Supplemental Material

Supplementary Table 1. Chemopreventive Effects of Demethylating Drugs in Rodent Models

Agent	References	Target site	System/carcinogen	Concentration/Dose	Results – Comments - Methods
5-aza-2'-deoxycytidine	Laird *et al*., 1995 [65]	intestine	*APC*$^{Min/+}$ mice	1 mg/kg, 1x per wk	↓ tumor formation, most effective when treatment started at 1 wk of age
	Lantry *et al.*, 1999 [301]	lung	NNK-treated mice	1 mg/kg, 3x per wk	↓ tumor formation
	Davis & Uthus, 2002 [94]	intestine	DMH-treated rats	1 mg/kg, 1x per wk	↓ aberrant crypts and aberrant crypt foci formation
	Belinsky *et al.*, 2003 [302]	lung	NNK-treated mice	0.5 mg/kg, 3x per wk	↔ no effect when applied alone, ↓ tumor formation in combination with *HDAC* inhibitor
	Belinsky *et al*., 2003 [302]	lung	NNK-treated *DNMT1*-deficient mice	0.25 mg/kg, 3x per wk	↓ tumor formation
	McCabe *et al.*, 2006 [303]	prostate	TRAMP mice	0.25 mg/kg, 2x per wk	↓ tumor formation, ↓ *MGMT* promoter methylation, ↑ *MGMT* mRNA expression
	Hellebrekers *et al.*, 2006 [304]	murine melanoma	xenograft	10 mg/kg/d	↓ tumor growth, angiostatic activity
	Tang *et al*., 2009 [114]	oral cavity	4-NQO-treated mice	250 μg/kg b.w. 2 x per wk for 15 wk	↓ No. of cancerous tongue lesions ↓ severity, in combination with low-dose retinoic acid reversal of 4-NQO-mediated effects on *RARβ2*, *COX-2* and *c-Myc* expression
Zebularine	Hellebrekers *et al.*, 2006 [304]	human colon cancer	xenograft	1000 mg/kg/d	↓ tumor growth
	Hellebrekers *et al*., 2006 [304]	murine melanoma	xenograft	1000 mg/kg/d	↓ tumor growth, angiostatic activity
	Yoo *et al*., 2008 [66]	small intestine	*APC*$^{Min/+}$ mice (male and female)	0.2 mg/ml in drinking water starting at 1 wk of age	↓ tumor formation in female mice ↓ DNA methylation at B1 short interspersed nucleotide elements (*SINE*) in small intestine and colon (pyrosequencing) ↓ *IGF2* promoter methylation in large intestine (MS-SnuPE) ↔ no effect on DNA methylation in other organs ↔ no effect on tumor formation and DNA methylation in male mice

Abbreviations: 4-NQO, 4-Nitroquinoline 1-oxide; *APC*$^{Min/+}$, mouse model with mutant *Adenomatous Polyposis Coli* developing multiple intestinal neoplasia; *COX2*, cyclooxygenase 2; *DMH,* dimethylhydrazine; *IGF2*, insulin-like growth factor 2; *MGMT*, O6-methylguanine-DNA methyltransferase; MS-SnuPE, Methylation Sensitive Single Nucleotide Primer Extension; NNK, Nicotine-derived nitrosamine ketone; *RARβ2*, retinoic acid receptor β2; TRAMP, transgenic adenocarcinoma of the mouse prostate

Supplementary Table 2. Methods to Measure DNA Methylation Changes Used in Chemoprevention Studies

Assay	Principle	Reference
Assessment of global 5meC level		
5meC-IHC	• 5meC-specific immunohistochemistry • generally not quantitative, but was modified in some studies to a semi-quantitative dot-blot method involving comparison with a DNA methylation standard • ELISA-based detection kits using 5meC-specific antibodies available	[258, 306]
HPLC	• requires large amounts of DNA.	[306]
LC-MS/MS	• HPLC coupled with tandem mass spectrometry	[306]
CE	• capillary electrophoresis, requires large amounts of DNA.	[306]
In vitro methyl acceptance capacity assay	• radioactive quantification of [^{3}H]-methyl-group incorporation into DNA by *in vitro* DNMT activity. Methylation status is inversely related to incorporated radioactivity	[306]
Detection of DNA methylation of selected target regions		
Methylation-sensitive restriction digestion	• detection by southern blotting.	[307]
Quantitative PCR/*Hpa*II resistance assay	• comparison of restriction digestion using a methylation-sensitive (e.g. *Hpa*II) and a methylation insensitive restriction enzyme (e.g. *Msp*I) followed by PCR amplification, which is indicative of the methylation status at the restriction site. • Disadvantages: limited availability of informative restriction sites, false positive results due to incomplete digestion, requirement of large amounts of high molecular weight DNA.	[307]
Methylation-specific PCR (MSP)	• Initial modification of DNA by sodium bisulfite treatment, which deaminates all unmethylated, but not methylated, cytosines to uracil. • Subsequent PCR amplification with primers specific for methylated versus unmethylated primer binding sites. • MSP is very sensitive, but was found to be prone to false positive results and overestimation of the number of methylated samples. • The *Methylight* assay developed from MSP as a quantitative high-throughput method.	[308-310]
COBRA	• Combined bisulfite restriction analysis assay • combination of bisulfite treatment, PCR amplification with methylation-insensitive primers, restriction digestion and a quantification step. • The method is quantitative, sensitive and amenable to small sample size	[311].
Bio-COBRA	• Modification of the COBRA assay that incorporates an electrophoresis step in microfluidics chips.	[312]
Bisulfite sequencing	• “Golden standard” of DNA methylation analysis, yielding single-nucleotide resolution information about the methylation status of a defined region of DNA. • requires cloning of the PCR product prior to sequencing for adequate sensitivity • time-consuming and expensive method not suitable for higher throughput	[313]
Bisulfite pyrosequencing	• Sequencing-by-synthesis approach. Nucleotide incorporation is monitored in real-time based on the conversion of pyrophosphate (PPi) released during the reaction into a bioluminometric signal. • After bisulfite treatment and methylation-insensitive PCR, the degree of methylation at each CpG position in a sequence is determined from the ratio of T and C. • A disadvantage of the method is its high cost.	[314]
MS-SnuPE	• ‘Methylation-sensitive single-nucleotide primer extension’ assay • bisulfite-converted and PCR-amplified DNA is used as a template for the primer extension reaction. MS-SnuPE-primers are annealed to the sequence up to the base pair immediately before the CpG site of interest. DNA polymerase then extends the primers one base pair into the C (or T) using terminating dideoxynucleotides. The C to T ratio is determined quantitatively, either by the use of radioactively labeled nucleotides used for the reaction. • A modification of the method uses ‘Matrix-assisted laser desorption ionization/time-of-flight’ (MALDI-TOF)-based mass spectrometry analysis to differentiate between the two primer extension products.	[315, 316]
MassARRAY	• quantitative methylation analysis based on MALDI-TOF mass spectrometric detection. Regions of bisulfite-converted DNA are amplified by PCR with tagged primers, *in vitro* transcribed into RNA and cleaved base-specifically by endoribonuclease. Mass spectra of cleavage products are obtained by MALDI-TOF mass spectrometry. Fragments differ in mass depending on the sequence changes introduced by the initial bisulfite treatment. • sensitive, requires only very small amounts of DNA, allows high-throughput quantification of DNA methylation in candidate regions in a 384-well format.	[317]

Assay	Principle	Reference
Analysis of genome-wide DNA methylation		
MeDIP, MCIp	• enrichment of methylated or unmethylated DNA fragments by 5meC-specific antibody (MeDIp) or methyl binding domain protein (MCIp) • analysis of genome-wide CpG methylation status by DNA array technology, or next generation sequencing (NGS).	[318, 319]
HELP	• '*Hpa*II tiny fragment enrichment by ligation-mediated PCR' assay for whole genome methylation analysis • DNA is separately digested with two restriction enzymes, *Hpa*II and its methylation-insensitive isoschizomer *Msp*I. The resulting DNA fragments are then amplified using a ligation-mediated PCR, labeled with fluorescent dyes and co-hybridized to DNA microarrays, allowing to discriminate hypomethylated loci (represented by both *Hpa*II and *Msp*I) from methylated loci (represented by *Msp*I only).	[320]
MSRF	• 'Methylation-sensitive restriction fingerprinting' • genomic DNA is digested with *Mse*I. *Mse*I cuts at TTAA sites rare in CG-rich regions ⇨ DNA is digested to short fragments with CG-rich regions remaining largely intact. • fragments are further digested with the methylation-sensitive restriction enzyme *Bst*U I, followed by PCR using different pairs of short arbitrary primers in the presence of radiolabeled [^{32}P]-dNTPs; fragments with methylated *Bst*U I restriction sites are amplified, whereas unmethylated fragments are cut and not amplified. • PCR products are size-fractionated by high-resolution polyacrylamide gel electrophoresis to detect differentially methylated fragments by comparison between two samples (e.g. tumor vs. normal, treated vs. untreated). • Differentially represented bands are identified by cloning of excised fragments and sequencing.	[321, 322]
DMH	• 'Differential methylation hybridization'; further development of MSRF • detection of differential methylation is based on comparative hybridization of radioactive or fluorescently-labeled PCR products to DNA microarrays.	[28, 323]

Supplementary Table 3. Chemopreventive Agents Targeting DNA Methylation *in vitro*

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
Folate	reviewed in Lamprecht & Lipkin, 2003 [81] Kim *et al.,* 2004 [83] Kim *et al.*, 2005 [84] Johnson *et al*., 2008 [70] Duthie, 2010 [82]	• maintenance of genomic stability • regulation of purine and pyrimidine biosynthesis ⇨ DNA biosynthesis, DNA repair, proliferation • synthesis of SAM from methionine ⇨ impact on DNA methylation		
NaSelenite, and seleno-compounds	Fiala *et al*., 1998 [92]	• ↓ DNMT activity		• nuclear extracts of human colon carcinoma; HPLC with radioflow detection. IC_{50} values 3.8, 8.1, 5.2 µM for selenite, benzyl selenocyanate, *p*-XSC
***p*-XSC**	Fiala *et al*., 1998 [92]	• ↓ DNMT activity in HCT116	• 1.25-40 µM (24 h)	• nuclear extracts of treated cells; IC_{50} ~20 µM
NaSelenite	Davis *et al.*, 2000 [93]	• ↑ genomic DNA methylation in Caco-2 cells	• 1, 2 µM (14 d)	• *Sss1* DNMT-mediated methyl-^{3}H-incorporation (*in vitro* methyl acceptance capacity of DNA)
		• ↑ *p53* promoter methylation	• 1, 2 µM (14 d)	• quantitative PCR for resistance to *Hpa*II-digestion indicating site specific methylation
	Davis *et al*., 2002 [94]	• ↑ genomic DNA methylation in HT29 cells	• 1, 2 µM (7 d)	• *Sss1* DNMT-mediated methyl-^{3}H-incorporation • (*in vitro* methyl acceptance capacity of DNA)
		• ↓ DNMT1 expression in HT29 cells	• 1, 2 µM (7 d)	
	Xiang *et al*., 2008 [96]	• ↓ DNMT1 and -3a mRNA (and protein) expression in LNCaP cells	• 1.5 µM (8 d)	• dual action on DNA methylation and histone acetylation
		• ↓ HDAC activity, ↑ histone H3K9 acetylation, ↓ H3K9 methylation	• 1.5 µM (7 d)	• Fluor de Lys Fluorescent Assay System (Biomol)
		• ↓ global DNA methylation	• 1.5 µM (7 d)	• dot blot with anti-5-methylcytosine antibody
		• ↓ promoter methylation and ↑ mRNA (protein) expression of *GSTP1, APC, CSR1*	• 0.5, 1.5 µM (7, 14 d)	• MSP
		• ↑ acH3, ↓ DNMT1 and H3K9me2 associated with *GSTP1* promoter		• ChIP
Retinoic acid (RA)	Di Croce *et al.*, 2002 [109]	• ↓ *RARβ2* promoter and exon 1 methylation in NB4 cells	• not stated (48 h)	• bisulfite sequencing
		• ↑ *RARβ2* mRNA expression in NB4 cells	• not stated (48 h)	• enhanced effect in combination with 5-Aza-dC
	Liu *et al.*, 2004 [111]	• ↓ *hTERT* promoter activity, ↓ telomerase activity, associated with progressive ↑ *hTERT* promoter methylation during RA-induced differentiation of human HT and HL-60 cells	• HL-60: 1 µM • HT: 2 µM • (up to 12 d)	• bisulfite sequencing
	Nouzova *et al*., 2004 [110]	• ↔ CpG island methylation during RA-induced NB4 cell differentiation • ↔ *RARβ* CpG island methylation	• 5 µM (5 d)	• differential methylation hybridization with CpG island microarrays • concomitant analysis of histone acetylation
NaButyrate	Spurling *et al.*, 2008 [125]	• ↑ of retinoic acid-mediated *RARβ2* mRNA activation in HCT116, HT29 cells	• 4 mM (24 h)	• better known as HDAC inhibitor
		• ↓ *RARβ2* promoter methylation in HCT116, HT29 cells	• 4 mM (24 h)	• COBRA; bisulfite sequencing; • sporadic pattern of demethylation in the absence of DNA synthesis, since NaB cause cell cycle arrest
		• ↔ amplification of inter-methylated sites (AIMS)	• 4 mM (24 h)	• no signs of global demethylation

(Table 3) Contd…..

