Ann Colorectal Res. 2015 September; 3(3): e30100. doi: 10.17795/acr-30100

Published online 2015 September 9. **Review Article**

Molecular Biomarkers of Colorectal Cancer: A Review of Published Articles From Iran

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Received 2015 May 19; **Revised** 2015 June 1; **Accepted** 2015 June 3.

Abstract

Context: Colorectal cancer is one of the most common cancers worldwide (the third most common cancer in the world) and is especially more common in Western countries; however, its incidence has been increased significantly during the last few years in Eastern countries such as Iran and considered as one of the five common cancers in this country. According to molecular pathways, numerous biomarkers have been identified for colorectal cancers which help patients' management.

Evidence aquisition: In this study, we tried to review published articles about the molecular biomarkers of colorectal cancer from Iran. We searched medical databases such as google scholar, Scopus, PubMed, Magiran, SID and Iran Medex for keywords of "colon cancer, KRAS, BRAF, mismatch repair gene, Microsatellite instability, molecular genetics, molecular pathogenesis, biomarker and Iran" to find studies published about colorectal cancers from Iran regarding molecular biomarkers.

Conclusion: This study showed that molecular biomarkers in colorectal cancer of Iranian patients are not so different from Western population.

Keywords: Biological Markers, Colorectal Cancer, Cancer

1. Introduction

Colorectal cancer is one of the most common cancers worldwide (the third most common cancer in the world), and is especially more common in Western countries; however, its incidence has been increased significantly during the last few years in Eastern countries such as Iran and considered as one of the five common cancers in this country (1). According to the annual reports of the Cancer Institute, colorectal cancer is the third common cancer in Iranian women and the fifth common cancer in Iranian men. Incidence rate of the disease has been increased during the past 25 years in Iran (2).

The other important finding about this cancer is significant surge of incidence in younger age population compared to older population (3). The underlying cause of this epidemiological increase can be due to changing in lifestyle and environmental factors; however, familial clustering and genetic predisposition should be considered (4). Colorectal cancers can be sporadic, familial and hereditary. HNPCC (hereditary nonpolyposis colon cancer) or Lynch syndrome is the most common form of hereditary colorectal cancer accounting for 5 - 10% of entire colorectal cancer population. Based on previous studies in Iran, clinical diagnosis of HNPCC was observed in 4.7% of probands in Tehran (5).

Molecular pathogenesis of colorectal cancer has been extensively studied and its molecular carcinogenesis has been identified far more than any other cancer. Recent advances in molecular biomarkers to personalize therapy contributed to a major progress in the treatment and prognosis of the disease (6). Many of these molecular changes and biomarkers are now a part of routine laboratory tests and some would be in the near future.

There are several molecular pathways leading to colon cancer. The three most important recognized pathways are 1) Genomic instability which can be somatic and germ line; several forms of genomic instability are chromosomal instability in tumor suppressor genes such as SMAD-4, TP53 and APC (Adenomatous polyposis gene); DNA repair defects (in mismatch repair genes) and aberrant DNA methylation are two other patterns of chromosomal instability; 2) Mutational inactivation of tumor suppressor genes mostly in APC (adenomatous polyposis gene), TP53, TGF-β tumor suppressor pathway, and mismatch repair genes (MMR) and 3) Activation of oncogenic pathways of RAS, BRAF, Phosphatidyl inositol 3-kinase (PIK-3) (7).

According to these molecular pathways, numerous biomarkers have been identified for colorectal cancers to help patients' management (8). In this study, we tried to review published articles about the molecular biomarkers of colorectal cancer from Iran. We searched medical databases such as google scholar, Scopus, PubMed, Magiran, SID and Iran medex for keywords "colon cancer, KRAS, BRAF, mismatch repair gene, Microsatellite instability,

molecular genetics, molecular pathogenesis, biomarker and Iran" to find studies published about the colorectal cancers from Iran regarding molecular biomarkers.

2. Microsatellite Instability (MSI)

Microsatellites or simple sequence repeats are repeating sequences of DNA. The accuracy of the genome is regulated by multiple mechanisms, one of which is the correction of DNA replication errors by DNA MMR mechanism. Alterations in MMR genes can cause MSI. During DNA replication, short segments of the repeated bases of DNA, which are found throughout the human genome (as microsatellites), are the subject of insertion or deletion types mutations that can change the length of these microsatellites (9).

In the previous reports of different geographic parts of the world, MSI is detected in 15% of colorectal cancers due to germ line mutation in one of the mismatch repair genes (MLH1, MSH2, MSH6 and PMS2) or to epigenetic silencing (methylation) of MLH1. About 3% of these are associated with HNPCC (hereditary nonpolyposis colon cancer) or Lynch syndrome and the other 12% are seen in sporadic colorectal cancer. On the other hand, 15% - 20% of sporadic and almost all (more than 90%) patients with Lynch syndrome show microsatellite instability (10).

