Significance of molecular diagnostics in medicine

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Abstract

The development of molecular biology has a significant role in the progress, improvement and precise diagnosis of many diseases in medicine. With the development of molecular biology, personal medicine is also developing. Thanks to molecular analyses, we have early detection of hereditary diseases, predisposition to them, their precise diagnosis, precise molecular and genetic detection, evaluation and precise determination of the best therapeutic effectiveness of which drug in the treatment of the diagnosed disease.Numerous infectious agents that can be cultured by classical microbiological analyzes can be early diagnosed by molecular analyses.

The only drawback of molecular analyzes is that they require expensively equipped laboratories, educated staff, and do not accurately provide the stage of the disease in which the examined patient is. Follow-up of patients is necessary, often by repeating the same analyses, which are expensive, in order to precisely determine the exact stage of the disease and predict its prognosis, when it comes to infectious agents.

Nevertheless, molecular diagnostics has a significant role in the prevention of carcinoma, allergic reactions, food intolerances, precise and accurate selection of drugs in the therapy of rare diseases and diagnostics of uncultivated microbiological agents.

Key words: molecular biology, molecular diagnostics, genome, microbiological agents

1. WHAT IS MOLECULAR BIOLOGY

Molecular biology is a branch of biology that studies the basic phenomena of life (metabolism, heredity, irritability) at the level of molecules that make up the cell. The field of molecular biology overlaps with the fields of interest of biology and chemistry, especially genetics and biochemistry (1).

Genome is a term used to denote the complete set of hereditary information in an organism. In those organisms whose bodies are made of cells, the hereditary information exists as DNA. Because there are two major groups of organisms - prokaryotes and eukaryotes - that differ in cell type, the organization of genomes is very different in these two groups (1,2).

Proteome - the entire set of proteins that is, or can be, expressed by a genome, cell, tissue, or organism at a given time. It is a set of expressed proteins in a given type of cell or organism, at a given time, under defined conditions. Proteomics is the study of the proteome (2).

2. WHAT IS MOLECULAR DIAGNOSTICS AND WHAT IS TESTED WITH MOLECULAR DIAGNOSTICS

Molecular diagnostics is a set of techniques used to analyze biological markers in the genome and proteome and how their cells express their genes as proteins, applying molecular biology to medical testing, used to diagnose and monitor disease, detect risk about malignant tumors and deciding which therapies will work best for each individual patient (3).

By analyzing the specifics of the patient and his disease, molecular diagnostics offers a view of personalized medicine. These tests are useful in a range of medical specialties, including infectious diseases, oncology, human leukocyte antigen typing (which investigates and predicts immune system function), coagulation, and pharmacogenomics - the genetic prediction of which drugs will work best (4).

Molecular diagnostics use in vitro biological assays such as polymerase chain reaction–enzyme-linked immunosorbent assay or fluorescent "in situ" hybridization (). The assay detects a molecule, often in low concentrations, that is a marker of disease or risk in a sample taken from a patient. Preserving the sample before analysis is critical. Manual handling should be minimized.

The fragile RNA molecule poses certain challenges. As part of the cellular process of expressing genes as proteins, it provides a measure of gene expression but is vulnerable to hydrolysis and degradation by the ever-present ribonuclease enzymes. Samples can be rapidly frozen in liquid nitrogen or incubated in preservation media (5).

The microarray chip contains complementary DNA of many interesting sequences. Complementary DNA fluoresces when hybridized to a corresponding DNA fragment in the sample (5).

Prenatal

Conventional prenatal tests for chromosomal abnormalities such as Down syndrome rely on analysis of the number and appearance of chromosomes - the karyotype. Molecular diagnostic tests, such as comparative genomic microarray hybridization, instead test a

DNA sample, and because of the cell-free DNA in plasma, could be less invasive, but as of 2013 is still an adjunct to conventional tests (6).

Treatment

Some of a patient's single nucleotide polymorphisms - small differences in their DNA - can help predict how quickly they will metabolize certain drugs; this is called pharmacogenomics (7). For example, the CYP2C19 enzyme metabolizes several drugs, such as the anticoagulant Clopidogrel, into their active forms.

Some patients possess polymorphisms at specific sites of the 2C19 gene that make them poor metabolizers of those drugs; doctors can test for these polymorphisms and find out if drugs will be fully effective for that patient (8).

Advances in molecular biology have helped show that some syndromes previously classified as a single disease are actually multiple subtypes with completely different causes and treatments. Molecular diagnostics can help diagnose subspecies - for example infections and cancers - or genetic analysis of a disease with an inherited component, such as Silver-Russell syndrome (8,9).