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
EGCG (and other green tea polyphenols)	Fang *et al*., 2003 [129]	• ↓ DNMT activity	• 5 - 50 μM	• KYSE 510[a] nuclear extracts; radioactive detection; inhibitory potential EGCG > ECG = MeEGCG > EGC = DiMeEGCG > EC
		• *in silico* modelling of DNMT binding		• H-bonding in the catalytic pocket through pyrogallol moiety
		• ↓ *p16, RARβ, MGMT, hMLH1* promoter hypermethylation, ↑ mRNA expression in human esophageal cancer KSYE 510 cells	• 5 - 50 μM • (12 h – 6 d)	• MSP
		• ↑ *RARβ, hMLH1* protein expression in KSYE 510 cells	• 20, 50 μM • (3 and 6 d)	
		• ↔ DNMT-1, DNMT3a, DNMT3b, MBD2 mRNA expression in KSYE 510 cells	• not stated	• no effect
		• ↓ promoter methylation and ↑ mRNA expression of *p16* in HCT116, *RARβ* in KYSE 150 and PC3	• 5, 20 μM • (6 d)	• MSP
	Chuang *et al.*, 2005 [138]	• ↔ methylation of *p16* promoter and *MAGE-A1, Alu, LINE* repetitive elements in T24, HT29, PC3 cells	• 20, 30 μM • (6 d)	• quantitative methylation analysis by Ms-SnuPE, pyrosequencing
		• ↔ mRNA re-expression of *p16* in T24, HT29, PC3 cells	• 20, 30 μM • (6 d)	• no effect
	Lee *et al*., 2005 [130]	• ↓ bacterial *Sss1* DNMT, human DNMT-1 activity	• 0.1 - 20 μM	• radioactive detection, IC_{50} = 0.47 μM
		• *in silico* modelling of DNMT binding		• importance of gallic acid moiety, DNMT binding is enhanced by Mg^{2+}
		• ↓ *RARβ* promoter methylation in MCF-7 and MDA-MB-231 cells	• 0.2 – 50 μM • (3, 6 d)	• nested MSP, slight reduction in methylation
	Stresemann *et al.*, 2006 [139]	• ↔ genomic 5meC content in TK6 and HCT116 cells	• 2, 10, 50 μM • (3 d)	• capillary electrophoresis; no effect
	Fang *et al*. 2007 [133]	• ↑ *p16, MGMT* mRNA re-expression in KSYE 510 cells	• 20 μM • (up to 40 d)	• long-term treatment
		• ↑ *RARβ, p16, DAPK* mRNA re-expression in KSYE 510 and CL13 murine lung tumor cells	• 10 μM • (5 + 1 d)	• additive or synergistic effect in combination with HDAC inhibitors or 5-aza-2'deoxycytidine
	Berletch *et al*., 2008 [134]	• site specific ↓ of *hTERT* promoter methylation, ↑ *hTERT* repressor E2F binding (ChIP), ↓ mRNA expression in MCF-7 cells	• 50, 100 μM • (up to 12 d)	• nested MSP; ChIP
	Kato *et al*., 2008 [135]	• ↓ *RECK* promoter methylation in HSC3, HSC4, SCC9, SCC25 oral squamous cell carcinoma cells, ↑ mRNA expression • ↓ MMP activity, ↓ invasion	• 5 – 50 μM • (up to 6 d)	• MSP
	Gao *et al*., 2009 [136]	• ↓ Wnt inhibitory factor (*WIF-1*) promoter methylation, ↑ mRNA expression, ↓ *Tcf/Lef* activity, ↓ cytosolic β-catenin level in H460 and A549 cells	• 5, 50 μM • (3 d)	• MSP, bisulfite sequencing
	Pandey *et al.*, 2009 [131]	• ↓ DNMT activity	• 5,10, 20 μM	• nuclear extracts of untreated LNCaP cells, ELISA-based detection (Epigentek)
		• ↑ expression of GSTP1 protein in LNCaP cells	• 10, 20 μM (up to 7d)	• ELISA

(Table 3) Contd…

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
Green tea polyphenols	Pandey *et al.*, 2009 [131]	• ↓ DNMT activity	• 5-20 μg/ml (3 d) 10 μg/ml (up to 7 d)	• nuclear extracts of untreated or treated LNCaP cells; ELISA-based detection (Epigentek)
		• ↓ DNMT mRNA and protein expression in LNCaP cells	• 1-10 μg/ml (3 d) 10 μg/ml (up to 14 d)	
		• ↓ methylation of proximal GSTP1 promoter in LNCaP cells	• 1-10 μg/ml (3 d) • 10 μg/ml (up to 14 d)	• MSP, bisulfite sequencing; • no effect on LINE-1 methylation
		• ↑ mRNA and protein expression of *GSTP1* in DU145 cells	• 2.5-10 μg/ml (3 d) • 10 μg/ml (up to 7 d)	
		• ↑↓ nuclear expression of methyl-binding domain protein 1 (MBD1), MeCP2 in LNCaP cells	• 10 μg/ml	• ↑ after 3 days, ↓ after 7 days
		• Association with *GSTP1* promoter: MBD2 >70% ↓; Sp1 >12-fold ↑; linked with ↓ HDAC activity, mRNA and protein expression, ↑ histone H3 and H4 acetylation	• 10 μg/ml (7 d)	• ChIP
Catechins and flavonoids: **catechin, epicatechin, EGCG, quercetin, fisetin, myricetin**	Lee *et al*., 2005 [130]	• ↓ bacterial *Sss1* DNMT and human DNMT1 activity, in the absence or presence of COMT	• 0.2 – 20 μM	• direct inhibition and indirect effects based on concomitant methylation of agents by COMT leading to reduced SAM/increased SAH levels. IC_{50} values for DNMT1 in the presence of COMT < 10 μM; EGCG < myricetin < quercetin < fistetin < catechin < epicatechin
Coffee polyphenols	Lee *et al*., 2006 [132]	• ↓ bacterial *Sss1* DNMT and human DNMT1 activity, in the absence or presence of COMT	• 5, 20 μM	• indirect effect, due to increased formation of SAH during *O*-methylation of catechol compounds. IC_{50} values < 5 μM
Caffeic acid, chlorogenic acid		• ↓ *RARβ* promoter methylation in MCF-7, MDA-MB-231 cells	• MCF-7: 1 - 50 μM (8 d) • MDA-MB-231: 0.2 – 20 μM (3 d)	• MSP
Flavonoids and polyphenols	Fang *et al.*, 2007 [133]	• ↓ DNMT activity	• 20, 50 μM	• KYSE 510 nuclear extracts; radioactive detection. Hydroxy-cinnamic acid, garcinol, luteolin > 50% inhibition at 50 μM
Apple polyphenols	Fini *et al.*, 2007 [140]	• ↓ *hMLH1, p14ARF, p16* promoter methylation, ↑ mRNA or protein expression in RKO, SW48 and SW480 colon cancer cells	• 2 μM catechin equivalents (up to 8 d)	• MSP; COBRA
		• ↔ DNMT-1, DNMT-3b mRNA, ↓ protein expression in RKO and SW480 cells	• 2 μM catechin equivalents (2 d)	
Dietary polyphenols:	Paluszczak *et al*., 2010 [298]	• ↓ DNMT activity	• 20, 40 μM	• nuclear extracts of untreated MCF-7 cells, ELISA-based method (Epigentek)
rosmarinic acid, ellagic acid		• ↔ DNMT mRNA and protein expression in MCF-7 cells	• 20, 40 μM	• no effect

(Table 3) Contd….

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
baicalein and others		• ↔ *RASSF1A, GSTP1, HIN1* promoter methylation in MCF-7 cells	• 20, 40 μM (3 d, 6 d)	• MSP; no effect
		• ↔ histone H3K9me2, H3K9me3, H3K27me3 methylation in MCF-7 cells	• 5, 10 or 20, 40 μM	• Western blotting; no effect
Genistein, soy isoflavones	Fang *et al.*, 2005 [163]	• ↓ human DNMT activity • ↓ HDAC 1 activity (max. 30% ↓ at 100 μM)	• 20 - 50 (100) μM	• nuclear extracts of untreated KSYE 510 cells, human recombinant DNMT1; radioactive detection. • Biochanin A and daidzein are less effective
		• ↓ *p16, RARβ, MGMT* promoter methylation, ↑ mRNA expression in KSYE 510 (KYSE 150, LNCaP, PC3) cells	• 2 - 20 μM (up to 6 d)	• MSP; enhanced activity by co-treatment with HDAC or DNMT inhibitors
Genistein	King-Batoon *et al*., 2008 [164]	• ↓ *GSTP1* promoter methylation, ↑ mRNA expression in MDA-MB-468 breast cancer cells. No effect in MCF-7 cells, no effect on RARβ promoter methylation in both cell lines.	• 3.125 μM 3x/wk (6 d)	• MSP
	Spurling *et al*., 2008 [125]	• ↓ *RARβ2* promoter methylation in HCT116 cells	• 25 μM (3 d)	• bisulfite sequencing
	Li *et al*., 2009 [166]	• ↓ DNMT-1, -3a,-3b protein expression in MCF-7 (partly in MCF10AT)	• 100 μM (3 d)	•
		• site-specific ↓ methylation at E2F-1-binding site in hTERT promoter in MCF-7 cells	• 50, 100 μM (3 d)	• ChIP-bisulfite sequencing
		• ↓ hTERT mRNA expression through ↑ E2F-1-, • ↓ H3K4me2-, ↑ H3K9me3 binding to hTERT promoter in MCF-7 and MCF10AT	• 50, 100 μM (up to 3 d)	• ChIP • (compare Table 3)
	Majid *et al*., 2009 [167]	• ↓ *BTG* promoter methylation, ↑ mRNA expression in A498, ACHN, HEK-293 renal cell carcinoma cell lines	• 25, 50 μM (3 d)	• bisulfite sequencing
		• ↓ DNMT activity, ↓ MBD2 binding, ↑ HAT activity, ↓ HDAC activity (ACHN cells only)	• 50 μM (3 d)	• nuclear extracts of treated cell lines; ELISA-based detection (Epigentek)
		• partly ↓ DNMT-1, 3a, 3b protein expression		• ELISA
		• ↑ acH3, acH4, H3K4me2, H3K4me3, RNA pol II binding to BTG promoter		• ChIP
Soy isoflavones	Vardi *et al*., 2010 [165]	• ↓ *GSTP1* and *EPHB2* promoter methylation, ↑ protein expression in three prostate cancer cell lines. • ↔ no effect on *BRCA1* and *RASSF1A* promoter methylation		• MSP, immunohistochemistry
Curcumin	Liu *et al*., 2009 [188]	• molecular docking studies		
		• ↓ bacterial *Sss1* DNMT activity	• 1 nM-100 μM	• ELISA-based fluorescence detection; demethoxy-derivatives are equally effective, tetra- and hexahydro-metabolites less active
		• 15-20% ↓ in global methylation in MV4-11 cells	• 3 and 30 μM (3 d)	• LC-MS/MS

(Table 3) Contd….

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
Nordihydro-guaiaretic acid (NDGA)	Cui *et al.*, 2008 [206]	• ↓ E-cadherin promoter methylation, ↑ E-cadherin mRNA and protein expression in SKBR3 and MDA-MB-435 human breast cancer cell lines and MDA-MB-435 xenografts	• 10-100 μM (7 d)	• MSP, bisulfite sequencing
	Cui *et al.*, 2008 [205]	• ↓ *p16* and E-cadherin promoter methylation, ↑ *p16* and E-cadherin mRNA and protein expression in RKO and T47D cell lines, linked with ↓ Cyclin D1 and ↓ pRB and ↑ cellular senescence	• 10-100 μM (3, 6 d)	• MSP, bisulfite sequencing
	Byun *et al.*, 2008 [207]	• ↔ *LINE-1* methylation in HepG2 cells	• 100 μM	• bisulfite pyrosequencing
Parthenolide (sesqui-terpene lac-tone)	Liu *et al.*, 2009 [178]	• molecular docking studies		
		• ↓ bacterial *Sss1* DNMT activity	• 0.1-100 μM	• ELISA-based fluorescence detection
		• ↓ DNMT mRNA and protein expression in MV4-11 and Kasumi-1 cells	• 3-10 μM (24 h)	• may be associated with cell cycle arrest and apoptosis induction
		• ↓ global methylation in MV4-11 and K562 cells	• 5-10 μM	• LC-MS/MS
		• ↓ *HIN-1* promoter methylation and ↑ mRNA expression in MCF-7 cells	• 10, 30 μM	• LC-MS/MS; bisulfite sequencing
Mahanine	Jagadeesh *et al.*, 2007 [300]	• ↓ *DNMT* activity in LNCaP, PC3 cells	• 1-3 μg/ml (3 d)	• ELISA-based EpiQuik DNMT assay kit (Epigentek) using nuclear extracts of treated cells
		• ↑ *RASSF1A* mRNA expression in various tumor cell lines (LNCaP, PC3, A431, A549, ASPC-1, HT29, MCF-7, SKOV-3)	• 1-3 μg/ml (3 d)	• RASSF1A down-regulates cyclin D1 expression
Mahanine derivative	Sheikh *et al.*, 2010 [200]	• ↑ *RASSF1A* mRNA expression in PC3 cells, ⇨ ↓ cyclin D1 mRNA expression	• 5 μM (3 d)	• synthetic fluorescent derivative of mahanine; epigenetic effects are accompanied by ↓ volume of PC3 xenograft tumors
		• ↓ DNMT activity in PC3 cells	• 2 μM (3 d)	
		• sequestration of DNMT3b in the cytosol	• 5 μM (6 h)	
		• ↔ amplification of inter-methylated sites (AIMS)	• 4 mM (24 h)	• no signs of global demethylation
Lycopene	King-Batoon *et al.,* 2008 [164]	• ↓ GSTP1 promoter methylation and ↑ mRNA expression in MDA-MB-468 cells, ↓ *RARβ, HIN1* promoter methylation in MCF10A cells, but not in MCF-7 cells	• 2 μM once (7 d) • 2 μM 1x/wk (14 d)	• MSP
Sulforaphane (SFN)	Merran *et al*., 2010 [225]	• ↓ DNMT1 and DNMT3a expression	• 2.5 -10 μM (6 d)	
		• ↓ telomerase activity and *hTERT* mRNA expression	• 2.5 -10 μM (6, 9 d)	
		• ↓ *hTERT* methylation at the CTCF binding site in exon 1	• 5, 10 μM (6 d)	• bisulfite sequencing
		• ↑ inhibition of cell growth, apoptosis induction in MCF-7, MDA-MB-231 cells, but not in MCF10A cells	• 5 – 20 μM (3, 6, 9 d)	• associated with chromatin modifications
Phenylethyl isothiocyanate (PEITC)	Wang *et al.,* 2007 [229]	• ↓ *GSTP1* promoter methylation and protein reexpression of GSTP1 in LNCaP-AD and –AI cells	• 0.5 - 2 μM (5 d)	• MSP; pyrosequencing. • dual action on DNA methylation and histone acetylation
		• ↑ GSTP1 protein expression/GST activity in LNCaP cells	• 2, 5 μM/0.5-4 μM (5 d)	

(Table 3) Contd…..

