observation on synergistic interaction between arsenic and cigarette smoke in enhancement of lung cancer development. Study by the division demonstrated that increased lung tumorigenesis indeed can be induced in A/J mice when these animals were co-exposed to arsenite and NNK, a component of cigarette smoke. Lung tumor incidence increased from 10% (animals treated with either arsenite or NNK) to 50% (animals co-treated with arsenite and NNK). This increased incidence (fivefold) is consistent with that observed in an epidemiological study reported in Taiwan.

The research team established several studies to better understand the disease mechanism. Two major carcinogens in cigarette smoke are NNK and BaP. In their studies, the team demonstrated that arsenic enhanced NNK and BaP metabolism via induction of CYP2A and CYP1A1 expressions and activations, respectively. Increased NNK metabolites and harmful DNA adducts (O6- and N7-MeG) were found in urine of animals co-exposed to NNK and arsenite. These metabolite markers may be helpful for human exposure studies. In summary, arsenite enhances the BPDE-DNA adduct-induced mutagenesis with no marked effect on repair of BPDE-DNA adducts, suggesting that arsenic, as combined with cigarette smoking, may act as a co-mutagen to promote the development of human lung cancer.

Over the past three years the division has shown that arsenite affects intracellular glutathione/redox status as well as NF-κB and AP-1 activities in vascular endothelial cells. It demonstrated arsenite enhances the TNF-α-induced adhesion expression via regulation of AP-1 and NF-κB activities in a glutathione-sensitive manner and showed that arsenite induces cytotoxicity by down-regulation of vascular endothelial nitric oxide synthase in vascular endothelial cells. Arsenite caused cell cycle arrest in a G_2/M phase that was accompanied by accumulation and/or phosphorylation of checkpoint-related molecules — including p53, Cdc25 phosphatases, and securin — in primary vascular endothelial cells. Moreover, study by the division indicates that TNF-α induces adhesion molecule expression and monocytes-endothelial monolayer binding mainly via activation of NF-κB in a glutathione-sensitive manner.

Methylated arsenic has been reported to be specifically carcinogenic toward lung tissues. Therefore, cigarette smoke or its components may also increase arsenic carcinogenicity in the lung by altering arsenic metabolism (methylation). AS3MT and GSTO1 are two major enzymes involved in arsenic metabolism. GSTO1 catalyzes the reduction of MMA(V), a rate-limiting step in arsenic biotransformation, while AS3MT is responsible for transferring methyl group to As(III) or MMA(III). An *in vitro* study has shown that BaP treatment increased the mRNA expression of GSTO1 by approximately 50% in both human and mouse hepatoma cell lines, but had no effects on AS3MT. Whether the change in GSTO1 expression results in different methylation capacity *in vivo* awaits confirmation in animals.

B. Persistent Organic Pollutants (POPs) Research Program

Polychlorinated biphenyls (PCBs) and dioxins are recognized environmental endocrine disruptors, which are environmentally persistent and may bio-accumulate in human bodies. These compounds, with half-lives of 7–14 years in humans, may result in various adverse effects, such as cancer, reproductive dysfunction, endocrine disruption (i.e., thyroid, growth, steroid hormones), and neuro-cognitive deficits. A new emerging and important endocrine disruptor, namely phthalate, was added in POPs research program for the

potential high exposure and sensitivity to the children.

In order to study the developmental effect associated with phthalate, the team utilized its birth cohort to first determine the phthalate internal doses in children aged 2–3 and 5–6 years. Second, the developmental effects and steroid hormone changes were examined according to the POPs and phthalate internal dose using a perspective cohort study design. The division found levels of PCDD/F and PCB levels in placenta comparable with those in studies in the United States, with mean levels of 12.9 TEq-pg/g lipid (SD = 4.9) for PCDD/Fs and 2.9 (SD = 1.6) for dioxin-like PCBs. A total of 118 newborns were studied with complete data, including dioxins/PCBs levels in the placenta and thyroid hormone status in the cord serum collected in 2003. Multivariate analyses showed independently and significantly decreased FT_4*TSH with increasing non-ortho PCBs ($r = -0.2$, $p<0.05$). DEHOM suggests that significant FT_4 feedback alterations to the hypothalamus result from in-utero exposure to non-ortho PCB. Insulin-like growth factor (IGF)-1, IGF binding globulin-3, and (thyroxine) × (thyroid stimulating hormone) (T_4*TSH) were significantly associated with increased placental weight and Quetelet Index (QI) in kg/m^2 (correlation coefficient: $r = 0.2 \sim 0.3$ $p<0.05$). Considering the vast existence of bio-accumulated dioxins and PCBs and the resultant body burden in modern society, the division suggests routine screening of both thyroid hormone levels and thyroid function in newborns.

Regarding mechanistic research, steroid hormones are essential to life and involved in a wide variety of physiological processes. Based on their regulatory properties, steroid hormones are classified into three types: mineralocorticoids, glucocorticoids, and sex steroids. The first two types are produced mainly by the adrenal gland. All steroids are derived from cholesterol via branches of conversion reactions catalyzed by cytochrome P450 hydroxylases and hydroxysteroid dehydrogenases. Tuning of steroid biosynthesis can alter the biological activities of steroids and yield beneficial or adverse effects. Our laboratory is particularly interested in the effects of PCBs and flavonoids on adrenal steroidogenesis.

The studies of pregnant women and their children help to promote education for a healthier next generation in Taiwan. The divisional experts — comprising epidemiologists, medical doctors, toxicologists, cell and molecular biologists, and biochemists — have formed a strong team to take a role in research that is not just leading in Taiwan but also important throughout the world.

C. Occupational Medicine Related Research

The division's occupational medicine program does not yet have its own clinical facilities at NHRI, so it established collaboration with Kaohsiung Medical University (May 2007) and National Taiwan University (May 2008). The collaboration with Kaohsiung Medical University has two branches: an occupational medicine physician training program and occupational-medicine-related research. Since Taiwan has a severe shortage of occupational medicine physicians and occupational diseases are often under-diagnosed in the nation, NHRI is actively recruiting candidates for occupational medicine specialties training. So far four occupational medicine physicians have enrolled in this new program. In its occupational-medicine-related research, the team at Kaohsiung Medical University has collected 296 adult asthmatic patients. Among them, 42 asthmatic cases (15%) were diagnosed as work-related asthma according to the 1995 ACCP criteria. In addition, 82 newly diagnosed lung cancer patients, 105 bladder cancer patients, and 325 community controls have been recruited. A detailed occupational-history and job-exposure matrix will be constructed to aid diagnosis of occupation-related cancers and to elaborate occupational risk factors.

Since June 2008, members of the division have been conducting research on occupational cancers and herniation of intervertebral disc (HIVD) at National Taiwan University. Case findings and confirmation of occupational lung, skin, and bladder cancers, as well as of leukemia, will be performed by retrospective review of patients admitted to National Taiwan University Hospital. Occupational risk factors of skin cancer will be studied by hospital-based case-control design. In addition, 375 HIVD cases and 375 controls will be recruited at National Taiwan University Hospital and hospitals in southern and central Taiwan. Occupational risk factors, rehabilitation programs, and return-to-work will be studied in this project.

D. Toxicogenomic Studies on Genes and Environmental Interaction for Prevention of Female Lung Adenocarcinoma in Taiwan

The mutagenicity potencies and aryl hydrocarbon receptor (AhR) activation activities of restaurant chimney exhaust, motorcycle exhaust, and cigarette smoke were evaluated with the Ames test and an AhR bioassay. From these three kinds of environmental

samples, restaurant exhaust contained the highest concentrations of polycyclic aromatic hydrocarbons (PAH). Motorcycle exhaust samples did not activate AhR *per se* but did increase TCDD's (a potent dioxin) AhR activating potency. Taken into account its cytotoxic and mutagenic effects, and its synergism with TCDD to induce AhR activity, MEP might be the most toxic risk factor among these three.

LC-MS/MS analytical methods for determination of naphthalene and 4-ABP induced DNA adducts, 1,2 naphthquinone (1,2-NPQ) protein adducts, and ttDDE exposure biomarkers have been developed. In this cross-sectional study, the team enrolled 206 workers in Chinese-style restaurants in northern Taiwan. Using log-transformed 8-OHdG as the dependent variable in multiple linear regression confirmed that cigarette smoking was the significant predictor (1.09-fold risk, $p = 0.001$) and that urinary 1-OHP was a marginal but significant predictor (1.06-fold risk, $p = 0.07$). These findings suggest that in addition to cigarette smoking, urinary 1-OHP reflected a small effect of COF exposure and oxidative DNA damage in workers in Chinese-style restaurants.

The genetic and epigenetic mechanisms for lung carcinogenesis induced by major components of COF, ttDDE, and benzo[a]pyrene (BaP), were investigated *in vitro* and *in vivo*. Exposure of COFs containing ttDDE may promote lung adenocarcinoma formation by causing DNA damages, epithelial hyperplasia, and chronic inflammation. BaP, the other COF component, may increase lung cancer risk by interacting with E_2 to produce genotoxic metabolites, OHE_2. Antioxidants such as NAC may prevent these tumorgenic effects induced by ttDDE and BaP in the lung.

E. Evidence-Based Health Policy Research and Development — the National Risk Center Initiative Pilot Project

Health risk assessment is the process of quantifying the probability of a harmful effect to individuals or populations from certain environmental exposures. Health problems caused by environmental pollution are a great challenge in Taiwan. The current risk assessment process (hazard identification, dose-response analysis, exposure assessment, and risk characterization) was carried out for three main issues in this research: probabilistic assessment of acrylamide intake from certain foods cooked at high temperature; associations between health effects and heavy-metal contamination in Changhua County, Taiwan; and evaluation of the health effects of air pollution and related regulatory policies in Taiwan, based on the hazard identification of $PM_{2.5}$ as a risk factor for CVD.

Results of acrylamide (AA) study showed that AA contents range from 7 to 105 ng/g in protein-based foods and from 6 to 321 ng/g in rice-based - less than in potato-based foods, which have the highest AA contents. Potato chips had the highest level in potato-based foods, at 8218–8240 ng/g. Similar to the potato-based foods, the fried fritter (*youtiao*), a wheat-flour-based product, also has a high AA content. AA concentrations in fritters range from 3674 to 4087 ng/g. Moreover, longer frying times and higher temperatures result in higher concentrations.

In a study of heavy metals, the geometric means of blood cadmium levels were 1.48 and 1.28 μg/L among men and women, respectively. In men, the blood cadmium level increased from 1.2, 1.4, 2.4, and 2.5 μg/L for non-smokers, ex-smokers, smokers of fewer than 21 cigarettes per day, and smokers of 21 or more cigarettes per day, respectively, after adjustment for age, occupation, and dietary habits ($p<0.001$ for trend test). In women, blood cadmium concentration was significantly ($p<0.01$) associated with decreasing mean corpuscular hemoglobin concentration (MCHC, $\beta = -0.90$) and increasing hematocrit ($\beta = 3.40$) after adjusting for ferritin level, serum creatinine, microglobulin, uric acid, and age. The odds ratios (ORs) of anemia increased to 5.14 ($p<0.05$) when concentrations of blood cadmium and β2-microglobulin were larger than 1.26 μg/L and 80.1μg/L, respectively. The cadmium levels were similar to those found in studies in the United States and Europe.

The results of the $PM_{2.5}$ study showed that $PM_{2.5}$ was lowest in Yilan County (annual average concentration was 22.68 $\mu g/m^3$) and highest in Kaohsiung (annual average concentration of 49.23 $\mu g/m^3$). If the $PM_{2.5}$ control standards are set at 15 $\mu g/m^3$, over 10 years an estimated 12,817 fewer people would die from lung cancer and 51,549 fewer people would die of cardiopulmonary diseases. If the standard is instead set at 12 $\mu g/m^3$, over 10 years 14,464 fewer people would die of lung cancer and 58,640 fewer people would die of cardiopulmonary diseases.

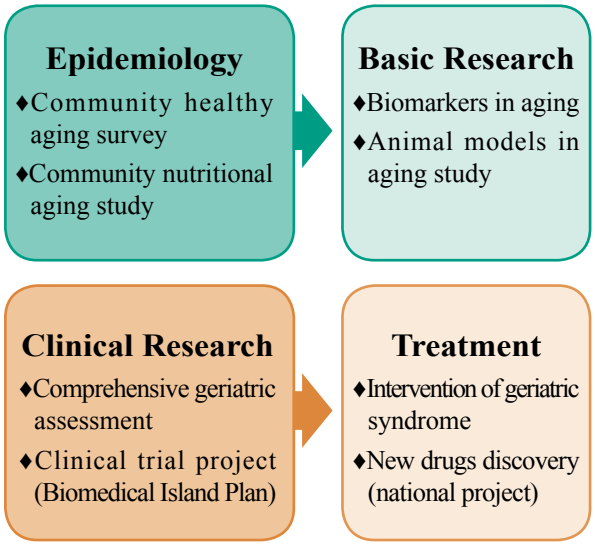
In addition, the efficacy of a nanoemulsion fuel additive to reduce emissions by motorcycles and automobiles will be demonstrated using the air samples collected from dilution tunnels connected to the exhaust pipes of automobiles and motor scooters.

Division of Geriatric Research

Mission

The Division of Geriatric Research strives to promote successful aging for the elderly in Taiwan through a wide spectrum of gerontology and geriatrics research based on various scientific models and programs. The division works toward this mission by designing and conducting a series of research programs to explore the issues of aging from clinical, epidemiological, and socioeconomic aspects; promoting geriatric subspecialist training program for elderly care and geriatric research at newly established geriatric departments and divisions at medical centers; and integrating all resources and research results for continual improvement of quality of life for the elderly.

Research Strategies



Major Progress

A. Epidemiology

1. Elderly cohort study on betel nut chewing and mortality

A total of 4,049 participants aged 60 years or older and 2,462 participants aged 50–66 years were enrolled in 1989 and 1996, respectively, in a population-based cohort study to determine long-term effects of betel nut chewing. Information regarding betel quid chewing and covariates were collected at baseline and updated at subsequent interviews. Proportional hazards analysis was performed to determine the effect of chewing on all-cause and cause-specific deaths. During a mean follow-up of 9.5 years, 2,309 deaths occurred. The division found that ever chewers were at higher risk of only total and cerebrovascular deaths, while increased chewing-years or quid-years appeared to be associated with increased mortality risk. Further studies are needed to better understand the possible mechanisms of death.

2. Taiwan isoflavone multicentric enrollment study

The use of traditional hormone replacement therapy for menopausal syndrome has been controversial due to its potential side effects. Phytoestrogen from soy-based foods has been proposed as an alternative to traditional hormone replacement therapy. Together with National Taiwan University Hospital, Changhua Christian Hospital, National Cheng Kung University Hospital, and members of the local medical community, the division has been investigating the effects of isoflavone on bone mineral density in Taiwanese women with postmenopausal osteopenia. A one-year study by the division observed that daily consumption of a maximum of 150 mg of isoflavone per day did not have any significant effects on bone mineral density. This study has been extended to a two-year observation with the daily dose increased to 300 mg to determine the therapeutic benefits of isoflavone on the maintenance of healthy bone mineral density, blood pressure, glucose, and lipids.

3. Taipei centenarian study — centenarian offspring sub - study

Based on the baseline Taipei Centenarian Study in 2005, the sub-study is conducted by the division to evaluate the natural children of centenarians on mortality risk factors — such as parents' age at death, prevalence of age - related disease, and functional status — assessed in different domains of function in

order to compare them with their spouses and other individuals. The division's collection of pre-designed questionnaires and saliva from the participants facilitate its comparison of centenarian offspring with controls on a variety of health outcomes, helping reveal the complex relationship between heredity and environment.

B. Clinical Research

1. Clinical Geriatrics Fellowship Training Program

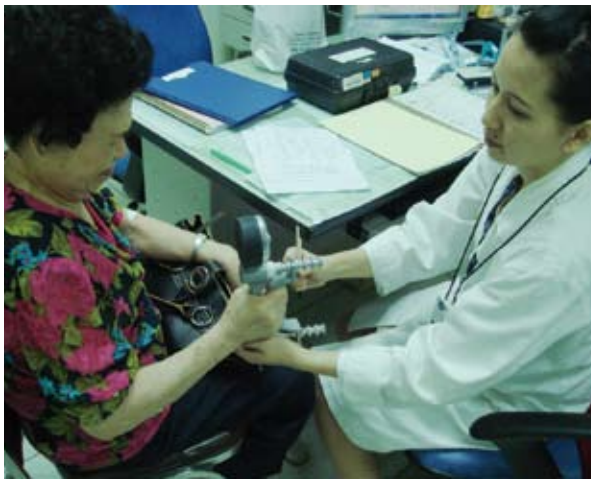
In collaboration with National Taiwan University Hospital, National Taipei College of Nursing, and Chang Gung Memorial Hospital, and with generous support from the Department of Geriatrics and Adult Development at Mount Sinai Medical Center, the division organized the pioneering NHRI Geriatrics Fellowship Training Program for clinical geriatricians in Taiwan. Despite the absence of any previous model for emulation and the scarcity of qualified geriatric faculty, the fellowship training program proved to be a success. Fifteen geriatricians completed the program between 2004 and 2006. In addition, the division published Taiwan's first clinical geriatrics training manual in February 2007 to facilitate the ongoing development of clinical geriatrics training in newly founded medical centers and further improve the quality of eldercare in Taiwan.

2. Clinical and laboratory predictors of all-cause mortality in older population

Although several studies have been conducted to explore how clinical and laboratory predictors are related to total mortality or cause-specific mortality in the elderly, the overall effect of these indicators on mortality has rarely been evaluated. A sample of 2,086 Kaohsiung residents age 65 or older and participating in population-based health examinations in 1995 and 1996 were followed up until the end of 2003. All participants completed medical history and underwent clinical assessment and 12 laboratory tests, including pulse rate, blood pressure, height, weight, serum level of cholesterol, triglyceride, creatinine, uric acid, as well as fasting blood glucose, hemoglobin, and red and white blood cell counts. The division found that systolic blood pressure, creatinine, uric acid, fasting blood glucose, and hemoglobin were statistically related to total mortality. This finding can be expected to assist geriatricians in evaluating patients' prognosis by establishing a mortality risk profile based on disease conditions and biomedical parameters.

3. Relationship of homocysteine levels to quadriceps strength, gait speed, and late-life disability in older adults

Elevated homocysteine is known to cause tissue injury by oxidative stress, endothelial damage, and protein homocysteinylation. It is also associated with multiple age-related problems, such as cardiovascular diseases, dementia, and osteoporotic fracture. Little, however, is known about the role of homocysteine in physical disability among older adults. The division elucidated that elevated homocysteine is associated with disability, instrumental activities of daily living, leisure and social activities, lower extremity mobility, and general physical activities. Homocysteine, however, has an inverse relationship to quadriceps strength and gait speed. Likewise, quadriceps strength seems to mediate the inverse association between homocysteine and gait speed. Elevated homocysteine is associated with multiple domains of disability, mediated in part by muscle strength and gait speed. The results suggest that homocysteine level may be an important indicator of performance status in older adults.



4. Cerebrovascular correlates of physical performance and cognitive function in old age

Disruption of frontal-subcortical circuits caused by impaired cerebral blood flow is often associated with carotid artery atherosclerosis and stenosis, while cerebral micro-angiopathic changes (cerebral white matter lesions or leukoaraiosis) are related to executive dysfunction, cognitive impairment, depression, and gait/balance problems. Based on the data collected from the annual health examination for the elderly, the study proposes to use carotid ultrasound and brain magnetic resonance imaging to evaluate carotid atherosclerosis and cerebral white matter lesions. Comprehensive examination of selected geriatric syndromes such as cognitive impairment, gait/balance disturbance, falls, and depression, will be performed. The division is examining how traditional cardiovascular risk factors — such as high blood pressure, diabetes mellitus, smoking, and high cholesterol, as well as cerebrovascular diseases, specifically carotid atherosclerosis and leukoaraiosis — will affect mood, cognition, and physical function in the elderly. Additional analyses will be performed to investigate the possible mediating roles of cerebrovascular diseases in the association between cardiovascular risk factors and geriatric syndromes.

5. Clinical assessment & treatment of frailty syndrome

Domestic research to define and measure frailty as a core concept of geriatric syndromes is warranted in Chinese society. As a core pathophysiologic phenomenon of declining functions during the aging process, frailty remains more a constellation of many conditions than a discrete clinical entity and is certainly in need of a precise scientific definition. Comprehensive geriatric assessment is a multi-dimensional and inter-disciplinary diagnostic process that aims to understand the medical, psychosocial, and functional problems of the frail elderly and to recommend a care plan for treatment and long-term follow-up. Currently 188 cases have been recruited in the experimental group and 112 cases in the control group. According to physical status, there are 36 frail cases, 103 pre-frail cases, and 49 non-frail cases. The study selects subjects for MRI spectrometry, with 57 such cases completed so far. Results relating to muscle power and MRI images in patients with various frailty status and laboratory data in the cohort elder patients will be analyzed and reported during 2008.

Division of Medical Engineering Research

Mission

The main missions of the Division of Medical Engineering Research are to maintain high standards in its research, further interdisciplinary and multi-institutional research collaboration, and promote the medical engineering industry in Taiwan with product-oriented research and development.

