

PERSONALIZED CLINICAL LABORATORY DIAGNOSTICS

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1. Abstract

Molecular diagnostics play an important role in development of personalized medicine and may be termed personalized diagnostics. This chapter shows the role of various diagnostic technologies in personalizing treatment. Besides polymerase chain reaction (PCR), several non-PCR methods, biochips/microarrays, and nanobiotechnologies play an important role. Technologies for biomarkers, single nucleotide polymorphisms (SNPs), and cytogenetics provide basic information for personalized medicine. Finally, integration of diagnostics with therapeutics is an important component of personalized medicine. Future challenges and prospects of personalized diagnostics are discussed.

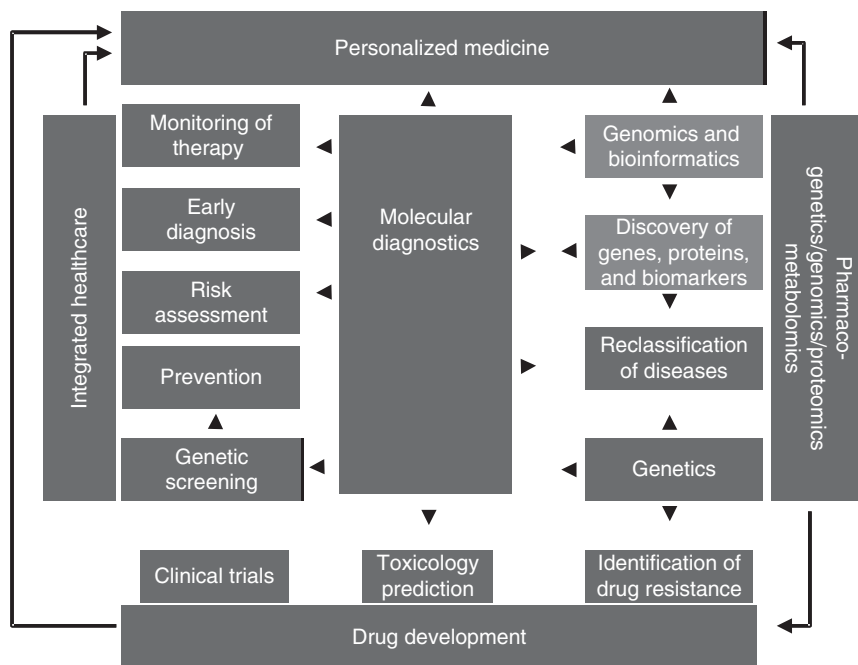
2. Introduction

Personalized medicine, also referred to as individualized therapy, simply means the prescription of specific treatments and therapeutics best suited for an individual taking into consideration both genetic and environmental factors that influence response to therapy [1, 2]. Genomic/proteomic technologies have facilitated the development of personalized medicines but other technologies such as metabolomics are also contributing to this effort. Any diagnostic procedure that facilitates the development of personalized medicine can be termed “personalized diagnostics.” Molecular diagnostics plays an important role in personalized medicine [3]. Role of molecular diagnostics in personalized medicine and interaction of various technologies is shown in [Fig. 1](#).

3. Basic Concepts of Personalized Medicine

3.1. PHARMACOGENOMICS AND PERSONALIZED MEDICINE

Pharmacogenomics applies the large-scale systemic approaches of genomics to drug discovery and development [2]. It also involves the study of the mechanisms by which drugs change the expression of genes, including drug-metabolizing enzymes, a phenomenon known as induction. Various



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FIG. 1. Role of molecular diagnostics in personalized medicine.

technologies enable the analysis of these complex multifactorial situations to obtain individual genotypic and gene expression information. These same tools are applicable to study the diversity of drug effects in different populations. Pharmacogenomics promises to enable the development of safer and more effective drugs by helping to design clinical trials such that nonresponders would be eliminated from the patient population and take the guesswork out of prescribing medications. It will also ensure that the right drug is given to the right person from the start. In clinical practice, doctors could test patients for specific SNPs known to be associated with nontherapeutic drug effects before prescribing in order to determine which drug regimen best fits their genetic makeup. Pharmacogenomic studies are rapidly elucidating the inherited nature of these differences in drug disposition and effects, thereby enhancing drug discovery and providing a stronger scientific basis for optimizing drug therapy on the basis of each patient's genetic constitution.

3.2. PHARMACOGENETICS AND PERSONALIZED MEDICINE

Pharmacogenetics, a term recognized in pharmacology in the pregenomic era, is the study of influence of genetic factors on action of drugs as opposed to genetic causes of disease [2]. Now it is the study of the linkage between the individual's genotype and the individual's ability to metabolize a foreign compound. The pharmacological effect of a drug depends on pharmacodynamics (interaction with the target or the site of action) and pharmacokinetics (absorption, distribution, and metabolism). It also covers the influence of various factors on these processes. Drug metabolism is one of the major determinants of drug clearance and the factor that is most often responsible for interindividual differences in pharmacokinetics.

The differences in response to medications are often greater among members of a population than they are within the same person or between monozygotic twins at different times. The existence of large population differences with small inpatient variability is consistent with inheritance as a determinant of drug response. It is estimated that genetics can account for 20–95% of variability in drug disposition and effects. Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to interindividual differences in the efficacy and toxicity of many medications.

Although interindividual variations in drug response result from effects of age, sex, disease, or drug interactions, genetic factors represent an important influence in drug response and efficacy and remain constant throughout life. This has led to the recognition of the discipline “pharmacogenetics” since the 1950s, which can be viewed as an integration of gene profiling and pharmaceutical chemistry. From this initial definition, the scope has broadened so that it overlaps with pharmacogenomics.

Pharmacogenomics, a distinct discipline within genomics, carries on that tradition by applying the large-scale systemic approaches of genomics to understand the basic mechanisms and apply them to drug discovery and development. Pharmacogenomics now seeks to examine the way drugs act on the cells as revealed by the gene expression patterns and thus bridges the fields of medicinal chemistry and genomics. Some of the drug response markers are examples of interplay between pharmacogenomics and pharmacogenetics; both are playing an important role in the development of personalized medicines. The two terms, pharmacogenetics and pharmacogenomics are sometimes used synonymously but one must recognize the differences between the two.

3.3. ROLE OF PHARMACOPROTEOMICS IN PERSONALIZED MEDICINE

Role of proteomics in drug development can be termed “pharmacoproteomics” [4]. Proteomics-based characterization of multifactorial diseases may help to match a particular target-based therapy to a particular marker

in a subgroup of patients. The industrial sector is taking a lead in developing this area. Individualized therapy may be based on differential protein expression rather than a genetic polymorphism.

