



**UNIVERSITI PUTRA MALAYSIA**

**MOLECULAR INVESTIGATIONS OF THE COMMON MITOCHONDRIAL  
DELETION (AMTDNA 89777 ) TUMORAL TISSUES AS COMPARED  
WITH ADJACENT NON-TUMORAL TISSUES FROM GASTRIC CANCER  
PATIENTS AT BAGHIYATALLAH HOSPITAL IN TEHRAN**

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**By**

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
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*Specially dedicated to,  
My beloved Father, Mother, Sister and My missing love  
For their invaluable love, understanding, tolerance, sacrifice, moral support*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**MOLECULAR INVESTIGATIONS OF THE COMMON MITOCHONDRIAL DELETION ( $\Delta$ MTDNA<sup>4977</sup>) IN TUMORAL TISSUES AS COMPARED WITH ADJACENT NON-TUMORAL TISSUES FROM GASTRIC CANCER PATIENTS AT BAGHIYATALLAH HOSPITAL IN TEHRAN**

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**January 2006**

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**Faculty: Medicine and Health Sciences**

The human mitochondrial genome has been completely sequenced and each gene has been identified and characterized. The human mtDNA is a supercoiled, double-stranded circular molecule of 16,569 base pairs in size which codes for 13 of the 87 proteins required for oxidative phosphorylation as well as the 12S and 16S rRNAs and 22 tRNAs required for protein synthesis in the mitochondria.

One of the common regions and hot spot of the mitochondrial genome so-called the common mitochondrial deletion ( $\Delta$ mtDNA<sup>4977</sup>) was investigated which is at nucleotide number 8470 to 13447. This deletion affects important genes, involved in OXPHOS (Oxidative Phosphorylation) such as ATPase 6, ATPase 8, Cytochrome Oxidase III, and NADH subunits ND3, ND4, ND4L, and ND5 which may have a strong metabolic disadvantage. The prevalence of the  $\Delta$ mtDNA<sup>4977</sup> deletion has been investigated in different cancers. However in this study, we screened the common



mitochondrial deletion to determine the prevalence of the common mitochondrial deletion in tumoral tissues as compared with adjacent non-tumoral tissues in gastric cancer by multiplex PCR amplification, polyacrylamide gel electrophoresis and Southern blot.

In order to investigate whether a high incidence of mutation exists in mitochondrial DNA of gastric cancer tissues, DNA isolated from these cells was used to amplify hypervariable regions ATPase8/6, COXIII, ND3, ND4 and ND5 of  $\Delta$ mtDNA4977.

In 107 cancer patients,  $\Delta$ mtDNA4977 was detected in 6 cases (5.60%) of the tumoral tissues and 18 cases (16.82%) of the non-tumoral tissues that were adjacent to the tumors. Levels of  $\Delta$ mtDNA4977 deletions were found to be more in non-tumoral tissues than in adjacent tumoral tissues. There was no correlation between the clinical parameters like age, sex, tumor location and tumor size of patients. However there was an obvious relationship with intestinal-type of gastric cancer.

The percentage of deleted genome in gastric tumoral tissues ranged from 25% to 74%. The level of the heteroplasmy was quantified by densitometry analysis with a Gel Doc 2000 BIO-RAD instrument (Hercules, CA, USA). The level of heteroplasmy was determined as ~25% to 74%.

Unknown genetic aspects, ambiguous environmental factors and Reactive Oxygen Species (ROS) can cause the  $\Delta$ mtDNA4977 mutation rate to be increased in gastric

cancer. The results suggest that percentage level of  $\Delta$ mtDNA4977 is less common and intolerable in tumoral tissue, probably because of high metabolism and ROS generation. We postulate that the cells initially had  $\Delta$ mtDNA4977, transformed to tumoral cell and the existence of this deletion confers metabolic disadvantage, thus cells that contain such mtDNA deletion would be overgrown by other cancer cells without this mtDNA deletion. As a result, the presence of  $\Delta$ mtDNA4977 will be low in tumoral cell.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KAJIAN MOLEKULAR TERHADAP DELESI BIASA MITOKONDRIAL ( $\Delta$ MTDNA<sup>4977</sup>) PADA SEL TUMOR BERBANDING DENGAN SEL SEBELAHAN BUKAN TUMOR DALAM KANSER PERUT PESAKIT-PESAKIT DI BAGHYATALLAH HOSPITAL IN TEHRAN**

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Genom mitokondria (mtDNA) manusia telah berjaya diuraikan jujukannya, dengan setiap gen telah dikenalpasti dan dicirikan. mtDNA mempunyai struktur molekul yang dipanggil supercoiled yang dibentuk oleh dua lapisan molekul DNA yang berpasangan di dalam gelungan yang bersaiz 16,569 basepair untuk penghasilan 13 daripada 87 protein untuk aktiviti yang dipanggil fosforilasi oksidatif, termasuk juga 12S dan 16S rRNA, dan 22 tRNA, di dalam mitokondria.