When it began in 2000 the division focused primarily on four traditional medical engineering research areas: biomechanics, biomaterials & tissue engineering, medical imaging, and biophotonics & instrumentation. While the division has continued to maintain its productive and award-winning research program in biomechanics, biomedical imaging has gradually become its main theme of research, resulting in two integrated research programs: interventional MRI and multi-modality molecular imaging platforms. These three research programs will help the division stay on top of technological innovations in medical engineering research.

At the beginning of 2007, the division was awarded a two-year product-development grant from the Executive Yuan's special "Medical Device Industry Priority Promotion Program." This has enabled the division to kick off the development of two promising medical imaging products — the MR guided high intensity focused ultrasound ablation system and a high frequency ultrasound system — taking mature technology developed within the division as well as with collaborating institutes, developing it into practical prototypes for animal or clinical testing, and preparing it for commercialization. The division established a team of product design engineers for this program. It believes the successful transition into practical products for the first two projects will help create a pipeline of innovative technology that will help build a biomedical imaging industry in Taiwan.

Major Progress

The division conducts research in the following four mission-oriented integrated programs:

- biomechanics and tissues engineering
- interventional MRI research
- multi-modality animal molecular imaging platform
- development of a biomedical imaging industry in Taiwan.

Below are some of the division's main accomplishments in 2007 and 2008.

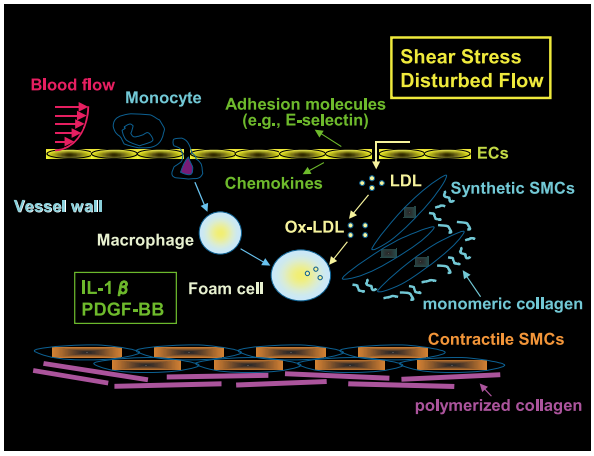
A. Biomechanics and Tissues Engineering

1. Genomic analysis and mechanotransduction in the focal origin of atherosclerosis: a spatial approach to endothelial and smooth muscle cell structure, gene expression, and function

Atherosclerosis is the principal contributor to the pathogenesis of heart attack and stroke. The key cell types involved in atherosclerosis include endothelial cells (ECs), smooth muscle cells (SMCs), and monocytes/macrophages. This project utilized genomic approaches to investigate the molecular and cellular mechanisms underlying the effect of disturbed flow on the pathogenesis of the focal origin of atherosclerosis.

The findings indicated that shear stress plays an inhibitory role in the pro-inflammatory gene expression in ECs located in close proximity to SMCs, thereby exerting anti-inflammatory effects on the vascular wall. Moreover, the study has elucidated the role of different levels of shear stress in modulating EC gene expression in response to tumor necrosis factor-α (TNF-α). The findings support the hypothesis that high levels of laminar shear stress serve anti-inflammatory and atheroprotective functions in vascular biology. In contrast, disturbed flow with a low and oscillatory shear stress elicits factors that induce EC expression of pathophysiologically relevant genes whose products may serve pro-inflammatory, pro-coagulant, proliferative, and pro-apoptotic functions and hence promote atherosclerosis.

The study also found that ECs co-cultured with SMCs embedded in collagen gels significantly increased the adhesion and transmigration of neutrophils, peripheral blood lymphocytes (PBLs), and monocytes under disturbed flow, particularly in



Shear stress regulates cell-cell interactions in atherogenesis.

the reattachment area. Co-culture of ECs and SMCs induced their expressions of adhesion molecules and chemokines, which contributed to the increased leukocyte adhesion and transmigration. These findings have provided insights into the mechanisms of leukocyte interaction with the vessel wall (composed of ECs and SMCs) under the complex flow environments found in regions of prevalence for atherogenesis.

The research group also demonstrated that the growth factor PDGF-BB and the cytokine IL-1β were cooperative in inducing phenotypic modulation of human aortic SMCs cultured on polymerized collagen from a contractile toward a synthetic phenotype. This SMC phenotypic modulation induced by PDGF-BB/IL- β was mediated by the receptor interaction and through the PDGFR- β/PI3K/Akt/mTOR/p70S6k signaling pathway.

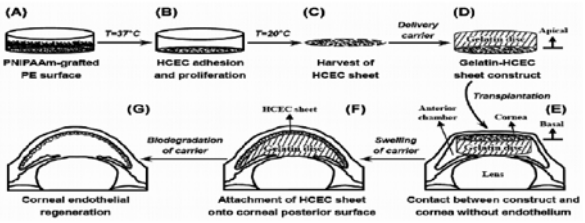
2. Damage of blood cells in cardiovascular devices: using helical stent as a model

The project aims to study blood cell damage in cardiovascular assist devices using a deformable helical stented conduit as a model. Multi-dimensional movements of the stented vessel wall were experimentally observed to include simultaneous stretching and twisting of the vessel wall *in vivo*. Structural dynamic methods were applied to analyze the stress distribution in the vessel wall and that in the moving blood cells. Setting up realistic and manageable boundary conditions in the study was a difficult challenge to overcome. The simulation that includes all possible geometrical and material nonlinearities is found difficult to converge in structural analysis. The group will develop a more realistic model to simulate the fluid-structure interaction, which can provide the coupling between the dynamics of blood flow and the dynamic vessel wall movements.

3. Investigation of the feasibility of "cell enhancement" by cultured human and rabbit corneal endothelial cells on a biodegradable membrane to improve the quality of cornea donors

At present, the source of cornea donors is limited in Taiwan. It is possible by enhancing the corneal endothelial cells (CECs) density of cornea donors to improve the availability and quality of donor corneas for surgery. The aim of this project is to test the feasibility of biodegradable membranes in transplanting corneal endothelial cells, applying this to restore human vision with the hope of reconstituting a structural and functional endothelial monolayer.

Results from a short-term study suggest that the transplanted cultured human CECs sheet could be integrated into rabbit corneas denuded of endothelium. In addition, the corneas have returned to a near normal thickness, indicating the usefulness of bioengineered human CECs sheet. The group has developed a novel strategy for corneal endothelial reconstruction with a bioengineered cell sheet. They have shown that it is possible to fabricate the bioengineered human corneal endothelium *in vitro* by the temperature-modulated detachment of cultured cell sheets from thermoresponsive supports. Based on the current methods of the group, adult human CECs monolayer (with normal morphology and viability) can be obtained without the need of cell carriers during cultivation. The group also found different regenerative abilities between central and peripheral of cornea endothelium cells; it may imply the existence of corneal endothelial committed stem cells in the corneal periphery.



A novel technology for corneal endothelial reconstruction with a bioengineered cell sheet by utilizing functional biomedical naterials.

B. Interventional Magnetic Resonance Imaging (iMRI) Research Program

The interventional magnetic resonance imaging (iMRI) research program began in 2005 and comprises eight component projects conducted by investigators in MED and those from National Taiwan University Hospital, Taipei Veterans General Hospital, and Chang-Gung Memorial Hospital. This program is both clinical driven and product oriented. High intensity focused ultrasound (HIFU) thermal ablation under magnetic resonance (MR) imaging guidance was identified to be the first product target for system integration; and the program has since established the system design and developed a research platform for preliminary animal testing. More importantly, this program has resulted in the disclosure and/or filing of up to five patents, a solid foundation for subsequent product development and commercialization efforts.

1. A general platform for *in vivo* MRI temperature monitoring of thermal ablation and hyperthermia procedures

A PC-based data processing platform has been constructed for MR-guided thermal ablation and hyperthermia procedures. It features a user-friendly graphic interface and can display a temperature map in real time during thermal treatment. A pig liver HIFU ablation experiment has been performed. The size and location of ablation lesion observed on sliced liver after the experiment are consistent with hot zones exceeding 50°C observed on the temperature images, confirming the accuracy of the MRI-guided thermal therapy system.

2. High intensity focused ultrasound treatment of hepatoma: guided by dynamic contrast enhanced MRI

The study group has developed DCE-MRI techniques for lung cancer. In 2007, the group developed another new imaging technique to measure protein denaturation and tested the technique in pork and liver specimens with HIFU operating in the MRI scanner. This novel technique allows them to study the relationship between physical effect (thermal dose) and biological effect (protein denaturation); a U.S. patent application has been filed for this. In the second half of 2007 the group continued to set up and perform MRI-guided HIFU treatment in animals. They began with the rabbit thigh as the target of heating and have implemented the burning operation successfully.

Figure 1.

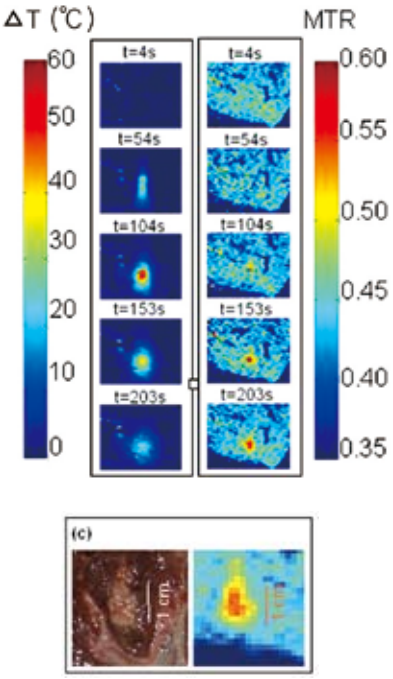
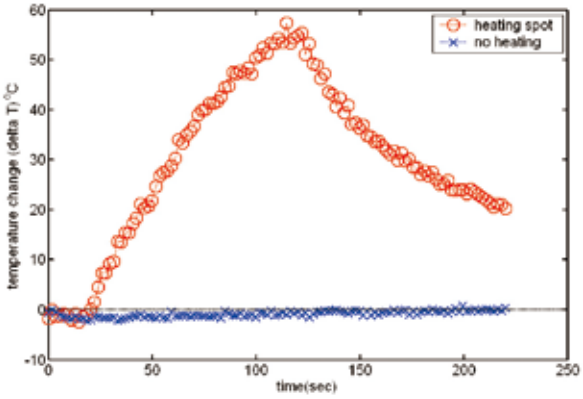


Figure 2.(a)



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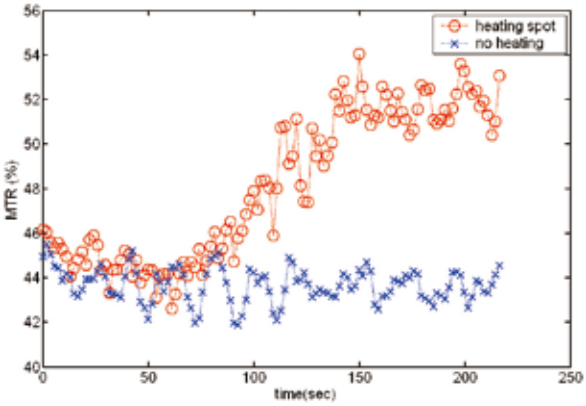
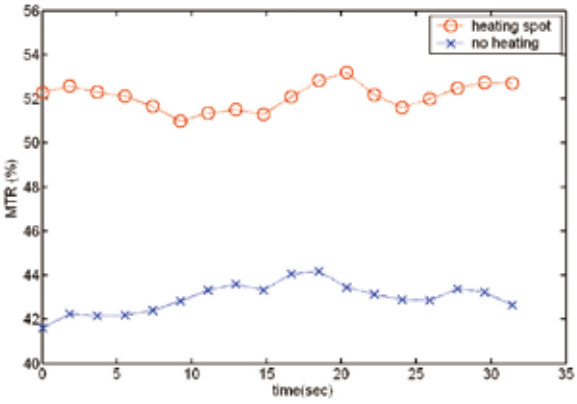


Figure 2.(c)



Pseudo-colored maps of temperature change (a) and MTR (b). Each picture represents different time points before HIFU heating (t=4 s), during heating (t=54 s, 104 s), and after heating (t=153 s, 203 s). Comparison of optical images of cut face of the heated tissue and the MTR map 2 min after turning off of HIFU (c). The time course of temperature change (a), and of MTR (b) in the heated (red) and non-heated areas. The time course of MTR 2 min after turning off the HIFU pulses (c).

3. Lesion formation and transformation during the treatment of experimental liver tumors using an MRI-guided high-intensity focused ultrasound system

In order to have better control of ablating lesions and reduce damage to adjacent normal tissue, this component project investigated the lesion-formation process during HIFU therapy. The team found that absolute temperature of 50°C and 6% change of MTR are the best parameters for depicting the borders of lesions after HIFU ablations. Moreover, MTR values are more stable immediately after an ablation, rather than during the ablation.

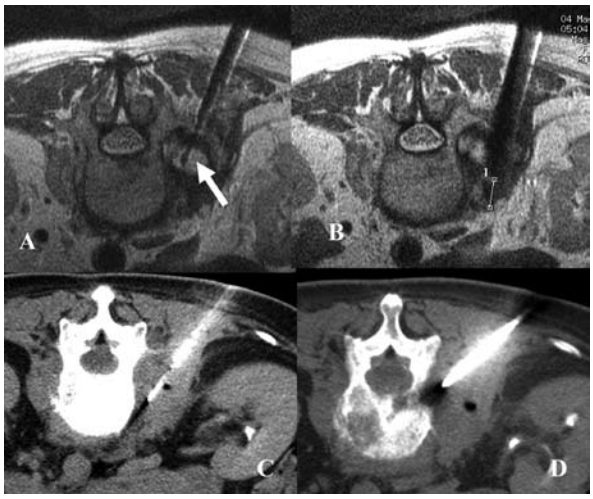
4. Investigation of HIFU (high intensity focused ultrasound) thermal therapy systems for tumor treatments

Imaging sampling rate, system movement, imaging data processing, and signal transmitting are the most important factors affecting tracking accuracy in the development of tracking algorithm and application of HIFU for moving tumors thermal therapy part, according to our initial results. The preliminary study indicated that HIFU with imaging tracking could have the acoustic power convergent at the desired location to produce better heating. The initial study showed that it is possible to quantitatively control the blood-brain barrier opening of rats by using an ultrasound transducer with injection of a contrast agent. *In vivo* experiments also showed the opening region occurs in the focal zone, as expected. The results have been published in *Medicine and Biology*.

The study also demonstrated that the proposed cylindrical ultrasound phased array can provide effective heating for breast tumor thermal therapy without overheating the skin and ribs within a reasonable treatment time. This was published in *Physics in Medicine and Biology*.

5. Preliminary studies of spine biopsy procedures under real-time MRI guidance

The purpose of this study is to develop clinical and basic test programs for MR-guided spinal biopsy using innovating imaging modalities and instruments, and to evaluate the efficacy of new products. In this project, the study group performed spine biopsy procedures in 16 patients using present closed MRI systems. The results show that MRI can be used to identify detailed tissue differences, localize true lesion, and contribute to good diagnostic accuracy and shorter procedure time. The researchers designed the preliminary MR guided biopsy phantom to analyze the artifacts of biopsy needle.



A 52-year-old female received MR guided and subsequently CT guided biopsy at L1-2 spinal level with proved diskitis. A and B show axial MR imaging (TR/TE = 3529/13) guided needle shadow in paraspinal abscess (arrow); C and D reveal CT guided biopsy in soft tissue window with less soft tissue contrast as compared with MR imaging.

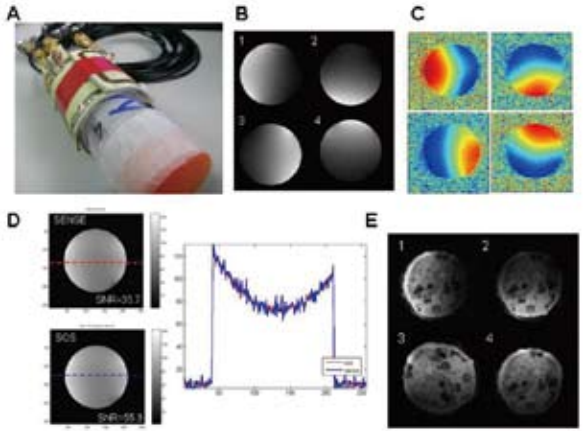
6. Enhanced fMRI signal for neurological interventional applications

In this component project, the BOLD (blood-oxygenation-level-dependence) sensitivity of functional MRI (fMRI) was enhanced by two methods: optimization of fMRI acquisition and data processing. In 2007, without the dedicated iMRI system, the pre-clinical test was examined using the 3T MRI on eighteen patients needing pre-surgical fMRI mapping. Furthermore, automation of CO_2 delivery and near real-time ICA processing has been optimized for clinical application. The CO_2 platform designed by this project has fulfilled the requirements for clinical application. It will benefit functional brain studies, such as pre-surgical mapping using BOLD-based fMRI, pre-operation evaluation for carotid stenting, radio-chemical therapy of brain tumors, and characterization of brain lesions.

7. Interventional MRI coil and system based on parallel imaging technique

The main purpose of this project is to design an RF receive coil that is compatible with the parallel imaging scheme and optimized for MRI-guided liver tumor ablation and/or biopsy procedures. The research team built the well-decoupled phased array coil and combined with parallel imaging approach to reduce the acquisition time by decreasing the k-space data numbers. The team also designed and fabricated a low input-impedance, low-noise pre-amplifier for the

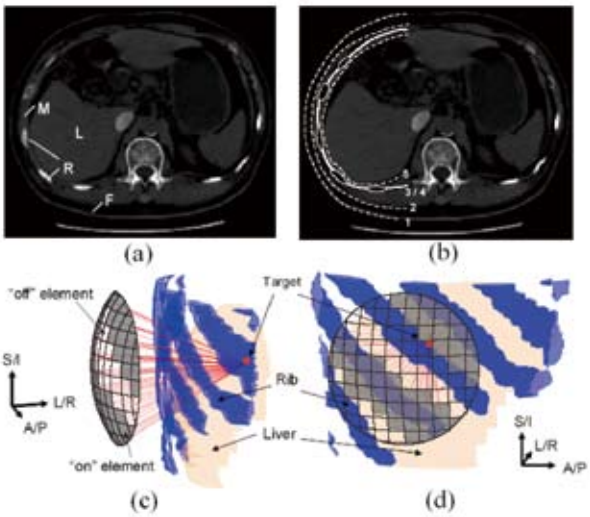
decoupling between the elements of the array coil with arbitrary geometry. By combining the above achievements, an open human-phased array coil was built for HIFU use.



(A) four-channel phased array coil for rat imaging (B) phantom images (C) coil sensitivity profiles of the four channels (D) SENSE and SOS reconstructed images and corresponding slice profiles (E) images of mice abdomen.

8. MR-imaging guided focused ultrasound therapy system for brain treatment: a feasibility study

High-intensity focused ultrasound can locally and reversibly increase the permeability of the blood–brain barrier (BBB), which can be detected using magnetic resonance imaging (MRI). This may be accompanied, however, by side effects such as microhemorrhage, erythrocyte extravasations, and even extensive hemorrhage. Although current contrast-enhanced T1-weighted MRI can be used to detect the changes in BBB permeability, its efficacy in detecting tissue hemorrhage after focused-ultrasound sonication remains limited. In this stage, we demonstrate the feasibility of using magnetic-resonance susceptibility-weighted imaging (MR-SWI) for identifying possible tissue hemorrhage associated with disruption of the BBB induced by focused ultrasound in a rat model. Brains of 42 Sprague-Dawley rats were subjected to 107 sonications either unilaterally or bilaterally. Localized BBB opening was achieved by delivering burst-mode focused ultrasound energy into brain tissue in the presence of microbubbles. Rats were studied by T2-weighted and contrast-enhanced T1-weighted MRI techniques, as well as by SWI. Tissue changes were analyzed histologically; and the extent of apoptosis was investigated with the terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling (TUNEL) method. The results demonstrated that SWI is more sensitive than standard T2-weighted and contrast-enhanced T1-weighted MRI techniques in detecting hemorrhages following brain sonication. Longitudinal study showed that SWI is sensitive to the recovery process of the damage and therefore could provide important and complimentary information to the conventional MR images. Potential applications, such as drug delivery in the brain, might benefit from this.



(a) CT sectional image showing the chest anatomy. L = liver, R = rib, M = intercostal muscle, F = fat. (b) Pseudo muscle–muscle interfaces were added on the CT image to connect the ribs for a continuous tissue interface layer for subsequent beam calculation (1 = water–fat, 2 = fat–muscle, 3 = muscle–muscle or muscle–frontal rib surface, 4 = muscle–muscle or dorsal rib surface–muscle, 5 = muscle–liver). (c, d) The reconstructed 3-D tissue interfaces and the phased-array orientation as well as the spatial arrangement of 2-D array elements from different views (the water–fat layer is not shown). The elements for which the normal vectors were blocked by ribs were deactivated (denoted as "off") to reduce the frontal rib acoustic energy absorption. S/I = superior/ inferior direction, A/P = anterior/ posterior direction, L/R = left/ right direction.

