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MOLECULAR EPIDEMIOLOGY (MJ WARGOVICH, SECTION EDITOR)

Epigenetics Offer New Horizons for Colorectal Cancer Prevention

Michael Schnekenburger · Marc Diederich

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Abstract In recent years, colorectal cancer (CRC) incidence has been increasing to become a major cause of morbidity and mortality worldwide from cancers, with high rates in westernized societies and increasing rates in developing countries. Epigenetic modifications including changes in DNA methylation, histone modifications, and non-coding RNAs play a critical role in carcinogenesis. Epidemiological data suggest that, in comparison to other cancers, these alterations are particularly common within the gastrointestinal tract. To explain these observations, environmental factors and especially diet were suggested to both prevent and induce CRC. Epigenetic alterations are, in contrast to genetic modifications, potentially reversible, making the use of dietary agents a promising approach in CRC for the development of chemopreventive strategies targeting epigenetic mechanisms. This review focuses on CRC-related epigenetic alterations as a rationale for various levels of prevention strategies and their potential modulation by natural dietary compounds.

Keywords Colorectal cancer · Epigenetic · DNA methylation · Histone modification · Non-coding · microRNA · Cancer prevention · Early detection · Biomarker · Predictive marker · Prognostic marker · Chemoprevention · Molecular epidemiology

Introduction

Colon and rectal cancers (colorectal cancer, CRC) represent globally, in terms of frequency, the third leading cause of cancer-related mortality (ie, after lung and breast cancer). Nevertheless, CRC incidence and mortality rates vary over 10-fold worldwide. Lowest incidence rates are observed in Africa and Asia and highest ones are found in Australia/New Zealand, North America, and Western Europe with a mortality rate of approximately 30%. Although incidence rates in developed countries are stabilizing, they are severely increasing in both developing countries and several areas historically at low risk [1]. Since the 1970s, CRC incidence in USA has continuously increased in the African-American population to become more frequent in this population than in Caucasians or other ethnic groups [2]. Similarly, data from migration population studies revealed that some ethnic groups are showing increased CRC incidence rate while they are migrating from low-risk to high-risk areas, to finally reach rates comparable to the host country [3–5]. Despite genetic variation, these epidemiological data strongly suggest a role of environmental and lifestyle factors deeply contributing to the etiology of CRC.

Although it is well accepted that genetic factors and inflammatory bowel disease place certain individuals at increased risk [6], various modifiable lifestyle factors have been identified related to CRC pathogenesis. Significant lifestyle risk factors are represented by sedentarity and changes in dietary habits, from a moderate to a Western-like enriched diet associated with high consumption of unsaturated fats and red meat, high intake of alcohol, and smoking.

Epigenetic mechanisms are fundamental to tightly regulated cellular processes. Epigenetic aberrations governing tumor suppressor gene (TSG) inactivation, oncogene activation, and chromosomal instability play a fundamental role in tumorigenesis including CRC. Epigenetic events are involved in all critical pathways and steps of carcinogenesis including tumor initiation, and some events are usually detectable before neoplastic transformation [7, 8, 9•, 10]. Nonetheless, it is well accepted that environmental and

M. Schnekenburger · M. Diederich (✉)
Laboratoire de Biologie Moléculaire et Cellulaire de Cancer,
Hôpital Kirchberg, 9, rue Edward Steichen,
L-2540, Luxembourg, Luxembourg
e-mail: marc.diederich@lbmcc.lu

dietary factors greatly influence epigenetic events. Moreover, the reversibility of epigenetic alterations stimulates the development of novel therapeutic approaches with an open field for development in cancer chemoprevention. Taking together, these observations suggest that improved early detection and dietary intervention are preventive approaches of choice to decrease CRC incidence.

In this review, we focus on epigenetic alterations associated with CRC, which offer promising novel biomarkers for early detection, with an emphasis on how these alterations can potentially be modulated by dietary compounds for preventive interventions.

Colorectal Carcinogenesis and Cancer Prevention

The vast majority of CRCs are a multistep-associated adenoma-carcinoma progression associated with successive clinico-histopathological stages. This transformation initially starts with a premalignant lesion, called aberrant crypt foci (ACF), rising from normal colonic mucosa, progressing to a premalignant lesion (ie, adenoma), and finally evolving to invasive adenocarcinoma and metastasis (Fig. 1). The tumor-node-metastasis (TNM) system stages CRCs depending on the extent of invasion of the intestinal wall (T), the degree of lymphatic node involvement (N), and whether there is presence of metastasis (M). Based on this system, CRC is ranked from 0 (in situ tumor confined to mucosa) to stage IV (presence of metastasis). Thus, an increasing ranking correlates to a more advanced cancer and likely a worse outcome [11].

Although most CRCs occur sporadically, the importance of inheritance associated with a family history of the disease is evaluated to approximately 25% [12]. Some well-defined syndromes associated with CRC pathobiology have been identified: hereditary non-polyposis CRC (HNPCC), familial adenomatous polyposis (FAP), and MUTYH-associated polyposis (MAP), which are caused by germline mutations in DNA mismatch repair (MMR) genes, adenomatous polyposis