Germline mutations in HNPCC show some novel types in Iranian population, which is different from western countries. Identification of these mutations is helpful in different populations and can help manage colorectal cancer in these populations by screening, prevention strategies and following up suspected HNPCC families (11, 12).

Polymorphism in certain genes of MLH1 in some reports have been identified in Iranian population, some of which can predispose people to colorectal cancers (13). MSI can be tested in colorectal cancer by immunohistochemistry (IHC) or molecular methods, both of which have limitations and advantages (13). The basic tests identify five markers (NR-21, BAT-26, BA T -25, NR-27 and NR-24) to devise the simplest diagnostic assay (9). Previous reports have shown significant diversity regarding the most common marker for microsatellite testing; BAT 25, BAT26 and NR21 have been reported in HNPCC and sporadic CRCs (9-28).

Table 1 shows the reports from Iran that studied the frequency of MMR genes in both hereditary and sporadic colorectal cancers. As it shows, the frequency of MSI in patients with HNPCC has been 29% - 62.5% (9-25), there are not so many studies about the frequency of MSI in sporadic CRC from Iran; however, the reported frequencies of MMR have been from 19.4% to 66.6% (9-25).

Another important clinical implication of MSI testing in sporadic CRC is its effect on responsiveness to therapy, stage and prognosis. Patients with MSI-H tumors had better survival rates compared with those with non-MSI-H (i.e., MSS, MSI-L) tumors. MSI-H was also found to be associated with lower tumor stage at diagnosis. Subsequently, MSI-H colon cancers have a more favorable survival compared with non-MSI-H tumors that was independent of tumor stage (23). The clinicopathologic reports from Iran in colon cancers with MSI are very controversial. In sporadic CRCs, Faghihi et al. reported more cases in distal and rectal part of colon in MSI-I cases. (21) The report by Moghbeli et al. is more compatible with other countries (20), i.e. MSI CRCs have been more common in females and older age group, also had lower stage and less lymph node metastasis as well as more proximal location (20). Brim et al. reported less chromosomal aberrations and high frequency of methylation in genes such as MMP2 in Sporadic MSI CRCs (22).

3. KRAS Mutation

Management of metastatic colorectal carcinoma (mCRC) has been considerably improved after discovering a number of novel drugs, including targeted agents like bevacizumab, cetuximab, and panitumumab. The overall survival of advanced disease has been considerably improved by adding newer targeted biologic agents. In the recent years, more studies have been

Table 1. Reported MMR Gene Mutations from Different Studies on Iranian Population

Author	Year	Study Population	Number of Cases	Methology	MSI[a]	MSI-H[a]	MSI-L[a]	MSS[a]	Most Common Instable Marker
Bishehsari et al. (16)	2006	HNPCC	12	PCR-Sequencing	100	100	None	0	-
		Sporadic	170		19.40	19.40		80.60	
Salehi et al. (17)	2008	HNPCC	32	PCR-SSCP-Sequencing	62.50	-	-	37.50	-
Haghighi et al. (19)	2010	HNPCC	78	PCR-sequencing	41	26.90	14.10	59	NR- 21
Moghbeli et al. (20)	2011	Sporadic CRC	67	PCR-sequencing	43.30	26.90	16.40	56.70	BAT-25
Shemirani et al. (9)	2011	HNPCC	80	PCR -sequencing	-	-	-	-	NR-21
		Sporadic CRC	80		-	-	-	-	
Faghihi et al. (21)	2012	Sporadic	96	PCR-sequencing	22.90	-	-	77.10	BAT-26
Brim et al. (22)	2014	Sporadic	27	PCR-Sequencing	66.60	14.80	18.50	44.40	-
Zeinalian et al. (24, 25)	2015	HNPCC	31	IHC and PCR-sequencing	29	19.40	9.60	61	BAT-26

[a]Data are presented as %.

focused on selecting patients who would benefit from these targeted therapies. This focuses on the role of the KRAS mutation in the growth and histopathology of the tumor, clinical outcomes and management choice of cancer of the large bowel (19, 20). This proto-oncogene, KRAS, is regularly mutated (30 - 50% in different surveys) in CRC. Roughly, 90% of the activating mutations that are influential solitary amino acid replacement in the GTPase pocket and guide a block of the activity of KRAS-p21 protein, are recognized in codons 12 (GGT) and 13 (GGC) of exon 1 and almost 5% in codon 61 (CAA) situated in exon 2. The most regularly found kinds of mutations are G > A and G > T transitions (21). KRAS testing has a vital improvement in the treatment of CRCs, especially after metastasis (19, 20, 22). There are very few studies about the frequency of K-ras mutation from the Middle East and Iran; consequently, in the present study, we reviewed KRAS mutation rate and spectrum in previous studies from Iran.

As Table 2 shows, the prevalence of KRAS mutation in Iran is similar to studies from other countries; the overall reported prevalence from different countries have been as low as 20 to 50%, and in the few studies from Iran, this was 12.5% - 37.4% (29-32). The most common KRAS mutation in most previous studies has been 12G - A, and in one study from south of Iran was 12 G - C (29-32).

