Target Therapy for Cancer: Anti-cancer Drugs Targeting Growth-Factor Signaling Molecules

What Can and Cannot Be Done Using a Microarray Analysis? Treatment Stratification and Clinical Applications in Oncology

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Received July 1, 2011

Ten years have passed since the emergence of microarray technology. Recent microarray procedures have provided reliable results on all platforms and have enabled highly reproducible gene expression measurements. Thus, nearly all technical matters regarding microarray measurements are thought to have been resolved. Treatment stratification for molecular-targeted drugs can now be achieved based on the presence of somatic mutations, gene amplification, and/or protein overexpression. However, no clinically available biomarkers have been identified for molecular-targeted drugs using microarray analysis. Microarray data as a database for the gene expressions of clinical samples may be a critical issue, especially for the development of molecular-targeted treatments. In addition, microarray analysis during early-phase clinical trials for molecular-targeted drugs is considered to provide critical information, including proof-of-concept and confirmation of the inhibition of the target molecule. Meanwhile, OncotypeDX® and MammaPrint® assays have been developed to determine the benefits of chemotherapy for breast cancer patients. These multigene-based assays are commercially available and have shown encouraging results for treatment stratification or decision-making for treatment using cytotoxic drugs in clinical settings. During the development of these assays, numerous samples and efforts were required to create a model using multi-center or inter-group investigations. Based on the success of these models, the development of further assays for determining multigene expressions is likely to increase in the future. In the present article, we introduce our data on mutant epidermal growth factor receptor (EGFR) signaling and amplification of fibroblast growth factor receptor 2 (FGFR2) using microarray analysis, and treatment stratification and clinical applications using gene expression profiles for cancer treatments are discussed.

Key words microarray; multigene-based assay; molecular-targeted drug; cytotoxic drug

1. INTRODUCTION

Recent advances in scientific technology have enabled the use of commercially available microarray technology to obtain quantitative data regarding the expression of numerous genes. Ten years have now passed since the emergence of microarray technology, and this technology can now be used routinely. During the past decade, the numbers of probes on microarray have increased to the level of so-called genome-wide gene expressions (ca. 50000 probes), and the reproducibility of such analyses has improved dramatically. Thus, microarray analysis undoubtedly exhibits powerful potential for hypothesis-free approaches to the identification of pertinent genes from amongst large numbers of genes. Recently, several microarray technology and multigene polymerase chain reaction (PCR)-based assay procedures have been used in clinical settings for decision-making purposes in the treatment of breast or colorectal cancer. Meanwhile, the growing data on microarray analyses have unexpectedly indicated that this technology cannot be used for all purposes. What can and cannot be done using gene expression profiles in the field of oncology? In the oncology field, microarray analysis appears to be a widely-used and valuable method to determine gene expression data at the whole-genome level. In this article, we introduce our data on mutant epidermal growth factor receptor (EGFR) signaling and amplification of fibroblast growth factor receptor 2 (FGFR2) using microarray analysis, and treatment stratification and clinical applications using gene expression profiles in cancer therapy are also discussed.

2. COMPARABILITY AND REPRODUCIBILITY OF MICROARRAY PROCEDURES

Early during the development of microarray technology, concordant results using different microarray platforms or different suppliers of microarray were not thought to be possible. For example, gene expression data were evaluated using the same RNA sample and three different suppliers of microarrays (Amersham, Affymetrix, and Agilent) and the results were reported in Nucleic Acids Res. in 2003. The correlation coefficients for each gene among the inter-supplier data were ca. 0.5, and only 20% of the genes produced concordant results among the inter-supplier data in experiments detecting gene expression changes. These results suggested that the data were unstable and were hampered by background noise. The authors concluded that establishing industrial manufacturing standards and further independent and thorough validation of this technology were needed.
In 2004, the U.S. Food and Drug Administration (FDA) recognized the importance of genetic data, such as microarray data, for the development of new drugs, and the FDA began to receive attached data when new drug applications were submitted. Therefore, the clinical application of microarray analyses became a reality. Quality controls for microarray data with regard to comparability and reproducibility were urgently needed for use in clinical settings. Consequently, the MicroArray Quality Control (MAQC) project was undertaken by a consortium of academic societies, industry members, and regulatory authorities to create guidelines for the standardization and quality of microarray data. These results were reported in *Nature Biotechnology* in 2006.2) The microarray data from seven commercially-available microarray platforms (Affymetrix, Amersham, Agilent, Applied Biosystems, Eppendorf, GE Healthcare, Illumina and the National Cancer Institute) were examined simultaneously using the same RNA sample. The correlation coefficients for each gene among the platforms and PCR-based data ranged from 0.86 to 0.93. No significant differences in the gene expression levels were observed between the one-color and two-color methods. The consortium also developed an evaluation method using external RNA controls. In addition, very similar genes were selected by inter-platform microarray analyses against the same RNA sample. Collectively, the authors concluded that recent microarray procedures are reliable for use with all platforms and provide highly reproducible gene expression measurements.5) These results suggest that purely technical matters regarding microarray measurements have been resolved.