C. Multi-modality Animal Molecular Imaging Platform

1. Development and characterization of liposome microbubbles

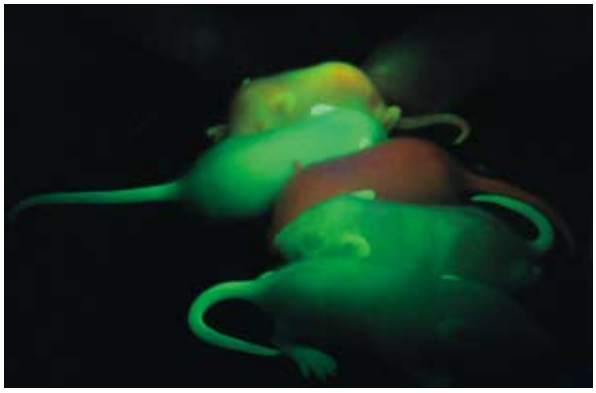
The potential bio-effects of microbubbles are important issues to cavitation-based therapy, such as thrombolysis, drug delivery, and gene delivery. The primary purpose of this project is to develop procedures for characterizing and analyzing various properties of microbubbles.

The research team successfully produced albumin-based microbubbles with encapsulated C_3H_8. Compared to other microbubbles (lipid based,

and/or different gas inside the bubbles), these microbubbles can last over 150 seconds for *in vivo* mouse imaging, produce strong cavitation effects, and improve B-mode image contrast. The team has also used these in-house microbubbles for *in vivo* mouse imaging and molecular imaging of angiogenesis. In addition, high-frequency contrast-enhanced ultrasound imaging with the albumin-based microbubbles has been demonstrated as an effective preclinical tool to identify the hemodynamic changes of hepatocellular carcinoma lesions in Hepatitis B virus X transgenic mice, demonstrating that these microbubbles can be reliably used for pre-clinical small animal research.

2. Conditional imaging reporter transgenic animal models for inflammatory diseases

This project specifically aims to generate novel RU486 inducible fluorescent Cre transgenic mice and analyze their tissue specificity and sensitivity, to generate and analyze the inducible hsp60 transgenic mice, and to generate and analyze the MCP-1 reporter mice, exploring applications of these transgenic mouse models in diagnosis and therapy. The key to the completion and the most exciting part of this project is to perform multimodality (optical, microPET, SPECT/CT) animal imaging.

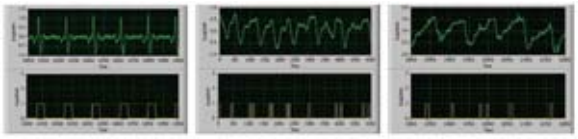


Fluorescent conditional transgenic mice generated in Lin Lab by *in vivo* DNA recombination.

The research team provided evidence that DsRed-T1, which functions in dimers, could be expressed in transgenic mice without the impairment in physiology. The study also demonstrated that massive expression of Hsp60 would result in heart failure. The combination of conditional hsp60 transgenic mouse and appropriate Cre mouse will greatly facilitate future studies on the complicated roles of HSP60 in triggering immunity, maintaining mitochondrial homeostasis, and mediating stress responses. MCP-1 participates in the early phase of inflammation and responsible for many respiratory and immune abnormalities. The transgenic mice model established in this study is a useful tool for the investigation of various MCP-1 related inflammation diseases.

3. Improvements on a microPET imaging system — development of cardiac and respiratory-gating devices

Positron emission tomography (PET) is a nuclear medicine imaging technology in which biochemical substrates relevant to life and life processes are labeled with positron-emitting radionuclides to trace and measure important *in vivo* functional and physiological information at the molecular and cellular levels. For experiments on small animals, the study group has developed monitoring platforms for the ECG, respiratory, body temperature, and blood pressure waveforms, and the level of oxygenation of blood (SaO_2). The group has also used ECG, respiration, and blood pressure signals to trigger the CCD camera for acquiring the images. The triggering signals could help non-displacement microPET image reconstruction.



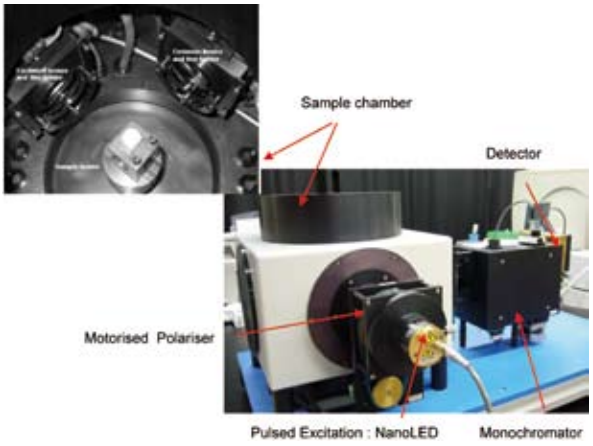
Above, left to right: ECG, respiratory, and blood pressure signals. Below: ECG, respiratory, and blood pressure gating signals.

4. Fluorescence resonance energy transfer (FRET)-based dynamic analysis on pro-inflammatory cytokines *in situ* following spinal cord injury

This study uses TNF-α as a marker to provide a novel quantitative FRET-based methodology for *in situ* dynamic monitoring of pro-inflammatory cytokines following spinal cord injury. The group has successfully expressed both DsRed-TNF and ECFP-TNF receptor fusion proteins in the AD 239 cell system. The antibody-antigen interaction incurred perturbation and energy depletion of excited QD-abTNFα, resulting in quenching of fluorescence lifetime in a TNFα concentration-dependent manner. It implicates the possible application of this design to quantification of cytokines, e.g. TNFα, induced following spinal cord injury.

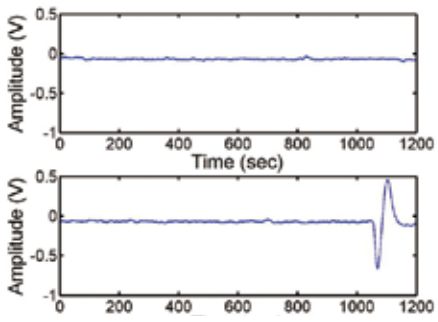
After the group reported the fabrication of chitosan-based membrane with nanopores, the membrane was used as a microdialysis probe with the I.D. ca. 650 μm and O.D. ca. 720 μm. Its biocompatibility was tested by implanting a piece of

the membrane material into rat brain and observing the viability of peripheral neural cells by nuclear staining evaluation. Based on the observation, the materials used for microdialysis probe should be safe for *in vivo* implantation.



5. Quantitative and qualitative analysis of DNA by electrical charge detection using micro electrophoresis chip

This study evaluates a novel concept regarding DNA fragments electrophoretic analyses by directly detecting electrical charges. A prototype of the portable electrophoresis device consists of a flow channel 35 mm long × 0.5 mm wide × 0.2 mm deep on an acrylic substrate. A detection circuit with amplification gain of 10,000 and analogous filter bandwidth between 0.1 Hz and 10 Hz has been developed. Preliminary results show the developed electrophoresis device can pick up the electrical signals of un-separated DNA fragments with total mass of 0.2 μg, and the magnitude is 0.6V. Micro-flow channels fabricated by an excimer-laser and low-noise amplifier with high gain, e.g. 100,000 are being processed for detecting very weak electrical signals of DNA.



Electrical signal versus time for only TBE solution (above graph) and for TBE solution with DNA markers (below graph).

6. Quantitative measurements in imaging small animals using positron emission tomography (PET)

As one of the most widely used modalities in molecular imaging, PET offers potentially high accuracy in quantitative measurements of the physiological and functional information that it can derive from the collected image data. This high accuracy results from high-quality image reconstruction and reliable compensations for various physical factors involved in PET image data formation. The physical factors that need to be considered in the imaging model include photon attenuation, scattering, random coincidence, detector resolution, and deadtime. This project has conducted a systematic investigation on attenuation and scatter corrections, especially the development of a new energy-dependent method for scatter correction in PET, and an implementation of the selected methods on the microPET R4 system for routine use. In addition, the research team has initiated an additional investigation of the effect of the limited spatial resolution within the field-of-view that is often non-uniform, especially toward the peripherals where the so-called parallax errors are prevalent. The project also intends to develop several pre-processing strategies in recovering the spatial resolution and incorporate these approaches with the selected image reconstruction algorithms.

The team believes these studies will improve substantially the accuracy of quantitative measurements in imaging of small animals using microPET R4. The methods developed for microPET R4 will also be useful for and can be applied to other PET systems for either human or animal scans.

D. Development of the Biomedical Imaging Industry in Taiwan

In 2007 a product engineering team was established to develop an MR-guided HIFU value-added product prototype and to conduct a preliminary study for the development of a high frequency ultrasound system. Both projects are on track to deliver their first prototypes next year.

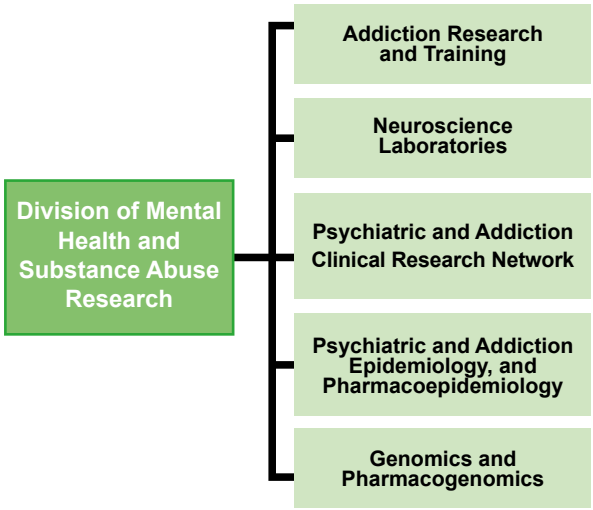
Division of Mental Health and Substance Abuse Research

Mission

The Division of Mental Health and Substance Abuse Research sets the following as its mission:

- conduct multidimensional translational research on problems related to mental health and addiction, integrating basic, clinical, and public health research traditions and approaches;
- develop effective, innovative interventions and prevention strategies for major psychiatric and addictive problems;
- establish a multi-step and multi-dimensional training system to attract basic, clinical, and public health researchers to pursue careers in neuroscience, psychiatric medicine, and addiction medicine.

Organization Chart



Major Progress

In just four years, the division has recruited a cadre of well-qualified investigators with diverse expertise, including in the neurosciences, and in clinical and population-based research (epidemiology and outcome research). The division's team has excelled not only in academic performance as measured by the conventional yardsticks — including publishing books, being published in book chapters and peer-reviewed journals, and receiving grants/contracts from major funding agencies such as the National Science Council and Department of Health — but also established infrastructures crucial for neuroscience and for psychiatric and addiction research, including the behavioral animal models, neurophysiology laboratory, tissue repository (DNA and cell line banks), therapeutic drug monitoring system, P-2 laboratory, the Taiwanese Psychiatric Research Network (TPRN) and the Taiwanese Addiction Research Network, centralized data management and analysis system. The division is also fostering international collaboration with major academic and research institutions, including the National Institution on Alcohol Abuse and Alcoholism (NIAAA), the National Institute on Drug Abuse (NIDA), the National Institute of Mental Health (NIMH), the University of California at San Diego, the University of California at Los Angeles, Harvard University, the University of Melbourne, and the University of Adelaide in Australia, as well as the University of London's Institute of Psychiatry. At the same time, the division also has been active with training/educational activities. Researchers who have been in the clinical psychiatry fellowship program, for example, have already made significant contributions to the development of the division's research programs, including the new geriatric and addiction research initiatives, and on track for distinguished careers, either with the division or with other major medical centers. All of the division's efforts have contributed to its ability to develop the two major initiatives with focus on addiction research and training, the "Multidimensional Integrated Translational Research on Addiction" (MITRA) and the "Taiwanese Addiction Fellowship Training Program" (TAFT).

A. Addiction Research and Training

Capitalizing on recent developments in methadone programs and the availability of the division's research infrastructures, basic, clinical, and public health research plans (MITRA), with opioids addictions and their treatment as the initial focus, the programs are fully integrated and will together examine the clinical phenomenology, comorbidity, medical consequences, outcome, treatment response, innovative intervention strategies, and mechanisms responsible for addiction and drug effects, utilizing established animal and cellular models. Concurrently, under the leadership of Dr. Ing Ho, the division has established an ongoing national forum with experts from the field as well as policymakers and administrators. Four workgroups evolved from the meetings, focusing on establishing standard operating procedures for clinical care and research, data linking and integration, policy and interagency collaboration, and development of innovative approaches. A series of meetings focusing on these issues has led to consensus building and the establishment of a clinical network for research purposes, with the finalization of standard operating procedures for different types of clinical research

ready for application, including plans for integrating genotyping and therapeutic drug monitoring into the protocols. At the same time, an integrated basic research program with seven of the laboratory-based researchers has been established; it will enable the team to examine different aspects of the effects of opioids on pregnant murines and their offspring. The team has made significant strides on all fronts. Clinically, the division has formed partnerships with practically all major players in the field, including the Department of Health's Bali Psychiatric Center, Chia-Nan Psychiatric Center, Taoyuan Psychiatric Center, and Tsao-Tung Psychiatric Center; the En-Tsu-Kong Medical Center, Kaohsiung; the Kaohsiung Kai-Shuan Psychiatric Center; the National Defense Medical Center; the Taipei City Psychiatric Center; and the Tzu Chi Medical Center. With these partners the division has formed the Taiwanese Addiction Research Network, which promotes clinically oriented research. The program currently focuses on opioid addictions; but the model as developed will be applied to studying other forms of addiction, including both illegal and legal substances, such as methamphetamine and betel nuts, as well as other addictive behaviors. Future efforts will also focus on issues interfacing addictive and other psychiatric disorders. Thus, projects will be developed to study populations concurrently suffering from psychosis or depression as well as substance abuse, making these "dual diagnosis" and "comorbidity" issues. The program is expected to contribute to a better understanding of mechanisms responsible for addiction-related problems, formulation of better treatment strategies, and an increase in evidence-based health policies for addiction problems.

The division plans for one aspect of the initiative, the Taiwanese Addiction Fellowship Training Program, to train a new generation of clinician-researchers (physician-scientists) who will serve as leaders for this new field. At the same time, they also will help to establish addictionology as a recognized medical discipline, thus further promoting care for and research on expanding populations with various kinds of addiction problems. In collaboration with the Taiwanese Society of Psychiatry and the newly formed Taiwanese Society of Addiction Sciences, the division has formed an executive committee whose members consist of many of Taiwan's leaders in this field, to oversee this initiative, with the Taiwanese Society of Psychiatry providing certification for trainees enrolled in the program. Such a certification process will serve as the backbone for the establishment of the addiction subspecialty. The division is currently finalizing the curriculum, which will include year-long weekly didactics and supervised clinical rotations, as well as research training with mentors who are world experts on topics of interest to the trainees. The plan makes use of not only faculty members from Taiwan with varied expertise but also international experts and scholars, who will come for lectures and bedside teachings. Mechanisms for the recruitment of trainees have been established; and the division has received enthusiastic responses from qualified applicants, ensuring that there will be a pool of talented young psychiatrists for this program. An inauguration ceremony is scheduled for October 21, 2008, immediately following the division's scientific review.

B. Geriatric Research Programs

In collaboration with colleagues from NHRI's Center for Health Policy Research and Development, Division of Geriatric Medicine, and Division of Biostatistics and Bioinformatics, the division helped formulate and plan two large-scale research projects focusing on Taiwan's geriatric population. These are the Healthy Aging Longitudinal Study in Taiwan and the Interventional Study of Geriatric Frailty, Osteoporosis, and Depression. The former is a long-term, multi-wave project that will follow a cohort of community-dwelling elderly to determine how genetic, environmental, and lifestyle risk factors determine the trajectory of aging, including frailty, cardiovascular functions, and neuropsychiatric conditions (cognitive function, depression, and propensity for substance use and abuse); members of the Division of Mental Health and Substance Abuse Research are in charge of one of the four subcomponents, "Neuropsychiatric Disorders in the Elderly: Risk Factors and Impact on Health." The latter of the two research projects is an intervention study aimed at developing strategies for the prevention, early identification, and early intervention of conditions commonly seen in the elderly, including osteoporosis, frailty, and depression. The division's colleagues developed the proposal for a subcomponent of the project titled "Effectiveness of a Collaborative Care Depression Program," which includes case management, referral, and a brief structured psychosocial intervention program for the management of depression. A pilot study is being conducted in Miaoli. The division plans to use the findings and experiences from this pilot study to further develop the program into a full-scale study to include elderly from different communities, such that the results would enable the division to more effectively provide prevention and early intervention for this population.

C. Infrastructures and Platforms

Considerable efforts have been made over the last four years toward establishing the division's neuroscience laboratories, which occupy some 20,000 square feet, and acquiring most of the basic equipment for them. Unique features of the laboratories include a cell lines and DNA repository facility currently holding more than 600 EBV-transformed frozen cells, and with the capacity to process several hundred more per year; a system for therapeutic drug monitoring, both for the development of new measurement methods and for the assays of clinical samples on a routine basis; and two fully equipped neurophysiology laboratories, which could be used for intra-neuronal electric recording as well as for studies on neurogenesis. Considerable efforts have been made to establish behavioral animal facilities for neuroscience research; these have much higher standards — including for lighting, temperature control, and animal handling procedures — than those required for the central animal facilities. In anticipation of the need to process biological samples that may be potentially infectious (e.g., those from heroin addicts with HIV and/or hepatitis infections), we also are setting up a P-2 laboratory for sample processing and storage.

Among all these developments, it may be particularly noteworthy that Dr. Zaodung Ling and his team have developed a unique developmental model that examines the cumulative effects of environmental assaults on the brain starting from the prenatal stage. This might be best for understanding many progressive neuropsychiatric disorders, including not only Parkinson's disease and Alzheimer's disease, but also conditions such as schizophrenia. The team demonstrated that, along with genetic predisposition prenatal activation of Toll like receptor by bacterial toxin lipopolysaccharide (LPS) is a causative for DA neuron loss, and therefore a potential etiology of PD. This is done in an animal model in which a small dose of LPS was given to pregnant rats. The offspring of such exposed females displayed a reduced number of DA neurons, brain innate immunity dysfunction, glutathione homeostasis disturbance, blood brain barrier dysfunction, hypersensitivity to subsequent toxin, and Lewy body formation (another pathological hallmark of PD).

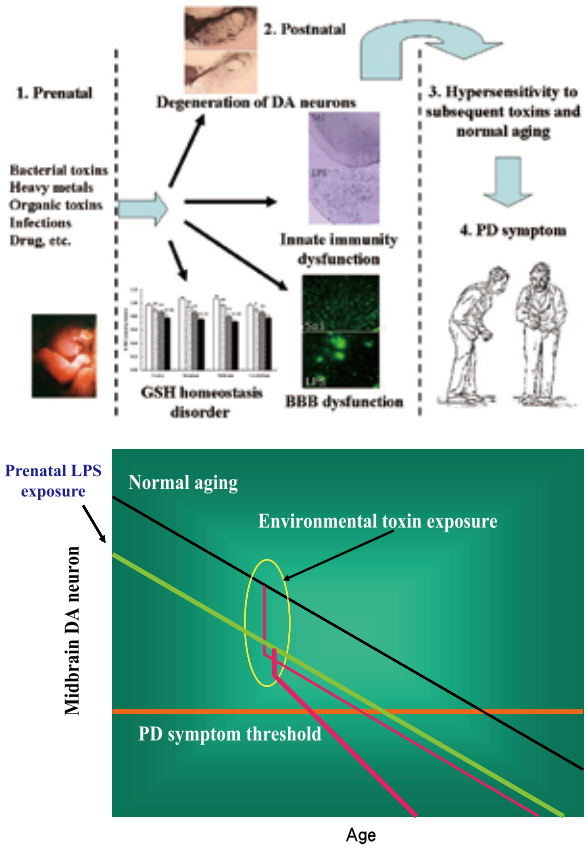


Figure 1. New discovery in etiology of Parkinson's disease (PD) — Early Toll like receptor activation as mechanism underlying PD etiology, by Dr. Zaodung Ling.

This prenatal theory of PD etiology has been published in several international journals and has been recognized by the National Institutes of Health and many research institutions worldwide.

D. The Taiwanese Psychiatric Research Network (TPRN)

Modeled after NHRI's Taiwanese Cooperative Oncology Group (TCOG), the network includes a number of medical centers and subspecialty psychiatric centers, which have been instrumental in carrying out the division's major clinical trials and pharmacogenomic projects, developing substantive and ongoing links with pharmaceutical companies, and promoting large-scale trials to address important clinical issues. TPRN also organized a large number of training conferences to help train both physicians and research nurses in clinical-trial research, as well as workgroups to establish consensus and clinical guidelines for major psychiatric conditions (refractory

schizophrenia, pharmacotherapy of schizophrenia, bipolar disorders, major depressive disorders, child psychiatry, and geriatric psychiatry). Using TPRN as the model, the division is developing a parallel network for addiction research, which has been tentatively named the Taiwanese Addiction Research Network. The two will overlap and support each other, as some of the participating clinical programs will be the same.

