CIRCULATING NUCLEIC ACIDS IN PLASMA AND SERUM

Essay

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BY

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Abstract

The recent interesting nucleic acide in plasma and serum has opened up Numerous new areas of investigation and new possibilities for Molecular diagnosis in oncology increased amounts of circulating DNA Have been found in a variety of disorders including canceraute immune Diseases , diabetic complications and infection .

Studies showed that there is an increasing level of circulating tumor Related DNA related to higher stages of disease and these finding have Important implication for detection , monitoring and prognosis of many Types of malgnacies .

Discovery of fetal DNA in maternal plasma has opened up new Possibilities for non invasive prenatal diagnosis and monitoring Quantitative aberration and a number of pregnancy associated disorders. An evolution in molecular techniques has recently allowed much better Characterization of circulating in these conditions and led to the Emergence of a new field of investigations Recent discoveries have Brought anew understanding of circulating DNA and show promise for The detdction and follow up of various disorders.

Key words : nucleic acids , cancer, fetal DNA, diabetes

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CONTENTS

List of figures:

Figure (14): Plasma DNA concentration in healthy subjects and diabetic subjects with and without retinopathy.

Figure (15): mRNA for rhodopsin in healthy subjects and diabetic subjects with and without retinopathy

List of Abbreviations:

ACR	Albumin Creatinine Ratio.
ACS	Acute Cronary Syndrome.
AMI	Acute myocardial infarction.
APC	Adenomatous polyposis coli.
ATP	Adenosine tri phosphate.
cDNA	Complementary DNA.
CVS	Chorionic villous sampling.
dATP	Deoxyadenine-triphosphate
dCTP	Deoxycytosine- triphosphate.
ddATP	Dideoxynucleotide
DEPC	Diethylpyrocarbonate
dGTP	Deoxyguanine- triphosphate.
DN	Diabetic nephropathy.
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphates
DR	Diabetic retinopathy.
ds DNA	Double stranded DNA.
dTTP	Deoxythymine-triphosphate.
DYZ1	Male-specific DNAsequences.
EBV	Epstein-Barr virus
ELISAs	Enzyme linked radio immune assay
EtBr	Ethidium bromide
FGFR3	Fibroblast growth factor receptor 3gene.
GEq	Genome equivalents
GIT	Guanidinium isothiocyanate.
HBV	Hepatitis B virus

HDN	Hemolytic disease of the new-born.
hTERT	Telomerase reverse transcriptase
hTR	Telomerase RNA template.
IgH	Immunoglobulin heavy chain-joining region.
LOH	loss of heterozygosity
mRNA	Messenger RNA.
NPC	Nasopharyngeal carcinoma.
NSTEMI	Non-ST elevation myocardial infarction.
Ms-PCR	Methylation-specific polymerase chain reaction
PCR	Polymerase chain reaction
PFE	Pulsed field electrophoresis.
Rh	Rhesus D gene.
RIAs	Radio immune assay
RNA	Ribonucleic acid
rRNAs.	Ribosomal ribonucleic acid
RT-PCR	Real-time polymerase chain.
SRY	A single-copy Y chromosome-specific sequence.
STEA	ST -elevation angina.
STEMI	ST- elevation myocardial infarction.

Introduction

Amazing development in biotechnology have taken place in the late twentieth century **(Isaacson,1999)** .When the decoding of the human genome , progress in stem cell research and gene therapy have been witnessed. Many of these advances would not have been possible without the many earlier landmark discoveries that unveiled the mysteries of genetics and paved the way for modern molecular diagnostics **(Alberts et al., 2002 & Garwin and Lincoln 2003)**.

Genetics began modestly when Mendel experimented with garden peas. His finding, published in 1866 and suggesting the concepts of alleles and genes as discrete units of heredity, essentially captured the most fundamental concepts in inheritance **(Bradbury, 2003).**

In1910 Morgan revealed that the units of heredity are contained within Chromosomes, but it was Avery in 1944 who confirmed through studies on bacteria that it was DNA (deoxyribonucleic acid) that carried the genetic information **(Nakao, 2001).**

Franklin and Wilkins (1950) studied DNA by x-ray crystallography, which subsequently led to the unraveling of the double-helical structure of DNA by Watson and Crick (1953). In the 1960s Smith demonstrated that DNA can be cleaved by restriction enzymes, which facilitated the subsequent development of recombinant DNA technologies **(Smith et al., 2003).**

Nathan furthered the work on restriction enzymes and was the first to construct genetic map. In1975the southern blot was invented, which allowed the detection of specific DNA sequences **(Collins et al., 2003).**

In 1977 DNA-sequencing methodologies were developed, and the first complete DNA-sequence of an organism, a bactriophage, was published.

Prenatal genetic diagnosis of sickle cell disease was first shown to be feasible by Kan and Change in 198. **Mullis and** co-workers developed the polymerase chain reaction (PCR) in 1985.

DNA microarrays, which allow the simultaneous interrogation of gene transcripts, became a reality in 1996. Remarkably, the draft human genome sequence was released in 2001and completed in 2003.