Genetic variations among patients, such as gene expressions and single nucleotide polymorphisms (SNPs), are considered to be a major cause of the widely varying responses to drugs among individuals during the clinical development of drugs. To increase the success rate of the development of new drugs, the U.S. FDA started a program for voluntary exploratory data submissions (VXDSs) without immediate regulatory impact in 2004. Among these data, microarray gene-expression technologies have been increasingly applied to elucidate the sources of variability in drug response.3)

3. MULTIGENE-BASED ASSAYS IN CLINICAL SETTINGS

More than fifty years have passed since the emergence of anti-cancer cytotoxic drugs in clinical settings, no single biomarker for predicting the responses to treatment with cytotoxic drugs or for stratifying treatments had been identified, unlike the biomarkers that have been identified for predicting responses to molecular-targeted drugs. The difficulty of identifying biomarkers for use in cytotoxic drug treatment may be explained by the numerous genes that cooperate and are involved in the drug target pathway. However, striking evidence has been reported for the treatment of breast cancer with cytotoxic drugs using a multigene-based approach (PCR-based and microarray-based assays) in clinical settings.

The OncotypeDX® assay was developed for distinguishing formalin-fixed, paraffin-embedded (FFPE) samples, which are relatively available in clinical settings. Initially, 250 candidate genes were selected based on data from medical literature and databases, and the expression levels of these genes were evaluated using three cohorts in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-20 trial. Among them, 16 genes that were correlated with the clinical outcome were identified and were used to build the final model. The recurrence score (RS) was determined based on the gene expression data, and the model was validated using independent samples from the NSABP B-14 trial.5) The RS ranged from 0—100 and was used to define low-risk (<18), intermediate risk (≥18 and ≤30), and high-risk (>30) groups. The Trial Assigning Individualized Options for Treatment (TAILORx) study, a prospective intergroup study, is presently ongoing with the objective of assessing the effectiveness of OncotypeDX® for determining whether hormonal therapy alone or chemotherapy and hormonal therapy should be used in patients belonging to the intermediate-risk group. The MammaPrint® assay is a commercially available custom microarray platform consisting of 70 genes. This assay requires the use of frozen tumor specimens. Seventy-eight breast cancer samples from patients who were premenopausal, younger than 55 years, did not have nodal metastasis, and who had not undergone prior systemic chemotherapy were used to examine the gene expression levels of 25000 genes using a microarray analysis. Seventy genes were selected as prognosis-related genes and were validated using the leave-one-out cross-validation method.5) The performance of this 70-gene assay was then evaluated in several subsequent studies.5,6,7)

The OncotypeDX® and MammaPrint® assays have provided encouraging results suggesting that multigene-based assays are a promising approach for treatment stratification or decision-making applications in clinical settings. In the development of assays, four steps are needed, as follows: (i) the selection of genes in a training cohort, (ii) cut-off determination and model building in an independent cohort, (iii) validation of the built model in an independent cohort, and (iv) a comparison between the multigene-based assay and the old standard (Fig. 1). Steps (i)
and (ii) may be performed using retrospectively collected samples, but steps (iii) and (iv) require a prospective study, indicating that numerous samples and efforts are required to build models in multi-center or inter-group investigations.

