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Simple Molecular Diagnostic Tools in Clinical Medicine

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Abstract

Background: Advances in genetic/genomic studies have been a robust driving force in the field of clinical medicine, particularly in the diagnostic field. Although ambitious efforts to elucidate all aspects of the genome have shown remarkable progress, there are numerous practical unmet needs in the clinic. To facilitate the use of genetic/genomic research outcomes for patient treatment, simple, inexpensive, and convenient tools are essential. However, accurate and precise outcomes cannot be compromised to achieve convenience, and the performance of the simple molecular diagnostic tools should fulfil all basic requirements.

Objective of the review: In this review, current trends in molecular diagnosis are described, and future perspectives are suggested.

Discussion and conclusions: In the future, an optimal diagnosis system that can analyze the body status with just with a single drop of blood is expected to be developed. However, a simple, inexpensive, and portable molecular diagnostic tool is currently required. It is not easy to predict which type of diagnostic tools will become dominant, but POCT with molecular diagnostic functions is a strong candidate.

Keywords: Molecular diagnosis; Medical genomics; Point of care test; Translational medicine

Abbreviations: FISH: Fluorescence *in Situ* Hybridization; PCR: Polymerase Chain Reaction; *MALDI-TOF:* Microarray-based Assay; Matrix-assisted Laser Desorption/Ionization-time of Flight; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; POCT: Point of Care Testing; LOC: Lab on a Chip.

Introduction

Searching for papers using the Medline search tool for the keywords 'genetics', 'genomic', and 'research' generates a list of more than 693, 558 papers. If 'diagnosis' is included rather than 'research', more than 228, 953 papers will be found. The importance of diagnosis in studies of genetics/genomics can be easily estimated based on these large numbers of papers. Advances in genetic/genomic studies have been a robust driving power in the field of clinical diagnosis [1]. Although ambitious efforts such as next-generation sequencing to understand the human genome made great progress, there are still strong practical needs such as simple diagnostic tools that can be used for clinical purposes. To achieve simple, inexpensive, and convenient but accurate diagnosis outcomes, the performance of molecular diagnostic methods should fulfill these basic requirements [2]. Before describing the simple molecular diagnosis tools, I will briefly summarize the trends in genetics/genomics in medicine.

Development of Technologies

Over the last two decades, developments in the science of genomics and enormous advances in genetic/genomic technologies have altered the understanding of the molecular features of diseases and improved disease diagnoses. Genetic/genomic technologies encompass a wide range of laboratory technologies that provide nucleic sequences, functions, and other information of genomes. With advances in these technologies, medical applications have accelerated and become less costly. The major technologies used in gene-related molecular diagnosis are the direct detection of nucleic acids such as fluorescence *in situ* hybridization (FISH), nucleic acid amplification such as polymerase chain reaction (PCR), microarray-based assays, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry of DNA, and nucleotide sequencing. New technologies and clinical knowledge have enabled significant progress in the diagnosis and management of diseases. Incorporation of genomics into medical practice revolutionizes this field.

Fish

FISH is a cytogenetic technique that has been used since the early 1980's. This technique detects and localizes specific nucleic acid sequences using fluorescent probes that bind specifically to a sequence of interest with a high degree of sequence complementarity, which can be detected under a fluorescence microscope. FISH is used to detect specific features in DNA and RNA targets in amniotic fluid cells, circulating tumor cells, and tissue samples. In medicine, FISH can be used for diagnosis, prognosis evaluation, or cancer remission examination. Although FISH has been used as a manual cytogenetic

method, automation by computer-assisted counting is commonly used and can be incorporated in the lab on a chip microfluidic device in the future.

PCR

PCR is a biochemical technology used to amplify a section of DNA by several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. PCR is commonly used in clinics for cancer diagnosis. PCR also allows for rapid and highly specific diagnosis of infectious diseases. The basis for PCR diagnostic applications in microbiology is in the detection of infectious agents and discrimination of non-pathogenic from pathogenic strains by detecting the presence of specific genes. Early detection of infectious pathogens gives physicians significant lead-time in treatment. PCR-based DNA quantitation techniques are also used to measure viral load.

There are numerous variations of PCR techniques that are used for dedicated purposes [3]. For example, allele-specific PCR is a diagnostic technique based on single-nucleotide variations. PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between a template and primer, and thus successful amplification with a single-nucleotide polymorphism (SNP)-specific primer indicates the presence of the specific SNP in a sequence.

Microarray

A microarray is a multiplex 2-dimensional assay fabricated on a glass or silicon substrate, which detects large amounts of biological material and is used as a high-throughput screening system. With the establishment of gene chip companies, such as Affymetrix, Agilent, Applied Microarrays, Array it, and Illumina, DNA microarray technology has become very sophisticated and widely used in medical applications such as gene expression profiling, SNP detection, and copy number analysis, among others [4].

Mass spectrometry of DNA

Analysis of DNA by spectrometry is achieved by performing the following processes: crystallization of the PCR product, ionization of crystal by laser, and ion detection based on product mass. As smaller molecules travel faster than larger molecules, time-of-flight measures the difference in time when different molecules hit the detector and the software calculates the mass of the fragments. MALDI-TOF can resolve mass differences of 16 Da. MALDI-TOF mass spectrometry of DNA is used to measure the amount of genetic target material and/or variations and is suitable for a variety of applications including somatic mutation profiling, genotyping, methylation analysis, molecular typing, and quantitative gene expression [5].

