Review Article

Genomic and Epigenetic Instability in Colorectal Cancer

Akram Safaei[1], Sara Sobhi[2], Mostafa Rezaei-Tavirani[3], Mohammad Reza Zali[4]

Abstract

Colorectal Cancer (CRC) is the third most common cancer in men and the second most common cancer in women worldwide. Both genetic and epigenetic alterations are common in CRC and are the driving force of tumorigenesis. Chromosomal instability, microsatellite instability and CpG island methylator phenotype pathways are responsible for genetic instability in colorectal cancer. Chromosomal instability pathway consists of activation of proto-oncogenes and inactivation of tumor suppression genes and Loss of Heterozogosity (LOH). In this review, we discuss genetic and epigenetic phenomena that can be suggested as biomarkers in colorectal cancer.

Keywords: Epigenomics; Genetic; Colorectal cancer

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1. Students' Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Gastroentrology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author: Mostafa Rezaei-Tavirani, PhD;
Associated Professor of Biophysics
Tel: (+98) 2122714248
Email: rezaei.tavirani@ibb.ut.ac.ir

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Introduction

Colorectal Cancer (CRC) is shown as the second most common leading causes of death from cancer in developed countries. Even though colorectal tumorigenesis is a complex process, epidemiological and experimental data indicate that change in some protein level has a role in the development of CRC. Colonoscopy is still the most accurate test for colorectal cancer screening; however, it is costly and is associated with procedure-related complications as well as poor patient compliance. In contrast, another commonly used colorectal cancer screening test, Fecal Occult Blood Testing (FOBT) is inexpensive and simple to perform, but has a relatively low sensitivity and specificity [1]. Advances in understanding the molecular pathology of colorectal cancer, has led to identification of promising early detection molecular markers for use in non-invasive colorectal cancer screening assays[2]. Cancers can be characterized by patterns of changes in gene expression. Genes that mediate tumorigenesis can be broadly characterized as oncogenes; which are activated by alterations, and tumor suppressor genes; which are inactivated during tumorigenesis [3]. Tumor suppressors restrain growth and proliferation, passage through the cell cycle, motility, invasion, or other functions related to stable differentiation. Genes that encode tumor suppressors are commonly inactivated by deletion, mutations, promoter methylation, or other changes in regulation. Colorectal Cancers (CRCs) develop gradually over a long period of time through the sequential accumulation of genetic alterations [4]. It is now appreciated that there are multiple molecular pathways to colon cancer, and that these pathways involve both mutations and epigenetic alterations. For example, serrated polyps are associated with microsatellite instability and aberrant DNA methylation, whereas tubular adenomas more commonly arise via inactivation of the Adenomatous Polyposis Coli (APC) tumor suppressor gene and concurrent genetic alterations resulting from chromosomal instability [5].

A biomarker is a substance that is objectively measured that indicates the presence of an abnormal condition within a patient and allows disease progression and/or therapeutic response to be monitored [6]. Biomarkers provide a powerful

and dynamic approach to understanding the spectrum of malignancies with applications in observational and analytic epidemiology, randomized clinical trials, screening, diagnosis and prognosis[7]. In this review, we will provide a scenery of the role of genetic and epigenetics in colorectal cancer, and will discuss applications of these epigenetic alterations as biomarkers for early detection, diagnosis, prognostication and management of patients with colorectal cancer.

Genetic mutations

Some studies suggested mutations in some gene are associated with colorectal cancer [8-11]. Mutation in APC gene cause to inactivation of APC that leads to activation of the Wingless/Wnt pathway, a common mechanism for initiating the polyp□cancer progression sequence [12]. KRAS and TP53 mutations as well as mutations in genes that regulate important cell signalling pathways such as the Transforming Growth Factor –β 1 [TGFB1] signalling pathway was reported [13]. Mutations in KRAS or BRAF occur in approximately 55–60% of colorectal cancer, aberrantly activating the MAPK signalling pathway, inducing proliferation and suppressing apoptosis [14, 15].