Proteomics will have a great impact on diagnosis during the first decade of the twenty-first century. By the end of the decade protein chip-based tests will be available for several diseases. Knowledge gained from genomics and proteomics will be combined to provide optimal detection of disease at an early stage for prevention or early intervention. Proteomics-based molecular diagnostics will have an important role in the diagnosis of certain conditions and proteomics-based medicines would be integrated in the total healthcare of a patient. Advantages of application of pharmacoproteomics in personalized medicine are as follows:

- Pharmacoproteomics is a more functional representation of patient-to-patient variation than that provided by genotyping.
- Because it includes the effects of posttranslational modification, pharmacoproteomics connects the genotype with the phenotype.
- By classifying patients as responders and nonresponders, this approach may accelerate the drug development process.

4. Molecular Diagnostic Technologies for Personalized Medicine

Following the discovery of PCR, molecular diagnostics has been expanding rapidly, particularly during the last decade. Several non-PCR and proteomics-based tests have joined the list which now runs over several hundred tests. A classification of clinical laboratory tests that are relevant to personalized medicine is shown in [Table 1](#). Tests in these categories may overlap as this classification is a mix of technologies and applications. Some of these technologies will be described briefly in the following text.

5. Role of PCR in Development of Personalized Medicine

Several PCR-based methods are used in the development of personalized medicine. An example is that of single-strand conformational polymorphism (SSCP) analysis. These can be used alone or in combination with other technologies. PCR-based methods are time-consuming and are used mostly in laboratory setting. For the practical development of personalized medicine, point-of-care testing is more suitable and rapid results are required such as in the office or out-patient management of infection, where

TABLE 1
A CLASSIFICATION OF CLINICAL LABORATORY TESTS AND THEIR APPLICATIONS RELEVANT TO
PERSONALIZED MEDICINE

Technologies
Polymerase chain reaction (PCR)-based methods, for example, single-strand conformational polymorphism (SSCP)
Non-PCR based methods: for example, direct molecular analysis without amplification
Enzyme-linked immunosorbent assay (ELISA)
Peptide nucleic acid (PNA) technology
Combined use of nucleic acid amplification and with methods such as immuno-PCR
Proteomics diagnostic technologies
Cytogenetics, for example, fluorescent <i>in situ</i> hybridization
Biochips and microarrays
Nanobiotechnology-based diagnostics
Single nucleotide polymorphisms (SNP) genotyping
Gene expression profiling
Transcriptome profiling studies
Sequencing, for example, multiplex DNA sequencing and whole genome sequencing
Applications
Biomarker detection and application for diagnostics as well as therapeutics
Pharmacogenomic tests
Pharmacogenetic tests
Pharmacoproteomic tests
Pharmacometabolic tests
Molecular toxicology: toxicogenomics and toxicoproteomics
To understand the molecular mechanisms of disease as basis for rational therapy
Discovery and development of personalized medicines
Diagnostics as companions to therapeutics
Monitoring of therapy as well as determining prognosis of disease

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identification of the microorganism is required before suitable treatment is prescribed and the patient leaves the office.

5.1. SSCP ANALYSIS

SSCP, an easy and sensitive PCR method, is one of the most popular methods for detection of mutations. The target sequence is amplified and labeled simultaneously by radioactive primers and nucleotides. The amplified product is then heated to dissociate the strands and subjected to nondenaturing polyacrylamide gel electrophoresis. The patient’s DNA is analyzed alongside normal samples on gels and mutations can be detected by differences in the respective band patterns seen on autoradiography. Putative mutant PCR products are cloned and sequenced to determine the nature of

SSCP difference. False negative results, however, cannot be excluded. Therefore, the absence of mutations cannot be proven by this technique. Automated capillary electrophoresis (CE) systems have now been introduced for SSCP analysis instead of conventional slab gel electrophoresis. SSCP analysis in combination with CE is a rapid, simple, sensitive, and high-throughput (HT) mutation-screening tool, and has been successfully applied for mutation detection involving human tumor suppressor genes, oncogenes, and disease-causing genes. The new technique has a great potential for mutation screening of large numbers of samples in clinical diagnosis. The use of SSCP analysis to discover and genotype SNPs has been widely applied to the genetics of hypertension, including both monogenic disorders such as Liddle's syndrome and polygenic disorders such as essential hypertension [5].

5.2. REAL-TIME PCR SYSTEMS

Some of the limitations have been addressed in, a number of real-time PCR systems are now on the market, which offer many general technical advantages over standard PCR, including reduced probabilities of variability and contamination, as well as online monitoring and the lack of need for postreaction analyses. There are currently five main chemistries used for the detection of PCR product during real-time PCR: DNA binding fluorophores, 5' endonuclease, adjacent linear and hairpin oligoprobes, and the self-fluorescing amplicons. Some of real-time PCR systems were developed with contemporary applications such as quantitative PCR (qPCR), multiplexing, and HT analysis in mind. In real-time PCR, the amount of product formed is monitored during the course of the reaction by monitoring the fluorescence of dyes or probes introduced into the reaction that is proportional to the amount of product formed, and the number of amplification cycles required to obtain a particular amount of DNA molecules is registered. Assuming a certain amplification efficiency, which typically is close to a doubling of the number of molecules per amplification cycle, it is possible to calculate the number of DNA molecules of the amplified sequence that were initially present in the sample. With the highly efficient detection chemistries, sensitive instrumentation, and optimized assays that are available today the number of DNA molecules of a particular sequence in a complex sample can be determined with unprecedented accuracy and sensitivity sufficient to detect a single molecule [6].

An example of usefulness of real-time PCR is for identification of pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) as well as methicillin-sensitive *S. aureus* (MSSA) and the accurate determination of antimicrobial sensitivities for prompt optimal patient therapy. Real-time PCR targeting the *S. aureus*-specific thermonuclease nuc gene and the

staphylococcal methicillin resistance determinant *mecA* gene has been used as a reliable indicator of the presence of MRSA and detection time for MRSA/MSSA isolates from positive blood cultures was dramatically reduced from 24 to 48 h required for routine tests to approximately 3 h [7]. Quantitative real-time PCR is useful in detection and staging of lung cancer as well as construction and validation of prognostic and predictive gene expression signatures [8].

Real-time qPCR can be applied to analysis of clinical samples to help stratification of patients in personalized medicine approach. It enables the measurement of gene expression or DNA copy number in specific cell types that are available only in a small quantity. Limitations of real-time PCR include that PCR product increases exponentially and variation increases with cycle number. There is an increased variation after transformation to linear values and increased risk of false negative results.