Salah satu daripada lokasi di dalam genom tersebut yang dikenali sebagai common mitochondrial deletion ( $\Delta$ mtDNA4977), yang terkandung di antara *basepair* 8470 dan 13447, telah diselidiki. Ketiadaan pada lokasi ini memberi kesan penting kepada gen-gen yang terlibat di dalam OXPHOS (*oxidative phosphorylation*) seperti ATPase 6, ATPase 8, Cytochrome Oxidase III, dan, subunit-subunit NADH - ND3, ND4, ND4L dan ND5, yang seterusnya menyebabkan masalah metabolik di dalam tubuh.

Berlakunya kehilangan  $\Delta$ mtDNA4977 telah diselidiki di dalam beberapa jenis kanser. Namun, di dalam penyelidikan ini kami mengenalpasti kehilangan ini untuk mencari kekerapannya pada sel tisu barah berbanding dengan sel tisu bukan barah pada barah usus dengan menggunakan teknik amplifikasi multiplex PCR, elektrophoresis gel polyacrylimide dan blot Southern.

Bagi menyelidik samada kewujudan kejadian mutasi yang tinggi dalam DNA mitokondrial pada tisu kanser perut, DNA telah diasingkan dari tisu ini dan di amplifikasikan bahagian-bahagian kepelbagaian tinggi ATPase8/6, COXIII, ND3, ND4 dan ND5 dari  $\Delta$ mtDNA4977. Dalam 107 pesakit kanser dikaji,  $\Delta$ mtDNA4977 telah dikesan pada 6 kes (5.60%) dari tisu tumor dan 18 kes (16.82%) dari tisu bukan tumor yang bersebelahan kepada tumor. Tahap delesi  $\Delta$ mtDNA4977 didapati lebih pada tisu bukan tumor berbanding dalam tisu tumor bersebelahan. Didapati tiada korelasi di antara parameter klinikal seperti umur, seks, lokasi tumor dan saiz tumor pada pesakit, walaubagaimanapun adalah jelas terdapat perhubungan dengan jenis intestin dari kanser perut.

Faktor genetic, alam sekitar yang tak diketahui dan Tindakbalas Spesis Oksigen(ROS) boleh sebabkan kadar mutasi  $\Delta$ mtDNA4977 meningkat dalam kanser perut. Keputusan mencadangkan aras  $\Delta$ mtDNA4977 adalah kurang biasa dan tidak bertolak ansur dalam tisu tumor, mungkin disebabkan oleh metabolisma dan ROS yang tinggi. Kami postulat yang sel pada mulanya ada  $\Delta$ mtDNA4977, telah bertukar pada sel tumor dan muncul delesi ini menjadikan metabolik tidak baik, jadi sel yang mengandungi delesi mtDNA



tersebut akan membesar dengan bantuan sel kanker tanpa delesi mtDNA. Dan hasilnya, kehadiran  $\Delta$ mtDNA4977 akan menjadi rendah dalam sel tumor.

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## LIST OF ABBREVIATIONS

$\Delta$ mtDNA <sup>4977</sup>	Common Mitochondrial Deletion
$\mu$ g	Microgram
$\mu$ l	Microlitre
$\mu$ M	Micrometer
AJCC	American Joint Committee on Cancer
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myelogenous Leukemia
APS	Ammonium Persulfate
ASR	Age Standardized Ratio
ATP	Adenosin Tri-Phosphate
bp	Base pair
COX	Complex
CT	Computerized tomography
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine Tetra-Acetic acid
EtOH	Ethanol
FPC	Familial polyposis coli
GI	Gastrointestinal
HCC	Hepatocellular Carcinoma



IARC	International Agency for Research on Cancer
IMHME	Iranian Ministry of Health and Medical Education
KAc	Potassium Acetate
Kb	Kilobase
M	Molar
MDS	Myelodysplastic Syndrome
mM	Millimolar
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
mtDNA	Mitochondrial DNA
mtEF	Mitochondrial Elongation Factor
NADH	Nicotine Amide Dehydrogenase
nDNA	Nuclear DNA
ng	Nanogram
OD	Absorbance of DNA
PAGE	PolyAcrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PHRI	Public Health Research Institute
POLRMT	Mitochondrial RNA Polymerase
RCC	Renal Cell Carcinoma
ROS	Reactive Oxygen Species
rpm	Revolutions per minute
rRNA	Ribosomal RNA