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
Phenylhexyl isothiocyanate (PHI)	Lu *et al*., 2008 [234]	• ↓ *p16* promoter methylation in RPMI8226 cells	• 0.5 μM (10 d)	• MSP
		• ↓ proliferation, ↑ cell cycle arrest in G1	• 0.5 μM (2, 4 d)	• dual action on DNA methylation and histone acetylation
Mithramycin A (MMA)	Lin *et al.*, 2007 [247]	• ↓ promoter methylation and ↑ mRNA of *SLIT2* and *TIMP3* (candidate anti-metastasis TSGs) in Cl1-5 cells	• 10 nM (14 d)	• MSP; bisulfite sequencing
		• *in-silico* docking to DMNT1 catalytic domain		• known to bind to GC- or CG-rich DNA sequences
		• ↓ DNMT1 protein expression, ↔ *DNMT1* mRNA in Cl1-5 and A549 cells	• 10 nM (14 d)	
		• ↓ cell migration *in vitro*		

[a]**Abbreviations and cell lines:** 5meC, 5-methyl cytosine; A431, human vulvar epidermoid carcinoma cell line; A498, human renal cell carcinoma cell line; A549, human lung adenocarcinoma epithelial cell line; ac-H3, acetylated histone H3; ac-H4, acetylated histone H4; ACHN, renal cell carcinoma cell line; AIMS, amplification of inter-methylated sites; Alu, repetitive element belonging to the short interspersed elements (SINE); *APC*, adenomatous polyposis coli; ASPC-1, human pancreatic tumor cell line; BSC, benzyl selenocyanate; *BTG*, B cell translocation gene; Caco-2, human colorectal adenocarcinoma cell line; ChIP, chromatin immunoprecipitation; Cl1-5, human lung adenocarcinoma cell line; CL13, murine lung tumor cell line; COBRA, combined-bisulfite restriction analysis; COMT, catechol-*O*-methyltransferase; *CSR1*, cellular stress response 1; d, day; *DAPK*, death-associated protein kinase; DiMeEGCG, dimethyl-epigallocatechin gallate; DU145, human prostate cancer cell line; EC, epicatechin; ECG, epicatechin gallate; EGC, epicatechin gallate; EGCG, epigallocatechin gallate; ELISA, enzyme-linked immunosorbent assay; *EPHB2,* ephrin 2*; GSTP1*, glutathione-S-transferase π; H3K27me3, histone 3 lysine 27 trimethylation; H3K4me2, histone 3 lysine 4 dimethylation; H3K4me3, histone 3 lysine 4 trimethylation; H3K9me2, histone 3 lysine 9 dimethylation; H3K9me3, histone 3 lysine 9 trimethylation; H460, human large-cell lung carcinoma cell line; HaCaT human keratinocytes; HCT116, human colorectal carcinoma cell line; HEK-293, renal cell carcinoma cell line; *HIN1*, high in normal-1; *hMLH1*, human mutL homolog1; HT, human teratocarcinoma cells; HT29, human colon adenocarcinoma cell line; HSC3, HSC4, human oral squamous cell carcinoma; *hTERT*, human telomerase reverse transcriptase; K562, human chronic myelogenous leukaemia cell line; Kasumi, human myeloid leukaemia cell line; KYSE 150, human oesophageal carcinoma cell line; KYSE510, human esophageal squamous cell carcinoma cell line; LC-MS/MS, liquid chromatography combined with tandem mass spectrometry; *LINE*, long interspersed elements; LNCaP, androgen-sensitive human prostate adenocarcinoma cell line; LNCaP-AD, androgen-dependent LNCaP; LNCaP-AI, androgen-independent LNCaP; *MAGE-A1*, human Melanoma-associated antigen 1; MBD1, methyl-binding domain protein 1; MBD2, methyl-CpG binding domain protein 2; MCF10AT, premalignant human breast epithelial cell line (T24 c-Ha-ras oncogene- transfected MCF10A cells); MCF-7, human breast cancer cell line; MDA-MB-231, human breast cancer cell line; MDA-MB-435, human breast cancer cell line; MDA-MB-468, human breast cancer cell line; MeCP2, methyl CpG binding protein 2; MeEGCG, monomethylated epigallocatechin gallate; *MGMT*, *O6*-Methylguanin-DNA-Methyltransferase; MMP, matrix metalloprotease; MSP, methylation-specific PRC; Ms-SnuPE, Methylation Sensitive Single Nucleotide Primer Extension; MV4-11, humane acute myelocytic leukemia cell line; NB4, acute promyelocytic leukemia cell line; PC3, human prostate cancer cell line; *pRB*, retinoblastoma protein; *p*-XSC, 1,4-phenylenebis(methylene)selenocyanate; RA, retinoic acid; *RARβ*, retinoic acid receptor β; *RASSF1A*, Ras association domain family 1 A; *RECK*, reversion-inducing-cysteine-rich protein with kazal motifs; RKO, human colon carcinoma cell line; SAH, *S*-adenosly-homocysteine; SAM, *S*-adenosyl-methionine; SCC9, SCC25, human oral squamous cell carcinoma; SKBR3, human ER-neg. breast cancer cell line; SKOV-3, human ovarian carcinoma cell line; *SLIT2*, slit homolog 2 protein; SW48, human colon adenocarcinoma cell line; SW480, human colon adenocarcinoma cell line; T24, human bladder carcinoma cell line; T47D, human breast cancer cell line; *Tcf/Lef*, T cell-specific transcription factor/lymphoid enhancer-binding factor; *TIMP-3*, tissue inhibitor of matrix metalloprotease; TK6, human B lymphoblastoid cell line; TSG, tumor supressor gene; *WIF-1*, Wnt-inhibitory factor 1; wk, week.

Supplementary Table 4: Modulation of DNA Methylation by Chemopreventive Agents in Rodent Models

Agent	References	Target site	System/carcinogen	Concentration/Dose	Results – Comments - Methods
Folate	Lamprecht & Lipkin, 2003 [81] Kim *et al*., 2004 [83] Kim *et al*., 2005 [84] Johnson *et al*., 2008 [70] Duthie, 2010 [82]	liver, colon	mouse, rat healthy individuals patients with colonic adenoma and colon cancer	various	summary of studies investigating DNA methylation in cell culture, rodent models and human intervention studies
NaSelenite or SeMethionine	Davis & Uthus, 2000 [93]	liver, colon	rats	selenium-deficient diet vs. 0.1 or 2 mg/kg diet for 6 wk	↓ global DNA methylation (*in vitro* methyl acceptance capacity of DNA) ↓ plasma homocysteine levels with Se-deficient diet ↑ with Se-supplementation
NaSelenite	Davis & Uthus, 2002 [94]	intestine	DMH-treated rats	selenium-deficient diet vs. 0.1 or 2 mg/kg diet for 12 wk	↓ aberrant crypt foci formation ↓ liver SAM/SAH ratio with ↑ Se-supplementation
	Davis *et al.*, 2003 [95]	intestine	rats	interaction of selenium-and folate-deficiency vs. selenium and folate supplementation (2 mg/kg diet for 12 wk	↑ aberrant crypt foci formation by Se in folate-deficient rats, ↓ aberrant crypt foci formation by Se in folate-supplemented rats ↓ global DNA methylation in Se-deficient rats (colon only) (*in vitro* methyl acceptance capacity of DNA)
Retinoic acid (RA)	Tang *et al*., 2009 [114]	oral cavity	4-NQO-treated mice	low dose: 100 µg/kg b.w. 2 x per wk, high dose: 1 mg/kg b.w. 1x per wk for 15 wk	↓ No. of cancerous tongue lesions
Vitamin E	Fischer *et al*., 2010 [118]	liver	rats	Vit. E-deficient (α-tocopherol < 1 mg/kg diet) vs. control (α-tocopherol 12 mg/kg diet) (6 months)	↔ global DNA methylation (5meC, ELISA kit Epigentek, Sigma Alrdich) ↔ promoter methylation of SDR5A1 and GCLM (MassARRAY)
EGCG Polyphenone E	Fang *et al.,* 2007 [133]	plasma, small intestine, liver	healthy mice	application of EGCG of green tea polyphenols at various doses and for various durations	moderate ↓ SAM by chronic administration through drinking fluid, no effect on SAH, homocysteine, methionine; stronger influence when given once at high doses
Green tea polyphenols	Volate *et al*., 2009 [142]	colon, small intestine	AOM-treated $APC^{Min/+}$ mice	0.6% in drinking fluid, starting at 8 wks of age	↓ tumor formation ↓ AOM-induced *RXR*α promoter methylation (bisulfite pyrosequencing) ↑ *RXR*α mRNA and protein expression in tumor tissue
	Morey Kinney *et al.*, 2009 [297]	prostate, gut, liver	TRAMP and wt mice	0.1-0.6% in drinking fluid	no inhibition of tumor formation no effect on 5meC levels (LC-MS/MS) and methylation of B1 repetitive element and *MAGE-a8* (bisulfite pyrosequencing) no effect on promoter hypermethylation of I*RX3*, *CACNA1A*, *CDKN2A*, *NRX2* (MassARRAY) no genome wide effect on DNA methylation (HELP assay)

(Table 4) Contd…..

Agent	References	Target site	System/carcinogen	Concentration/Dose	Results – Comments - Methods
Genistein	Day *et al.*, 2002 [173]	brain, kidney, liver, spleen, prostate, testes	healthy male mice	300 mg/kg AIN 93G starting at 8 wks of age	3/900 clones from prostate hypermethylated after genistein treatment, no change in liver (methylation-sensitive restriction digestion and mouse differential methylation hybridization (mDMH) with radioactive detection)
	Dolinoy *et al*., 2006 [174]	tail, brain, kidney, liver	A^{vy} mice	250 mg/kg AIN 93G; *in utero* and post-natal exposure until day 21	↑ methylation at 6 CpG sites of the A^{vy} intracisternal A particle (IAP) murine retrotransposon at post-natal day 21 and 150 (bisulfite sequencing); ↓ obesity
	Tang *et al*., 2008 [176]	uterus	healthy mice, intact and ovarextomized (OVX)	50 mg/kg bw genistein, neonatal exposure on days 1-5, analysis at day 19 and after 6 and 18 month. Comparison with diethylstilbestrol treatment	persistent promoter hypomethylation of nucleosomal binding protein 1 (*Nsbp*1) (methylation-sensitive restriction fingerprinting (MSRF); bisulfite sequencing); correlation with ↑ *Nsbp1* mRNA expression
Soy isoflavones (genistein + daidzein)	Guerrero-Bosagna *et al*., 2008 [175]	pancreas, liver	healthy mice	2% soy isoflavone extract in diet; pre- and post-natal exposure of offspring	↓ methylation differences between male and females of skeletal α-Actin (*ActaI*) in liver at post-natal day 42 (bisulfite sequencing); ↑ sexual maturation
Nordihydro-guaiaretic acid (NDGA)	Cui *et al*., 2008 [206]	human breast cancer	xenograft	100 mg/kg, 3x per wk	↓ tumor volume, reactivation of *E-cadherin* protein expression
Parthenolide	Liu *et al*., 2009 [178]	human leukemia	xenograft	10 mg/kg 4 mg/kg, 5x per d	↓ tumor volume ↓ global DNA methylation (LC-MS/MS) ↓ DNMT protein expression
Mahanine derivative	Sheikh *et al.*, 2010 [200]	prostate	xenograft	10 mg/kg i.p. every other day for 28 d	↔ toxicity up to 550 mg/kg b.w. ↓ tumor volume by 40%
Phenethylisothio-cyanate (PEITC)	Wang & Chiao, 2010 [231]	prostate	TRAMP and wt mice	15 μmol daily by gavage for 13 wk	↓ tumor incidence and severity ↓ *MGMT* promoter methylation (MSP) in tumor tissue
Celecoxib and DFMO	Pereira *et al*., 2004 [257]	colon	AOM-treated rats	Celecoxib 500 mg/kg DFMO 100, 1000 or 3000 mg/kg diet 1 or 4 days prior to death at wk 37	AOM-treated control: global DNA hypomethylation, ERα promoter hypermethylation in tumor DNA (dot-blot with 5-MeC-antibody, bisulfite sequencing) reversal of global hypomethylation by celecoxib or high dose DFMO (dot-blot with 5-MeC-antibody) time- (and dose-)dependent ↓ ERα promoter methylation and ↑ mRNA expression by both compounds alone and in combination (bisulfite sequencing)