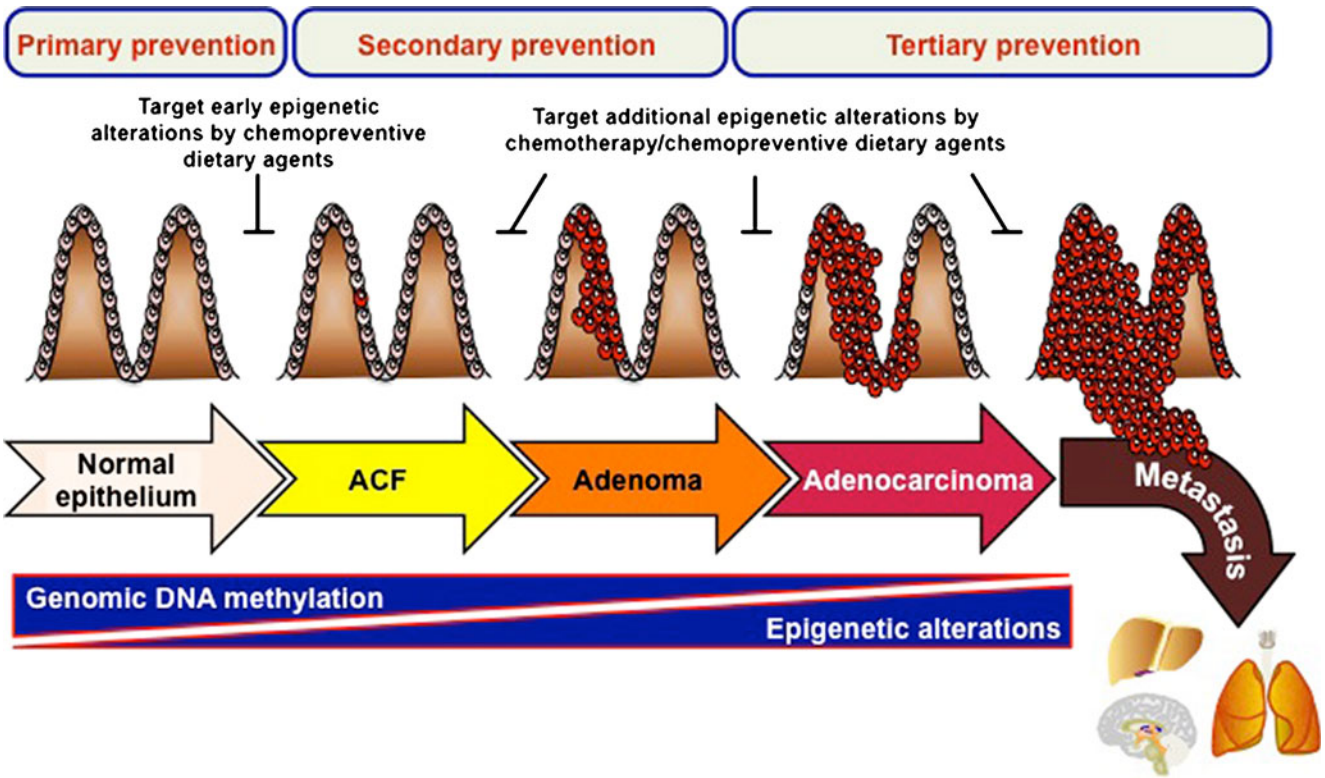

Fig. 1 Colorectal cancer (CRC) progression as a model for epigenetic alteration cascade and prevention strategies. CRC development is initially starting by a premalignant lesion, called aberrant crypt foci (ACF), rising from normal colonic mucosa, progressing to a premalignant lesion (adenoma) and then to invasive adenocarcinoma, and finally evolving to metastatic adenocarcinoma. Epigenetic alterations are largely contributing to CRC initiation and adenoma-carcinoma progression. These alterations are characterized by global genomic DNA hypomethylation leading to genomic instability and oncogene activation concomitantly to an increase of CpG island promoter hypermethylation-mediated silencing of tumor suppressor genes. These changes are accompanied by an increase of aberrant histone modification profiles and miRNA signatures reinforcing oncogenic activation and tumor suppressor loss associated with CRC progression. Consequently, epigenetic alterations represent promising targets for CRC prevention. Early epigenetic aberrations represent interesting targets for primary prevention, especially through chemoprevention by dietary epigenetic modulators, as well as for secondary prevention as early biomarkers of CRC initiation. Modifications occurring at later stages may be targeted by chemotherapeutic interventions as well as chemopreventive agents to limit or block disease progression (secondary and tertiary prevention activities)

coli (APC) TSG, and MUTYH gene, respectively; plus a number of relatively rare polyposis syndromes [13].

Mechanisms underlying the adenoma-carcinoma sequence have been identified for their contribution to CRC pathogenesis in relation to alterations of TSG and oncogene functions. Among these mechanisms, genomic instability represented by two "genotypic" subtypes pathways associated with somatic mutations are frequently identified: chromosomal instability (CIN) and microsatellite instability (MSI) [9•, 13]. Although CIN is observed in approximately 85% of CRC cases, the initiating mechanism is still poorly understood. The most common cytogenic abnormalities observed in CIN are mutations of APC gene, which occur in the majority of sporadic CRCs and also very early in adenoma development, and chromosome aberrations such as loss of heterozygosity of 5, 17p, and 18q. The latter contains the deleted in colorectal cancer (DCC) TSG. MSI is characterized by the inactivation of genes implicated in mismatch repair (MMR) system leading to subsequent mutations in the microsatellite repeat sequences of genes linked to tumor progression [13]. In addition to somatic mutations, epigenetic alterations are also particularly common in CRC. Epigenetics is defined as the heritable changes in gene expression patterns that occur without a change in the primary DNA sequence. This field encompasses DNA methylation, histone modifications and chromatin remodeling, and non-coding RNA-mediated interference [9•, 10, 13].

After years of research it appears that the best way to avoid the burden of cancer might be prevention. Under the general concept of prevention, several levels of approaches are encompassed [14]. Avoiding exposure to potential carcinogens or life risk factors is associated with the primary level of prevention. However, preemptive behavior prevention is not limited to this aspect. Indeed, chemoprevention, ie, the use of natural agents in healthy individuals without signs of premalignancy, falls also in this category. Secondary prevention corresponds to early detection of tumor-related abnormal changes aiming to prevent cancer development. Screening tests are included in this category, which require robust clinical biomarkers for early diagnosis. Finally, tertiary prevention consists to control cancer development to a more advanced-stage or reoccurrence after treatment and reduce adverse health effects.

Given the fact that epimutations are potentially reversible, the major field of applications regarding epigenetics might be cancer prevention. Accordingly, epimutations represent secondary prevention biomarkers by their precocity in carcinogenesis processes (ie, before neoplastic transformation). Primary to tertiary prevention may be achieved through chemoprevention, with dietary agents controlling epigenetic (re)programming, to either prevent or reverse premalignant stem cell phenotypes (Fig. 1).

Epimutations in CRC: Biomarkers and Targets for Prevention

DNA Methylation in CRC

In humans, DNA methylation occurs at the 5′ position of the pyrimidine ring of the cytosine residues within CpG dinucleotides through addition of a methyl moiety to form 5-methylcytosines. This process is catalyzed by three DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B) using the cofactor *S*-adenosyl-methionine (SAM). Although CpG dinucleotides represent approximately 1% of the human genome, they are unequally distributed across the genome and are clustered in small DNA stretches. These CpG-rich regions, called CpG islands (CGIs), are usually present near promoters and exogenic regions. CGIs are usually unmethylated in normal differentiated cells, whereas CpGs located in intergenic regions are methylated [8, 15].

In cancer, promoter CGI of numerous TSGs are found to be densely methylated, which results in transcriptional silencing. Interestingly, these epimutations may be cancer type-specific and tumor stage-specific. Thus, methylation patterns can be considered as biomarkers for diagnosis, prognosis, as well as prediction and monitoring of therapy response [8, 10, 15, 16]. Therefore, the identification of these cancer-associated methylation signatures is really critical for cancer prevention purposes.

Recent studies show that CRC is strongly associated with aberrant DNA methylation profiles, which has been linked to the origin and progression of the disease. The list of epimutations is growing quickly with the use of developing technologies allowing genome studies. To date, a long list of TSGs involved in numerous signalization pathways and cellular processes were found frequently methylated in CRC (Table 1). Noteworthy, a widespread contribution of DNA methylation participates in the disruption of β-catenin–dependent Wnt signaling pathway, which plays an important role in colorectal tumor development [9•, 13]. Moreover, methylation can affect coding and non-coding genes (eg, microRNA, miRNA) participating in loss of tumor suppressor functions. Remarkably, most of these methylated genes are investigated as potential biomarkers for preventive or therapeutic purposes. However, the methylation prevalence varies substantively depending on the considered genes as well as between studies regarding a same gene. The discrepancy between studies, like the case of CDKN2A (p16), for which methylation ranged from 10% to 58% depending on reports [17–19], could be explained by the phenotype of patients constituting these cohorts as well as how clinical parameters were included in these analyses. Indeed, certain genes are found frequently methylated in specific CRC subgroups, such as AXIN2 found preferentially associated with MSI tumors [20].