S	Svedberg
SDS	Sodium Dodecyl Sulfate
SOD	Superoxide Dismutase
SSC	NaCl, Trisodium Citrate (Citric Acid)
SSCP	Single-Stranded Conformation Polymorphism
Taq	Thermus aquaticus thermostable DNA
TBE	Tris-Borate-EDTA
TE	Tris EDTA buffer
TEMED	N,N,N',N'-Tetramethyl-Ethylenediamine
TFAM	Mitochondrial Transcription Factor A
TFB1M	Mitochondrial Transcription Factor B1
TFB2M	Mitochondrial Transcription Factor B2
tRNA	Transfer RNA
UICC	Union Internationale Contre le Cancer
V	Volt

## CHAPTER 1

### INTRODUCTION

Cancer is a serious health problem worldwide, imposing a large economical and psychological burden as well as loss of life and productivity (Whelan *et al.*, 1993). Despite a decrease in the annual incidence and mortality rates over the past 50 years, gastric cancer remains the second cause of cancer-related death. As with other cancers, the etiology of gastric cancer is unknown (Rocco and Franco, 2004).

Lots of effort and money have been put in the fields of clinical, epidemiological, pharmacological, and biological research on cancer in the recent decades. Although we have witnessed dramatic progress in the field, but still there is a long way to go. Cancer is the third most common cause of death in Iran, accounting for 14% of the total death toll (Naghavi, 2001).

It stands just after cardiovascular events and accidents (46% and 17% of the total death toll respectively) according to the latest census of the Iranian Ministry of Health and Medical Education (IMHME). Overall, gastrointestinal (GI) cancers cause about half of all cancer deaths in Iran. Of the 17,450 GI cancers recorded in the IMHME report, 7,560 (43.3%) were gastric cancers (Naghavi, 2001). In other words, of every 100 people dying of cancer in Iran, 22 die of gastric cancer, and six from esophageal cancer. Unfortunately, both of these cancers come to medical attention when they are rather advanced and limited or no effective therapies are available for them. Theoretically,



these cancers may be treatable in their early stage; therefore, finding them at the earliest possible stage may subject them to effective therapy.

The Public Health Research Institute (PHRI) of Tehran University and the International Agency for Research on Cancer (IARC) jointly established a cancer registry in the city of Babol in Mazandaran Province in the Caspian littoral in 1969 (Joint Iran and IARC , 1977). The registry mainly covered the two provinces of Gilan and Mazandaran, and the nearby city of Ardabil. Information gathered by this cancer registry soon showed an unusually high incidence of esophageal cancer in the Caspian Littoral. Interestingly the distribution was rather uneven. The western parts of the Littoral (Ardabil) had a high incidence (age standardized ratio (ASR): 20.1 and 6.2 per 100,000 population for men and women, respectively), while the central parts had a lower incidence (ASR: 13.0 and 2.3 per 100,000 for males and females, respectively), and the eastern parts had an unusually high incidence (ASR: 165.5 and 195.3 per 100,000 for men and women, respectively)(Habibi,1965;Haghighi and Nasr,1971; Kemet and Mahboubi, 1972; Mahboubi *et al.*, 1973; Joint Iran and IARC, 1977). This was especially evident in the Turkman plain in the East of the littoral having the above-mentioned ASR, which was among the highest ever recorded in the world. Another point of interest was that esophageal cancer was more common among Turkman females.

A recent cancer surveillance study performed by the Ministry of Health of the Research Institute of Iran (Ministry of Health and Medical Education., 2000) revealed that gastric adenocarcinoma is the most common fatal cancer in Iran with a wide variation of death

rate in different provinces. The mortality rate in Bushehr, southern Iran (Persian Gulf Coast), is 3.6 per 100,000 per year as compared to a rate of 8.4 per 100,000 in East Azarbaijan, northern Iran. According to a study in 1971, (Haghighi and Nasr, 1971) antral adenocarcinoma was considered the predominant type of gastric cancer in Iran. Some unpublished data from Ardabil in Eastern Azarbaijan on the west coast of the Caspian Sea, however, reported a higher frequency of gastric cardia cancer. This descriptive, endoscopic study was designed to further explore the anatomic subsite of different GI cancers in this high prevalence gastric cancer area.

In Malaysia, of the three main ethnic groups, Malays, Chinese and Indians, the incidence of gastric ulcer was highest among the Chinese, next the Indians; however, the number of gastric cancers were too few for analysis of its racial incidence (Paramarajah, 1985). Other researches indicated that the incidence of surgery for gastric cancer in Singapore increased during the period 1951 to 1980 (males from 3.5 to 8.7 per 100,000 per year; females from 0.5 to 4.2 per 100,000 per year). This increase occurred mainly during the first decade of the study and was confined to persons aged 55 and above. Chinese had the highest incidence, followed by Indians and then Malays (Kang, 1988).

Goh and Parasakthi (2001) had evaluated 2,381 subjects for *Helicobacter pylori* prevalence and found that it varied from different areas of study and ranged from a low of 26.4% in blood donors from Kota Baru to a high of 55.0% in Kota Kinabalu. The most striking differences, however, were noted in the prevalence rates among different