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# Lung cancer
## A modified epigenome

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**Key words:** epigenetic, lung cancer, DNA methylation, histone modification

**Abbreviations:** NSCLC, non small cell lung cancer; CDKi, cyclin-dependent-kinase inhibitor; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; DNMT, DNA methyltransferase; miRNA, microRNA

Epigenetic is the study of heritable changes in gene expression that occur without changes in DNA sequence. This process is important for gene expression and genome stability and its disruption is now thought to play a key role in the onset and progression of numerous tumor types. The most studied epigenetic phenomena includes post-translational modifications in DNA and histone proteins as well as microRNAs expression. As epigenetic aberrations are potentially reversible, their correction has emerged as a potential strategy for the treatment of cancer. This review highlights the roles of chromatin epigenetic modifications and of microRNAs expression in lung tumorigenesis and discusses the emerging epigenetic therapies which are being developed for the treatment of lung cancer.

Cancers are traditionally viewed as genetic disorder. However it has become increasingly evident that aberrant epigenetic modifications also play major roles in the tumorigenic process. Epigenetic defines heritable and reversible modifications of gene expression without changes in the nucleotidic sequence. The three most studied epigenetic phenomena are (1) DNA methylation, (2) chromatin modifications (including post-translational modifications of histones and chromatin modifying complexes) and (3) non-coding RNAs mediated regulations. Epigenetic processes are finely tuned, undergo many regulations in response to environment and involve all the signaling pathways described so far. Epigenetic plays a crucial role in the control of nuclear architecture and gene activity and constitute one of the basis of the biological diversity. In this review, we focus on the growing number of publications describing the huge aberration of the epigenetic landscape in lung cancer cells (**Table 1**). Additionally, we outline advances in the potential use of these epigenetic events for cancer diagnostic, prognostic and targeted epigenetic therapy.

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### DNA Methylation and Lung Cancer

DNA methylation is the best known and the most widely studied epigenetic modification. Methylation is the only covalent DNA modification described in mammals and is restricted to cytosines which are followed by guanines residues, a motif called CpG dinucleotide.[1] While CpG dinucleotides seem depleted from the vast majority of the genome, they are found enriched in regions known as "CpG islands." Typically, CpG islands are genomic regions of at least 200 bp and up to several Kb in length characterized by a high GC percentage. CpG islands are mainly found near or at the transcription start site within the promoter of ~40% of mammalian genes. CpG islands play a major role in the process of transcriptional regulation, the unmethylated status of a CpG-island correlating with the ability of a gene to be transcribed in the presence of the required co-regulators. Most CpG islands are usually unmethylated in normal cells allowing gene transcription. In contrast, CpG sites are usually methylated throughout the genome. Methylation is a normal physiological function in the cell, involved in embryonic development,[2] genomic imprinting[3] and chromosome-X inactivation.[4] In cancer, frequent alterations in DNA methylation are observed and include: (1) locus-specific hypermethylation (at CpG island) which often occurs at tumor suppressor gene loci and leads to the loss of their expression, (2) genome-wide hypomethylation mainly found in the body of genes and in DNA repetitive sequences leading to genomic instability, and (3) altered DNA methyltransferases (DNMTs) expression.

In human lung cancer, promoter DNA hypermethylation is involved in the silencing of various tumor suppressor genes. The best studied example is the case of the CDKi $p16^{INK4a}$ (CDKN2A), which promoter hypermethylation prevents the negative control exerted by $p16^{INK4a}$ on RB phosphorylation thereby promoting cell cycle progression. $p16^{INK4a}$ hypermethylation is considered as one of the earliest event in lung tumorigenesis and increases constantly with disease progression.[5,6] Other examples include H-CADHERIN (CDH13),[7] 14-3-3σ,[8] DEATH ASSOCIATED PROTEIN KINASE 1 (DAPK1),[9] RAS ASSOCIATION DOMAIN FAMILY 1 gene (RASSF1A),[10] CASPASE-8,[11] RETINOIC ACID RECEPTOR β-2 (RAR-β), TISSUE INHIBITOR of METALLOPROTEINASE 3 (TIMP3),

