A Study on Tumor Suppressor Genes Mutations Associated with Different Pathological Colorectal Lesions.

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By

Salwa Naeim Abd El-Kader Mater

M.Sc. in Biochemistry, 2002

Biological Applications Department
Nuclear Research Center
Atomic Energy Authority

Under the supervision of

Prof. Dr. Amani F.H Nour El-Deen

Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

Prof. Dr. Abdel Hady Ali Abdel Wahab

Professor of Biochemistry and Molecular Biology
Cancer Biology Department
National Cancer Institute
Cairo University

Prof. Dr.Mohsen Ismail Mohamed

Professor of Clinical Pathology Biological Applications Department
Nuclear Research Center
Atomic Energy Authority

Dr. Azza Salah Helmy

Assistant Professor of Biochemistry
Biochemistry Department
Faculty of Science
Ain Shams University

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تحت إشـــــراف

ا.د/ أماني فاروق حسين نور الدين
أستاذ الكيمـياء الحيوية
قسم الكيمـياء الحيوية
كلية العلوم ـ جامعة عين شمس

أ.د /عبدالهادي على عبدالوهـاب
أستاذ الكيمياء الحيوية والبيولوجيا الجزئية
قسم بيولوجيا الأورام
معهد الأورام القومي ـ جامعة القاهرة

ا.د/ محسن إسماعيل محمد
أستاذ الباثولوجيا الإكلينيكية
شعبة تطبيقات النظائر المشعة
مركز البحوث النووية ـ هيئة الطاقة الذرية

د / عزة صلاح حلمي
أستاذ مساعد الكيمياء الحيوية
قسم الكيمياء الحيوية
كلية العلوم ـ جامعة عين شمس

قســـــــــم الكيمياء الحيويـــة
كليـــــــة العلـــــــــوم
جامعـــة عيـــــــن شمس
2011

Abstract

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the Western world. In Egypt; there is an increasing incidence of the disease, especially among patients ≤40 years age. While CRC have been reported in low incidence rate in developing countries**,** it is the third most common tumor in male and the fifth common tumor in females in Egypt. Early diagnosis and surgical interference guarantee long survival of most CRC patients. Early diagnosis is impeded by the disease onset at young age and imprecise symptoms at the initial stages of the disease. As in most solid tumors, the malignant transformation of colonic epithelial cells is to arise through a multistep process during which they acquire genetic changes involving the activation of proto-oncogenes and the loss of tumor suppressor genes. Recently, a candidate tumor suppressor gene, KLF6, which is mapped to chromosome 10p, was found to be frequently mutated in a number of cancers. There are some evidences suggesting that the disruption of the functional activity of KLF6 gene products may be one of the early events in tumorgenesis of the colon. The main objective of the present study was to detect mutational changes of KLF6 tumor suppressor gene and to study the loss of heterozygosity (LOH) markers at chromosome 10p15 (KLF6 locus) in colorectal lesions and colorectal cancer in Egyptian patients. The patients included in this study were 83 presented with different indications for colonoscopic examination. Selecting patients with colorectal pre-cancerous lesions or colorectal cancer was done according to the results of tissue biopsy from lesion and adjacent normal. The patients were classified into three main groups; (GI) Cancerous group, (GII) polyps group including patients with adenomatous polyps (AP), familial adenomatous polyps (FAP) and hyperplastic polyps (HP) and (GIII) Inflammatory Bowel Diseases (IBD) including patients with ulcerative colitis (UC) and Crohn's disease (CD). Purified DNAs which were extracted from the tissue samples, PCR amplified and subjected to the following examinations: **1-** Detection of KLF6 mutations by using SSCP-silver staining technique and DNA sequencing by using BigDye Terminator v3.1Cycle Sequencing kit using Biosystem automated sequencer (The ABI PRISM 3100 Genetic Analyzer). **2-** Determination of Loss of heterozygosity (LOH) on chromosome 10p15 regions (KLF6-locus) by using three microsatellite markers which includes KLFM1, KLFM2, and KLFM4. Data from the present study could be

summarized as follows: In GI, 55.3% of cases had abnormalities in KLF6 gene (mutations and LOH). LOH was detected in 29% of investigated samples while KLF6 mutations were detected in 44% of cases. In GII, 57% of cases had abnormalities in KLF6 gene (mutations and LOH). LOH was detected in 55% of investigated samples while mutations of KLF6 gene were detected in 26% of investigated samples. In GIII, 50% of samples had abnormalities in KLF6 gene (mutations and LOH). LOH was detected in 36.4% of investigated samples while mutations of KLF6 gene were detected in 27.3% of investigated samples. Most of the mutations reported were of the missence and /or Transversion type and were almost in exon 2. In conclusion, our data highlight for the first time a role of KLF6 gene in the progression of Egyptian colorectal carcinogenesis where the results suggest that KLF6 gene alteration is involved in the progression of Egyptian colorectal carcinogenesis from both sporadic adenomatous polyps and ulcerative colitis pathways. Detecting mutational sites differing from that detected in western populations may be a characteristic of Egyptian CRC due to environmental and genetic factors. The detections of such genetic abnormalities may also be used as a marker for the early uncovering of colon cancer cases. It is recommended that those who have pre-neoplastic colon lesions in which the KLF6 gene has mutated or lost its heterozygosity should experience more frequent colonoscopic examinations for the detection of doubtful malignant changes. The present study also paves the way to further research needed to elucidate the possible role of KLF6 protein in the transactivation of other genes involved in cell cycle regulations and apoptosis. It also supports the possibility of using KLF6 gene as a target for gene therapy in colorectal cancer.

