

September 17-18, 2009, Beijing, China

IFPT'6

Progress on Post-genome Technologies

— Proceedings of the 6'th International Forum
on Post-genome Technologies(IFPT '6)

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SOUTHEAST UNIVERSITY PRESS

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江西省图书馆



11241765

Southeast University Press



图书在版编目(CIP)数据

后基因组技术进展:第六届国际后基因组生命科学技术学术论坛论文集=Progress on Post-genome Technologies: Proceedings of the 6'th International Forum on Post genome Technologies(6' IFPT);英文/罗国安等主编. —南京:东南大学出版社,2009.9
ISBN 978-7-5641-1842-6

I. 后... II. 罗... III. 基因组—国际学术会议—文集—英文 IV. Q343.1-53

中国版本图书馆 CIP 数据核字(2009)第 161023 号

Progress on Post-genome Technologies: Proceedings of the 6'th International Forum on Post-genome Technologies
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Printed in Nanjing, China
ISBN 978-7-5641-1842-6

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Organized by

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Southeast University
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Northwest University

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Life-surveyor Project of Japan
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Huadong Research Institute for Medicine and Biotechnics

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PREFACE

Human body consists of many organs where many cells work together to perform their functions. Although massive molecular data are averages over ensembles of cells, the question is whether all the cells in an organ work in the same way. In a cancer tissue, it has been pointed out that a small amount of cancer stem cells play important roles to reconstruct a cancer tissue again even after destroying the cancer tissue by anti-cancer drugs. However, the details have not been clarified because of the difficulty of analyzing very small number of cancer stem cells. It has been also pointed out that the heterogeneity of ES cells might be important for keeping their totipotency. For understanding a real life system, we need new tools to analyze individual cells besides the averaged biological data. We are at a new frontier where we understand functions of organs based on the information on individual cells using new tools.

Seven years ago, we started IFPT to promote the interdisciplinary and international collaborations in the post genome era. Beijing is the capital of China and has many beautiful places. I hope all the participants will relax and enjoy the meeting in Beijing and hopefully have chances for collaborations.

Hideki Kambara

PhD, Professor

Co-Chairman of IFPT'6

Hitachi Ltd. / Tokyo University of Agriculture and Technology

August 27, 2009



Dr. Kambara Hideki, the founder of IFPT, is the Fellow of Hitachi Ltd., the visiting professor of Tokyo University of Agriculture and Technology. He graduated from the University of Tokyo in 1967, and received his doctorate degree in 1972 from the University of Tokyo. He honored The Asahi Prize for “development of a high performance DNA sequencer” in 2004, the National Medal of Honor with Purple Ribbon in 2003, “Star of Asia” by BusinessWeek’s in 2002, the 48th Okochi Memorial Grand Technology Prize for “development of the capillary array DNA sequencer” in 2002, and Commendation by the Minister of Education, Culture, Sports, Science & Technology to Persons of Scientific &

Technological Research Merits, for “research on fluorescence detection method DNA base sequencing device” in 2001. Dr. Kambara has published over 80 papers on mass spectrometry and DNA analysis, and holds more than 100 patents in Japan and U. S. A. His research interests includes atmospheric pressure ionization mass spectrometry; field desorption ionization and collision induced decomposition mass spectrometer for biological molecules; molecular secondary ion mass spectrometry; combined system of liquid chromatograph and mass spectrometer; fluorescent DNA sequencer; capillary array DNA sequencer; DNA expression profile analysis; and instruments for DNA diagnostics.

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NOVEL SEPARATION AND IDENTIFICATION TECHNIQUES FOR PROTEOME STUDY

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With the in-depth research in proteomics, more and more requirements have been put forward on the development of novel separation and identification techniques for proteome study.

To improve the identification capacity of low abundance proteins, two strategies have been proposed, depletion of high abundance proteins and enrichment of low abundance ones. For the former case, with SCX HPLC as the first dimension, and 8 parallel columns of RPLC as the second dimension, multidimensional array chromatography has been proposed to remove multiple high abundance proteins simultaneously according to UV absorbance, by which after the removal of 58 high abundance proteins from human liver tissue, the identified protein number was increased from 451 to 1213. For the latter case, various novel materials have been synthesized to achieve high selective enrichment of target proteins/peptides, including protein imprinted polymers, immobilized metal affinity chromatography materials and magnetic mesoporous materials.

To improve the separation capacity of complex proteome samples, several platforms have been established. Besides multidimensional array chromatography for peptides and proteins separation, with microenzymatic reactors as interfaces to hyphenate proteins and peptides separation, multidimensional HPLC and CE based integrated platforms were proposed, by which high resolution, high accuracy and high throughput analysis of proteome could be achieved.