**Table 1.** Major epigenetic changes in lung cancer

| Epigenetic changes | Targets | Cancer type | Ref | Proposed or effective therapy |
|---|---|---|---|---|
| **DNA methylation** | | | | |
| CpG islands hypermethylation | $p16^{INK4a}$ | NSCLC | 5, 6, 36, 37–39 | |
| | CDH13 | NSCLC | 7, 39 | |
| | 14-3-3s | SCLC, NSCLC | 8 | Demethylating agents (41) |
| | DAPK1 | SCLC, NSCLC | 5, 9, 38 | |
| | RASSF1A | SCLC, NSCLC | 10, 39 | DNA methyltranferase inhibitors |
| | Caspase8 | SCLC, NSCLC | 11 | |
| | RAR-b | NSCLC | 12 | Demethylated agents in combination with HDACi |
| | TIMP3 | NSCLC | 12 | |
| | MGMT | NSCLC | 12, 36, 37 | |
| | ECAD | NSCLC | 12 | Demethylated agents in combination with chemotherapy |
| | GSTP1 | NSCLC | 12 | |
| | APC | NSCLC | 38, 39 | |
| | FHIT | NSCLC | 38 | |
| Genome wide hypomethylation | | NSCLC | 21–24 | |
| **Chromatin modifications** | | | | |
| Histone modification | | | | |
| Acetylation | H2AK5ac | NSCLC | 54 | HDACi (56, 60) |
| | H3K9ac | NSCLC | 54 | |
| | H3K18ac | NSCLC | 42 | HDACi in combination with ionizing radiation (57) |
| | H4K5ac | NSCLC | 44 | |
| | H4K8ac | NSCLC | 44 | |
| | H4K12ac | NSCLC | 44 | HDACi in combination with Chemotherapy or radiotherapy (56, 58–60) |
| | K4K16ac | NSCLC | 44 | |
| Methylation | H3K4me2 | NSCLC | 42, 54 | |
| | H4K20me3 | NCSLC | 44 | |
| Chromatin remodelling complexes | | | | |
| | BRG1 | NSCLC | 63–67 | |
| **Micro-RNAs** | | | | |
| Reduced expression | Let7 | NSCLC | 72 | Chromatin modifying drugs (HDACi…..) |
| | miR-128b | NSCLC | 75 | |
| Overexpression | miR-17-92 | NSCLC | 70, 76 | synthetic oligonucleotides |
| | miR-155 | NSCLC | 71 | anti-miRNA oligonucleotides (antagomirs) |
| | miR-21 | NSCLC | 92 | |

$O^6$-METHYLGUANINE DNA METHYLTRANSFERASE (MGMT), E-CADHERIN (ECAD) and GLUTHATIONE S-TRANSFERASE P1 (GSTP1).[12] As those genes are involved in a broad range of biological processes, promoter DNA hypermethylation appears as a key event in lung carcinogenesis. Consistently, genome wide analyses have pointed to the fact that the extend of promoter DNA hypermethylation is probably under-appreciated.[13-16] By using a high-throughput global expression profiling approach, Shames et al. recently identified 132 genes that are methylated with high penetrance in lung cancer cells.[16] More strikingly, the analysis performed by Brena et al. supports the notion that 4.8% of all CpG island promoters might be aberrantly methylated, suggesting that the expression of about 1,400 genes might be disturbed in lung cancer.[13] Overexpression of DNA methyltranferases DNMT1, DNMT3A and DNMT3B has been described in NSCLC especially among smoker patients, and correlates with hypermethylation of tumor suppressor gene such as $p16^{INK4a}$, FHIT and RARβ.[17,18] Furthermore, polymorphisms that influence expression of the DNMT3B gene have been connected with risk of lung cancer.[19,20] Genome wide hypomethylation may also have a role in the onset and development of lung cancer. Global DNA hypomethylation is highly associated with the progression of lung tumors and is already detected in the normal part of the lung from cancer patient as compared to a normal individual.[21] Hypomethylation status within exons 5–8 of p53 from peripheral blood DNA has been reported as a relevant predictor of lung cancer among male smokers.[22] Furthermore,

extensive DNA hypomethylation at repetitive sequences has also been observed in lung tumors.[23,24]

Accumulating evidence argues that epigenetic gene silencing through DNA hypermethylation can actually predispose to mutational events.[25] Because of its spontaneous hydrolytic deamination under physiological conditions, methylated cytosine is considered as a potent endogenous mutagen. Whereas methylated cytosine represents only 1% of the bases in the mammalian genome, it is estimated that it might be at the origin of as much as 30% of all transition mutations found in human disease such as cancers.[26,27] Methylation of CpG sites has been reported to facilitate the binding for benzo[a]pyrene found in cigarette smoke, leading to the formation of major DNA damage hotspots in human lung cancer.[28,29] This has been particularly well-illustrated for the occurrence of some hotspot mutations of the p53 tumor suppressor gene in lung tumors.[30,31] Other studies have shown that silencing through promoter hypermethylation of DNA repair genes such as MGMT may predispose to mutation of key genes such as p53 and K-RAS.[32-34] Although this has not been demonstrated in lung cancer it can be envisaged that such mechanism could exist as silencing of MGMT has been associated with p53 mutation in these tumors.[35]

The development of squamous cell carcinoma can be predicted by $p16^{INK4a}$ and MGMT promoter methylation up to three years before clinical diagnosis[36,37] and DNA methylation may serve as a marker for the early detection of lung cancer when detected in the sputum of the patient.[5,38] In a multivariate model, Brock et al. have recently shown that promoter methylation of CDKN2A, CDH13, RASSF1A and APC is associated with early recurrence in patients with stage I NSCLC.[39] Altogether, theses studies highlight promoter methylation as a promisive epigenetic approach for early detection and prognosis of NSCLC. In addition, demethylating drugs have a great and promising clinical potential as their use restores the expression of epigenetic silenced genes and inhibits tumor cell growth.[40] They induce manageable short-term side effects at doses showing therapeutic efficacy[40] although their long-term effects remain to be fully evaluated. The 5-aza-2'-deoxycytidine demethylating agent has been reported to increase the survival of NSCLC patients in the absence of prior chemotherapy, up to 6 years in some cases.[41] Current investigations are aimed at combining epigenetic therapies (i.e., hypomethylating agents with histone deacetylase inhibitors) and at attempting to integrate epigenetic therapy with more standart therapy.