In addition to the geriatric research programs described above, the following items in the public health domain may be particularly worthy of notice:

1. The establishment of a cohort for the longitudinal follow-up of pre-teens for their substance abuse problems
2. The establishment of an ongoing research group called the Psychopharmaco-Epidemiological Research Group (PERG), which includes psychiatrists, epidemiologists, health economists, and biostatisticians. Utilizing the National Health Insurance Database, the group has focused on issues related to the utilization patterns and health risks of benzodiazepines, and has identified trends of potential abuse and misuse, as well as associated health risks, such as hip fractures and accidents.
3. The division also played a significant role in the project that will produce the groundbreaking health policy report, "Healthy People 2020," particularly in the two chapters addressing substance abuse and stress and coping (as well as resilience).

Progress of Individual Projects

All projects have been progressing well, with some starting to yield interesting and significant results. Some of the highlights are described below.

- **Epidemiology of Child and Adolescent Mental Disorders in Taiwan**

  Principal Investigator: Chuan-Yu Chen

  The study has reached its expected goals, showing data suggesting that the treated prevalence of autism and other developmental disorders has increased over time, that autism has been identified at progressively earlier ages, and that autism is associated with comorbidity with other behavioral and medical disorders. There also are significant urban-rural differences in these measures, suggesting a disparity in services favoring the urban settings. As early diagnosis and intervention are crucial for the care of these children, the discrepancy is an issue that requires the attention of clinicians and policymakers.

- **Clinical Pharmacogenomics of Antidepressant Response**

  Principal Investigator: Keh-Ming Lin

  With the addition of two other clinical sites (at Mackay Memorial Hospital and Far Eastern Memorial Hospital) the recruitment of subjects has accelerated considerably, such that we are already approaching the mid-point of the project's projected sample size. Preliminary analysis shows that the majority of the patients responded well to the interventions. Data analysis and plans for publications, with identification of topics, were discussed in a recent Investigators' Meeting. In the meantime, a number of review articles and book chapters have been completed and are currently in press.

- **Functional Genomic Study of Psychoactive Drug Targets**

  Principal Investigator: Chia-Hsiang Chen

  The group has finished the drug treatment of mice with four different drugs acting on the CNS, and extracted the RNA from cortex. Currently the group is carrying out microarray analysis of gene expression profiles in the cortex of these animals. The team has been productive in publications.

- **Systematic Mutation Screening of Glutamate Receptor Genes in Schizophrenia**

  Principal Investigator: Daniel Ding-Lieh Liao

  The laboratory portion of the project, with mutation screening focusing on the c-terminal coding regions and 3'UTR of four genes (GRIN2A–GRIN2D), is progressing well and will be completed by the end of this year, as planned. Cumulative effects of multiple rare mutations on schizophrenia will be studied statistically. Dr. Liao has continued to be productive with publications.

- **Amisulpride Augmentation Therapy for Clozapine-Resistant Schizophrenic Patients: a 14-week randomized, double-blind, and placebo-controlled trial**

  Principal Investigator: Sheng-Chang Wang

In this multi-center clinical trial, the team has collaborated with four major psychiatric hospitals in northern and eastern Taiwan. The most challenging part is to set up the standard operating procedures for case enrollment, data collection, and sample transportation for all of the collaborating hospitals. The establishment of the standard operating procedures and working relationships represents a tremendous achievement that will serve as a prototype for similar future projects, including those on addiction research. In the first year, we have screened most of the eligible subjects from the collaborative hospitals. We will start the second phase of clinical intervention in the following months. A number of new research ideas have been generated in the process. For example, clozapine levels show that most patients may have been inadequately treated; also, whether input from our TDM system would result in better outcome for these patients, converting them from "clozapine resistant" to "clozapine responsive" patients, could be a side project that could result in clinically useful insights.

- **Pharmacogenetic Study of Interaction between Antipsychotics and Smoking Cessation**

  Principal Investigator: Tsuo-Hung Lan

  The project has recruited 150 smoking volunteers with schizophrenia who completed the clinical interventions after consent forms were completed. The team expects to recruit at least 200 subjects in total in the next year. A paper based on data collected on the readiness of smoking cessation in schizophrenic patients was published in the *American Journal of Psychiatry* in May 2007.

- **Prospective and Retrospective Pharmacogenetic Profiling of Antipsychotics-Induced X-syndrome and Diabetes**

  Principal Investigator: El-Wui Loh

  This project, which represents a collaboration of the division with two major psychiatric hospitals, collected relevant clinical data and DNA samples for nearly 700 schizophrenic patients. A three-stage association approach has been adopted, starting with 29 candidate genes and yielding a total of 168 SNPs. Stage 1 resulted in the identification of five genes, which are then included in stage 2. In the second stage two genes — the PPARG gene (3 of 4 taqSNPs) and the IKBKB gene (1 of 3 taqSNP) — were revealed to be associated with glucose level after adjustment by age and sex. Genotyping of TaqSNPs of the phase I metabolism genes have also been completed. Statistical analysis is being conducted. Results will receive further scrutiny; and plans for publication will follow.

- **Role of Hippocampal Neurogenesis in Major Depression: a comparative study of newly generated neurons and existing old neurons**

  Principal Investigator: Sabrina Wang

  The P.I. has established a unique rat model for studying the effect of chronic mild stress in three strains of rats with differential neuroimmune and neuroendocrine profiles. Dr. Wang also established a well-functioning neurophysiology laboratory needed for the project. Although difficulties with the animal facilities imposed significant delays, the project has progressed well, with most of the goals for this year already reached.

- **Regulatory Mechanisms Underlying the Microglial Choice in Synthesizing Either Pro- or Anti-Inflammatory Cytokines**

  Principal Investigator: Rongtai Wei

  The project has demonstrated strain-difference in microglial immune responses, and the sequential production of pro- and anti-inflammatory cytokines by individual microglial cells in response to immune challenges. A manuscript titled "Microglia of Inflammation-Susceptible Lewis Rats Exhibit Inherently Hyperreactive Inflammatory Responsiveness: comparison with microglia of resistant Fischer rats" is currently being revised.

- **TLR's Early Activation as a Mechanism of Psychoneurological Diseases**

  Principal Investigator: Zaodung Ling

  The project uses a unique model developed by the P.I. to study the process of neurodegeneration during early stages of development when clinical symptoms are absent. The model thus is effective

in studying the pathogenesis in manners mimicking human neurodegenerative diseases. Problems with the animal facility were overcome by using a different facility, so the project is still on schedule. The brains collected in the collaborator's lab are under processing. The data will be collected and analyzed by the end of this year.

- **Microglial Innate Immunity in Alzheimer's Disease**

  Principal Investigator: Feng-Shiun Shie

  The studies have been progressing well. Findings suggest that activation of peroxisome proliferator-activated receptor gamma (PPARγ) in primary microglia using co-treatment with 15-deoxy-delta (12,14)-prostaglandin J2 (15-d PGJ2) and its synergistic partner, 9-cis retinoic acid (RA), promoted Aβclearance while inhibiting Aβ-mediated immune activation. The effects of the co-treatment on Aβ clearance were PPARγ-dependent; the anti-inflammatory effects, however, were independent of PPARγ activation. In addition, Aβ-activated microglia reduced secretion of pro-nerve growth factor, and its effects were reversed to basal levels by the co-treatment in a PPARγ-independent manner. These data suggest that multiple mechanisms may underlie the beneficial effects of the co-treatment and are not limited to PPARγ activation only.

Division of Molecular and Genomic Medicine

Mission

The mission of the Division of Molecular and Genomic Medicine is to improve health and prevent disease through better understanding of the molecular and genetic bases of diseases prevalent in Taiwan. The division applies modern genetic and molecular technologies to conduct high-quality research in genes and molecular mechanisms involved in diseases. It works toward providing the health care system with clinically relevant solutions through fundamental and translational research leading to improved patient management as well as disease prediction and prevention and therapy.

Toward this mission, the division has both short- and long-term goals.

Short-Term Goals

- use various genomic mapping and scanning approaches to identify genes altered in cancers common in Taiwan
- use various molecular profiling and cDNA expression approaches to identify differentially expressed genes for functional studies in diseases
- investigate the growth and oncogenic signal pathways involved in the development and progression of cancers common in Taiwan
- establish mechanisms through which molecular and genomic research can be applied to clinical practice or biotechnology development

Long-Term Goals

- develop and bring in state-of-the-art molecular and genomic technologies and research activities to identify genes and molecular mechanisms involved in diseases prevalent in Taiwan
- facilitate integrated research efforts of NHRI by working closely with other divisions
- rapidly disseminate laboratory findings and provide molecular and genomic expertise to researchers within NHRI
- promote health-related molecular and genomic research in Taiwan by serving as a resource center and by forging complementary, synergistic collaborations with other investigators
- provide clinically relevant solutions to the health care system through our research and improve patient management by disease prediction and prevention

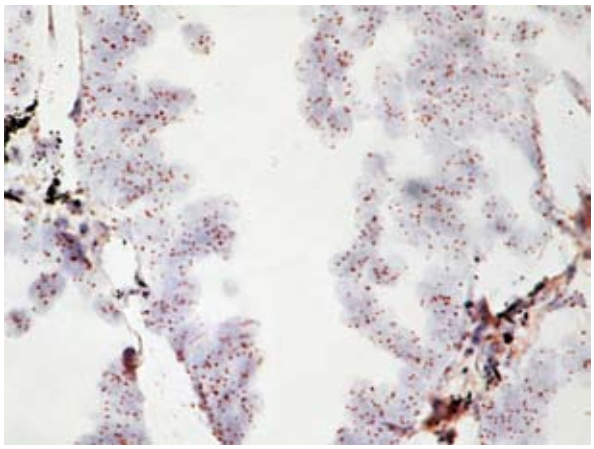
Major Progress

Research activities in the Division of Molecular and Genomic Medicine can be broadly divided into three areas: clinical genomics, functional genomics, and technology development and support.

A. Clinical Genomics

1. Increased epidermal growth factor receptor (*EGFR*) gene copy number is strongly associated with *EGFR* mutations and adenocarcinoma in non-small cell lung cancers: a chromogenic *in situ* hybridization study of 182 patients

To evaluate the association of epidermal growth factor receptor (*EGFR*) gene copy number with *EGFR* and *k-ras* mutation status as well as tyrosine kinase inhibitor (TKI) sensitivity in non-small cell lung cancer (NSCLC), *EGFR* gene copy number of 182 NSCLC tumor specimens were analyzed by chromogenic *in situ* hybridization (CISH). *EGFR* and *k-ras* mutation analyses were also performed for, respectively, 176 and 157 of the 182 patients. Additionally, 36 patients in this study had received TKI monotherapy. The tumor was considered to be CISH positive if the gene copy number was ≥ 5 signals per nucleus in ≥ 40% of tumor cells. CISH-positive tumors were strongly associated with adenocarcinoma (56.8%) compared with squamous cell carcinoma (15.9%) ($P<0.0001$). The CISH-positive tumors were also strongly associated with *EGFR* mutations (78%) compared with wild type (20.2%) ($P<0.0001$). Only six tumors had *k-ras* mutations. None had *EGFR* mutation; and only one was CISH positive. In the patients treated with TKI, *EGFR* mutation was strongly associated with TKI responsiveness (22 of 25 responders) ($P<0.0001$); but the CISH-positive tumors were only marginally significant (18 of 25 responders) (P=0.0665). Patients with *EGFR* mutations or CISH-



positive tumors were all associated with longer median survival, although not at statistically significant levels. The results suggest increased *EGFR* copy number is highly correlated with *EGFR* mutation in adenocarcinoma. Although it is less correlated with TKI responsiveness when compared with *EGFR* mutations, it still could be a good alternative molecular predictive marker for TKI responsiveness, since CISH can be done on paraffin section and is much quicker than DNA sequencing.

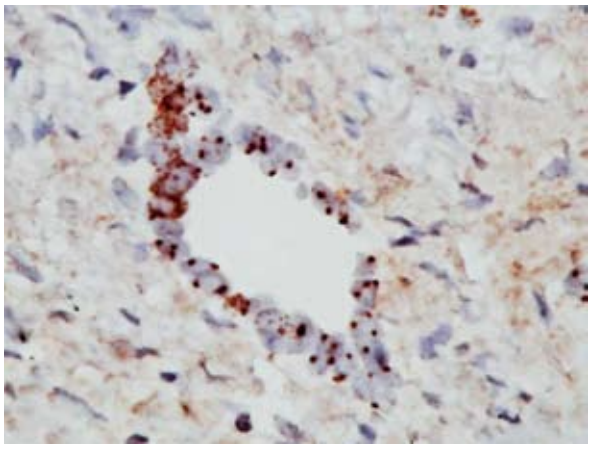


Figure 1. Adenocarcinoma of lung with increased EGGER gene copy numbers (>5 copies) by chromomeric in site hybridization.

2. Genomic Research Center at NHRI: enabling technology for comparative analysis of microbial pathogen

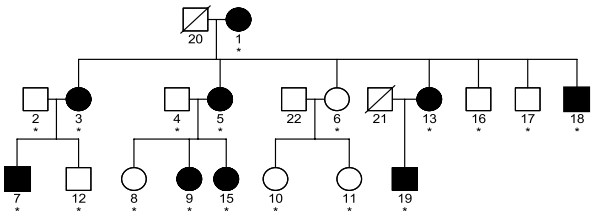


Figure 2. Autosomal dominant form of cutaneous lichen amyloidosis. Patients are shown as filled square (male) or circle (female). Asterisk indicates individuals that gave DNA for this genetic study. A mutation was found in the OSMR gene on human chromosome 5 for all the patients in this pedigree.

The division has applied state-of-art genomic technology to conduct genomic studies on human genetic disorders, bacterial pathogens, and common cancers in Taiwan. Specifically, the study group has established standard protocols for the Illumina genotyping system, the Roche 454 re-sequencing system, and the NimbleGen comparative genomic hybridization (CGH), and has conducted genomic research on disease gene mapping, microbial genomics, and cancer genomics. The division has supported human genetics research projects such as cutaneous amyloidosis, avascular necrosis, hyperuricemia (for disease gene mapping and functional study), and the chromosomal instability of lung adenocarcinoma and hepatocellular carcinoma (for cancer genomics).

- For SNP discovery in 46 Taiwanese Aborigines (23 gout patients, 23 controls), the group has finished DNA sequencing of 683 PCR reactions involving 39 genes in the 4q region. A candidate gene for hyperuricemia was identified.
- The study detected TP53 gene mutation and discovered a link between TP53 mutation and chromosomal 4q LOH.
- The group finished initial mapping of disease gene loci for autosomal dominant form of cutaneous lichen amyloidosis and assigned a subset of families to chromosome 5.

B. Functional Genomics

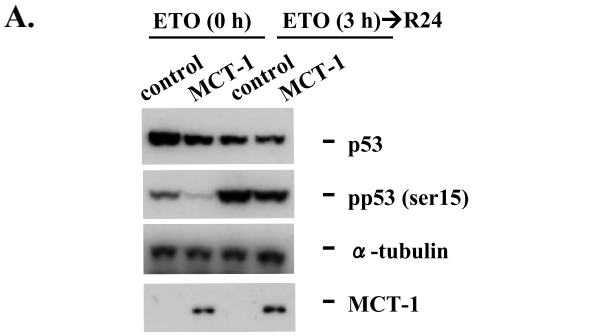
Functional genomics is the research area where the division applies high-throughput technology for systematic analysis of the human genome to identify targets for further studies on the function of specific groups of genes or the regulation of gene expression from a genomic region. Research progress made in this area includes:

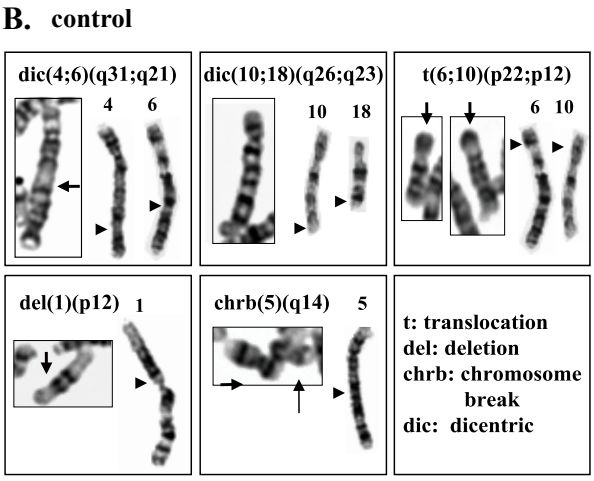
- IR-induced G2-M arrest in K562 cells was impaired by RNAi-mediated knockdown of Bcr-Abl. The group also transfected Bcr-Abl into Pro-B cells and found that the IR-induced G2-M arrest was enhanced, suggesting that Bcr-Abl may play a role in modulating G2 arrest in CML cells.
- EGFR is a receptor tyrosine kinase involved in many important cellular events, including cell proliferation, differentiation, migration, and survival. Aberrant EGFR signaling has been found to cause various types of malignancy. The group found that different EGFR mutations cause constitutive receptor activation through distinct mechanisms.
- Researchers found that phosphatase NEAP regulates neuronal signaling and differentiation.
- Pleiotropic cytokine transforming growth factor-β (TGF-β) is a prototypic member of a large family of cytokines that control numbers of fundamental cellular behaviors, such as cell proliferation, differentiation, and apoptosis during embryogenesis as well as in mature tissues. Smad proteins, major mediators of TGF-β

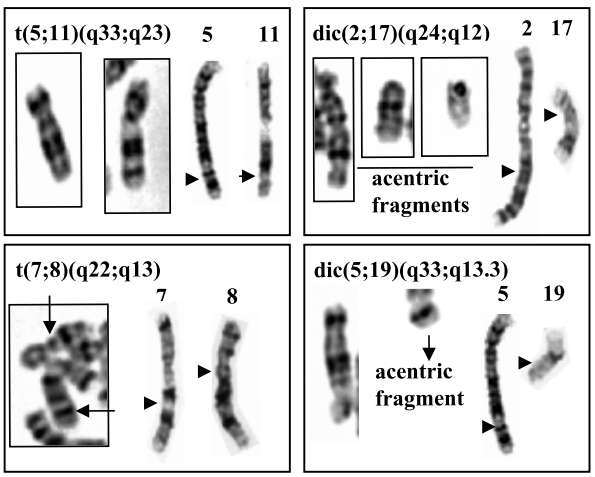
signal transduction, may change their properties during carcinogenesis. By searching for proteins that interact with Smad(s) in a proline-directed phosphorylation-sensitive manner, the study found that protein isomerase Pin1 can bind to Smad3 and that Pin1 activates the transacting ability of transcription factor ATF-2.

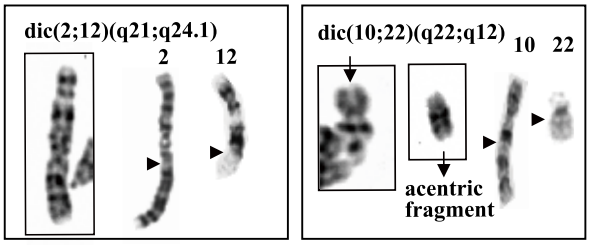
1. Implications of MCT-1 oncogene in breast carcinogenesis

MCT-1 (multiple copies in T-cell malignancy) oncogene is overexpressed in human breast carcinomas, lung cancers, and lymphomas. MCT-1 is a nucleus/cytoplasm shuttling protein, preferentially highly expressed at cytoplasm of the cancer tissues. MCT-1 oncoprotein is involved in cell cycle regulation









D. ETO-induced chromosome aberrations

control cells		Events	Ratio
Deletion	12	1	3.3%
Breakage	2, 5, 5, 10, 17, 17, 21, 22, der(9), del(1)	10	33.3%
Translocation	t(2;?), t(6;10), t(7;11), dic(1;13), dic(2;4), dic(4;6), dic(der(9);X), dic(10;18), dic(X;16),	9	30%
MCT-1 overexpressing cells			
Deletion	1, 5, 6, der(9), 18, 2	6	20%
Breakage	dup(1), i(8), 4, 2, 1, 7, 11, X	8	26%
Translocation	t(2;?), t(5;11), t(6;22), t(6;22), t(7;8), t(7;22), t(10;22), t(18;21), t(X;13), t(X, 20), t(2;7), dic(del(1);15), dic(5;6), dic(5;19), dic(8, 14), dic(10;22), dic(2;12), dic(2;17), dic(2;X)	19	63%

Figure 3. Constitutive expression of MCT-1 promotes chromosomal instability. MCF-10A were treated with ETO for 3 h, ETO (3 h), and then cultured in the ETO-free medium for another 24 h, ETO (3 h)R24. The total p53 and phospho-ser15-p53 amounts are relatively reduced in MCT-1 expressing cells than in controls (A). Cytogenetic G-banding analyses of ETO-induced chromosomal abnormalities are represented by the following abbreviations: t, translocation; del, deletion; chrb, chromosome break; and dic, dicentric (B-C). Chromosomal aberrations are quantitatedin MCT-1 expressing and control cells (D). In the response to ETO, more chromosomal translocations and deletions are induced by increasing MCT-1.

and translational control. The project data indicate that ectopic expression of MCT-1 malignantly transforms human mammary epithelial cells. The project's preliminary results are as follows:

- The study clarified that MCT-1's oncogenic effects in deregulation of genome surveillance system and impairment of cell cycle checkpoints are involved in p53 destabilization, DNA misrepair, chromosome aberrations, and increasing genome amplification and translocations.
- MCT-1 protein expression status is increased mainly in cytoplasm of breast cancer tissues as compared with paired non-neoplastic tissues with preferential confinement in nuclear compartment. Thus, MCT-1 protein mobilization could be associated with its activity in tumorigenesis.
- MCT-1's functional roles in cell survival and cell proliferation in AKT and MAPK signaling cascades, as well as in induction of EGFR and HER2 protein expression in mammary cells, are under investigation.
- MCT-1 over-expression in non-oncogenic mammary epithelial cells not only transformed the cells but also disrupted normal acini-like spheroid morphogenesis.
- Hyper-activation of cell survival and proliferation signaling pathways and dysfunction of genome surveillance system are implicated as the potential molecular basis for MCT-1's tumorigenic activity.