Table 1 Epimutations associated with colorectal cancer based on experimental data from patients[a]

Epigenetic event	Name	Locus	Function/targets	Note[b]	Comments
Hypermethylation	ADAMTS5	21q22.1-q22	Protease	NA	Increase of methylation level in CRC
	ADHFE1	8q12.3	Alcohol dehydrogenase	NA	Increase of methylation level in CRC
	ALX4	11p11.2	Homeobox gene	85/64	Adenoma vs CRC
	APBA1	9q13-q21	Intracellular signaling	16–28	
	APBA2	15q11-q12	Intracellular signaling	22/26	Stage I+III vs IV
	APC	5q22.2	Wnt signaling	21	
	APC2	19p13.3	Wnt signaling	100	
	AXIN2	17q24.1	Wnt signaling	29	Associated with MSI tumors
	B4GALNT1	12q13.3	Lipid metabolism	100	
	B4GALNT2	17q21.3	Lipid metabolism	50	Correlated with EBV-associated gastric carcinomas
	BARX1	9q12	Homeobox gene	56	
	BMP3	4q21.21	Bone and cartilage formation	72/60	Adenoma vs CRC
	BNIP3	10q26.3	Apoptosis	66	
	BOLL	2q33.1	Development	NA	Increase of methylation level in CRC
	CACNA1G	17q22	Calcium metabolism	39	
	CASR	3q21.1	Calcium metabolism	9/69/90	Adenoma vs CRC vs lymph node metastatic tissues
	CCNA1	13q13.3	Cell cycle	100	
	CD109	6q13	Complement system	33	
	CDH1	16q22.1	Cell adhesion	51	
	CDH13	16q23.3	Cell adhesion	32–66	Poor prognosis
	CDH2	18q12.1	Cell adhesion	45	
	CDH4	20q13.3	Cell adhesion	78	
	CDKN2A (p14)	9p21.3	Cell cycle	34	
	CDKN2A (p16)	9p21.3	Cell cycle	10–58	
	CDKN2B (p15)	9p21.3	Cell cycle	68	
	CDX1	5q33.1	Homeobox gene	100	
	CHFR	12q24.33	Cell cycle	26–63	Associated with disease recurrence
	CNRIP1	2p14	G protein-coupled receptor	91/94	Adenoma vs CRC
	CNTFR	9p13.3	Cytokine signaling	22	
	CPAMD8	19p13.12	Innate immunity	90	
	CXCL12	10q11.21	Cytokine signaling	62	
	DAPK1	9q21.33	Apoptosis	43	
	DCC	18q21.2	Putative TSG	81/83	Adenoma vs CRC (20% in normal)
	DFNA5	7p15.3	Unknown	65	
	DKK1	10q21.1	Wnt signaling	13–35	Associated with MSI tumors
	DKK2	4q25	Wnt signaling	65	
	DKK3	11p15.3	Wnt signaling	35	
	DKK4	8p11.2-p11.1	Wnt signaling	20	
	DLC1-i4	8p22	Putative TSG	100	
	DLEC1	3p22.2	Putative TSG	38	Poor prognosis
	EFEMP1	2p16.1	Cell migration	39	Poor prognosis
	EGFR	7p11.2	Cytokine signaling	58	Poor prognosis
	EN1	2q13-q21	Homeobox gene	33	
	EphA1	7q32-q34	Intercellular signaling	49	Poor prognosis
	EphA5	4q13.1	Intercellular signaling	53	
	EphA7	6q16.1	Intercellular signaling	49	More frequent in moderately differentiated adenocarcinomas
	EPHB2	1p36.12	Intercellular signaling	53	
	ESR1	6q25.1	Hormonal signaling	31	
	EVL	14q32.32	Cell migration	60	
	EYA2	20q13.1	Development	44/51	Adenoma vs CRC
	EYA4	6q23	Development	70	

Table 1 (continued)

Epigenetic event	Name	Locus	Function/targets	Note[b]	Comments
	FAM127A	Xq26	Unknown	58	
	FBN1	15q21.1	ECM component	69/79	Adenoma vs CRC
	FBN2	5q23.3	ECM component	90	
	FLNC	7q32.1	Cell migration	30	
	FOXL2	3q23	Transcription factor	50	
	GAS7	17p13.1	Development	NA	Increase of methylation level in CRC
	GATA4	8p23.1	Transcription factor	70	Independent of clinicopathologic features
	GATA5	20q13.33	Transcription factor	79	Independent of clinicopathologic features
	GPNMB	7p15	Development	100	
	GPR101	Xq25-q27.1	G protein-coupled receptor	40	
	GRID1	10q22	Glutamate receptor	60	
	GRIN2A	16p13.2	Glutamate receptor	82	
	GSPT2	Xp11.22	GTPase	21	
	GUCY1A2	11q21-q22	Intercellular signaling	50	
	HACE1	6q16.3	Stress response	28	
	HIC1	17p13.3	Transcriptional repressor	35/42	Adenoma vs CRC
	HLTF	3q24	Transcription factor	18–34	
	HOXB13	17q21.32	Homeobox gene	40	
	HRK	12q24.23	Apoptosis	36	
	HUS1	7p12.3	Cell cycle	22	
	ID4	6p22.3	Transcription factor	46	
	IGF2	11p15.5	Development	22	
	IGFBP3	7p12.3	Hormonal signaling	25	
	IGFBP7	4q12	Hormonal signaling	18/23	Adenoma vs CRC
	IKZF1	7p12.2	Transcriptional activator	30–82	% increase with Duke's stages
	INA	10q24.33	Development	35/65	Adenoma vs CRC
	INHBB	2q14.2	Inhibin	30	
	IRF8	16q24.1	Transcription factor	43	
	ITGA4	2q31.3	Cell adhesion	75/92	Adenoma vs CRC
	KCNK12	2p16.3	Potential potassium channel	41	
	KLF4	9q31.2	Transcription factor	25	
	LAMA1	18p11.31	Cell migration	100	
	LRRC3B	3p24.1	Putative TSG	77	
	MAL	2q11.1	Proteolipids	84/91	Adenoma vs CRC
	MGMT	10q26.3	DNA repair	20–60	
	miR-1-1	20q13.33	Translational repression	50	
	miR-9-1	1q22	Translational repression	50	Associated with the presence of lymph node metastasis
	miR-34a	1p36.22	Translational repression	74	
	miR-34b/c	11q23.1	Translational repression	99	
	miR-124-1	8p23.1	Translational repression	75	
	miR-129-2	11p11.2	Translational repression	83	
	miR-137	1p21.3	Translational repression	100	
	miR-148	NA	Translational repression	65	
	miR-342	14q32.2	Translational repression	67/86	Adenoma vs CRC
	miR-345	14q32.2	Translational repression	87	
	miR-373	19q13.42	Translational repression	88	
	MLH1	3p22.2	DNA repair	18–22	Poor prognosis
	MMP2	16q12.2	Protease	95	
	MYOD1	11p15.1	Transcription factor	69	
	NDRG2	14q11.2	Putative TSG	27	
	NDRG4	16q21	Putative TSG	70–86	
	NEURL	10q25.1	Putative TSG	31	