On the other hand, microarray and multigene-based assays have not been successful, with the exception of applications for breast cancer. Subramanian and Simon clearly stated multiple serious problems in the design and analysis of many of the clinical studies using microarrays.10) They proposed a guideline for the design, analysis, and evaluation of prognostic signature studies that emphasized the importance of focused study planning to address specific medically important questions. They also proposed the use of unbiased analysis methods to evaluate whether the resulting signatures provide evidence of medical utility beyond standard-of-care-based prognostic factors, and explained why most clinical studies using microarray analyses have failed.10)

4. MICROARRAY AND MOLECULAR-TARGETED DRUGS

Detecting changes in signal transduction using microarray analysis requires special attention when interpreting the results. Somatic mutations of the EGFR tyrosine kinase in non-small cell lung cancer have been shown to be associated with hyper-responsiveness to a selective EGFR tyrosine kinase inhibitor. We previously examined the difference in signal transduction between wild-type and mutant EGFR forced-expressing stable cell lines using a microarray analysis.11,12) The results indicated that early growth response 1 (EGR1) was markedly upregulated by more than 10-fold in the mutant EGFR-overexpressing cell line (Fig. 2A). The constitutive upregulated EGR1 expression in the mutant EGFR cells was suppressed by exposure to 20 nM of EGFR-tyrosine kinase inhibitor AG1478 or MEK inhibitor U0126, indicating that the upregulation arose from the activation of the EGFR-MEK signal transduction (Figs. 2B, C). Of note, the microarray analysis detected a change in the upregulated expression of transcription factor EGR1, which is the most downstream molecule in the EGFR signaling pathway, as a final response, but it could not detect any change in upstream molecules. The result suggests that microarray analysis can detect changes in gene expression levels, but not phosphorylation status in the signaling pathway.

Regarding the clinical applications of microarray analyses for the development of molecular-targeted treatments in oncology, the use of microarray data for clinical samples as a database appears to be a critical issue. Retrospective druggable or gene-of-interest expression analyses using expression data for clinical samples are easy to perform.13–15) We previously used microarray analyses to identify prognostic biomarkers for gastric cancer using endoscopic biopsy samples.16) Retrospective gene expression analyses of clinical samples revealed that several gastric cancers overexpressed...
EGFR, HER2 and FGFR2, presumably because of gene amplification (data not shown). Based on this data, further analysis may enable the identification of a specific subpopulation with gene amplification, and may contribute to the development of a novel molecular-targeted therapy for patients with gastric cancer (Fig. 3). In addition, numerous microarray data have been deposited in the publicly available Gene Expression Omnibus (GEO: http://www.ncbi.nlm.nih.gov/ gds).

Meanwhile, microarray analyses can have a strong impact on proof-of-concept (POC) studies for early-phase clinical trials. In a phase 1 study of vorinostat (suberoylanilidehydroxamic acid [SAHA]), Garcia-Manero et al. demonstrated that proliferation-associated genes were down-regulated after therapy and that antioxidant gene expression may confer vorinostat resistance, similar to the results of preclinical studies using microarray analyses against peripheral blood samples from patients receiving vorinostat therapy.17) We are presently analyzing similar data for SAHA and other drugs, and consider this approach to be promising for phase 1 studies (manuscript in preparation).

5. CONCLUSIONS AND PERSPECTIVE

What can and cannot be done using microarray analyses has become clear as a result of the significant technical and statistical improvements that have been made. Microarray analyses have been partially modified and are already in use as multigene-based assays in clinical settings for deciding whether the addition of chemotherapy may benefit patients. Furthermore, microarray analyses remain a powerful and unique tool for identifying genes using a hypothesis-free approach and for the systematic creation of gene expression databases. To further develop clinical applications, considerable effort, including clear clinical purposes, specific study designs, and numerous samples, will be required. However, such efforts may ultimately benefit cancer therapy.

Acknowledgements This study was supported by the Third-Term Comprehensive 10-Year Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

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Fig. 3. Schema for the Development of Molecular-Targeted Therapies Using Microarray Analysis in Gastric Cancer

The identification of overexpressing molecular targets in clinical samples using microarray data may warrant further investigation in future clinical trials.