BIOMARKER-BASED CANCER THERAPY PERSONALIZATION

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The efficacy of cancer treatments varies significantly from case to case, even if their tumors are categorized into the same histological subtypes and stages. Thus it would be necessary to identify a biomarker that could provide evidence about the probability of benefit or toxicity from a specific therapy. For example, the RET proto-oncogene gene mutation, which is an important target in cancer therapies. Technologies such as microarray, high-speed sequencing, and mass spectrometry have advanced rapidly in recent years, allowing comprehensive analyses of the cancer genome and proteome. It is anticipated that the application of these technologies will contribute to the discovery of new cancer biomarkers.

Antibody-based proteomics is a concept that emerged in alliance with the progression of genome-wide antibody production projects. The completion of proteome-scale antibody libraries is expected to enable not only rapid verification of biomarkers, but also direct screening of antibodies by high-throughput assays. Here we report new methods that can screen antibodies on the basis of their reactivity with a large number of patient samples with reasonable comprehensiveness and throughput: automated quantification of immunofluorescence tissue microarray and immunofluorescence reverse-protein microarray (RPPM). Using these antibody-based proteomics technologies, we were able to identify biomarkers that can predict the efficacy of combinational chemotherapy, radiotherapy, and molecular targeting therapy. We also discuss the potential application of the antibody-based proteomics technologies to early-phase drug development.

DISCOVERY OF FUSION GENES

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For molecular targeted therapy, an accurate selection of patients who benefit from therapy, i.e. a precise detection of the target molecule in tumor tissues, is most important. We developed a sensitive anti-ALK immunohistochemistry, the intercalated antibody enhanced polymer (iAEP) method. The iAEP method has enabled efficient and sensitive detection of EML4-ALK and has helped in identifying novel ALK fusions: KIF5B-ALK, SQSTM1-ALK, PPHBP1-ALK in lung cancer, lymphoma and inflammatory myofibroblastic tumor, respectively, and TPM3-ALK and EML4-ALK in inflammatory myofibroblastic tumor, respectively, and TPM3-ALK and EML4-ALK in inflammatory myofibroblastic tumor, respectively.

EML4-ALK is well known to be significantly overexpressed in several human tumors, and we have developed a novel fluorescence probe (gGlu-HMRG) for EML4-ALK, which is quickly activated by GGT and yields an over 350-fold increase in fluorescence signal compared with the quenched state. gGlu-HMRG showed a large fluorescence increase in several cancer cell lines, but not in a normal cell line. Indeed, tumor cells in a mouse model of peritoneal metastases were successfully visualized with high signal contrast between the tumor and background. The activation occurred within 1 min of spraying the probe, and the signal was strong enough to be detected even with our naked eyes. This probe is believed to be practical for clinical application during surgical or endoscopic procedures.

RAPID IN VIVO CANCER IMAGING BY TOPICALLY SPRAYING A NEWLY DESIGNED ACTIVATABLE FLUORESCENCE PROBE

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Fluorescence imaging is one of the most powerful techniques currently available for continuous observation of dynamic responses in living cells and animals. For highly efficient development of novel fluorescence probes, we have established several rational strategies for controlling the properties of visible light to NIR-excitable fluorophores based on the intramolecular photoinduced electron transfer. These strategies are quite powerful and versatile and, indeed, based on these strategies, we have succeeded to develop a series of fluorescence probes for reactive oxygen species, ions, and various enzymatic activities. Recently, we have established another strategy to control the fluorescent properties of various fluorophores based on intramolecular spirocyclization. For example, if one of the amino groups of hydroxymethyl rhodaminegreen (HMRG), a new rhodaminegreen derivative bearing a hydroxymethyl group instead of the original carboxylic group, is amidated with an amino acid, the resultant product is colorless in neutral pH buffer due to the preferred spirocyclization. This compound can be a highly sensitive fluorescence probe for the target aminopeptidase, because it is selectively reactive towards the target enzyme to yield highly fluorescent HMRG.

