Epidemiology and Molecular Genetics of Colorectal Cancer in Iran: A Review

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Although the incidence of colorectal cancer in Iranian older age subjects is currently very low compared to Western population, the younger generation is experiencing an accelerated rate approaching the Western rates and the burden of disease will increase dramatically in near future. The high frequency of positive family history of colorectal cancer in Iranian patients indicates that a significant number of colorectal cancers in Iran arise in family members and relatives of colorectal cancer patients. It is clear that the familial clustering of colorectal cancer is more often seen in younger probands and cancer located in the right side of the colon. These epidemiologic findings call for a broader attempt to promote public awareness and screening strategies in those families with a member affected by colorectal cancer, especially at younger age or with proximal tumors.

Based on our present understanding, the possibility of preventing or curing most colon and rectal cancers is now plausible. The molecular biology of colon cancer has been the subject of many researches and is better understood than those for any other solid cancer and have established an important example for cancer research. It is now clear that colorectal cancer develops as the result of genetic and epigenetic alterations that lead to malignant transformation of normal mucosa. In spite of these scientific progresses and the fact that screening can reduce the rate of death by detecting early cancer or premalignant polyps, the rate of screening is very low globally and negligible in Iran and many other developing countries which is due to cost, resistance by physicians, patients, and the healthcare system. In Iran screening should at least be started in family members at earlier age with colonoscopy as the preferred modality of screening method.

Keywords: Colorectal cancer • epidemiology • Iran • molecular genetics

Introduction

Colorectal cancer (CRC) is a leading cause of death in the Western world.1 Given the high incidence and mortality of the disease in Western populations, CRC has been extensively studied in these countries. While much attention has been appropriately drawn to the upper gastrointestinal cancers in hot spot zones of Caspian Littoral in Iran, the face of CRC in the country has remained unknown for years. This neglect to study CRC has partly been due to the very low incidence of this cancer observed and reported in studies back in 1970s. The risk of developing CRC is influenced by both environmental and genetic factors; almost all economic indices show that, since the 1979 Iranian revolution, living standards have improved dramatically across the entire country (Statistical Center of Iran, 2007, World Bank report 2006) and the country has experienced rapid development in socioeconomic status during the past three decades with significant lifestyle changes like sedentary lifestyle and the diet rich in fat and meat, and poor in cereals and fiber typical of Western population. Recent epidemiologic studies in Iran2,3 have

References

1. [Citation 1]

2. [Citation 2]

3. [Citation 3]
shown a rapid increase in the rate of CRC while there are still no preventive measures established. Elucidating the epidemiologic trend of CRC in Iran and adopting the most appropriate evidence-based screening method is necessary in order to prevent morbidity and mortality for this lethal and preventable cancer in the country.

Epidemiology

According to the estimated cancer incidence and mortality rates from recently published population-based cancer registry data covering about 22% of Iranian population,1,2,4,5 each year more than 51,000 cases of cancer are diagnosed and 35,000 deaths due to cancer occur in Iran which is the second highest number of cancer deaths in Eastern Mediterranean Region of WHO. CRC is the third most common cancer in males with the age adjusted rate (ASR) of 8.3 per 10^5, and fourth most common cancer in females with an ASR of 6.5 per 10^5. According to this study, the estimated number of new cases of CRC in Iran is 3641 each year, from which 2262 die of CRC annually, accounting for approximately 6.3% of all cancer deaths in Iran.5

The first comprehensive study on the incidence and age distribution of CRC in the country was reported by our group using a five-year data from the same population-based cancer registries.6 We found that Iran is still a low-risk country for CRC, particularly in older population. Though the ASR in young Iranian and the US population is close (Table 1), the rates are much lower in older Iranians (Figure 1). However, the results of this study revealed a significant increase in the incidence of CRC compared with the previously reported rates. The observed trend was in line with the results of two local cancer registries in other parts of Iran. A marked increase in the incidence of CRC has been shown in Shiraz from 3.96 per 10^5 population per year in 1970 – 1980 to 6.92 in 1990 – 2000.7 In Tehran CRC incidence was reported to increase sharply by 82% during the last 30 years.8 According to a recently published pathology-based national Iranian cancer registry, the ASR for CRC in males has increased from 5.5 to 8.2.5