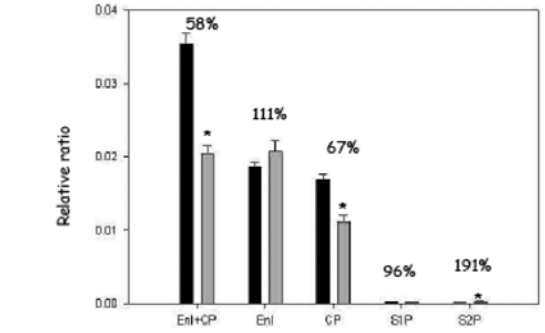
2. Expression and activity profile of Met and HGF-induced gene in human cancer: role of Amphiregulin and Sprouty in tumor growth and metastasis

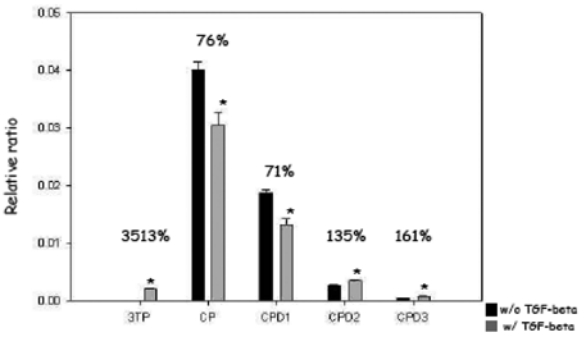
The fundamental goal of this study is to utilize the HGF-MET ligand-receptor pair as the model system to dissect pathways that mediate the crucial aspects of cancer metastasis. Met, the receptor for HGF, is a tyrosine kinase and a key regulator of signaling pathways that control important biological functions including cell growth and differentiation. The AREG gene initially identified through microarray analysis is an EGFR ligand.

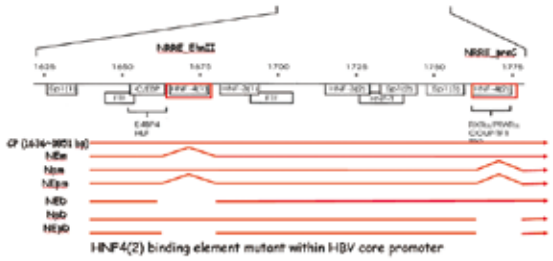
- Utilizing various research tools, including the RNAi approach, the team observed that inhibition of AREG or EGFR resulted in the loss of HGF-induced biological functions. The results indicate that the EGFR pathway is indispensable for many biological functions mediated by HGF and Met.
- The study group demonstrated that DKK2 could antagonize the WNT signalling pathway and function as a tumor suppressor gene.

3. Inhibitory effect of cytokines on HBV replication

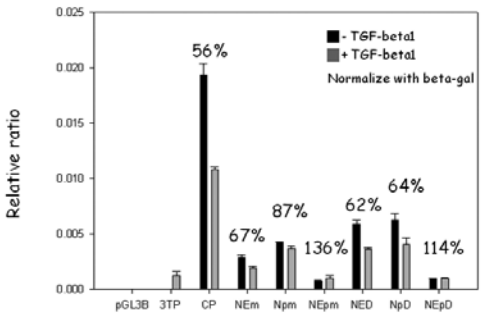
Chronic hepatitis B is one of the most serious viral infections in humans worldwide. More than 350 million people in the world suffer from this; and chronic hepatitis B is a high risk factor for cirrhosis and hepatocellular carcinoma.Examining the regulatory mechanisms of HBV replication that can suppress viral replication during HBV infection is an important effort to control this disease.



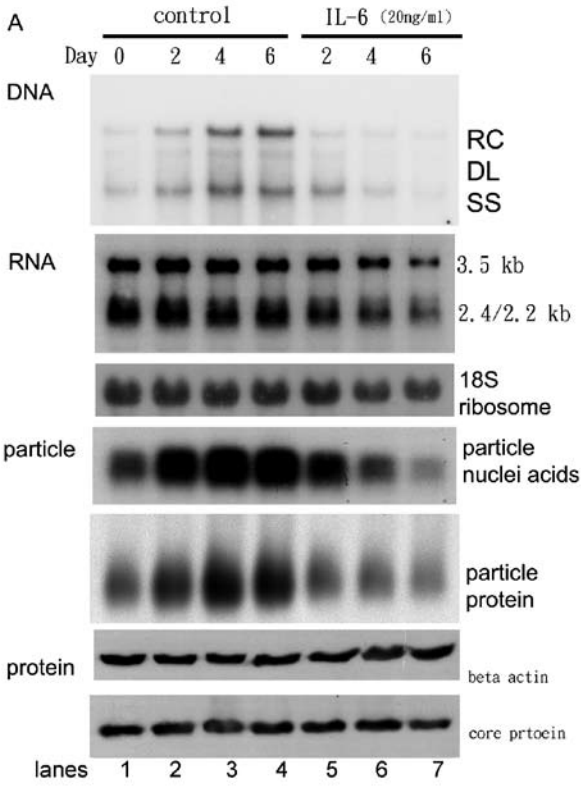




TGF-b1 responsive elements: HNF4 binding sites



- The study group established an HBV-producing cell line (1.3ES2) sensitive to the antiviral activities of cytokines. After screening of several hepatitis-elevated cytokines, the group found that TGF-b1 could inhibit HBV replication at the physiological concentration. The results indicate that TGF-b1 might exert its antiviral effect primarily by specifically reducing the activity of HBV core promoter and subsequently decreasing the expression of core protein and pgRNA, the template of the viral genome.
- The major responsive element to TGF-b1 was characterized to be the HNF4 binding site within the core promoter. TGF-b1 could reduce the expression level of HNF4 and induce dedifferentiation of cells, which subsequently resulted in the suppression of HBV replication.
- IL-6 might exert its antiviral effect primarily through inhibition of viral encapsidation or capsid maturation. The study results indicate that the antiviral effect of IL-6 was dependent on HBx protein and closely related to the activation of the PI3K signaling pathway. The mechanisms involved in the effects of TGF-b1 and IL-6 appear to be quite different.



C. Technology Development and Support

1. Endomesoderm gene regulation network in zebrafish

Knowledge of gene regulatory networks is fundamental for an understanding of the mechanisms of any biological phenomena. In the post-genomic era, it is important to understand the function of genomes by decoding the genomic regulatory network. The overall goal of this project is to establish the gene regulatory networks underlying the mesendoderm development in zebrafish and to use colorectal cancer cell lines combined with Q-PCR to screen anti-cancer drugs.

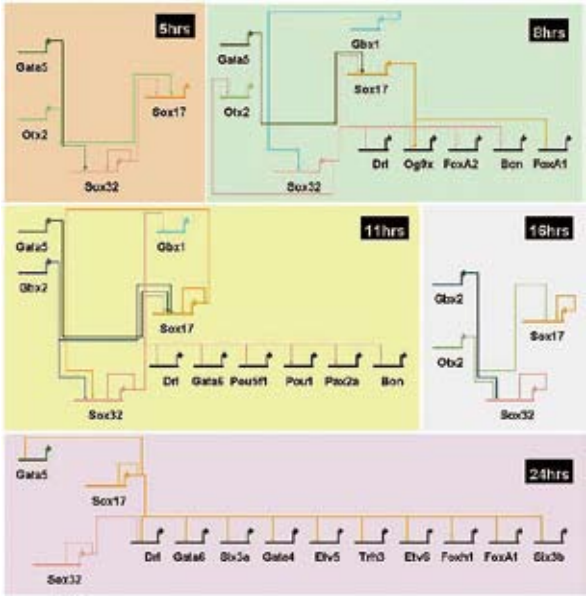
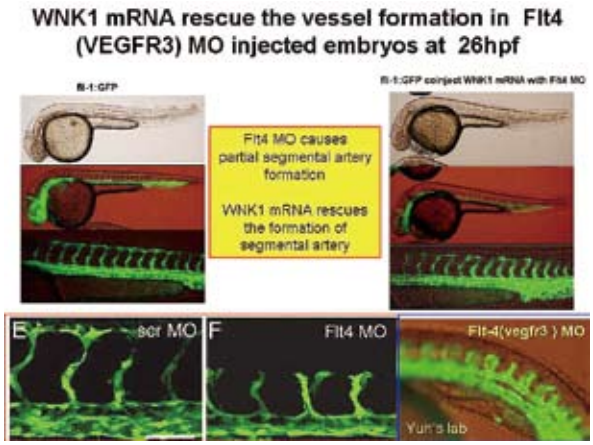


Figure 4. The Sox32 and Sox17 subcircuit network is divided into different areas during processing of the developmental stages. Our selected time-point of 5 hpf was late blastula; 8 hpf was middle gastrula; and 11 hpf, 16 hpf, and 24 hpf were segmentation periods. The relation of gene interaction is defined from our data, and then summarized as different time-points. The information about the role of Sox32 and Sox17 is summarized in a systematic manner. The stages are labeled with different colors: 5 hpf, orange; 8 hpf, cyan; 11 hpf, yellow; 16 hpf, gray; and 24 hpf, amethyst. The gene interactions are linked with a line: arrow line, gene positive regulated target gene; blunt line, regulation relation. The summarized relation of networks built downstream relies on Sox32 and Sox17 as verified by gene-specific MO perturbation screening and the upstream inputs of Sox32 and Sox17 by perturbation screening for other gene-specific MOs.



- The study group used bioinformatics methods searching for spatial and temporal expression patterns from ZFIN, and created a databank for that information.
- The group mapped completely the transcription factors and signal transduction pathways for the zebrafish endomesoderm through integrating the literatures, reporting spatial and temporal expression patterns and using BioTapestry software to integrate the information to build zebrafish embryonic genetic regulatory networks. The manuscript is under preparation.
- The architecture of those networks was verified by perturbation, using morpholino to knockdown one of 35 candidates, and by the Q-PCR method to measure the profile change of other gene expressions for determining the relationship between those genes. The group injected morpholinos against Gata5, Gata6, FoxA2, Sox32, POU1, Otx2, FoxA1, Six3a, Six3b, Gbx1, and Gbx2. Using the Q-PCR results of those morphants on four genes — Gat5, Gata6, Sox32, and FoxA2 — the group has mapped the gene regulatory networks.
- For the linkage of endomesoderm formation and carcinogenesis, the group has found that there are 16 oncogenes and 8 tumor suppressor genes from the expression profiles of the human counterpart genes in 3 colorectal cancer cell lines. The samples were collected with Drs. Wang Hwei-Ming and Chiang Feng-Fan from the division of colorectal surgery at Taichung Veterans General Hospital.
- The study found the expression of tumor suppressor genes increased and the expression of oncogenes decreased after treatment with anti-cancer drugs. This can be an easy and fast in-vitro screening system in search for new anti-cancer drugs.

D. Collaboration

1. Collaboration with other NHRI research units

The Division of Molecular and Genomic Medicine is working to expand its collaboration with other divisions of NHRI, such as the National Institute of Cancer Research and the divisions Division of Biotechnology and Pharmaceutical Research. The division has already collaborated with the latter on establishing assays and screening schemes for product development.

2. Collaboration with local institutes in the Hsinchu area

The division believes that it can provide an excellent training environment for students, who represent an important driving force of research. To formally engage with the universities in the area, the division is working to establish a program at National Tsing Hua University focusing on biotechnology in medicine. Members of the division have offered lectures and laboratory courses to students at local universities.

3. Participation in the National Research Program of Genomic Medicine

The research in cancers (liver and lung), microbial genomics, and innovative technology are in line with those of the National Research Program of Genomic Medicine. Through this program, the division can apply for grant support as well as join national efforts studying diseases that are especially relevant to the health of the residents of Taiwan.

4. Engagement with the health care system and biotechnology industry

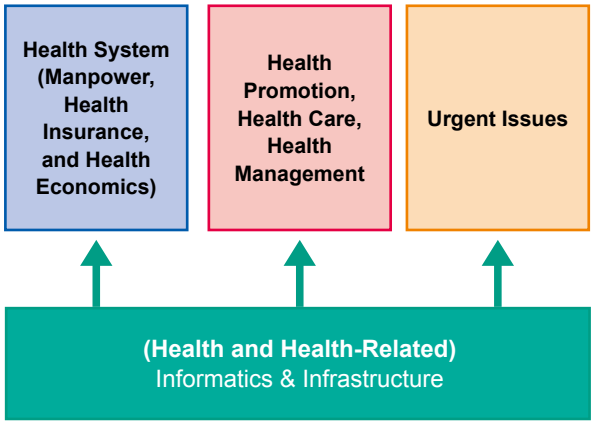
The government and public increasingly would like to know how research spending has benefited the society. As NHRI is supported by Taiwan's govevnment via Department of Health, the division is obligated to keep in mind the need of the health care system. Not only should we focus our research based on disease prevalence but also target our projects on the specific issues of the health care system. Additionally, the research activities should be linked to development in the biotechnology industry, in which the division will constantly pay attention to the trends of the industry, for example, biomarkers for prevention and diagnosis of diseases.

Center for Health Policy Research and Development

Mission

Functioning as a health policy think tank, the Center for Health Policy Research and Development identifies urgent issues in public health, the medical system, health education, and manpower resources to propose evidence-based recommendations to help policymakers improve the quality of health policies and thus enhance the nation's health. The center recruits experts from different professions to promote health-related industry and has established a communication network to aid cooperation with leading universities and health institutions in order to facilitate public health research. At the same time, the center promotes international collaboration and exchange to better understand global trends in health policy and increase the visibility of the center.

Research Framework



To advance the development and efficacy of health policies and optimize the allocation of manpower and various resources, the center focused on two major domains in 2007: long-term developmental projects, which involve planning future research, and mission-oriented projects, which are often aimed at helping the government policy makers address timely needs.

Major Progress

A. Health System: Manpower, Health Insurance, and Health Economics

1. Private supplemental health insurance in Taiwan

This project investigates the interrelation in Taiwan between the supply and demand of supplemental private health insurance and its development in response to National Health Insurance policy. A total of 3002 randomly sampled members of the public were interviewed in the 2006 survey, with the results inputted into a database. Questions concerning financial status, health condition, investment in private health insurance, perceptions of health insurance, and utilization of the National Health Insurance system were included. This database is able to provide evidence-based, practical, and valuable information for policy making and strategic planning for both the National Health Insurance (NHI) and private health insurance networks. One thousand questionnaires each from Taiwan's two largest cities, Taipei and Kaohsiung, were collected in 2007 and are being analyzed.

2. Use patterns and pharmacoeconomic evaluation of psychotropic medications

This project used the longitudinal NHI Database of 2002–2004, which includes 200,432 randomly selected individuals as well as selected individuals who received any second-generation antipsychotic medications or new antidepressants between 2002 and 2004, inclusive. Among the 1,325 individuals receiving second-generation antipsychotic medications, 5%, 23%, 23%, 13%, and 36% were aged 0–17, 18–34, 35–49, 50–64, and 65 and over, respectively. Men and women were equally distributed in this sample. Among the 4,354 individuals receiving new antidepressants, 5%, 27%, 30%, 19%, and 39% were aged 0–17, 18–34, 35–49, 50–64, and 65 and over, respectively. Men and women accounted for 39% and 61%, respectively, of this sample.

Using the same database, researchers found that 203 (7%) of the 2,807 individuals who received any antidepressants during 2002–2004 were newly diagnosed with hypertension. The preliminary results indicate that citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, and venlafaxine, are not associated with an increased risk of hypertension, unlike conventional antidepressants.

This project also used the Longitudinal NHI Database of 2000–2004, which includes those admitted to hospitals between 1996 and 2001 due to mental disease(s). Between September 2002 and December 2004, 17,526 subjects received antipsychotic treatment; and 442 (3%) of them were newly diagnosed with diabetes mellitus. The preliminary results indicate that clozapine and olanzapine are more associated with an increased risk of diabetes mellitus among schizophrenic patients than first-generation antipsychotics. Risperidone and quetiapine, however, were not associated with such a risk.

3. Physician supply and demand analysis

This project projected future demand for medical services and certain specialists. The center analyzed the National Health Insurance database to learn the average medical resource utilization of orthopedic surgeons, general surgeons, and ENT surgeons in 2002–2004, and then used Miller's mathematical model to estimate the growth of manpower in 2007–2021. Based on the estimates of medical utilization and assuming all doctors retire at the age of 65, in 2021 Taiwan will need 1577 orthopedic surgeons, 6411 general surgeons, and 1412 ENT surgeons. The center's projections are that by that same year — with annual increments of 50 orthopedic surgeons, 170 general surgeons, and 66 ENT surgeons — Taiwan will have 1403 orthopedic surgeons, 6054 general surgeons, and 2239 ENT surgeons.

4. A prospective study on Diabetes Management through an Integrated Delivery System (DMIDS)

This project aims to improve quality of diabetic care, especially by achieving better diabetes control by integrating a community-based delivery system. In 2003 a total of 1223 patients with diabetes were recruited, of whom 617 were randomly assigned to the experimental group and 606 to the control group. Patients in the control group receive usual care by the primary physician, while those in the intervention group received additional diabetes-related educational consultations with case managers every three or four months. A process evaluation in the third year showed satisfactory rates of adherence to the program, with the overall biannual biomarkers follow-up exam, annual ophthalmological exam, annual foot exam, annual EKG exam, and adherence to scheduled health education achieving rates of 89%, 91%, 96%, 94%, and 76%, respectively.

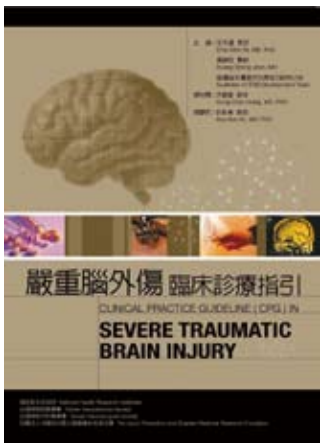
The two-year preliminary results showed that the HbA1cs in the intervention group is significantly better than that in the control group after 6 months, 18 months, and 24 months, especially in the group with initial HbA1cs between 8% and 10%. This demonstrates that regular education consultation is generally effective in improving glycemic control. Although the blood pressure and lipid profiles have not reached the expected goals, this project does prove the effective roles of case managers in the care for type 2 diabetic patients in communities.

5. Effectiveness evaluation of well-child care in Taiwan

This project aims to obtain helpful information for formulating policies to advance and monitor the quality of well-child care. A database containing information on the provision of well-child care and quality monitoring in developed countries has been established. The information shows that most developed countries emphasize free provision of well-child care to their citizens. This finding supports the Bureau of Health Promotion's advocacy of well-child care in Taiwan. Two reports on the investigation on the circumstances of well-child care provision in Taiwan, and the quality and the substitution between physicians' and nurses' time in the provision of such care, have been written. The health care utilization data for children born between 1997 and 2005 have also been put into a longitudinal database.

B. Health Promotion, Health Care, and Health Management

1. Development of clinical practice guidelines on major diseases in the National Health Insurance database



This project has coordinated major medical professional associations to promote evidence-based medicine (EBM) and formulate guidelines for EBM practice. In August 2007 the center released its first clinical practice guidelines in this area, for severe traumatic brain injuries. Other guidelines — including for acute myocardial infarction, benign hypertrophy of the prostate, pneumonia, osteoarthritis, hemodialysis, hypertension, and chronic virus hepatitis B and C — have been completed and are undergoing revision by internal and external audits. The guidelines will be posted on the center's Evidence Based Clinical Guidelines information platform, http://ebpg.nhri.org.tw, to promote their use and facilitate international interchange.

2. Child and adolescent behaviors in long-term evolution (CABLE): second phase

In 2001 eighteen primary schools were randomly selected from Taipei City (representing urban areas) and Hsinchu County (representing rural areas). A total of 2,218 first graders (cohort 1) and 2,075 fourth graders (cohort 2) with parental permissions completed the questionnaires. Annual follow-up cross-

sectional surveys — focusing on aspects such as health behavior, mental health, family interaction, and peer relationships — have been implemented since 2001.

A database has been built, statistical analyses performed, and papers written using these cross-sectional and longitudinal data.

Analysis indicates that the period around the time children enter junior high school is a critical one. During that stage, children perform more negative health behaviors than positive health ones. Furthermore, some behaviors showed different trajectories, depending on gender and area. More observation is needed to confirm the developmental trends of each behavior and other important issues to understand the factors of different issues for intervention. The study is ongoing.

