NANOBIO-TECHNOLOGY IN MOLECULAR DIAGNOSTICS

Current Techniques and Applications

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horizon bioscience

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By

Prof. K.K. Jain
MD, FRACS, FFPM
Jain PharmaBiotech
Basel, Switzerland

horizon bioscience

Horizon Bioscience
32 Hewitts Lane
Wymondham
Norfolk NR18 0JA
U.K.

www.horizonbioscience.com

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AUTHOR'S BIOGRAPHY

Professor K. K. Jain is a neurologist/neurosurgeon by training and has been working in the biotechnology/biopharmaceuticals industry for several years. He received graduate training in both Europe and USA, has held academic positions in several countries and is a Fellow of the Faculty of Pharmaceutical Medicine of the Royal Colleges of UK. Currently he is a consultant at Jain PharmaBiotech. Prof. Jain is the author of over 350 publications including 11 books and 44 special reports, which have covered important areas in biotechnology, gene therapy and biopharmaceuticals. His publications include several articles on nanobiotechnology.

ABBREVIATIONS

Aβ	Amyloid beta
AFM	atomic force microscopy
ATP	adenosine triphosphate
bDNA	branched DNA
CGH	comparative genomic hybridization
ds	double stranded
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FISH	fluorescent in situ hybridization
FITC	fluorescein isothiocyanate
FRET	fluorescence energy transfer
GFP	green fluorescent protein
HCV	hepatitis C virus
HBV	hepatitis B virus
LC	liquid chromatography
MAbs	monoclonal antibodies
MRI	magnetic resonance imaging
MS	mass spectrometry
Nm	nanometer
PCR	polymerase chain reaction
QD	quantum dot
RLS	resonance light scattering
RNA	ribonucleic acid
rRNA	reporter RNA
RT	reverse transcriptase
ss	single stranded
SNP	single nucleotide polymorphism
SPR	surface plasmon resonance

PREFACE

Molecular diagnostics has been evolving rapidly during the past decade and has an impact on the practice of medicine as well as many other applications including drug discovery. Advances in biotechnology have been incorporated into molecular diagnostics. There has been a distinct trend in miniaturization with development of biochips and microfluidics. This trend has continued with the development of nanotechnology. Nanotechnologies are now being applied to molecular diagnostics to refine and extend the limits of detection. As the introductory chapter on molecular diagnostics shows, there are a large number of technologies and only a fraction of these have yet been affected by introduction of nanobiotechnology. There is a tremendous scope for further development.

This book gives an introduction of nanobiotechnology relevant to molecular diagnostics, a field that has been termed nanodiagnostics. Current state of development of nanodiagnostic technologies including nanobiochips and nanobiosensors is reviewed. Besides important applications in clinical diagnostics, the role of molecular diagnostics in drug discovery is also described.

This book was derived and expanded from the special report on nanobiotechnology by the author. The voluminous literature relevant to this topic was reviewed and 180 selected references are included in the bibliography. It will be useful for those developing nanobiotechnology, clinical laboratories, researchers in molecular diagnostics and scientists involved in drug discovery in the pharmaceutical industry. Financiers of nanotechnology have a scientific interest in the new developments and this book will be a source of useful information including development of technologies in the commercial sector.

K. K. Jain MD

Basel, Switzerland

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Abstract

Clinical application of molecular technologies to elucidate, diagnose and monitor human diseases is referred to as molecular diagnosis. Basics of molecular diagnostic technologies are described as an introduction to application of nanobiotechnologies for refining the tests. One example is molecular labels. Many types of nucleic acids require a secondary detection technology, e.g. a label, because a nucleic acid does not have intrinsic properties that are useful for direct high-sensitivity detection. Currently available labeling technologies have limited sensitivity and efficacy, which can be improved by nanoparticle technologies.

Introduction

Clinical application of molecular technologies to elucidate, diagnose and monitor human diseases is referred to as molecular diagnosis. It usually refers to the use of nucleic acid technologies that use DNA, RNA (ribonucleic acid), genes or proteins as bases for diagnostic tests. In a broader sense molecular diagnostics also includes the use of non-nucleic acid technologies such as monoclonal antibodies and enzyme-linked immunosorbent assay (ELISA). The historical evolution of molecular diagnostics relevant to nanobiotechnology is shown in Table 1-1.