Table 1 (continued)

Epigenetic event	Name	Locus	Function/targets	Note[b]	Comments
	NEUROG1	5q31.1	Putative TSG	36	
	NPY	7p15.1	Putative TSG	NA	Increase of methylation level in CRC
	NRCAM	7q31.1	Cell adhesion	50	
	NTNG1	1p13.3	Development	70	
	NTRK2	9q21.33	Differentiation	100	
	OSMR	5p13.1	Cytokine signaling	55/89/90	Mucosa adjacent to CRC vs colorectal polyps vs carcinoma
	PAPSS2	10q23.2	Development	100	
	PDLIM4	5q31.1	Development	85/70	Adenoma vs CRC
	PPM1E	17q23.2	Phosphatase	55	
	PRKD1	14q12	Kinase	20	
	PROM1	4p15.32	Putative TSG	62	
	PTGIS	20q13.1-q13.3	Prostaglandin signaling	30/44	Adenoma vs CRC
	PTGS2	1p25.2-3	Inflammation	72	
	PTPRD	9p23	Phosphatase	50	
	RAB32	6q24.3	Ras signaling	56	MSI tumors
	RARβ	3p24.2	Hormonal signaling	33–85	
	RASSF1A	3p21.2	Ras signaling	41/57	Stage I/III vs IV
	RASSF2	20p13	Ras signaling	42	
	RASSF5	1q32.1	Ras signaling	NA	Increase of methylation level in CRC
	RECK	9p13.3	Putative TSG	44	
	RUNX3	1p36.11	Transcription factor	27–63	Poor prognosis
	SCTR	2q14.1	G protein-coupled receptor	81	
	SFRP1	8p11.21	Wnt signaling	95–100	
	SFRP4	7p14.1	Wnt signaling	100	
	SH3TC1	4p16.1	Putative TSG	40	
	SLC5A8	12q23.1	Solute carrier	80	
	SLC6A15	12q21.31	Solute carrier	NA	Increase of methylation level in CRC
	SLIT2	4p15.2	Cell migration	72	
	SMO	7q32.1	G protein-coupled receptor	21	
	SNCA	4q21.3-q22	Dopamine signaling	53/66	Adenoma vs CRC
	SOCS1	16p13.13	Cytokine signaling	22	
	SOX17	8q11.23	Transcription factor	86/89–100	Adenoma vs CRC
	SPARC	5q33.1	ECM component	100	
	SPG20	13q13.3	Putative TSG	78/89	Adenoma vs CRC
	SST	3q28	Hormonal signaling	90	
	ST3GAL6	3q12.2	Putative TSG	44	Correlated with EBV-associated gastric carcinomas
	STARD8	Xq13.1	Putative TSG	55	
	SYNE1	6q25.2	Putative TSG	95	
	SYT6	1p13.2	Calcium metabolism	64	
	TAC1	7q21.3	Hormonal signaling	95	
	TCERG1L	10q26.3	Putative TSG	100	
	TFPI2	7q22	ECM component	NA	Increase of methylation level in CRC
	TIMP3	22q12–13	ECM component	23	
	TMEFF2	2q32.3-q33	Cell proliferation	77	
	TP73	1p36.33	Cell cycle control (G1-S)	63	
	TUBG2	17q21	Cell migration	71	
	TUSC3	8p22	Putative TSG	66	Associated with ulcerative colitis
	TWIST1	7p21.1	Transcription factor	NA	Increase of methylation level in CRC
	UNC5C	4q22.3	Development	64/76	Adenoma vs CRC
	VIM	10p13	Cell migration	91/77	Adenoma vs CRC
	WIF-1	12q13.13	WIF-1	100	Very limited number of samples

Table 1 (continued)

Epigenetic event	Name	Locus	Function/targets	Note[b]	Comments
	WNT5a	3p14.3	Wnt signaling	20	Associated with MSI and BRAF V600E mutation
	WRN	8p12	DNA repair	29	
	WT1	11p13	Transcription factor	58	
	ZNF569	19q13.12	Transcription factor	40	
Hypomethylation	C7orf50	7p22.3	Unknown	NA	
	CARD14	17q25.3	NF-κB signaling	NA	
	CCDC116	22q11.21	Transcriptional regulator	NA	
	CDH3	16q22.1	Cell adhesion	77	
	CSRP1	1q32.1	Development	NA	
	EPHX4	1p22.1	Cell detoxification	NA	
	GPR109A	12q24.31	G protein-coupled receptor	NA	
	GPSM1	9q34.3	G protein signaling	NA	
	GRAP	17p11.2	Intracellular signaling	NA	
	H19	11p15.5	Putative TSG	18	
	HIST1H2BO	6p22.1	Histone	NA	
	IGF2	11p15.5	Development	35	Poor prognosis
	L1CAM	Xq28	Cell adhesion	NA	
	LAMB1	7q22	ECM component	NA	
	LILRA4	19q13.4	Cytokine signaling	NA	
	LINE1	NA	Retrotransposon	NA	Associated with MSI and CIMP tumors
	MAEL	1q24.1	piRNA system	NA	
	MIRLET7BHG	22q13.31	Long non-coding RNA	NA	
	NRXN1	2p16.3	Cell adhesion	NA	
	NUP50	22q13.3	Macromolecule trafficking	NA	
	S100A4	1q21.3	Cell cycle	NA	
	S1PR4	19p13.3	G protein-coupled receptor	NA	
	SFT2D3	2q14.3	Transport and trafficking	NA	
	SLC39A4	8q24.3	Solute carrier	NA	
	SLC6A18	5p15.33	Solute carrier	NA	
	SLC6A6	3p25.1	Solute carrier	NA	
	TIAM1	21q22.1	Cell migration	NA	Associated with metastasis
miRNA	let-7 family	NA	DLD-1, c-Myc, K-RAS	−	Poor prognosis
	miR-1-1	20q13.33	TAGLN2	−	
	miR-9-1	1q22		−	
	miR-10b	2q31.1		−	
	miR-15b	3q25.33		+	
	miR-16	NA	Wip1	−	
	miR-17	13q31.3	E2F1	+	Poor prognosis, MSS tumors
	miR-18a	13q31.3	K-RAS	+	Without lymph node metastasis
	miR-18b	Xq26.2		+	Without lymph node metastasis
	miR-19a	13q31.3	PTEN	+	Without lymph node metastasis
	miR-19b	NA		+	
	miR-20a	13q31.3	BNIP2	+	MSI
	miR-21	17q23.1	Cdc25A, MSH2, PTEN, RECK, TIMP3	+	Poor prognosis, decrease of chemotherapy response, MSI tumors
	mir-24	NA	DHFR	−	
	miR-25	7q22.1		+	
	miR-26b	2q35	EphA2	−	
	miR-29a	7q32.3		+	
	miR-29b	NA		+	
	miR-30a	6q13	Beclin 1	−	
	miR-30c	NA		−	