Tomlinson et al. examined 550 k SNPs in 930 cases of CRC with familial histories of the disease, and identified rs6983267 at 8q24.21 as the most common SNP associated with CRC [16]. This finding was confirmed by the additional screening of 7,334 cases of CRC, which gave an Odds Ratio (OR) of 1.27 [P = 1.27 9 10-14][17]. Zenka et al investigated 100,000 SNPs in 7,480 cases of CRC, and discovered that SNPs at 8q24 [OR 1.18, P = 1.41 9 10-8] as well as at 9q24 [OR 1.14, P = 1.32 9 10-5] were associated with the incidence of CRC [18] . Kupfer et al also reported the significance of rs10795668 at 10p14, which was associated with CRC [19]. Tenesa et al reported SNPs in a screen of more than 14,500 CRC cases, finding that 11q23 [rs3802842: OR 1.1, P = 5.8 9 10-10], 18q21 [rs4939827: P = 5.8 9 10-10], and 8q24 [rs7014346: OR 1.19, P = 8.6 9 10-26] were associated with CRC [20]. In a study of CRC patients, Pittman et al identified 11q23 [rs3802842: OR 1.17, P = 1.08 9 10-12] as an important SNP [21]. One study in Japan was found that three SNPs—rs6983267, rs10808556 on 8q24, and rs10411210 on 19q13—were significantly correlated with the incidence of CRC in Japan [22-26]. In a Swedish-based cohort, von Holst et al reported 11loci that were associated with an increased or decreased risk of colorectal cancer, including 8q23.3 [rs16892766], 8q24.21 [rs6983267], 9p24 [rs719725], 10p14 [rs10795668], 11q23.1 [rs3802842], 14q22.2 [rs4444235], 15q13.3 [rs477 9584], 16q22.1 [rs9929218], 18q21.1 [rs4939827], 19q13.1 [rs10411210], and 20p12.3 [rs961253]. Of those 11 loci, 8q23.3, 8q24.21, 10p14, 15q13.3, and 18q21.1 showed statistically significant odds ratios similar to the previously published findings. Also, it was reported polymorphism in insulin pathway role in potential risk for a disorder but, such data are highly heterogeneous [27-29].

1. Microsatellite instability:

Microsatellites are DNA sequences in which a short motif of 1–5 nucleotides are tandemly repeated ten to hundred times. Microsatellites are prone to mutation during replication due to transient split of the two helical strands and slippage of the DNA polymerase complex at re annealing, which generate an insertion or deletion loop depending on slippage direction. Unless such mismatch is corrected, the loss or gain of repeated units on the daughter strand results in length variation termed microsatellite instability [MSI][30]. Instability manifests as small increases or decreases ["instability"] in the number of repeats in microsatellites throughout the genome because of defects in Mismatch Repair (MMR) genes. These unrepaired alterations contribute to carcinogenesis along a distinct pathway [the MSI pathway] that differs from the chromosomal instability [31]. Approximately 15% of Colorectal Cancers (CRC) display MSI owing to either epigenetic silencing of MLH1 or a germline mutation in one of the mismatch repair genes MLH1, MSH2, MSH6 or PMS2 [32]. Discovery of MSI in colorectal tumors has increased awareness of the diversity of colorectal cancers and implications for specialized management of patients [33]. It became apparent that a subset of colorectal tumors were characterized by a large number of mutations at microsatellite sequences [34]. Several genes affected by MSI were then identified that encoded regulators of cell proliferation [GRB1, TCF-4, WISP3, activin receptor-2, insulin-like growth factor-2 receptor, axin-2, and CDX], the cell cycle or apoptosis [BAX, caspase-5, RIZ, BCL-10, PTEN, hG4-1, and FAS], and DNA repair [MBD-4, BLM, CHK1, MLH3, RAD50, MSH3, and MSH6] that provided an important roll into the carcinogenetic pathway [35].

Colorectal tumors with MSI have an increase in the number of point mutations compared to cancer cells without MSI for example mutations in □catenin that make it unable to interact with APC protein [36]. Microsatellite instability occurs in approximately 15% of colon cancers and results from inactivation of the mutation Mismatch Repair (MMR) system by either MMR gene mutations or hypermethylation of the MLH1 promoter [37]. MSI promotes tumorigenesis through generating mutations in target genes that possess coding microsatellite repeats, such as TGFBR2 and BAX [38].

2. Loss of chromosome:

Another promising prognostic marker is allelic loss of chromosome, which is highly prevalent in CRC [39-41]. For example the long arm of chromosome 18 contains several genes of potential importance in CRC pathogenesis and progression. Among the genes located on 18q are the DCC tumor

suppressor gene, which codes for a neutrin-1 receptor important in cell adhesion and apoptosis; the SMAD4 gene, which codes for a downstream signal transducer in Transforming Growth Factor [TGF]-β 1 signalling involved in tumor suppression; and the SMAD22 gene, involved in endodermal differentiation [39, 42].

Chromosomal abnormalities in CRC have been studied by multiple groups using either Comparative Genomic Hybridization (CGH) or array Comparative Genomic Hybridization (aCGH) [43, 44]. There are three known pathways in CRC pathogenesis: Chromosomal Instability (CIN), Microsatellite Instability (MSI), and the CpG Island Methylator Phenotype (CIMP) pathways [45]. Some of the believed consequences of CIN are loss of tumor suppressor genes and amplification of oncogenes in the affected regions [46, 47]. Truncating Apc mutations can lead to both quantitative and qualitative ploidy changes in primary mouse cell lines, mainly due to kinetochore and centrosome abnormalities [48].