Table 1. Age-adjusted rates per 100,000 in Iran and the US (The US rates have been calculated from the five-year age-specific SEER data from 1995 – 1999 and age structure of the US during the same years.)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Iran</th>
<th>The US</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0 – 14</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>15 – 24</td>
<td>0.6</td>
<td>0.8</td>
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<tr>
<td>25 – 34</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>35 – 44</td>
<td>4.8</td>
<td>6.8</td>
</tr>
<tr>
<td>45 – 54</td>
<td>17.7</td>
<td>13.2</td>
</tr>
<tr>
<td>55 – 64</td>
<td>34.9</td>
<td>27.1</td>
</tr>
<tr>
<td>≥65</td>
<td>37.3</td>
<td>29.2</td>
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Remarkable increases in the incidence of CRC have been reported in other populations in the past decades. Many Asian countries with traditionally low incidence of CRC have already experienced a rapid rise in their CRC incidence comparable now to rates from Western populations. The age-standardized rate in Miyagi Prefecture in Japan has increased from 10.0 per 100,000 person-years to 60.0 per 100,000 person-years in men and 40.0 per 100,000 person-years in women during 39 years.9 Similar trends have been reported in Singapore, Hong Kong, Taiwan, and urban China.9 Changes in dietary habit and lifestyle, which have been primarily shown by immigration studies to affect the incidence of CRC,10,11 are proposed to be the reason underlying the increase of CRC incidence in Asian countries.12 Sedentary lifestyle and a diet rich in fat and meat, and poor in cereals and fiber, typical of Western population, are suggested to increase the incidence of CRC.13,14 Epidemiologic studies in Iran also show a significant decrease in physical activity and an increase in fat consumption in Iranian population during the past decade.15–17

The ongoing transition in dietary habit and lifestyle, and the observed rise of CRC rates in Iran, in the light of lessons learned from Asian countries, herald a higher incidence of the disease in the country in near future. This prediction is further supported by a recent study on cancer incidence rates in Iranian immigrants to British Columbia.
Cancer incidence rates computed from population-based cancer registries in Iran and BC cancer registry in Canada showed a two-fold rise in CRC incidence among female Iranian immigrants.

However, viewing the CRC incidence rise solely as a direct consequence of westernization of diet and lifestyle may be an oversimplification suggested by clinicopathologic and molecular studies on CRCs from south-east Asia. These studies have shown that in addition to the CRCs originating from adenoma, another group of these tumors originated from flat lesions without any adenomatous elements in their vicinity. More interestingly, these latter tumors showed a different pathologic and molecular pattern from that of adenomatous tumors and were more invasive than the polypoid carcinomas pointing out to their potential distinct carcinogenic pathways. Although this type of CRC comprises up to one third of the tumors in south-eastern Asian countries, it is not frequently observed in Western CRCs.

This indicates a need for comprehensive clinical and molecular studies on CRC in each population.

Family studies and clues for genetic predisposition

CRC, similar to many other solid tumors, is a disease of aging with the majority of cases arising after 65 years of age. Several epidemiologic studies from Iran have shown that the proportion of young CRC patients is considerably higher than Western countries. Early-onset CRC (less than 40 years of age at the time of diagnosis) comprises almost one fifth of all CRC cases in the country. This feature is different from that of Western countries where the rates of early-onset CRC vary from 2% to 8%. The high proportions of young CRC cases seen in Iran could be partly explained by the young age-structure of the country (Table 1) and relatively low rates of CRC in older individuals (Figure 1). It is possible that changing environmental risk factors during recent years could contribute to the greater CRC incidence observed in younger rather than older age group in Iran. In particular, increased CRC rates during the last 20 years might be attributable to the substantial decrease in physical activity coupled with excess energy intake and change in dietary habit, and might now be reflected in younger population who shared these exposures during childhood and younger adulthood. However, although we cannot rule out some shared environmental exposures as the explanation for the higher incidence of CRC in younger Iranian, these results might also suggest a genetic predisposition for CRC in Iran.

