

Rajnee Kanwal and Sanjay Gupta

J Appl Physiol 109:598-605, 2010. First published Mar 4, 2010; doi:10.1152/jappphysiol.00066.2010

You might find this additional information useful...

This article cites 61 articles, 21 of which you can access free at:

<http://jap.physiology.org/cgi/content/full/109/2/598#BIBL>

Updated information and services including high-resolution figures, can be found at:

<http://jap.physiology.org/cgi/content/full/109/2/598>

Additional material and information about *Journal of Applied Physiology* can be found at:

<http://www.the-aps.org/publications/jappl>

This information is current as of September 10, 2010 .

HIGHLIGHTED TOPIC | *Epigenetics in Health and Disease*

Epigenetics and cancer

Rajnee Kanwal¹ and Sanjay Gupta^{1,2,3}¹Departments of Urology and Nutrition, Case Western Reserve University, ²University Hospitals Case Medical Center, and ³Case Comprehensive Cancer Center, Cleveland, Ohio

Submitted 20 January 2010; accepted in final form 3 March 2010

Kanwal R, Gupta S. Epigenetics and cancer. *J Appl Physiol* 109: 598–605, 2010. First published March 4, 2010; doi:10.1152/jappphysiol.00066.2010.— Epigenetic modifications are central to many human diseases, including cancer. Traditionally, cancer has been viewed as a genetic disease, and it is now becoming apparent that the onset of cancer is preceded by epigenetic abnormalities. Investigators in the rapidly expanding field of epigenetics have documented extensive genomic reprogramming in cancer cells, including methylation of DNA, chemical modification of the histone proteins, and RNA-dependent regulation. Recognizing that carcinogenesis involves both genetic and epigenetic alterations has led to a better understanding of the molecular pathways that govern the development of cancer and to improvements in diagnosing and predicting the outcome of various types of cancer. Studies of the mechanism(s) of epigenetic regulation and its reversibility have resulted in the identification of novel targets that may be useful in developing new strategies for the prevention and treatment of cancer.

methylation; histone modification; microRNA

Glossary

5aC	5-azacytidine	H4K20me3	Histone H4 trimethyl lysine 20
5adC	5-aza-2'-deoxycytidine	HDAC	Histone deacetylase
APC	Familial adenomatous polyposis	HMTases	Histone methyltransferases
BRCA1	Breast cancer 1, early onset	HOXD4	Vertebrate Hox paralogy group 4
CDC42EP5	CDC42 effector protein (Rho GTPase binding) 5	HP1	Heterochromatin protein 1
CDH13	H-cadherin	JMJD3	JmjC-domain protein
CST6	Human cystatin M/E gene	LOH	Loss of heterozygosity
DNMT	DNA methyltransferase	LOI	Loss of imprinting
DSB	Double strand break	MBD	Methyl-CpG-binding domain
EZH2	Enhancer of zeste homolog 2	MeCP	Methyl-cytosine binding protein
GSTP1	Glutathione S-transferase P1–1 isoform	methyl	
H3ac	Acetylated histone H3	H3K4	Histone H3 lysine 4 methylation
H3K20me1	Mono-methylated histone H3 at lysine 20	methyl	
H3K27me1	Histone H3 monomethyl lysine 27	H3K9	Histone H3 lysine 9 methylation
H3K27me2	Histone H3 dimethyl lysine 27	MGMT	O ⁶ -methylguanine-DNA methyltransferase
H3K36me3	Trimethylation of lysine 4, 9 and 36 of histone H3	MKK4	Mitogen-activated protein kinase kinase 4
H3K4me1	Mono-methylated histone H3 at lysine 4	MLH1	Mismatch repair gene 1
H3K4me2	Di-methylated histone H3 at lysine 4	MYOD1	Myogenic determination gene
H3K4me3	Histone H3 trimethylated at lysine 4	NME	Metastasis-suppressor nme gene
H3K79me3	Tri-methylated histone H3 at lysine 79	PARP	Poly-(ADP)ribosyl polymerase
H3K9me1	Mono-methylated histone H3 at lysine 9	PCNA	Proliferating cell nuclear antigen
H3K9me2	Dimethylated histone H3 lysine 9	PML-RAR	Promyelocytic leukemia-RAR fusion protein
H4ac	Acetylated histone H4	PRDM14	Zinc finger SET domain gene
H4K16ac	Acetylated histone H4 at lysine 16	RA	Retinoic acid
		RAR	Retinoic acid receptor
		RARB2	Retinoic acid receptor B2
		RASSF1A	Human Ras association domain family 1A gene
		Rb	Retinoblastoma
		SIRT	Silent information regulator type 1

Address for reprint requests and other correspondence: S. Gupta, Dept. of Urology, Case Western Reserve Univ., Univ. Hospitals Case Medical Center, 10900 Euclid Ave., Cleveland, OH 44106 (e-mail: sanjay.gupta@case.edu).

SWI/SNF	Family of ATPase-dependent chromatin remodeling determinants
TIMP3	Tissue inhibitor of metalloproteinases 3
TSA	Trichostatin A
TSG	Tumor suppressor gene
TSP1	Thrombospondin 1
uPA	Urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor
VHL	Von Hippel-Lindau
WRN	Werner gene

CANCER IS THE MANIFESTATION of both genetic and epigenetic modifications (1, 2, 16–18). Although cancer initiation and progression are predominantly driven by acquired genetic alterations, it is becoming clear that microenvironment-mediated epigenetic perturbations play important roles in neoplastic development (27). Epigenetics is defined as heritable changes in gene expression activity and expression that occur without alteration in DNA sequences but which are sufficiently powerful to regulate the dynamics of gene expression (37). The key processes that are responsible for epigenetic regulation are DNA methylation, modifications in chromatin (covalent modification of core histones), nucleosome positioning (physical alteration), and posttranscriptional gene regulation by noncoding RNA (micro-RNAs) (16). A number of well characterized epigenetic modifications are linked to aberrant gene functions and altered patterns of gene expression that play critical roles in the pathobiology of cancer.

