Recent Advances of Diagnoses and Therapeutics in Practical Medicine

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Graduate School of Biomedical Sciences,
Hiroshima University

1. Introduction

In the biomedical science, a large quantity of research budget and researchers' efforts has been served to improve health and quality of life of people. I would like to show, during the course of this international workshop, some recent progress made in practical medicine which has been directly or indirectly influenced by myself; a medical doctor of internal medicine, respirologist, and clinical biologist. These may give the members of the 21st century COE program "Nanoelectronics for Terra-Bit Information Processing" some idea on how to apply nanotechnology in the field of practical medicine.

There are five major strategies in developing practical medicine. First, the discovery and development of new medical drugs have dramatically improved medical care, such as penicillin for bacterial infection, digitalis for heart failure, and corticosteroids for allergic diseases. These are mostly carried out by pharmacologists. Second, the discovery of unknown molecules and novel functions of known and unknown molecules in human cells is a quite important new area for medical scientists. Successful results produce the establishment of new diagnostic procedures and new drugs. Third, development of new procedures by inspired thinking, such as new operation methods, are also very important. Fourth, the application of recently developed industrial technologies will make a great improvement in experimental biology and medical devices. Fifth, the establishment of clinical evidences, such as epidemiologic study and translational research, is the final gateway to success for scientific efforts to be applied in practical medicine.

2. Discovery of unknown molecules and their clinical application

There are three effective methods in finding unknown molecules from human cells that may be crucial in the cause of root diseases. For example; in finding unknown molecules crucial for the occurrence of malignant formation, three major strategies are used. Namely, monoclonal antibody production, DNA microarray and proteomics. Using these methods, the differences of expression levels and functions between normal and malignant cells can be found. According to the results, many diagnostic and therapeutic tools have been made. A lot of new molecules have been discovered by the monoclonal antibody research dating from 1974, when the method was invented. DNA microarray is a prevailing method that is strongly expected for the progress in medicine. Proteomics is promising, but the method of use is controversial at present.

3. Discovery of KL-6 and its clinical application

We discovered KL-6 in 1984 [1]. KL-6 is used widely used as a serum disease marker for interstitial pneumonia and pulmonary fibrosis in the national medical security system authorized by the Ministry of Health, Labour and Welfare, Japan, at present. Though interstitial pneumonia is a disease that is difficult for most medical doctors to diagnose, the popularization of KL-6 made the disease be diagnosed much easier than before; i.e., most interstitial pneumonia might have been diagnosed as bacterial and viral pneumonias previously and such erroneous diagnosed diseases became to be diagnosed correctly. KL-6 is measured in more than 40000 serum samples per month in Japan. Though some research reports on the usefulness of KL-6 in European and American people has been published, they are not able to share the up-to date benefits of KL-6, because the clinical measure is limited only to Japan. Serum KL-6 level is less than 500 U/ml in healthy individuals and most patients with respiratory diseases other than interstitial pneumonia, but the level is more than 500/ml in most patients with active interstitial pneumonia, as shown in Fig.2. The mechanism for the increase of KL-6 in serum might be supposed as the enhanced epithelial-capillary permeability caused by pulmonary alveolar epithelial damage (Fig. 3).

4. Three-dimensional culture of the cartilage and transplantation

This study and clinical application has been made by Dr. Mitsuo Ochi, Professor of Orthopedics, Hiroshima University Hospital, who has been my classmate from junior-high through the Medical course of Hiroshima University School of Medicine for twelve years. He is the first doctor in the world who began to develop the three-dimensional culture of chondrocytes making regenerating cartilage for the

therapy of cartilage defects in elbow and knee joints. The originality exists in the three-dimensional culture and its clinical application as shown in Fig. 4 [2].

5. Visualization of the peripheral lung structure with synchrotron radiation computed tomography

This study is made using the synchrotron of Spring-8 in Hyogo, Japan, by Dr. Hirohiko Ikura and his colleagues, at the Department of Radiology, Ehime University Hospital, who was a student of the post-graduate course in my laboratory when I worked in Ehime University. Since synchrotron radiation has ultra brightness ($1x10^8$-fold higher than medically used X-ray), a monochromated beam can be used in CT. The detectability of high resolution CT in medical use is 200 μm, but the synchrotron CT (SRCT)10 μm. Using SRCT, Therefore, we can see the second lobule in the lung tissue and alveolar septa as shown in Fig. 5.

6. Discovery of new serum tumor markers by DNA microarray

ADAM8 was recently found to be a promising serum tumor marker for lung cancer detection as shown in Fig. 6 using the method of DNA microarray by Dr. Ishikawa N. and Prof. Nakamura Y, et al [3]. at the Institute of Medical Science, the University of Tokyo. They used highly elaborate devices to find differences in mRNA expression level between normal and cancer cells. By their methods, 23040 genes are able to be examined simultaneously.

7. Medical Research Center for Environmental Effects on Japanese Emigrants, Hiroshima University (Director: Nobuoki Kohno, M.D.)