Inherited susceptibility to CRC

During the last 15 years, several single-gene Mendelian diseases have been recognized which account for at least part of the familial aggregation detected in population studies of CRC. The most
common recognized predisposing factor for CRC is the mutations in the DNA mismatch repair (MMR) genes which can cause hereditary nonpolyposis colorectal cancer (HNPPC) or Lynch syndrome. Other syndromes that predispose to CRC are due to mutations in the base-excision repair gene MYH, and familial adenomatous polyposis (FAP) which is due to mutations in the APC gene; together these conditions account for fewer than 5% of CRC cases and are inherited in an autosomal dominant fashion, that are associated with a very high risk of developing colon cancer.

One of the major clinical hallmarks of this inherited susceptibility is the occurrence of the CRC at a young age.

In a study aimed at characterizing the profile of familial CRC aggregation in Iranian patients, we observed a relatively high frequency of familial clustering of CRC among Iranians. There were significant differences in distribution of tumor sites between those with and without family history of CRC. In patients with positive family history of CRC the most frequent affected site of colon was the right side. In this series the clinical diagnosis of HNPPC was established in 4.7% of probands, which is relatively high in comparison with most Western populations, reporting a frequency of 1.7% to 4%. Moreover, family history of CRC along with clinical diagnosis of HNPPC was significantly more prevalent among younger Iranian patients. The high frequency of family history of cancer among Iranian CRCs, observed and reported by our group, has been investigated and observed separately by another recent study. The familial clustering of CRC among Iranian CRCs along with the high proportion of young CRC cases in the country are important clues suggesting the need for genetic studies at the molecular level in this population.

A significant proportion of CRC clustering, especially those fulfilling Amsterdam Criteria, has been attributed to defects in genes involved in MMR. MMR genes encode proteins for correcting DNA nucleotide base mispairs and small frame shifts that frequently occur during DNA replication. Inactivation of MMR system, mostly due to the hereditary event, leads to the genome instability classically traced back by alterations in the short repetitive markers in the genome namely “microsatellite instability” (MSI).

In an attempt to characterize the molecular basis of the observed clustering of CRC, we examined 200 Iranian CRCs for microsatellite status. After DNA extraction and purification from microdissected normal and tumor from each patient, two mononucleotide repeats, BAT25 and BAT26, shown to be the most sensitive markers for detecting high MSI status (MSI-H) and...
regarded as sufficient for the identification of CRCs with MMR defect, were analyzed by PCR and fragment analysis. MSI status was observed in 23% of the cases and was more frequent in early-onset (age <40) CRCs than in later-onset tumors, and in tumors located in the proximal colon than in distal tumors (Figure 2).

The relatively high percentage of MSI—an indirect marker of genetic predisposition in CRCs—in our series, and its predominance in early-onset and proximal tumors are in concordance with our previously cited clinical findings indicating a high percentage of familial clustering of CRC among Iranian CRCs, especially in early-onset and right-sided tumors. Alterations in MMR genes, among which hMLH1 and hMSH2 are the mostly mutated genes, have been identified to be responsible for MSI status, and the genetic predisposition to CRC particularly in HNPCC families. Therefore, immunohistochemical (IHC) analysis was performed in the same series of patients to examine the expression of MMR proteins (including hMLH1 and hMSH2). The result of this study has not been reported yet, but is in line with our MSI analysis.

Furthermore, we are analyzing the fulfilled Amsterdam Criteria patients along with the patients with MSI for germ line mutations in hMLH1 and hMSH2. The entire coding regions and flanking intronic regions of hMLH1 (58 kb with 19 coding exons) and hMSH2 (73 kb with 16 exons) are being screened for mutations. The result of this study would give us an insight of the prevalence of MMR gene mutations in Iranian HNPCC which could be used for implementing preventive strategies in familial CRCs.

Molecular genetics of sporadic CRC
Apart from the hereditary syndromes in CRC whose genetic bases are relatively well-defined, most colorectal tumors do not occur in the families and are not the consequence of a single genetic alteration.

Over the past two decades, molecular studies on CRC have increased our knowledge about the genetic changes involved in the malignant colorectal transformation process. Somatic mutations in APC, K-ras, and p53 were proposed to act sequentially in progression from normal mucosa to carcinoma in CRC. This model contributes to the somatic cell transformation most importantly in sporadic cases of CRCs. A recent study shows that a total of 189 “candidate” cancer genes with a large number of previously uncharacterized cancer genes can alter in CRC. Most of the identified candidate genes had never been found mutated in cancer before. These new findings indicate that CRC is a more complex genetic disease than was anticipated in the past.