Abbreviations: AOM, azoxymethane; A^{vy} mice, Agouti viable yellow mice; CACNA1A, Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit; CDKN2A, Cyclin-dependent kinase inhibitor 2A; DFMO, d,l-α-difluoromethylornithine; DMH, 1,1-Dimethylhydrazin; ERα, Estrogen receptor α; IAP, intracisternal A particle; GCLM, regulatory subunit of γ-glutamylcystein synthase; IRX3; Iroquois homeobox 3; MAGE-a8, Melanoma Antigen, Family A, 8; mDMH, mouse differential methylation hybridization; MSRF, methylation-sensitive restriction fingerprinting; NRXN2, Neurexin 2; NSBP1, Nucleosomal binding protein 1; OVX, ovariectomized; RXRα, retinoic X receptor α; SAH, S-Adenosyl-*L*-homocysteine; SAM, S-Adenosyl-*L*-methionine; SDR5A1, 5-α-steroid reductase type 1; Se, selenium; TRAMP, transgenic adenocarcinoma of the mouse prostate

Supplementary Table 5. Modulation of DNA Methylation by Chemopreventive Agents in Human Studies

Agent	References	Study population/design, samples	Genes analysed	Results – Comments - Methods
Folate, green vegetables, multivitamins	Stidley *et al*., 2010 [85]	Cohort-based study with 1100 participants (75% f, 25% m); sputum samples with exfoliated aerodigestive tract cells	*p16, MGMT, RASSF1A, DAPK, GATA4, GATA5, PAX5α, PAX5β*	Protection against methylation (MSP): folate, OR: 0.84 per 750 μg/d, 95% CI 0.72-0.99 leafy green vegetable consumption per 12 monthly servings, OR: 0.83, 95% CI 0.74-0.93 multivitamins, OR: 0.57, 95% CI 0.40-0.83
Retinoic acid	Sirchia *et al*., 2002 [108]	13 breast cancer patients, clinical 3-wk Phase 1B trial; tumor samples	*RARβ2*	↔ no effect on *RARβ2* promoter methylation (MSP) and mRNA expression.
Resistant starch and/or *Bifidobacterium lactis*	Worthley *et al*., 2009 [126]	20 m/f volunteers; randomized, double-blind, placebo-controlled 4-wk crossover trial; normal rectal biopsies	methylation levels of 5 genes with hyper-methylation related to ageing: *ESR1, GATA5, HIC1, HPP1, SFRP1* 11 genes with cancer-related hyper-methylation: *MLH1, CDKN2A, MINT1, MINT2, MINT31, CACNA1G, IGF2, RUNX3, NEUROG1, SOCS1, MGMT* *LINE1* repetitive element	only *MINT2* promoter methylation levels influenced to various degrees by intervention (*Methylight* qMSP)
Green tea intake	Yuasa *et al*., 2009 [146]	106 gastric cancer patients (m/f), microdissected tumor cells	*CDX2, BMP2, p16, CACNA2D3, GATA5, ER*	↓ frequency of *CDX2* and *BMP2* promoter methylation by ≥ 7 cups/day of green tea (MSP)
Soy isoflavones	Qin *et al*., 2009 [177]	34 healthy pre-menopausal women; prospective, randomized, double-blind intervention trial with 40 or 140 mg of isoflavones daily through one menstrual cycle; breast tissue samples obtained by mammary ductoscopy	*p16, RASSF1A, RARβ2, ER, CCDN2*	↓ *RARβ2* and *CCND2* promoter methylation in women with low, ↑ promoter methylation in women with high circulating post-treatment serum genistein levels (n.s.) (nested qMSP)

Abbreviations: *BMP2*, bone morphogenetic protein 2; CACNA2D3, calcium channel, voltage-dependent, alpha 2/delta subunit; *CACNA1G*, $Ca_v3.1/\alpha1G$ T-type calcium channel gene; *CCDN2*, cyclin D2; *CDKN2A*, cyclin-dependent kinase inhibitor 2A (also known as $p16^{INK}$); *CDX2*, caudal type homeobox transcription factor 2; *DAPK*, death-associated protein kinase; *ER*, estrogen receptor; *ESR1*, estrogen receptor gene; *GATA4*, GATA-binding protein 4, 5; *HIC1*, Hypermethylated in cancer1; *HPP1*, hyperplastic polyposis1; *IGF2*, insulin-like growth factor 2; *LINE1*, Long Interspersed Nucleotide Element; *MGMT*, O6- methylguanine-DNA methyltransferase; *MLH1*, MutL homolog 1; *NEUROG1*, neurogenin1; OR, odds ratio; *p16*, cyclin-dependent kinase 4 inhibitor 2A; *PAX5β*, paired box gene 5; qMSP, quantitative methylation specific PCR; *RARβ2*, retinoic acid receptor β2; *RASSF1A*, Ras association (RalGDS/AF-6) domain family member 1; *RUNX3*, runt-related transcription factor 3; *SFRP1*, Secreted frizzled-related protein 1; *SOCS1*, Suppressor of cytokine signaling 1

Supplementary Table 6. Methods for the Analysis of Histone Modifying Enzymes and Chromatin Modifications

Assay	Principle	Reference
Methods to measure enzymatic activities and histone modifications on chromatin		
Histone extraction and analysis	• Histones are extracted from cell nuclei under acidic conditions with HCl treatment and collected after TCA precipitation. • Acid urea (AU) gel electrophoresis separates differently modified histone isoforms based on the charge. However, histone variants have only minor sequence variation, so the AUT and 2D AUT gels are frequently utilized by adding triton X-100 in AU gels to release the hydrophobic regions of histones and enhance the resolution. • SDS-PAGE followed by immunoblotting with antibodies specific allows detection of pan-acetylated histone H3, H4, or site-specific histone modifications. • Reversed-phase HPLC • Mass spectroscopy (MS) to identify protein, determine the abundance, and distinguish the modifications of histone variants.	[324]
HDAC activity (radioactive detection)	• HDAC enzymes remove acetyl groups from acetylated substrates. For determination of activity or inhibition, HDAC enzymes are preincubated with test agents or vehicle, and then the reaction is initialized by adding radioactive substrates with [^{3}H]acetyl-groups, either enzymatically labeled by HAT or chemically acetylated onto H3 or H4 peptides. Deacetylation process is then quenched by adding HCl, and the released [^{3}H] acetic acid is extracted with ethyl acetate and scintillation counted for radioactivity.	[273, 325, 326]
HDAC activity (non-isotope detection)	• The commercial Fluor-de-Lys HDAC activity assay kit is often used to analyze HDAC activity. A synthetic peptide substrate containing an acetylated lysine side chain is incubated with the HDAC enzymes for *in vitro* deacetylation, which generates the free amino group for reacting with a fluorophore in the developer solution, leading to fluorescence emission for measurement with florescence reader. • SIRT deacetylase assay is performed similarly except that nicotinamide is added to developer solution prior to quenching the reaction.	[221, 325]
HAT activity (radioactive detection)	• HAT catalyzes the transfer of acetyl groups from acetyl-CoA to histones. HAT enzyme, purified core histones, and inhibitor or vehicle are mixed. The reaction is initialized by addition of [^{3}H]acetyl-CoA substrate. The reaction mixture is either blotted onto filter paper or resolved by SDS-PAGE, and the activities are assessed by scintillation counting or autoradiography.	[275, 327, 328]
HAT activity (non-isotope detection)	• A commercial colorimetric HAT activity assay kit utilizes acetyl-CoA as a cofactor. Active HAT releases the CoA molecule for the production of NADH to react with a soluble tetrazolium dye. The reaction is detected with a spectrophotometer.	[7]
Histone lysine methyltransferase (HKMT) activity (radioactive detection)	• The Histone lysine methyltransferase enzyme, as well as histone substrate, is preincubated with inhibitors or vehicle; and then [^{3}H]S-adenosyl-*L*-methionine is added to initiate the reaction. The reaction products are then TCA-precipitated, resolved on 15% SDS-PAGE, and subjected to autoradiography.	[305, 328].
Histone demethylase activity of LSD1	• With an H3K4me2 peptide as substrate, the LSD1 demethylase activity is determined by measuring the amount of chemiluminescence from the production of H_2O_2, which is generated when the cofactor $FADH_2$ is converted to FAD during LSD1 dependent demethylation. Signal intensities are integrated and calibrated against H_2O_2 standards. The histone substrates before and after reaction could be analyzed by Western blotting with specific H3K4me2 antibodies.	[288, 289]
Chromatin immunoprecipitation (ChIP)		
ChIP	• ChIP analysis is performed to analyze the binding of specific proteins associated with a specific DNA region by shearing formaldehyde-fixed cellular chromatins followed by immuno-precipitation with specific Ac-H3, Ac-H4, or other antibodies. • After immunoprecipitation, the recovered chromatin fragments are de-crosslinked and purified for PCR analysis for enrichment in the promoter, transcription start site, or coding regions. Alternative analysis can utilize microarray chips for genome-wide analyses (ChIP-on-chip; ChIP-chip).	[329-331]

Abbreviations: 2D-AUT, 2-dimentional acid urea triton; Ac-H3 and Ac-H4, acetylated histone H3 and H4; AU, acid urea; AUT, acid urea triton; CoA, Co-enzyme A; FAD, Flavin adenine dinucleotide; H_2O_2, hydrogen peroxide; H3K4me2, dimethylated histone H3 lysine 4; HAT, histone acetyltransferases; HCl, hydrochloric acid; HDAC, histone deacetylase; HKMT, histone lysine methyltransferase; HPLC, high performance liquid chromatography; LSD1, lysine specific demethylase 1; NADH, nicotinamide adenine dinucleotide; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SIRT, sirtuin; TCA, trichloroacetic acid

Supplementary Table 7. Chemopreventive agents Targeting HDAC and SIRT Activity *in vitro*

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
β-Methylseleno-pyruvate (MSP) α -Keto- γ -methylseleno-butyrate (KMSB)	Nian *et al*., 2009 [99]	• ↓ HDAC activity, ↑ ac-H3, • ↑ expression of $p21^{WAF1}$ mRNA and protein, • ↑ $p21^{WAF1}$ promoter activity in HCT116 and HT-29 cells	10, 50 μM (0.5 - 48 h)	Fluor-de-Lys HDAC kit (Biomol) using recombinant HDAC1, 8, and nuclear extracts from colon cancer cells ChIP Ac-H3K9 and Ac-H3K18 on p21 promoter p21 promoter luciferase assay
		• ↑ G_2/M arrest and apoptosis, ↑ cleaved caspase-3, -6, -7, -9 and PARP.		
		• Molecular modelling		MSP: competitive inhibitor of *HDAC8*
Retinoic acid (RA)	Sirchia *et al*., 2002 [108]	• ↑ acetylation at *RARβ* P2 promoter in T47D (P2 unmethylated), but not in MCF7 cells (P2 methylated)	1 μM	ChIP-acH4 *RARβ2* status: expressed (Hs578t cells); not expressed (T47D, MCF7 cells)
		• ↑ *RARβ2* transcript (T47D, but not MCF7) *in vitro* and in tumor xenografts *in vivo* • ↓ T47D colony formation *in vitro,* • ↓ T47D xenograft growth	1 μM 2.5 mg/kg bw	
		• ↑ acetylation at *RARβ2* in cells with methylated *RARβ2* P2 (MCF-7 and MDA-MB-231 cells) only in combination with TSA • ↑ growth inhibition , ↑ apoptosis	TSA 30-330 nM RA 1 μM (24 -48 h)	ChIP Ac-H3, Ac-H4; *in situ* cell death and horseradish peroxidase detection kit (Roche) Reactivation needs simultaneous administration of both RA and TSA; synergistic effects by combination
		• ↑ *RARβ2* transcription in breast cell line HCC 712; prostate: PC-3, DU 145, and LNCaP; larynx Hep2	TSA 30-330 nM RA 1 μM	all with partially methylated or methylated *RARβ2* P2 promoter
	Nouzova *et al*., 2004 [110]	• ↑ phenotypical changes of CD11b expression in NB4 cells; ↑ terminal differentiation, ↓ proliferation • ↑ gene expression of *RARβ, CD11b, HCK, OS-9, HOXA1*, *c-myc, c-myb, hTERT* • >100 single-copy CpG islands (within 1kb of TSS) of known genes become hyperacetylated in DNA from RA-treated NB4 • ↑ ac-H4 in *HOXA1* gene • ↑ ac-H4 not only in single copy genes but also in satellite DNA in RA-treated NB4 cell • ↔ detectable changes in genomic methylation	5 μM (72 h)	Flow cytometric analysis of CD11b population. Affymetrix U133A expression microarray analysis ChIP-chip analysis using Ac-H4 ChIP kit (Upstate) followed by CpG island microarray analysis.
	Liu *et al*., 2004 [111]	• ↑ terminal differentiation in HT and HL-60 cells	HL60 1 μM HT 2 μM (12 d)	
		• ↓ *hTERT* promoter activity in differentiating HT cells	2 μM (3, 6, 9, 12 d)	Promoter luciferase assay
		• ↓ H3K9ac in *hTERT* promoter	HL60 1 μM; HT 2 μM (12 d)	ChIP
	Love *et al*., 2008 [293]	• ↓ proliferation, ↑ differentiation in HL-60 cells. • ↑ CD11b expression • ↓ hTERT mRNA, ↓ telomerase activity	2 μM (6, 12 d)	
		• ↑ apoptosis, • ↑ mRNA levels of DNMT3a, ↓ DNMT1, 3b in HL-60 cells	2 μM (up to 12 d)	
		• ↓ DNMT1, ↓ H3K9ac binding at the *hTERT* promoter.		ChIP