3. Building a patient-safety system — culture survey

This project analyzes patient safety culture by leadership. The sample comprises managers of study hospitals. Dr. J.B. Sexton's Safety Attitude Questionnaire was translated into Chinese, with pretesting performed in March. Cronbach's α of six dimensions of the Safety Attitude Questionnaire had a result of 0.75 to 0.85 (teamwork climate, safety climate, job satisfaction, stress recognition, perceptions of management, and working conditions). A total of 116 study hospitals — including 9 medical centers, 32 regional hospitals, 64 district hospitals, and 11 psychiatric hospitals — participated in this study. The team sent 4,386 questionnaires and received 3,185 responses, a response rate of 72.6%. Dr. Sexton came to NHRI to discuss the results and taught the research team how to deal with raw data for broader publishing. According to the results, the scores for patient safety culture were not very positive (3.64–3.99).

4. Prevalence, risk factors, medical utilization, and health behavior of chronic kidney disease in Taiwan

A total of 1,223 participants were recruited for this designated diabetic cohort. Preliminary results show 35.7% of the cohort has diabetic nephropathy and that the risk factors contributing to diabetic nephropathy include maleness, old age, relatively low education, longer diabetic duration, exposure to smoking, poor control of blood pressure, and blood sugar. Those who had poor diabetic control (HbA1c≥9%) had 3.19 times ($P<0.0001$) higher risk to develop proteinuria than those who had HbA1c<9%. Smoking status is also significantly related to diabetic nephropathy. For male smokers with more than 30 pack-year exposure to smoking, the risk of proteinuria is 2.48 times ($P=0.025$) higher than that for non-smokers.

C. Health and Health-Related Informatics & Infrastructure

1. National Health Interview Survey — data analysis and linkage

In 2007, this project has prepared data releases, wrote research briefs, published articles in a special issue of the Journal of Taiwan Public Health, prepared data linkages, and analyzed new data, comparing it with the results of previous surveys.

The three reports the team published in 2007 covered changes in oral-health-related behaviors of the general population between 2001 and 2005 in Taiwan, the falls of the elderly between 1999 and 2005, and the relationship between occupation and alcohol drinking in those ages 18–64. All three reports provided important information for promoting health and preventing diseases.

This project also issued news releases to educate the general public on how to have a healthy and happy Chinese New Year, manage a stroke, increase dental care, prevent falls in the elderly, and control asthma. This information was distributed to the media and public health professionals.

2. Health data center for primary prevention promotion

Since 2001 this project has worked to construct and maintain a health database that facilitates research in the field of primary prevention. The database already contains data on population and mortality from 1971 to 2004, a cohort who received employer-sponsored medical examination programs between 1989 and 1992, and the 2001 Taiwan National Health Interview Survey. It also includes annual reports of the cancer registry, by the Monopoly Bureau, and by the Department of Health. Other information will be collected and added later, including mortality statistics from Japan, Hong Kong, Singapore, South Korea, and the United States, as well as those countries' respective population data and health related survey data. The center continues to assist those performing primary prevention research in public health, epidemiology, and health policy.

3. Establishment and application of a health geographic information system: a health e-Penghu community health resources decision support system

A Geographic Information System (GIS) provides a suite of tools to examine health outcomes' relationships to location and the distribution of service providers, with respect to health and demographic

characteristics and other GIS reference layers. It enables researchers and policy makers to better understand the distribution of resources and health outcome, and improves the ability of decision-makers to understand the implications of economic, social, and demographic factors on the distribution of services. Together with the Division of Environmental Health and Occupational Medicine, the center is currently studying two such projects: the National Risk Center Initiative Pilot Project and the Analysis of Temporal and Spatial Pattern of Influenza-like Illness using Sentinel Surveillance Database through a GIS System.

4. Establishment of an evidence-based medicine research & development center and East-Asian Cochrane network

In order to enrich resources for evidence-based medicine and accelerate clinical applications, NHRI has subscribed to the Cochrane Library, an EBM database, and made this available to researchers throughout Taiwan, including at 61 regional hospitals. Since December 2007 the center has been working with some 300 physicians from 17 medical centers to translate the information in the Cochrane Library into Chinese, to make it better available to local users.

Together with the Taiwan EBM Association, the center co-hosted the Second Asia-Pacific EBM Network Conference in 2007 with the hope of identifying common ground and cooperating with other researchers in other countries in East Asia. At the end of the conference some 400 attendees from 16 countries reached a consensus to establish the East Asia Cochrane Alliance. The Center for Health Policy Research and Development has joined Guidelines International Network as an organizational member, which will help the Department of Health strengthen its international medical cooperation, its global disease prevention plan's health promotion, and its international visibility, thus improving the domestic research capacity.

5. Development of a health policy e-academy

Having knowledge easily accessible in digital format and having a digital learning environment have become crucial throughout the sciences. The center has established four such platforms: the Learning and Content Management System (LCMS) of the Health Policy e-Academy, a Web-based video conference system, a video conference reservation system, and the Health Policy e-Academy Portal. So far 33 special lectures, 7 e-learning curricula, and 13 e-books have been digitalized and made available through the e-academy.

D. Urgent Issues

1. Healthy People 2020

In order to construct a roadmap to improve health for all in Taiwan by 2020, the center and the Department of Health have been conducting a joint two-year project, "Healthy People 2020." The project plans to increase life expectancy and decrease health inequalities in Taiwan through health policies. Therefore, based on the model of health determinants, public health experts, health professionals, and health administrators have gathered to discuss how to promote and improve people's health in Taiwan. These experts have been tasked into four groups — social environment, healthy lifestyle, quality healthcare, and focus populations — to examine health problems and set up health policies according to their respective expertise. The first edition of "Healthy People 2020" and "Healthy People 2020 Executive Summary" were published in May 2008.

2. Prevention of betel nut hazards

Beginning in March 2006 the committee held several meetings to discuss betel nuts in Taiwan and related problems. The following year, after gathering opinions from experts in health, academia, and government, the committee produced a report, "The Prevention of Betel Nut Hazards — the Feasibility of Betel Nut Taxes." This report outlines the betel nut industry and the problems associated with betel nuts. It also reviews the benefits of laws, regulations, and policies in the reduction of betel nut use. Moreover, the report evaluates the feasibility of betel nut taxes recommends what steps to take next in the battle against the hazards of betel nuts. The final report was distributed to relevant authorities.

E. Policy Recommendations

In 2007, the Center for Health Policy Research and Development drew up policy recommendations on strategies for Taiwan's bid to join the World Health Organization, strategies for Taiwan's participation in APEC, strategies for health aid to foreign countries, the design of performance-evaluating indicators for medical missions, the framework of a strategic task force for international health cooperation, the feasibility of betel nut taxes, and physician's education, practices, and national health expenditure. These recommendations have been delivered to the relevant offices in the public sector, including the Office of the President, National Security Council, Department of Health, Council of Agriculture, Ministry of Foreign Affairs, and International Cooperation Development Fund.

F. Health Informatics Research and Development

New technological advances in data extraction and knowledge discovery have revolutionized health informatics. The center has been a part of this through its research projects on health informatics systems and its maintenance of important data banks, such as the following:

- Health Data Bank: http://hdata.nhri.org.tw
- Health Geographic Information System: http://hgis.nhri.org.tw
- Children and Adolescent Behaviors in Long-term Evolution: http://cable.nhri.org.tw/
- National Health Interview Survey (NHIS): http://nhis.nhri.org.tw/
- A Prospective Study on Diabetes Management through an Integrated Delivery System (DMIDS): http://dmids.nhri.org.tw/
- Evidence Based Practices Guidelines: http://ebpg.nhri.org.tw/index.aspx
- Healthy Policy e-Academy: http://ehealthpolicy.nhri.org.tw/hpea.htm

G. International Health Research Cooperation

1. Establishment of an academic network for cooperation in international health policies

The purpose of this project is to develop cooperative relationships with international organizations and think tanks from developed countries through joint research on international health issues, and to subsequently establish an academic network for cooperation in international health strategies. Toward these ends, in 2007 the center participated in international conferences and interacted with experts from important international health-policy and health-security think tanks in the Asia-Pacific and European regions.

Five policy recommendations have been drawn up: strategies for Taiwan's bid to join the World Health Organization, to gain greater participation in APEC, and to provide health aid to foreign countries; the design of performance-evaluating indicators for medical missions; and the framework for a strategic task force for international health cooperation.

To study the implementation of Taiwan's local health and medical care system in remote areas, the center also conducted field surverys of Ren'ai Township in Nantou County and Xiulin Township in Hualian County. Based on these surveys and other information, the center has produced plans for a fact-finding tour for foreign guests and those in relevant sectors who wish to learn from Taiwan's successful public health infrastructure and unique experiences.

2. Holding international annual conferences

▶ **Eighth Asia-Pacific Conference on Tobacco or Health**

More than four hundred participants from thirty-nine countries gathered to discuss tobacco control at this three-day conference co-organized by NHRI.

▶ **Tenth European Health Forum, Gastein**

The director of the Center of Health Policy Research and Development, Professor Kuo, was invited to co-chair and co-organize the gathering's forum on pharmaceuticals and the European Union.

▶ **Second Asia-Pacific Evidence-Based Medicine Network Conference 2007**

NHRI and the Taiwan Evidence-Based Medicine Association co-hosted the Asia-Pacific EBM Network Conference, November 16–18, 2007.

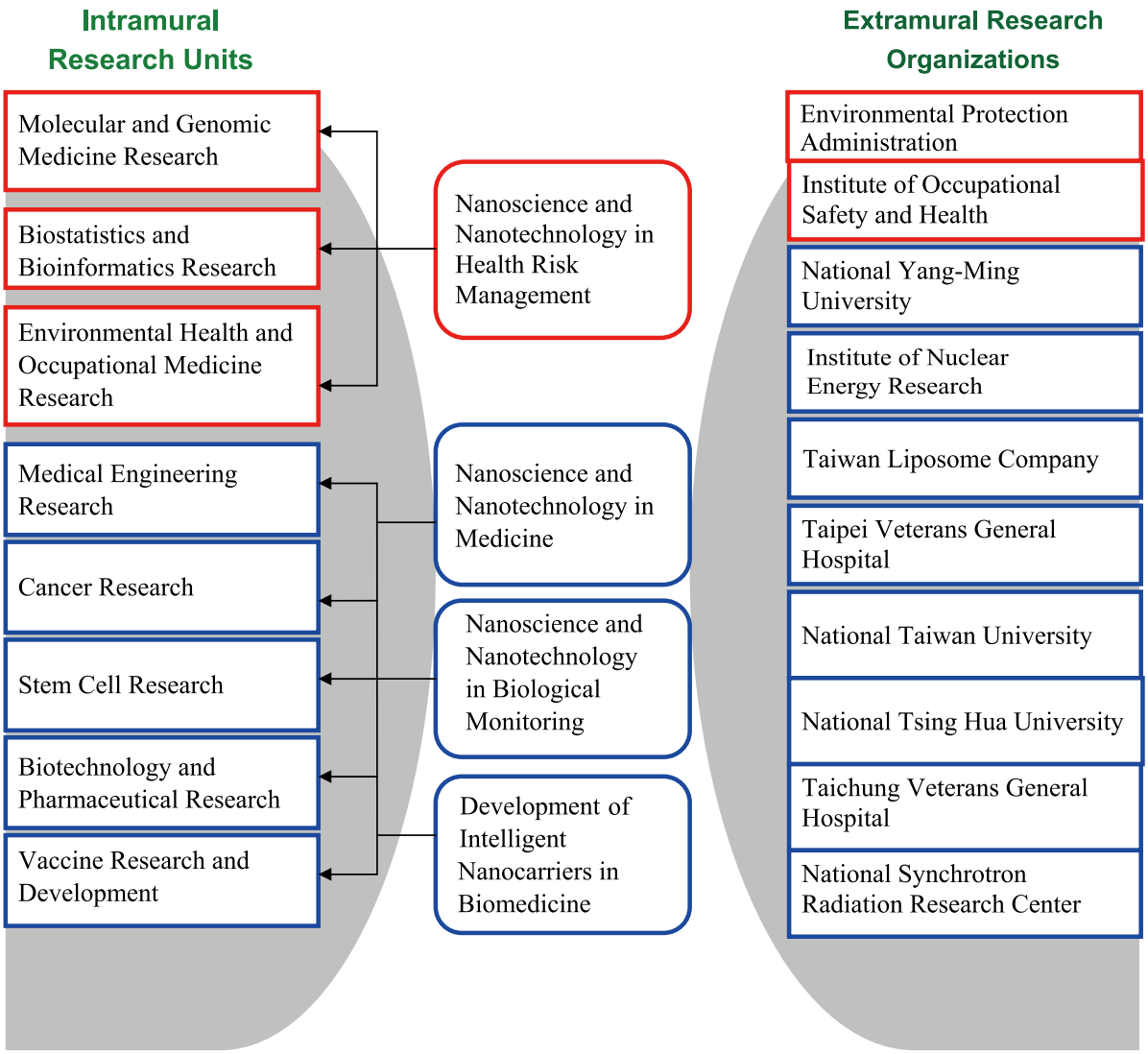
Center for Nanomedicine Research

Mission

The Center for Nanomedicine Research at NHRI was established in 2004. The center has several main missions:

- discover new techniques and applications for nanotechnology
- integrate and coordinate intramural and interdivisional nanomedicine research at NHRI
- integrate and coordinate extramural nanomedicine research activities through collaboration with universities, hospitals, and centers for medical research
- educate and incubate research manpower in the nanomedicine field of Taiwan
- provide a platform for exchange of research experience and activities for nanomedicine researchers
- promote the relevant application of nanotechnology to significant medical problems.

Infrastructure for Collaboration



Major Progress

A. Research on Nanotargeted Radiopharmaceuticals for Cancer Imaging and Therapy

Nanotechnology has great potential for applications in a wide range of fields, including cancer prevention, diagnostics, and therapeutics. Recent advances in drug delivery — using nanoliposomes and nano-immunoliposomes through passive and active targeting, and multifunctional and multivalent targeting on tumor sites — as well as research on diagnostic molecular imaging, create new opportunities for the application of nanotechnologies in novel anticancer diagnostics and therapeutics development with not only higher specificity and efficacy but also fewer toxic effects. The center has Taiwan's first integrated alliance research and development project on the development of anticancer molecular nano-biotargeted therapeutic radiopharmaceuticals for treatment of tumors and ascites. Research advancement has been carried out from novel nanotargeted drug design and formulation to laboratory preclinical animal model studies. This project has focused on the development and preparation of passive and active nanotargeted therapeutics with liposome and immunoliposome nanoparticles to deliver radio-therapeutic and radiochemo-therapeutic payloads selectively targeting tumors and ascites, and to study the biodistribution, pharmacokinetics, radiation dosimetry, therapeutic efficacy, and dosing of nanotargeted therapeutics. *In vivo* molecular imaging was applied to study drug targeting and therapeutic response. This groundbreaking cancer nanomedicine initiative has created opportunities for novel anticancer drug research and development, applications, and interdisciplinary personnel training in Taiwan. Institutes around the world have contributed to the project.

The center's overall research goals from 2004 to 2008 include: (1) research and development of novel bifunctional and bimodality passive and active nanotargeted radiopharmaceuticals; (2) preclinical biodistribution, pharmacokinetics, radiation dosimetry, and pharmacodynamic studies of nanotargeted therapeutics for treatment of malignant tumor and ascites animal models; (3) *in vivo* nuclear and optical molecular imaging studies of tumor targeting and therapeutic response of novel bifunctional and bimodality nano-targeted therapeutics.

Optimized drug development processes will be explored and compared. Possible translation research with nanotargeted radiopharmaceuticals from bench to bedside for treatment of malignant ascites will be identified in future investigations. The new integrated platform technologies will not only enhance the development of cancer-curing drugs, but also help accelerate the discovery and development of drugs for other diseases.

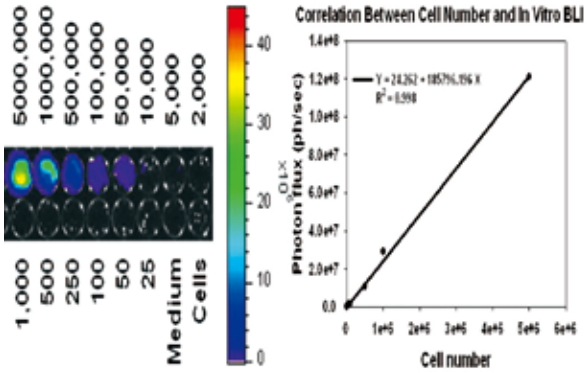


Figure 1. *In vitro* bioluminescence of CT-26/tk-luc tumor cells expressing luciferase. CT-26/tk-luc were diluted from 5×10^6 down to 25 cells and scanned for one minute post luciferin addition. Wells containing cells (no luciferin) and medium only served as negative controls. A good correlation between cell number per well and bioluminescence (photon/s/well) was established, with $R^2 = 0.998$.

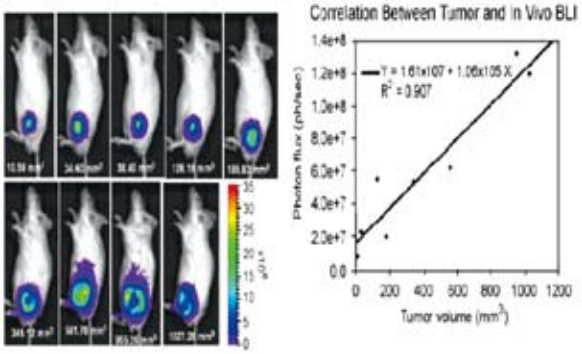


Figure 2. Monitoring subcutaneous animal tumor growth *in vivo* with bioluminescence imaging. CT-26/tk-luc cells were injected subcutaneously into BALB/c mice (n = 4). Tumor growth in animal model was monitored and quantified weekly by BLI and caliper measurements. A good correlation of BLI to tumor volume was observed ($R^2 = 0.908$).

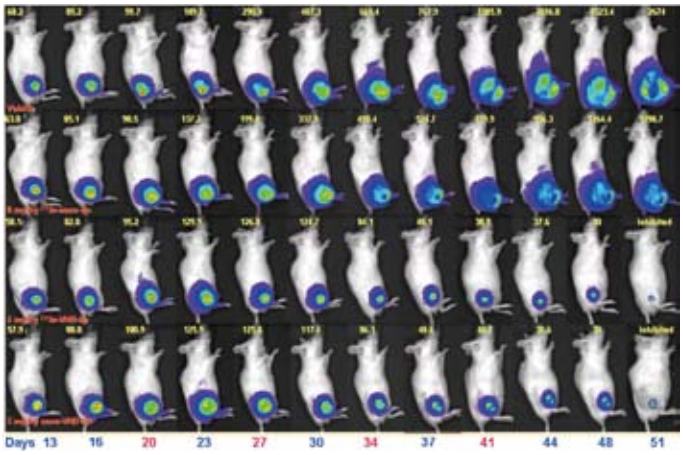


Figure 3. *In vivo* bioluminescence imaging of HT-29/luc tumor bearing SCID mice. HT-29/luc tumor cells (2×10^6) were transplanted subcutaneously into dorsal region of right thigh of male SCID mice and followed by i.v. injection of passive nanotargeted ^{111}In-NanoX/VNB-liposomes at the indicated times. Significant therapeutic efficacy was found in both the bimodality radiochemo-therapeutics of ^{111}In-VNB-liposomes (^{111}In 100μCi × 4 and VNB 5 mg/kg × 4) and chemo-therapeutics of NanoX-VNB-liposomes (VNB 5 mg/kg × 4) groups.

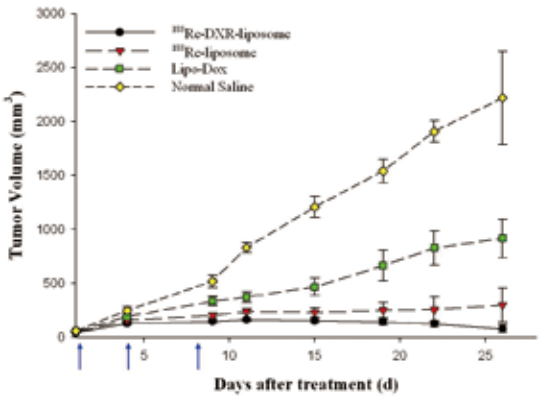


Figure 4. Demonstration of the therapeutic effectiveness of tumor growth inhibition by passive nanotargeted radio-therapeutics of ^{188}Re-liposome and bimodality nanotargeted radiochemo-therapeutics of ^{188}Re-DXR-liposome on CT-26 solid tumor bearing in Balb/c mice animal model.

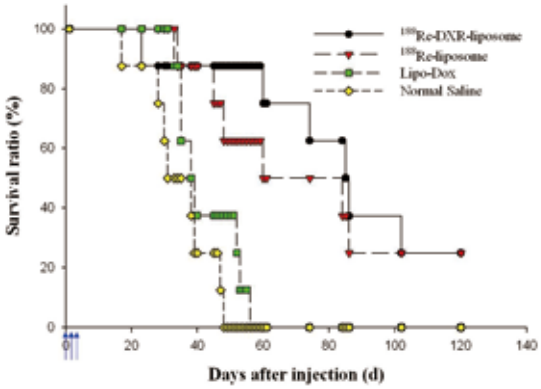


Figure 5. Illustration of the therapeutic efficacy by survival curve for mice treated by passive nanotargeted radio-therapeutics of ^{188}Re-liposome and bimodality nanotargeted radiochemo-therapeutics of ^{188}Re-DXR-liposome on CT-26 solid tumor bearing in Balb/c mice animal model.