LOH is defined as loss of one of the two copies or alleles of a gene. Often the remaining allele is affected by a mutation. Contrary to the common types of transmembrane receptors, DCC (Deleted in Colorectal Carcinoma, DCC is a "conditional tumor suppressor gene") blocks cell growth in the absence of its ligand, netrin-1. Approximately 70% of CRCs show LOH in the DCC gene region. Netrin-1 is produced deep in the crypts of the colorectal mucosa. When the DCC gene is mutated, netrin-1 will not bind to DCC transmembrane protein, resulting in abnormal cell survival [49].

Epigenetic Biomarkers

Epigenetic alterations play a major role in the initiation and progression of Colorectal Cancers (CRCs). Even in the hereditary CRCs, cancer progression is the result of the progressive accumulation of both genetic and epigenetic alterations. Chromatin remodeling through histone modification is an important mechanism of epigenetic gene dysregulation in human cancers [50]. The epigenetic mechanisms currently believed to play a role in cancer include:
1)DNA methylation of cytosine bases in CG rich sequences, called CpG Islands; 2) post-translational modifications of histones, which are proteins that form the nucleosomes, which regulate the packaging structure of the DNA (called chromatin); 3) micro RNAs and non coding RNAs; and 4) nucleosome positioning [51].

1. DNA hypomethylation:

Significance of the global hypomethylation, and aberrant CpG island hypermethylation was not immediately evident leaving open the idea that the epigenetic alterations in cancers, including colorectal cancers are merely bystander phenomenon in the cancer genome [52]. The aberrant hypermethylation of genes appears to be a common molecular mechanism for silencing tumor suppressor genes and can contribute to cancer formation through the transcriptional repression of these genes [53, 54]. Recently, LINE-1 hypomethylation has shown promise as a prognostic marker for shorter disease free survival in proximal colorectal cancer [55]. DNA hypomethylation is important for genome stability; then it may cause strand breaks and mutagenesis through alterations in chromatin conformation, which increase the accessibility of the DNA to DNA-damaging agents promoting genomic instability [56]. Some results show that global genome hypomethylation occurs in the gastritis level [57]. DLEC1, located at 3p22.3, is a common tumor suppressor locus with frequent genetic abnormalities in multiple cancers. It was found frequently silenced by promoter methylation in colorectal and gastric cancers in a tumor-specific manner. Tumor-specific promoter methylation makes this gene a biomarker for tumor early diagnosis [58]. Some studies show the combined methylation status of P16, P14, HLTF (Helicase-Like Transcription Factor), SOCS1 (Suppressor of Cytokine Signalling-1), CDH13 (H-cadherin), RUNX3 (a member of the human runt-related transcription factor family) and CHFR (Checkpoint with FHA and RING finger) in 58 primary colorectal carcinomas [59]. Silencing of SFRP (Secreted Frizzled-Related Protein1) by promoter methylation causes constitutive activation of the Wnt/Bcatenin signalling pathway, which is associated with several tumors as well as CRC [60]. Hypermethylation of promoter regions in colorectal cancer occurs early in some genes such as MLH1 , VIM and SEPT9 , these methylated genes are being used as the basis for early detection markers [61].

At this time, stool-based methylated VIMENTIN (mVim) is a clinically validated marker for early detection of colorectal cancer that is now commercially available in the United States under the name ColoGuard assay (LabCorp)[62]. The test exploits the fact that the Vimentin gene (VIM) is aberrantly methylated in a majority of colorectal cancers (53–84%). This early detection test is a PCR-based assay that simultaneously measures methylated VIM as well as DNA integrity and has reported a sensitivity of 83% and a specificity of 82%, with approximately equal sensitivity in stage I-III colorectal cancer patients [63].

2. Histone modification:

Another epigenetic change is chromatin modification, specifically, covalent modifications of the histone proteins [64]. The epigenetic status of histones have been demonstrated to influence transcription, DNA repair, and replication [65].Different combinations of histone tail modifi cations influence transcription by affecting chromatin structure [66]. Histone acetylation is a hallmark of active regions while hypoacetylated histone tails are found in transcriptionally inactive euchromatic or heterochromatic regions [67]. Histone covalent modifications can be affected by oncogenic RAS pathways to regulate the expression

of target genes like Cyclin D1 or E-cadherin and that the dynamic balance of opposing histone-modifying enzymes is critical for the regulation of cell proliferation [68]. Histone H3-K9 modification status was also closely related to cancer-related genes that controlled epigenetically [69]. Alterations in H3K9 and H3K27 methylation are correlated with aberrant gene silencing in many types of cancer [70, 71]. Results have been suggested that aberration of the global H3K9me2 level is an important epigenetic event in colorectal tumorigenesis and carcinogenesis involved with gene regulation in neoplastic cells through chromatin remodeling [50]. The changes of histone methylation (histone modification) in cancer can due to chromosomal translocation, amplification, deletion, overexpression or silencing [72, 73].