## Chromatin Modifications in Lung Cancer

The fundamental unit of chromatin is called the nucleosome. This specialized regulatory structure consists of 147 bp of genomic DNA wrapped around an histone octamere composed of two copies of each of the core histones H2A, H2B, H3 and H4. Each of these core histone possesses a globular domain as well as an amino-terminal tail which protrudes outside of the nucleosome and in that way is accessible to numerous nuclear regulators. Amino-terminal tails of histone proteins are subjected to wide variety of post-translational covalent modifications such as acetylation, methylation, phosphorylation and ubiquitinylation. These modifications can either favor (e.g., acetylation, methylation) or inhibit (e.g., methylation) the access to the chromatin and are involved in all DNA-based processes described so far. Clearly, global alterations of histone modification patterns have the potential to affect the structure and integrity of the genome and to disrupt normal patterns of gene expression. Several protein complexes involved in transcription regulation function by modifying histones or altering chromatin structure. They are mainly represented by Histones AcetylTransferases (HATs)/Histones Deacetylases (HDACs) and Histones methyltransferases (HMTs)/Histones Demethylases (DHMTs) complexes that determine the level of acetylation and methylation respectively of the amino-terminal domains of nucleosomal histones associated with them, and by ATP-dependent complexes such as SWI/SNF which use the energy of ATP hydrolysis to locally disrupt or alter the association of histones with DNA. The last years have supported the notion that histones modifications may contribute to tumorigenesis[42,43] and have shown an altered expression pattern of histone and chromatin modifying enzymes in human tumors.

**Histones modifications.** Recently, we demonstrated the existence of a global post-translational modifications profile of histone H4 in a comprehensive panel of normal lung tissue and primary lung tumors. In this study, cancer cells exhibited a gain of H4K5ac and H4K8ac and a loss of H4K12ac, H4K16ac and H4K20me3.[44] Interestingly, loss of H4K20me3 already occurs in early precursors lesions of squamous cell carcinoma and increases with disease progression. The finding that these changes occurs so soon in the course of lung tumorigenesis indicate that they might be relevant steps in the transformation process. Some clues as to how these cancer specific histone modifications arise are emerging. The demonstration that loss of H4K20me3 correlates with decreased expression of the histone methyltransferase SUV4-20H2,[44] fits well with a recent study showing that Suv4-20 h-double-null mice have lost nearly all H4K20me3 and H4K20me2 states.[45] Similarly, reduced mRNA levels of the MYST Tip60 HAT[46] could explain the hypoacetylation of Histone H4K16, as this enzyme mediates the H4K16 acetylation.[47,48] Aberrant expression of other histone modifying enzymes has also been reported to discriminate lung tumor samples from their normal counterparts. For instance, mutations and deletions of the CBP HAT gene,[49] variable levels of HDAC1-10[50,51] or overexpression of the MAPJD and JMJD2C HDMs have been described.[52,53] Whether such aberrant expression patterns could correlate with specific histone modifications remains to be determined.

Analysis of histone modifications is able to cluster the tumor samples according to their histological type suggesting that post-translational modifications of histones could be a nice alternative for the diagnosis of lung cancer.[44] Changes in global level of individual histone modifications also influence prognosis. Loss of H4K20me3 correlates with reduced survival in patients with stage I adenocarcinoma.[44] The epigenetic pattern of H3K4me2, H2AK5ac and H3K9ac influences the clinical outcome of NSCLC patients, especially in early-stage tumors.[54] The cellular levels of H3K4me2 and H3K18ac have also been reported as independent predictors of clinical outcome in lung adenocarcinoma,

and may be general predictors of clinical outcome in adenocarcinomas of different tissue origins.[42,55] Further studies are needed to analyze whether distinct combinations of histone modifications might define "tumor signature profiles" that could be used as complementary diagnostic tools, prognostic factors and predictors of responses to treatment. Inhibitors of HDAC (HDACi) have emerged as novel and promising anticancer agents[56] and several molecules have been shown to increase the cytotoxic effects of radiation in NSCLC by decreasing DNA repair efficiency and promoting cell death.[57] HDACi also display benefits when used in combination with standard NSCLC chemotherapeutic agents and are likely to be a novel approach for the treatment of NSCLC because of an anti-growth activity against NSCLC cells.[58,59] Phases I and II clinical trials with HDACi in the treatment of advanced NSCLC have been completed[56] and ongoing clinical trials are exploring the use of many new HDACi singly or as part of a combination with others therapeutic modalities such as chemotherapy or radiotherapy.[60] Clearly, HDACi have a specific antitumor effect and thorough studies analyzing the full potential and mechanism of these drugs with regards to optimal dose, schedule, patient selection and combination strategies would allow the development of molecules with more effective therapeutic effect.