We have been conducting the Hawaii-Los Angeles-Hiroshima Study since 1970, mainly to determine the effects of environmental changes on various diseases by comparing Japanese-Americans with native Japanese subjects. Japanese-Americans living in Hawaii and Los Angeles who originated mainly from Hiroshima, Japan, and are genetically identical with native Japanese (Fig. 7). Through this study, we made several clear observations about Japanese-Americans. First, Japanese-Americans were highly exposed to a westernized lifestyle; in other words, a relatively high fat and simple carbohydrate diet with low physical activity as compared to native Japanese. Second, the prevalence of type 2 diabetes among Japanese-Americans and death from ischemic heart disease among Japanese-American diabetes patients was higher. Third, the serum fasting insulin level as well as the insulin level after a glucose load, was higher among Japanese-Americans, even when the serum glucose levels were not statistically different as compared to native Japanese. Accordingly, Japanese-Americans were thought to have a high insulin resistance status. Fourth, the intima-media wall thickness of the cartoid artery is apparently worse in Japanese- Americans than native Japanese as shown in Fig.8 [3]. In conclusion, it appears that for genetically pure Japanese people environmental factors are important for the development of metabolic diseases such as diabetes mellitus and cardiovascular disease.

8. Conclusion

Recent progresses in practical medicine which have been witnessed by the author have been shown here today. The author is hoping that this presentation will possibly make the researchers attending this workshop have some worthwhile research ideas in the field of application of nanotechnology in regards to practical medicine.

References

[1] Kohno N, et al., New serum indicator of interstitial pneumonitis activity: sialylated carbohydrate antigen KL-6. Chest **96**: 68-73, 1989.

[2] Ochi M, et al., Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. J Bone Joint Surg Br. **84**: 571-8, 2002.[3] Ishikawa N. et al., ADAM8 as a novel serological and histochemical marker for lung cancer. Clin Cancer Res, 2004 (in press)

[4] Watanabe H, et al., Westernization of lifestyle markedly increases carotid intima-media wall thickness (IMT) in Japanese people. Atherosclerosis 166:67-72, 2003.

1.	Monoclonal antibody production:	Antigenic Determinant of Protein, Peptide, & Carbohydrate
2.	Genomics (DNA microarray):	mRNA, DNA
3.	Proteomics:	Protein, Peptide

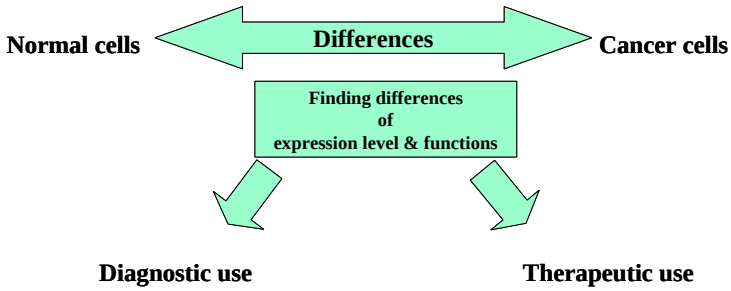


Fig. 1: Strategies to find unknown molecules and known molecules which are important for cancer development.

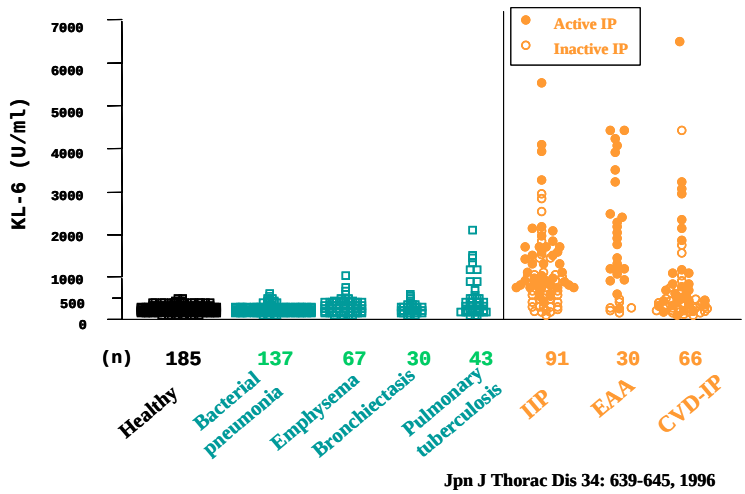


Fig. 2 : Serum KL-6 levels in Respiratory Diseases.

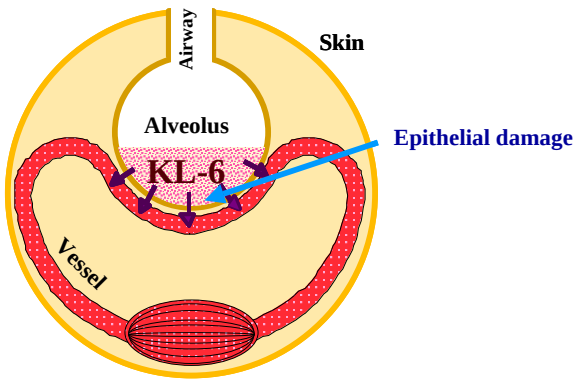


Fig. 3: KL-6 is absorbed into circulation, and the absorption levels are co-related with the alveolar-capillary permeability maybe reflecting epithelial injury.