	Phipps *et al.*, 2009 [112]	● ↓ proliferation, morphological changes at day 6 in SK-BR-3 cells	2 μM (6, 12 d)	branch formation, apoptotic bodies, nuclear granules, and cytoplasmic shrinkage
		● ↓ anchorage-independent growth in SK-BR-3 cells	2 μM (14 d)	
		● ↑ % apoptotic cells by day 6, then return to the level of day 0.	2 μM (up to 12 d)	
		● ↔ *hTERT* promoter methylation in SKBR3 cells	2 μM (up to 12 d)	bisulfite sequencing
		● ↓ H3K9ac in *hTERT* promoter in SK-BR-3 cells ● ↓ telomerase activity	2 μM (up to 12 d)	ChIP
Butyrate	Wu *et al.*, 2001 [294]	● ↑ ac-H3 and ac-H4 in HT29 cells	5 mM (8-24 h)	AUT gel electrophoresis of nuclear histone extracts. sustained and prolonged histone hyperacetylation may relate to chemopreventive effects, compared to transient effects induced by TSA
	Nakata *et al.,* 2004 [295]	● ↓ *Bcl-2*, ↑ *DR5*, ↑ *caspases 8* and *10* activities in Jurkat cells	10 mM (24 h)	Promoter luciferase assay
	Myzak *et al.,* 2006 [39]	● ↑ *p21* mRNA; cell cycle arrest at both G_1 and G_2/M phases, ↓ *cyclin D1, B1* in CCRF-CEM cells	2 mM (2-8 h)	
		● de-repression *of p21* promoter through increase histone acetylation and disruption of the Sp1/Sp3 binding followed by HDAC recruitment.		ChIP
		● ↓ *c-myc* mRNA		
Soy isoflavones: Genistein, daidzein	Hong *et al*., 2004 [159]	● ↑ *ERα*-mediated core histone acetylation	12.5 nM to 12.8 μM	Histone acetylation assay using purified chromatin and [^{3}H]acetyl CoA
Genistein	Basak *et al*., 2008 [162]	● ↓ *AR* protein by proteosomal degradation in LNCaP cells ● ↓ *PSA* mRNA levels	1-50 μM (72 h)	ChIP
		● ↓ nuclear translocation of *AR* in LNCaP cells	25 μM	
		● ↑ acetylation of HSP90 and dissociation of *AR* from HSP90	1, 10, 25 μM (72 h)	
		● ↓ HDAC protein levels; ↓ nuclear localization	1, 10, 25 μM (72 h)	HDAC6 siRNA recapitulates the effect on AR protein level
		● ↑ *HDAC6* protein level by 17-β-estradiol (E2)	1 nM E2 (24, 48 h)	Genistein-mediated reduction of HDAC protein levels may be due to anti-estrogenic activity
	Li *et al.*, 2009 [166]	● ↓ *hTERT* mRNA ● ↓ cell growth without inducing apoptosis in MCF-10AT and MCF-7	50, 100 μM (72 h)	
		● ↓ telomerase activity; ↓ *hTERT* promoter activity, ● ↓ expression of *DNMT1, 3a, and 3b* in MCF-7, but not *DNMT3a and 3b* in MCF10AT.		Telomeric repeat amplification protocol (TRAP) assay Promoter luciferase activity assay
		● ↑ binding of *E2F1*, ↓ *c-Myc*, H3K4me2, but ↑H3K9me3, and ↔ ac-H3 in *hTERT* promoter		ChIP: E2F1, c-myc, H3K4me2, H3K9me3, Ac-H3
	Jawaid *et al*., 2010 [299]	● ↓ H3 and ↓ acetylation response after HDACi treatment	10 nM (40 - 60 d)	Low dose, long term culture model LTGT-long term genistein treatment

		• ↑ procaspase 9 in long-term genistein treatment in MCF7 cells • LTGT-MCF-7 cells show ↓ growth rate, ↓ response to mitogens (EGF), TSA, and apicidin.		
Curcumin	Chen *et al*., 2007 [186]	• ↓ proliferation in Raji cells		IC_{50}: 25 μM (24 h)
		• ↓ *p300* and *HDAC1* mRNA and protein level • ↓ HDAC3 protein, ↓ Notch 1 protein	12.5 μM (24 h)	
Sulforaphane (SFN)	Myzak *et al.,* 2004 [221]	• ↓ *HDAC* activity, ↑ global H3 and H4 acetylation • ↑ local H4 acetylation in *p21* promoter in HEK293 and HCT116 cells. • ↑ *p21* protein level	15μM (47 h)	TOPflash reporter assays HDAC activity assay (Fluorde-Lys HDAC activity assay, Biomol) using nuclear extracts ChIP assay SFN-cysteine is the active metabolite (IC_{50}=15 μM)
	Myzak *et al.,* 2006 [222]	• ↓ HDAC activity, ↑ global histone acetylation, ↑ ac-H4 in the promoters of *p21* and *Bax* • ↑ protein expression of *p21* and *Bax*, • ↑ caspase3 activity, G_2/M cell cycle arrest in BPH-1, PC3, and LNCaP cells.	15 μM (48 h)	Fluor-de-Lys HDAC activity assay (Biomol) using total cell lysate proteins ChIP analysis: Ac-H4 binding on p21 and Bax promoter Multi-caspase activity assay (Guava technologies)
	Gibbs *et al*., 2009 [224]	• ↑ acetylation of HSP90 and dissociation of *AR* from HSP90 in LNCaP and VCaP cells	20 μM (4 h)	HSP90 is acetylated by HDAC6
		• ↓ *AR* protein by proteasomal degradation	5 – 20 μM (12, 24 h)	HDAC6 overexpression rescues the SFN-induced AR protein degradation; HDAC6 siRNA recapitulates SFN's effect on AR protein level
		• ↓ *PSA* mRNA in LNCaP	5 – 20 μM (12, 24 h)	
		• ↓ mRNA levels of PSA and TMPRSS2-ERG in VCaP	5 – 20 μM (24 h)	
		• ↓ *AR* binding on ARE of *PSA* and *TMPRSS2* in LNCaP and VCaP	20 μM (24 h)	ChIP
	Meeran *et al.,* 2010 [225]	• ↓ proliferation and colony forming potential in MCF-7, MDA-MB-231, but not MCF10A	5 – 20 μM (3, 6, 9 d)	
		• ↓ *hTERT* mRNA expression; and ↓ telomerase activities in MCF-7 and MDA-MB-231, • ↔ *hTERT* mRNA; ↔ telomerase activity because of low basal activity in MCF-10A	10 μM (6 d)	telomerase activity
		• ↑ ac-H3, H3K9ac in MCF-7 and MDA-MB-231, but not in MCF-10A		
		• ↑ ac-H3, H3K9ac • ↑ ac-H4, ↑ H3K9me3 and H3K27me3 in *hTERT* promoters of both MCF-7 and MDA-MB-231 • ↑ac-H4, ↔ H3K9me3 and H3K27me3 in MCF-10A • ↑ *MAD1* repressor binding; ↓ *c-Myc* activator binding; ↑ *CTCF* repressor binding in E-box sites of *hTERT* promoters in all MCF-7, MDA-MB-231, and MCF-10A cells	2.5, 5, 10 μM (6 d)	ChIP
		• ↓ HDAC activity in MCF-7 and MDA-MB-231; only slight decrease in MCF-10A • ↔ HAT activity	2.5, 5, 10 μM (6 d)	Colorimetric HDAC activity assay (Active motif) Colorimetric HAT activity assay (Epigentek)

Phenylethyl isothiocyanate (PEITC)	Wang *et al.,* 2008 [230]	• cell cycle arrest at G_1 phase, ↑ *p27* and *p21* without changing *p53* level in LNCaP cells	0.5-1 μM (24 h)	
		• ↑ ac-H3 in *p21* promoter	10 μM (24 h)	ChIP: Ac-H3
		• ↑ H3K4 methylation and ↓ H3K9 methylation	1, 10 μM (30 h)	
		• ↓ *c-Myc* expression (5 μM) and ↓ *c-Myc* binding to the Sp1 transcriptional complex in *p21* promoter (10 μM)	5, 10 μM (24 h)	Sp1-binding site *p21* oligo pull-down assay
Phenylhexyl isothiocyanate (PHI)	Beklemisheva *et al.*, 2006 [235]	• ↓ *HDAC1* protein level, ↓ HDAC1/2 activity • ↑ ac-H3, ac-H4, H3K14ac • ↑ *p21* and cell cycle arrest at G_1 phase • ↑ apoptosis in LNCaP cells	1, 5, and 20 μM (6 h)	cell-free assay detecting HDAC 1 and 2 (Color de Lys, Biomol)
		• ↑ ac-H3 association with *p21* promoter	20 μM (24 h)	ChIP: Ac-H3
	Ma *et al.,* 2006 [236]	• ↓ *Bcl-2* protein in HL-60 cells	1, 20, 40 μM (14 h)	
		• ↓ *HDAC1* and ↑ *p300* protein level • ↓ *HDAC1/2* activities • ↑ ac-H3, ac-H4, H3K14 ac, H3K4 methylation • ↑ H3K4 methylation, ↓ H3K9 methylation • ↑ *p21*, *p27* protein levels in HL-60 cells	5, 20, 40 μM (7 h)	cell-free assay detecting HDAC 1 and 2 (Color de Lys, Biomol)
		• ↑ ac-H3 association in *p21* promoter	20, 40 μM (7 h)	ChIP: Ac-H3 (K9, K14)
		• ↑ apoptosis	0.01, 10, 20, 40 μM (5 d)	TUNEL staining in cytospin preparations No significant apoptosis in mononuclear cells from normal peripheral blood and bone marrow
	Lu *et al.,* 2008 [234]	• ↑ ac-H3	0.5, 2 μM (72 h)	
		• ↑ *p21* protein level	5 μM (24 h, 48 h)	
		• p16 DNA hypomethylation	0.5, 1, 2 μM (10 d)	MSP
		• ↓ VEGF production in RPMI8226 myeloma cells	0.1, 5, 10 μM (24 h, 48 h)	
		• Disruption of mitochondria membrane potential	5μM, 10μM, 48h	JC-1 dye staining
	Huang *et al.,* 2007 [237]	• ↑ *p300/CBP*, ac-H3, ac-H4 in Molt4 cells • ↑ H3K4 methylation, ↓ H3K9 methylation • ↑ cell cycle arrest at G_0/G_1 phase, apoptosis	N/A (only abstract)	
	Huang *et al.,* 2010 [239]	• ↑ ac-H3, ac-H4 • ↑ H3K4 methylation, ↓ H3K9 methylation • ↑ apoptosis, ↓ proliferation in SMMC-7721 cells	N/A (only abstract)	
	Xiao *et al*., 2010[238]	• significant ↑ ac-H3 and ac-H4 • ↑ apoptosis in bone marrow cells of 10 AML patients	10 μM (3 h, 7h)	
Diallyldisulfide (DADS)	Druesne-Pecollo *et al*., 2008 [244]	• transient ↑ ac-H3 and/or ac-H4 in leukemia and colon, liver, breast and prostate cancer cell lines • ↑ *p21* mRNA and protein level • ↑ cell-cycle arrest, differentiation, apoptosis	variable 200 μM – 1 mM (0.5 h – 24 h)	review article

Allylmercaptan (AM)	Nian *et al.*, 2008 [243]	• competitive inhibition of HDAC activity	24 μM	Fluor-de-Lys HDAC activity assay (Biomol) using HDAC8 purified enzyme and nuclear extracts of treated or untreated cells cells
		• ↓ HDAC activity with HT29 nuclear extract	20 μM	
		• ↑ ac-H3 and ac-H4 (10 min up to 72 h) • ↑ ac-H3 and Sp3 binding in *p21* promoter (4 h) • ↑ p53 binding in the distal enhance of *p21* (24 h) • ↑ $p21^{Waf1}$ expression and G_1 cell cycle arrest (3 h – 72 h)	0.5-2 mM	ChIP
	Nian *et al.*, 2009 [220]			review article on isothiocyanates and AM
Apicidin	Han *et al.*, 2000 [249]	• Anti-proliferative activity in various ccancer cell lines	48 h	IC_{50} values in low μg/ml range
		• ↓ DNA synthesis, G_0/G_1 cell cycle arrest		
		• altered morpology		
		• ↑ ac-H4 in HeLa cells		
		• ↓ HDAC activity		nuclear extracts, radioactive detection
		• ↑ gelsolin, *p21* protein level, in HeLa cells • ↓ *pRB* phosphorylation	0.1 – 2 μg/ml (24 h)	↔ *cyclin D1, Cdk2, HDAC1, p53*
		• ↑ ac-H4, ↓ HDAC activity, ↓ DNA synthesis, proliferation irreversible	1 μg/ml, up to 150 h after removal	in contrast, butyrate-mediated effects are reversible
	You *et al.*, 2008 [250]	• ↔ DNMT1 protein in each phase of cell cycle before treatment, ↓ DNMT1 expression at both mRNA and protein levels, ↔DNMT3a and 3b, ↑ *p21* mRNA and protein, G1 phase cell cycle arrest in HeLa cells. • ↑*p21* and ↓ DNMT1 protein in NCCIT, MCF7, and HCC1954 cells	1 μM (24 h)	Serum starvation and stimulation of cell population; analyzed with flow cytometry Pharmacological inhibitor mevinolin, which arrest cells at G_0/G_1 phase Suppression of DNMT1 by apicidine is independent of cell cycle arrest, as both apidicin and mevinolin arrest HeLa cells at G_1 phase whereas mevinolin showed no effect on DNMT1 expression.
		• ↓ Pol II recruitment in the initiation and coding region of DNMT1 promoter, but not GAPDH promoter.		ChIP: pol II, on initiation and coding regions of DNMT1 promoter
		• ↑ global Ac-H3 and Ac-H4, but ↑ hypoacetylation of H3 and H4 on the initiation site of DNMT1 promoter; • ↓ H3K4me3 at TSS, but ↑ H3K9me3 and H3K27me3 on the initiation site of DNMT1 promoter but not upstream of TSS. • ↑ binding of pRB, followed by binding of HDAC1 and dissociation of PCAF to the pRB/E2F binding site of DNMT1 promoter.		ChIP-Ac-H3, Ac-H4, pRB, E2F1, HDAC1, P/CAF, H3K4me3, H3K9me3, H3K27me3
Resveratrol	Howitz *et al.*, 2003 [264]	• ↑ catalytic activity of SIRT1	100 μM	
		• ↑ cell survival after exposure to ionizing radiation by stimulating SIRT1-dependent deacetylation of p53	0.5 μM	reversal of effect at 50 μM
		• ↑ Sir2 in yeast	2-5 μM	
		• ↑ DNA stability, extending lifespan by 70%	10 μM	
Dihydrocoumarin (DHC)	Olaharski *et al.* 2005 [272]	• ↓ Sir2p activity in yeast	500-750 μM	Gal-Sir2 phenotype yeast lethal phenotype assay
		• ↓ activities of human SIRT1 and SIRT2		Recombinant SIRT1 and SIRT2 deacetylase assay using [^{3}H]acetylated H4 peptide *SIRT1* IC_{50}: 208 μM; *SIRT2* IC_{50}: 295 μM
		• ↑ *p53* acetylation, apoptosis and senescence phenotype in human TK6 cells	1-5 mM	