The molecular/genetic markers in CRC could be affected by environmental and/or genetic background, and thus studying and comparing CRCs from populations with different epidemiologic features of the disease can help us better understand the underlying mechanism of CRC tumorigenesis. The source of current data on the molecular alterations in CRC has been mostly from studies on Western populations, and little information available on molecular features of tumors from countries with different epidemiologic pattern of the disease such as Iran. Our group screened tumor samples of Iranian CRCs for K-ras mutations at codons 12, 13, and 61 by sequencing analysis. Clinical data and MSI analysis were used to correlate frequency and the spectrum of K-ras mutations. Interestingly, CRCs with suspected genetic predisposition (HNPCC or MSI) were associated with specific type of mutation in K-ras.

We also compared our results with those observed in a series of CRC patients from Italy, a country with remarkably higher incidence of CRC. This comparison showed a significant difference in mutation spectra namely the G to A transition in the second base of codon 13 of K-ras with significantly lower frequency in Iranian series. This type of mutation has been previously shown to be correlated with high consumption of refined grain—a dietary pattern directly associated with the CRC risk, and common in Italy—whereas the main staple in Iran is wheat-based with a variety of unrefined, unleavened, whole-wheat breads which could partly explain the lower incidence of CRC in Iran.

In another study, we focused on p53 alteration in the Iranian CRCs by both p53 gene mutation analysis of exons 5 – 8 using PCR-direct sequencing and analysis of protein expression by IHC. This study showed that p53 mutations in the Iranian CRCs occur as frequently as in the non-Iranian series indicating the significant role of p53 alteration in CRC progression independent of the population of study. However, we found for the first time that proximal and distal tumors harbor different p53 mutational spectra when the site and
type of mutation is taken to account. Distal CRCs showed a higher frequency of G to A transitions at CpG whereas G to A transitions at non-CpGs were more frequent in proximal tumors. Comparing the distribution of mutated codons also revealed different spectra between the two sites. As we have shown in our series (Figure 2), and was previously proposed, tumors from proximal and distal part of the large intestine may undergo different tumorigenesis pathways. On the other hand, different types of mutations at p53 such as G to A transitions at CpGs vs. non-CpGs can be involved in alternative mutagenic mechanisms. Therefore distinct types of P53 mutations between proximal and distal tumors, observed in our study, could be expected.

Interestingly, although the CpG mutations were less frequent in proximal part, mutations at codon 213—one of the CpG sites of p53 gene—stood as the most frequent mutation site in the proximal part. Notably, reports on esophageal cancers from Iran had shown distinct type of mutations in esophageal squamous cancers from Iran with reportedly high frequency of the mutations at first nucleotide of codon 213. These findings prompt further studies to search for possible environmental influences on mutational pathways involved in carcinogenesis of gastrointestinal cancers in Iran.

Another pathway in CRC is the methylation of CpG islands in the promoter regions of the key genes involved in cell cycle or MMR system, and the subsequent transcriptional silencing and inactivation of these genes. This, therefore, is a good example of the environmental influence, including nutrition on the gene expression, and studying this pathway in the view of different epidemiology of the disease and dietary profile in our population would be of importance.

A recent study on the associations between a common polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR C677T), the circulating levels of folate/vitamin B12, and promoter methylation of tumor-related genes including P16, hMLH1, and hMSH2 among sporadic CRCs in Iran have shown that there is a positive association between high serum folate/vitamin B12 levels with promoter methylation of those genes and risk of CRC, but in association with the particular MTHFR polymorphisms, and not all the genotypes. They also found that this effect could be different based on the tumor location and age of the individual.

The authors concluded that caution should be practiced in the mandatory fortification of cereals with folic acid. One of the strengths of this report is the population they studied and the fact that none of the patients used vitamin supplementation in their life, allowing for the measurement of the association of the serum methyl donor and methylation status in physiologic range. While previous studies of this type have been done in Western populations where individuals are exposed to several different supplements during their life which make it sometimes more tedious to search for associations.