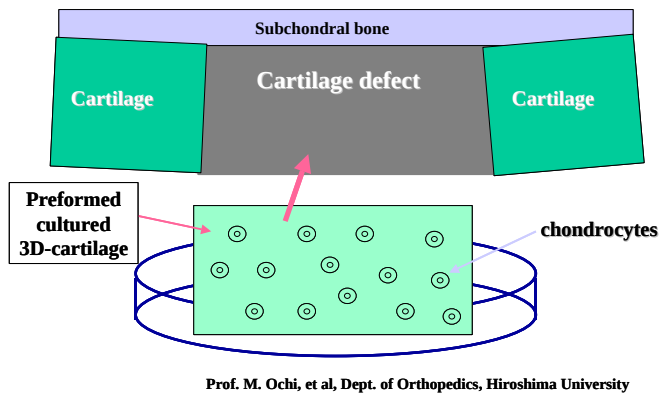
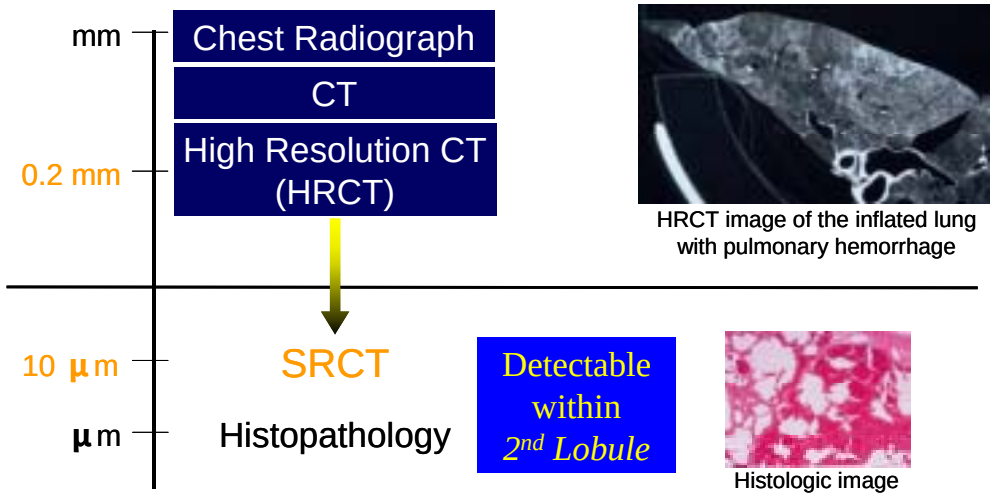
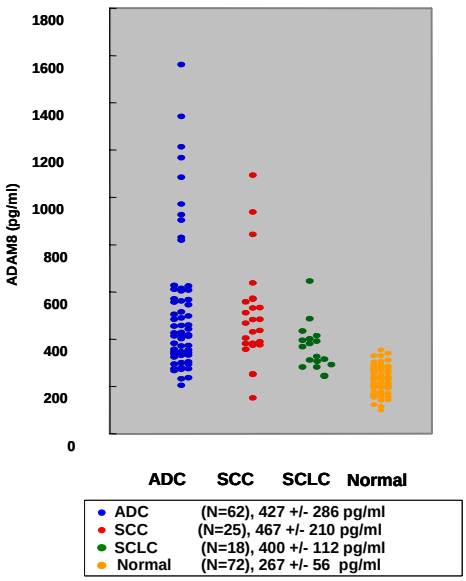


Fig.4: Three-dimensional culture and transplantation.



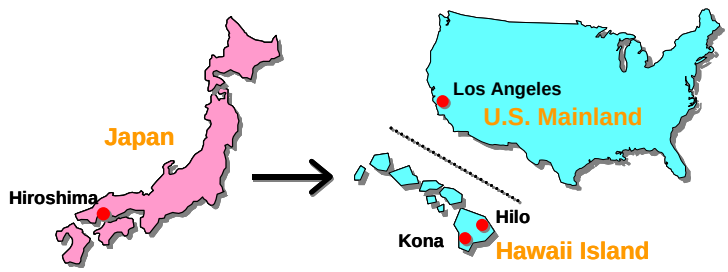
Hirohiko Ikura, MD, Dept. of Radiology, Ehime University

Fig.5: Detectability of Lung Lesions with Various Modalities.



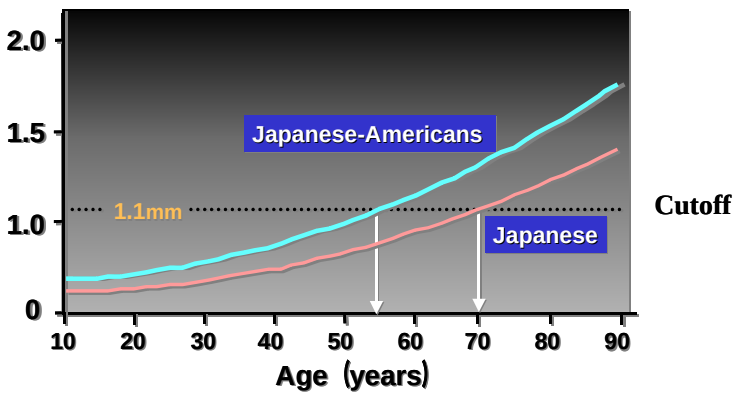
Ishikawa N, Kohno N, Nakamura Y, et al. Clin Cancer Res (in press).

