Molecular Diagnosis Of Hypermethylation Genes As Biomarkers In Colorectal Adenocarcinoma

Thesis

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By

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Table of contents

List Of Tables

List Of Figures

List Of Abbreviations

15-PGDH	**15-prostaglandin dehydrogenase**
APC	**Adenomatous Polyposis Coli**
CEA	**Carcinoembryonic Antigen**
CGI	**CpG islands**
CIMP	**CpG island methylator phenotype**
CIN	**chromosomal instability**
CRC	**Colorectal Cancer**
Ct	**Cycle threshold**
DCBE	**Double contrast barium enema**
DNA	**Deoxy ribonucleic acid**
EGF	**Epidermal Growth Factor**
EGFR	**EGF Receptor**
FAP	**Familial adenomatous polyposis**
FOBT	**Fecal occult blood test**
GTC	**Guanidine thiocyanate**
HNPCC	**Hereditary non-polyposis colorectal cancer**
IBD	**Inflammatory bowel disease**
IRF8	**IFN regulatory factor 8**
IRS2	**Insulin Receptor Rubstrate 2**
MAPK	**Mitogen-activated protein kinase**
mCRC	**Metastatic CRC**
MGMT	**O6-methylguanine- DNA methyltransferase**
MMR	**Mismatch repair**

MSI	**Microsatellite instability**
MSP	**Methylation specific polymerase chain Reaction**
MSS	**Microsatellite stable**
MHC-complex	**Major histocomptability complex**
NHL	**Non-hodgkin lymphoma**
NCI	**National Cancer Institute**
OSMR	**Oncostatin M receptor-β**
OS	**overall survival**
PB	**peripheral blood**
PCR	**Polymerase chain reaction**
PI3K	**phosphatidy linositol 3-kinase**
RefSeq	**Reference sequence**
RQ	***Relative Quantification***
UC	**Ulcerative colitis**
UICC	**International Union Against Cancer**
VEGF	**Vascular endothelial growth factoR**
VC	**Virtual colonoscopy**
WHO	**World Health Organization**
Wnt	**Wingless gene family**

Abstract

Background: In Egypt, colorectal carcinoma (CRC) is the sixth common cancer in males and the fifth among females. Widely studied epigenetic event in colorectal carcinogenesis is the hypermethylation of CPG islands associated with regions of tumor –suppressor genes.CGI methylation-induced gene silencing occurs early and is functionally linked with carcinogenesis. KRAS and BRAF mutations have been associated with silencing of MGMT and MLH1 genes respectively.

Method: The present study was performed to examine the quantitative gene expression of wild types of KRAS and BRAF and their corresponding epigenetic MGMT and MLH1 respectively to evaluate its role as tumour markers of CRC. We used colonic tissue biopsies of twenty six patients proven to have colorectal adenocarcinoma. For each patient we examine two samples, one from the colonic lesion and the other from adjacent normal colonic tissue. The methylation status of BRAF and KRAS was evaluated by methylation-specific qPCR (Methyl-Light)

Results: Wild types of both BRAF and KRAS were expressed (100%, 26/26) at both malignant and normal adjacent tissues with high significant difference in malignant tissues than normal tissue. Quantitation of BRAF was more significant than KRAS. Epigenetic hypermethylation of MLH1 and MGMT were highly significant expressed at malignant tissues and there was a highly significant positive correlation between qPCR of wild types BRAF and KRAS and their corresponding epigenetic MLH1 and MGMT respectively.

Conclusion:

Our observations suggest that BRAF and KRAS epigenetic hypemethylation may contribute for diagnosis of colorectal carcinogenesis at an early stage and for proper follow up and prognosis.

Key words:

BRAF, KRAS hypermethylation, qPCR , CRC, and MGMT,MLH1 epigenetics.

INTRODUCTION AND AIM OF THE WORK

Introduction:

Colorectal carcinoma is the third most common cause of cancer-related deaths worldwide. Although great proceedings have been made in diagnosis and treatment, still 40-50% of colorectal cancer patients die of the disease within five years of diagnosis***(Compton et al .,2000)***.Despite improvements in the therapeutic armamentarium for metastatic CRC (mCRC), the 5- year overall survival (OS) remains poor, with a median survival of 18 to 21 months ***(Sargent et al .,2005).*** Additional drugs, as well as further insights about the mechanisms of resistance, are needed to improve clinical outcome ***(Meyerhardt et al., 2005).*** The local and systemic activation and regulation of the immune system by malignant cells during carcinogenesis is highly complex with involvement of the innate and acquired immune system ***(Jarnicki et al., 2006)***. Despite the fact that malignant cells do have antigenic properties their immunogenic effects are minor. The causes of a weak immune response to malignant cells are multifarious and subsumed in the term "tumor immune escape". Important single mechanisms of the immune escape are down regulation of MHC-class I complex, loss of co-stimulatory surface antigens, decreased expression of apoptosis inducing death receptors (e.g. Fas/TRAIL receptor) on malignant cell, and loss of tumor infiltrating

cytotoxic T cells by tumor induced apoptosis ***(Elkord et al., 2008)***. An additional, very important aspect of the "tumor immune escape" during carcinogenesis is a significant disturbed cellular immune response ***(Khong et al .,2002)***.