Table 1. Gene as risk factors for colorectal cancer

Name	Function	Pathway or interaction	Location	Ref
Genetic Safaei et al	**mutation**			
APC	Tumor suppressor gene	Wnt signalling pathway	5q21-q22	[74]
TGF	Proliferation, cellular differentiation ,immunity	The SMAD pathway or the DAXX pathway	19q13	
Kras	Proliferation and suppressing apoptosis	MAPK signalling pathway	12p12.1	[75]
B-RAF	Cell division, differentiation	MAP kinase/ERKs signalling pathway	7q34	[76]
MSI				
MLH1	DNA mismatch repair	Repair interact with Exonuclease 1 MSH4, PMS2, Myc, , MBD4.	3p21.3	[77]
PMS2	DNA mismatch repair	Interact with MLH1.	7P12	[78]
IGF2	Acts as a signalling antagonist	Signalling antagonist interact with IGFBP3 and Transferrin.	11p15.5	[79]
aXin 2	Regulation of the stability of beta-catenin	Wnt signalling pathway interact with GSK3B.	17q23-q24	[80]
FAS	Induces apoptosis on binding Fas ligand.	Apoptosis pathways	10q24.1	[81]
RAD50	DNA double-strand break repair	Interact with RINT1, MRE11A, TERF2IP, Nibrin, TERF2 and BRCA1.	5q31	[82]
LOH				
DCC	DCC tumor suppressor gene lead to proliferation and cell migration.	Three signalling states on [ligand-bound, migration and proliferation], off [ligand-unbound, apoptosis inducing] and absent [lack of signal]	18q21	[83]
SMAD4 and SMAD2	A transcription factor cell proliferation, apoptosis, and differentiation	Cell signalling and TGF-□ pathways	18q21.1	[84]
NTN1	Axon guidance and cell migration during development	Interact with DCC.	17p13-p12	[85]
DNA	**Methylation**			
P16	Tumor suppressor gene and Cyclin-Dependent Kinase(CDK)inhibitor	CDK4/ CDK6 pathway mechanism in cell cycle	9p21	[86]
SOCS1	Regulate cytokine signalling	Interact with Janus kinase 2 Growth hormone receptor, CD117, IRS2.	16p13	[87]
CDH13	Cell–cell contacts, dynamic regulation of morphogenetic processes	Beta-catenin/Wnt pathway	16q2	[88]
RUNX3	Transcription factors, functions as a tumor suppressor	Interact with TLE1.	1p36.1	[89]
SEPT9	Tumor cell migration and invasion	Interact with SEPT2and SEPT7.	22q13.2	[90]
VMT	The major cytoskeletal component of mesenchymal cells	Growth regulated signalling	10p-10q23	[91]

3. MicroRNA:

Another type of epigenetic event is driven by microRNAs (miRNAs), short, non-coding RNAs, that regulate the translation of several genes binding to their 3_UTR regions [92]. It has been suggested that miRNAs may prevent colon cancer cell proliferation through KRAS regulation [93-95]. MiRNA upregulation or downregulation may play a role in CRC, but the mechanisms involved in this process are still unclear. MiRNA gene promoter sequences contain numerous p53 binding sites, an important tumor suppressor gene whose activity is lost in colorectal tumors [96]. Down regulation of hsa-miR-143, one of the most frequent miRNA alteration described in colon cancer [97-99]. Decreased expression of miRNAs 124a, let-7a-3 and 10a CRCs is directly related to the increased methylation because these miRNA genes are located near a CpG island [92]. MiRNA-34b/c is found to be epigenetically silenced in many colon cancer cell lines and primary CRC tumors [100].

Conclusion

Genetic alteration in CRC tumors has been extensively studied, and it continues to evolve. Advances in understanding of chromatin structure, histone modification, transcriptional activity, DNA methylation, gene mutation have lead to an integrated approach to the role of genetic and epigenetics in carcinogenesis. The deeper understanding of the mechanisms of colorectal cancer cell, genetic alteration and epigenetic phenomenon open a light attitude to define prognosis of this common cancer.

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Conflict of Interest

The authors have no conflict of interest in this article.

Authors' Contribution

The subject selection and article structure made and wrote by Mostafa Rezaei-Tavirani, Akram Safaei and Sara Sobhi. Mohamad Reza zali provided many useful consultations. Finally; all authors commented on the manuscript and approved it as well.

References

1. Smith RA, Cokkinides V, Brooks D, Saslow D, Brawley OW. Cancer screening in the United States, 2010: a review of current American Cancer Society guidelines and issues in cancer screening. CA: a cancer journal for clinicians. 2010;60[2]:99-119.

2. Ahlquist DA, Sargent DJ, Loprinzi CL, Levin TR, Rex DK, Ahnen DJ, et al. Stool DNA and occult blood testing for screen detection of colorectal neoplasia. Ann Intern Med. 2008;149[7]:441-50.

3. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100[1]:57-70.

4. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. Science Signalling. 2007;318[5853]:1108.

5. Noffsinger AE. Serrated polyps and colorectal cancer: new pathway to malignancy. Annual Review of Pathological Mechanical Disease. 2009;4:343-64.

6. Issaq HJ, Blonder J. Electrophoresis and liquid chromatography/tandem mass spectrometry in disease biomarker discovery. Journal of Chromatography B. 2009;877[13]:1222-8.

7. Kumar S, Mohan A, Guleria R. Biomarkers in cancer screening, research and detection: present and future: a review. Biomarkers. 2006 Sep-Oct;11[5]:385-405.

8. Fleming N, Jorissen RN, Mouradov D, Christie M, Sakthianandeswaren A, Palmieri M, et al. SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer. Cancer research. 2012.

9. Abdul Murad NA, Othman Z, Khalid M, Abdul Razak Z, Hussain R, Nadesan S, et al. Missense Mutations in MLH1, MSH2, KRAS, and APC Genes in Colorectal Cancer Patients in Malaysia. Digestive Diseases and Sciences.1-10.

10. Je EM, Lee SH, Yoo NJ. Somatic mutation of a tumor suppressor gene BAP1 is rare in breast, prostate, gastric and colorectal cancers. Apmis. 2012.

11. Von Holst S, Picelli S, Edler D, Lenander C, Dalén J, Hjern F, et al. Association studies on 11 published colorectal cancer risk loci. British journal of cancer. 2010;103[4]:575-80.

12. Vogelstein B, Fearon E, Hamilton S. Genetic alterations during colorectal-tumor development. Journal of Occupational and Environmental Medicine. 1989;31[10]:815.

13. Derynck R, Akhurst RJ, Balmain A. TGF-□ signaling in tumor suppression and cancer progression. Nature genetics. 2001;29[2]:117-29.

14. Nosho K, Irahara N, Shima K, Kure S, Kirkner GJ, Schernhammer ES, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. PLoS One. 2008;3[11]:e3698.

15. Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, et al. Evaluation of a large, population-based sample supports a CpG

island methylator phenotype in colon cancer. Gastroenterology. 2005;129[3]:837.

16. Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, Spain S, et al. A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24. 21. Nature Genetics. 2007;39[8]:984-8.

17. Tomlinson IPM, Webb E, Carvajal-Carmona L, Broderick P, Howarth K, Pittman AM, et al. A genome-wide association study identifies colorectal cancer susceptibility loci on chromosomes 10p14 and 8q23. 3. Nature Genetics. 2008;40[5]:623-30.

18. Zanke BW, Greenwood CMT, Rangrej J, Kustra R, Tenesa A, Farrington SM, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. Nature Genetics. 2007;39[8]:989-94.

19. Kupfer SS, Anderson JR, Hooker S, Skol A, Kittles RA, Keku TO, et al. Genetic heterogeneity in colorectal cancer associations between African and European Americans. Gastroenterology. 2010;139[5]:1677-85. e8.

20. Tenesa A, Farrington SM, Prendergast JGD, Porteous ME, Walker M, Haq N, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. Nature Genetics. 2008;40[5]:631-7.

21. Pittman AM, Webb E, Carvajal-Carmona L, Howarth K, Di Bernardo MC, Broderick P, et al. Refinement of the basis and impact of common 11q23. 1 variation to the risk of developing colorectal cancer. Human molecular genetics. 2008;17[23]:3720-7.

22. Tuupanen S, Niittymäki I, Nousiainen K, Vanharanta S, Mecklin JP, Nuorva K, et al. Allelic imbalance at rs6983267 suggests selection of the risk allele in somatic colorectal tumor evolution. Cancer research. 2008;68[1]:14-7.

23. Tuupanen S, Turunen M, Lehtonen R, Hallikas O, Vanharanta S, Kivioja T, et al. The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling. Nature Genetics. 2009;41[8]:885-90.

24. Jaeger E, Webb E, Howarth K, Carvajal-Carmona L, Rowan A, Broderick P, et al. Common genetic variants at the CRAC1 [HMPS] locus on chromosome 15q13. 3 influence colorectal cancer risk. Nature Genetics. 2007;40[1]:26-8.

25. Thompson CL, Plummer SJ, Acheson LS, Tucker TC, Casey G, Li L. Association of common genetic variants in SMAD7 and risk of colon cancer. Carcinogenesis. 2009;30[6]:982-6.

26. Houlston RS, Webb E, Broderick P, Pittman AM, Di Bernardo MC, Lubbe S, et al. Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. Nature Genetics. 2008;40[12]:1426-35.

