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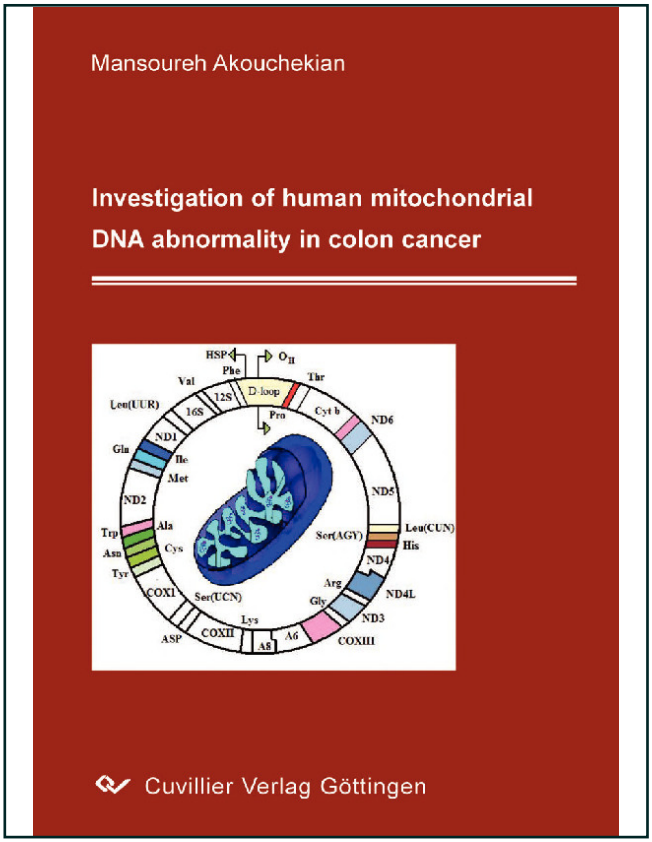
Investigation of human mitochondrial DNA abnormality in colon cancer



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1 Introduction

Each human cell contains a nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Mitochondria are small cytoplasmic organelles that contain their own DNA, which is a circular genome of 16,569 bp. Mitochondria are unique, because they are responsible for the major energy producing system of the cell, the oxidative phosphorylation enzyme pathway (OXPHOS). This process is accomplished by a series of protein complexes, collectively known as the respiratory chain, encoded by both nDNA and mtDNA. The complete respiratory chain contains at least 87 polypeptides, 13 of which are encoded by mtDNA, which is known for having a high acquired mutation rate at least 10 times higher than that reported for nDNA. It is generally accepted that the high mutation rate of mtDNA is caused by the lack of protective histones, inefficient DNA repair systems and continuous exposure to mutagenic effects of oxygen radicals generated by oxidative phosphorylation (Miyazono, *et al*., 2002). An association between mtDNA mutations and neurologic or metabolic disorders has previously been reported (Wallace, 1992, Armstrong, *et al.,* 2000). However most of the mutations are neutral polymorphisms which have accumulated sequentially in maternal lineages, creating groups of related mtDNA haplotypes.

Tumor development is often associated with mtDNA mutations and alterations in mitochondrial genomic function. Mutations in the mtDNA have been reported to occur in human cancers (Burgart, *et al.*, 1995, Fliss, *et al.,* 2000, Habano, *et al.*, 2000, Brandon, *et al*., 2006).

The mitochondrial electron transport chain has been recognized as one of the major cellular generators of reactive oxygen species (ROS), which include hydrogen peroxide (H_2O_2), the hydroxyl free radical ($\cdot OH$) and superoxide ($O_2\cdot^-$) (Liu, 2002). ROS have deleterious side effects, as they cause damage to nucleic acids, proteins and lipids. Since mitochondrial DNA and membranes are directly exposed to the ROS produced during cellular respiration they are especially vulnerable. ROS are involved in various pathological processes such as carcinogenesis and neurodegeneration.

The aims of this work were to detect new mtDNA mutations, deletions or insertions in patients with CRC to determine the prevalence of known mtDNA mutations, and to estimate the role of mtDNA polymorphisms and haplogroups as a risk factor for CRC.