EPIGENETIC MECHANISMS IN CANCER

DNA methylation. DNA methylation is an essential component of the epigenetic machinery in regulating gene expression and interacting with nucleosomes that control DNA packaging, affecting entire domains of DNA (14). In mammalian cells, DNA methylation occurs at the 5' position of the cytosine ring within CpG dinucleotides via addition of a methyl group to form 5-methyl cytosine (31). The modification at 5-methyl cytosine is catalyzed by enzymes known as DNA methyl transferases (DNMTs). There are three main DNMTs, one of which is DNMT1, the major maintenance enzyme that preserves existing methylation patterns following DNA replication by adding methyl groups to the hemi-methylated CpG sites (50). DNMT3a and DNMT3b are de novo methyltransferases that preferentially target unmethylated CpGs to initiate methylation; they are highly expressed during embryogenesis and minimally expressed in adult tissues (4). Another family member, DNMT-3L, lacks intrinsic methyltransferase activity; it interacts with DNMT3a and 3b to facilitate methylation of retrotransposons (15). Although DNA methylation regulates gene expression in normal tissues through genomic imprinting and female X-chromosome inactivation, these processes are significantly altered in cancer due to loss of imprinting (LOI). For example, LOI is the earliest genomic lesion observed in Wilms' tumors and in stem cell populations in other organs and tissues, ultimately leading to additional downstream genetic and epigenetic perturbations (50).

In humans, 50–70% of all CpG sites are methylated, primarily in the heterochromatin regions, and these methylated CpG islands are believed to be critical for the control of gene silencing and chromosomal stability (22–26). In contrast, euchromatin CpG islands remain locally unmethylated, allowing

access to transcription factors and chromatin-associated proteins for the expression of most housekeeping genes and several other regulatory genes (56). Specifically, in cancer cells, global hypomethylation is accompanied by hypermethylation of localized promoter associated CpG islands, which are usually unmethylated in normal cells (36). This unique pattern of individual gene methylation is the characteristic commonly observed in tumor suppressor genes in most types of human cancers, and serves as a surrogate for point mutations or deletions to cause transcriptional silencing of tumor suppressor genes (28). Several detailed and informative reviews of the association between DNA methylation and cancer are available (14, 40, 50, 51, 60).

In addition to DNA methylation, it has been shown that methylated DNA binding proteins (MBDs) can bind to methylated cytosine, forming a complex with histone deacetylase (HDAC) that leads to chromatin compaction and gene silencing. To date, six methyl-CpG-binding proteins, including MECP2, MBD1, MBD2, MBD3, MBD4, and Kaiso, have been identified in mammals (13). MECP2 binds methylated DNA *in vitro* and *in vivo* (40). It contains a methyl-CpG-binding domain (MBD) at its amino terminus and a transcription repression domain (TRD) in the middle. MBDs1–4 were cloned on the basis of their sequence homology to MECP2 in the MBD, and all except MBD3 bind preferentially to the methylated CpG islands. In addition, MECP2, MBD1, and MBD2 function as transcription repressors. MBD4 is a DNA glycosylase and is involved in DNA mismatch repair. Kaiso, which lacks an MBD domain, binds methylated CGCG through its zinc-finger domain (13). Different methyl-CpG binding proteins may recruit different chromatin-remodeling proteins and transcription-regulatory complexes to methylated DNA targets in the genome. Furthermore, it has been demonstrated that nucleosome remodeling complex (NuRD) can methylate DNA by interacting with DNA methylation binding protein MBD2, which directs the NuRD complex to methylate DNA (51). These and other more recent findings have established that DNA cytosine methylation, covalent modification of histones, and nucleosome remodeling are linked together and are critical components of epigenetic gene regulation. A list of DNA methylation genes that are altered in various human cancers is shown in Table 1.

Histone modification. In addition to direct methylation of DNA, chromatin structure is influenced by various histone modifications, which also play important roles in gene regulation and carcinogenesis (17, 18). Chromatin proteins serve as building blocks to package eukaryotic DNA into higher order chromatin fibers. Each nucleosome encompasses ~146 bp of DNA wrapped around an octamer of histone proteins. These octamers consist of double subunits of H2A, H2B, H3, and H4 core histone proteins (61). The histone proteins coordinate the changes between tightly packed DNA (heterochromatin) that is inaccessible to transcription and exposed DNA (euchromatin) that is available for binding to and regulation of transcription factors (38). These changes occur due to structural characteristics of the nucleosome that are known as "histone tails," which extend from the core octamer. These tails consist of N-termini of the histone proteins and are the major sites for posttranslational modifications. The list of potential modifications includes acetylation, methylation, phosphorylation, ADP-ribosylation, ubiquitination, sumoylation, and biotinylation.

Table 1. DNA methylation genes altered in various human cancers

Gene	Cancer Type	Alteration
DNA methyltransferase		
DNMT 1	colorectal, and ovarian cancer	upregulation and mutation
DNMT3b	colorectal, colon, breast, ovarian, oesophageal cancers, squamous cell carcinoma or multiple	upregulation
Methyl-CpG-binding proteins		
MeCP2	prostate cancer, Rett syndrome	upregulation, mutation
MBD1	prostate, colon, and lung cancers	upregulation, mutation
MBD2	prostate, colon, lung cancers	upregulation, mutation
MBD3	colon, and lung cancer	upregulation, mutation
MBD4	colon, stomach, endometrium cancers	mutation
Kaiso	colon, Intestinal, lung cancers	upregulation

DNMT, DNA methyltransferase; MBD, methyl-binding domain protein.

The majority of these modifications takes place at lysine, arginine, and serine residues, and within the histone tails of H3 and H4 core proteins. These modifications are reversible and are controlled by groups of enzymes (17, 30). Histone deacetylases (HDACs) and histone acetyl transferases (HATs) are the key enzymes responsible for these reversible modifications. HDACs and HATs comprise a large group of enzymes that are classified into several families and control various physiological functions of the cells (26, 58). Extensive literature is available on this topic, and it is impossible to sufficiently cover all classifications and modifications in depth in this review. A list of the histone-modification genes that are altered during carcinogenesis is presented in Table 2.