6. Combined PCR-Enzyme-Linked Immunosorbent Assay (ELISA)

PCR is limited with respect to gene quantification because of the exponential amplification of DNA, the tendency to occasionally amplify nonspecific DNA, and the semiquantitative character of such common DNA measurement techniques as Southern blotting or densitometry. Combined PCR–ELISA CR has been developed for overcoming the limitation of quantitative gene analysis. This method combines certain features of PCR and ELISA techniques for accurate, high-precision measurements of hybridization with sequence-specific probes. Unlike Southern blotting, the combined PCR–ELISA enables complete removal of competing DNA strands prior to hybridization and does not have the background problems associated with membranes. The quantification of the input DNA using this method is independent of the number of PCR amplification cycles, and can be calculated automatically with a standard laboratory ELISA plate reader. With these advances over current technology, the gene quantification method should find wide application in a variety of diagnostic and research applications in clinical chemistry, microbiology, and genetics. Applications relevant to personalized medicine include:

- Diagnosis and typing of infectious diseases.
- HLA typing for organ transplantation and autoimmune disease diagnosis.
- Cancer diagnosis by oncogene detection.
- Diagnosis of genetic diseases.
- Monitoring of inflammatory diseases by assaying cytokine gene expression.

7. Non-PCR Methods

7.1. ARRAYED PRIMER EXTENSION (APEX)

APEX reaction is a straightforward and robust enzymatic genotyping method in which hundreds to thousands of variations in the genome are simultaneously analyzed in a single multiplexed reaction. It differs from allele-specific hybridization in that the genotype information in APEX is obtained by single base extension, performed by a specific DNA polymerase, together with four different dye terminators [9]. This approach ensures highly specific discrimination without allele-specific hybridization, because the primer to be extended anneals just adjacent to the DNA base that needs to be identified. Selection of primers for specific sites or their consecutive placement in tiled format, shifting them by one base, enables SNP analysis, mutation detection, or resequencing of the DNA template. It also permits the analysis of insertions, deletions, splice variants, gene copy numbers, or CpG islands within the genome for gene methylation studies, by performing additional bisulfite reactions. Advantages for this method over usual hybridization strategies are:

- Reduced mismatching due to intercession of the polymerase.
- Increased resistance to oligonucleotide failure sequences.
- Tolerance of a greater range of hybridization conditions.

8. Direct Molecular Analysis Without Amplification

Direct analysis of single biological molecules is the key to the next generation of revolutionary technologies. Some technologies can directly analyze individual molecules of DNA, RNA, and proteins, without PCR amplification. They can replace traditional molecular biology techniques, which are hampered by the need for amplification and bulk fluorescence, with the accuracy and sensitivity of direct measurements based on single molecules. Additional advantages of the platform include:

- Sensitivity in the femtomolar range.
- No need for amplification (e.g., PCR) or enzymatic procedures, eliminating a major source of cost and bias.
- Small sample material requirements.
- Flexibility across sample types (e.g., DNA, RNA, and protein) and assays.

9. SNP and Personalized Medicine

Small stretches of DNA that differ in only one base are called SNP and serve to distinguish one individual's genetic material from that of another. SNPs comprise some 80% of all known polymorphisms. Among the roughly

3-billion nucleotide base pairs (i.e., the “letters”) that make up the genetic code, SNPs occur with a frequency of one per 500 base pairs so that there are approximately 6 million SNPs. Each gene contains approximately 5 coding SNPs, which likely effect the expression of the currently estimated 20,000–25,000. Identification of SNPs is important as it helps in understanding the genetic basis of common human diseases. In the absence of functional information about which polymorphisms are biologically significant, it is desirable to test the potential effect of all polymorphisms on drug response. More than 9 million SNPs have been already generated in public databases using a large number of methods but only a small fraction of these are well characterized and validated [10].

High-resolution genome-wide association studies using panels of 300,000 to 1 million SNPs aim to define genetic risk profiles of common diseases. These studies provide an opportunities to explore pathomechanism of human diseases that are unbiased by previous hypotheses or assumptions about the nature of genes that influence complex diseases. Many genetic variants identified as risk factors for diseases by such studies have been localized to previously unsuspected pathways, to genes without a known function.

In the absence of functional information about which polymorphisms are biologically significant, it is desirable to test the potential effect of all polymorphisms on drug response. Potential uses of SNP markers include drug discovery and prediction of adverse effects of drugs. SNPs have the following relation to an individual’s disease and drug response, which indicates their usefulness for personalized medicine:

- SNPs are linked to disease susceptibility.
- SNPs are linked to drug response, for example, insertions/deletions of ACE gene determine the response to beta blockers.
- SNPs can be used as biomarkers to segregate individuals with different levels of response to treatment (beneficial or adverse) in clinical settings.

Genotyping for complex diseases may be insufficient to predict whether a person is at risk for a particular disease. One tries to associate SNPs with disease, but if no SNP in a certain gene predicts disease, further interest in the gene or protein or enzyme is lost.

9.1. SNP GENOTYPING

A classification of technologies used for detection and analysis of SNPs is shown in [Table 2](#). The technologies are described in more detail elsewhere [11].

Desirable characteristics of a genotyping technology are: (1) robust performance and accuracy across a variety of circumstances, (2) HT performance, and (3) low cost. Sequencing offers the highest degree of specificity

TABLE 2
CLASSIFICATION OF TECHNOLOGIES FOR SNP ANALYSIS

DNA chips and microarrays
DNA sequencing
Electrochemical DNA detection
Fluorescence-detected 5'-exonuclease assays
Hybridization assays
Mass spectrometry (MS)-based methods, for example, matrix-assisted laser desorption ionization time of flight MS (MALDI-TOF MS)
Nanotechnology-based methods
Non-PCR methods: arrayed primer extension (APEX)
PCR-based methods
Peptide nucleic acid (PNA)-based methods
Pyrosequencing
Restriction-fragment-length polymorphism (RFLP)
Single molecular fluorescence technology
Zinc finger proteins

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and selectivity. For ten or fewer SNPs and sample numbers in the thousands, the current gold standard is TaqMan real-time PCR (Applied Biosystems). MassARRAY system (SEQUENOM), a mass spectrometry-based platform suitable for high throughput and up to 1000 SNPs. Pyrosequencing (Biotage AB), a sequencing-by-synthesis method, can be used for up to 100 SNPs. Affymetrix provides the densest coverage at the whole-genome level with its GeneChip Human Mapping 500K Array Set and Affymetrix GeneChip® Scanner 3000 MegAllele, based on Molecular Inversion Probe Technology (obtained by acquisition of ParAllele™ BioScience), enables the highest level of multiplexing that is commercially available as well as increase throughput with low capital investment. Illumina is supplementing its current 100K chip with a 250K chip. Restriction fragment length polymorphism analysis is laborious and hit-and-miss as success depends on whether the restriction enzyme recognizes particular SNPs. It is relatively inexpensive, which makes it appropriate for a small number of SNPs and a small number of samples. New methods for SNP genotyping are being investigated.

