Extended Abstracts of the 2003 International Conference on Solid State Devices and Materials, Tokyo, 2003, pp. 352-353

F-5-1 (Invited)

DNA Chips and Their Medical Applications

Paolo Fortina[1], Larry J. Kricka[2] and Saul Surrey[3]

[1]Center for Translational Medicine, Department of Medicine, Jefferson Medical College, Thomas Jefferson University
406 Medical Office Building, 1100 Walnut Street, Philadelphia, PA 19107, USA
Phone: +1-215-955-0683 E-mail: paolo.fortina@jefferson.edu
[2]Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania
3400 Spruce Street, Founder Pavilion 7.103, Philadelphia, PA 19104, USA
[3]Cardeza Foundation for Hematologic Research, Jefferson Medical College, Thomas Jefferson University
702 College Building, 1015 Walnut Street, Philadelphia, PA 19107, USA

In 1997, the film GATTACA exemplified a future in which a DNA diagnostic test taken after birth determined the protagonist's biological future. The individual was informed from an evaluation of his genome that he would live only a few decades since there was a 99% chance of his developing heart failure.

While this was a science fiction movie, today efforts are growing towards implementation of clinical molecular genetic tests as the Human Genome Project is exposing our molecular individuality [1]. Indeed, since the completion of the DNA sequence determination of the human genome, we have entered the post-genome era where we seek to characterize genes, provide functional assignment and identify those that are diagnostic or prognostic of disease [2]. Functional genomics studies are assigning function to the estimated 30-40,000 human genes and identifying common human polymorphic variations [3]. In addition, new data on the relationship between an individual's genome and the environment is changing our concept of disease. This abundance of gene-based data will unlikely result in a GATTACA-like scenario; however, a new-era of gene-based medicines is quickly materializing. In the '80s, when researchers discovered the β^{39} or the ΔF508 mutations, linking them to beta thalassemia and cystic fibrosis, respectively, it seemed reasonable to expect one gene, one mutation, one diagnosis. Today we know that several hundred genes may underlie illnesses such as cancer, cardiovascular disease and hereditary disorders [4]. Even previously classified simple recessive diseases involve interaction with gene modifiers, which play unanticipated roles in altering clinical presentation [5].

Molecular diagnostics is becoming critical to every specialty, and DNA testing is ordered increasingly in clinical settings. In cancer alone, the potential is stunning as new technologies allow re-classification of tumors according to their molecular profile, sometimes permitting prediction of outcome and/or therapeutic response [6]. New targets for therapy and novel diagnostic markers for a variety of different diseases are being discovered at a unprecedented pace [7].

Therefore, the ability to analyze simultaneously multiple nucleic acid sequences for variation in a rapid and accurate fashion is becoming pivotal in all areas of medicine. Introduction of biochips in the clinical laboratory has resulted in the potential for detecting nucleic acid variation on a genome-wide scale (Figure 1). Microarrays are critical tools in medicine, which involve surface-bound molecules, referred to as probes, and solution-phase molecules referred to as targets [8]. Target(s) can be specific or complementary to the probe(s) and can be used in a multiplex fashion to assess genetic variation. (*e.g.*, single nucleotide polymorphism).

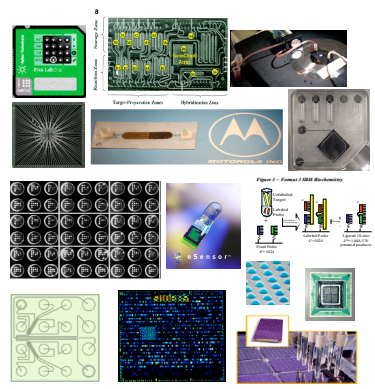

Figure 1: DNA chips

Differences of a single nucleotide polymorphism or SNP occur approximately once every 1000 nucleotides. SNPs are frequently used as markers linked to risk of developing a specific disease, but not themselves contributing to a disease-causing genetic change [9]. Therefore, each SNP can act as a landmark allowing one to pinpoint a region of interest for investigating whether a given region is associated with a specific disease. Eventually, it will be possible to screen people to discover their unique disease susceptibilities. Most likely, a complete catalog of characterized human diversity will be one of the most valuable resources of the human genome project.