Noncoding RNAs. Noncoding RNAs were initially noted to perform catalytic functions in facilitating RNA splicing, but it was later recognized that they participate in the epigenetic phenomenon of posttranscriptional gene modification (11, 53). They are also known as nonprotein coding RNA or microRNA, and they are 21–23 nucleotides in length. Approximately 1,000 miRNA genes have been computationally predicted in the human genome, with each miRNA targeting multiple protein coding transcripts. Although miRNA are vital to normal cell physiology their misexpression has been linked to carcinogenesis, and miRNA profiles are now being used to classify human

cancers (8, 44, 48). A list of some of the miRNAs whose expression is altered during carcinogenesis is presented in Table 3. The influence of miRNA on the epigenetic machinery and the reciprocal epigenetic regulation of miRNA expression suggest that its deregulation during carcinogenesis has important implications for global regulation of epigenetics and cancer. Several detailed and informative reviews of the association between miRNA and cancer are available (44, 47, 53).

EPIGENETIC SWITCHING IN CANCER

It is evident that discrete genetic alterations in neoplastic cells alone cannot explain multistep carcinogenesis whereby tumor cells are able to express diverse phenotypes during the complex phases of tumor development and progression. In fact, cancer cells have an altered epigenotype compared with the tissues from which they arise. The epigenetic switch is characterized by changes in the level and placement of DNA methylation and histone modification, and these changes influence the phenotype of the neoplastic cells (12, 16). Many cancer cells acquire altered levels of expression of epigenetic enzymes, but the products of their reaction do not match the phenotype, suggesting that there are other factors affecting their activity (18, 19). For several decades it has been known

Table 2. Histone modification genes altered in various human cancers

Histone Deacetylases	Cancer type	Alteration
HDAC1	colorectal cancer, cervical dysplasias, endometrial stromal sarcomas, gastric, prostate and colon cancer	upregulation/downregulation
HDAC2	multiple gastric carcinomas, colon cancer	upregulation/mutation
HDAC3	colon and prostate cancer	upregulation
HDAC4	prostate, breast and colon cancers	upregulation/downregulation/mutation
HDAC5	colon cancer, AML	repression
HDAC6	breast cancer, AML	upregulation
HDAC7	colon cancer	upregulation
HDAC8	colon cancer	upregulation
SIRT1	colon cancer	upregulation/downregulation
SIRT2	glioma	downregulation, deletion
SIRT3	breast cancer	upregulation
SIRT4	AML	downregulation
SIRT7	breast cancer, thyroid carcinoma	upregulation
Histone acetyl transferases		
p300	colorectal, breast, ovarian, hepatocellular cancers and oral carcinomas	mutation, translocation, deletions
CBP	colon, breast, and ovarian cancers, AML	mutation, Translocation, deletions
pCAF	colon	mutation
MOZ	hematological cancers	translocation
MORF	hematological and uterine cancers, leiomyomata	translocation
Tip60	Colorectal and prostate cancer	downregulation, translocation

AML, acute myeloid leukemia; HDAC, histone deacetylases; SIRT, sirtuins; MOZ, monocytic leukemia zinc finger protein; MORF, MOZ related factor.

Table 3. *MicroRNA alteration in various human cancers*

MicroRNAs	Target Gene(s)	Cancer Type	Alterations
miR-127	Bcl-6	bladder cancer	upregulation
miR-124	CDK6	colon cancer	upregulation
miR-223	NFI-A, MEF2C	acute myeloid leukemia	upregulation
miR-34b/34c	p53 network, CDK6, E2F3	colon cancer	upregulation
miR-17, miR-92	c-MYC	lung cancer	upregulation
miR-372,miR-373	RAS, p53 ,CD44	testicular germ cell tumor and breast cancer	upregulation
miR-21	PDCD4,PTEN,TPM1,RECK, TIMP3, BCL2	glioblastoma, breast, lung, prostate, colon and cervical cancer	upregulation
miR-155	RHOA	Burkitt's lymphoma, breast ,colon, and lung cancers	upregulation
miR-146	NF-κB	breast, pancreas and prostate cancers	upregulation
miR-92b	PRMT5	brain primary tumors	upregulation
miR-520	CD44	breast cancer	upregulation
miR-10b	HOXD10	metastatic breast cancer	upregulation
miR-9	CDH1	breast cancer	upregulation
miR-127, miR-199a	BCL6, E2F1	cervical cancer	upregulation
miR-421	CBX7, RBMXL1	gastric cancer	upregulation
miR-1228,miR-195, miR30b, miR-32, miR345	CDKN2A,NF2,andJUN	Malignant mesothelioma (MM)	upregulation
miR-190, miR-196	HGF	pancreatic cancer	upregulation
miR-125	AKT, ERBB2-4, FGF, FGFR, IGF, MAPKs, MMP11, SP1, TNF, VEGF	breast	upregulation
miR-126	CRK1,PIK3R2,SPRED1, VCAM1	breast and lung cancer	downregulation
miR-146a, miR-146b	ROCK1, IRAK1,TRAF6	prostate cancer and papillary thyroid carcinomas	downregulation
miR-340, miR-421, miR-658	MYC, RB, PTEN	lymph node metastasis and gastric cancer	downregulation
let-7a-3	RAS, IGF-II	lung and ovarian cancer	downregulation
miR-221, miR-222	CDKN1C/P57 and CDKN1B/P27	hepatocellular carcinoma	downregulation
miR-9	NF-κB	ovarian and lung cancer	downregulation
miR-218, miR-145	PXN	breast, lung and prostate cancer	downregulation
miR-25, miR-32, miR-142	ITGAα1	lung cancer and solid tumor	downregulation
miR- 124, miR-183	ITGBβ1	lung cancer	downregulation
miR-143	ERK5	cervical cancer	downregulation
miR-372, miR-373	LATS2	testicular germ cell cancer	downregulation
miR-181	VGFR	lung cancer	downregulation
miR-370	MAP3K8	MzChA-1, KMCH-1, cholangiocarcinoma	downregulation
miR-342	ER, PR and HER2	breast and colon cancer	downregulation
miR-145	ER	colon and breast cancer	downregulation
miR- 124, miR-183	ITGB1 β	lung cancer	downregulation