Cambinol	Heltweg *et al.,* 2006 [273]	• ↓ activities of human SIRT1, 2 • ↔ activity of human SIRT5; ↔ activity against SIRT3		Deacetylase activity assay using purified GST-SIRT1, 2, 3, and 5 (SIRT 4, 6, 7 not available); [^{3}H]ac-H4 peptide substrate; detected by scintillation counting SIRT1 IC_{50}: 56 μM; SIRT2 IC_{50}: 59 μM; SIRT5 IC_{50}: >300μM
		• ↑ *ac-p53* • ↑ chemosensitization to etoposide of NCI H460 cells • ↔ on cell cycle on its own	Etoposide 100 nM Cambinol 10 μM (72 h)	
		• ↑ apoptosis in Namalwa cells	10, 20, 50 μM (48 h)	

Abbreviations: A2058, human amelanotic melanoma cell line*;* A375, human amelanotic melanoma cell line; *AR*, androgen receptor; ac-H3, acetyl histone H3; ac-H4, acetyl histone H4; *APC*, adenomatous polyposis coli; AUT, Acid-urea-triton; *Bmi-1*, BMI1 polycomb ring finger oncogene; Caco2, human epithelial colorectal adenocarcinoma cell; CCRF-CEM, leukemic lymphoblasts; CD11b, macrophages monocyte antigen; *CDK*, cyclin dependent kinase; ChIP, chromatin immunoprecipitation; *c-Myb*, protooncogene c-Myb; *c-Myc*, c-Myc transcription factor and oncogene; *CREBBP*, *CREB* binding protein; *CTCF*, CTCF zinc finger protein; DU-145, human prostate carcinoma cell line; DuPro: human prostatic carcinoma cell line; DNMT, DNA methyltransferase; *E2F1*, *E2F1* transcription factor; E-box, E-box DNA binding sequence; *ER*, estrogen receptor**;** *EGF*, epidermal growth factor; *Ezh2*, enhance of zeste polycomb protein; *Fzd*, frizzled protein; G361, human melanoma cells; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *GSTP*1, glutathione transferase π 1; H3K4me, H3K4me2, H3K4me3, histone H3 lysine 4 mono, di, tri- methylation; H3K9ac, H3K9me, H3K9me2, H3K9me3, histone H3 lysine 9 acetylation, mono, di, tri-methylation; H3K27me, H3K27me2, H3K27me3, histone H3 lysine 27 mono, di, tri-methylation; H3K18ac, histone H3 lysine 18 acetylation; H4K12ac, H4K16ac, histone H4 lysine 12 and histone H4 lysine 16 acetylation; HaCaT, human keratinocyte cell line; HAT, histone acetyl transferase; HCC2185, human breast cancer cell line; HCC712, human breast cancer cell line; HCC1954, human breast cancer celll lin; HCT-116, human colon epithelial adenocarcinoma cell line; HCK, HCK tyrosine protein kinase; HDAC, histone deacetylase; HEK293, Human Embryonic Kidney 293 cell; HeLa human cervical epithelial carcinoma cell line; HeP2, human laryngeal carcinoma; HL-60, Human promyelocytic leukemia cells; *HOXA1*, Homeobox A1; HRP, horse radish peroxidase; *HSP90*, heat shock protein 90; HT29, human colon adenocarcinoma grade II cell line; *hTERT*, human telomerase reverse transcriptase; *Id*, inhibitor of differentiation or DNA binding; LNCaP, human prostate adenocarcinoma cell line; *LSD1*, lysine specific demethylase 1; *MAD1*, Mitotic spindle assembly checkpoint protein MAD1; MCF-7, human breast adenocarcinoma cell line; MCF-10A, immortalized human breast epithelial cell line; MDA-MB-231, human breast adenocarcinoma cell line; Molt4, human acute lymphoblastic leukemia cell line; NB4, acute promyelocytic leukemia cell line; NCCIT, human teratocarcinoma cells; NCI-H460, human large-cell lung carcinoma cell line; *Notch-1*, notch transmembrane protein-1; *OS-9*, osteosarcoma amplified 9; $p16^{INK4a}$, cyclin-dependent kinase inhibitor 2A; $p21^{WAF1}$, cyclin-dependent kinase inhibitor 1A; *p27*, cyclin-dependent kinase inhibitor 1B; *p53*, tumor protein p53; *p300,* p300 protein acetyltransferase; *PCAF*, P300/CBP-associated factor; *pRB*, retinoblastoma protein; *PARP*, Poly (ADP-ribose) polymerase; PC-3, human prostate cancer epithelial cell line; Pol II, RNA polymerase II; *PSA*, prostate specific antigen; RA, retinoic acid; Raji, B-cell lymphoma cell line; RAR, retinoic acid receptor; RARE, RAR response element; RBP2, Retinol-binding protein 2; SCC13, human SCC-13 squamous cell carcinoma; SCC25, human oral squamous cell carcinoma; *SIRT*, Sirtuin 1, silent mating type information regulation 2 homolog 1; SK-BR-3, human breast carcinoma cell line; SMMC-7721, human hepatoma cells; *Suz12*, Polycomb protein SUZ12; T47D, human ductal breast epithelial tumor cell line; TK6, human B lymphoblastoid cells; TSA, trichostatin; TSS, transcription start site; VCaP, an immortalized vertebral-cancer of the prostate cell; WRE, Wnt response element

Supplementary Table 8: Chemopreventive agents targeting histone acetyltransferases (HAT) *in vitro*

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
Genistein	Majid *et al*., 2008 [160]	• ↑ expression of *p21, p16*^{INK4a} at mRNA and protein levels, • ↓ expression of cyclins A2, B2, and E2 proteins; • ↔ expression of *p27*^{CIP1} in LNCaP and DuPro cells; • Cell cycle arrest at G_0/G_1 phase in LNCaP, and G_2/M arrest in DuPro	10, 25 μM (96 h)	
		• ↑ ac-H3, and ac-H4, • ↑ H3K4me2 close to TSS of *p16* and *p21*, but no H3K9me2 detected in LNCaP, DuPro, and RWPE cells	10, 25 μM (96 h)	ChIP-Ac-H3, Ac-H4, H3K4me2, H3K9me2
		• ↑ HAT expression at mRNA level including *p300, PCAF, CREBBP, HAT1*		
Curcumin	Balasubramanyam *et al.,* 2004 [184]	• ↓ activity of *p300/CBP*, but not of *PCAF* • ↓ acetylation of H3, H4 by *p300/CBP* • ↓ *p300*-dependent *p53* acetylation		*p300* IC_{50}: 25 μM; *PCAF* IC_{50}: >>100μM
	Kang *et al.*, 2005 [185]	• ↓ ac-H3 and ac-H4; ↔ HDAC activity *in vitro*	50, 100 μM (24 h)	[^{3}H]acetate incorporation assay with crude histones acid-extracted from cellular lysates
		• ↓ crude HAT activity	20 – 100 μM (20 h)	Total HAT preparations from curcumin-treated Hep3B lysates. *p300* overexpression partially attenuates and dominant negative *p300* construct transfection partially mimics the effect of curcumin on HAT activity
Anacardic acid	Balasubramanyam *et al.*, 2003 [275]	• ↓ *p300* and *PCAF* HAT activities		HAT assays using purified *PCAF* and *p300* Inhibition kinetics using [^{3}H]acetyl CoA *p300* IC_{50}: 8.5 μM (non-competitive inhibitor) *PCAF* IC_{50}: 5.0 μM
	Sun *et al.*, 2006 [276]	• ↓ Tip60 HAT activity in HeLa and HEK293T	IC_{50} 9 μM	HeLa cell extracts
		• transient supression of Tip60-dependent activation of ATM and DNA PKcs protein kinases in 293T cells	30 μM	
		• sensitization of HeLa cells to cytotoxic effects of ionizing radiation	30-100 μM	colony formation assay
	Sung *et al*., 2008 [277]	• ↓ constitutive and inducible *NF-κB* activity in KBM-5, H1299, Jurkat, DU-145, SCC4 cells	25 μM (4 h)	induced by TNF-α and a series of other inducers
		• ↓ activation of IκBα kinase, ↓ *IκBα* phosphorylation and degradation	25 μM (4 h)	
		• ↓ acetylation and nuclear translocation of *p65*	25 μM (4 h)	abrogated by downregulation of *p300* HAT
		• ↓ expression of NF-κB-dependent anti-apoptotic, proliferation and metastasis-related proteins *IAP1, XIAP, Bcl-2, Bcl-xL, c-FLIP, cyclinD1, c-Myc, Cox-2, VEGF, ICAM-1, MMP-9* induced by TNF-α		
		• potentiation of apoptotic effects of TNF-α, cisplatin, doxorubicin		
Garcinol	Balasubramanyam *et al., 2004* [279]	• ↓ *p300* and *PCAF* HAT activities *in vitro* and in HeLa cells	100 μM (24 h)	First cell-permeable HAT inhibitor *p300* IC_{50}: 7 μM, *PCAF* IC_{50}: 5 μM, mixed type inhibitor

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
		• hyperacetylation of H4 and H2B		
		• ↑ apoptosis	30, 70, 100 μM (24 h)	DNA laddering, nuclear fragmentation
		• ↓ global gene expression in HeLa cells	100 μM (24 h)	microarray analysis
	Prasad *et al*., 2010 [280]	• potentiation of TRAIL-induced apoptosis in HCT116 cells, sensitation of TRAIL-resistent cells	15 μM (12 h)	prevented by pre-treament with N-acetylcysteine
		• ↑ death receptor 4 and 5 protein level in HCT116 and other cell lines	5-20 μM (4-48 h)	effects are abrogated by downregulation of DR4 and DR5 by siRNA
		• ↓ expression of anti-apoptotic proteins *survivin, XIAP, Bcl-2, c-FLIP$_{L/S}$* • ↑ expression of proapoptotic *Bid, Bax,* cytochrome c release		prevented by pre-treament with N-acetylcysteine
		• ↑ generation of ROS	5-20 μM (1 h)	prevention of apoptosis induction by pre-treament with N-acetylcysteine
Ursodeoxycholic acid	Akare *et al.,* 2006 [283]	• ↑ differentiation and senescence by ↓ histone acetylation in HCT116 cells • ↑ expression of *E-cadherin*, *CK8, 18, 19*	500 μM (8, 24 h)	morphological changes β-galactosidase staining as a senescence marker
		• ↓ telomerase activity		TRAP assay
		• ↑ hypoacetylation of histones • ↔ HDAC activity *in vitro*		non-isotopic Bio-Vision HDAC and HAT colorimetric kits with HCT116 whole cell lysates
		• ↔ HDAC1 protein levels; ↑ *HDAC6* mRNA		HDAC6 overexpression induces senescence

Abbreviations: ac-H3, ac-H4, acetylated histone H3 and H4; *CK8, 18, 19*, cytokeratin 8, 18, and 19; *HAT*, histone acetyl transferase; HCT116, human colon epithelial adenocarcinoma cancer cell line; Hep3B, human hepatocellular carcinoma cell line; *p300*/CBP, p300 protein acetyl transferase; *PCAF*, P300/CBP-associated factor; TRAP assay, telomerase repeat amplification protocol

Supplementary Table 9: Chemopreventive agents targeting histone methylation *in vitro*