2 Review of the literature

2.1 Mitochondria

Mitochondria are organelles that are situated in the cytoplasm of eukaryotic cells. They are involved in various physiological processes such as intermediary metabolism and cellular signaling events. The name "mitochondrion" was coined by C. Bender in 1898, and is derived from the Greek *mitos* meaning thread and *chondrion* meaning granule. These small cytoplasmic organelles have been known as "the powerhouses of the cell" for long time. That means the most well-known and best-characterized function of mitochondria is the production of adenosine triphosphate (ATP) through oxidative phosphorylation. This process is accomplished by a series of protein complexes, collectively known as the respiratory chain, encoded by both nDNA and mtDNA. ATP is the unique energy source for performing a wide range of cellular functions. During the last few decades researchers have realized the involvement of mitochondria in other cellular functions as well apoptosis (Desagher & Martinou 2000), cell division (von Wangenheim & Peterson 1998), and possibly aging (Melov 2000, Rustin *et al*., 2000). Our understanding of the role of mitochondria in human diseases is continuously growing.

A typical human cell has several hundred mitochondria, which convert energy to forms that can be used to drive cellular reactions. Mitochondria are thought to derive from α-proteobacteria (Gray *et al*., 1999). It is believed that throughout their evolution mitochondria transferred most of their genes to the nucleus, and, in turn, received various “eukaryotic” genes of the host cell. It is accepted now that mitochondria contain approximately 700 different proteins and provide the cell with important functions such as energy production and apoptosis (Gaucher *et al.,* 2004).

2.1.1 Structure of mitochondria

Mitochondria are 0.5-1 μm in size and are bounded by two membranes, the outer (OM) and inner (IM) membranes, separated by a space called the intermembrane space (IMS). The OM completely encloses the mitochondria, while the IM surrounds a space called the matrix. The outer membrane is permeable to molecules of 10,000 daltons or less in molecular weight. It contains non-selective membrane channels and is composed of equal amounts of protein and lipid.

The IM composition is about 20% lipid and 80% protein, and it has the highest protein fraction among cell membranes. The IM is highly impermeable, and therefore virtually all molecules and ions require special transporters. The mitochondrial inner membrane is organized into convoluted invaginations that project into the matrix and are called cristae (Lodish, 2000). The IM contains the enzyme complexes of oxidative phosphorylation for the synthesis of ATP.

The internal structure of mitochondria is traditionally depicted as a "baffle model" in which the cristae of the IM form folds similar to the bellows of an accordion (Sjöstrand 1956). The mitochondrial matrix contains hundreds of enzymes, including those required for the oxidation of pyruvate and fatty acids and those, which are active in the tricarboxylic acid (TCA) cycle. The matrix also contains several identical copies of the mtDNA, mitochondrial ribosomes, tRNAs and various enzymes required for the transcription and translation of mitochondrial genes. There are contact sites between the outer and inner mitochondrial membranes; these sites are formed during the transport of nuclear-encoded precursor proteins into mitochondria.

2.2 The mitochondrial genome

The human mitochondrial DNA was the first human "chromosome" that has been completely sequenced in 1981. It was reported to be a circular double stranded molecule 16,569 base pairs in length (Anderson *et al*., 1981). mtDNA has two strands, a guanine rich heavy (H strand) and a cytosine rich light (L strand), and encodes 13 proteins, 22 transfer RNAs (tRNA) and two ribosomal RNAs (rRNA), 12SrRNA and 16SrRNA (Fig 2.1). Due to the presence of many copies per cell, it corresponds to about 1% of the total DNA content in an average cell. The mitochondrial genome of mammals is organized extremely economically, showing a gene organization that is very compact without any introns (Fernandez-silva *et al*., 2003).

The only non-coding segment of mtDNA in all vertebrates is the displacement loop (D-loop) or control region, a region of 1124 bp (position 16024 – 576) on the mtDNA. The control region contains the origin of replication of the H-strand (OH) and the promoters for L and H-strand transcription. Both strands of the mammalian circular mtDNA genome are transcribed from a single major promoter. The D-loop is the region with the most variable sequence among different species, even though it harbors some well conserved elements (Sbisa *et al*., 1997).