Fig.6: ADAM8 as a serum tumor marker for lung cancer.



We have been conducting the Hawaii–Los Angeles–Hiroshima Study since 1970, mainly to determine the effects of environmental changes on various diseases by comparing Japanese-Americans with native Japanese subjects.

Fig.7: Medical Research Center for Environmental Effects on Japanese Emigrants, Hiroshima University (Director: Nobuoki Kohno, MD,PhD).



Watanabe H et al: Atherosclerosis, 2003

Fig.8: Comparison of carotid intima-media wall thickness (IMT) between Japanese-Americans and Japanese.

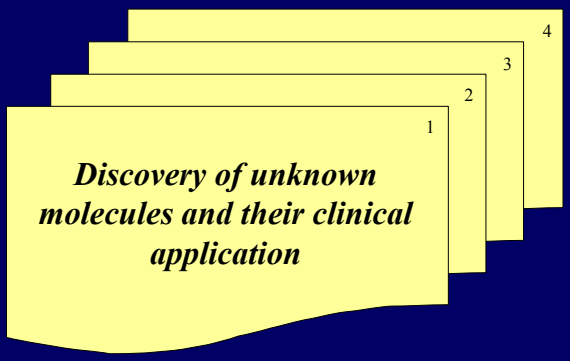
The third Hiroshima International Workshop on Nanoelectronics for Tera-Bit Information Processing

Recent advances of diagnoses and therapeutics in practical medicine

Nobuoki Kohno, MD, PhD
Professor
Department of Molecular and Internal Medicine
Graduate School of Biomedical Sciences
Hiroshima University

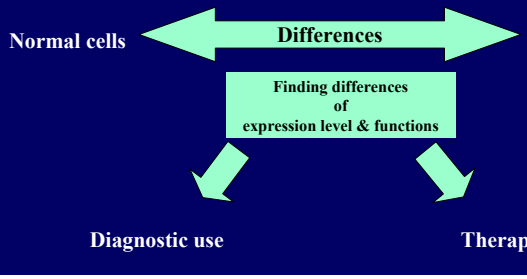
Strategies to develop practical medicine

1) Discovery and development of new drugs (mainly by pharmacologists)
2) Discovery of unknown molecules and novel functions of known molecules
   - Diagnostic use
   - Therapeutic use
3) Development of new procedures
4) Application of recently developed technologies
5) Establishment of clinical evidence
   - Epidemiologic study
   - Translational research



Strategies to find unknown molecules and known molecules which might be important for cancer development

1.	Monoclonal antibody production:	Antigenic Determinant of Protein, Peptide, & Carbohydrate
2.	Genomics (cDNA microarray):	mRNA, DNA
3.	Proteomics:	Protein, Peptide

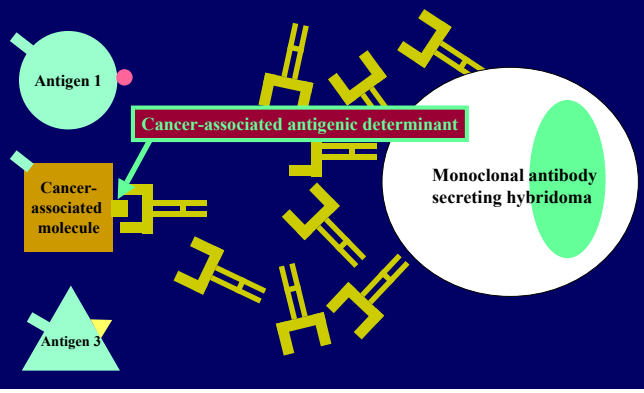


Monoclonal antibody production

The discovery of KL-6 as a serum marker for interstitial pneumonia

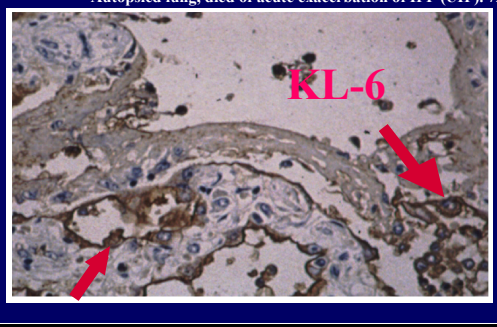
Nobuoki Kohno, et al.
Hiroshima University

The production of monoclonal antibodies by hybridoma is theoretically infinite



We established KL-6 monoclonal antibody

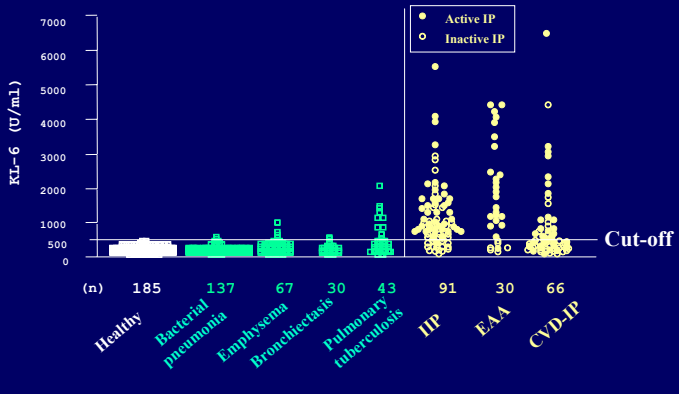
Autopsied lung, died of acute exacerbation of IPF (UIP). 72 y, male



KL-6 antigens existed also in human serum.