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
Epigallocatechin gallate (EGCG)	Balasubramanian *et al.*, 2010 [147]	• ↓ protein levels of *BMI-1*, *SUZ12*, and *EZH2* in SCC-13, HaCaT, and A431 cells • ↓ total H3K27me3 and ↓ survival in SCC-13 cells • cell cycle arrest by ↓ Cdks 1, 2, 4, and cyclins D1, E, A, and B1; and ↑ *p21* and *p27* proteins • ↑ cleavage of PARP and caspases 9, 8, and 3; ↑ Bax, and ↓ Bcl-xL proteins in SCC-13 cells • *BMI*-overexpression reverses the effects of EGCG	60 µM (24 h)	
Chaetocin	Greiner *et al.*, 2005 [305]	• ↓ *SUV39* activity • ↓ H3K9me2 with human *SUV39* enzyme		Inhibitor of SUV39 histone lysine methyltransferase (preferential methylation of H3K9 methylation) IC_{50}: 0.8 µM
	Cherrier *et al.*, 2009 [286]	• ↓ H3K9me3 at the *p21* promoter • ↑ *p21* promoter activity, cell cycle arrest	75-200 nM (24 h)	CHIP
	Lakshmikuttyamma *et al*., 2010 [287]	• ↑ $p15^{INK4B}$ and *E-cadherin* re-expression without promoter demethylation in myeloid leukemia cells	50 – 200 nM (12 h)	in contrast to re-expression induced by promoter demethylation and reduced association of *SUV39* with promoter region by DNMT inhibitor 5-aza-2'-deoxycytidine
		• ↓ H3K9me2 and H3K9me3 at *p15* and *E-cadherin* promoter	100 nM (12 h)	ChIP
		• ↑ cell cycle arrest and apoptosis	25-100 nM (48 h)	mimicked by SUV39H1 downregulation by shRNA
Polyamine analogues: PG11144 (cis) PG11150 (trans)	Huang *et al.,* 2009 [288]	• ↓ *LSD1* lysine demethylase activity		*LSD*1 catalyses demethylation of mono- and di-methylated H3K4 *In vitro* LSD1 activity assay with 5 µM H3K4me2 peptide; IC_{50} ~ 5 µM for both cis/trans agents competitive inhibition kinetics against LSD1
		• ↓ proliferation of RKO and HCT-116 cells • ↑ apoptosis	2.5 -5 µM (48 h)	
		• ↑ global H3K4me and H3K4me2, but not H3K4me3 in HCT116 and RKO cells • ↑ *SFRP1* and *SFRP2* expression in HCT116 cells (at 24 h) • ↑ H3K4me, H3K4me2, but ↓ H3K9me2, H3K9ac, H4K16ac levels at *SFRP2* promoter	1, 5, 10 (24, 48 h)	ChIP
		• ↑ De-repression of *SFRP1, 2*, and *4* in HCT-116 cells • ↑ association of H3K4me and H3K4me2; ↓ H3K9me2 at promoters of *SFRP1* and *2*, ↔ H3K4me3 association • ↔ *LSD1* binding at the promoters of *SFRP1* and *2* • ↓ H3K9ac and H4K16ac at *SFRP1* promoter • slightly ↑ H3K9ac, ↔ H4K16ac at *SFRP2* promoter	10 µM (24 h)	ChIP

Agent	First author [Reference]	Activity	Concentration (Incubation Time)	Methods for epigenetic analyses – Comments
n-3 Polyun-saturated fatty acid (n-3 PUFA) DHA, EPA	Dimri *et al*., 2010 [291]	• ↓ *EZH2* protein level but not mRNA level in MCF7, MDAMB231, T47D cells • ↓ H3K27me3 and H3K9me3 • ↑ *E-cadherin* and *IGFBP3* • ↓ invasion ability	80 μM (3 – 8 h)	• Cell culture was 24 h starved in 0.5% serum medium Matrigel invasion assay

Abbreviations: *BMI,* B-cell-specific Moloney murine leukemia virus integration site 1 (histone methyltransferase); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; *EZH2*, Enhancer of Zeste 2 (histone methyltransferase); HCT-116, human colon epithelial adenocarcinoma cell line; HKMT, histone lysine methyltransferase; H3K9ac, histone H3 lysine 9 acetylation; H4K16ac, histone H4 lysine 16 acetylation; H3K4me, H3K4me2, histone H3 lysine 4 mono-, and di- methylation; H3K9me, H3K9me2, H3K9me3, histone H3 lysine 9 mono-, di-, and tri-methylation; H3K27me3, histone H3 lysine 27 trimethylation; *IGFBP3*, insulin growth factor binding protein 3; *LSD1*, lysine specific demethylase 1 (histone lysine demethylase); MDA-MB-231, human breast adenocarcinoma cell line; T47D, human ductal breast epithelial tumor cell line; MCF7, human breast adenocarcinoma cell line; RKO, human colon carcinoma cell line; *SUV39*, histone methyl transferase SUV39; *SFRP1* and 2, secreted frizzled related protein 1 and 2

Supplementary Table 10: Chemopreventive effects of histone modifying agents *in vivo*

Agent	References	Target site	System/carcinogen	Concentration/Dose	Results – Comments - Methods
Sulforaphane (SFN)	Myzak *et al.*, 2006 [227]	colon mucosa	wt mice	SFN and SFN-NAC, 10 µmol /animal as a single dose by gavage (6 h)	• ↓ HDAC activity in colon mucosa at 6 h (Fluor-de-Lys HDAC activity assay, Biomol) • SFN and SFN-NAC have similar extents of HDAC inhibition • ↑ Ac-H3 and Ac-H4 in SFN-NAC group
				SFN, 10 µmol /animal as a single dose by gavage (24, 48 h)	• transient ↑ ac-H3 and ac-H4 at 6 h and 24 h • ↑ p21 at 24 and 48 h
		ileum, colon, prostate, and PBMC	wt mice	~ 6 µmol SFN/day (10 wk)	• ↑ acetylated histones and ↑ $p21^{WAF1}$ expression • ↓ HDAC activity in prostates; • ↑ global histone acetylation and local histone acetylation at p21 and Bax promoters. • ChIP for Ac-H3, Ac-H4 binding to *p21* and Bax promoter (polyp samples)
		ileum, colon	$APC^{Min/+}$ mice	~ 6 µmol SFN/day (10 wk)	• ↓ tumour multiplicity in $APC^{Min/+}$ mice • ↑ ac-H3 in promoters of p21 and bax; ↑ Bax protein expression in ileum polyps (ChIP)
Sulforaphane (SFN)	Myzak *et al*., 2007 [228]	prostate	Xenografts	7.5 µmol/animal (21 d)	• ↓ growth of PC3 xenograft (40%); ↑ global acetylation • ↓ HDAC activities in xenografts, prostates, and MBCs • Non-significant ↑ Ac-H3 and Ac-H4 in PC3 xenografts, but is signififcant in prostates; significant ↑ Bax expression in PC3 xenografts and prostates • Fluor-de-Lys HDAC activity assay (Biomol)
Broccoli sprouts	Dashwood *et al.,* 2007 [226]	human PBMC		Single dose, 68 g broccoli sprouts (~105 mg SFN or ~570 g mature broccoli); PBMC collected at 0, 3, 6, 24, and 48 hr	• transient ↓ HDAC activity and ↑ ac-H3 and ac-H4 in PBMC at 3 - 6 h, but returned to normal at 24 and 48 h
Diallyl disulfide (DADS)	Druesne-Pecollo *et al*., 2007 [245]	Colon	Rat	200 mg/kg, gavage or intracaecal perfusion	• ↑ transient ac-H4 (total and at specific lysine residues)
OSU-HDAC42	Sargent *et al.,* 2008 [332]	Prostate	TRAMP mice	25 mg/kg/day, diet	• ↓ progression of prostate cancer • ↓ progression to poorly-differentiated carcinoma (24 wk) • ↑ ac-H3, E-cadherin and *p21*; ↓ synaptophysin • reversible hematological alterations and testicular degeneration • ↔ body weight in drug-treated mice
			PC3 xenograft	25 mg/kg/day, diet	• ↓ Ki67, ↑ ac-H3, ↑ caspase 3 cleavage
Cambinol	Heltweg *et al*., 2006 [273]	Burkett lymphoma xenograft	Daudi Burkitt lymphoma xenograft	100 mg/kg/ iv or i.p. daily for 2 wks	• ↓ tumor growth
Polyamine analogues PG11144 (cis) PG11150 (trans)	Huang *et al*., 2009 [288]	Colon	HCT116 xenograft	Single agent: PG11144 10 mg/kg, i.p., twice/wk for 3 wks Combination: PG11144 10 mg/kg,	• ↑ H3K4me2 in xenografts • ↓ tumor growth inhibition by single agent treatment with either PG11144 or 5-Azacytidine; the combination treatment almost completely inhibits tumor growth

Agent	References	Target site	System/carcinogen	Concentration/Dose	Results – Comments - Methods
				i.p., twice/wk; plus 5-Aza 2 mg/kg, i.p., 5x per wks	

Abbreviations: ac-H3 and ac-H4, pan acetylation of histone H3 and H4; *APC*, adenomatous polyposis coli; *Bax*, *Bcl-2* associated protein X; *Bcl-2*, B-cell lymphoma 2; ChIP, chromatin immunoprecipitation; HDAC; histone deacetylase; i.p., intraperitoneal injection; i.v. intravenous injection; Ki67, Ki67 cancer antigen protein; MBC: mononuclear blood cell; $p21^{WAF1}$, cyclin-dependent kinase inhibitor 1A; *p53*, tumor protein p53; PBMC, peripheral blood mononuclear cell; PC3, human prostate cancer epithelial cell line; PCNA, proliferating cell nuclear antigen; *TIMP-1, 2* metallopeptidase inhibitor 1, and 2; *uPA*, urokinase, plasminogen activator

Supplementary Table 11: Chemopreventive agents targeting miRNAs *in vitro* and *in vivo*

Agent	First author [Reference]	up-regulated miRNAs/targets	down-regulated miRNAs/targets	Methods – Comments
Folate	Kutay *et al*., 2006 [86]	*let-7a-2, miR-101b-2, miR-103-2, miR-106, miR-106a-1, miR-106b-1, miR-130, miR-130a, miR-130a-1, miR-17, miR-172a-2, miR-20, miR-20-1, miR-21, miR-21-1, miR-219-1, miR-23a, miR-23b, miR-24, miR-320-2, miR-328-1, miR-93, miR-99b* • verification of *let-7a, miR-21, miR- 23, miR-130, miR-190, miR-17-92*	*miR-122, miR-123, miR-125b-1, miR-125b-2, miR-192-1, miR-192-2, miR-215, miR-26a-1, miR-26a-2, miR-26a* • verification of *miR-122* in rat liver hepatocellular carcinoma and in human primary hepatocellular carcinoma	• microarray with 245 human and mouse miRNAS • male Fisher rats-fed folic acid, methionine, and choline-deficient (FMD) diet for up to 54 wks, develop hepatocellular carcinoma
	Marsit *et al*., 2006 [87]	*miR-183, miR-191, , miR-205, , miR-22, miR-221, miR-222, miR-24, miR-345, miR-34a, miR-361, miR-422b, miR-99a* • *miR-222:* upregulation confirmed in human peripheral blood from individuals with low folate intake	*miR-198, miR-210*	• microarrays of 385 known human miRNAs • human lymphoblastoid cells under folate-deficient growth conditions • levels return to normal in complete medium
NaSelenite	Sarveswaran *et al,*. 2010 [97]	*miR-34b*, *miR-34c*, but not *miR-34a*		• 2.5 μM selenite in LNCaP cells • ↑ apoptosis • ↑ total p53 and p-p53, • ↑ p21, Bax, DR5 as p53 target genes • all effects abrogated by p53 siRNA
Retinoic acid	Garzon *et al*., 2007 [106]	*miR-15a, miR-15b, miR-16-1, miR-223, miR-342, miR-107, let-7a-3, -7c, -7d* • *miR-107* target *NFI-A* confirmed • essential proximal NF-κB binding site identified for *let-7a-3/let-7b* cluster transactivation	*miR-181b*	• miRNA microarrays • NB4 cells treated with 100 nM RA for 4 days
		•	•	•
	Rossi *et al*., 2010 [107]	• mir-215, mir-223, mir-186 • 34 long ncRNAs (several verified by qRT-PCR)	• mir-017, mir-193, mir-195, mir-025, let-7a-1 • 24 long ncRNAs (several verified by qRT-PCR)	• microarray analysis of 243 miRNAs and 492 human genes transcribing for putative long ncRNAs • NB4 cells differentiated with 0.5 μM RA
Vit. E	Gaedicke et al., 2008 [117]		miR122a and miR-125b in rat liver, in comparison with animals on control diet	• male rates kept on Vit. E-deficient diet for 6 month
NaButyrate	Chen *et al*., 2008 [127] (abstract only)	17 miRNA up-regulated at day 6 and 9, compared to day 0	• 22 miRNAs downregulated at day 6 • 27 miRNAs downregulated at day 9	• Microarray analysis • embryonic stem cells induced to differentiate by NaButyrate for up to 9 days • 15 differntially expressed miRNAs keep contact with HDACs
EGCG	Tsang *et al*., 2010 [148]	*let-7a, let-7b, let-7c, let-7d, miR-16, miR-18b, miR-20a, miR-25, miR-92, miR-93, miR-221, miR-320, miR-377* • verified by qRT-PCR: *let-7a, miR-16, miR-221* • *miR-16*-mediated downregulation of Bcl-2 confirmed by precursor and inhibitor transfection	*miR-10a, miR-18a, miR-19a, miR-26b, miR-29b, miR-34b, miR-98, miR-99b, miR-129, miR-138, miR-181d, miR-182, miR-186, miR-193b, miR-196a, miR-196b, miR-199a, miR-200a, miR-205, miR-210, miR-217, miR-222, miR-302b, miR-302c, miR-335, miR-342, miR-361, miR-373, miR-376a, miR-409, miR-422, miR-423, miR-425, miR-450, miR-*	• miRNA microarray analysis with 328 miRNAs • HepG2 cells treated for 24 h with 100 μM EGCG