Analysis of molecular changes associated with predisposition to various cancers requires detection of specific gene mutations, detection of gene dosage changes, identification of rare fusion-gene transcripts, analysis of polymorphic repeat profiles, and/or monitoring of differentially expressed genes. Therefore, to distinguish single nucleotide differences, array-based

approaches have been designed which employ ASO-based hybridization of labeled solution-phase targets, polymerase-catalyzed extension of arrayed or solution-phase oligonucleotide probes, array-based tiled sequencing strategies with overlapping probes, use of padlock probes and combination of polymerase chain reaction and ligase detection with zip-code hybridization and/or array-based hybrid capture using bar-coded array-bound oligonucleotides [10]. In those instances in which expression profiling is required, arrays of oligonucleotides or cDNA clones are used, providing data on the comparative expression of genes in normal and diseased states or for monitoring effects of drug treatment [11].

Microarray analysis of gene expression enables the identification of genes whose expression is modified by disease or environmental exposures. Eventually, any type of drug-related therapy will be targeted to get expression patterns back in homeostasis. Therefore, drug discovery will evolve as a result of the ability to measure compound response as a function of both genetic differences and gene expression variables, which will lead to discovery of new drugs. Furthermore, microarray technologies are useful tools for profiling thousands of single nucleotide polymorphisms (SNPs) in order to correlate genotypes with disease risk, toxicity assessment and drug response.

Today, a number of companies provide novel tools including platforms for low-, medium- and high-density arrays for high-throughput analysis. Options of buying ready-made chips are expanding and a perspective on commercially available instrumentation will be presented with emphasis on their advantages and disadvantages. Set-up and operational costs as well as sensitivity, accuracy, reproducibility, cost-effectiveness and validation through comparisons with standard methodologies will be discussed. Specifically, advantages and disadvantages of commercial platforms for DNA/RNA analysis such as the Affymetrix GeneChip GenFlex Tag Array System, MALDI-TOF, Illumina, Nanogen NanoChip™ Research System and Pyrosequencing PSQ™ 96 will be presented. Additional devices such as the Hitachi Thermal Gradient chip and Motorola eSensor, which have yet to be commercialized will also be addressed.

Point-of-care testing (POCT) also has assumed increasing importance as an alternative to clinical laboratory-based testing. Today, POCT includes glucose monitoring, pregnancy and ovulation testing, drug abuse testing, urine and blood tests performed by cartridges, dipsticks and flow-through cassettes at the bedside, in the private physician's office or at home. Future miniaturization is focused on development of hand-held, portable microanalyzers for personal use. Critical to realization of these devices will be the level of miniaturization achievable, the quality control issues and the ethical aspects.

Although uses for biochips in clinical laboratories have increased, there are still obstacles associated with their routine use [12]. Simplicity of assay design, equipment and supply costs, operating complexity, sensitivity, accuracy, reproducibility and validation in addition to governmental approval represent issues still outstanding [13].

Eventually, measurement of protein levels will be required for a comprehensive understanding of diseases and will require proteomics-based devices for testing [14]. Finally, more recently developed nanoscale-based technologies will be used to probe DNA structure and facilitate single cell and single molecule detection [15].

References

[1] W.W. Grody, Methods Mol. Biol. 170, 53-69 (2001)
[2] L. Peltonen and V.C. McKusick, Science 291, 1224-1229 (2001)
[3] E.F. Petricoin III, et al. Nat. Genet. Supplement 32, 474-479 (2002)
[4] C.H. Chung et al., Nat Genet Supplement, 32, 533-540, (2002)
[5] J.H. Nadeau, Nat. Rev. Genet. 2, 165-174 (2001)
[6] T.R. Golub, et al., Science 286, 531-537 (1999)
[7] D.L. Gerhold, et al., Nat Genet Supplement, 32, 547-552, (2002)
[8] P. Fortina, et al., Technology options and applications of DNA microarrays. In: Biochip Technology (Cheng, J. and Kricka, L.J., eds.) pp. 185-216, Harwood Academic Publishers (2001)
[9] A.-C. Syvanen, Nat. Rev. Genet. 2, 930-942 (2001)
[10] P.-Y. Kwok, Annu. Rev. Genomics Hum.Genet. 2, 235-258 (2001)
[11] D.K. Slonim, Nat Genet Supplement, 32, 502-508, (2002)
[12] E. Dequeker, et al., Nat. Rev. Genet. 2, 717-723 (2001)
[13] E. Dequeker and J.J. Cassiman, Nat. Genet. 25, 259-260 (2000)
[14] G. MacBeath, Nat Genet Supplement, 32, 526-532, (2002)
[15] K.K. Jain, Expert Rev. Mol. Diagn. 3, 153-161 (2003)