List of Abbreviations

ACF	aberrant crypt foci
AFP	Alpha Fetoprotein
AP	adenomatous polyps
APC	Adenomatous polyposis coli gene
APS	Ammonium persulfate
bp	Base pair
BTEB1	basic transcription factor element binding protein
BTEB2	basic transcription factor element binding protein 2
CD	Crohn's disease
CEA	Carcinoembryonic Antigen
CIN	Chromosomal instability
COPEB	core promoter element-binding protein
CPBP	core promoter binding protein
CPBP	Core Promoter Binding Protein
CPE	core promoter element
CRC	Colorectal cancer
DCBE	Double contrast barium enema
DCC	Deleted in colorectal cancer gene
ddH_2O	Deionized distilled water
DNA	Deoxi-ribonucleic acid
DRE	Digital rectal exam
EDTA	Ethylene Diamin tetraacetate
ER	Estrogene receptor
FAP	familial adenomatous polyps
FKLF	fetal erythroid Kruppel-like factor
FOBT	Fecal occult blood test
Ha-MSV	Harvey murine sarcoma viruses
IBD	Inflammatory Bowel Disease
IRB	institutional review board
Kd	Killo Dalton
Ki-MSV	Kirsten murine sarcoma viruses
KLF6	Krüppel-like factor 6
KLF-family	Krüppel-like transcriptional factors family
Lis-SSCP-loading buffer	low ionic strength- Single-strand conformation polymorphism- loading buffer
LOH	Loss of heterozygosity
MMR	DNA mismatch repair
MSI	micro satellite instability
OD	Optical density

PCR-LIS-SSCP-silver staining	polymerase chain reaction-low ionic strength-Single-strand conformation polymorphism- silver staining method
PSA	Prostate Specific Antigen
PSG	Pregnancy-Specific Glycoprotein genes
PSG	pregnancy-specific glycoprotein
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
TAE	Tris-Acetate-EDTA buffer
TBE	Tris-Borate-EDTA buffer
TE buffer	Tris EDTA buffer
TEMED	N,N,N$^{/}$,N$^{/}$-tetramethylene diamine
TGF β	transforming growth factors β
U.C	ulcerative colitis
UC-CRC pathway	Ulcerative colitis- colorectal cancer pathway
UKLF	ubiquitous Kruppel-like factor
UTR	Un-translated regions
uPA	urokinase type plasminogen activator
10p15	Short arm of chromosome 10 (locus 15)

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INTRODUCTION AND AIM OF THE WORK

Introduction:

Normal development is a balance process, which includes proliferation and cell death. Indeed both proliferation and apoptotic cell death are very complex process that involves the participation of many genes. In both events, the tumor suppressor genes are the most important and studied genes ***(Jung & Messingm, 2000).***The carcinogenic procedure is a multistep process involving several genetic alterations that eventually ends in malignant transformation. The study of carcinogenic procedure is very important, because it does not only shed light on some critical steps in the progress of carcinogenesis but may also provide a rational approach for early diagnosis of cancer ***(Mendoza-Rodriguez & Cerbon, 1995).***

Although some diagnostic markers are available that are assayable from blood or tissue samples, e. g. Carcinoembryonic Antigen (CEA), Alpha Fetoprotein (AFP) or Prostate Specific Antigen (PSA), the assays using these markers have not, to date, been markedly predictive of the presence of cancer in these individuals, as verified by other clinical diagnoses. The sensitivity and specificity of these assays has been disappointingly low. Time-consuming and labor-intensive clinical assessments (e. g. palpations, x-rays, mammograms, biopsies) have remained the accepted methods for diagnosing cancer. Thus, a need exists for a biomarker that is predictive of the presence of cancer or of an increased risk of developing a cancer in the individual. In particular, a need exists for a marker and an assay to measure the presence and amount of this marker for individuals with an early stage of cancer. If such diagnostic test is available, early treatment with beneficial outcomes would be more likely than at present ***(Srivastava and Gopal-Srivastava, 2002).***

One of the most important discoveries in cancer biology was the cancer arise as a result of cumulative genetic changes in cells. The progression of a tumor from normal cells to pre- cancerous ones, to cancer and then to local invasion and finally metastasis is the result of the clonal expansion of cells that have acquired a selective growth advantage, which allows them to outnumber neighboring cells. This