**Alteration of chromatin-remodeling complexes.** BRG1 is a catalytic component of the SWI/SNF chromatin-remodeling complex and regulates gene expression by disrupting DNA-histone interactions at the nucleosomes in an ATP-dependent manner.[61] This protein has been proposed to be a tumor suppressor and mice studies support a role for BRG1 loss in lung cancer development. Indeed, inactivation of BRG1 enhances the tumorigenic effect of carcinogens and induces the development of lung adenomas.[62] In human lung cancer, loss of heterozygosity surrounding the BRG1 loci and somatic point mutations of BRG1 have been described.[63,64] As a result, loss of BRG1 protein is observed in some NSCLC[65] and correlates with reduced survival.[66,67]

## Micro-RNAs for Major Biological Insights in Lung Cancer

Micro-RNAs are small non-coding RNAs initially transcribed as large RNA precursor (called pri-miRNA) which are processed into a ~70 nucleotide pre-miRNA and exported to the cytoplasm to undergo final processing steps to obtain a mature miRNA of ~22 nucleotides length.[68] Depending on the degree of homology to their 3'UTR target sequence, miRNAs induce translational repression or degradation of mRNAs. It is estimated that 1,000 miRNAs are transcribed and that 30% of the human genome is under miRNA regulation, one miRNA being able to modulate post-transcriptionally hundreds of downstream genes. In this regard, miRNAs control a wide range of biological processes including apoptosis, development, proliferation and differentiation.[69] High-throughput analyses have highlighted aberrant miRNAs expression profiles in an increasing range of human cancer types[70-72] and all these studies support the same view: the alterations seen in cancer cells that express miRNAs consist of both overexpressed and downregulated miRNAs. Therefore, miRNAs may function either as tumor suppressors or oncogenes and the genomic abnormalities found to influence their activity are the same as those described for protein-coding genes. To date, both "in vivo" and "in vitro" studies demonstrate a deregulation of miRNA expression in lung cancer and highlight them as useful diagnostic, pronostic and therapeutic tools.

A growing number of miRNAs has been found aberrantly expressed in lung cancer and our understanding of miRNAs expression patterns and function in normal and lung cancer cells is just starting to emerge. One of the first miRNAs identified is Let7 which appears to be important in lung cancer. Indeed, overexpression of let-7 inhibits Ras protein expression[73] and represses proliferation of lung cancer cells both "in vitro" and "in vivo,"[74] identifying let-7 as a tumor suppressor. This is confirmed by clinical data as reduced expression of Let-7 miRNA is observed in primary lung tumors.[72] Other miRNAs with tumor suppressor function include miR-128b which is a direct negative regulator of the EGFR oncogene and which expression is lost in lung tumors.[75] As an example of oncogenic miRNAs is the miR-17-92 cluster which upregulation is observed in lung cancer cells.[70,76] Overexpression of miR-17-92 positively stimulates cell proliferation[76,77] inhibits differentiation of lung epithelial progenitor cells in transgenic mice.[78] Expression of the E2F1 transcription factor is negatively regulated by miR-17-92,[79] suggesting that its differential pattern in lung tumors[80] could rely on aberrant expression of miR-17-92. Predicted regulatory targets of the miR-17-92 cluster also include the PTEN and RB2 tumor suppressors[81] that are known to play important roles in lung cancer. More fundamentally, abrogation of global miRNA processing through targeted silencing of components of the miRNA machinery promotes lung tumorigenesis[82] suggesting that global decrease of miRNA expression causally contributes to the transformed phenotype. Mice exposed to cigarette smoke exhibit variations in miRNA profiles expression (including let-7 and the p53 tumor suppressor responsive miRNA miR-34) especially during the weaning period.[83,84] These results demonstrate that miRNA alterations occur as an early response to environmental carcinogens "in vivo", before the onset of cancer. The precise mechanisms regulating miRNA expression in lung tumors is largely unknown but the few existing studies suggest that genetic and epigenetic alteration might affect miRNA status. MicroRNA-128b is located on chromosome 3p, which allelic loss is the most frequent and earliest genetic event in lung carcinogenesis. The cluster miR-17-92 is located at chromosome 13q31 in a region amplified in lung cancer. Expression of miRNA-124a is epigenetically silenced by DNA hypermethylation[85] and DNMTi restores miRNA-124a expression. Abnormalities in miRNA-processing genes might also be involved in aberrant miRNA pattern as decreased levels of Dicer expression are observed in lung tumors with a significant prognostic impact on the survival of surgically treated patients.[86]