Almost the entire H strand is used as a template when producing RNA information for the L strand sequence, and similarly an extensive part of the L strand is used to produce RNA. These polycistronic transcripts are cleaved at precise sites to produce tRNAs, rRNAs and mRNAs. Genes coding for the protein

ND6 and tRNAs for glycine (Gly), alanine (Ala), asparagine (Asn), cysteine (Cys), tyrosine (Tyr), serine (Ser), glutamine (Glu) and proline (Pro) are transcribed from the L chain. The genes encoding the ATPase 6 and 8 proteins are partly overlapping, and in addition, several tRNA genes overlap by 1-3 nucleotides. The frequency of RNA transcription is regulated by nuclear encoded proteins (Ojala *et al*., 1981, Christianson & Clayton 1986, Jeong-Yu & Clayton 1996).

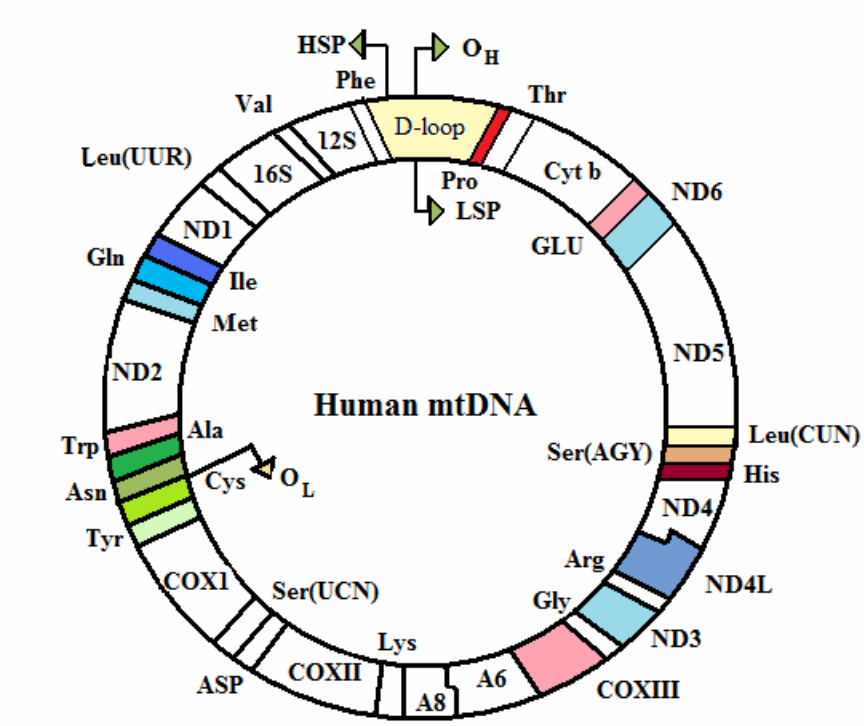

Figure 2.1: Human mitochondrial genome

The mitochondrial genome has several unique features relative to the nuclear genome. The organization of mtDNA is different from that of nDNA as listed in the following: The genetic code of mtDNA is different, replication of mtDNA is independent of the cell cycle, mtDNA is maternally inherited, and the mtDNA pool of a cell is randomly segregated to daughter cells. Also, a certain level of mutant DNA is required before it affects the function of a cell or tissue. This is called threshold effect. The mutation rate of mtDNA is higher than that of nDNA, and each cell harbors a high number of copies of mtDNA molecules, in contrast to

only two to four copies of nDNA. In the following some mitochondrial characteristics are mentioned in details.