Furthermore, to achieve high sensitive identification of proteomes, emphasis has been put on the development of novel matrices and targets of MALDI-TOF MS, chemical derivatization techniques to improve ionization capacity of phosphopeptides and data mining methods to improve MS identification capacity.

ACKNOWLEDGEMENT

The authors are grateful for the financial support from National Basic Research Program of China (2007CB914100).

Prof. Zhang Yukui



Prof. Yukui Zhang obtained his bachelor degree of science in 1965 from Nankai University, China. Then he began his research career in Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS). In 2003, he was selected as The Member of Chinese Academy of Sciences.

In the past years, Prof. Zhang has been working on the research of fundamental theory and the development of new techniques of chromatography, including gas chromatography (GC), high performance liquid chromatography (HPLC), membrane chromatography and capillary electrophoresis (CE). Many research results, such as the development of HPLC and membrane chromatography columns, multi-variance separation theory of chromatography and intelligent HPLC, have won several prizes from CAS. From 1990s, his research is focused on the high efficient separation and high sensitive detection of biomolecules, devoting himself to multidimensional separation, including 2D-GC, 2D-HPLC, 2D-CE and their hyphenation with mass spectrometry. Development of on-line concentration techniques and synthesis of new kinds of fluorescent dyes have been carried out as well.

Now Prof. Zhang is the member of Degree Committee of CAS, Director of Chromatography Specific Interest Committee of Chinese Chemistry Society, President of Chinese Chromatography Society, Editor-in-chief of Chinese Journal of Chromatography, and editor on board of Journal of Chromatography A. He has undertaken many national projects. Up till now, he has published over 400 papers, written 7 books and applied more than ten patents. In addition, he has brought up over 20 graduate students, and most of whom now become professors, active in the field of chromatography all around the world.

APPLICATION OF RNA CHECK: GENE EXPRESSION PROFILES OF DISSECTED SPECIMEN FOR PREDICTION OF METASTASES OF COLON CANCER

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ABSTRACT

We have been studying RNA, using microarrays, with more than 1000 dissected cancers. We randomly picked up data with 150 stage II colon cancers, and subjected them to training studies for markers that can predict metastasis after the operation. Fifty-five probes for prediction of metastases were obtained in multivariate analyses.

We then took another set of 150 samples of the colon cancer at stage II for verification, and ran a blind test using the 55 marker probes. The result showed that the accuracy of prediction was 76%, negative predictive value 90%, positive predictive value 31%.

Metastases of cancers are the worst and most serious problems for both patients and doctors. At the same time, unnecessary adjuvant therapies after dissection of cancers are yet other problems for patients, as such treatments seriously hurt patient's quality of life. Our RNA check studies can be of value in solving these problems. We are now looking for collaborators for larger scale analyses and with different ethnics.

ACKNOWLEDGMENT

This work was supported in part by a grant-in-aid from Japanese Science and Technology Agency



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He graduated the University of Tokyo, Faculty of Science in 1956. After he granted the Ph. D. from the Graduate School of University of Tokyo in 1961, he commenced research as a Research Associate at Kanazawa University, School of Medicine and at Kyushu University School of Medicine. From 1964 to 1967, he was a research fellow at the Biological Laboratories, Harvard University. From 1967 to 1968, he was a Research Fellow at Department of Biochemistry, Stanford University Medical School. He was appointed Associate Professor in Department of Biochemistry, Kyushu University in 1968, and in 1975 Professor in Laboratory of Molecular Genetics, Osaka University. In 1975, after attending the Asilomar Conference, he became a Fellow at Department of Biophysics, Princeton University. He was a Professor in Institute of Molecular and Cellular Biology, Osaka University in 1982, and Director of the Institute from 1987 to 1997. Since 1997, he was a Professor at Nara Advanced Institute of Science and Technology and Vice Director at International Institute of Advanced Studies. Through 1991 to 1994, he served as a Vice President of Human Genome Organisation (HUGO). He now is President at DNA Chip Research Inc, since April 1998.

NUCLEIC ACID MASS SPECTROMETRY: A TECHNIQUE WITH UNUSUAL SENSITIVITY AND PRECISION

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ABSTRACT

There are four major advantages in detecting RNA or DNA, after PCR amplification of specific loci, by mass spectrometry (MS) instead of by fluorescence. MS detection is about 100 times more sensitive than conventional real time (rt) PCR because it is an end point method so that more cycles of amplification can be used. MS quantification is at least ten times more precise than RT-PCR because sequence matched internal standards can be used to compensate for intrinsic PCR noise and MS can analyze many loci at once, together with positive or negative controls as needed because each peak in a spectrum is in essence a separate color and hundreds of peaks can be analyzed simultaneously. Finally, MS can provide actual DAN sequence information for amplicons as long as 1 kb. These advantages enable a number of applications that are difficult, or impossible by RT-PCR or even conventional DNA sequencing. A number of these applications will be described including the detection and quantification of somatic oncogene mutations in cancer biopsies, non-invasive prenatal diagnostics in which fetal nucleic acids are detected and quantified in the mother's blood serum, diagnostics of human papilloma virus (HPV) sequences and viral loads, and analysis of the clade structure in patients infected with hepatitis C virus (HCV). While the equipment cost of nucleic acid mass spectrometers is much higher than RT-PCR instrumentation, the cost of using it is much less because no labeled nucleic acid probes are required. Hence in the long term, using nucleic acid mass spectrometry is quite cost effective.