An infectious disease

Molecular diagnostics have been used to identify infectious diseases such as chlamydia, influenza virus and tuberculosis or specific strains such as the H1N1 virus or SARS-CoV-2 (10,11). Genetic identification can be fast; for example, a loop-mediated isothermal amplification test diagnoses the malaria parasite and is crude enough for developing countries (12).

But despite these advances in genome analysis, in 2013 infections were still more often identified by other means - their proteome, bacteriophage, or chromatographic profile (13). Molecular diagnostics have also been used to understand the specific strain of the pathogen - for example by discovering which drug resistance genes it possesses - and hence which therapies to avoid (13).

In addition, analyzes based on metagenomic sequencing can be carried out with the so-called "next generation" to identify pathogenic organisms without bias (14).

Disease risk management

A patient's genome may include an inherited or random mutation that affects the likelihood of developing a disease in the future (15). For example, Lynch syndrome is a genetic disease that predisposes patients to colon and other cancers; early detection can lead to careful follow-up that improves the patient's chances of a good outcome (16).

Cardiac risk is indicated by biological markers and screening can measure the risk that a child will be born with a genetic disease such as cystic fibrosis (17). Genetic testing is ethically complex: patients may not want the stress of knowing their risk.[40] In countries without universal health care, known risk can increase insurance premiums (18).

Cancer

Cancer is a change in cellular processes that causes a tumor to grow out of control (19). Germ cells sometimes have mutations in oncogenes, such as KRAS and CTNNB1 (β-catenin). Analyzing the molecular signature of cancer stem cells - DNA and its expression

levels via messenger RNA - allows doctors to characterize cancer and choose the best therapy for their patients (19).

As of 2010, assays involving an array of antibodies against specific protein marker molecules are new technology; there are hopes for these multiple assays that could measure many markers at once (20). Other potential future biomarkers include micro RNA molecules, which cancer cells express more than healthy ones (21).

Cancer is a disease with excessive molecular causes and constant evolution. There is also disease heterogeneity even within an individual. Molecular studies of cancer have demonstrated the importance of driver mutations in tumor growth and metastasis (19). Many sequence variation detection technologies have been developed for cancer research.

These technologies can mainly be grouped into three approaches: polymerase chain reaction, hybridization, and "next-generation" sequencing (22). Currently, many polymerase chain reaction and hybridization assays are approved by the United States Food and Drug Administration as in vitro diagnostics (23). Next-generation sequencing assays, however, are still at an early stage in clinical diagnostics (24).

In order to make a molecular diagnostic test for cancer, one of the important issues is the detection of variations in the DNA sequence. Tumor biopsy samples used for diagnostics always contain only 5% of the target variant compared to the wild-type sequence. Also, for noninvasive applications from peripheral blood or urine, the DNA test must be specific enough to detect mutations at variant allele frequencies of less than 0.1% (25).

There are also microchips that use a hybridization mechanism to diagnose cancer. More than a million different probes can be synthesized on an array with Affymetrix's Genechip technology with a detection limit of one to ten copies of messenger RNA (23).

Optimized microarrays are generally considered to produce reproducible relative quantitation of different targets (22). Currently, the Food and Drug Administration has already approved a number of diagnostic assays that use microchips: Agendia's MammaPrint assays can inform the risk of breast cancer recurrence by profiling the expression of 70 genes associated with breast cancer (7).

The Autogenomics INFNITI CYP2C19 assay can profile genetic polymorphisms, whose effects on therapeutic response to antidepressants are large;[53] and Affymetrix's CytoScan Dx can assess intellectual disabilities and congenital disorders through chromosomal mutation analysis (23).

In the future, cancer diagnostic tools will likely focus on "next-generation" sequencing. By using DNA and RNA sequencing for cancer diagnosis, the technology in the field of molecular diagnostic tools will be developed better.

Although the throughput and cost of “next-generation” sequencing has decreased dramatically over the past 10 years by approximately 100-fold, we remain at least 6 orders of magnitude away from performing genome-wide deep sequencing (22).

Currently, Ion Torrent has developed some "next-generation" sequencing panels based on translational AmpliSeq, for example, the Oncomine Comprehensive Assay (26). They aim to use deep sequencing of cancer-related genes to detect rare sequence variants.

A molecular diagnostic tool can be used to assess cancer risk. For example, the BRCA1/2 test from Myriad Genetics assesses women for lifetime risk of breast cancer (19). Also, some types of cancer are not always used with clear symptoms. It is useful to screen people when they are not showing obvious symptoms so that cancer can be detected in the early stages.