CDK6, cyclin D kinase 6; MEF2C, myocyte enhancer factor 2C; NFIA, Nuclear factor 1 A-type; p53, tumor protein 53; RAS, Rat Sarcoma; CD44, cluster differentiation 44; Pdcd4, programmed cell death 4; TPM1, tropomyosin 1; PTEN, phosphatase and tensin homologue; BCL2, B-cell lymphoma 2 protein; RECK, reversion Inducing cysteine rich protein kazal motif; ROHA, ras homolog gene family member A; NF-κB, nuclear factor-κappaB; PRNT5, protein arginine N-methyltransferase 5; HOXD10, homeobox D10; CDH1, Cadherin-1; CBX7, chromobox 7; RBMX L1, RNA binding motif protein X-linked; CDKN2A, cyclin-dependent kinase inhibitor 2A; NF2, neurofibromatosis, type 2; HGF, hepatocyte growth factor; ERBB2-4' or (HER4), human epidermal growth factor Receptor 4; JUN, janus N-terminal kinases; FGFR, fibroblast growth factor receptor; MAPKs, mitogen-activated protein kinase; MMP11, matrix metalloproteinase11; VEGF, vascular endothelial growth factor; TNFα, tumor necrosis factor-alpha; CRK1, Cdc2-related kinase1; PIK3R2, phosphatidylinositol 3-kinase regulatory subunit beta; SPRED1,sprouty-related, EVH1 domain containing 1; VCAM, vascular cell adhesion molecule; ROCK1, rho-associated, coiled-coil containing protein kinase 1; IRAK1, interleukin-1 receptor associated kinase-1; TRAF6, TNF receptor associated factor 6; Rb, retinoblastoma; IGF-II, insulin-like growth factor 2; PXN, paxilin; ITGβ1, integrin beta-1; ERK5, extracellular signal-regulated kinase 5; LATS2, large tumor suppressor homolog 2; ER, estrogen receptor; PR, progesterone receptor.

that tumor cells possess a global hypomethylated genome, while at the same time focal cytosine methylation has increased in specific places of the genome (2, 52). In normal cells, CpGs within repetitive DNA elements and coding regions of the genes are methylated, whereas in tumor cells LINE-1 repeats, satellite DNA, and moderately repeated DNA sequences become unmethylated, whereas genes containing CpG clusters become hypermethylated, rendering them transcriptionally silent (2, 52). Proportional changes in histone modifications are also observed in cancer (17). It has been demonstrated that H3K4me3, H3K4me2, and H3ac are heavily enriched around the transcriptional start sites of genes with slightly lower enrichment of H3K4me1 and H4ac. Furthermore, enrichment of H3K4me3, H3K4me2, and H3ac at the transcriptional start site has been shown to positively correlate with the extent of

gene activity (55). Aberrant distributions of histone modifications H3K4me1, 2, 3, and H3K36me3 correlate with gene activation with high levels of enrichment around the transcription start site, whereas H3K9me1, H3K20me1, and H3K27me1 correlate with gene expression due to elevated levels of these markers localized downstream from the transcription start site and throughout the entire transcribable region (35). In contrast, high levels of H3K27me2, 3, and H3K79me3 and modest levels of H3K9me2 and 3 are linked to gene repression or silencing. Fraga et al. (20) first demonstrated that loss of H4K16ac and H4K20me3 is a common hallmark of human cancer cells associated with DNA hypermethylation at repetitive sequences. In addition, deamination of methylated cytosine forms thymine, creating a lesion that is difficult to correct because the DNA repair mechanisms cannot easily discrimi-

nate which base is correct in the resulting G:T mismatch. At the translational level, the first direct link between miRNAs and cancer was demonstrated by Carlo Croce's group in 2002 (7, 8, 56), describing the loss of miR-15 and miR-16 in 13q14-deleted chronic lymphocytic B-cell leukemia. Epigenetic modifications in cancer could also affect the stability of the genome, providing a link between the organization of the genome and its replication and repair. Much of the descriptive work has demonstrated the nature of such alterations; however, the precise cause of the epigenetic switch in cancer is still elusive.

EPIGENETIC ALTERATIONS IN CANCER

In human cancers, aberrant epigenomics are known to contribute to various phases of neoplastic development including initiation, promotion, invasion, metastasis, and chemotherapy resistance (10, 16, 23, 25). It was recently proposed that more than 300 genes and gene products are epigenetically altered in various human cancers, and these alterations have been linked to proliferative changes, cellular atypia, dysplasia, carcinoma in situ, invasive malignancy, metastatic malignancy, and therapy-resistant malignancy (23, 25). Epigenetic silencing of genes can affect cancer at various stages (19). The epigenetic changes in gene expression and their pathologic correlation is a result of overlapping changes in genes expression, but some of them may be associated with particular stages of cancer development. For example, the gene that encodes the cell cycle inhibitor p16^{INK4A} and the DNA repair genes MLH1 and BRCA1 are some susceptible genes that undergo early methylation-associated silencing that correlates with neoplastic transformation (3, 23, 28). Several other genes such as MLH1, VHL, WRN, and BRCA1 that are inactivated by CpG island hypermethylation in transformed cells have anti-proliferative roles and in many instances there are familial cancer cases with associated germ-line-like mutations in these genes. In breast and prostate cancer, the RASSF1A promoter is frequently methylated, and likewise GSTP1 is methylated in the neoplastic cells of more than 90% of cases of prostate cancer (18, 32). These observations support the concept that epigenetic changes can promote malignant transformation.