Agent	First author [Reference]	up-regulated miRNAs/targets	down-regulated miRNAs/targets	Methods – Comments
			484, miR-491, miR-494, miR-497, miR-505, miR-507, miR-516, miR-517c, miR-518a, miR-518c, miR-519, miR-522, miR-524, miR-526 • verified: *miR-18a, miR-34b, miR-193b, miR-222, miT-342*	
Genistein, Soy Isofalvones	Parker *et al*., 2009 [169]	• UL3A cells: *miR-122a, miR-137, miR-196a, miR-204, miR-206, miR-217, miR-331, miR-449b, miR-454, miR-501, miR-515,* and *miR-578* • UL3B cells: *miR-517c, and miR-7* • both UL-3A and 3B: *miR-135* and *miR-765*		• microarray with 467 miRNAs • UL-3A, UL-3B ovarian cancer cell lines incubated with 5 μM genistein for 48 h
	Li *et al.,* 2009 [170]	• up-regulation of *let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, miR-200b, miR-200c* by isoflavone treatment, which are down-regulated in resistant vs. sensitive cell lines • *miR-200b, miR-200c* involved in EMT regulation • up-regulation of *miR-200* target *E-cadherin* mRNA	• downregulation of slug (transcription factor involved in EMT	• μParaFlo microfluidic chips with 711 miRNAs • gemcitabine-resistant human pancreatic cancer cell lines • treatment with 25 μM isoflavones for 48 h
	Sun *et al*., 2009[172]		• *miR-27a* with concomitant weak up-regulation of target ZBTB10	• C918 human uveal melanoma cell treated with genistein at 25-200 μM • inhibition of xenograft growth by intervention with 25, 50, 100 mg/kg b.w.
	Li *et al*., 2010 [197]	• re-expression of *miR-146a* by isoflavone treatment inhibits invasive capacity, with concomitant downregulation of EGFR, IRAK-1, NF-κB, MTA-2	• ↓ *miR-146* in pancreatic cancer cells compared to normal pancreatic duct cells	• Colo357 and Panc-1 • treatment with 25 μM isoflavone mixture
	Majid *et al*., 2010[171]	• up-regulation of *miR-1296* by genistein treatment with concomitant down-regulation of MCM genes and *CDK2, CDK7, CDT1*	• ↓ *miR-1296* in prostate cancer	• LNCaP, PC3 cells treated with 25 and 50 μM genistein alone and in combination with TSA
Curcumin	Sun *et al*., 2008 [189]	*miR-19a, miR-20a, miR-23a, miR-23b, miR-25, miR-26a, miR-27a, miR-92, miR-93, miR-98, miR-103, miR-181a, miR-181b, miR-181d, miR-204, miR-374, miR-510* • verified upregulation of *miR-22* with concomitant downregulation of targets ERα and transcription factor Sp1 • confirmation by transfection with sense and anti-sense *miR-22* oligonucleotide	*miR-7, miR-15b, miR-21, miR-22, miR-24, miR-34a, miR-140, miR-146b, miR-148a, miR-195, miR-196a, miR-199a** • verified downregulation of *miR-196*	• miRNA microarrays based on uParaflow microfluidic technology • BxPC-3 pancreatic cancer cells treated with 10 μM curcumin for 72 h
	Ali *et al*., 2010 [190]	• miR-200b, miR-200c	• *miR-21* with concomitant induction of PTEN	• BxPC-3 and MIAPaCa-E pancreatic cancer cells treated with 4 μM curcumin for 72 h
	Zhang *et al*., 2010 [191]	• 4 miRNAs upregulated	• *miR186** • transfection with *miR-186* inhibitor induces apoptosis • overexpression of *miR-186** protects from curcumin-induced apoptosis	• Microarray analysis with 342 human miRNAs • A549/DDP cells treated with 15 μM curcumin for 48 h, resulting in induction of apoptosis

Agent	First author [Reference]	up-regulated miRNAs/targets	down-regulated miRNAs/targets	Methods – Comments
Ellagitannin	Wen *et al*., 2009 [193]	*let-7e, miR-194, miR-302a, miR-346, miR-373, miR-370, miR-424, miR-433, miR-452, miR-510, miR-512-5p, miR-513, miR-518c, miR-518f-526a, miR-519e, miR-525, miR-526b* • verified by qRT-PCR*: let-7e, miR-370, miR-373*, mir-526b*	*let-7a, let-7c, let-7d, let-7f, let-7i, miR-542-3p, miR-299-3p, miR-200a** • verified by qRT-PCR: *let-7a, let-7c, let-7d*	• m´miRCURY LNA miRNA Array with probes for 452 human miRNAs • HepG2 cells treated with 50 µg/ml ellagitannin for 6 -24 h
Diindolyl methane (DIM)	Li *et al.,* 2009 [170]	• re-expression of *let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, miR-200b, miR-200c* by DIM treatment, which are down-regulated in resistant vs. sensitive cell lines • *miR-200b, miR-200c* involved in EMT regulation • up-regulation of *miR-200* target *E-cadherin* mRNA	• downregulation of *slug* (transcription factor involved in EMT) and *ZEB1*	• µParaFlo microfluidic chips with 711 miRNAs • gemcitabine-resistant human pancreatic cancer cell lines • treatment with 25 µM DIM for 48 h
Indole-3-carbinole (I3C)	Izzotti *et al*., 2010 [232]	significantly up-regulated by intervention in rat lung in comparison with ECS-treated animals: *let-7b, miR-10a, miR-26a, miR-30c, miR-34b, miR-99b, miR-122a, miR-123-prec, miR-124a-prec, miR-125a-prec, miR-222-prec*	down-regulated in rat lung by ECS: *let-7a, let-7b, let-7c, let-7f, miR-10a, miR-26a, miR-30a, miR-30c, miR-34b, miR-34c, miR-99b, miR-122a, miR-123-prec, miR-, 24a-prec, miR-125a-prec, miR-125b, iR-140s, miR-145-prec, miR-146-prec, miR-191-prec, miR-192, miR-219-prec, miR-222-prec, miR-, 223-prec*	• microarray with probes for 484 rodent miRNAs • treatment of rats with I3C: 2500 mg/kg diet; 25 g diet/animal/day • pre-treatment for 3 days before exposure to ECS for 4 weeks • miRNA analysis in lung tissue
	Li *et al.*, 2010 [197]	• re-expression of *miR-146a* by DIM treatment inhibits invasive capacity, with concomitant downregulation of *EGFR, IRAK-1, NF-κB, MTA-2*	• ↓ *miR-146* in pancreatic cancer cells compared to normal pancreatic duct cells	• Colo357 and Panc-1 • treatment with 25 µM DIM for 48 h
PEITC	Izzotti *et al*., 2010 [232]	significantly up-regulated by intervention in rat lung in comparison with ECS-treated animals *let-7a, let-7b, let-7c, let-7f, miR-10a, miR-26a, miR-30c, miR-34b, miR-99b, miR-122a, miR-123-prec, miR-124a-prec, miR-125a-prec, miR-125b, miR-140s, miR-145-prec, miR-146-prec, miR-191-prec, miR-192, miR-222-prec, miR-223-prec* • enhanced inducing potential in combination with I3C	down-regulated in rat lung by ECS *let-7a, let-7b, let-7c, let-7f, miR-10a, miR-26a, miR-30a, miR-30c, miR-34b, miR-34c, miR-99b, miR-122a, miR-123-prec, miR-, 24a-prec, miR-125a-prec, miR-125b, iR-140s, miR-145-prec, miR-146-prec, miR-191-prec, miR-192, miR-219-prec, miR-222-prec, miR-, 223-prec*	• microarray with probes for 484 rodent miRNAs • treatment of rats with PEITC: 500 mg/kg diet; 19.2 g diet/animal/day • pre-treatment for 3 days before exposure to ECS for 4 weeks • miRNA analysis in lung tissue
	Izzotti *et al*., 2010 [233]	PEITC/sham liver: *miR-34c, miR-299, miR-452* PEITC/ECS in comparison with ECS lung: *let-7a, let-7c, mir-26a, miR-125b* liver: *miR-297a, miR-297b, miR-466b, miR-466f, miR-467a, miR-467d, miR-467e*	PEITC/sham lung: *miR-181, miR-466a, miR-666, miR-706, miR-708* liver: *miR-26a, miR-125a, miR-142, miR-200b, miR-323, miR-331, miR-338, miR-466a, miR-551* PEITC/ECS in comparison with ECS lung: *miR-29b, miR-31, miR-135b, miR-200b, miR-382* liver: *miR-153, miR-292, miR-322, miR-323, miR-376b, miR-463, miR-470, miR-687, miR-697, miR-719, miR-874*	• microarray with probes for 576 mouse miRNAs • exposure to ECS or sham (no exposure) started within 12 h after birth • treatment of mice with PEITC: 1000 mg/kg diet after weaning • miRNA analysis in lung and liver tissue
MithramycinA	Bianchi *et al*., 2009 [248]	• *miR-210,* correlates with K562 differentiation concomitant with α- and γ-globin mRNA induction • upregulation of *miR-210* in normal		• miRNA micro-array analysis with 470 probes for human miRNA • K562 cells induced to differentiate with mithramycin

Agent	First author [Reference]	up-regulated miRNAs/targets	down-regulated miRNAs/targets	Methods – Comments
		erythroid precursor cells γ-globin mRNA induction		(20-40 nM) for up to 7 days • normal erythroid precursor cells
Resveratrol	Tili *et al*., 2010 [270]	*miR-1, miR-30c-1, miR-146b-5p, miR-194-2, miR-206, miR-323, miR-340, miR-363-5p, miR-494, miR-497, miR-560, miR-565, miR-572, miR-574, miR-615, miR-622, miR-638, miR-639, miR-663, miR-801* • ↑ *miR-663*, with concomitant downregulation of *TGFβ1* • ↑ *TGFβR1, TGFβR2* • ↑ *PTEN* • ↑ *E-cadherin, SMAD7*	*miR-16-1, miR-17, miR-21, miR-23a, miR-23b, miR-25, miR-26a, miR-29c, miR-30d, miR-30a-3p, miR-30e-5p, miR-92a-2, miR-100-1/2, miR-102, miR-103-1, miR-103-2, miR-146a, miR-181a2, miR-196a1, miR-205, miR-340, miR-424, miR-565, miR-594, miR-629, miR-631, miR-657, miR-659* • target genes include *Dicer1, PDCD4, PTEN*, effectors of the *TGFβ* signaling pathway • *SMAD2/3/4* promoter activity	• miRNA micro-array analysis • SW480 cells treated with 50 μM resveratrol for 14 h
	Tile *et al*., 2010 [271]	*miR-663*	*miR-155* ↓ AP-1 activity and the levels of *JunB* and *JunD*; ↓ LPS signaling	• THP-1 cells treated with 50 μM resveratrol for 14 h
n-3 PUFA	Davidson *et al*., 2009 [292]	tumor vs. normal mucosa: *miR-132, miR-224, miR-34a, miR-223, miR-146b, miR-335, miR-218, miR-1, miR-146a, miR-99a, miR-10b, miR-100, miR-142-3p, miR-126, miR-214, miR-451, miR-125b, miR-34c, miR-199a, miR-193a, miR-142-5p, miR-497, miR-365, miR-199b, miR-195, miR-21, miR-650* fish-oil diet in comparison to tumor control: *let-7d, miR-15b, miR-107, miR-191, miR-324-5p* • upregulation of *PTEN* upon transfection of HCT-116 with anti-*miR-21*	tumor vs. normal mucosa: *miR-32, miR-181c, miR-148a, miR-204, miR-429, miR-182, miR-324-3p, miR-425, miR-96, miR-205, miR-200a, miR-200c, miR-107, miR-190, miR-141, miR-192, miR-375, miR-194, miR-215* • verification of *miR-107* downregulation in HCT-116 by anti-*miR-107* transfection	• microarray analysis of 368 mature miRNAs using TaqMan Human MicroRNA Panel Low-Density Array • male rats on n-3 (fish-oil) or n-6 (corn-oil) diet, combined with two types of fibre (pectin or cellulose) • treatment with AOM to induce colon cancer

Abbreviations: BxPC-3, human pancreatic adenocarcinoma cell line; C918, human uveal melanoma cells; CDK2, CDK7, cyclin-dependent kinase 2, 7; CDT1, chromatin licensing and DNA replication factor 1; Colo357, Human Pancreatic Adenosquamous Carcinoma cell line; DR5, death receptor 5; ECS: environmental cigarette smoke; EGFR, Epidermal growth factor receptor 5; EMT, epithelial-mesenchymal-transition; HepG2, human hepatoma cell line; IRAK-1, Interleukin-1 receptor-associated kinase 1; K562, human erythromyeloblastoid leukemia cell line; LNCaP, androgen-sensitive human prostate adenocarcinoma cells; MCM, minichromosome maintenance gene family; MIAPaCa-E, human pancreatic carcinoma cell line; MTA-2, metastasis associated protein 2; NB4, human promyelocytic leukemia cell line; NF-1A, nuclear factor 1; NF-κB, nuclear factor κB; PC-3, prostate cancer cell line; Panc-1, pancreatic carcinoma cells; PTEN, Phosphatase and Tensin homolog; SMAD, Sma and Mad related proteins; SW480, human colon adenocarcinoma cell line; TGFβR1, transforming growth factor β receptor 1; THP-1, human acute monocytic leukemia cell line; UL3A, UL3B, ovarian cancer cell lines; ZBTB10, zinc finger and BTB domain containing 10.