Sequencing

Nucleotide sequencing is the process of determining the precise order of the nucleotides, adenine, guanine, cytosine, and thymine, within a genome. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. The high demand for low-cost sequencing has driven the development of high-throughput sequencing or next-generation sequencing technologies that parallelize the sequencing process, producing thousands or millions of sequences concurrently. The use of genomic technologies enables patient diagnosis and treatment based on information regarding an individual's entire DNA sequence. This method will become part of mainstream healthcare practice in the near future. However, knowledge and experience is slowly gained by clinical research leaders, and the process of embedding this new practice in high-quality healthcare has been gradual and difficult. In addition, a lack of reliable and mature technologies for clinicians prevents next-generation sequencing from becoming a popular medical diagnostic tool.

Application of Genomics in Medicine

By translating basic genetic/genomic knowledge and insights into the development of new healthcare interventions for drug development, disease prevention strategies, and diagnostic methods, laboratory studies have contributed to the progression of clinical medicine. Molecular diagnosis is applied in clinics to refine diagnoses and understanding diseases from the perspective of molecular signatures, individualizing clinical care based on the molecular differences, predicting drug effects, tracking epidemics of infectious diseases, and developing new optimized therapies. Major areas in which molecular diagnosis are used include prenatal genetic testing, detection of infectious pathogens, prediction of disease risk, mutation studies in diseases such as cancer, and pharmacogenomics in drug development, among others [3].

In some cases, genetic/genomic discoveries have revolutionized disease definition and have been used to establish patient care strategies. One area of particular interest is the development of new drugs that can be prescribed according to the patient's genetic makeup, such as somatic mutations. It is also possible to identify subjects who carry gene variants associated with a greater risk of some cancers so that targeted surveillance strategies can be developed for the individual patient and their close relatives. Some clinicians still believe that genomic medicine will only be applied in the future. However, increasing numbers of targeted or individualized drugs are reaching the market and the numbers of registered Phase III trials enlisted in www.clinicaltrials.gov site suggest that the trend of genomic medicine will not only continue, but also be accelerated.

Many obstacles remain to be overcome. First, there are no sufficiently reliable tools for genomic medicine. Even next-generation sequencing requires an upgrade in the technical skills of users. Additionally, not all genes have been examined in detail. In addition to genes, the discovery of non-coding DNAs and new types of RNAs have placed a greater burden on researchers to elucidate genome functions. Large-scale data analyses are not complete and must be further developed.

Simple Molecular Diagnostic Tools

At present, the most popular molecular diagnosis tools are PCR-based assays. PCR assays are used to measure gene expression, detect mutations, and detect infectious pathogens in clinical samples. As the cost of sequencing is rapidly decreasing, the use of sequencing technologies has become one of the hottest topics in molecular diagnosis. However, how to translate the results of this method into patient care requires further analysis. As PCR and sequencing technologies require expensive instruments and well-trained personnel to operate the devices, it is currently not easy for bedside clinicians to implement these methods. Therefore, there remains a strong need for simple and inexpensive molecular diagnostic tools in the clinic.

Point of care testing

Point of care testing (POCT) involves medical testing at the site of patient care or simple medical diagnosis tools that can be used at the bedside. POCT includes blood glucose test, blood gas and electrolytes analysis, rapid coagulation testing, rapid cardiac markers diagnostics, drugs of abuse screening, urinary strip testing, pregnancy testing, fecal occult blood analysis, food pathogens screening, hemoglobin diagnostics, infectious disease testing, and cholesterol screening, among others. Most of these tests are based on enzymatic reactions or immune reactions; currently, very few genetic POCT tools are available.

The advantages of POCT methods are their convenience, quick response, and low cost. Rapid and immediate responses allow patients, physicians, and care teams to make immediate clinical management decisions. Recently, POCT devices have become connected to hospital electronic medical record systems, and the results can be shared instantaneously with all members of the medical team through the software interface to enhance communication by decreasing turnaround time [6,7].

Lab on a chip

Lab on a chip (LOC) and microfluidic chips have been intensively studied because of their potential role as a POCT method for molecular diagnosis [8]. This type of device integrates one or several laboratory functions on a single small chip [9]. LOCs require extremely small fluid volumes at approximately the picoliter level. The advantages of LOC are (1) low fluid volume consumption and lower reagent cost, (2) faster analysis and response times, (3) better reaction control, (4) smaller system size, (5) medium- to high-throughput analysis, (6) lower fabrication costs, and (7) safer and simple platform. The disadvantages of LOC are (1) they are currently in a technically immature stage, (2) require motor power to drive solution through high-resistance channels, (3) have low signal to noise ratios, (4) lack accuracy and precision, and (5) are relatively costly. In the near future, when LOC methods overcome these weaknesses, they will become widely used in the clinic.

Conclusion

In the future, an optimal diagnosis system that can analyze the body status with just with a single drop of blood is expected to be developed. However, a simple, inexpensive, and portable molecular diagnostic tool is currently required. It is not easy to predict which type of diagnostic tools will become dominant, but POCT with molecular diagnostic functions is a strong candidate. Currently, most POCT devices have stand-alone type features. Thus, they require additional supporting systems for sample preparation, clinical data integration, and automatic processing [10]. However, in the future, all of these systems will be integrated into a single system to facilitate users' convenience.

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