10. Genetic Variations in the Human Genome Other Than SNPs

Although many studies have been conducted to identify SNPs in humans, few studies have been conducted to identify alternative forms of natural genetic variation. These include insertions and deletions (INDELs) as well as copy number variations (CNVs) in the genome.

10.1. INDELS IN THE HUMAN GENOME

A map of 415,436 INDELS have been mapped in the human genome that signal a little-explored type of genetic difference among individuals [12]. INDELS are an alternative form of natural genetic variation that differs from the much-studied SNPs. Both types of variation are likely to have a major impact on humans, including their health and susceptibility to disease. Whereas SNPs are differences in single chemical bases in the genome sequence, INDELS result from the insertion and deletion of small pieces of DNA of varying sizes and types. If the human genome is viewed as a genetic instruction book, then SNPs are analogous to single letter changes in the book, whereas INDELS are equivalent to inserting and deleting words or paragraphs. INDELS were discovered using a computational approach to reexamine DNA sequences that were originally generated for SNP discovery projects. INDELS are distributed throughout the human genome with an average density of one INDEL per 7.2 kb of DNA. Variation hotspots were identified with up to 48-fold regional increases in INDEL and/or SNP variation compared with the chromosomal averages for the same chromosomes. The map is being expanded.

INDELS already are known to cause human diseases. For example, cystic fibrosis is frequently caused by a 3-base pair deletion in the CFTR gene, and DNA insertions called triplet repeat expansions are implicated in fragile X syndrome and Huntington's disease. Transposon insertions have been identified in hemophilia, muscular dystrophy, and cancer. INDEL maps will be used together with SNP maps to create one big unified map of variation that can identify specific patterns of genetic variation to help predict the future health of an individual. The next phase of this work is to figure out which changes correspond to changes in human health and develop personalized treatments.

10.2. VARIATION IN COPY NUMBER IN THE HUMAN GENOME

CNV of DNA sequences is functionally significant but has yet to be fully ascertained. A study showing that ~12% of human genes vary in the CNV of DNA sequences they contain—a finding that contradicts previous assumptions that the DNA of any two humans is 99.9% similar [13]. The discovery indicates that CNV could play a larger role in genetic disease than previously thought, with broad implications in genetic diagnostic testing. Some CNVs are already known to be associated with disease, including AIDS, inflammatory bowel disease, lupus, cataracts, arterial disease, and schizophrenia. The findings could change the direction of future genetic disease research, which has primarily focused on SNPs. Some diseases are caused by CNV rather than SNPs.

10.3. STRUCTURAL VARIATIONS (SVs) IN THE HUMAN GENOME

Structural changes are extremely common in human populations. Genetic variation among individual humans occurs on many different scales, ranging from gross alterations in the human karyotype to a SNP. More bases are involved in structural changes in the genome than are involved in single-base-pair changes.

Although the original human genome sequencing effort was comprehensive, it left regions that were poorly analyzed. Later investigations revealed that, even in healthy individuals, many regions in the genome show SVs, which involve kilobase- to megabase-sized deletions, duplications, insertions, inversions, and complex combinations of rearrangements. A study offers a new view of what causes the greatest genetic variability among individuals suggests that it is due to the presence of structural changes that cause extended segments of the human genome to be missing, rearranged, or present in extra copies, rather than to single point mutations [14]. This study was designed to fill in the gaps in the genome sequence and to create a technology to rapidly identify SVs between genomes at very high resolution over extended regions.

10.4. MAPPING AND SEQUENCING OF SV FROM HUMAN GENOMES

The first high-resolution map showing the structural variants (SVs) that exists in the human genome has been published [15]. Using a clone-based method, the complete DNA sequences of eight people of diverse geographic ancestry was examined: four of African descent, two of Asian descent, and two of western European descent. The DNA sequence of those eight people was compared to the DNA sequence derived from the Human Genome Project, which is known as the reference sequence. This map provides a comprehensive picture of the normal pattern of SV present in these genomes, refining the location of 1695 SVs that were more than about 6000 base pairs long; 50% of these were seen in more than one individual and lay outside regions of the genome previously described as SV. The researchers discovered 525 new insertion sequences, ranging in size from a few thousand to 130,000 base pairs, which are not present in the human reference genome, and many of these are variable in copy number between individuals. Complete sequencing of 261 SVs revealed considerable locus complexity and provides insights into the different mutational processes that have shaped the human genome.

In various parts of human genome, some people have segments of DNA sequence that other people do not have. Large genetic regions may be flipped in one person compared with another and these differences can influence a person's susceptibility to various diseases. These data provide a standard for

genotyping platforms and a prelude to future individual genome sequencing projects. The results also indicate that the human genome sequence is still incomplete that sequencing of additional genomes will be required to fill the remaining gaps. The eight people studied are part of a much larger group whose genomes will be sequenced as part of the 1000 Genomes Project, an international effort to sequence the genomes of people from around the world.

In order to understand SV, it is also essential to develop new technologies designed to detect genetic differences among people. For example, SNP biochips, whether used in research or in clinical applications, need to reflect this SV to find links between particular gene variants and diseases. Currently available biochips would miss an association for nearly half of these sites. Besides their potential applications, the new results provide a wealth of data to explore hypotheses and make discoveries as we now have eight new reference human genomes.

11. Role of Biomarkers in Personalized Medicine

Development of personalized medicine is closely linked to biomarkers, which may serve as the basis for diagnosis, drug discovery, and monitoring of diseases. This process of personalization starts at the development stage of a medicine and is based on pharmacogenomics, pharmacogenetics, and pharmacoproteomics. Biomarkers are becoming more critical in the process of discovering and developing new drugs and in determining additional uses for established drugs. Biomarkers are ushering in the age of personalized medicine. One example is the use of cancer biomarkers in development of personalized therapy of cancer [16].

Identification and characterization of a large number of genetic polymorphisms (biomarkers) in drug metabolizing enzymes and drug transporters in an ethnically diverse group of individuals may provide substantial knowledge about the mechanisms of interindividual differences in drug response. Pharmacogenetics is used in preclinical investigations for biomarkers of drug-response or drug-induced toxicity, identification of genes with variants that may define patient populations, identification of proteins as potential biomarkers, or the comparison of the response in human and clinical animal models. Application of pharmacogenetic biomarkers should be able to predict adverse reactions in clinical trials.

