

Foreword

The introduction of light microscopy in 1872 by Ernst Abbe and Carl Zeiss was one of the first revolutionary steps in the diagnosis of human diseases. It was possible to determine associated structural defects by morphological analysis of tissues and single cells, resulting in the development of major classifications and subgroup definitions. These have been revised many times during the last 130 years and continue to have an huge impact on modern diagnostics. For decades during the last century, light microscopy was one of the most important methods available for the clinical diagnosis of tumors and for describing morphological changes associated with widespread disorders, such as diabetes and heart disease.

From chromosomal analysis in the 1960s, molecular biological methods, polymerase chain reaction, and immunological methods, such as immunofluorescence, which enables us to define the surface marker profile of single cells, have been introduced into disease diagnosis. Examples are: the discovery of the Philadelphia-Chromosome t(9;22) as the main feature of chronic myeloid leukemia (CML); the association of HLA-DR subtypes with specific diseases (e.g., HLA B27 in patients with Bechterew's disease); and mutations of the APC gene in patients with colon cancer. By using such single genetic markers, the risk stratification of these diseases has been improved, resulting in more specific treatment with better clinical long-term outcomes. In particular, for CML, it was possible to use the well-characterized molecular defect to design the first target-specific drug (STI571, ImatinibTM), dramatically improving the treatment options for patients with Ph+ leukemias.

Twenty years ago, in 1985, I was asked to provide a review article entitled "Cell surface markers in leukemia: biological and clinical correlations" (Thiel, E., *Crit. Rev. Oncol. Hematol.* 1985: 209–260). Introduction of cell

surface marker analysis by flow cytometry into clinical bone marrow and peripheral blood samples from patients with acute leukemia (in particular those with acute lymphoblastic leukemia (ALL)), extended the classification of ALL from three morphological defined subgroups (L1–L3 as defined by the French–American–British Cooperative group) to about ten different subtypes. Discrimination between B-lineage and T-lineage ALL, as well as determination of high-risk ALL subtypes, enabled us to introduce the risk-adapted treatment plan for patients with ALL. The consequence of this was, on the one hand, diversity of therapeutic regimens in ALL, but on the other hand, improvement of disease-free long-term survival in a number of patients to more than 60%. Successful immunophenotyping has triggered therapeutic advantages in patients with ALL over the last two decades.

Recently available microarray technology is a powerful new tool for assessing the expression of a large number of genes in a single experiment. Its most important use in medical science is for characterizing global gene expression profiles, specific for disease subgroups or ones which have prognostic value (e.g., for disease progression). This can speed up the identification of diagnostic and prognostic markers. Furthermore, gene expression profiling can help predict responses to different pharmacological treatments, resulting in therapeutic stratification for individual patients. This book summarizes the most recent work on gene expression profiling in clinical medicine, including diabetes, heart diseases and tumor diseases. From identifying previously known and unknown subtypes of disease and making a correlation with survival, researchers have moved on to constructing distinct gene models for predicting the clinical outcome or response to specific therapeutic agents. This may change treatment strategies in the future, resulting in more individualized therapy for all kinds of human diseases.

Clinical implications of microarray technique have been reflected recently in two other examples. First, Supplement 6 to vol. 37 of *Nature Genetics* in June 2005, entitled “The Chipping Forecoast III” summarizes recent technical advantages and predictive power of microarray data analysis. Second, is a most important development for clinicians awaiting the introduction of gene expression profiling into the clinical routine. In July 2005 the first international multicenter trial (MILE – Microarray Innovations in Leukemia), conducted by one of the contributors to this

volume (Professor Torsten Haferlach, Munich) and initiated by the European LeukemiaNet started to analyze 4000 clinical samples from patients with all subtypes of leukemias, by traditional diagnostic methods (including morphology, immunology, molecular genetics) and by gene expression profiling using microarrays. This trial will be the first attempt to correlate results from standard diagnostics in leukemia with those from gene expression analysis. Diagnosis of leukemias by microarrays can be expected to achieve the level of clinical application very shortly. Just as the introduction of light microscopy revolutionized diagnosis in human disease, I would predict that gene expression profiling will change our understanding of disease classification and prognosis evaluation dramatically over the next few years.

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Caution: PCR can help to detect the expression of single genes within one reaction. By optimizing the volume of PCR, the number of genes which can be detected within one reaction could be increased to a maximum of six by using fluorescence labeled primers or probes. High-throughput analysis of multiple genes, e.g. in hundreds of patients samples by PCR is very time consuming and requires a lot of technical and personnel power. As an example, it would require about 675 days of work (24 hours a day) to analyze all the human genes which are known at this time by PCR using a singleplex reaction.

In 1998, Patrick O'Brien published the first paper about a new technique which could be used to simultaneously analyze gene expression of 45 genes within an experiment by using a microarray which was prepared by high-speed robotic printing of complementary DNAs on glass slides. The detection of gene expression using such a "microarray" which is commonly called a "DNA- or RNA-chip" requires a high resolution scanner which can detect the fluorescence signals from each of the complementary cDNAs with a high sensitivity and specificity. Finally, each of the fluorescence signals is