The aggressive cancer phenotype has also been shown to be regulated, in part, by epigenetic mechanisms (10). Metastatic and therapy-resistant behaviors of cells involve associations between genetic and epigenetic events and alterations in multiple pathways that contribute to distant metastasis (10, 25, 43). The regulatory networks that function at various level cause changes in gene expression in both tumor and host cells, influencing transcription, translation, methylation, and a multitude of other processes (43, 49). The initiation of these events is the result of impairment of a large number of tumor suppressor genes that have the ability to trigger pro-angiogenic and metastatic properties in affected malignant cells (10). For example, downregulation of the angiogenesis inhibitor TSP1 and NM23, which is encoded by nonmetastatic cells, NME1, NME2, and MKK4, is repressed during this process (10). Additional genes that are deregulated in cancer are the tissue inhibitor of metalloproteinases such as TIMP3 (promoter hypermethylation during cancer), which binds to the VEGF-2 and inhibits angiogenesis. Some other genes that participate in metastasis are uPA, calcium binding proteins, and S100P,

which facilitates tissue invasion and is associated with a poor prognosis in breast and prostate cancers (10). Upregulation of these genes occurs due to hypomethylation and unmasking of the promoter, which correlates with poor clinical outcome (43, 60).

During metastasis the epigenetic model proposed is that the host microenvironment exerts an initial inhibitory constraint on tumor growth, which is followed by acceleration of tumor progression through complex "cell-matrix" interactions (25). For example, Hu et al. (29) demonstrated distinct epigenetic changes in cultured epithelial and myoepithelial cells and in stromal fibroblasts from normal breast tissue and breast carcinomas, suggesting that aberrant epigenomics in the stroma are unique and discrete from their associated carcinoma cells. Of the five genes they examined, three (PRDM14, HOXD4, and CDC42EP5) were found to be methylated in carcinoma cells, whereas estrogen receptor PGR and 17 β -estradiol metabolizing enzyme HSD17B4 are concomitantly methylated in the stromal tissue. Furthermore, the signaling pathway leading to hypermethylation of the CST6 gene is induced by the activated serine/threonine kinase Akt1 pathway. Activation of Akt1 signaling causes DNA methylation and also recruits DNA methyltransferase and represses histone modifications to the promoter of CST6, an event that contributes to epigenetic silencing. These events illustrate how epigenetic events influence cancer development and progression. A list of genes that are epigenetically silenced during various stages of human cancer are shown in Table 4.

EPIGENETIC BIOMARKERS

Epigenetic markers have shown promise in establishing the diagnosis and prognosis of various human cancers (21). The methylated DNA sequences represent potential biomarkers for diagnosis, staging, prognosis, and monitoring of response to therapy. DNA methylation markers hold a number of advantages over other biomarkers, specifically their stability, their ability to be amplified at relatively low costs, and their restriction to limited regions of DNA methylation (37). Epigenetic markers can be detected in resected tumors and in body fluids. For example, the occurrence of hypermethylated CDH13, MYOD1, MGMT, p16INK4b, and RASSF1A genes varies significantly among cancer types; their presence can be detected in body fluids as well as in plasma DNA (47). Hypermethylated cancer genes can also be detected in urine sediments and may prove useful in detecting bladder cancer. A more sensitive and specific screening test for prostate cancer is being evaluated for use in testing urine and plasma DNA. This diagnostic test targets a single methylated gene GSTP1 (14). In addition, combined hypermethylation assays for small number of genes such as RASSF1A, RARB2, APC, and GSTP1 have been used to help discriminate between benign and cancerous changes in the prostate. The field of DNA methylation-based markers for prognosis and diagnosis is still emerging and its widespread use in clinical practice has yet to be implemented (23, 30).

Repetitive DNA elements such as short and long interspersed nuclear elements (SINEs and LINEs) and other repetitive sequences are often hypomethylated in human cancers (60). However, the utility of global hypomethylation as a prognostic marker in clinical practice remains undetermined.

Table 4. *Epigenetically silenced genes and their pathways in various human cancers*

Pathway	Gene(s)	Cancer Type
Cell cycle	p15INK4B, p16INK4A, p14ARF, RB, RASSF1, p27KIP1, p21WAF1/CIP1, and RB	hepatic, lung, colorectal, gliomas, head and neck, squamous cell, breast, gastrointestinal, hepatocellular, salivary gland, retinoblastoma, bladder, pancreatic, melanoma, prostate cancers and hematological cancers
DNA repair	MLH1, MGMT, BRCA1, RASSF1, WRN	colorectal, endometrial, gastric, squamous cell, melanoma, testicular, esophageal, liver cancers
Transcriptional regulation	VHL, RIZ1, HIC1, BEX2	gall bladder, gastric, thyroid, esophageal, renal, oral cancers
Cell adhesion	CDH1, CDH13	breast, lung, and stomach cancers, leukemia
Cell adhesion, invasion, metastasis,	SERPINB5 (MASPIN)	breast, thyroid, prostate, melanoma
	SFRP1	
Cell growth and proliferation	MDG1	breast, gastric
Transcription factor	CEBPA, GATA4, GATA5, ID4, RUNX3	endometrial, lung, hepatoma, colon, nasopharyngeal, bladder cancers
Signal transduction	RARB2	esophageal and breast cancer
DNA Damage	14-3-3 σ , GADD45G	breast, gastric, liver, lung, skin, ovarian, neuroblastoma, lymphoma, cervical, nasopharyngeal cancers
Carcinogen detoxification	GSTP1	prostate, breast, and renal cancers
Apoptosis	DAPK, TMS1	cervical, lung, glioma, esophageal, mesothelioma cervical, prostate cancers and hematologic cancer
Nuclear export	APC	colorectal, endometrial, breast, lung, stomach, melanoma
Hormone receptor	ER, PR, AR, PRLR and TSHR	breast, prostate, thyroid cancers

MLH1, mismatch repair gene 1; MGMT, O⁶-methylguanine-DNA methyltransferase; MDG1, mammary-derived growth inhibitor 1; BRCA1, breast cancer 1; RASSF1, ras association (RalGDS/AF-6) domain family member 1; WRN, Werner syndrome RecQ helicase like; VHL, Von Hippel-Lindau 1; RIZ1, retinoblastoma protein-interacting zing-finger gene; HIC1, hypermethylated in cancer-1; BEX2, brain expressed X-linked2; RB, retinoblastoma; CDH1, cadherin-1; MDG1, microvascular endothelial differentiation gene 1; CEBPA, CCAAT enhancer binding protein alpha; GATA4, GATA binding protein 4; ID4, DNA-binding protein inhibitor; RUNX, runt-related transcription factor 3; RARB2, retinoic acid receptor B2; GADD45G, growth arrest and DNA-damage-inducible protein GADD45 gamma; GSTP1, glutathione S-transferase pi 1; DAPK1, death-associated protein kinase; TMS1, target of methylation-induced silencing1; APC, adenomatous polyposis coli; ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; PRLR, prolactin receptor; TSHR, thyroid stimulating hormone receptor.