Pharmacogenomics can now be redefined as the study and application of DNA-, and RNA-based biomarkers to predict how an individual's genetic inheritance affects the body's response to a drug. Pharmacogenomic biomarkers should be able to predict drug efficacy in clinical trials. Discovery and

validation of pharmacogenomics biomarkers can lead to the development of pharmacogenomic tests that can be used to personalize therapy.

12. Application of Biochip Technology in Developing Personalized Medicine

Biochip is a broad term indicating the use of microchip technology in molecular biology and can be defined as arrays of selected biomolecules immobilized on a surface. DNA microarray is a rapid method of sequencing and analyzing genes. An array is an orderly arrangement of samples. The sample spot sizes in microarray are usually less than 200 μm in diameter. It is comprised of DNA probes formatted on a microscale (biochips) plus the instruments needed to handle samples (automated robotics), read the reporter molecules (scanners), and analyze the data (bioinformatic tools).

Microarrays allow a look at very subtle changes in many genes at one time. They provide a snapshot of what genes are expressed or active, in normal and diseased cells. When normal cells or tissues are compared to those known to be diseased, patterns of gene expression can emerge, enabling assessment of the severity of the disease and identification of genes that can be targeted for therapy. This is how microarrays can potentially be used to develop personalized medical treatments. Microarray technology not only helps to make sense of the vast amount of genomic information but also enables its application to the patient by early detection of disease and prediction of drugs response in individuals. Although some problems of standardization and integration with electronic records remain, microarrays are promising for efficient, cost-effective, and personalized approaches to human health care. Numerous biochip technologies are available for clinical applications. The best known are the GeneChip and the AmpliChip CYP450.

12.1. GENECHIP

Affymetrix has developed an approach in which sequence information is used directly to design high-density, two-dimensional arrays of synthetic oligonucleotides. The GeneChip (Affymetrix) probe arrays are made using spatially patterned, light-directed combinatorial chemical synthesis and contain up to hundreds of thousands of different oligonucleotides on a small glass surface. The arrays have been designed and used for quantitative and highly parallel measurements of gene expression, to discover polymorphic loci and to detect the presence of thousands of alternative alleles. The HG-U133 Plus 2.0 Array, gives the protein-coding content of the human genome on a single piece the size of a fingernail.

12.2. AMPLICHIP CYP450

AmpliChip CYP450 (Roche Molecular Diagnostics) is used with the GeneChip 3000Dx microarray system of Affymetrix. It was cleared by the regulatory authorities for marketing in the USA and the EU as an *in vitro* laboratory diagnostic test in December 2004. The microarray chip (also referred to as “probe microarray”) contains millions of tiny DNA molecules and the test is performed using DNA that is extracted from a patient’s blood. DNA sequence is determined based on the sequence of the probe molecule to which the DNA is most similar. AmpliChip CYP450 contains more than 15,000 different oligonucleotide probes to analyze both the sense and the antisense strands of an amplified target DNA sample [17].

AmpliChip CYP450 provides comprehensive coverage of gene variations, which play a role in the metabolism of approximately 25% of all prescription drugs. The chip has high built-in sensitivity for analyzing 29 polymorphisms and mutations for the 2D6 gene and two polymorphisms for the 2C19 gene, thereby increasing the probability of more accurately determining the genotype and phenotype. Accurately genotypes over 99% of the world’s population. AmpliChip CYP450 test is intended to be an aid for physicians in individualizing treatment doses for patients on therapeutics metabolized through these genes.

12.3. PROTEIN BIOCHIPS

ProteinChip (Vermillion Inc.) has a role in proteomics comparable to that of GeneChip in genomics. It is based on SELDI (surface-enhanced laser desorption/ionization) process, which has four parts as applied to patient samples:

1. Patient sample of proteins is processed on the ProteinChip Array.
2. Enhance the “signal-to-noise” ratio by reducing chemical and biomolecular “noise” (i.e., achieve selective retention of target on the chip by washing away undesired materials).
3. Read one or more of the target protein(s) retained by a rapid, sensitive, laser-induced process (SELDI) that provides direct information about the target (molecular weight).
4. Process (characterize) the target protein(s) at any one or more locations within the addressable array directly *in situ* by engaging in one or more on-chip binding or modification reactions to characterize protein structure and function. Software produces map of proteins, revealing expression of marker protein with color change in the patient sample as compared to the control sample.

ProteinChip system was the first complete tool for disease-focused protein biology. The ProteinChip system uses small arrays or plates with chemically or biologically treated surfaces to interact with proteins. Unknown proteins are affinity captured on treated surfaces, desorbed and ionized by laser excitation, and detected according to molecular weight. Known proteins are analyzed using on-chip functional assays. For example, chip surfaces can contain enzymes, receptor proteins, or antibodies, enabling on-chip protein-protein interaction studies, ligand binding studies, or immunoassays. With state-of-the-art ion optic and laser optic technologies, the ProteinChip System detects proteins ranging from small peptides of less than 1000 Da up to proteins of 300 kDa or more and calculates the mass based on time-of-flight. The system includes ProteinChip arrays and reagents consumed in the process, the chip reader, software to analyze results, and proprietary database to enable comparison between phenomic and genomic data. New ProteinChip arrays have been packaged into a series of application-specific kits to enhance ease-of-use for the biologist performing protein analysis. Vermillion's new ProteinChip Biomarker System enables clinical researchers to rapidly discover, characterize, and validate predictive protein biomarkers and biomarker patterns in their own laboratories.

Protein biochip technology has been used for cytokine profiling to predict responsiveness to etanercept in rheumatoid arthritis [18]. This fulfills a need for predicting a clinical response to a tumor necrosis factor-alpha (TNF- α) blocking agent. Another example of application is the Evidence Cardiac Panel, an automated protein biochip microarray system used for biomarker-based diagnosis of acute myocardial infarction, which enables the simultaneous determination of creatine kinase MB, myoglobin, glycogen phosphorylase BB, heart-type fatty acid-binding protein, carbonic anhydrase III, and cardiac troponin I [19].

13. Role of Nanobiotechnology-Based Diagnostics in Personalized Medicine

Use of nanobiotechnology in molecular diagnostics and can be termed "nanodiagnostics." Nanotechnology is defined as the creation and utilization of materials, devices, and systems through the control of matter on the nanometer (one billionth of a meter)-length scale. Various nanotechnologies and their applications in life sciences are described in detail elsewhere under the term "nanobiotechnology" [20]. Numerous nanodevices and nanosystems for sequencing single molecules of DNA are feasible. Because of the small dimension, some of the applications of nanobiotechnology in molecular diagnostics fall under the broad category of biochips/microarrays but

are more correctly termed nanochips and nanoarrays. Advances in nano-diagnostics will facilitate the development of personalized medicine by:

- Improving the sensitivity and extending the present limits of molecular diagnostics.
- Discovery of biomarkers by nanodiagnostics will facilitate clinical trials of personalized medicines and monitoring of effects of therapy.
- Integration of diagnosis and therapy, for example, quantum dots (QDs) for localization of cancer plus delivery of therapeutics.