Table 1-1: Historical evolution of molecular diagnostics

Year	Landmark/ Reference
1909	Phoebus Levene, an American chemist studying yeast, discovered deoxyribose – the D in DNA. In 1920, he identified the chemical bases of genome – adenine, cytosine, guanine and thymine
1920	The expression "Genom" used in German for the haploid chromosome set," which, together with the pertinent protoplasm, specifies the material foundations of the species
1944	DNA shown to carry genetic code in pneumococci (Avery 1944)
1953	Identification of the double-stranded structure of the DNA (Watson and Crick 1953)
1969	Discovery of in-situ hybridization for gene location by labeled RNA probes (Gall & Pardue 1969)

1975	Monoclonal antibody (MAb) technology (Köhler & Milstein 1975)
1980s	DNA probes: segments of DNA labeled with radioactive markers
1985	Discovery of polymerase chain reaction (Mullis et al 1986)
1986	Development of fluorescent in situ hybridization (FISH) technique (Pinkel et al 1986)
1988	DNA biosensor: electrochemical detection of DNA was carried out by the use of a fluoride ion selective electrode and stripping voltammetry (Downs et al 1988)
1990	First publication on in situ polymerase chain reaction (Haase et al 1990)
1991	Synthesis of DNA on a silicon chip – birth of the biochip (Fodor et al 1991).
1991	Wedding of molecular biology and cytogenetics and molecular cytogenetics (Lichter et al 1991)
1992	Branched DNA technology used to quantify HIV levels (Urdea et al 1993)
1992	Discovery of aptamers – single-stranded DNA molecules (Bock et al 1992)
1993	First publication on real-time PCR (Higuchi et al 1993)
1994	Potential of use of nanotechnology for biosensors (Sleytr et al 1994)
1995	Applications of proteomics (PROTEins expressed by a genOME) in molecular diagnostics
1998	Discovery of Locked Nucleic Acids (LNA), a novel class of DNA analogues, with potential applications in molecular diagnostics (Kumar et al 1998)
2005	X-ray crystallography used to determine the 3D structures of nearly all the possible sequences of DNA at atomic level and create a map of DNA structure, facilitating the study of gene function.

There are over six hundred such diagnostic systems. Detailed description of molecular diagnostic technologies is given elsewhere (Jain 2005). Many new technologies such as nanotechnology have been used to refine molecular diagnostics. The focus of this report is on application of nanobiotechnology to molecular diagnosis and basic techniques of molecular diagnostics will be described in this chapter. Polymerase chain reaction (PCR) is the most important of these technologies. There are several modifications of PCR and non-PCR diagnostic technologies as well.

The polymerase chain reaction

The polymerase chain reaction (PCR) is a method of nucleic acid analysis for producing large amounts of a specific DNA fragment of a defined sequence and length from a small amount of a complex template. It can selectively amplify a single molecule of DNA or RNA several million-fold in a few hours. Use of this technology enables the detection and analysis of specific gene sequences in a patient's sample without cloning. Analyses can be performed on even a few cells from body fluids or in a drop of blood. Thus, PCR eliminates the need to prepare large amounts of DNA from tissue samples. PCR has

revolutionized molecular diagnostics. Apart from laboratory diagnosis, it has affected genomics and biotechnology as well.

Basic principles of PCR

PCR is based on the enzymatic amplification of a fragment of DNA that is flanked by two "primers"--short oligonucleotides that hybridize to the opposite strands of the target sequence and then prime synthesis of the complementary DNA sequence by DNA polymerase (an enzyme). The chain reaction is a three-step process – denaturation, annealing, and extension – that is repeated in several cycles. At each stage of the process, the number of copies is doubled from two to four, to eight, and so on. The reactions are controlled by changing the temperature using a special heat-stable Taq polymerase. After 20 cycles, roughly 1 million copies exist, or enough material to detect the desired DNA by conventional means such as color reaction.

RNA can also be studied by making a DNA copy of the RNA using the enzyme reverse transcriptase. Such an approach enables the study of mRNA in cells that use the molecule to synthesize specific proteins or the detection of the genome of RNA viruses. PCR has been fully automated via use of thermal cycling. It is a fast, sensitive, and specific test with applications in diagnosis of various diseases described in following chapters.

Target selection

Several strategies are available for selecting a genetic target to be amplified so as to detect an infectious disease organism. For example, genes that contain both conserved and variable sequence regions may be targeted. In such a case, specificity may be obtained either at the amplification (primer) or detection (probe) stage. The target may also consist of a virulent gene that is uniquely responsible for distinguishing pathogenic from closely related nonpathogenic strains, types, or species.

Detection of amplified DNA

The first detection methods used with PCR were radioactively labeled probes that identified specific amplified sequences. With improvements in specificity, it became possible to visualize amplified DNA of the predicted size directly by examining its fluorescence after staining. Probes have now been converted to nonisotopic colorimetric