Histone modification patterns also provide prognostic and diagnostic information in cancer (55). Repressive chromatin structures characterized by particular histone modifications such as H3K9, H3K27, and H4K20 methylation may precipitate DNA methylation (34). Generalized changes in chromatin structure and histone modification also occur; for example, increased H3K4 dimethylation and H3K18 acetylation activation are reported to be associated with poor prognosis (34). The major limitation is that it is unknown to what extent these changes correlate with alterations in gene activity in cancer cells. For instance, in addition to the H3K27 methylase EZH2, the corresponding demethylase JMJD3 has been reported to be overexpressed in metastatic prostate cancers (3). Likewise, overexpression of the histone deacetylase HDAC1 is regularly observed in prostate cancers harboring the major TMPRSS2-ERG fusion. These observations need to be assessed in larger patient cohorts for further validation before implementation in clinical practice (3, 54).

More recently, miRNAs have also been proposed as potential epigenetic biomarkers in the diagnosis of cancer (11, 45). Some miRNAs, such as miR-199a, miR-200a, miR-146, miR-214, miR-221, and miR-222 have been found to be upregulated, whereas miR-100 is downregulated in human cancers (47). The miRNA let-71 was recently designated as a tumor suppressor and miR-429, miR-200a, and miR-200b were found to be clustered on a single primary transcript regulated by the epithelial-to-mesenchymal transition (48). Studies have shown that two other miRNAs, miR-21 and miR-181a, can be used to identify the presence or absence of malignant phenotypes. A group of 27 miRNAs have been shown to be significantly associated with chemotherapy response and have been proposed as possible prognostic and diagnostic biomarkers, similar to DNA methylation biomarkers (41, 45). Excellent re-

views of this topic are available and have addressed the diagnostic and prognostic promise of epigenetic markers.

EPIGENETIC THERAPY AND PREVENTION

The epigenome of a cancer cell is characterized by a global pattern of DNA hypomethylation and alterations in miRNA profile and histone modification at various lysine and serine residues (12). Conceptually, epigenetic changes are reversible and consequently are rational targets for therapeutical approaches. Two main classes of epigenetic drugs, namely DNA methyltransferase (DNMT) inhibitors and histone deacetylase (HDAC) inhibitors, are currently used in clinical trials for the treatment of cancers (40). DNMT inhibitors 5-azacytidine and 5-aza-2'-deoxycytidine, which are both analogs of cytosine, have demonstrated clinical activity at low doses against hematological neoplasms. However, these nucleoside analogs show poor activity against solid tumors and are associated with severe toxic side effects. These drugs target the epigenome in a non cell-specific way and therefore modify the methylation patterns in tumor cells as well as in normal cells. Several new DNMT anti-sense (MG98) and small molecule RG108 inhibitors are currently under development, which are more specific and less toxic (33, 39).

The second class of agents is the HDAC inhibitors, which offer more promising targets for epigenetic anticancer therapy (5, 9). Interest in histone modifications has grown over the last few years with the discovery and characterization of a large number of histone-modifying molecules and protein complexes (9). Clinical trials show that HDAC inhibitors are well tolerated; can inhibit HDAC activity in peripheral mononuclear cells and tumors, and more importantly, have clinical activity with objective tumor regression. It is postulated that histone

acetylation is associated with activation of gene transcription (6). The HDAC inhibitors butyrate, trichostatin A (TSA), depsipeptide, oxamflatin, MS-275, and SAHA induce expression of several cell cycle regulatory molecules that inhibit cell-cycle progression, acting to block cyclin-dependent kinase activity and, as a consequence, causing cell-cycle arrest (5, 6). Furthermore, inactivation of tumor suppressor genes by aberrant DNA methylation of the promoter region is complemented by another epigenetic event that alters the structure of chromatin—the hypoacetylation of lysines in histones, brought about by histone deacetylases. Because cross-talk can occur between DNA methylation and histone deacetylation, a combination of these two epigenetic modifications represents an interesting approach for therapeutic intervention. Inhibitors of these two pathways in combination have been shown to produce a synergistic reactivation of tumor suppressor genes and an enhanced antineoplastic effect against tumor cells (34).

The use of miRNAs as potential therapeutic targets has been examined in several studies that have shown that specific miRNA deregulations (both overexpression and downregulation) in cancer cells is associated with pathogenic effect (11, 44, 45). Studies have demonstrated that reducing the expression levels of miR-10b, miR-21, the miR-146 family, miR-155, miR-373, and miR-520c in solid tumors by locked nucleic acid anti-miRNAs or antagomirs, or reexpressing miR-126, miR-148a, miR-206, miR-335 and miR-200 family by mimic miRNAs could be initially tested and validated in preclinical settings and then, if successful, could be considered for Phase I clinical trials alone or in combinations with existing regimens (44, 45). Additionally, manipulation of miRNA levels may also be a means of altering DNA methylation and therefore miRNA therapy in combination with DNMT inhibitors may be a better combination. However, there is a need to refine and optimize these approaches. There are a number of reports and review articles pertaining to the utility of various DNMT, HDAC inhibitors, and miRNAs in the management of various hematological and solid tumors that are beyond the scope of this review.