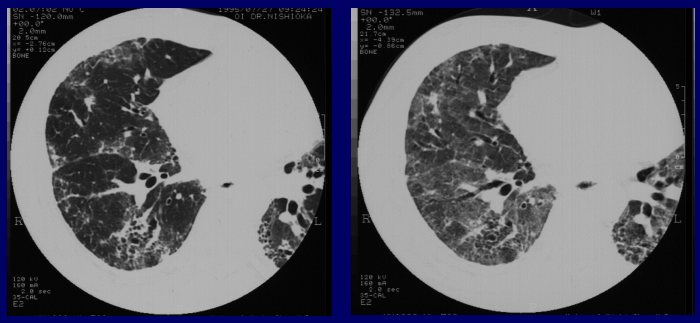
Serum KL-6 levels in interstitial pneumonia (IP)

Jpn J Thorac Dis 34: 639-645, 1996



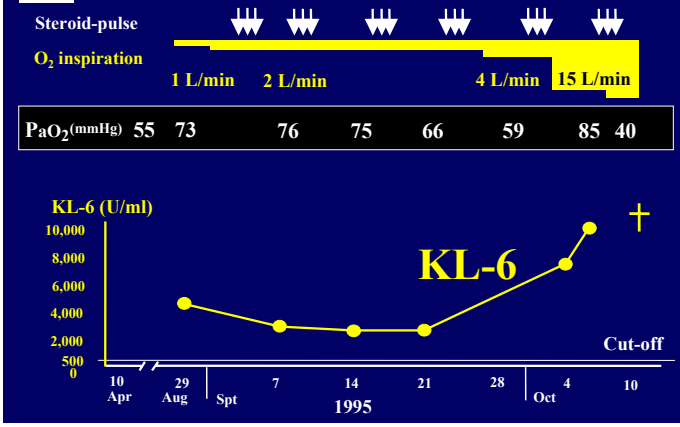
61 y, man: IPF, died of acute exacerbation

High resolution computed tomograpy (HRCT) image



7/27/95 9/29/95

61 y, M: idiopathic pulmonary fibrosis, died of acute exacerbation



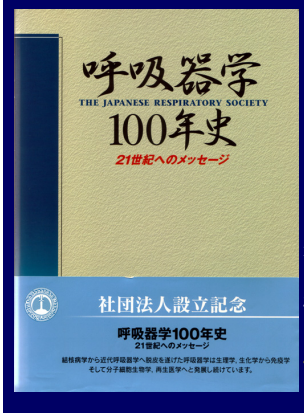
Drugs possibly causing drug-induced pneumonia (Drug-induced interstitial pneumonia)

Pharmaceuticals and Medical Devices Agency, Japan, Apr. 2004

- ✧ As adverse effects ··· 1232 drugs
- ✧ As severe adverse effects ··· 50 drugs
  - Chinese herb medicine "Sho-sai-ko-to"
  - Interferons α, α-2a, α-2b, β, β-1b, γ-1a, γ-n1
  - Gefitinib (Anti-cancer molecular targetting drug)
  - Amiodarone (Anti-arrhythmia)
  - Some anti-cancer cytotoxic drugs
  - Leflunomide (Disease-modifying anti-rheumatic drug)

KL-6 is at present used as a diagnostic serum marker for interstitial pneumonia in the national health security system authorized by the Ministry of Health, Labour and Welfare, Japan, from 2000.

KL-6 was evaluated as one of honorable discoveries achieved by Japanese researchers



Four Japanese researchers were presented in the preface of the 100 year commemorative book of the Japanese Respiratory Society. (written by the late Prof. Hiomi Honma)

1. 1966 Invention of flexible fiber bronchoscope by Dr. M. Ikeda, et al.
2. 1967 Discovery of immunoglobulin E (IgE) by Dr. K. Ishizaka
3. 1982 Invention of high resolution computed tomography by Dr. Y. Tohdo
4. 1985 Discovery of KL-6 by Dr. N. Kohno, et al.

cDNA microarray

Seeing expression levels of 23,040 different genes (messenger RNAs) in one lot

Ishikawa N, Nakamura Y, et al.
The Institute of Medical Science,
The University of Tokyo

cDNA and mRNA hybridization

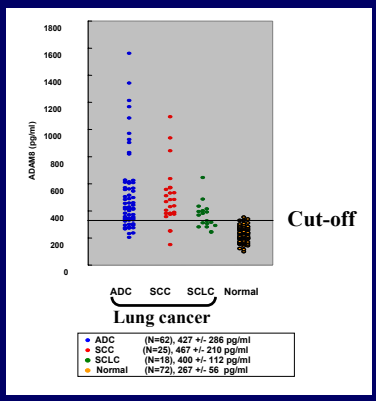
DNA	RNA
A = T	A = U
G = C	G = C

mRNA
U-A-C-G-U-A-C-G-U-A-C-G---AAAAAAA

cDNA --------A-T-G-C-A-T-G-C-A-T-G-C---

Ishikawa N, Kohno N, Nakamura Y, et al.
Clin Cancer Res (in press)
The Institute of Medical Science, The University of Tokyo