Charles R. Cantor, Ph. D.

Charles Cantor is a founder, Chief Scientific Officer, and Member, Board of Directors, at SEQUENOM, Inc. He is also founder of SelectX Pharmaceuticals, a drug discovery company based in the Boston area. He is co-director of the Center for Advanced Biotechnology at Boston University, and professor of Biomedical Engineering. Dr. Cantor has held positions at Columbia University and University of California at Berkeley, and was also director of the Human Genome Center of the Department of Energy at Lawrence Berkeley Laboratory. He has published more than 400 peer-reviewed articles, has been granted more than 60 patents, and co-authored a three-volume textbook on Biophysical Chemistry and the first textbook on Genomics; *The Science and Technology of the Human Genome Project*. He sits on the advisory boards of more than 20 national and international organizations and is a member of the National Academy of Sciences.

DEVELOPMENT OF DNA ANALYSIS TECHNOLOGIES —WHAT COMES NEXT—

Kambara Hideki

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DNA analysis technologies have been developed greatly in these ten years. Capillary array DNA sequencers have been contributed to the completion of the human genome project. The sequenced genome data can be a standard map for sequencing individual human genomes. Consequently, the human genome sequencing has become much easier than before. The long base read was required and very important before the completion of the human genome sequencing, however, the requirement has reduced by the completion. Even the sizes of sequenced DNA fragments are short, they can be successfully connected each other by coupling with the map. Massive parallel DNA sequencers based on a step-by-step nucleotide incorporation reaction with millions of reaction chambers have been developed recently. Even though the readable base lengths with the new systems are not so large, they work well with the map. They give extremely high throughput compared with a capillary array DNA sequencer based on gel electrophoresis. Individual human genome sequencing with a new system will be carried out in one day at low cost in near future.

We will have huge data as to genomes, transcriptomes and proteins. However, many things may still remain unsolved because the obtained data are averages over ensembles of cells or tissues. Important information on a real life system may be masked by averaging. We may need more precise data from individual cells. So much data have been obtained to now and much more data will be obtained in future. It may be good to understand a local event. However, it may confuse people to understand a whole event. We may need a system which can help us to understand the whole event.

We started a project called "life surveyor" to develop tools for analyzing single-cells or small number of cells for obtaining the precise data. We have developed various probes for monitoring bio-molecules in single-cells, tools for analyzing metabolites with small number of cells, micro-devices to handle and analyze single-cells, and transcriptomes in single-cells. The project has ended in March this year. The tools developed in the project include a tool for analyzing gene expressions in single-cells. It shows that gene expression levels obtained with single-cells are fluctuated from cell to cell even if the cells are carefully and equally controlled. The standard deviation for the measurement itself is much smaller than the observed fluctuations. It indicates that the fluctuations might be due to the individualities of cells.

Although all the cells in a tissue have been equally treated, individual cells might have their own different roles in the tissue, just like people in a human society, and might give different gene expressions. Massive parallel single-cell analysis will give new insight into a cell community in a tissue and it will give important information for understanding a real life system. The technology will be used to various medical applications because it has been clarified that small number of cells play important roles frequently in cancer tissues or in differentiation processes.

Besides the detail analysis of cells, it seems necessary to develop a tool to understand a whole human body based on various molecular data. It will encourage people who are interested in system biology where they are trying to understand a real life system based on molecular science.

In the medical field, there are two types of medicines, the western medicine and the oriental medicine originated in China. The western medicine is scientific, local, anatomical, theoretical and deductive. It is very powerful and successfully applied to various diseases. The oriental medicine is philosophic, global, phenomenological, empirical and inductive. Some people are trying to combine the western medicine and the oriental medicine to realize an advanced medicine. This is similar to the change occurring in the life science from analyzing parts to understanding a total system. In both cases, we need a new information system just like Google Earth where an image of earth appears at first and then we can see the detail map and photos we are interested which may be called Google Body. Google Body is a visualization tool for human body situation which will include various molecular science data, various medical data including data obtained with the western medicine as well as oriental medicine. If Google Body