For example, the ColoGuard test can be used to screen people over the age of 55 for colon cancer (19,20,21). Cancer is a long-standing disease with different stages of progression, and molecular diagnostic tools can be used to predict cancer progression. For example, the OncoType Dx test from Genomic Health can assess breast cancer risk.

Their technology can inform patients to seek chemotherapy when needed by examining RNA expression levels in breast cancer biopsy tissue (26).

3. SIGNIFICANCE OF MOLECULAR DIAGNOSTICS IN MEDICINE AND MEDICAL DIAGNOSTICS (THROUGH CONVENTIONAL MEDICINE BACTERIA, VIRUSES, FUNGI, SO ON TO GENETIC ANALYSIS, GENETIC CHANGES, IN THE ORGANISM, TO TIMELY DETECTION OF GENETIC DISEASES)

Modern bacterial classification relies on genome linkage. Genetic variation in bacterial populations poses a major challenge to taxonomic classification, and recently several bacterial species have been reclassified based on interspecies genome comparison.

These were facilitated by next-generation sequencing technologies and advances in genome comparison approaches that led to the rearrangement of different bacterial species and a revolution in the microbial classification system. One of the results of these studies is the development of suitable DNA barcodes as a reliable and economical method for the identification of different bacterial genera (17,20).

To refine this further, we applied a genome-wide comparison approach across 1104 bacterial genome assemblies (excluding plasmids) to identify unique genomic segments among intra-species genome assemblies (24).

Using extensive bioinformatics analysis, we identified species-specific genomic regions and designed unique primers for 100 different species (belonging to 62 genera) involving 62 pathogenic and 13 opportunistic pathogenic bacterial species and built a database (http://slsdb.manipal. edu/bact/).

These species-specific genomic regions will have a major impact on in silico and molecular methods aimed at bacterial classification and identification. They may also serve as better DNA barcodes than the markers currently used to distinguish bacteria, and may also find application in a variety of translational research programs (25,26).

4. ADVANTAGES IN RELATION TO CLASSIC MICROBIOLOGY

Advantages of microbiological analysis on a molecular basis have significant advantages in relation to classical microbiology. One of the significant advantages is the early detection of

infection caused by uncultivated microorganisms, whether they are bacteria, viruses or parasites. It is a type of pattern. Genetic analyzes are important for the purpose of early prevention, diagnosis, and if not complete prevention, then alleviation of the clinical picture.

Molecular analyzes provide accurate and precise information on the action of individual drugs against certain agents, on each organism separately - personalized pharmacology.

5. DEFICIENCIES REGARDING CLASSIC MICROBIOLOGY

Molecular biology, in addition to all the positive analyzes that are applicable in clinical microbiology, also has its drawbacks. With analyzes at the molecular level, we do not have a realistic picture of the amount of pathological agents (bacteria, viruses or fungi) in the examined material, and due to the lack of cultivation, we do not have antibacterial sensitivity. So we cannot get a complete picture of therapy with molecular diagnostics. However, the gold standard in clinical microbiology is classical microbiology. The importance and invaluable advantage of molecular biology is in genetics, pharmacy and in microbiology when non-cultivable or highly infectious microorganisms are suspected, the cultivation or isolation of which requires special working conditions. One of the disadvantages for molecular biology is that it requires expensive equipment, the reagents used for the analyzes are very expensive and the trained staff are strictly educated for this type of analysis.

6. BENEFITS OF MOLECULAR DIAGNOSTICS

Molecular and genomic approaches have revolutionized medical microbiology by offering faster and more accurate diagnostic techniques for infectious diseases. Traditional methods, which include culturing microbes and biochemical testing, are time-consuming and may not detect antibiotic-resistant strains. In contrast, molecular and genomic methods, including polymerase chain reaction (PCR)-based techniques and whole-genome sequencing, provide rapid and precise detection of pathogens, early-stage diseases, and antibiotic-resistant strains. These approaches have advantages such as high sensitivity and specificity, the potential for targeted therapies, and personalized medicine (3,7,22).

However, implementing molecular and genomic techniques faces challenges related to cost, equipment, expertise, and data analysis. Ethical and legal considerations regarding patient privacy and genetic data usage also arise. Nonetheless, the future of medical microbiology lies in the widespread adoption of molecular and genomic approaches, which can lead to improved patient outcomes and the identification of antibiotic-resistant strains. Continued advancements, education, and exploration of ethical implications are necessary to fully harness the potential of molecular and genomic techniques in medical microbiology.

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