27. Safaei A, Karimi K, Arkani M, Rostami F, Arbabi E, Vahedi M, et al. Association of adiponectin gene [rs 2241766] polymorphism and colorectal cancer in Tehran, Iran. Medical Science Journal of Islamic Azad Univesity-Tehran Medical Branch. 2012;22[2]:110-5.

28. Arkani M, Safaei A, Karimi K, Arbabi E, Rostami F, Iman M, et al. Association of the insulin gene polymorphism and colorectal cancer. Koomesh. 2012;13[2]:172-6.

29. Arkani M, Safaei A, Karimi K, Vahedi M, Mohebi SR, Fatemi SR, et al. Association of IGF-1 gene [rs5742612] polymorphism with colorectal cancer. Journal of Sabzevar University of Medical Sciences. 2012;19[2]:109-15.

30. Aaltonen LA, Peltomäki P, Mecklin JP, Järvinen H, Jass JR, Green JS, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer research. 1994;54[7]:1645-8.

31. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138[6]:2073-87. e3.

32. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer—the stable evidence. Nature Reviews Clinical Oncology. 2010;7[3]:153-62.

33. Söreide K, Janssen E, Söiland H, Körner H, Baak J. Microsatellite instability in colorectal cancer. British journal of surgery. 2006;93[4]:395-406.

34. Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin JP, et al. Clues to the pathogenesis of familial colorectal cancer. Science [New York, NY]. 1993;260[5109]:812.

35. Duval A, Hamelin R. Mutations at Coding Repeat Sequences in Mismatch Repair-deficient Human Cancers Toward a New Concept of Target Genes for Instability. Cancer research. 2002;62[9]:2447-54.

36. Aust DE, Terdiman JP, Willenbucher RF, Chang CG, Molinaro□Clark A, Baretton GB, et al. The APC/□□catenin pathway in ulcerative colitis–related colorectal carcinomas. Cancer. 2002;94[5]:1421-7.

37. Moghbeli M, Moaven O, Dadkhah E, Farzadnia M, Roshan N, Asadzadeh-Aghdaee H, et al. High frequency of microsatellite instability in sporadic colorectal cancer patients in Iran. Genetics and Molecular Research. 2011;10[4]:3520-9.

38. Grady WM. Genomic instability and colon cancer. Cancer and Metastasis Reviews. 2004;23[1]:11-27.

39. Lurje G, Zhang W, Lenz HJ. Molecular prognostic markers in locally advanced colon cancer. Clinical colorectal cancer. 2007;6[10]:683-90.

40. Ratto C, Sofo L, Ippoliti M, Merico M, Doglietto GB, Crucitti F. Prognostic factors in colorectal cancer. Diseases of the colon & rectum. 1998;41[8]:1033-49.

41. Shankaran V, Wisinski KB, Mulcahy MF, Benson AB. The role of molecular markers in predicting response to therapy in patients with colorectal cancer. Molecular Diagnosis & Therapy. 2008;12[2]:87-98.

42. Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. Journal of Clinical Oncology. 2006;24[33]:5313-27.

43. Nakao M, Kawauchi S, Furuya T, Uchiyama T, Adachi J, Okada T, et al. Identification of DNA copy number aberrations associated with metastases of colorectal cancer using array CGH profiles. Cancer genetics and cytogenetics. 2009;188[2]:70-6.

44. Sandberg AA, Meloni-Ehrig AM. Cytogenetics and genetics of human cancer: methods and accomplishments. Cancer genetics and cytogenetics. 2010;203[2]:102-26.

45. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology. 2010;138[6]:2059-72.

46. Knösel T, Petersen S, Schwabe H, Schlüns K, Stein U, Schlag P, et al. Incidence of chromosomal imbalances in advanced colorectal carcinomas and their metastases. Virchows Archiv. 2002;440[2]:187-94.

47. Watanabe T, Wu TT, Catalano PJ, Ueki T, Satriano R, Haller DG, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. New England Journal of Medicine. 2001;344[16]:1196-206.

48. Alberici P, Fodde R. The role of the APC tumor suppressor in chromosomal instability. 2006.

49. Ogino S, Nosho K, Irahara N, Shima K, Baba Y, Kirkner GJ, et al. Prognostic significance and molecular associations of 18q loss of heterozygosity: a cohort study of microsatellite stable colorectal cancers. Journal of Clinical Oncology. 2009;27[27]:4591-8.

50. Nakazawa T, Kondo T, Ma D, Niu D, Mochizuki K, Kawasaki T, et al. Global histone modification of histone H3 in colorectal cancer and its precursor lesions. Human pathology. 2012;43[6]:834-42.

51. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010;31[1]:27-36.

52. Suzuki H, Watkins DN, Jair KW, Schuebel KE, Markowitz SD, Chen WD, et al. Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. Nature genetics. 2004;36[4]:417-22.