It is becoming apparent that miRNA expression profiles confer important clues for clinical diagnosis and prognosis of human lung cancer. MicroRNA microarray analyses have identified statistical profiles which could discriminate lung cancers from non-cancerous lung tissues, as well as molecular signatures that differ

according to tumor histology.[71,87] Also interesting is the recent identification of Has-miR-205 as a highly specific marker for squamous carcinoma[88] suggesting that a clinical diagnostic assay based on miR-205 expression levels could aid for differential diagnosis of NSCLCs. Aberrant miRNA expression can be used as a marker for the diagnosis of NSCLC in sputum specimen[89] and detection of miRNA expression in peripheral blood or in serum is a good indicator of its expression in the tumor sample.[90,91] As miRNAs are more stable than mRNA and more tissue specific than DNA, their measurement could provide a novel promising non invasive approach to discriminate between normal and cancer patient samples. Several miRNAs are reported to be associated with the clinical outcome of lung cancer. Reduced Let-7 expression correlates with shorter survival in both univariate an multivariate analyses[71,72] and is an independent prognostic factor for the stage of the disease.[72] Overexpression of miR-155 correlates with a poor prognosis when all clinical variables are considered together.[71] Overexpression of miR-21 is an independent negative prognostic factor for overall survival in NSCLC patients.[92] Importantly, a five-miRNA signature (let-7a, miR-221, miR-137, miR-372 and miR-182) has been recently associated with survival and cancer recurrence in NSCLC patients.[93] Remarkably, this signature is valuable even after patients stratification by stage or histology.

The potential for using miRNAs in lung cancer therapy is now being explored. Let-7 overexpression confers radio-sensitivity to lung cancer cells "in vitro."[94] miR-128b LOH, a direct regulator of EGFR, correlates with clinical response and survival following gefitinib treatment.[75] miR-221, miR-222 and miR-17-92 alter the phenotype of lung cancer cells and sensitize them to cytotoxic agents.[76,77,95] Such results offer the experimental bases for the use of miRNAs as therapeutic targets. Further experiments are needed to uncover the emerging power of small non-coding RNAs to improve lung cancer therapeutics, and would have significant consequences for cancer patients in the clinical area.

## Concluding Remarks

It is becoming increasingly clear that disruption of epigenetic processes promotes lung tumor development and growth, and evidence is accumulating that epigenetic changes may account for some of the heritable effects of cigarette smoking. The management of aberrant epigenetic states as a way to target early tumor development as well as tumor progression is therefore a logical therapeutic approach. In the future, developing new strategies to avoid the pleiotropic properties of anti-cancer drugs, such as DNMTi or HDACi, will be of particular interest. Indeed, the side effects of these both compounds could have unscheduled consequences in term of genes expression, in that they may display growth-promoting effects on tumor cells. Otherwise, as the number of miRNAs increases constantly and the expression of many of them is reduced in lung cancer cells, targeting miRNA is also a promising strategy in term of cancer treatment and further studies are required to uncover the potential usefulness of chromatin modifying drugs in restoring the loss of expression of tumor suppressor miRNAs. In this context, administration of synthetic oligonucleotides that mimic endogenous miRNAs, might also be used to treat specific tumor types. Conversely, targeting oncogenic miRNAs through administration of anti-sense oligonucleotides, called AMO (anti-miRNA oligonucleotides) will become into focus, given that the use of antagomirs, which are AMOs conjugated with cholesterol, has recently emerged as an efficient approach to inhibit miRNA activity.[96] In the coming years, a more complete dissection of the cellular and molecular pathways controlled by epigenetic process will undoubtedly provide novel insights into tumor related mechanisms and will highlight promising fields for the development of novel therapies to fight lung cancer.

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**References**

1. Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 2007; 39:457-66.
2. Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 1992; 69:915-26.
3. Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. Nature 1993; 366:362-5.
4. Heard E, Clerc P, Avner P. X-chromosome inactivation in mammals. Annu Rev Genet 1997; 31:571-610.
5. Belinsky SA, Palmisano WA, Gilliland FD, Crooks LA, Divine KK, Winters SA, et al. Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. Cancer Res 2002; 62:2370-7.
6. Belinsky SA, Nikula KJ, Palmisano WA, Michels R, Saccomanno G, Gabrielson E, et al. Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. Proc Natl Acad Sci USA 1998; 95:11891-6.
7. Sato M, Mori Y, Sakurada A, Fujimura S, Horii A. The H-cadherin (CDH13) gene is inactivated in human lung cancer. Hum Genet 1998; 103:96-101.
8. Osada H, Tatematsu Y, Yatabe Y, Nakagawa T, Konishi H, Harano T, et al. Frequent and histological type-specific inactivation of 14-3-3sigma in human lung cancers. Oncogene 2002; 21:2418-24.
9. Tang X, Khuri FR, Lee JJ, Kemp BL, Liu D, Hong WK, et al. Hypermethylation of the death-associated protein (DAP) kinase promoter and aggressiveness in stage I non-small-cell lung cancer. J Natl Cancer Inst 2000; 92:1511-6.
10. Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat Genet 2000; 25:315-9.
11. Hopkins-Donaldson S, Ziegler A, Kurtz S, Bigosch C, Kandioler D, Ludwig C, et al. Silencing of death receptor and caspase-8 expression in small cell lung carcinoma cell lines and tumors by DNA methylation. Cell Death Differ 2003; 10:356-64.
12. Zochbauer-Muller S, Fong KM, Virmani AK, Geradts J, Gazdar AF, Minna JD. Aberrant promoter methylation of multiple genes in non-small cell lung cancers. Cancer Res 2001; 61:249-55.
13. Brena RM, Morrison C, Liyanarachchi S, Jarjoura D, Davuluri RV, Otterson GA, et al. Aberrant DNA methylation of OLIG1, a novel prognostic factor in non-small cell lung cancer. PLoS Med 2007; 4:108.
14. Rauch TA, Zhong X, Wu X, Wang M, Kernstine KH, Wang Z, et al. High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer. Proc Natl Acad Sci USA 2008; 105:252-7.
15. Zhong S, Fields CR, Su N, Pan YX, Robertson KD. Pharmacologic inhibition of epigenetic modifications, coupled with gene expression profiling, reveals novel targets of aberrant DNA methylation and histone deacetylation in lung cancer. Oncogene 2007; 26:2621-34.
16. Shames DS, Girard L, Gao B, Sato M, Lewis CM, Shivapurkar N, et al. A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. PLoS Med 2006; 3:486.
17. Lin RK, Hsu HS, Chang JW, Chen CY, Chen JT, Wang YC. Alteration of DNA methyltransferases contributes to 5'CpG methylation and poor prognosis in lung cancer. Lung Cancer 2007; 55:205-13.
18. Kim H, Kwon YM, Kim JS, Han J, Shim YM, Park J, et al. Elevated mRNA levels of DNA methyltransferase-1 as an independent prognostic factor in primary nonsmall cell lung cancer. Cancer 2006; 107:1042-9.
19. Lee SJ, Jeon HS, Jang JS, Park SH, Lee GY, Lee BH, et al. DNMT3B polymorphisms and risk of primary lung cancer. Carcinogenesis 2005; 26:403-9.