It has been established that environment, diet, and lifestyle factors contribute to cancer development by inducing both epigenetic and genetic changes that, in combination with genetic makeup, result in the disruption of key cellular processes leading to neoplasia (58). The best studied example is the relationship between dietary methionine and DNA methylation (59). As an essential amino acid, methionine plays a central role in epigenetic regulation by serving as a methyl donor for methylation reactions. In the process of cytosine methylation, DNMT enzyme converts SAM to S-adenosylhomocysteine (SAH); therefore, an optimal supply of SAM or removal of SAH is essential for normal establishment of genome-wide DNA methylation patterns. CpG methylation patterns are largely erased in the early embryos and then re-established in a tissue-specific manner (52). Therefore, early embryonic development may represent a sensitive stage, and dietary and environmental factors that affect DNA methylation reaction and the activity of DNMTs may result in permanent fixation of aberrant methylation patterns (27). Another potential mechanism by which environmental and dietary exposures affect the epigenome may involve transposable elements (16, 19). Transposons are groups of mobile genetic elements that, when activated, may cause genetic mutations and transcriptional noise (52). They are shown to be heavily methylated and

transcriptionally silent in somatic cells. Although it is well documented that the activation of transposable element-derived promoters may be a consequence of perturbed DNA methylation, transposable elements have been shown to be activated by different kinds of cellular stress (27). Therefore, stress induced by environmental and dietary agents may activate transposable elements, leading to altered establishment and maintenance of epigenetic states. Different classes of HDACs may also be altered by environmental and dietary agents. Interestingly, certain dietary chemopreventive agents such as butyrate, diallyl disulfide, and sulforaphane have demonstrated HDAC inhibitory activity (42). This is highlighted by a recent study demonstrating that resveratrol, a molecule produced by a variety of plants and the most potent inhibitor of SIRT1, a member of the sirtuin family of NAD-dependent deacetylases, improves health and extends life span (23). Certain dietary agents such as green tea polyphenols and phenethyl isothiocyanate have shown dual actions as DNMT as well as HDAC inhibitors in cancer cells (46, 57). The dual action of these agents on both DNA and chromatin was more effective than 5'-aza-2'-deoxycytidine, or TSA, suggesting that they may be better epigenetic modifiers for cancer prevention, achieved through dietary intervention.

CONCLUSIONS AND FUTURE DIRECTION

The importance of epigenetics in cancer has been recognized and interest in the field has grown dramatically over the last few years. Recent advances in epigenomic approaches allow mapping of the methylation/acetylation state and miRNA levels in the genome with high accuracy, which will help in the identification of biomarkers for various diseases. An understanding of the link between epigenetic deregulation and cancer will help in designing better treatment strategies. Additionally, the intrinsic reversibility of epigenetic alterations represents an exciting opportunity for the development of novel strategies for cancer prevention.

ACKNOWLEDGMENTS

We apologize to those investigators whose original work could not be cited owing to the space limitations.

GRANTS

The original work from author's laboratory outlined in this review was supported by United States Public Health Service Grants RO1 CA-115491 and R21 CA-109424.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. *Nat Rev Cancer* 7: 834–846, 2007.
2. Baba S, Yamada Y, Hatano Y, Miyazaki Y, Mori H, Shibata T, Hara A. Global DNA hypomethylation suppresses squamous carcinogenesis in the tongue and esophagus. *Cancer Sci* 100: 1186–1191, 2009.
3. Barradas M, Anderton E, Acosta JC, Li S, Banito A, Rodriguez-Niedenfuhr M, Maertens G, Banck M, Zhou MM, Walsh MJ, Peters G, Gil J. Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS. *Genes Dev* 23: 1177–1182, 2009.
4. Berletch JB, Phipps SM, Walthall SL, Andrews LG, Tollefsbol TO. A method to study the expression of DNA methyltransferases in aging systems in vitro. *Methods Mol Biol* 371: 81–87, 2007.

