
بسم الله الرحمن الرحيم
وَأَنْزَلَ اللهُ
عَلَيْكَ الْكِتَابَ
والْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ
تَكُنْ تَعْلَمُ
وَكَانَ فَضْلُ
اللهِ عَلَيْكَ
عَظِيمًا

MOLECULAR BIOLOGICAL SCREENING AS A MARKER OF PREMALIGNANT STAGE IN DIFFERENT COLONIC DISORDERS

Thesis

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LIST OF ABBREVIATIONS

Abbrev. No.	Full term
FOBT	Fecal ocult blood testing
APC	Adenomatous polyposis coli.
CEA	Carcino embrionic Antigen
CRC	Colorectal cancer.
K-ras	Kirsten rat sarcoma
MSI	Micro satellite instabilty
p53	Protein with molecular weight ~53 kDa.

INTRODUCTION

Worldwide, colorectal cancer has the third and fourth highest incidence and the fifth and fourth highest mortality in females and males, respectively ***(Parkin et al., 2005).*** Despite advances made, the efficacy of therapy has reached a plateau, making early diagnosis fundamental to reduce morbidity and mortality, especially as it is known that patients diagnosed at early stages show long-term survival ***(Desch et al., 2005).***

The most widely used screening technique for colorectal cancer is the fecal occult blood test (FOBT). However, this simple, inexpensive, and noninvasive test is heavily prone to produce not only false-positive results but also false-negative results because colorectal tumors bleed intermittently ***(Bromer et al., 2005).*** On the other hand, colonoscopy, which has very high diagnostic accuracy in terms of both sensitivity and specificity, is characterized by a moderate compliance because it is invasive and not without potentially adverse events. Its use is limited to second-level diagnostic tests within screening programs ***(Bast et al., 2001).***

Numerous serum markers, such as carcinoembryonic antigen (CEA), carbohydrate antigen 19-9, and lipid-associated sialic acid, have been investigated in colorectal cancer, but their low sensitivity has induced the American Society of Clinical Oncology to state that none can be recommended for screening

and diagnosis and that their use should be limited to postsurgery surveillance ***(Kopreski et al.,2000).***

The search, therefore, continues for markers that match the diagnostic accuracy of fecal occult blood test but are less invasive than colonoscopy for the early detection of colon cancer ***(Fernandes et al., 2005).***

Free circulating DNA has produced interesting results for lung and breast cancer. The few studies of this marker done for colon cancer have shown higher levels of circulating DNA in patients than in healthy individuals ***(Thijssen et al., 2002)***, but its diagnostic relevance has not been adequately investigated. The biological characterization of circulating DNA in the blood of patients has shown that an important component derives from tumor cells ***(Johnson et al., 2002)***. Although the mechanism of DNA release into the blood is unknown, cell apoptosis or necrosis, as well as the active release of DNA, have been hypothesized ***(Stroun et al., 2001)***.

Fecal DNA tests: DNA from colorectal neoplasms is shed in the stool where it can be isolated and tested for the presence of changes acquired during carcinogenesis ***(Calistri et al., 2003)***. Not all genetic abnormalities associated with colorectal cancer can be included in the test, however, and false negative results occur ***(Lenhard et al., 2005).***The implications of "false positives," abnormal DNA testing in patients who are not found on colonoscopy to have colonic lesions, is uncertain,

and some may indicate upper gastrointestinal neoplasms or premalignant genetic abnormalities in a portion of the bowel mucosa ***(Levin et al., 2006).***

A stool DNA-based screening test for colorectal cancer, PreGen-Plus, is commercially available. An entire bowel movement must be collected for PreGen-Plus testing, and specimens must be shipped with an ice pack. Many patients are likely to find the process more complex or bothersome than FOBT ***(Woolf et al., 2004).*** The test is expensive relative to all other tests except colonoscopy, and it may be less cost-effective than colonoscopy. This technology is not currently recommended in screening guidelines, due mainly to its high cost and low sensitivity; test modifications are in development ***(Davies et al., 2005).***

Role of RAS gene:

K-ras is the most frequently mutated RAS Gene in human CRC. The importance of ras to colorectal tumorigenesis is underscored by the finding that CRC cells in which a mutated ras gene has been removed or replaced lose their ability to form tumors in nude mice ***(Imperiale et al., 2004).***

The ras oncogenes encode a family of small proteins with homology to G-proteins that regulate cellular signal transduction by acting as a one-way switch for the transmission of extracellular growth signals to the nucleus. These proteins normally cycle between an inactive GDPbound state, and an

active GTP-bound state. Ras mutations, typically point mutations, leave the protein resistant to GTP hydrolysis by GTPase, resulting in a constitutively active GTP bound protein, and a continuous growth stimulus. Posttranslational modification of the ras protein by the enzyme farnesyl transferase is necessary for activation, a fact that has been exploited for therapeutic purposes ***(Bienz et al., 2000).***

Ras mutations are found in up to 50 percent of sporadic CRCs, and 50 percent of colonic adenomas larger than 1 cm; they are rarely seen in smaller adenomas. At least two reports suggest that they are more common in proximal colon cancers than in more distal colorectal primaries. K-ras has also been implicated in the process of tumor invasion and metastasis ***(Uthoff et al., 2001).***

The lack of mutations in smaller adenomas suggests that ras mutations are acquired during later adenoma progression. However, ras mutations are not limited to dysplastic colonic lesions. Up to 100 percent of nondysplastic aberrant crypt foci (ACF, believed to be the first intermediate between normal colonic mucosa and the adenomatous polyp), and 25 percent of hyperplastic polyps have ras mutations, but their significance is unclear ***(Goss et al., 2000).***

The identification of ras mutations in CRC is of potential clinical relevance for both screening and therapy:

- The detection of ras mutations in fecal material is a potentially sensitive screening method for the early diagnosis of CRC. However, the addition of ras to a panel of other DNA markers (including APC, p53, BAT26 and L-DNA) does not improve the specificity of fecal genetic testing, and subsequent studies using the entire panel of DNA markers have not been as promising as initial reports suggested ***(van de et al., 2002).***

- The therapeutic potential of agents that target the ras signal transduction pathway (e.g., farnesyl transferase inhibitors), is being explored in patients with CRC whose tumors contain ras mutations ***(van de et al., 2002)***.

AIM OF THE WORK

To test the effectiveness of using molecular biological testing as screening method for premalignant stage in different colonic lesions.

CANCER COLON

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in both men and women. In 2011, an estimated 141,210 new cases are expected to be diagnosed, and 49,380 deaths from CRC are expected to occur ***(American Cancer Society, 2011).***

Two kinds of observations indicate a genetic contribution to CRC risk: (1) increased incidence of CRC among persons with a family history of CRC; and (2) families in which multiple family members are affected with CRC, in a pattern indicating autosomal dominant inheritance of cancer susceptibility ***(Sandler et al., 2003).***

About 75% of patients with CRC have sporadic disease, with no apparent evidence of having inherited the disorder. The remaining 25% of patients have a family history of CRC that suggests a genetic contribution, common exposures among family members, or a combination of both. Genetic mutations have been identified as the cause of inherited cancer risk in some colon cancer–prone families; these mutations are estimated to account for only 5% to 6% of CRC cases overall. It is likely that other undiscovered major genes and background genetic factors contribute to the development of CRC, in conjunction with nongenetic risk factors ***(American Cancer Society, 2011).***