13.1. NANOSCALE SINGLE CELL OR MOLECULE IDENTIFICATION

Nanotechnology has facilitated the development of methods for detection of single cell or a few molecules. Some examples are as follows:

- Nanolaser scanning confocal spectroscopy, with capability of single cell resolution, can be used to identify a previously unknown property of certain cancer cells that distinguishes them from closely related normal cells [21].
- Nanoproteomics: detection of a single molecule of protein.
- Nanoarray devices for detection of a single molecule of DNA.
- Biobarcode assays [22].
- Carbon nanotube transistors for genetic screening.
- Nanopore technology [23].

13.2. NANOPARTICLES FOR MOLECULAR DIAGNOSTICS

13.2.1. *Gold Nanoparticles for Diagnostics*

Small pieces of DNA can be attached to gold particles no larger than 13 nm in diameter. The gold nanoparticles assemble on to a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used, the technique can detect millions of different DNA sequences simultaneously.

The current nonoptimized detection limit of this method is 20 femtomolar. Gold nanoparticles are particularly good labels for sensors because a variety of analytical techniques can be used to detect them.

13.2.2. *Quantum Dots*

QDs are inorganic fluorophores that offer significant advantages over conventionally used fluorescent markers. Advantages are:

- Broad excitation spectra
- Fluorescence is stable
- Simple excitation with no need for lasers
- Simple instrumentation

- Red/infrared colors enables whole blood assays
- High sensitivity

Applications of QDs for molecular diagnostics include the following:

- Cancer
- Genotyping
- Whole blood assays
- Multiplexed diagnostics
- DNA mapping
- Immunoassays and antibody tagging
- Detection of pathogenic microorganisms

The most important potential applications of QDs are for cancer diagnosis. Luminescent and stable QD bioconjugates enable visualization of cancer cells in living animals. QDs can be combined with fluorescence microscopy to follow cells at high resolution in living animals. QDs, coated with a polyacrylate cap and covalently linked to antibodies for immunofluorescent labeling of breast cancer marker Her2

Carbohydrate-encapsulated QDs with detectable luminescent properties are useful for imaging of cancer. Finally, QDs enable integration of diagnostics with therapeutics.

Another application of QDs is for viral diagnosis. Rapid and sensitive diagnosis of respiratory syncytial virus (RSV) is important for infection control and efforts to develop antiviral drugs. Current RSV detection methods are limited by sensitivity and/or time required for detection, which can take 2–6 days. This can delay effective treatment. Antibody-conjugated nanoparticles rapidly and sensitively detect RSV and estimate relative levels of surface protein expression [24]. A major development is use of dual-color QDs or fluorescence energy transfer nanobeads that can be simultaneously excited with a single light source.

A QD system can detect the presence of particles of the RSV in a matter of hours. It is also more sensitive, allowing it to detect the virus earlier in the course of an infection [25]. When an RSV virus infects lung cells, it leaves part of its coat containing F and G proteins on the cell's surface. QDs have been linked to antibodies keyed to structures unique to RSV's coat. As a result, when QDs come in contact with either viral particles or infected cells they stick to their surface. In addition, colocalization of these viral proteins was shown using confocal microscopy.

13.2.3. *Nanobiotechnology for Molecular Imaging in Living Subjects*

Nanobiotechnology has been used in various biomedical imaging modalities, that is, optical imaging, computed tomography, ultrasound, MRI, single-photon-emission computed tomography, and positron emission

tomography. Targeting ligands, imaging labels, drugs, and therapeutic agents can all be integrated into nanoparticles to enable targeted molecular imaging and molecular therapy [26].

Iron nanoparticles, 15–20 nm in size, having saturation magnetization, have been synthesized, embedded in copolymer beads of styrene and glycidyl methacrylate (GMA), which were coated with poly-GMA by seed polymerization [27]. The resultant Fe/St-GMA/GMA beads had diameters of 100–200 nm. By coating with poly-GMA, the zeta potential of the beads changed from -93.7 to -54.8 mV, as measured by an electrophoresis method. This facilitates nonspecific protein adsorption suppression, as revealed by gel electrophoresis method, which is a requisite for nanoparticles to be applied to carriers for bioscreening.

Nanoparticles are used as labeling molecules for bioscreening. Superparamagnetic nanoparticles are useful for cell tracking cells and for calcium sensing. Ferrofluids (Immunicon's CellTracksTM Technology) consists of a magnetic core surrounded by a polymeric layer coated with antibodies for capturing cells. A family of calcium indicators for MRI is formed by combining a powerful superparamagnetic iron oxide nanoparticle-based contrast mechanism with the versatile calcium-sensing protein calmodulin and its targets [28].

Superparamagnetic nanoparticles measuring 2–3 nm have been used in conjunction with MRI to reveal small and otherwise undetectable lymph-node metastases. Ultrasmall superparamagnetic iron oxide enhances MRI for imaging cerebral ischemic lesions. A dextran-coated iron oxide nanoparticle enhances intracranial tumors by MRI for more than 24 h.

14. Role of Cytogenetics in Personalized Medicine

The term “cytogenetics” has been classically used for studies of the cellular aspects of heredity. It has been used mainly to describe the chromosome structure and identify abnormalities related to disease. Besides clinical diagnostics, cytogenetics has been used for basic genomic research as well. Localizing specific gene probes by fluorescent *in situ* hybridization (FISH) combined with conventional fluorescence microscopy has reached its limit. The term “cytogenetics” should be replaced by “cytomics,” which means that the structural and functional information is obtained by molecular cell phenotype analysis of tissues, organs, and organisms at the single cell level by image or flow cytometry in combination with bioinformatic knowledge extraction concerning nuclei acids, proteins, and metabolites (cellular genomics, proteomics, and metabolomics) as well as cell function parameters like intracellular pH, transmembrane potentials, or ion gradients. Cytogenetics is

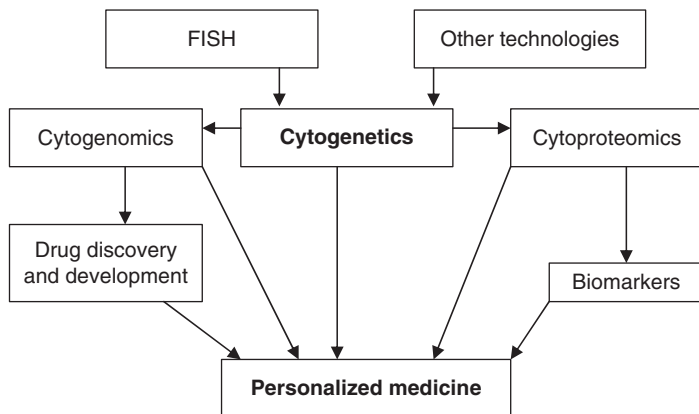


FIG. 2. Relation of cytogenetics to personalized medicine.

related to other technologies in the same way as genetics with the difference that everything is at cell level. Relation of cytogenetics to personalized medicine is shown in [Fig. 2](#).