This is a brief account on a fraction of the many great discoveries that shaped modern genetics and molecular diagnostics.

LANDMARK DEVELOPMENT

The discovery of extra cellular nucleic acids in the circulation was reported by **Mandel and Metais (1948)**.These investigators observed the presence of circulating DNA and RNA in the plasma of healthy and sick individuals .This work was only a few years following the demonstration that DNA is the material of inheritance and this report even preceded the paper on the double –helical structure of DNA (**Wallace, 2001**) .It was unfortunate, therefore, that there was essentially no interest in circulating nucleic acids in the subsequent decade following this pioneering work (Lo, 2001)

Research on circulating DNA resumed following the discovery of high levels of circulating DNA in patients with systemic lupus erythematoses **(Tan et al., 1966).**

Further interest in this field was developed following the demonstration that increased concentrations of DNA in the serum could be detected in patients with cancer. It was further shown that the concentration of circulating DNA was higher in individuals with metastatic disease and , in some cases , the levels of circulating DNA decreased with successful anticancer therapy **(Leon et al., 1977),** due to technological limitations at that time, the precise cellular origin of the extra cellular DNA in cancer patients could not be determined **(Lo, 2001a).**

Stroun et al., 1989 suggested that the circulating DNA that is found in cancer patients demonstrates certain characteristics of tumor DNA this important suggestion was shown to be correct when two groups reported the presence of tumor-associated oncogene mutations in the plasma of patients with myelodysplastic syndrome ,acute myelogenous leukemia **(Vasioukhin et al.,1994)** and pancreatic cancer ,these reports were powerful evidence that tumor cells indeed release their DNA into the circulation .Apart from the inherent biological interest of this phenomenon ,this observation also opened up exciting possibilities for tumor detection and monitoring **(Sorenson et al.,1994).**

significant progress in plasma/serum DNA research was achieved when two groups simultaneously reported the presence of tumor –associated micro satellite alterations in the plasma and serum of cancer patients **(Chen et al., 1996 and Nawroz et al., 1996)** thus, microsatellaite alterations, such as loss of heterozygosity (LOH), could be found in the plasma and serum and could be shown to match those occurring in the primary tumors. The observation of LOH in the plasma and serum was particularly impressive as it suggested that, in these cases, most of the plasma/serum DNA was tumor-derived **(Chen et al., 1996).**

Aim of the work

Was under taken to study circulating nucleic acids in plasma and serum with special emphasis on their release ,clearance and function as related to health and in different disease states. Through a light on the different methods of their assay and quantification.

Molecular composition and structure of DNA and RNA

The physicochemical properties and functions of nucleic acids are largely governed by the composition and structure of DNA and RNA.

Structure of DNA:

A single molecule of DNA is a polymer consisting of a backbone of invariant composition and of side groups arranged in a variable sequence. The polymer is synthesized from monomers (nucleotides) composed of the sugar deoxyribose phosphate residue, and a purine or a pyrimidine base. The purines are adenine (A) and guanine(G) and the pyrimidine bases are cytosine (C) and thymine (T).The four nucleotide building blocks of DNA are abbreviated dATP(deoxyadenine-triphosphate) ,dGTP (deoxyguanine- triphosphate) ,dCTP (deoxycytosine-triphosphate),and dTTP(deoxythymine-triphosphate) respectively Fig. (1) **(Grewal and Moazed, 2003)**.

Nucleotides are joined by phosphodiester bonds that link the 5-phosphate group of one to the 3-hydroxyl group of the next, there are no 3-3or 5-5 linkage; thus the sugar and phosphate moieties compose the nonspecific portions of the molecule.The sequence of the bases varies from molecule to molecule and uniquely identifies each DNA polymer, which determines the identity and function of the protein product that the DNA encodes **(Jones and Takai, 2001).**

Although the purines and pyrimidines are of different composition and sizes, when in the proper orientation, adenine forms hydrogen bonds with thymine and guanine forms hydrogen bonds with cytosine to form planar structures of similar dimensions. A portion of each nucleotide is hydrophobic contribute to the energetically favorable secondary structure of DNA as it is found in its native form: a right-handed, double stranded helix **(Venter et al., 2001).**

The planar base pairs stack in the inside of the helix, 10 bases per turn, whereas the hydrophilic sugar phosphate backbone forms non covalent bonds with surrounding water molecules. For the two DNA polymers to form the proper hydrogen bonds between the bases, two requirements must be fulfilled: the polymers must run in opposite directions (antiparallel) as defined by the free hydroxyl groups at each end (3-5 vs. 5-3) and the sequences of each molecule must be such that A: T and G: C hydrogen bonds are always formed (base pairing). Two DNA strands that meet this requirement are called complementary **(Lander et al., 2004).**

Owing to base pairing and the double –helical conformation, double stranded DNA (ds DNA) is an exptionally stable molecule, retention of the base pairs in the inner portion of the helix prevents disruption by water molecules **(Horn and Peterson, 2002).**

The helical conformation places each identical orientation within the molecule and forms the same secondary monomer in bonds as every other monomer **(Khorasanizadeh, 2004).** This secondary