●Basic Stage

(Fiscal Year 2005-2007)

Yokohama Waterfront Area

Proteomics on Disease-associated Cells Using New Technology System

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Life Sciences
IT
Environment
Nanotech Materials
Others

Project Promotion

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Core Research Organization

Yokohama City University

Major Participating Research Organizations

Industry…Toyo Kohan Co., Ltd., SUS Corporation, Toray Industries, Inc., FANCL CORPORATION, CellFree Sciences Co.,Ltd., DNA Chip Research Inc.
Academia…Yokohama City University
Government…Kihara Memorial Yokohama Foundation for the Advancement of Life Sciences

Aim of research and development

This project's aim is to detect rapidly and exhaustively proteins that relate to such diseases as ovary/prostate cancer, aging, food allergy and neural network formation using newly developed proteome analysis technology in collaboration with networked researchers in this area. We will also try to detect disease-associated proteins efficiently after improving conventional analytical method, and then, will clarify the relationship between disease and proteins that are detected by analyzing their expression pattern and functions. On the other hand, we will also evaluate the relationship between candidate molecules, which are obtained from disease model animal and from cells, and disease.

In addition, we will analyze interaction between disease-associated intranuclear proteins, which are expressed in a large quantity followed by the signal of cDNAs picked up from the library, and drug candidate compounds. Moreover, we will collect necessary information for the drug discovery through the analysis of the 3-D complex structure that is formed upon mutual interaction. Based on the above research results, we will try to create diagnostic reagents, diagnostic systems, functional foods, cosmetics and new drugs.

Contents of research

1. Detection and identification of disease-associated proteins

We will identify more than 50 kinds of proteins, whose quantity changes along with incidence of diseases (mainly cancer), using mass spectrometric method after separating and detecting them with fluorescent differential gel electrophoresis and nano liquid chromatography. Among them, 5-10 kinds of candidate proteins for diagnostic marker will be selected. On the other hand, efficiency for the drug discovery research will be attained by using the identified markers, which are available for the drug's mode of action and toxicological tests. We will try to develop new drugs through the screening of compounds that react with disease-associated proteins.

2. Functional analysis of disease-associated proteins

We will establish the evaluation system that correlates with diseases by detecting new diagnosis markers and the candidate molecule for the application to the disease diagnosis such as cancer, to the development of treatment and to the regenerative remedy. We will analyze the functions of proteins obtained from patient's biopsy sample, and then clarify the relationship between proteins and disease. In addition, we will conduct proteome analysis exhaustively by using tissue stem cells and tissues from disease model animal so that we may find candidates of new protein markers for the diagnosis and of molecule-targeted proteins.

3. Structural analysis of complex compound

After identifying intranuclear cDNAs that relate to human diseases, we will construct expression system subjected to the cDNAs picked up from the library, and then prepare the corresponding proteins in a large quantity. The 3-D structures of the proteins are determined using X-ray and NMR method.

We will estimate the protein function based on its structure, and identify the targeted compound that binds to the corresponding protein.

In addition, after estimating and designing the compound that regulates the function of the corresponding protein, we will identify the compound that binds to the protein through flow-type NMR method. We will determine the 3-D complex structure between protein and identified compound using X-ray and NMR method. Based on the above, the basic data of compounds for the drug candidate are prepared.

The main study results

1. Detection and identification of disease-associated proteins

As a result of detection of the protein, which is specific to the clear ovary cell cancer, followed by the protein analysis, we detected a few increased proteins including L-lactase dehydrogenase B chain and annexin A4 in this cell. On the other hand, we also succeeded to identify some decreased proteins. As for annexin A4, the increased expression of the specific gene and protein was found in the high-grade clear ovary cell cancer. In addition, we detected a few proteins whose expression was accompanied by prostate cancer and Kawasaki-disease. Moreover, we developed a new technology that could detect all at once 1,800 kinds of proteins, which exist in the human serum, on a single gel plate.

2. Functional analysis of disease-associated proteins

The following are the results of this research group.

1) Based on the proteome analysis using specimen derived from human breast and prostate cancer, we tried to find marker proteins for the diagnosis of breast cancer at an early stage.
2) Detection of new disease-associated molecules focusing on Pin I and angiotensin III that are suggested to have some relationship with cancer.
3) Establishment of breast cancer mouse model at an early stage and of cultured cell line expressing a series of the factors (forexample; PAR-1h) that are necessary for the formation of epithelial cell polarity.

3. Structural analysis of complex compound

Upon taking human targeted cDNAs out of their library, we constructed about 90 kinds of E. coli expression systems. It was succeeded to express targeted proteins with more than 80% efficiency using both cell and cell-free system. Among them, we could obtain two kinds of proteins that are usable for the NMR structural analysis.