The broader scope of biology at cell level can be covered by terms such as cytogenomics, cytomatabolomics, and cytoproteomics. Because of its important role in diagnosing disease at molecular level, cytogenetics is an important part of molecular diagnostics and can be referred to as molecular cytogenetics. Cytogenetics has been refined with use of nanobiotechnology.

14.1. CYTOMICS AS A BASIS FOR PERSONALIZED MEDICINE

Cytomics enables differential molecular cell phenotyping between diseased and healthy cells. Molecular pathways can be explored in this way including the detection of suitable target molecules, without detailed *a priori* knowledge of specific disease mechanisms. This is useful during the analysis of complex diseases such as infections, allergies, rheumatoid diseases, diabetes, or malignancies. The top-down approach reaching from single cell heterogeneity in cell systems and tissues down to the molecular level seems suitable for a human cytome project to systematically explore the molecular biocomplexity of human organisms. The analysis of already existing data from scientific studies or routine diagnostic procedures will be of immediate value in clinical medicine, for example, as personalized therapy by cytomics [29].

At the single-cell level in conjunction with data-pattern analysis, high-content screening by image analysis or flow cytometry of clinical cell- or tissue-section samples provides differential molecular profiles for the

personalized prediction of therapy-dependent disease progression in patients. The molecular reverse-engineering of these molecular profiles, which is the exploration of molecular pathways, backwards, to the origin of the observed molecular differentials, by systems biology has the potential to detect new drug targets in knowledge spaces, typically inaccessible to traditional hypotheses [30]. Furthermore, predictive medicine, by cytomics in stratified patient groups, opens a new way not only for personalized medicine but also for the early detection of adverse drug reactions in patients.

14.2. STUDY OF CHROMOSOMES BY ATOMIC FORCE MICROSCOPY

Both AFM and scanning near-field optical microscopy have been used to obtain local information from G-bands and chromosomal probes. The final resolution allows a more precise localization compared to standard techniques, and the extraction of very small amounts of chromosomal DNA by the scanning probe is possible. This method is also focused on the combination of biochemical and nanomanipulation techniques, which enable both nanodissection and nanoextraction of chromosomal DNA.

14.3. QD FISH

The photostability and narrow emission spectra of nonorganic QD fluorophores make them desirable candidates for FISH to study the expression of specific mRNA transcripts. A method for direct QD labeling of modified oligonucleotide probes using streptavidin and biotin interactions increased sensitivity of multiple-label FISH [31]. This technique also gives excellent histological results for FISH combined with immunohistochemistry. QD's broad absorption spectra allowed different colored probes specific for distinct subnuclear genetic sequences to be simultaneously excited with a single excitation wavelength and imaged free of chromatic aberrations in a single exposure.

A rapid method for the direct multicolor imaging of multiple subnuclear genetic sequences utilizes novel QD-based FISH. A Texas red dye gamma-satellite probe produces fluorescent foci at the periphery of interphase nucleus and labels every centromere in metaphase chromosomes [32].

15. Integration of Molecular Diagnostics and Therapeutics

Integration of molecular diagnostics and therapeutics is an important part of personalized medicine. In a combined system for diagnosis and therapeutics, the term diagnosis will broadly include screening for identification of risk

factors whereas therapeutics would also include monitoring of therapy. A key factor that will drive the integration of diagnostics and therapeutics is the availability of improved and more precise diagnostic methods, which are easy to perform and are not expensive. As discovery of disease genes progresses, the genes may form the link between diagnosis and gene-based medicines.

Although the companion tests for therapeutic products themselves will be technically simple and most likely test for SNP variants, issues surrounding their development, regulatory approval, marketing and reimbursement remain to be established. Therapy based on diagnosis is applicable to early, acute or chronic stages of a disease. The patient may be treated by a medication determined to be safe and effective on the basis of molecular diagnostics. Not only would the cause of the illness be better defined by the molecular diagnosis, the most effective specific medication for a disease in a particular patient can be selected. Appropriate diagnostic tests can facilitate the frequent monitoring of the effects of therapy to verify the success by objective measurements and to detect the failure of therapy as early as possible so that appropriate changes in treatment can be instituted. Molecular diagnostic methods are an important part of monitoring of therapy. Some companion diagnostics for drugs are already in the market.

16. Concluding Remarks and Future Prospects

Gene-based molecular diagnostics is changing the practice of medicine and will continue to do so for the foreseeable future [33]. Pharmacogenomics and pharmacogenetics are providing the basis for the development of molecular diagnostics to improve drug selection, identify optimal dosing, maximize drug efficacy, or minimize the risk of toxicity. Rapid advances in basic research have identified many opportunities for the development of personalized treatments for individuals and/or subsets of patients defined by genetic and/or genomic tests. However, the integration of these tests into routine clinical practice remains a major multidisciplinary challenge. A rapid, low-cost access to each person's genomic information is the key to enabling molecular diagnostics and, ultimately, personalized medicine.

Genomics-based molecular profiling and related technologies may impact on the delivery of healthcare even before genomics-based drugs enter the market. Identification of genetic factors affecting the prognosis of disease is likely to be of most clinical relevance. Relationships of known genes, such as BRCA1 and BRCA2, with risk factors will be clarified; permitting evidence based preventive action in people at high genetic risk and better quantification of risk in family members. Greatest progress will be made in understanding the genetic contribution to the intermediate phenotypes linking genes and disease,

and thus the biology of the disorder, as in atherosclerotic disease. The greatest impact of personalized medicine will be in the treatment of cancer, infections, cardiovascular diseases, and neurological disorders. Molecular diagnosis of cancer including genetic profiling would be widely used for personalized treatment of cancer by the year 2015.

Detection of biomarkers would be greatly improved and facilitate development of diagnostics combined with therapeutics. However, further work is needed to fully optimize some diagnostic technologies for clinical laboratory setting as well as for POC applications.