ADAM8 as a serum tumor marker for lung cancer

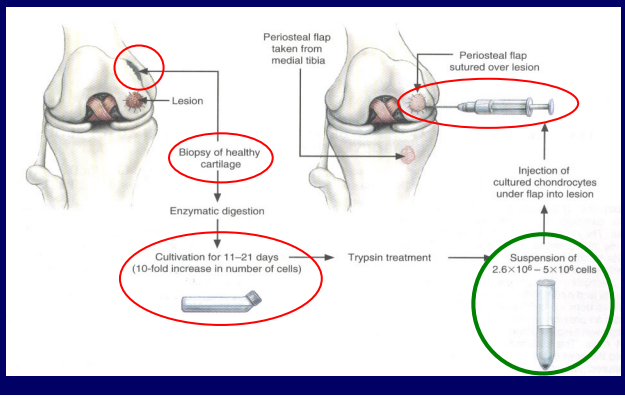


Cartilage repair with tissue-engineered cartilage created under three dimensional culture

Developed by Prof. M. Ochi, M.D.,
Dept. of Orthopedics, Hiroshima University

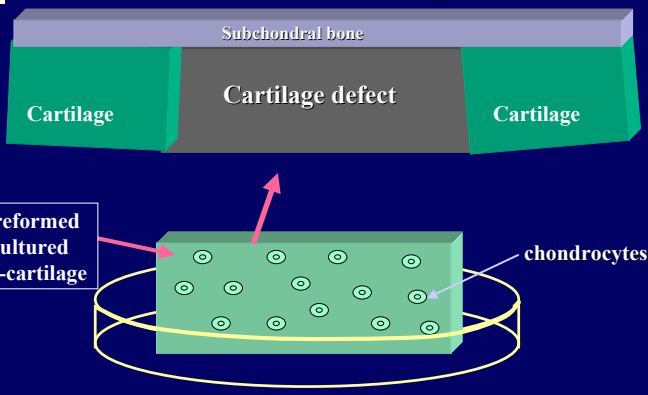
Autologous Chondrocyte Implantation (ACI)

(Brittberg, Peterson, et al, New Eng J Med, 1994)

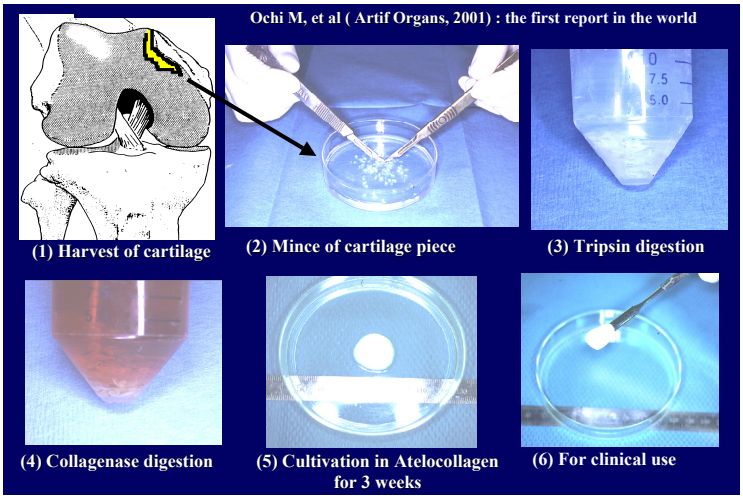


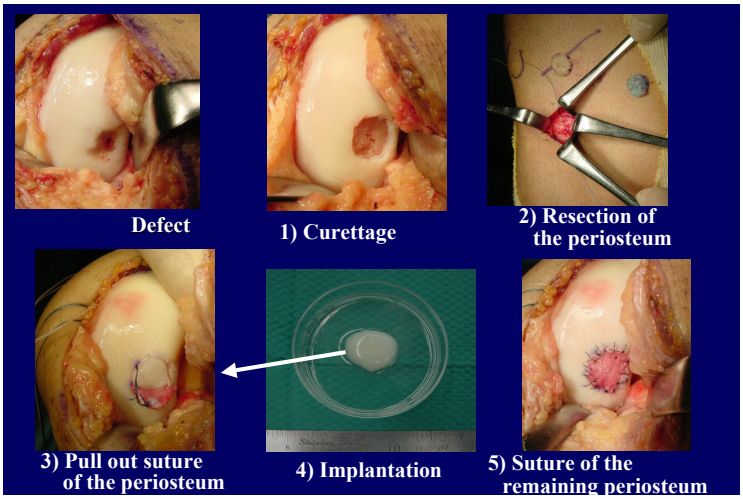
Prof. M. Ochi, et al, Dept. of Orthopedics, Hiroshima University