53. Tsai HC, Baylin SB. Cancer epigenetics: linking basic biology to clinical medicine. Cell research. 2011;21[3]:502-17.

54. Schuebel KE, Chen W, Cope L, Glöckner SC, Suzuki H, Yi JM, et al. Comparing the DNA hypermethylome with gene mutations in human colorectal cancer. PLoS genetics. 2007;3[9]:e157.

55. Ahn JB, Chung WB, Maeda O, Shin SJ, Kim HS, Chung HC, et al. DNA methylation predicts recurrence from resected stage III proximal colon cancer. Cancer. 2011;117[9]:1847-54.

56. Migliore L, Migheli F, Spisni R, Coppedè F. Genetics, cytogenetics, and epigenetics of colorectal cancer. Journal of Biomedicine and Biotechnology. 2011;2011.

57. Najjar Sadeghi R, Zojaji H, Mohebbi SR, Chiani M, Vahedi M, Mirsattari D, et al. Evaluation of global genome methylation in gastritis lesion and its correlation with clinicopatological findings. Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics. 2009;17[11-12]:11-2.

58. Ying J, Poon F, Yu J, Geng H, Wong A, Qiu G, et al. DLEC1 is a functional 3p22. 3 tumour suppressor silenced by promoter CpG methylation in colon and gastric cancers. British journal of cancer. 2009;100[4]:663-9.

59. Ramirez N, Bandres E, Navarro A, Pons A, Jansa S, Moreno I, et al. Epigenetic events in normal colonic mucosa surrounding colorectal cancer lesions. European Journal of Cancer. 2008;44[17]:2689-95.

60. An B, Kondo Y, Okamoto Y, Shinjo K, Kanemitsu Y, Komori K, et al. Characteristic methylation profile in CpG island methylator phenotype□negative distal colorectal cancers. International Journal of Cancer. 2010;127[9]:2095-105.

61. Lao VV, Grady WM. Epigenetics and colorectal cancer. Nature Reviews Gastroenterology and Hepatology. 2011.

62. Itzkowitz SH, Jandorf L, Brand R, Rabeneck L, Schroy PC, Sontag S, et al. Improved fecal DNA test for colorectal cancer screening. Clinical Gastroenterology and Hepatology. 2007;5[1]:111-7.

63. Itzkowitz S, Brand R, Jandorf L, Durkee K, Millholland J, Rabeneck L, et al. A simplified, noninvasive stool DNA test for colorectal cancer detection. The American journal of gastroenterology. 2008;103[11]:2862-70.

64. Jenuwein T, Allis CD. Translating the histone code. Science Signalling. 2001;293[5532]:1074.

65. Esteller M. Epigenetics in cancer. New England Journal of Medicine. 2008;358[11]:1148-59.

66. Zamore PD, Haley B. Ribo-gnome: the big world of small RNAs. Science Signalling. 2005;309[5740]:1519.

67. Matsubara N. Epigenetic regulation and colorectal cancer. Diseases of the Colon & Rectum. 2012;55[1]:96.

68. Mazón Peláez I, Kalogeropoulou M, Ferraro A, Voulgari A, Pankotai T, Boros I, et al. Oncogenic RAS alters the global and gene-specific histone modification pattern during epithelial–mesenchymal transition in colorectal carcinoma cells. The International Journal of Biochemistry & Cell Biology. 2010;42[6]:911-20.

69. Yamada N, Nishida Y, Tsutsumida H, Hamada T, Goto M, Higashi M, et al. MUC1 expression is regulated by DNA methylation and histone H3 lysine 9 modification in cancer cells. Cancer research. 2008;68[8]:2708-16.

70. Nguyen CT, Weisenberger DJ, Velicescu M, Gonzales FA, Lin JCY, Liang G, et al. Histone H3-lysine 9 methylation is associated with aberrant gene silencing in cancer cells and is rapidly

reversed by 5-aza-2□-deoxycytidine. Cancer research. 2002;62[22]:6456-61.

71. Valk-Lingbeek ME, Bruggeman SWM, van Lohuizen M. Stem cells and cancer: the polycomb connection. Cell. 2004;118[4]:409-18.

72. Yun J, Johnson JL, Hanigan CL, Locasale JW. Interactions between epigenetics and metabolism in cancers. Frontiers in Oncology. 2012;2.

73. Yamada N, Hamada T, Goto M, Tsutsumida H, Higashi M, Nomoto M, et al. MUC2 expression is regulated by histone H3 modification and DNA methylation in pancreatic cancer. International Journal of Cancer. 2006;119[8]:1850-7.

74. Rosin-Arbesfeld R, Townsley F, Bienz M. The APC tumour suppressor has a nuclear export function. Nature. 2000;406[6799]:1009-12.

75. Attisano L, Wrana JL. Signal transduction by the TGF-beta superfamily. Science Signalling. 2002;296[5573]:1646.

76. Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified as a mutational target. Biochimica et Biophysica Acta [BBA]-Reviews on Cancer. 2003;1653[1]:25-40.