20. Shen H, Wang L, Spitz MR, Hong WK, Mao L, Wei Q. A novel polymorphism in human cytosine DNA-methyltransferase-3B promoter is associated with an increased risk of lung cancer. Cancer Res 2002; 62:4992-5.
21. Anisowicz A, Huang H, Braunschweiger KI, Liu Z, Giese H, Wang H, et al. A high-throughput and sensitive method to measure global DNA methylation: application in lung cancer. BMC Cancer 2008; 8:222.
22. Woodson K, Mason J, Choi SW, Hartman T, Tangrea J, Virtamo J, et al. Hypomethylation of p53 in peripheral blood DNA is associated with the development of lung cancer. Cancer Epidemiol Biomarkers Prev 2001; 10:69-74.
23. Pfeifer GP, Rauch TA. DNA methylation patterns in lung carcinomas. Semin Cancer Biol 2009; 19:181-7.
24. Chalitchagorn K, Shuangshoti S, Hourpai N, Kongruttanachok N, Tangkijvanich P, Thong-ngam D, et al. Distinctive pattern of LINE-1 methylation level in normal tissues and the association with carcinogenesis. Oncogene 2004; 23:8841-6.
25. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. Nat Rev Genet 2006; 7:21-33.
26. Tornaletti S, Pfeifer GP. Complete and tissue-independent methylation of CpG sites in the p53 gene: implications for mutations in human cancers. Oncogene 1995; 10:1493-9.
27. Jones PA, Rideout WM, 3rd, Shen JC, Spruck CH, Tsai YC. Methylation, mutation and cancer. Bioessays 1992; 14:33-6.
28. Smith LE, Denissenko MF, Bennett WP, Li H, Amin S, Tang M, et al. Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. J Natl Cancer Inst 2000; 92:803-11.
29. Yoon JH, Smith LE, Feng Z, Tang M, Lee CS, Pfeifer GP. Methylated CpG dinucleotides are the preferential targets for G-to-T transversion mutations induced by benzo[a]pyrene diol epoxide in mammalian cells: similarities with the p53 mutation spectrum in smoking-associated lung cancers. Cancer Res 2001; 61:7110-7.
30. Denissenko MF, Chen JX, Tang MS, Pfeifer GP. Cytosine methylation determines hot spots of DNA damage in the human p53 gene. Proc Natl Acad Sci USA 1997; 94:3893-8.
31. Magewu AN, Jones PA. Ubiquitous and tenacious methylation of the CpG site in codon 248 of the p53 gene may explain its frequent appearance as a mutational hot spot in human cancer. Mol Cell Biol 1994; 14:4225-32.
32. Jacinto FV, Esteller M. Mutator pathways unleashed by epigenetic silencing in human cancer. Mutagenesis 2007; 22:247-53.
33. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene $O^6$-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 1999; 59:793-7.
34. Esteller M, Risques RA, Toyota M, Capella G, Moreno V, Peinado MA, et al. Promoter hypermethylation of the DNA repair gene O(6)-methylguanine-DNA methyltransferase is associated with the presence of G:C to A:T transition mutations in p53 in human colorectal tumorigenesis. Cancer Res 2001; 61:4689-92.
35. Wu JY, Wang J, Lai JC, Cheng YW, Yeh KT, Wu TC, et al. Association of $O^6$-methylguanine-DNA methyltransferase (MGMT) promoter methylation with p53 mutation occurrence in non-small cell lung cancer with different histology, gender and smoking status. Ann Surg Oncol 2008; 15:3272-7.
36. Zochbauer-Muller S, Lam S, Toyooka S, Virmani AK, Toyooka KO, Seidl S, et al. Aberrant methylation of multiple genes in the upper aerodigestive tract epithelium of heavy smokers. Int J Cancer 2003; 107:612-6.
37. Palmisano WA, Divine KK, Saccomanno G, Gilliland FD, Baylin SB, Herman JG, et al. Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer Res 2000; 60:5954-8.
38. de Fraipont F, Moro-Sibilot D, Michelland S, Brambilla E, Brambilla C, Favrot MC. Promoter methylation of genes in bronchial lavages: a marker for early diagnosis of primary and relapsing non-small cell lung cancer? Lung Cancer 2005; 50:199-209.
39. Brock MV, Hooker CM, Ota-Machida E, Han Y, Guo M, Ames S, et al. DNA methylation markers and early recurrence in stage I lung cancer. N Engl J Med 2008; 358:1118-28.
40. Issa JP. DNA methylation as a therapeutic target in cancer. Clin Cancer Res 2007; 13:1634-7.
41. Momparler RL, Eliopoulos N, Ayoub J. Evaluation of an inhibitor of DNA methylation, 5-aza-2'-deoxycytidine, for the treatment of lung cancer and the future role of gene therapy. Adv Exp Med Biol 2000; 465:433-46.
42. Seligson DB, Horvath S, McBrian MA, Mah V, Yu H, Tze S, et al. Global levels of histone modifications predict prognosis in different cancers. Am J Pathol 2009; 174:1619-28.
43. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 2005; 37:391-400.
44. Van Den Broeck A, Brambilla E, Moro-Sibilot D, Lantuejoul S, Brambilla C, Eymin B, et al. Loss of histone H4K20 trimethylation occurs in preneoplasia and influences prognosis of non-small cell lung cancer. Clin Cancer Res 2008; 14:7237-45.