Microclinical trials can be utilized for the purpose to gain pharmacokinetic information on a tentative drug candidate in human using labelled compound(s) and AMS or imaging technology using PET, or non-labelled compound(s) and LC/MS/MS, and to select drug candidate by ensuring adequate properties in early stage. In 2008, the NEDO (New Energy and Industrial Technology Development Organization) project entitled ‘Establishment of Evolitional Drug development with the Use of Microdosings and PET Imaging’ was adopted. In this project, the quantitative prediction method on drug absorption, distribution, metabolism and excretion (ADME) modeling and simulation will be applied to human to validate this methodology. That is, based on the in vitro data on metabolism, transport and binding with animal and human tissues, the drug concentration–time profiles in the plasma and target tissues such as the brain, tumor, liver and kidney will be predicted and the validity of the predictions will be investigated by using clinical studies under its microdose and therapeutic dose conditions. PET is a promising approach to determine the functional change of transporters associated with genetic polymorphisms or drug–drug interactions. Labelled PET probes have been developed for specific transporters in this NEDO project. In addition, in 2010, the Ministry of Education, Culture, Sports, Science and Technology (MEXT) has launched second program, the ‘J-AMP; Japan Advanced Molecular Imaging Program’, using the two major centers (RIKEN, NIRS). In this project, research is undertaken focusing on the cancer and dementia fields to contribute to the development of new drugs and biomarkers as diagnostic tools.

Here, in this presentation, I will share with you our recent progress in the use of the analysis of plasma clearance of drugs and PET imaging to evaluate the transporter function in vivo.

References

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**JS1 – 5 PHARMACO-METABOLOMICS FOR ANTICANCER DRUGS**

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Metabolomics is a biochemical phenotype of cellular events after gene expression, transcription and translation, and recently it has been included as a new ‘omics’ science, as a new field toward the understanding of global system biology. Since there are numerous networks with thousands of metabolites in human cells, a key technology for metabolomics is the separation analysis of these abundant metabolites. The mass spectrometry (MS) plays a central role for identification and quantification of metabolites in biological fluids. LC-MS is useful for studying hydrophobic metabolites, while the capillary electrophoresis (CE)-MS is a powerful method for analyzing charged compounds such as weak anions and cations.

Cancer metabolomics is an emerging field in cancer biology. Cancer-specific metabolic reprogramming such as the Warburg effect is considered to facilitate cell growth and division. Several signaling pathways implicated in cell proliferation also regulate metabolic pathways that incorporate nutrients into biomass, rather than ATP production.

We have firstly applied the CE-TOFMS metabolomic analysis to the pharmacology. Pharmaco-metabolomics investigates the drug action on the global metabolomic profiling and pathways. A comprehensive metabolomic fingerprint of cancer cells is expected to differentiate response or non-response to the drug therapy. Our recent findings regarding two metabolic inhibitors (5-FU and gemcitabine) are presented as examples. The metabolomic analyses can create new findings on cancer biology, pharmacology as well as potential biomarkers for drug action in the post-genome era.

**JS1 – 6 BIOMARKER RESEARCH IN LUNG CANCER AND ITS FUTURE IN JAPAN**

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Currently, lung cancer is regarded as a mixture of heterogeneous diseases rather than single entity of the disease in terms of prognosis and therapeutic response. The discovery of EGFR mutations in 2004 as a predictive factor for EGFR tyrosine kinase inhibitors (TKI) (gefitinib or erlotinib) really opened the door of new era of individualized approach to lung cancer.

It is noteworthy that two randomized phase 3 studies that compared gefitinib with platinum doublet therapy for patients with EGFR mutation (WJTOG 3405 and NEJ 002) were first of the trials for molecularly selected patients. They both successfully showed significantly longer PFS in the gefitinib arm. To extrapolate this finding to postoperative situation, randomized clinical trial comparing gefitinib with cisplatin plus vinorelbine as adjuvant treatment for resected stage IB–IIIA patients with EGFR mutation (IMPACT trial) is ongoing.

EML4-ALK fusion was found in 2007. It soon became evident that crizotinib, an ALK-TKI, was very active for this type of lung cancer in a phase I/II study conducted in United States and Korea. Crizotinib was quickly approved at the end of March 2012 that was within 5 years from the target discovery. In the mean time, the Japanese Lung Cancer Society published ‘Guidance for ALK gene testing in lung cancer patients’ to distribute right knowledge on diagnosis of ALK+ lung cancer. Newly identified targets such as DDR2 mutation, FGFR1 amplification in squamous cell carcinoma, or ROS1, RET fusions in adenocarcinoma also appear to belong to the same oncologic driver as EGFR or ALK. Targeted therapies for these are being developed.

However, acquired resistance inevitably emerges after ~10 months even in such patients. There is an urgent and unmet clinical need to develop countermeasures for acquired resistance based on biomarkers of resistance. Despite great enthusiasm for driver gene targets, biomarkers for cytotoxic chemotherapy such as ERCC1, RRM1, TS etc. have not draw adequate attention of Japanese oncologists. Retrospective validation using samples from patients enrolled in clinical trials (LETS, WJOG4107) are being carried out. In this process, biobank infrastructure is being developed.

In this symposium, I would like to introduce the current situation of biomarker research in lung cancer and discuss future direction.