Table 1 (continued)

Epigenetic event	Name	Locus	Function/targets	Note[b]	Comments
	miR-31	9p21.3	FIH-1	+	Poor prognosis
	miR-32	9q31.3		+	
	miR-33a	22q13.2		+	
	miR-34a	1p36.22	Bcl2, CDK4/6, E2F3, MET, SIRT1	−	
	miR-34b/c	11q23.1	Tp53	−	
	miR-92a	NA		+	MSS tumors
	miR-93	7q22.1		+	
	miR-95	4	SNX1	+	
	miR-96	7q32.2		+	
	miR-99a	21q21.1		−	
	miR-101	NA	COX-2	−	MSI tumors
	miR-106a	Xq26.2	E2F1	+	
	miR-106b	7q22.1	CDKN1A (p21)	+	Without lymph node metastasis
	miR-124-1	8p23.1		−	
	miR-125a	19q13.41		−	
	miR-125b	NA		+	Poor prognosis
	miR-126	9q34.3	p85β	−	Associated with metastasis
	miR-127	14q32.2		−	
	miR-129-2	11p11.2		−	
	miR-103b	NA		+	
	miR-133a	NA		−	
	miR-133b	6p12.2	c-Met	+	
	miR-135a	NA	APC	+	
	miR-135b	1q32.1	APC	+	Without lymph node metastasis
	miR-137	1p21.3	Cdc42, LSD-1	−	
	miR-139	11q13.4	β–Catenin	−	
	miR-140	16q22.1	HDAC4	−	
	miR-141	12p13.31	TGF-β1	+	
	miR-142	17q22		−	MSS tumors
	miR-143	5q32	DNMT3A, Erk5, K-RAS	−	Decrease of chemotherapy response, associated with metastasis
	miR-145	5q32	FLI1, IRS1, STAT1, YES	−	MSI tumors
	miR-146b	10q24.32		−	MSS tumors
	miR-155	21q21.3	MLH1, MSH2, MSH6	+	With lymph node metastasis
	miR-181b	NA		+	Decrease of chemotherapy response
	miR-182	7q32.2		+	
	miR-183	7q32.2	Klf4, Sox2, BMI1	+	
	miR-191	3p21.31		−	
	miR-192	11q13.1	DHFR, TS, TYMS	−	Decrease of chemotherapy response
	miR-195	17p13.1	Bcl-2	−	
	mir-196a	NA	AKT	−	Increase metastasis potential
	mir-196b	7p15.2		+	Without lymph node metastasis
	miR-200a	1p36.33	ZEB1, ZEB2, MLH1, MSH2	+	Associated with metastasis
	miR-200b	1p36.33	MLH1, MSH2	+	Associated with metastasis
	miR-200c	12p13.31	TGF-β2, ZEB1, ZEB2, BMI1, PTPN12	+	Poor prognosis, associated with metastasis
	miR-203	14q32.33	Klf4, Sox2, BMI1	+	
	miR-212	17p13.3		−	MSS
	miR-215	1q41	DHFR, TS, TYMS	−	Decrease of chemotherapy response
	miR-217	2p16.1		−	MSS
	miR-223	Xq12		+	
	miR-224	Xq28		+	Without lymph node metastasis
	miR-301b	22q11.21		+	Without lymph node metastasis

Table 1 (continued)

Epigenetic event	Name	Locus	Function/targets	Note[b]	Comments
	miR-320	8p21.3		−	Poor prognosis
	miR-328	16q22.1		−	
	miR-335	7q32.2		+	Without lymph node metastasis
	miR-342	14q32.2	DNMT1	−	
	miR-345	14q32.2	BAG3	−	
	miR-373	19q13.42	LATS2, CD44, RAB22A	−	
	miR-374a	Xq13.2		+	Without lymph node metastasis
	miR-378	5q32		−	Without lymph node metastasis
	miR-378*	5q32		−	Without lymph node metastasis
	miR-422a	15q22.31		−	
	miR-424	Xq26.3		+	Without lymph node metastasis
	miR-432*	14q32.2		+	MSI tumors
	miR-451	17q11.2	MIF	−	Poor prognosis
	miR-455	9q32		−	MSI tumors
	miR-484	16p13.11		−	MSI tumors
	miR-486	8p11.21		−	
	miR-492	12q22		+	MSI tumors
	miR-497	17p13.1		−	
	miR-498	19q13.42		−	Poor prognosis
	miR-510	Xq27.3		+	MSS tumors
	miR-513	NA		+	MSS tumors
	miR-542	Xq26.3		+	
	miR-552	1p34.3		+	
	miR-592	7q31.33		+	MSS tumors
	miR-675	11p15.5	Rb	+	

CIMP, CpG island methylator phenotype; ECM, extracellular matrix; MSI, microsatellite instability; MSS, microsatellite stable; TSG, tumor suppressor gene.

[a] Only hypermethylated genes with methylation prevalence≥20% in CRC patients and≤10% in normal mucosa were reported. Gene symbols and chromosome location are in accordance with www.genecards.org.

[b] For DNA hypermethylation/hypomethylation, number represent prevalence (%) in CRC; for miRNAs, - and + mean down-regulated and up-regulated in CRC compared to normal mucosa, respectively; NA means "not applicable."

Besides its diagnostic potential, methylated genes are associated with a number of clinical features correlated with poor prognosis (DLEC1, EFEMP1, EphA1, EGFR, MLH1, CDH13) [19, 21–24], Epstein-Barr virus–associated gastric carcinomas (B4GALNT2, ST3GAL6) [25]. In contrast, some methylated genes (GATA4, GATA5) are found methylated independently of clinicopathologic features [26].

Some genes are not, at least alone, good biomarkers for CRC since they are frequently methylated in other cancer types such CDKN2A (p16), found methylated across various tumors [10, 16]. In contrast, APC2, B4GALNT1, CCNA1, CDX1, GPNMB, LAMA1, NTRK2, PAPSS2, TCERG1L, and SFRP4 genes are found methylated near 100% of patients tested [19, 27–29]. Therefore, these genes could represent promising CRC biomarkers, similarly to the methylation of detoxification enzyme GSTP1, which is a hallmark of prostate cancer, even though data suggest it may also occur in other cancers [16, 30]. Nevertheless, it confirms that epigenetic silencing is far more common than mutations (see for review of mutation frequencies [13]). Interestingly, numerous genes are gradually methylated during colorectal carcinogenesis. By example, CASR is found methylated at 9%, 69%, and 90% in adenoma, carcinoma, and lymph node metastatic tissues, respectively [31]. Intriguingly, some CRC patients accumulate methylation abnormalities in a large number of genes. This CRC subset is defined with CpG island methylator (CIMP) phenotype characterized by clinicopathological and genetic (chromosomal instability) features, which are the consequence of hypermethylation-mediated TSG silencing involved in the malignant transformation of colonic tissue [32]. In sporadic MSI tumors, hypermethylation-mediated silencing of MMR genes such as MLH1 is common [19, 23, 24].