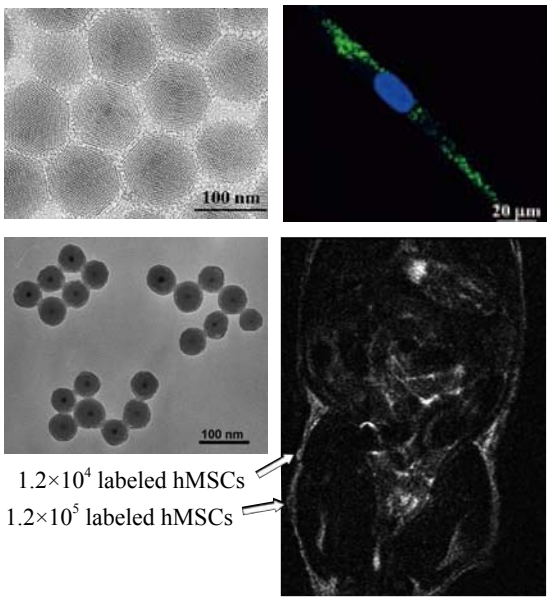
B. Studies of Stem Cell Biology Using Nanotechnological Approaches

Therapeutic uses of stem cells in replacing damaged endogenous cell populations have received a great deal of attention. To distinguish whether cellular regeneration originates from an exogenous cell source, the development of techniques to track therapeutic stem cells in patients is crucial.

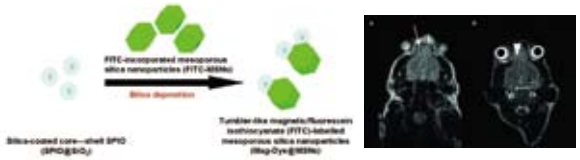
Nanotechnology is undergoing explosive development on many fronts. In biomedicine, for example, nanotechnology plays an innovative role in various applications, including imaging, drug delivery, biomarkers, and biosensors. In stem cell tracking, the use of superparamagnetic nanoparticles composed of magnetic resonance imaging (MRI) contrast agents (e.g. iron oxide and gadolinium, Gd) is the most common. Stem cell tracking by these nanoparticles, however, has two major serious limitations: their low cellular internalization efficiency and their harmful effects on the biological functions of stem cells. Therefore, the major goal of this research is to develop a useful nanoparticle with highly efficient cellular internalization and biocompatibility.

The project had several main achievements:

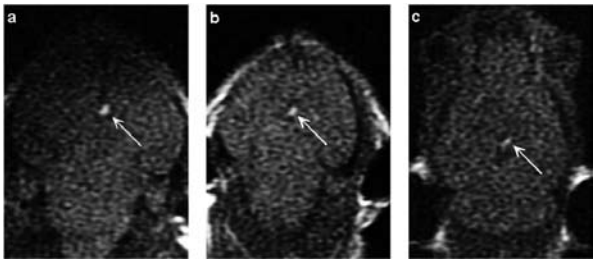
1. A novel vector composed of mesoporous silica nanoparticles (MSNs) with diameters of 110 nm displayed the advantages of biocompatibility, durability, and higher efficiency in internalization and suggested the potential application of MSNs in stem cell tracking.
2. Fluorescein isothiocyanate (FITC)-incorporated silica-coated core-shell superparamagnetic iron oxide nanoparticles — SPIO@SiO2(FITC), with diameters of 50 nm — were developed as a bi-functionally magnetic vector that can efficiently label stem cells for MRI.



3. Tumbler-like magnetic/FITC-labeled MSN, Mag-Dye@MSNs, have been developed. These are composed of SPIO@SiO2 nanoparticles co-condensed with FITC-MSNs.



4. Dual-functional Gd-FITC MSNs (Gd-Dye@MSNs), which possess fluorescence and paramagnetism, are developed and show potential for effective T1-enhancing trackers in human mesenchymal stem cells.



C. *In Vivo* Implantable Dual-functional Microdevice for Simultaneous Phtoenergy Transmission/Defection and Neurochemical Delivery/Analysis

An implantable, dual-function needle-type microprobe (500 μm o.d.) composed of a microdialysis probe and 13 optical fibers was designed and constructed (combo-microprobe). This combo-microprobe is capable of *in vivo* neurochemical delivery/analysis and photoenergy transmission/detection. Implantable microdevices have been intensively developed for diagnostic, monitoring, and delivery purposes in living subjects, including applications toward the central nervous system.

The combo-microprobe was implanted into anesthetized rat brains and used to simultaneously observe the cerebral ischemia-induced increase in extracellular glutamate concentration, which was performed by the microdialysis perfusion and analysis, and the increase in blood–brain barrier permeability,

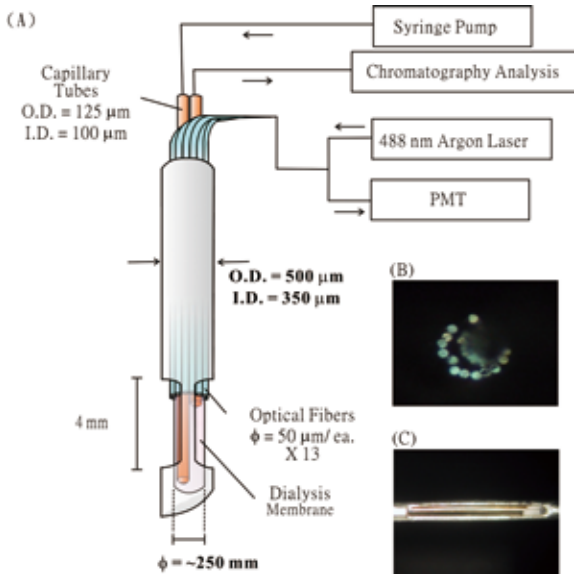


Figure 6. Combo-microprobe schematic drawing.

which was performed by the optical fibers to monitor the extravasated fluorescent nanospheres. This combo-microprobe can be applied to various *in vivo* biomedical investigations in the future. While the fiber optic probe can provide channels for photoenergy transmission/detection, microdialysis can be used simultaneously for neurochemical delivery/analysis in a specific organ under physiological stimulation — a breakthrough integration of two important functions.

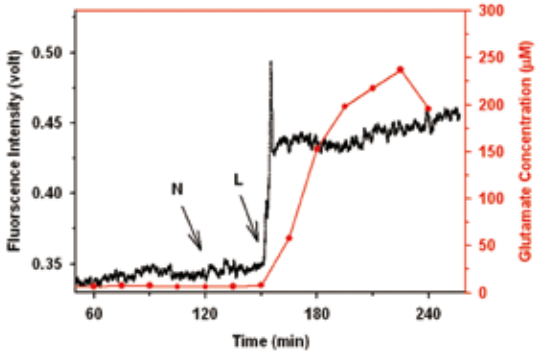


Figure 7. Combo-microprobe implanted into an anesthetized rat brain to simultaneously monitor the extravasation of pre-administered fluorescent nanospheres (continuous line) from cerebral vasculature and the level of glutamate (dotted line) following the ischemic insults. "N" is the time point for injection of the nanosphere solution. "L" is the time point for cerebral ligation.

D. Application of Organic/Inorganic Nanocarriers in Biomedicine Thermal Sensitive Liposome Encapsulated Iron Oxide Nanoparticles Used in Controlled Drug Release and Mri Diagnosis

The center has developed a hybrid organic/inorganic nanocarrier platform to help handle diagnosis, therapy, targeting, delivery, and controlled release. Generally, organic nanocarriers are biocompatible, biodegradable, and easy to formulate. On the other hand, inorganic nanoparticles always have some unique physical properties and are useful in diagnosis. Researchers of the center have developed a hybrid nanocarrier platform that combines the advantages of both kinds of material. For example, some of the hybrids use thermal-sensitive liposomes encapsulated with iron oxide nanoparticles. The liposomes, which are easily modified for targeting delivery *in vivo*, are used to carry hydrophobic and hydrophilic drugs, while the iron oxide nanoparticles are an MRI contrast agent for diagnosis. Also, an embedded heat source under AC magnetic field can lead the controlled release of drug from thermal-sensitive liposomes.

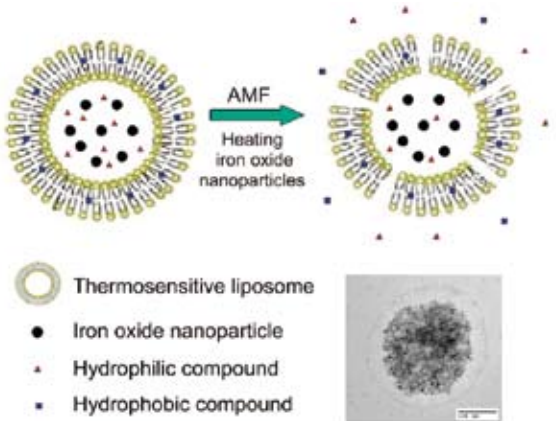


Figure 8. Schematic of controlled drug release from thermal-sensitive liposomes through heating-embedded iron oxide nanoparticles via a non-invasive alternative magnetic field (AMF).

E. Pharmacokinetic and Safety Assessments on Quantum Dots — Computational and Ultrastructural Toxicology of a Nanoparticle, Quantum Dot 705, in Mice

The center conducted pharmacokinetic and toxicology studies on quantum dot 705 (QD705) in male ICR mice for up to 6 months after a single intravenous dose. Time-course sacrifices were carried out at 1, 4, and 24 hours; 3, 7, 14, and 28 days; and 6 months on groups of six mice per time point. Mass balance studies were also carried out at 24 hours, 28 days, and 6 months. Using inductively coupled plasma mass spectrometry (ICP-MS), various tissues, urine, and feces were analyzed for cadmium (Cd111), which is a major (46%) component of QD705. Based on these experimental studies, a physiologically based pharmacokinetic (PBPK) computer simulation model was developed with excellent predictive capability for the time-dependent kinetic and distributional changes of QD705 in tissues. QD705 persisted and accumulated in spleen, liver, and kidney for at least 28 days with little or no disposition but was gradually and partially eliminated by 6 months. Although histological alterations of spleen, liver, and kidney are unremarkable as viewed by light microscopy, investigations using electron microscopy on numerous renal samples revealed definite mitochondrial alterations in renal tubular epithelial cells at 28 days and 6 months postdosing. Health implications and potential beneficial applications of QD705 are suggested.

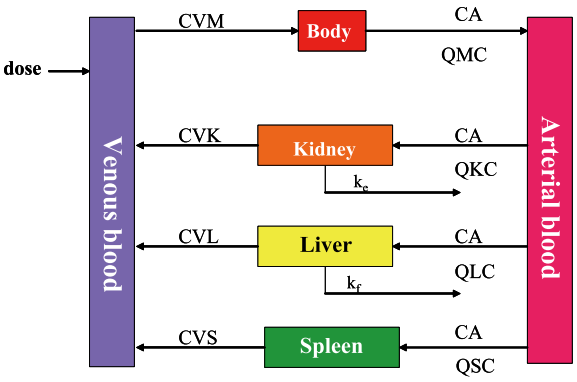


Figure 9. A conceptual PBPK model for QD705 in mice. CVM, CVK, CVL, and CVS represent QD 705 concentrations in venous blood, kidneys, liver, and spleen, respectively. CA is QD 705 concentration in arterial blood. QMC, QKC, QLC and QSC represent blood flow to body, kidneys, liver, and spleen, respectively.

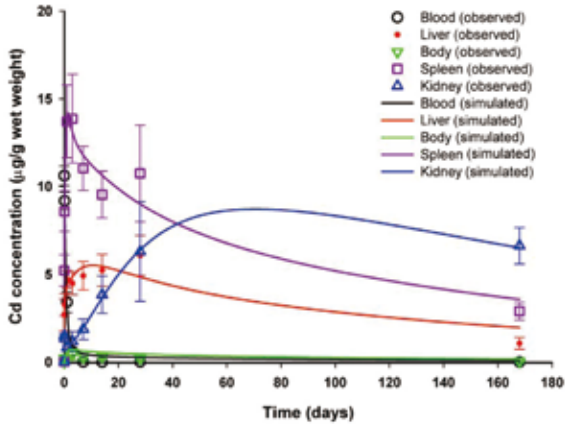


Figure 10. Comparison of PBPK model simulations with experimental tissue concentrations of QD705 6 months following an intravenous dosing

F. Nanoscience and Nanotechnology on Health Risk Assessment – Standardized Evaluation Platform for Nano-safety and Nano - Pharmaceuticals Assessment

The center plays a leading role in investigations into the interaction between nanomaterials and biological systems. In 2007, the center constructed an exposure chamber system for evaluating potential health risks of nano-aerosols upon animals and cells.

The exposure chamber system comprises three main parts: double isolated containers to prevent unexpected exposure, a nanoparticle generator to produce homogeneous and narrowly distributed aerosols and nano-pharmaceuticals, and a particle classifier and counter to monitor size distribution and concentration in real-time. By means of these features, the system can quantitatively define the physical parameters of target airborne nanoparticles.

The nanoparticle exposure chamber system, which features a highly integrated operation interface and newly developed protocols, can be customized to establish infrastructure and provide intelligent inputs for both nano-safety and nano-pharmaceutical assessments.

Vaccine Research and Development Center

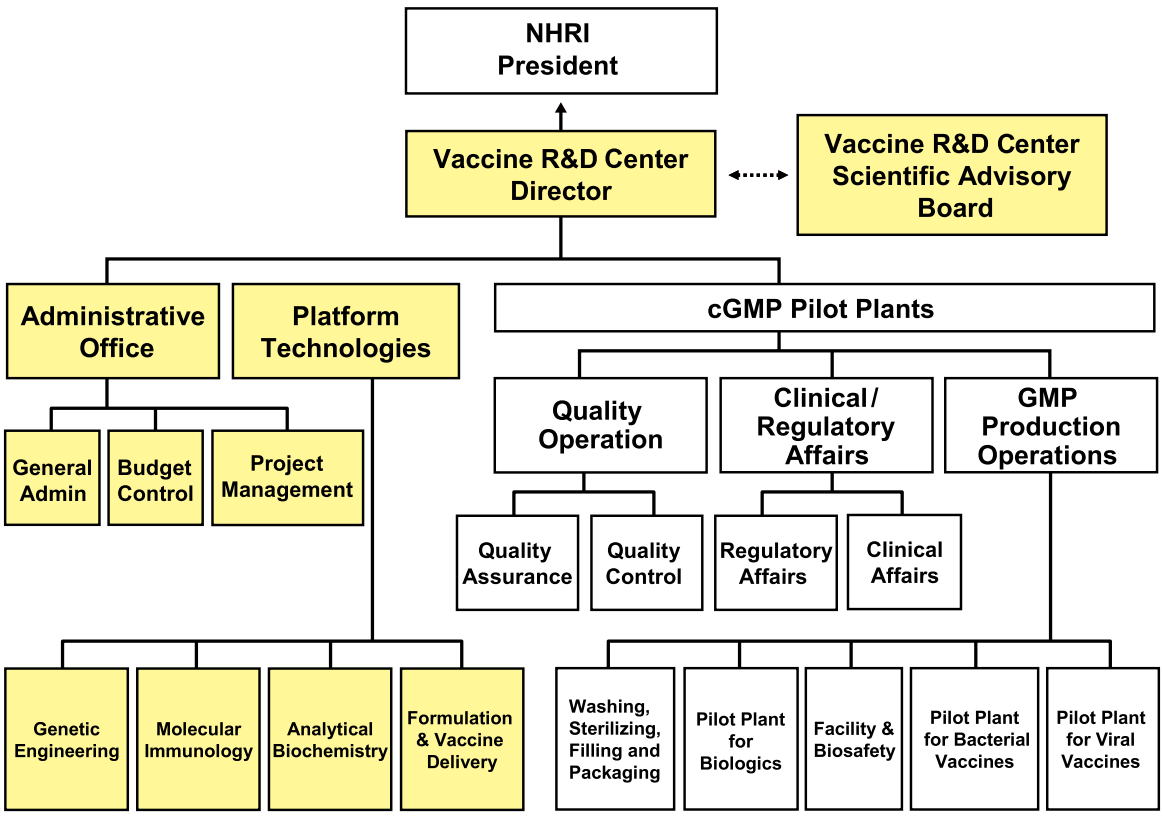
Mission

The Vaccine Research and Development Center works to develop vaccine-manufacturing capability, strengthen the foundation of vaccine research and development in Taiwan, implement vaccine-related government policies, and respond to emergency requests for vaccines against emerging infectious diseases.

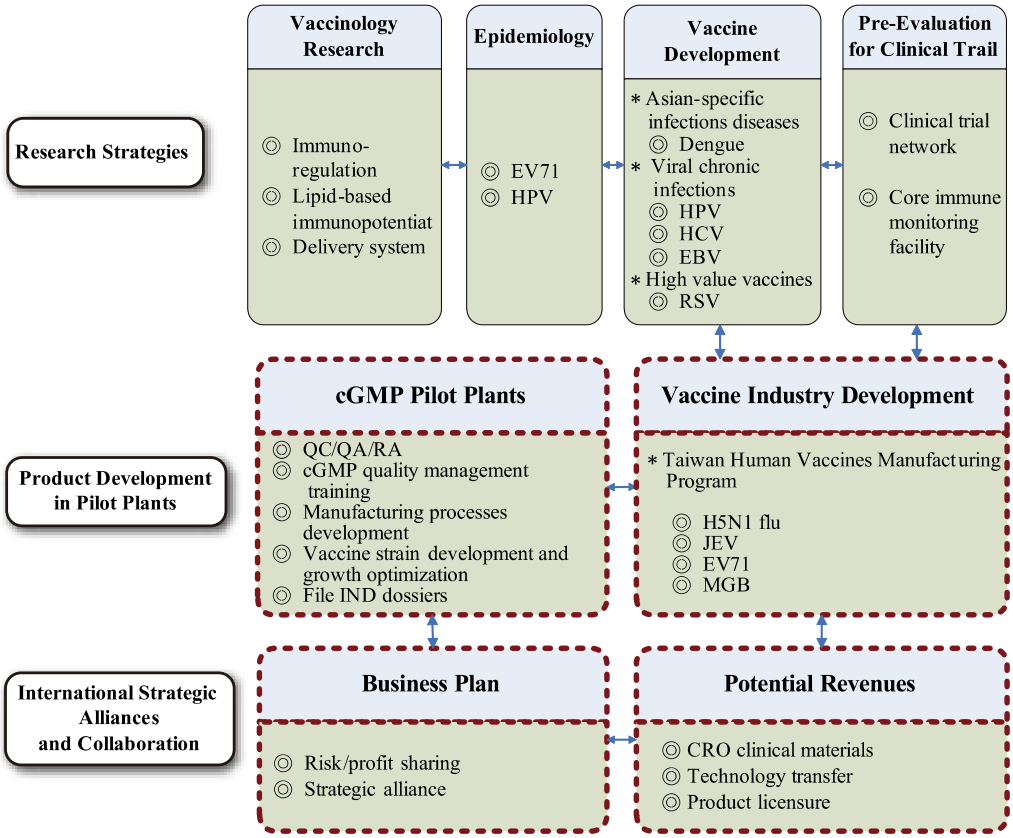
With the aid of government funding, the Vaccine Research and Development Center has already completed the first phase of a three-year development plan. This includes the following steps:

- Integrate limited resources and cGMP expertise to establishing a dedicated team, which will research vaccines against important regional diseases and develop different vaccine platform technologies (see the organization structure below)
- Build GMP pilot plant and successful transfer the products and technology from the Taiwan Centers for Disease Control to the Vaccine Research and Development Center
- Collaborate with international partners to implement training courses in vaccinology, project management for biotechnology, and cGMP quality management for educating local young scientists
- Form strategic alliances and collaborate with local universities and/or small biotechnical companies to bring vaccine candidates to phase 1 and 2 clinical trials in Taiwan and Asia
- Establish an emergency vaccine production P2+ facility in the NHRI research building, train staff in vaccine manufacturing facility located in Taiwan Centers for Disease Control (CDC) with cGMP knowledge and ability to respond to Taiwan Government emergency requests for vaccines against pandemic diseases like H5N1 flu.

Organizational Structure



Strategic Plans



For the second phase of its development, the vaccine center has a global strategic plan, which is divided into three sections: innovative vaccinology research programs, cGMP pilot plant and product-development programs, and strategic alliances and business-development plans.

1. Establishing innovative vaccinology research programs

In the second phase, the vaccine center's R&D program will be run by the high performance matrix project management systems. Each project has a fully justified rationale, SWOT analysis, defined milestones, and timelines for deliverables that are performed by competent staff from different platform technologies (see below) and monitored by the project-management team.