45. Schotta G, Sengupta R, Kubicek S, Malin S, Kauer M, Callen E, et al. A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. Genes Dev 2008; 22:2048-61.
46. ME LL, Vidal F, Gallardo D, Diaz-Fuertes M, Rojo F, Cuatrecasas M, et al. New p53 related genes in human tumors: significant downregulation in colon and lung carcinomas. Oncol Rep 2006; 16:603-8.
47. Kouzarides T. Chromatin modifications and their function. Cell 2007; 128:693-705.
48. Vaquero A, Sternglanz R, Reinberg D. $NAD^+$-dependent deacetylation of H4 lysine 16 by class III HDACs. Oncogene 2007; 26:5505-20.
49. Kishimoto M, Kohno T, Okudela K, Otsuka A, Sasaki H, Tanabe C, et al. Mutations and deletions of the CBP gene in human lung cancer. Clin Cancer Res 2005; 11:512-9.
50. Osada H, Tatematsu Y, Saito H, Yatabe Y, Mitsudomi T, Takahashi T. Reduced expression of class II histone deacetylase genes is associated with poor prognosis in lung cancer patients. Int J Cancer 2004; 112:26-32.
51. Sasaki H, Moriyama S, Nakashima Y, Kobayashi Y, Kiriyama M, Fukai I, et al. Histone deacetylase 1 mRNA expression in lung cancer. Lung Cancer 2004; 46:171-8.
52. Italiano A, Attias R, Aurias A, Perot G, Burel-Vandenbos F, Otto J, et al. Molecular cytogenetic characterization of a metastatic lung sarcomatoid carcinoma: 9p23 neocentromere and 9p23-p24 amplification including JAK2 and JMJD2C. Cancer Genet Cytogenet 2006; 167:122-30.
53. Suzuki C, Takahashi K, Hayama S, Ishikawa N, Kato T, Ito T, et al. Identification of Myc-associated protein with JmjC domain as a novel therapeutic target oncogene for lung cancer. Mol Cancer Ther 2007; 6:542-51.
54. Barlesi F, Giaccone G, Gallegos-Ruiz MI, Loundou A, Span SW, Lefesvre P, et al. Global histone modifications predict prognosis of resected non small-cell lung cancer. J Clin Oncol 2007; 25:4358-64.
55. Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M, et al. Global histone modification patterns predict risk of prostate cancer recurrence. Nature 2005; 435:1262-6.
56. Gridelli C, Rossi A, Maione P. The potential role of histone deacetylase inhibitors in the treatment of non-small-cell lung cancer. Crit Rev Oncol Hematol 2008; 68:29-36.
57. Cuneo KC, Fu A, Osusky K, Huamani J, Hallahan DE, Geng L. Histone deacetylase inhibitor NVP-LAQ824 sensitizes human nonsmall cell lung cancer to the cytotoxic effects of ionizing radiation. Anticancer Drugs 2007; 18:793-800.
58. Komatsu N, Kawamata N, Takeuchi S, Yin D, Chien W, Miller CW, et al. SAHA, a HDAC inhibitor, has profound anti-growth activity against non-small cell lung cancer cells. Oncol Rep 2006; 15:187-91.
59. Loprevite M, Tiseo M, Grossi F, Scolaro T, Semino C, Pandolfi A, et al. In vitro study of CI-994, a histone deacetylase inhibitor, in non-small cell lung cancer cell lines. Oncol Res 2005; 15:39-48.
60. Cang S, Ma Y, Liu D. New clinical developments in histone deacetylase inhibitors for epigenetic therapy of cancer. J Hematol Oncol 2009; 2:22.
61. Sudarsanam P, Winston F. The Swi/Snf family nucleosome-remodeling complexes and transcriptional control. Trends Genet 2000; 16:345-51.
62. Glaros S, Cirrincione GM, Palanca A, Metzger D, Reisman D. Targeted knockout of BRG1 potentiates lung cancer development. Cancer Res 2008; 68:3689-96.
63. Medina PP, Carretero J, Fraga MF, Esteller M, Sidransky D, Sanchez-Cespedes M. Genetic and epigenetic screening for gene alterations of the chromatin-remodeling factor, SMARCA4/BRG1, in lung tumors. Genes Chromosomes Cancer 2004; 41:170-7.
64. Girard L, Zochbauer-Muller S, Virmani AK, Gazdar AF, Minna JD. Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. Cancer Res 2000; 60:4894-906.
65. Medina PP, Sanchez-Cespedes M. Involvement of the chromatin-remodeling factor BRG1/SMARCA4 in human cancer. Epigenetics 2008; 3:64-8.
66. Fukuoka J, Fujii T, Shih JH, Dracheva T, Meerzaman D, Player A, et al. Chromatin remodeling factors and BRM/BRG1 expression as prognostic indicators in non-small cell lung cancer. Clin Cancer Res 2004; 10:4314-24.
67. Reisman DN, Sciarrotta J, Wang W, Funkhouser WK, Weissman BE. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. Cancer Res 2003; 63:560-6.
68. Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. Nat Rev Cancer 2006; 6:259-69.
69. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6:857-66.
70. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006; 103:2257-61.
71. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006; 9:189-98.
72. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 2004; 64:3753-6.
73. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, et al. RAS is regulated by the let-7 microRNA family. Cell 2005; 120:635-47.
74. Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, et al. The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res 2007; 67:7713-22.