Concomitant with DNA promoter CGI hypermethylation-mediated silencing, global genomic hypomethylation is

observed in CRC. This hypomethylation is usually associated with oncogene activation and genetic instability. Accordingly, an increasing list of genes were identified as hypomethylated in CRC patients, such as CCDC116, SFT2D3, MAEL, and H19/IGF2, which could also be used as biomarkers to reinforce CRC detection [33••, 34]. Furthermore, a recent study suggests that long interspersed nuclear element-1 (LINE-1) hypomethylation could be used as a predictive biomarker of chemotherapy response to fluoropyrimidines in CRC patients [35].

Finally, DNMT expression might also be used as a marker, since overexpression of DNMT1 mRNA was reported in 42% of CRC [36].

All together, these events may represent powerful biomarkers for secondary prevention and risk stratification in CRC. Accordingly, these markers represent promising targets for therapeutic/chemopreventive interventions.

miRNA in CRC

MiRNA pathway is an additional epigenetic mechanism implicated in the regulation of tightly regulated biological processes. MiRNAs are endogenous short non-coding RNAs (~22 nucleotides) that post-transcriptionally regulate mRNA expression levels in a sequence-specific manner. MiRNAs bind sequences located essentially in 5′ and 3′ untranslated regions of target genes degrading mRNA or blocking translation. Increasing amount of evidence reveals that miRNA expression signature dysregulations are associated with carcinogenesis, suggesting miRNAs might act as a novel class of oncogenes or TSGs [8, 10, 37].

An increasing number of reports indicate that miRNA dysregulations are important in colorectal carcinogenesis. Table 1 summarizes these alterations based on experimental data from patients. MiRNome signatures revealed that miRNA affected many tumor-suppressive and oncogenic pathways implicated in CRC pathobiology, including β-catenin/Wnt signaling (miR-135a, -135b, -139, -145) [38•, 39, 40], apoptosis (miR-34a, -133b, -195) [38•, 41], differentiation (miR-141, -200c) [42–44], p53 signaling (miR-34b/c) [45], proliferation (K-RAS signaling: let7 family, miR-18a, -143, -200c) [38•, 41, 46], cell cycle control (miR-34a, -192, -215, -675) [38•, 41, 47], and migration, invasion, and metastasis (miR-126, -143, -196a, -200a, -200b, -200c, -373, -520c) [38•, 41, 44]. MiRNA pathway may also modulate DNA methylation (miR-143, -342) [48, 49].

In addition, miRNA alterations are correlated to a number of clinicopathologic features and outcomes related to CRC pathogenesis. MiR-21 is a representative example, since high levels of expression are associated with lymph node positivity, increased metastasis propensity and advanced tumor stages associated with worse overall survival [50, 51]. Additional miRNAs, including miR-17, -31, -125b, -126, -143, -196a, -200c, -320, -451, and -498, were identified as associated to an increase of metastasis potential, a decrease of disease-free survival, and a poor prognosis [38•, 40–42, 44, 46, 52, 53].

Several studies have identified miRNA expression signatures associated with MSS or MSI CRC phenotypes. These include miR-17, -92, -142, -146b, -212, -217, -510, -513, and -592 associated with MSS, whereas miR-20a, -101, -145, -432*, -455, -484, -492, and -625 were higher in MSI-H tumors [39, 52, 54]. Furthermore, four miRNAs (miR-31, -224, -552, and -592) were identified as able to discriminate between MMR-proficient and MMR-defective adenocarcinomas [40].

All together, these data suggest that CRC-specific miRNA expression signatures are common events, which are representative of CRC-related genetic instability and may be a key event for tumor onset and development. Accordingly, miRNA expression signatures have great and valuable potential for diagnostic and prognostic purposes.

For the past decades, 5-fluorouracil (5-FU) has been and still is the most commonly used chemotherapeutic agent in CRC treatments. However, a significant fraction of patients are refractory or become resistant to 5-FU–based chemotherapies. A growing body of evidence is revealing the importance of miRNA alterations in the modulation of tumor response to 5-FU treatments. For instance, miR-92, -143, and -215, by impairing 5-FU–induced apoptosis [55], could be implicated in the resistance to 5-FU developed by CRC patients presenting low level of expression of miR-92, -143, and -215 [38•, 41]. In addition, miR-21, which plays a central role in colon cancer pathogenesis by targeting many TSGs with elevated expression in advanced tumor stages, was described as an independent predictive marker associated with poor survival and for which overexpression predicts a poor response to therapy [50, 51]. Finally, a recent study suggests that miRNA SNPs rs7372209 and rs1834306 in miR-26-a-1 and miR-100 genes, respectively, affect the clinical outcome of 5-FU–treated CRC patients [56]. These data suggest that miRNA signatures have a potential as marker to predict chemotherapy response.

It has been suggested that, in addition to DNA hypermethylation-mediated silencing of miRNAs (Table 1) [45, 53, 57, 58••, 59–61], alterations of proteins involved in miRNA processing is observed in CRC. Indeed, Papachristou et al. [62] reported that the nuclear ribonuclease Drosha and the cytoplasmic ribonucleases Dicer and Ago2 are possibly implicated in colorectal carcinogenesis and that Dicer could influence tumor progression to advanced stages.

Taken together, these findings demonstrate that miRNome alterations represent promising candidates to develop specific and sensitive biomarkers in CRC pathology with opportunities for primary to tertiary prevention levels.

Histones and Histone-Modifying Enzymes in CRC

An additional layer of epigenetic regulation of gene expression is represented by histone tail post-translational covalent modifications. Core histone (H2A, H2B, H3, H4) N-termini are modified by phosphorylation, acetylation, methylation, ubiquitylation, sumoylation, citrullination, β-N-acetylglucosamination, deimination, and ADP-ribosylation. Altogether, these dynamic and reversible modifications establish a "histone code" regulating chromatin structure and activity. The better understood modifications are acetylation of lysine and methylation of arginine and lysine residues. The acetylation/deacetylation reactions are catalyzed by histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively. Similarly, methylation/demethylation processes are driven by histone methyltransferase (HMTs) and histone demethylases (HDM). While acetylation occurs as a single addition, methylation exists at various levels on the same residue (ie, mono-, di-, and tri-methylation) [63, 64].