Further studies on this pathway is needed especially in the setting of the observed relatively high percentage of MSI tumors in Iranian CRCs, given the fact that the majority of MSI could be the result of silencing of hMLH1 expression secondary to the methylation of hMLH1 promoter region.

Finally, molecular markers in CRC such as mutations, polymorphisms, chromosomal alterations, protein expressions, and growth factors have been shown to significantly influence the disease progression and/or response to therapy. A study on locally-advanced rectal cancer from Iran treated with radiotherapy has shown that epidermal growth factor receptor (EGFR) expression may serve as a predictor of pathologic response to treatment in these tumors and thus could be used to identify a subgroup of high-risk patients who may benefit from more therapeutic modalities.

Two large retrospective reports on CRCs from Iran tried to identify the prognostic factors focusing on clinical characteristics of the tumors. The grade and stage of the tumor, and regional lymph node and distant metastasis were among the variables which were found by both studies to independently affect the prognosis. Although the patient’s age at diagnosis was found by one report as a prognostic factor, it was not of prognostic significance in another report. In none of these reports the location of the tumor, previously introduced by some studies as a prognostic factor in CRCs, was found of significance in the disease prognosis. Conflicting results on prognostic factors in CRC by different survival studies highlight the complexity of the task of determining the outcome of CRC on a retrospective study design. More importantly, given the presence of several carcinogenetic pathways in CRC, stratifying these tumors based only on clinical characteristics might be an under-
estimation. Therefore, prospective studies along with studies of molecular markers in CRC are necessary for predicting the course of the disease and choosing the appropriate treatment.

**Future prospects**

Our studies indicate that although CRC incidence in Iran is currently low, the population is in transition and may be experiencing an acceleration of the disease burden in near future. This hypothesis is further supported by the results of a study by Haghighi and colleagues on 801 large intestines from southern Iran in 1976. The authors found a much lower rate of adenomatous polyps in large bowels of Iranians over a 22-year period (1952 – 1973) compared to Americans of the same age, both in younger and older ages. Currently, CRC rates are still much lower in older Iranians; however, the rates are close in young Iranians and Americans. Therefore, we may see a cohort effect, and in the next decades, the CRC rates in older Iranian population may become close to those from high-risk countries such as the US.

The high frequency of positive family history of CRC in Iranian patients indicates that a significant number of CRCs in Iran arise in family members and relatives of CRC patients. Furthermore, we have shown that the familial clustering of CRC is more frequent in younger probands and right-sided tumors. These findings call for a broader attempt to promote public awareness and screening strategies in those families with a member affected by CRC especially at younger age or with proximal tumors. Screening should be started in family members at earlier age with colonoscopy as the preferred modality of screening method.

It has been shown that CRC in the young patients appears to be more aggressive, to present with advanced stages, and to have poorer pathologic findings. Given the fact that there are a lot of young-onset CRCs in Iran, healthcare providers should have heightened awareness when caring for early-onset CRCs.

We have shown that the K-ras mutational spectrum could be differentially influenced by genetic and environmental factors. A particular type of K-ras mutation which is linked to high consumption of refined grain is significantly lower among Iranian CRCs. This could be mainly due to the Iranian diet which is based on unrefined whole-wheat breads. This needs to be further supported by population-based nutritional studies.

Wholegrain foods have been shown to reduce the risk of several types of neoplasms, particularly of the digestive tract, including CRC. The current transition in Iranian dietary habit to diets richer in refined grain such as white bread, rice, and pasta as the main staple should be prevented by educating the public about their hazards.

By analyzing p53 alterations in the CRCs from Iran, we have shown different spectra of p53 mutations associated with alterations in K-ras and microsatellites, and also between distal and proximal tumors that further indicate distinct mutagenesis pathways of different types of p53 mutations, and also different tumorigenesis mechanisms between the sites of the large intestine.

Further molecular analysis is being performed at both somatic and germline levels. An ongoing prospective study on consecutive series of CRC patients from two main referral hospitals in Tehran has been started by our group, to collect proper samples and data for larger and more detailed molecular studies.

Extensive studies at clinical and molecular levels through collaboration between different centers in the country, along with international collaboration for more extensive molecular studies will help to unfold the mechanisms underlying the CRC carcinogenesis and find sensitive markers for early diagnosis and more effective therapy.

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