4. BRAF Mutation

BRAF is the last discovered member of RAF family proteins in MAPKinase signaling pathway, which acts with KRAS as a downstream serine threonine kinase effector downstream to epidermal growth factor receptor (EGFR), promoting cell proliferation (33). The most prevalent mutation in the BRAF gene in all cancers involves transversion of thymidine to adenosine at nucleotide position 1799 of exon 15, leading to conversion of GTG codon (Valine) to GAG (Glutamic acid), labeled as V600E, accounting for more than 90% of the observed mutations in this gene (33, 34). V600E BRAF gene mutation is associated with older age, female gender, proximal colon location, poor differentiation, mucinous histology, infiltrating lymphocytes and advanced stage (35, 36). BRAF mutations occur more frequently in MSI and CIMP-H (Cpg island methylator phenotype-high) CRCs and only rarely with MSS CRCs and mutually exclusive with KRAS mutations (35-37). BRAF seems to be an independent negative prognostic factor in CRCs (38, 39). In some studies, BRAF gene mutation analysis has been suggested for exclusion of HNPCC Syndrome (40, 41). Two monoclonal antibodies, Cetuximab and Panitumumab, target EGFR, and have been approved to treat metastatic CRC (42). Analysis of mutational status of the BRAF gene is recommended before initiating these new targeted therapies in metastatic CRC patients (42, 43). Most previous reports from different parts of the world showed that frequency of BRAF gene mutation is usually low. The frequency of BRAF mutation has been reported as low as zero in Thailand (44) and Mexico (45) to 12.2% in Australia (46). Studies from Western countries such as the USA (United States of America) reported the prevalence of about 9% - 10% (47). There are very few studies from Iran. Table 3 shows previous studies from Iran with BRAF mutation prevalence from 0 to 3.7% (18, 30, 48, 49).

5. P53

TP53 is the pivotal mediator of growth arrest and apoptosis in response to DNA damage. It stops cell cycle in damaged cells until alteration is properly repaired, otherwise it starts apoptosis cascade in damaged cells. Human P53

Table 2. Reported KRAS Mutation in Previous Studies on Iranian Population

Study	Year	Number of Cases	Methodology	Prevalence of KRAS Mutation
Shemirani (29) [a]	2011	48	PCR-Sequencing	12.5%
Naghibalhossaini (30)	2011	86	PCR-RFLP	28%
Bishehsari (16) [a]	2006	182	PCR-Sequencing	37.4%
Sobhani (31) [a]	2010	59	PCR-Sequencing	20.3%
Omidifar (32) [a]	2015	100	PCR-Sequencing	32%

[a]Most common mutation 12 G - A.

Table 3. Reported BRAF Mutation in the Previous Studies in Iranian Population

Study	Year	No of CASES	Methodology	Percentage
Brim (18)	2008	53	PCR-Sequencing	2%
Naghibalhossaini (30)	2011	110	PCR-RFLP	0
Ghaffarpour (48)	2011	27	PCR-Sequencing	3.7%
Javadi (49)	2014	100	PCR-Sequencing	0

gene is composed of 11 exons and codes a protein with 393 amino acids (50). About 13 types of polymorphisms have been described in this gene (51). These polymorphisms can affect colorectal cancer risk, prognosis and response to treatment (52, 53).

There are about 9 studies from Iran regarding different roles of P53 gene in colorectal cancer. One of the most frequent studied polymorphism has been in codon 72, exon 4. The studies regarding this polymorphism have shown that different people with different genotypes in this codon can have more risk of developing colorectal cancer in patients from Iran (54-56). There are also reports about the prognosis and response to specific treatment in patients with some mutations in exons 4, 5 and 6 (57, 58). This biomarker is still not a part of recommended biomarker panel for patients with colorectal cancers; however, some studies recommended evaluation of specific mutational analysis in P53 to predict response to treatment and prognosis (59).

6. Other Biomarkers in Colorectal Cancer

In addition to the above mentioned most common and important molecular biomarkers of colorectal cancer, there are other studies regarding less common and known biomarkers in colorectal cancer of Iranian patients in both genes of hereditary cancers such as APC (adenomatous polyposis coli) gene and sporadic CRCs such as Smad-7, EGF (epidermal growth factor) and MGMT (O^6-ethylguanine-DNA methyltransferase) (60-63). There are very few studies in Iranian population regarding these newly recommended biomarkers such as NRAS and PIK-3CA to be the source of decision about prognosis or risk of CRC in Iranian patients with this cancer.

7. Conclusions

CRC is one of the most common cancers in Iranian population, which emphasizes the need to find better methods to screen, diagnose and treat patients with this cancer. Molecular biomarkers can help in prediction of the risk of CRC in people, early diagnosis of this cancer, treat patients more efficiently and safely and at last increase the survival of patients with this cancer.

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