There is now clear evidence that aberrant histone modification profiles are closely connected to tumorigenesis. Indeed, dysregulated activity or expression of histone-modifying enzymes as well as their aberrant recruitment by cytogenetic alterations (eg, leukemia-associated fusion proteins) participate in cancer development by inducing aberrant regulation of oncogenes and/or TSGs, and affecting genome stability and/or chromosome segregation [10, 64, 65]. Although our knowledge about histone code and histone-modifying enzymes is incomplete, some data suggest their implications in CRC. A study from Weichert et al. [66] revealed that HDAC1, HDAC2, and HDAC3 are overexpressed in 36.4%, 57.9%, and 72.9% of CRC cases, respectively. Interestingly, the expression was significantly enhanced in strongly proliferating and poorly differentiated tumors. Thus, high HDAC expression levels are associated with reduced patient survival, with in addition, HDAC2 expression being a prognostic factor for survival [66]. HDAC2 overexpression is accompanied by H4K12 and H3K18 acetylation and correlates with adenoma-carcinoma progression [67]. HDAC1 increase was confirmed in another study reporting an upregulation of two HATs: K(lysine) acetyltransferase 2B (KAT2B, CBP) and p300. KAT2B overexpression was associated with long-term survival, whereas p300 overexpression was correlated with a poor prognosis [68]. Interestingly, the class III HDAC sirtuin 1 is overexpressed in 37% of CRC patients and is mainly associated with MSI and CIMP-high phenotypes [69•]. Finally, it was demonstrated that the expression of the cell-cycle regulator p21 is lower in CRC associated with widespread histone H3 hypo-acetylation in chromatin. These observations were connected to the development and progression of CRC but not with tumor biological behaviors [70].

Dysregulation of enzymes involved in histone methylation is also observed in CRC. Indeed, the HMT suppressor of variegation 3–9 homolog 1 (SUV39H1) is overexpressed in 25% of CRC patients and its expression is significantly associated with DNMT1 expression [36]. Furthermore, the histone H3 lysine 4-specific HMT suppressor of variegation, enhancer of zeste, and trithorax (SET)1 is over-expressed in colon tumor cells, where its expression promotes cell proliferation and survival [71]. Moreover, the multiple myeloma SET domain (MMSET) HMT and putative oncoprotein is overexpressed in CRC patients with a worse 5-year survival. Recently, MMSET expression was associated with a good prognostic value in colon cancer and is more pronounced in early stages of colon carcinogenesis (dysplasia) than in adenocarcinomas [72]. Noteworthy, the histone H3 lysine 9-specific HDM, Jumonji domain containing 1A (JMJD1A) was reported as a useful biomarker for hypoxic tumor cells [73]. In humans, enhancer of zeste homolog 2 (EZH2) overexpression-mediated gene silencing has been identified in numerous tumor types associated with H3K27me3 widespread high levels in chromatin. Recent evidence demonstrated that EZH2 overexpression is a common feature of CRC (observed in 87% of cases) [74]. Finally, it was suggested that oncogenic RAS pathways could modulate histone modifications to influence the expression of target genes involved in the regulation of cell proliferation [75]. Accordingly, overexpression of the HMT SET and MYND domain-containing protein 3 (SMYD3) has been reported in mutated K-RAS CRC patients [76].

Taken together these data suggest that histone modification profiles and histone-modifying enzymes could be used as marker as well as therapeutic/chemopreventive targets in CRC and therefore play a role in CRC prevention.

Chemoprevention, Epigenetics, and CRC

Epigenetic mechanisms by their potential reversibility represent interesting targets in CRC for chemopreventive approaches using dietary agents. Accumulating evidence suggests that natural molecules/nutrients present in our diet might modulate epigenetic events in humans. Table 2 summarizes compounds identified in various in vitro and in vivo tumor models that may exert their chemopreventive potential by targeting epigenetic mechanism(s). The current knowledge about some naturally occurring compounds, which may play a significant role in CRC chemoprevention related to epigenetic modulation, is discussed below.

Curcumin is well recognized for its chemopreventive and therapeutic properties in vitro and in vivo against many tumor types. Curcumin decreases inflammation cell proliferation, invasion, and angiogenesis, triggers apoptosis, and sensitizes tumor cells to cancer therapies [77–79]. These protective properties could be, at least partially, mediated by a modulation of epigenetic events. While no study was performed in

Table 2 Compounds present in diet acting as epigenetic modulators

Dietary agent	Food source	Potential epigenetic target
3,3′-diindolylmethane	Broccoli, cauliflower (indole-3-carbinol metabolite)	Histone modifications, miRNAs
6-methoxy-2E,9E-humuladien-8-one	Ginger	Histone modifications
Allicin	Garlic	Histone modifications
Allyl mercaptan	Garlic	Histone modifications
Anacardic acid	Cashew nuts	Histone modifications
Apigenin	Parsley, celery	DNA methylation
Biochanin A	Soy	Histone modifications
Butein	Toxicodendron vernicifluum	Histone modifications
Butyrate	Gut flora–mediated fermentation of dietary fiber	Histone modifications
Caffeic acid	Coffea	Histone modifications
Catechin	Green tea	Histone modifications
Chlorogenic acid	Coffea	Histone modifications
Cinnamic acid	Cinnamon	Histone modifications
Coumaric acid	Cinnamon	Histone modifications
Curcumin (diferuloylmethane)	Turmeric	Histone modifications, miRNAs
Daidzein	Soy	Histone modifications
Delphinidin	Cranberries, Concord grapes, pomegranates	Histone modifications
Diallyl disulfide	Garlic	Histone modifications
Dihydrocoumarin	Sweet clover (Meliotus officinalis)	Histone modifications
(-)-Epigallocatechin gallate	Green tea	DNA methylation, histone modifications, miRNAs
Equol	Soy	Histone modifications
Fisetin	Strawberries	DNA methylation
Flavone	Mandarin	Histone modifications
Folate	Leafy vegetables, beans, peas, lentils, eggs, liver	DNA methylation, histone modifications
Garcinol, isogarcinol	Garcinia indica	Histone modifications
Genistein	Soybean	DNA methylation, histone modifications, miRNAs
Hesperidin	Citrus	DNA methylation
Isoliquiritigenin	Licorice	Histone modifications
Isothiocyanates	Broccoli	Histone modifications, miRNAs
Kaempferol	Apples, nuts, tea, onions	Histone modifications
Luteolin	Celery, parsley	Histone modifications
Lycopene	Tomatoes and various fruits	DNA methylation
MCP30	Bitter melon	Histone modifications
Myricetin	Walnuts and various berries, fruits, and vegetables	DNA methylation
Naringenin	Citrus	DNA methylation
Phloretin	Apples	DNA methylation
Piceatannol	Grapes (resveratrol metabolite)	Histone modifications
Polyphenon B	Green and black tea	Histone modifications
Pomiferin	Maclura pomifera	Histone modifications
Protocatechuric acid	Olives	DNA methylation
Quercetin	Apples, tea, onion, nuts, berries	DNA methylation, histone modifications
Resveratrol	Grapes	Histone modifications
Rosmarinic acid	Rosemary	DNA methylation
S-allylmercaptocysteine	Garlic	Histone modifications
Sanguinarine	Opium poppy	Histone modifications
Silibinin	Milk thistle	Histone modifications
Sinapinic acid	Sinapis (mustard)	DNA methylation
Sulforaphane	Broccoli	DNA methylation, histone modifications
Syringic acid	Red grapes	DNA methylation
Theophylline	Green and black tea	Histone modifications
Ursolic acid	Basil	Histone modifications
Selenium	Nuts, cereals, meat, fish, eggs, kidney	DNA methylation, histone modifications

colon cells, curcumin is a well-known inhibitor of p300/KAT2B HAT activity [80]. Furthermore, it was shown that curcumin modulates the miRNA pathway. Specifically, curcumin inhibits miR-21 expression via AP-1 leading to a decreased proliferation and metastasis potential in CRC [81].