2.2.1 Special features of mtDNA

The mitochondrial genome has some distinctive characteristics that are different when compared to the nuclear genome. The first unique feature is the use of a differing genetic code, for this reason the cytosolic translation machinery is not able to translate mitochondrial mRNAs or vice versa (Table 2.I). Second, nuclear genes follow a Mendelian pattern of inheritance, whereas mitochondrial genes are maternally inherited. Third, the nuclear genome is either haploid or diploid while the mitochondrial genome is polyploid since there are thousands of mtDNA molecules per cell. When only one form of mtDNA exists in a cell, the state is called homoplasmy; when two or more co-exist, the state is called heteroplasmy. Another difference of the mtDNA molecule is the higher mutation rate. mtDNA is positioned in the vicinity of the OXPHOS system, which is considered to be the major producer of reactive oxygen species (ROS). This location might explain the increased mutation rate (Wallace, 2005).

Cells contain a high copy number of mitochondrial genomes, the number varying from one cell type to another, between 1,000 and 10,000 copies per cell (Larsson & Clayton 1995, Lightowlers *et al*., 1997). Normally, short-lived human cells such as sperm or leukocytes have a low mtDNA copy number, whereas long-lived cells (e.g. skeletal muscle, brain cells, oocytes) tend to have a high copy number of mtDNA. One hypothesis has suggested that high copy number may protect mtDNA from the accumulation of a critical threshold of mutations (Chinnery & Samuels 1999). One more different between mtDNA and nDNA is the high mutation rate in mtDNA. The rate of mutation in mtDNA is 10 to 17 times faster than that in nuclear genes (Neckelmann *et al.,* 1987, Wallace *et al.,* 1997).

2.2.2 Mitochondrial Haplogroups

Mitochondrial haplogroups are determined by polymorphisms that occurred tens of thousands of years ago and form high-prevalence population-specific substitutions today. These haplogroups are defined by ancient mutations (Wallace, 2003). The changes appeared and survived, therefore, they could not be deleterious mutations. Most of them probably had not any phenotypic effect and were neutral. Some of them had a beneficial effect and were positively selected. However, this positive effect was related to a particular environment and nowadays, in other environmental conditions, may have different effects on the

phenotype (Mishmar, 2003, Wallace, 2003, Ruiz-Pesini, 2004). In the other words human haplogroups are defined by special polymorphisms in human mitochondrial DNA. These haplogroups trace the matrilineal inheritance of modern humans back to the human origins in Africa and reflect the subsequent human migrations across the earth.

Haplotypes are subclusters of haplogroups, and the polymorphisms that determine them are less prevalent and have occurred more recently. Most of the polymorphisms determining haplogroups are continent-specific (Wallace, 1994).
Haplogroups could have important implications for understanding of the relationship between mutability of the mitochondrial genome and disease (Ozawa, 1991, Shoffner, 1993). There is growing evidence that certain mtDNA Haplogroups are associated with distinct disorders (Brown, 1995, Torroni, 1997).

2.3 Mitochondrial DNA replication

The replication and transcription of Mitochondrial DNA take place within the mitochondria. It occurs independently from the cell cycle and from nuclear DNA replication.
Factors involved in mtDNA metabolism are nuclear-encoded, including mtRNA polymerase, mtDNA polymerase γ (Pol γ) and most of the potential factors that regulate mtDNA replication, mtDNA transcription and mtRNA processing (Moraes, 2001).
The generally accepted model for mtDNA replication consists of an asymmetric synthesis. This means that the two mtDNA strands replicate in an asynchronous manner from two independent origins. The synthesis starts at one of the multiple origins of replication of the heavy strand (O_H) at the D-loop region using a short RNA primer. mtDNA synthesis continues until reaching the origin of replication of the light strand (O_L), which is situated approximately two thirds away around the mtDNA molecule. At this time, the synthesis of the light strand starts (Garesse & Vallejo, 2001). Because Pol γ requires short mtRNA primers; replication depends on mitochondrial transcription. Studies with a recombinant human DNA polymerase have estimated that one round of mtDNA replication takes about 1 h (Graves *et al*., 1998).

It was suggested that cells use different synthesis mechanisms under specific physiological conditions (Holt *et al*., 2000). However, these results remained controversial among scientists (Bowmaker *et al*., 2003, Bogenhagen & Clayton, 2003, Holt & Jacobs, 2003, Brown *et al*., 2005).