The emerging fields of metabonomics (metabolite profiling to identify genotype-phenotype associations) and phenomics might offer solutions to anticipating and decreasing risk for adverse drug reactions in each individual patient but tests based on these approaches are not expected to become generally available to the practicing clinician for at least the next 5 years

Within the next decade, measurement devices based on nanotechnology, which can make thousands of measurements very rapidly and very inexpensively, will become available. Future trends in diagnostics will continue in miniaturization of biochip technology to nano range. The most common clinical diagnostic application will be blood protein analysis. Blood in systemic circulation reflects the state of health or disease of most organs. Therefore, detection of blood molecular fingerprints, will provide a sensitive assessment of health and disease.

REFERENCES

- [1] K.K. Jain, Personalised medicine, *Curr. Opin. Mol. Ther.* 4 (2002) 548–558.
- [2] K.K. Jain, *Textbook of Personalized Medicine*, Springer, New York, 2009.
- [3] K.K. Jain, From molecular diagnostics to personalized medicine, *Expert Rev. Mol. Diagn.* 2 (2002) 89–91.
- [4] K.K. Jain, Role of pharmacoproteomics in the development of personalized medicine, *Pharmacogenomics* 5 (2004) 239–242.
- [5] Y. Dong, H. Zhu, Single-strand conformational polymorphism analysis: basic principles and routine practice, *Methods Mol. Med.* 108 (2005) 149–157.
- [6] M. Kubista, J.M. Andrade, M. Bengtsson, et al., The real-time polymerase chain reaction, *Mol. Aspects Med.* 27 (2006) 95–125.
- [7] G.M. Hogg, J.P. McKenna, G. Ong, Rapid detection of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* directly from positive BacT/Alert((R)) blood culture bottles using real-time polymerase chain reaction: evaluation and comparison of 4 DNA extraction methods, *Diagn. Microbiol. Infect. Dis.* 61 (2008) 446–452.
- [8] M. Skrzypski, Quantitative reverse transcriptase real-time polymerase chain reaction (qRT-PCR) in translational oncology: lung cancer perspective, *Lung Cancer* 59 (2008) 147–154.
- [9] J. Pullat, A. Metspalu, Arrayed primer extension reaction for genotyping on oligonucleotide microarray, *Methods Mol. Biol.* 444 (2008) 161–167.
- [10] S. Kim, A. Misra, SNP Genotyping: technologies and biomedical applications, *Annu. Rev. Biomed. Eng.* 9 (2007) 289–320.

- [11] K.K. Jain, *Molecular Diagnostics: Technologies, Markets and Companies*, Jain Pharma-Biotech Publications, Basel, (2009) pp. 1–910.
- [12] R.E. Mills, C.T. Luttig, C.E. Larkins, et al., An initial map of insertion and deletion (INDEL) variation in the human genome, *Genome Res.* 16 (2006) 1182–1190.
- [13] R. Redon, S. Ishikawa, K.R. Fitch, et al., Global variation in copy number in the human genome, *Nature* 444 (2006) 444–454.
- [14] J.O. Korbel, A.E. Urban, J.P. Affourtit, et al., Paired-end mapping reveals extensive structural variation in the human genome, *Science* 318 (2007) 420–426.
- [15] J.M. Kidd, G.M. Cooper, W.F. Donahue, et al., Mapping and sequencing of structural variation from eight human genomes, *Nature* 453 (2008) 56–64.
- [16] K.K. Jain, Cancer biomarkers: current issues and future directions, *Curr. Opin. Mol. Ther.* 9 (2007) 563–571.
- [17] K.K. Jain, Applications of AmpliChip CYP450, *Mol. Diagn.* 9 (2005) 119–127.
- [18] S. Fabre, A.M. Dupuy, N. Dossat, et al., Protein biochip array technology for cytokine profiling predicts etanercept responsiveness in rheumatoid arthritis, *Clin. Exp. Immunol.* 153 (2008) 188–195.
- [19] M.M. Mion, E. Novello, S. Altinier, et al., Analytical and clinical performance of a fully automated cardiac multi-markers strategy based on protein biochip microarray technology, *Clin. Biochem.* 40 (2007) 1245–1251.
- [20] K.K. Jain, Applications of nanobiotechnology in clinical diagnostics, *Clin. Chem.* 53 (2007) 2002–2009.
- [21] P.L. Gourlay, J.K. Hendricks, A.E. McDonald, et al., Mitochondrial correlation microscopy and nanolaser spectroscopy—new tools for biphotonic detection of cancer in single cells, *TCRT* 4 (2005) 585–592.
- [22] Y.P. Bao, T.F. Wei, P.A. Lefebvre, et al., Detection of protein analytes via nanoparticle-based bio bar code technology, *Anal. Chem.* 78 (2006) 2055–2059.
- [23] M. Rhee, M.A. Burns, Nanopore sequencing technology: research trends and applications, *Trends Biotechnol.* 24 (2006) 580–586.
- [24] A. Agrawal, R.A. Tripp, L.J. Anderson, S. Nie, Real-time detection of virus particles and viral protein expression with two-color nanoparticle probes, *J. Virol.* 79 (2005) 8625–8628.
- [25] E.L. Bentzen, F. House, T.J. Uteley, et al., Progression of respiratory syncytial virus infection monitored by fluorescent quantum dot probes, *Nano Lett.* 5 (2005) 591–595.
- [26] W. Cai, X. Chen, Nanoplatforams for targeted molecular imaging in living subjects, *Small* 3 (2007) 1840–1854.
- [27] M. Maeda, C.S. Kuroda, T. Shimura, et al., Magnetic carriers of iron nanoparticles coated with a functional polymer for high throughput bioscreening, *J Appl. Phys.* 99 (2006) 08H103.
- [28] T. Atanasijevic, M. Shusteff, P. Fam, A. Jasanoff, Calcium-sensitive MRI contrast agents based on superparamagnetic iron oxide nanoparticles and calmodulin, *PNAS* 103 (2006) 14707–14712.
- [29] G. Valet, Cytomics, the human cytoome project and systems biology: top-down resolution of the molecular biocomplexity of organisms by single cell analysis, *Cell Prolif.* 38 (2005) 171–174.
- [30] G. Valet, Cytomics as a new potential for drug discovery, *Drug Discov. Today* 11 (2006) 785–791.
- [31] P. Chan, T. Yuen, F. Ruf, et al., Method for multiplex cellular detection of mRNAs using quantum dot fluorescent in situ hybridization, *Nucleic Acids Res.* 33 (2005) e161.
- [32] L.A. Bentolila, S. Weiss, Single-step multicolor fluorescence in situ hybridization using semiconductor quantum dot-DNA conjugates, *Cell Biochem. Biophys.* 45 (2006) 59–70.
- [33] J.E. Finan, R.Y. Zhao, From molecular diagnostics to personalized testing, *Pharmacogenomics* 8 (2007) 85–99.