5. Bots M, Johnstone RW. Rational combinations using HDAC inhibitors. *Clin Cancer Res* 15: 3970–3977, 2009.
6. Bruserud O, Stappes C, Ersvaer E, Gjertsen BT, Rynningen A. Histone deacetylase inhibitors in cancer treatment: a review of the clinical toxicity and the modulation of gene expression in cancer cell. *Curr Pharm Biotechnol* 8: 388–400, 2007.
7. Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M, Dell'Aquila ML, Alder H, Rassenti L, Kipps TJ, Bullrich F, Negrini M, Croce CM. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proc Natl Acad Sci USA* 101: 11755–11760, 2004.
8. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 101: 2999–3004, 2004.
9. Cang S, Ma Y, Liu D. New clinical developments in histone deacetylase inhibitors for epigenetic therapy of cancer. *J Hematol Oncol* 2: 22, 2009.
10. Chiang AC, Massague J. Molecular basis of metastasis. *N Engl J Med* 359: 2814–2823, 2008.
11. Chuang JC, Jones PA. Epigenetics and microRNAs. *Pediatr Res* 61: 24R–29R, 2007.
12. Clark SJ. Action at a distance: epigenetic silencing of large chromosomal regions in carcinogenesis. *Hum Mol Genet* 16, Spec No 1: R88–95, 2007.
13. Clouaire T, Stancheva I. Methyl-CpG binding proteins: specialized transcriptional repressors or structural components of chromatin? *Cell Mol Life Sci* 65: 1509–1522, 2008.
14. Dehan P, Kustermans G, Guenin S, Horion J, Boniver J, Delvenne P. DNA methylation and cancer diagnosis: new methods and applications. *Expert Rev Mol Diagn* 9: 651–657, 2009.
15. Deplus R, Brenner C, Burgers WA, Putmans P, Kouzarides T, de Launoit Y, Fuks F. Dnmt3L is a transcriptional repressor that recruits histone deacetylase. *Nucleic Acids Res* 30: 3831–3838, 2002.
16. Ducasse M, Brown MA. Epigenetic aberrations and cancer. *Mol Cancer* 5: 60, 2006.
17. Ellis L, Atadja PW, Johnstone RW. Epigenetics in cancer: targeting chromatin modifications. *Mol Cancer Ther* 8: 1409–1420, 2009.
18. Esteller M. Epigenetics in cancer. *N Engl J Med* 358: 1148–1159, 2008.
19. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. *Nat Rev Genet* 7: 21–33, 2006.
20. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG, Pérez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MA, Ahn N, Imhof A, Caldas C, Jenuwein T, Esteller M. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet* 37: 391–400, 2005.
21. Gargiulo G, Minucci S. Epigenomic profiling of cancer cells. *Int J Biochem Cell Biol* 41: 127–135, 2009.
22. Grewal SI, Moazed D. Heterochromatin and epigenetic control of gene expression. *Science* 301: 798–802, 2003.
23. Gronbaek K, Hother C, Jones PA. Epigenetic changes in cancer. *Apmis* 115: 1039–1059, 2007.
24. Guarente L, Picard F. Calorie restriction—the SIR2 connection. *Cell* 120: 473–482, 2005.
25. Gupta GP, Massague J. Cancer metastasis: building a framework. *Cell* 127: 679–695, 2006.
26. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet* 10: 32–42, 2009.
27. Herceg Z. Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors. *Mutagenesis* 22: 91–103, 2007.
28. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 349: 2042–2054, 2003.
29. Hu M, Yao J, Cai L, Bachman KE, van den Brule F, Velculescu V, Polyak K. Distinct epigenetic changes in the stromal cells of breast cancers. *Nat Genet* 37: 899–905, 2005.
30. Iacobuzio-Donahue CA. Epigenetic changes in cancer. *Annu Rev Pathol* 4: 229–249, 2009.
31. Issa JP, Kantarjian HM. Targeting DNA methylation. *Clin Cancer Res* 15: 3938–3946, 2009.
32. Jones PA, Baylin SB. The epigenomics of cancer. *Cell* 128: 683–692, 2007.
33. Jung Y, Park J, Kim TY, Park JH, Jong HS, Im SA, Robertson KD, Bang YJ, Kim TY. Potential advantages of DNA methyltransferase 1 (DNMT1)-targeted inhibition for cancer therapy. *J Mol Med* 85: 1137–1148, 2007.
34. Kondo Y. Epigenetic cross-talk between DNA methylation and histone modifications in human cancers. *Yonsei Med J* 50: 455–463, 2009.
35. Kouzarides T. Chromatin modifications and their function. *Cell* 128: 693–705, 2007.
36. Laird PW. Cancer epigenetics. *Hum Mol Genet* 14, Spec No 1: R65–76, 2005.
37. Lin HJ, Zuo T, Chao JR, Peng Z, Asamoto LK, Yamashita SS, Huang TH. Seed in soil, with an epigenetic view. *Biochim Biophys Acta* 1790: 920–924, 2009.
38. Lund AH, van Lohuizen M. Epigenetics and cancer. *Genes Dev* 18: 2315–2335, 2004.
39. Lyko F, Brown R. DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. *J Natl Cancer Inst* 97: 1498–1506, 2005.
40. McCabe MT, Brandes JC, Vertino PM. Cancer DNA methylation: molecular mechanisms and clinical implications. *Clin Cancer Res* 15: 3927–3937, 2009.
41. Metias SM, Lianidou E, Yousef GM. MicroRNAs in clinical oncology: at the crossroads between promises and problems. *J Clin Pathol* 62: 771–776, 2009.
42. Myzak MC, Dashwood RH. Histone deacetylases as targets for dietary cancer preventive agents: lessons learned with butyrate, diallyl disulfide, and sulforaphane. *Curr Drug Targets* 7: 443–452, 2006.
43. Nguyen DX, Massague J. Genetic determinants of cancer metastasis. *Nat Rev Genet* 8: 341–352, 2007.
44. Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA. MicroRNAs—the micro steering wheel of tumour metastases. *Nat Rev Cancer* 9: 293–302, 2009.
45. Olson P, Lu J, Zhang H, Shai A, Chun MG, Wang Y, Libutti SK, Nakakura EK, Golub TR, Hanahan D. MicroRNA dynamics in the stages of tumorigenesis correlate with hallmark capabilities of cancer. *Genes Dev* 23: 2152–2165, 2009.
46. Pandey M, Shukla S, Gupta S. Promoter demethylation and chromatin remodeling by green tea polyphenols leads to re-expression of GSTP1 in human prostate cancer cells. *Int J Cancer* 26: 2520–2533, 2010.
47. Paranjape T, Slack FJ, Weidhaas JB. MicroRNAs: tools for cancer diagnostics. *Gut* 58: 1546–1554, 2009.
48. Peter ME. Let-7 and miR-200 microRNAs: guardians against pluripotency and cancer progression. *Cell Cycle* 8: 843–852, 2009.
49. Piris A, Mihm MC Jr. Mechanisms of metastasis: seed and soil. *Cancer Treat Res* 135: 119–127, 2007.
50. Plass C, Soloway PD. DNA methylation, imprinting and cancer. *Eur J Hum Genet* 10: 6–16, 2002.
51. Razin A, Kantor B. DNA methylation in epigenetic control of gene expression. *Prog Mol Subcell Biol* 38: 151–167, 2005.
52. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science* 293: 1089–1093, 2001.
53. Rouhi A, Mager DL, Humphries RK, Kuchenbauer F. MiRNAs, epigenetics, cancer. *Mamm Genome* 19: 517–525, 2008.
54. Schulz WA, Hoffmann MJ. Epigenetic mechanisms in the biology of prostate cancer. *Semin Cancer Biol* 19: 172–180, 2009.
55. Seligson DB, Horvath S, McBrien MA, Mah V, Yu H, Tze S, Wang Q, Chia D, Goodglick L, Kurdistani SK. Global levels of histone modifications predict prognosis in different cancers. *Am J Pathol* 174: 1619–1628, 2009.
56. Villa R, De Santis F, Gutierrez A, Minucci S, Pelicci PG, Di Croce L. Epigenetic gene silencing in acute promyelocytic leukemia. *Biochem Pharmacol* 68: 1247–1254, 2004.
57. Wang LG, Beklemisheva A, Liu XM, Ferrari AC, Feng J, Chiao JW. Dual action on promoter demethylation and chromatin by an isothiocyanate restored GSTP1 silenced in prostate cancer. *Mol Carcinog* 46: 24–31, 2007.
58. Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* 40: 897–903, 2008.
59. Waterland RA. Assessing the effects of high methionine intake on DNA methylation. *J Nutr* 136: 1706S–1710S, 2006.
60. Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. *Biochim Biophys Acta* 1775: 138–162, 2007.
61. Zhang Y, Ng HH, Erdjument-Bromage H, Tempst P, Bird A, Reinberg D. Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev* 13: 1924–1935, 1999.