Three-dimensional culture and transplantation



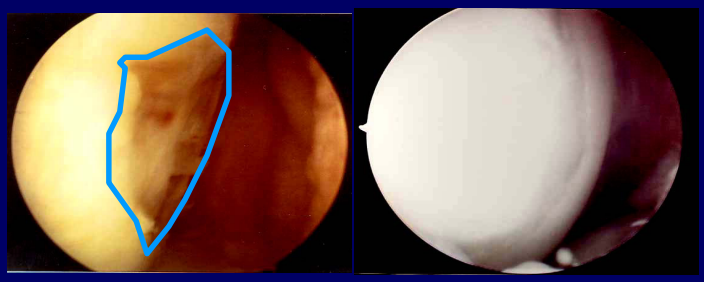
Ochi M, et al (Artif Organs, 2001) : the first report in the world





Prof. M. Ochi, et al, Dept. of Orthopedics, Hiroshima University

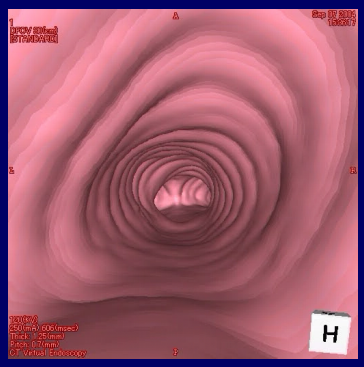
Case 1: 13 year-old boy, OCD of the med. femoral condyle

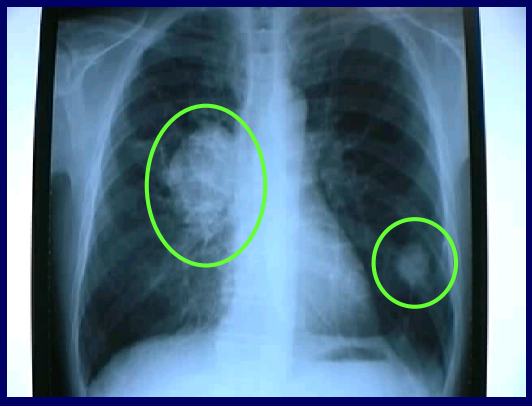


4
3

Clinical application of recently developed technologies

Virtual bronchoscopy by the reconstitution of high resolution computed tomography (HRCT) images of the lung





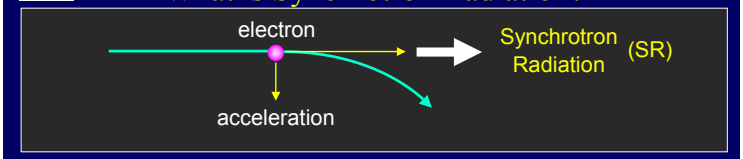
Visualization of the Peripheral Lung Structure with Synchrotron Radiation CT

SPring-8 (Hyogo, Japan)
circumference 1,400 m
diameter 450 m

Hirohiko Ikura, MD, et al.

Department of Radiology,
Ehime University
School of Medical

What is Synchrotron Radiation?



- White beam (from infrared to hard X-ray)
- Ultra brightness ($\times 10^8$ to medically used X-ray)

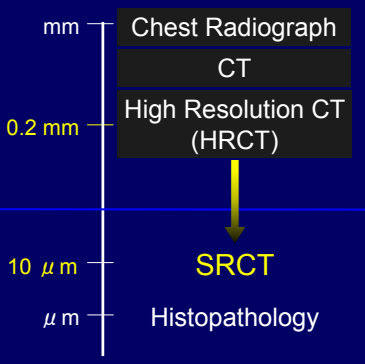
↓

Monochromated beam

↓

- High directionality
- Negligible geometrical blur

Detectability of Lung Lesions with Various Modalities



4

Establishment of clinical evidence

The project for health promotion
「Ken-minn Genki－Baizou Project」

by Hiroshima University co-operated with the Corporations of Hiroshima Prefecture, Hiroshima City and Higashi-Hiroshima City
(2002-2004)

As the special enterprise for social contribution granted by the Ministry of Education, Culture, Sports, Science and Technology, Japan

Director: Nobuoki Kohno, M.D., Ph.D.

The project for health promotion

Subjects:
Well-controlled diabetics
at the out-patient clinics

A → **The instructed group**
Education & exercise
with the instruction

B → **The control group**
Education & examinations
without the instruction

The study protocol

Entry 3rd 6th 12th month

Observational items
Physical exams
Athletic ability
Endothelial function
Atherosclerosis
Pulmonary function

Nutritional survey

Lifecorder®
(Electric pedometer)