(a) Genetic Engineering Platform

(b) Molecular Immunology Platform

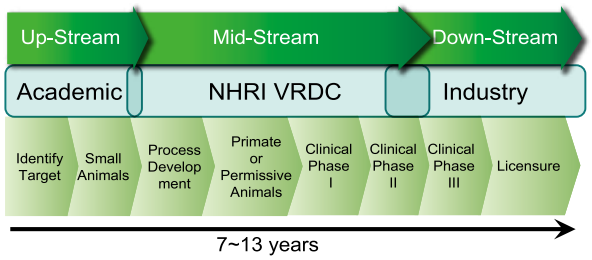
(c) Vaccine Formulation and Delivery Systems Platform

(d) Bioanalytical Chemistry Platform

2. Establishing cGMP pilot plant as a business unit

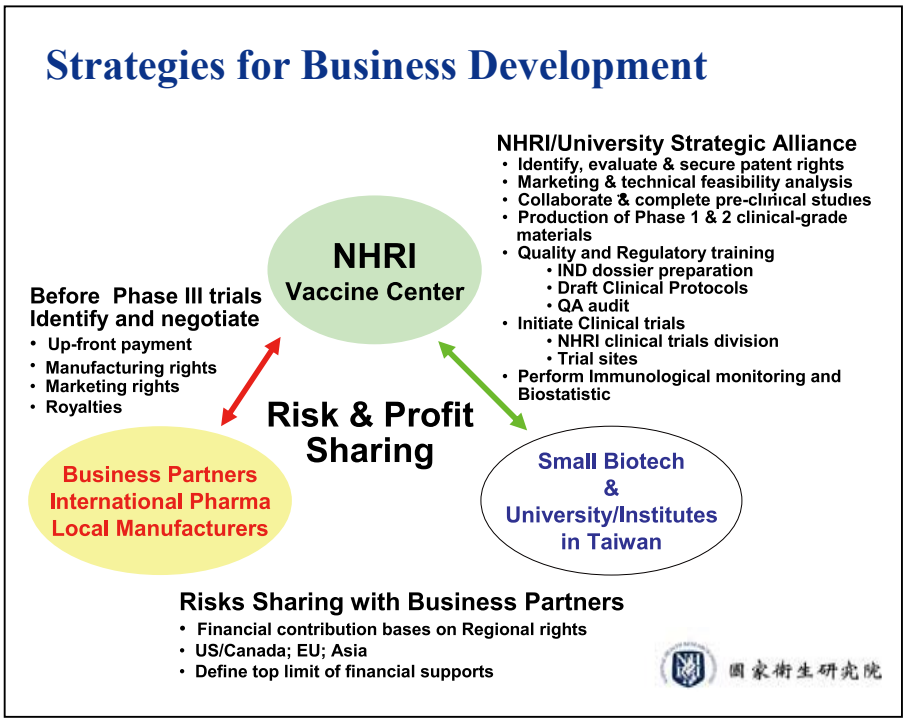
With a critical mass of experienced GMP biological production staff, and the completion of the infrastructure and facility validation in 2008, the cGMP pilot plant can play an important and leading role in Taiwan's vaccine industry (see the diagram below). The vaccine center can provide service to assist any institute that wants its vaccine candidates to enter phase 1 human clinical trials.

Roles of NHRI's Vaccine Center



3. Establishing a risk/profit-sharing business development model

To ensure that the vaccine center will have additional financial support and continued revenues from outside, the Vaccine Research and Development Center is establishing a risk/profit-sharing business development model, as shown in the diagram below. In this business development model, the vaccine center will first identify potential vaccine candidates from either academia or small biotech companies. The center will take the "risk" to embark on pre-clinical studies and manufacture GMP vaccine candidates for human phase 1 clinical trials. During this product-development process, the vaccine center will sign product development agreements with its partners. Within the product development agreements, the vaccine center will contribute the cost of pre-clinical studies, filing the investigational new drug (IND) dossiers, and manufacturing clinical trial materials. The center and its partners will co-apply for grants from Taiwan's Ministry of Economic Affairs to cover the cost of clinical trials. In return, the vaccine center will secure the manufacturing rights and the royalty payments when the vaccine candidate becomes a commercial product.



Major Progress

A. Immune-Regulatory Platform and Its Application in Vaccine Research and Development

1. Dendritic cells (DCs) play a central role in the initiation and regulation of immune responses. DCs have been developed for the treatment of cancer, infectious diseases, and autoimmune diseases. The research team preliminarily established the working system, including the sources of DCs and the assay system. The sources of DCs include the bone marrow-derived DC (BMDC) culture and the expansion of 2 DC lines, while the assay system includes state-of-the-art techniques in molecular and cellular biology.
2. This aims to clarify the roles of TGF-β/IL-10 in modulating immune-suppressive activity of hMSCs. The researchers observed that uMSCs could promote the generation of CD4+CD25+ regulatory T cells; activate Smad2; up-regulate cell cycle controlling factors p15, p19, p21, and p27; and then alter the profile of association between cyclins and CDKs. The effects on T cells caused by uMSCs are greatly similar to those mediated by TGF-β, particularly TGF-β1-mediated suppression. Events induced by uMSCs could be interfered by adding neutralizing TGF-β1 antibodies to the co-culture system. The researchers concluded that uMSCs induce CD4+CD25+ regulatory T cells and modulate the immune system by secreting TGF-β1 and activating the downstream signaling pathways.
3. This project focused on the development of an Asian dominant HPV serotype vaccine. Warts and cervical cancer are strongly associated with HPV infection. The center is collaborating with clinical physician Dr. Cheng of National Taiwan University to identify HLA-A2, HLA-A11, and HLA-A24–restricted CTL epitopes on E6 and E7 oncoprotein using MHC tetramer staining (HPV52 and HPV58).

B. Lipid - Based Immunopotentiator and Delivery System for Novel Vaccine Development

1. In order to prove the effectiveness of lipopeptide vaccination in humans, the research group used humanized HLA transgenic mice as animal models to mimic the effect in humans. The group established three HLA transgenic mice in the center (HLA-A2, A11 and A24). And the group found a novel HLA-A11 epitopes located on HPV18 E6 using HPV associated cervical cancer patients' PBMCs.
2. Focusing on the development of Asian-specific HPV serotype vaccine, the center has developed major platforms for developing CTL epitopes-base vaccines. The center obtained a research service contract (NT$500,000) from PDS Biotechnology Corp., a liposome company interested in these platform technologies, to perform HPV vaccine candidates in HLA transgenic mice.

C. Development of Dengue Vaccine

In dengue vaccine development, the research group aligned domain III (EPIII) of the four dengue virus serotypes to obtain consensus amino acid sequences. Deduced consensus peptides were used as peptide-based vaccine targets. The group found that the selected peptide vaccine candidate was able to elicit cross-neutralization antibody responses against dengue viruses, at least of serotypes 2 and 4. They also expressed consensus ED III (CED III) in the *E. coli* system, and recombinant CED III was able to induce an antibody response. Neutralization assays for serotypes 1–4 are in progress.

D. Mucosal Genetic Vaccine Against Respiratory Infectious Diseases

Human RSV has been recognized as the leading cause of lower respiratory infections in children and increasingly documented as an important pathogen in the elderly. The team attempts to develop a vaccine for this by expressing structural proteins in an adenoviral delivery vector in order to protect the elderly against RSV infection. After 10 infections, splenocytes from B6 mice immunized with rAd-LacZ, rAd-F0, rAd5-F0ΔTM, live RSV, or inactivated RSV were isolated and stained with antibodies for effector memory T cells population analysis.

Preliminary results have shown that the number of CD4+/CCR5+/CD62L+ memory T cells increased in the spleen of mice immunized with rAd-F0 but not in the control or the others' viruses infected mice. In contrast, rAd-F0 immune splenocytes exhibited an increased number of negative immunoregulator, CD25+/FoxP3+ regulatory T cells (Treg). These experiments have revealed that enhancement of cellular immunity and anti-F antibody responses could be achieved by using rAd-F genetic vaccination. In addition, long-lasting immunity can be induced in order to protect natural RSV infection.

E. Epidemiology Platform and Clinical Trial Network for Vaccine Development

1. To design clinical trials of new vaccines, age-specific disease burden and epidemiological characteristics need to be clearly understood. Clinical spectrum of EV71 infection ranges from mild hand-foot-mouth disease to severe cases with central nervous system and cardiopulmonary involvements. The project group has established a serological assay for measuring neutralizing antibody titers against EV71 virus with the help of the Taiwan Centers for Disease Control. The group measured neutralizing antibody titers in the 12 sera collected from 4 families. The sero-positive rates (antibody titer ≥ 1:8) in mother, newborn baby, and 6-month-old baby were 75% (3/4), 75% (3/4), and 0% (0/4), respectively.
2. Professor Michel Klein (chairman of Vaccine Center SAB, former vice president of Aventis-pasteur, and former CEO of CANVAC) spent April–July 2007 at NHRI presenting his course "Vaccinology: all you want to know about vaccines," which served as a forum for educating local and NHRI young scientists in vaccine concepts and technology.

F. Human Vaccines Research, Development, and Self-manufacturing Programs

1. Establish infrastructure and facilities to conduct vaccine research and development to meet local needs

- ***Infrastructure***

The Vaccine Research and Development Center has more than one hundred staff, including the center's director, ten principal investigators (P.I.s), a chief operations officer, five section managers, and more than 70 technical specialists and research assistants (RAs) for product development, research, and center administration. In addition, the center has nine postdoctoral fellows, five students, and four project-

specific contracted research assistants. The product development and manufacturing section is running in Zhunan while the Vaccine Center cGMP pilot plant is being completed. At the same time, the virology and bioanalytical laboratories are being set up to perform product characterization required for avian flu vaccine development. In addition, the clinical and regulatory affairs section has been established and is actively preparing pre-IND filing for avian flu vaccine trials. The center is still actively recruiting P.I.s with expertise in bioanalytical chemistry, molecular virology with an interest in animal model development, fermentation bioprocesses, and vaccine formulation to strengthen our vaccine-development capability.

- ***Construction of the pilot plant***

After Fu-tai Co. declared in August 2007 that it had finished the civil and electrical engineering of the pilot plant, NHRI organized a task force to thoroughly examine the work to date. On June 19, 2008, NHRI reported to the Department of Health that hardware construction of the civil and electrical engineering of the pilot plant was complete.

Although the facilities and utilities of the pilot plant have been finished, validating that all the equipment in the production area meets the requirement of PIC/S requires time. The staff is working with validation consultants to ensure the pilot plant will be fully validated before the end of 2008.

The center is currently setting up the quality system of QA with six categories from warehouse to product release, with assistance of the practical guidance from experts dispatched from Parenteral Drug Association Taiwan Chapter (TPDA). It expects the government to issue its plant license and a cGMP certificate by the end of 2008.

- ***Traditional vaccines production and technology transfer from Taiwan CDC***

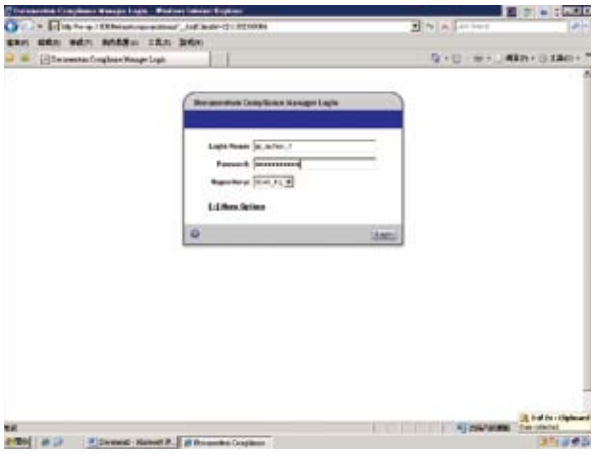
To ensure that domestic demand for traditional vaccines (BCG and antivenom antisera) is fully met, and to avoid any negative impact on the health and life of people in Taiwan, all technological producers have all already moved back to the pilot plant from Taiwan CDC. The center trains the new personnel so that they will be able to complete the entire validation process for the new cGMP pilot plant. All QC, QA, PMO, RA, CA, viral pilot plant, bacterial pilot plant, and central service staff are currently working at the new facility in Zhunan.

Figure 1. Freeze dryer.

Figure 2. Depyrogenation oven.

- ***Electronic document management system (EDMS) project***

To ensure quality, safety, and potency, the production processes of vaccines and biological products are strictly regulated by national and international regulatory authorities. In compliance with GXP regulations, the documents for control and management of research, product development, manufacturing processes, and license application have been clearly defined. Therefore, a well-controlled document management system such as EDMS is an important component of the quality management system of the Vaccine Center. After evaluating different vendors, the center selected the Documentum Technology as the EDMS. The purchase and bidding processes for EDMS software and hardware were completed in late 2006. It has just completed the IQ and OQ; and the PQ validation process of the EDMS system is being initiated.



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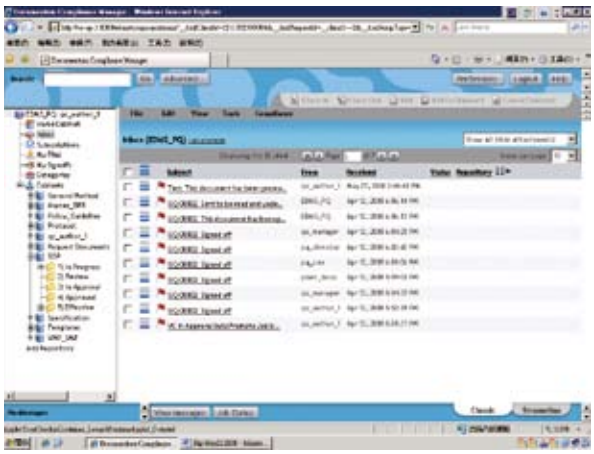

Window of EDMS

2. Establishing state-of-the-art vaccinology technology strategic alliances and joint ventures to produce cGMP-grade vaccines candidates for clinical trials in Taiwan and Asia

The vaccine center has formed strategic alliances — including with GSK, Sanofi Pasteur, and NIID of Japan; CANVAC, VGH/NYMU, and BCCDC of Canada; IVI of Korea; Indian Immunological Ltd. of India; IMOAE of the United States; and ADImmune of Taiwan — to acquire state-of-the-art vaccinology expertise, establish cGMP facilities, and assist the center in initiating clinical trials in Taiwan and elsewhere in Asia. GSK has offered training courses to help NHRI staff organize and conduct clinical trials. The EV71 and JEV vaccine development groups have visited the National Institutes of Infectious Diseases (NIID) of Japan and gleaned firsthand information about the phase 3 trials of JEV vaccines performed in Japan and the latest developments in EV71 vaccines. In collaboration with GE Healthcare Life Sciences, in May 2007 the center offered a one-day training course in vaccine manufacturing processes and technology. The vaccine center, National Yang Ming University (NYMU), and VGH Research are discussing collaboration to monitor vaccine efficacy in clinical trials and investigate immunomodulatory mechanisms in acute and chronic persistent viral infections, as well investigating opportunities to create a forum to train clinicians in "translational research."

3. Serving as the forum for training and educating local young scientists in vaccine-related biotechnology

- ***Training course on cGMP compliance and management***

To increase its visibility in both the biotechnology industry and academia, the vaccine center has organized cGMP compliance and management training modules with the assistance of PharmEng and in collaboration with NHRI's Incubator Center. The training course was well received in 2006 and

had more than fifty attendees, including staff from BFDA, the Taiwan Centers for Disease Control, and the pharmaceutical industry. Those who complete the full training course are presented a certificate issued jointly by PharmEng and NHRI.

- ***Training courses in vaccinology and vaccine manufacturing technologies***

The center is collaborating with Taipei Science and Technology University and PharmEng Consultant Inc. to offer a Master of Science degree course, Quality Management for Biologics and Bioengineering. It is hoped that this course will serve as a forum for training and educating local young scientists in vaccine-related biotechnologies. Also, in collaboration with the GE Healthcare Life Science, the center offered a one-day training course in May 2007 in vaccine manufacturing processes and technology. Both the director and deputy director of the Vaccine Research and Development Center continue to give lectures and write articles about vaccines and vaccinology. And P.I.s at the center have served as consultants to organizations including the Centers for Disease Control and Department of Health about healthcare issues, such as strategies for pandemic flu vaccine development and prevention, and how best to craft the nation's flu vaccine manufacturing and supply.

The center not only offers courses of its own and contributes to classes elsewhere but also serves as a site for training courses by outside specialists. For example, Dr. Michel Klein, the former senior vice president of Sanofi Pasteur and CEO of CANVAC, is spending three months in Taiwan to share his expertise in vaccinology with NHRI staff.

4. Preparing for emergency requests for vaccines against pandemic diseases and bioterrorism

To implement the government's policy of Taiwan becoming self sufficient in the manufacturing of vaccines, strengthening the infrastructure for vaccine research and development, and responding to any threat from emerging infectious diseases, the vaccine center has integrated the necessary resources and expertise from all relevant fields to establish a national vaccine research and development team dedicated to domestic research and production of important vaccines. Because of public health concerns, disease-prevention requirements, and the need to respond should there be a pandemic outbreak in Taiwan, the program concentrates on four vaccines, namely those for the H5N1 influenza virus, the enterovirus type 71, the Japanese encephalitis virus (JEV), and meningococcal B. The ultimate goal of this program is to prepare vaccine candidates for entering phase I and II clinical trials for at least three of the targeted vaccines in the next five years.

- ***H5N1 influenza vaccine development:***

The center has already completed several important steps toward development of an H5N1 influenza vaccine:

(1) validation of MDCK master cell bank (MCB), end of production cells (EPC) and NIBRG-14 master virus seed (MVS), with neither endogenous nor entragenous contaminants present;

(2) tumorigenicity test for MDCK EPC, with the results showing negative tumorigenicity for our MDCK cells;

(3) preclinical animal toxicity tests on both rodent and non-rodent animal models (using rats and rabbits, respectively), with the results indicating that test animals treated with vaccine bulk adjuvanted with aluminum phosphate ($AlPO_4$) did not develop severe systemic adverse effects;

(4) stability test on vaccine bulk pre-mixed with adjuvant aluminum phosphate ($AlPO_4$) showed 6-month stability of $AlPO_4$-adjuvated vaccine bulk in 4°C storage;

(5) construction of the pilot plant and equipment I/OQ.

The center's tests on MDCK EPC are scheduled to be completed by July 2009. Moreover, it will prepare production-related technical document for IND submission and initiate a phase I clinical trial in 2009.

- ***EV71 Vaccine Development***

Taiwan's Centers for Disease Control transferred the EV71 project to NHRI in 2007. The project's overall goals include establishing a manufacturing process for Vero cell-based EV71 vaccine, and manufacturing clinical trial materials and conducting phase I/II trials for technology transfer. In 1999, the Centers for Disease Control imported Vero cells from ATCC to establish a master cell bank (MCB) and a working cell bank (WCB). The center is currently establishing its own MCB and WCB at the pilot plant.

Taiwan's Centers for Disease Control has selected two seed viruses: one from genotype B4 and one from genotype C4. The center is evaluating the growth efficiency, immunogenicity, and cross-reactive antibody responses of these two vaccine strains. It has produced three batches of virus at the 20L scale in 2007 using the roller bottle technology transferred from the Taiwan CDC. Because roller bottle technology is labor intensive and hard to scale up, it is currently developing a bioreactor manufacturing process.

An experiment method of 50% tissue culture infectious dose ($TCID_{50}$) is used to quantify live virus titers, which is a semi-quantitative assay compared to the plaque assay. In addition, a non-standardized ELISA is used to quantify vaccine antigens, that is subjected to intra- and inter-assay variability. Therefore, two QC assays, including a plaque assay for quantifying live virus titers and a standardized ELISA for determining the amount of viral VP1 antigen, are currently being evaluated.

- ***JEV vaccine development***

Material and technology transfers from the Taiwan

CDC for the JEV project are ongoing. The center has already produced three lots of virus using the Beijing stain; and the staff from NHRI is now capable of independently operating the production process of JEV vaccine.

Since the former master virus seed established by the CDC was found to be contaminated, a new virus seed was needed. The Beijing virus seed was re-cloned; and fast-growing clones were selected. One hundred vials of new virus seed stock of the Beijing strain of JEV grown on Vero cells in the presence of serum-containing medium were prepared. The virus titer is 5×10^8 $TCID_{50}$/mL. In addition, an MOU was signed in May 2007 between NHRI and Indian Immunologicals Ltd., to jointly collaborate on JEV vaccine development. The center is currently establishing the master cell bank and working cell bank in the NHRI pilot plant.

- ***Neisseria meningiditis B (MGB) vaccine development***

The massive production of rAg473 in *E. coli* has been proven feasible. As estimated from a 5L-scale fermentation using animal-component-free media, a typical yield of 500 mg of purified rAg473 can be obtained for preclinical studies. The team has developed several new processes to improve the quality of the vaccine, including the chemical conditions for

extracting rAg473 from bacterial membranes and the selection process for high-expression clones. Since rAg473 is lipidated (as assessed by mass spectroscopy of trypsin-digests) and anchored in the outer membrane of the bacteria, LPS contamination is a very serious safety problem. Therefore, ultra-filtration technology was used, successfully, to remove LPS. The research lots of rAg473 have been used to immunize mice and shown to induce strong immune responses with high antibody titers, even without aluminum adjuvant. The enhancement of immunogenicity by lipidation of rAg473 was demonstrated by testing different forms of rAg473, including the non-lipidated one as control. The team's data indicate that lipoprotein Ag473 is a promising vaccine candidate.

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