Global gene expression profiling of clinical response to therapy has provided a useful means for biomarker and novel target discovery in several solid tumours ***(Minna et al., 2007).***Combination therapy (radiotherapy and chemotherapy) is the standard of care for both early and advanced disease ***(Davies et al .,2008).***

The transformation of normal mucosa to carcinoma is driven by the acquisition of mutations affecting genes involved in the control of cell proliferation, apoptosis and DNA repair. It is increasingly recognized that epigenetic changes, in which transcriptional silencing occurs independently of any change in DNA sequence, also play an important role. The most widely studied epigenetic event in colorectal carcinogenesis is the hypermethylation of CpG islands (CGI) associated with the promoter and first exon regions of tumor-suppressor genes ***(NJ Belshaw et al., 2008)***. CGI methylation-induced gene silencing, occurs early, and is functionally linked

with carcinogenesis, it offers the potential to provide biomarkers to assess an individual's risk of having or developing neoplasia ***(NJ Belshaw et al., 2008)***. KRAS mutation has been shown to be associated with epigenetic silencing of O6-methylguanine- DNA methyltransferase (MGMT), which is known to encode a DNA repair protein that removes potentially carcinogenic and cytotoxic alkyl adducts from the O6 position of guanine ***(Halford et al .,2005)***. Alterations in the MGMT gene impair the ability of the MGMT protein to remove alkyl groups from the O6 position of guanine. Therefore, alterations in the MGMT gene are thought to increase the mutational rate and the risk of cancer ***(Estelleret al., 2000)***. On the other hand, BRAF mutations have also been reported in hyperplastic polyps and serrated adenomas ***(Kambara et al., 2004)***. Subsequently, BRAF mutations have been shown to be associated with the epigenetic silencing of MLH1 but not with germ line mutation of mismatch repair genes **(*Domingo et al .,2005).***

AIM OF THE WORK:

In the current study, we will investigate epigenetic alterations in colorectal cancers by using quantitative real time PCR to measure DNA methylation**.**

REVIEW OF LITERATURE

Chapter One

Colorectal Cancer

1.1.Overview

Colorectal cancer (CRC) is the third most common malignant neoplasm worldwide and one of the leading causes of cancer death in women and men in the United States ***(Jemal et al., 2005)***. The lifetime incidence of CRC among women and men at average risk reaches 6% or one in 18 . ***(Parkin et al., 1999)***.The number of new CRC cases and reported deaths has declined in recent years due to improved screening and diagnostic methods, but it is estimated that approximately 783,000 new cases are diagnosed annually worldwide ***(Parkin et al .,1999)***. Over 95% of CRC are adenocarcinomas, and approximately half of all persons with CRC develop local recurrence or distant metastasis during the course of their illness. The median survival time for these latter patients can vary from 4 to 22 months. The mainstay of treatment for metastatic or recurrent CRC is chemotherapy, although small numbers of patients can undergo surgery or other forms of locoregional treatment. Advanced CRC has long been considered more resistant to chemotherapy. ***In Egypt*** colorectal carcinoma is one of the most common malignant neoplasms ***(Zalata et al., 2000).*** It was considered to be the fourth most common malignant neoplasm representing 6.1% of cancers in Egypt (after bladder, breast carcinoma and lymphoma) ***(El-Bolkainy et al., 1991).*** Recently, according to National Cancer Institute (NCI) statistics, in males CRC ranks the sixth most common cancer after bladder, liver, NHL, lung and leukemia, while in females it ranks the fifth common caner after breast, NHL, leukemia and liver cancer ***(El Attar et al., 2005).***

The median age of CRC cases in Egypt is 48 years for both males and females ***(El Attar et al., 2005).***Unlike western countries where CRC is prevalent among elderly people, 35% of the Egyptian patients with CRC are under 40 years of age. Moreover, the histopathological criteria and the mutational profile of the tumors diagnosed in Egypt are different from those of the western countries. In general they are of high histological grade and stage and they carry more mutations ***(Soliman et al., 1998, and Bahnassy et al., 2002)***