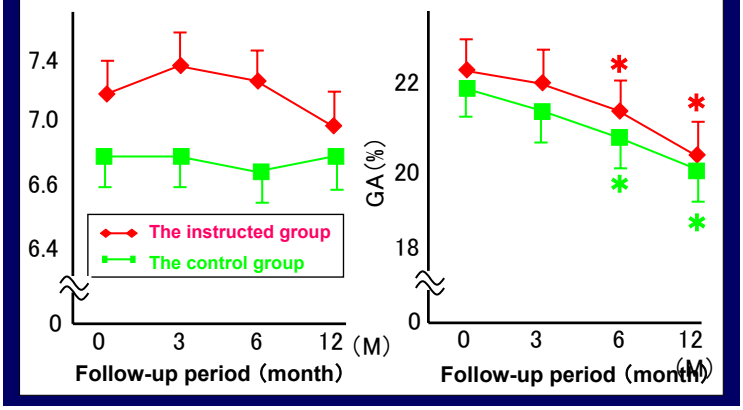
Pulse Wave Velocity

Carotid Echography

Athletic Ability

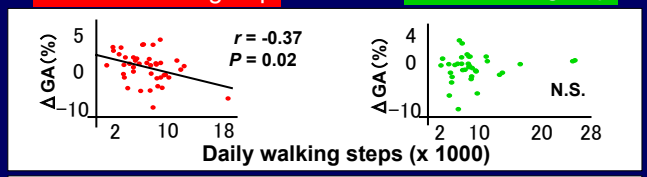
Lifecorder®
(Electric pedometer)

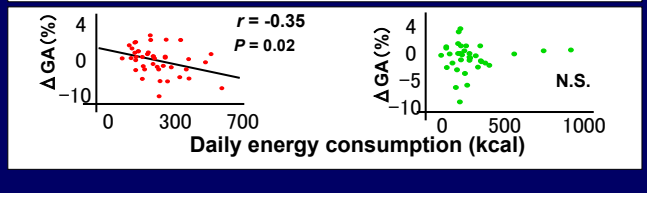
Chronological changes of HbA_{1c} & Glyco-albumin (GA)



Relationship between the change of GA and the quality of exercise

The instructed group | The control group

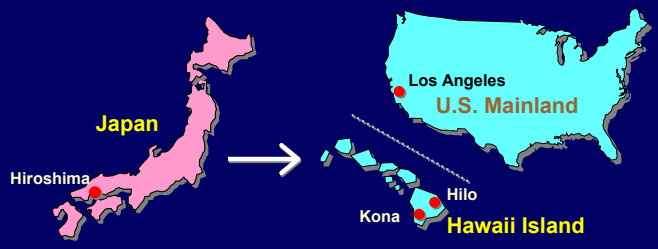




The Medical Research Center for Environmental Effects on Japanese Emigrants, Hiroshima University

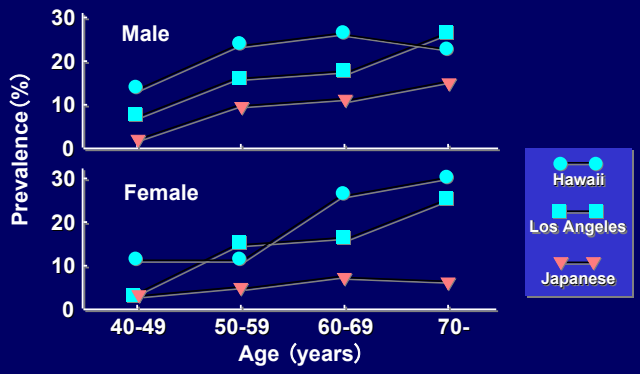
Director: Nobuoki Kohno, MD, PhD

The Hawaii–Los Angeles–Hiroshima Study



We have been conducting the Hawaii–Los Angeles–Hiroshima Study since 1970, mainly to determine the effects of environmental changes on various diseases by comparing Japanese-Americans with native Japanese subjects.

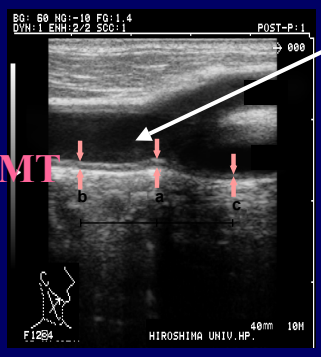
Prevalence of diabetics among Japanese-Americans or Japanese (1980's)



Hara H et al: Diabetes Res Clin Pract 24: S37, 1994.

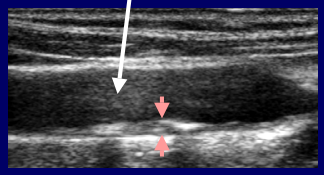
Carotid intima-media wall thickness (IMT)

Case A

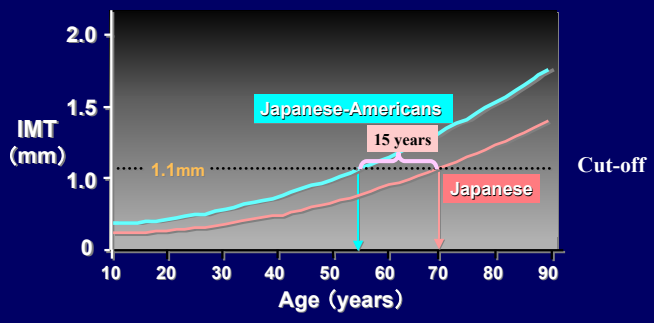


Carotid artery

Case B



Comparison of carotid intima-media wall thickness (IMT) between Japanese-Americans and Japanese



Watanabe H et al: Atherosclerosis, 2003