75. Weiss GJ, Bemis LT, Nakajima E, Sugita M, Birks DK, Robinson WA, et al. EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines. Ann Oncol 2008; 19:1053-9.
76. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005; 65:9628-32.
77. Matsubara H, Takeuchi T, Nishikawa E, Yanagisawa K, Hayashita Y, Ebi H, et al. Apoptosis induction by antisense oligonucleotides against miR-17-5p and miR-20a in lung cancers overexpressing miR-17-92. Oncogene 2007; 26:6099-105.
78. Lu Y, Thomson JM, Wong HY, Hammond SM, Hogan BL. Transgenic overexpression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. Dev Biol 2007; 310:442-53.
79. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005; 435:839-43.
80. Eymin B, Gazzeri S, Brambilla C, Brambilla E. Distinct pattern of E2F1 expression in human lung tumours: E2F1 is upregulated in small cell lung carcinoma. Oncogene 2001; 20:1678-87.
81. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003; 115:787-98.
82. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 2007; 39:673-7.
83. Izzotti A, Calin GA, Steele VE, Croce CM, De Flora S. Relationships of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J 2009.
84. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. Nature 2007; 447:1130-4.
85. Lujambio A, Ropero S, Ballestar E, Fraga MF, Cerrato C, Setien F, et al. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res 2007; 67:1424-9.
86. Karube Y, Tanaka H, Osada H, Tomida S, Tatematsu Y, Yanagisawa K, et al. Reduced expression of Dicer associated with poor prognosis in lung cancer patients. Cancer Sci 2005; 96:111-5.
87. Liang Y. An expression meta-analysis of predicted microRNA targets identifies a diagnostic signature for lung cancer. BMC Med Genomics 2008; 1:61.
88. Lebanony D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi K, et al. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol 2009; 27:2030-7.
89. Xie Y, Todd NW, Liu Z, Zhan M, Fang H, Peng H, et al. Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. Lung Cancer 2010; 67:170-6.
90. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008; 18:997-1006.
91. Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer 2009; 10:42-6.
92. Markou A, Tsaroucha EG, Kaklamanis L, Fotinou M, Georgoulias V, Lianidou ES. Prognostic value of mature microRNA-21 and microRNA-205 overexpression in non-small cell lung cancer by quantitative real-time RT-PCR. Clin Chem 2008; 54:1696-704.
93. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh S, et al. MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell 2008; 13:48-57.
94. Weidhaas JB, Babar I, Nallur SM, Trang P, Roush S, Boehm M, et al. MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. Cancer Res 2007; 67:11111-6.
95. Garofalo M, Quintavalle C, Di Leva G, Zanca C, Romano G, Taccioli C, et al. MicroRNA signatures of TRAIL resistance in human non-small cell lung cancer. Oncogene 2008; 27:3845-55.
96. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature 2005; 438:685-9.