77. Bronner C, Baker S, Morrison P, Warren G, Smith L, Lescoe M, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is. Nature. 1994;368[6468]:258-61.

78. Baker SM, Bronner CE, Zhang L, Plug AW, Robatzek M, Warren G, et al. Male mice defective in the DNA mismatch repair gene< i> PMS2</i> exhibit abnormal chromosome synapsis in meiosis. Cell. 1995;82[2]:309-19.

79. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nature Reviews Cancer. 2004;4[7]:505-18.

80. Miller JR, Hocking AM, Brown JD, Moon RT. Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca2+ pathways. Oncogene. 1999;18[55]:7860.

81. Suda T, Nagata S. Purification and characterization of the Fas-ligand that induces apoptosis. The Journal of experimental medicine. 1994;179[3]:873-9.

82. Hopfner K-P, Karcher A, Shin DS, Craig L, Arthur LM, Carney JP, et al. Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. Cell. 2000;101[7]:789-800.

83. Zaphiropoulos PG, Gailani MR, Vorechovsky I, Holmberg E, Smyth I, Pressman C, et al. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell. 1996;85:841-51.

84. Whitman M. Smads and early developmental signaling by the TGF□ superfamily. Genes & development. 1998;12[16]:2445-62.

85. Alcantara S, Ruiz M, De Castro F, Soriano E, Sotelo C. Netrin 1 acts as an attractive or as a repulsive cue for distinct migrating neurons during the development of the cerebellar system. Development. 2000;127[7]:1359-72.

86. Kotake Y, Cao R, Viatour P, Sage J, Zhang Y, Xiong Y. pRB family proteins are required for H3K27 trimethylation and Polycomb repression complexes binding to and silencing p16INK4a tumor suppressor gene. Genes & development. 2007;21[1]:49-54.

87. Alexander WS, Hilton DJ. The role of suppressors of cytokine signaling [SOCS] proteins in regulation of the immune response. Annu Rev Immunol. 2004;22:503-29.

88. Philippova M, Ivanov D, Tkachuk V, Erne P, Resink T. Polarisation of T-cadherin to the leading edge of migrating vascular cells in vitro: a function in vascular cell motility? Histochemistry and cell biology. 2003;120[5]:353-60.

89. Bae S-C, Choi J-K. Tumor suppressor activity of RUNX3. Oncogene. 2004;23[24]:4336-40.

90. Xu S, Jia Z-F, Kang C, Huang Q, Wang G, Liu X, et al. Upregulation of SEPT7 gene inhibits invasion of human glioma cells. Cancer investigation. 2010;28[3]:248-58.

91. Guarino M, Rubino B, Ballabio G. The role of epithelial-mesenchymal transition in cancer pathology. Pathology. 2007;39[3]:305-18.

92. Yang L, Belaguli N, Berger DH. MicroRNA and colorectal cancer. World journal of surgery. 2009;33[4]:638-46.

93. Wu WKK, Law PTY, Lee CW, Cho CH, Fan D, Wu K, et al. MicroRNA in colorectal cancer: from benchtop to bedside. Carcinogenesis. 2011;32[3]:247-53.

94. Slaby O, Svoboda M, Fabian P, Smerdova T, Knoflickova D, Bednarikova M, et al. Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology. 2007;72[5-6]:397-402.

95. Ng EKO, Chong WWS, Jin H, Lam EKY, Shin VY, Yu J, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut. 2009;58[10]:1375-81.

96. Xi Y, Shalgi R, Fodstad O, Pilpel Y, Ju J. Differentially regulated micro-RNAs and actively translated messenger RNA transcripts by tumor suppressor p53 in colon cancer. Clinical cancer research. 2006;12[7]:2014-24.

97. Nakajima G, Hayashi K, Xi Y, Kudo K, Uchida K, Takasaki K, et al. Non-coding microRNAs hsa-let-7g and hsa-miR-181b are associated with chemoresponse to S-1 in colon cancer. Cancer Genomics-Proteomics. 2006;3[5]:317-24.

98. Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced Accumulation of Specific MicroRNAs in Colorectal Neoplasia11Note: Susan M. O'Connor and Nicholas G. van Holst Pellekaan contributed equally to this work. Molecular Cancer Research. 2003;1[12]:882-91.

99. Ragusa M, Majorana A, Statello L, Maugeri M, Salito L, Barbagallo D, et al. Specific alterations of microRNA transcriptome and global

network structure in colorectal carcinoma after cetuximab treatment. Molecular cancer therapeutics. 2010;9[12]:3396-409.

100. Faber C, Kirchner T, Hlubek F. The impact of microRNAs on colorectal cancer. Virchows Archiv. 2009;454[4]:359-67.