Butyrate is an essential short-chained fatty acid (SCFA) for the colon epithelia formed from bacteria-fermented dietary fibers. Butyrate triggers growth arrest, differentiation, and/or apoptosis in many in vitro and in vivo precancerous and tumor cell models including CRC cell lines [82–84]. These biological effects leading to carcinogenesis suppression have been proposed to account for the chemopreventive properties of butyrate and to be mediated by HDAC inhibition–induced histone hyperacetylation [83, 84]. Furthermore, butyrate was identified as the most potent HDAC inhibitor among various SCFAs tested in colon carcinoma cells. In the same study, cinnamic acid, coumaric acid, and caffeic acid also showed HDAC inhibitory activities [85].

(-)-Epigallocatechin gallate (EGCG), the major polyphenol in green tea, has been extensively studied both in vitro and in animal models of carcinogenesis and is well recognized for its chemopreventive properties. EGCG seems to have DNA-demethylating properties since it can induce the reactivation of some methylation-silenced TSGs in various tumor models including human colon cancer cells, limiting their proliferation and invasiveness [86, 87].

Isothiocyanates such as sulforaphane (SFN) are sulfur phytonutrients abundant in broccoli reported to present chemopreventive properties in CRC. SFN has been initially found to inhibit in vitro HDAC activity in human colon cancer cells and then in numerous other models [88, 89]. In vivo, a study demonstrated that APCmin/+ mice with SFN-enriched diet have reduced tumor development associated with an increased histone acetylation and p21 expression [90]. Remarkably, in humans, consumption of 68 g broccoli resulted in a significant inhibition of blood HDAC activity 3 h following intake [91]. Furthermore, prolonged exposure to SFN induces a decrease of various class I and selected class II HDAC proteins and especially HDAC3 [92].

3,3′-diindolylmethane (DIM) is a digestive metabolite of indole-3-carbinol, which is found in vegetables such as broccoli or cauliflower. DIM strongly decreases the expression of the anti-apoptotic protein survivin and enhances the effect of butyrate on both apoptosis in colon cancer cells and prevention of FAP in APCmin/+ mice. These effects were accompanied by a drastic decrease of HDAC1, HDAC2, and HDAC3 expression [93], which could be explained by selective DIM-induced proteasomal degradation of class I HDACs (HDAC1–3, and 8), leading to p21 and p27 overexpression. These data may account for DIM's capability to trigger G2-cell cycle arrest and apoptosis [94].

Garlic-derived sulfur compounds such as diallyl disulphide (DADS) or allyl mercaptan (AM) are known for their HDAC inhibitory potential. Thus, these compounds induce total histone hyperacetylation in colon cancer cells as well as CDKN1A promoter-associated histone hyperacetylation, which is responsible for p21 overexpression and correlated with a G2/M-cell cycle arrest [89, 95]. Remarkably, epidemiological data suggest that garlic consumption decreases risks of CRC. Thus, it is believed that the effect of these sulfur compounds on HDAC account for their anticarcinogenic and chemopreventive properties.

Quercetin has been shown to activate the class III HDAC sirtuin 1 (SIRT1) and to be a potent antitumor agent by decreasing proliferation, and triggering G2/M-cell cycle arrest and apoptosis in cancer cells [96, 97]. In addition, a study revealed that quercetin demethylates CDKN2A promoter in colon cells [98]. Therefore, quercetin might present protective properties against CRC.

Finally, folate and selenium are common nutrients reported to influence epigenetic events. Epidemiological studies support the link between low folate concentrations and increased CRC risk [99]. Folate is the main source of methyl group necessary for the production of SAM, a universal cofactor in methylation reactions. Thus, defects in folate metabolism or intake lead to hypomethylation of genomic DNA or proto-oncogene and alterations of histone methylation patterns associated with genomic instability in colon cells [83]. Selenium has also been reported to alter epigenetic mechanisms, providing a rationale for its potential chemopreventive efficacy. Indeed, it was shown that colon DNA from rats fed a selenium-rich diet was hypomethylated, whereas low-selenium diet increases DNA methylation of the TSG von Hippel-Lindau [100]. These data were linked to selenium propensity to inhibit DNMT1 activity and protein expression in colon cells [101]. Furthermore, organoselenium metabolites of Se-methyl-L-selenocysteine and L-selenomethionine methylselenopyruvate induce HDAC inhibition–dependent histone H3 acetylation in colon cancer cells associated with an induction of p21 expression, which could account for G2/M cell cycle arrest and apoptosis [102]. Therefore, unbalanced and improper consumption of these nutrients might have an injurious impact on colorectal carcinogenesis.

Conclusions and Perspectives

Since epigenetic alterations are reversible, they were initially considered as interesting targets for chemotherapy using DNMT and HDAC inhibitors such as 5-aza-2′-deoxycytidine (decitabine) and suberoylanilide hydroxamic acid (SAHA, vorinostat), respectively. These compounds induce pleiotropic biological effects including regulation of cell growth, differentiation, autophagy, senescence, and apoptosis. Additionally, they sensitize cells to classical chemotherapeutic agents and they mostly act synergistically as antitumor agents against cancer

cells [10, 63, 103, 104]. Nonetheless, the use of such pharmacological epigenetic modulators is associated with some dose-limiting toxicities such as neutropenia and thrombocytopenia observed with SAHA or nonspecific cytotoxic effects observed with nucleoside analogues DNA demethylating agents inherent to their incorporation into DNA. In the perspective to reduce these drawbacks, natural compounds might represent a good alternative to identify safer epigenetic modulators. Accordingly, increasing evidence about the impact of environment on epigenetics as well as early occurrence of epimutations in carcinogenesis make us reconsider epigenetic events as promising preventive targets. However, to reach these attractive perspectives, we need to improve our current knowledge of CRC-associated early epigenetic changes, for early detection and to define promising epigenetic targets for chemoprevention. In addition, a clear impact of such chemopreventive strategies is needed, which requires a better rationale of studies to determine detail mechanisms, and assess safety and efficient doses for humans. Nevertheless, epigenetics and chemoprevention by dietary modulators is well associated with targeted therapy and personalized oncology and should ultimately aid to decrease CRC incidence and mortality rate.

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- • Of importance
- •• Of major importance

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