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INVITED REVIEW

Epigenetics of lung cancer

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Epigenetics of lung cancer
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Abstract: Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence. It has a role in determining when and where a gene is expressed during development. Perhaps the most well known epigenetic mechanism is DNA methylation whereby cytosines at position 5 in CpG dinucleotides are methylated. Histone modification is another form of epigenetic control, which is quite complex and diverse. Histones and DNA make up the nucleosome which is the structural unit of chromatin which are involved in packaging DNA. Apart from the crucial role epigenetics plays in embryonic development, transcription, chromatin structure, X chromosome inactivation and genomic imprinting, its role in an increasing number of human diseases is more and more recognized. These diseases include cancer, and lung cancer in particular has been increasingly studied for the potential biological role of epigenetic changes with the promise of better and novel diagnostic and therapeutic tools.

Key words: xxxx, xxxx, xxxx.

INTRODUCTION

Lung cancer is a major public health problem in the world and remains a leading cause of cancer-related deaths but its impact in developed and developing Asian countries is also devastating.[1–3] It is thought that over 1.2 million people died from lung cancer in 2001, with the majority coming from low- and middle-income countries.[4]

It is well known that cancer is a genetic disease, in that aberration of key genetic and thereby molecular pathways are critical for carcinogenesis. Such key events have been called hallmarks of cancer and have recently been reviewed.[5] There is also increasing evidence for a major role of epigenetic aberrations in lung and many other cancers. Why epigenetic alterations occur in cancer is not well understood although theories include this response being induced from sustained cellular stress.[6] This review seeks to provide the reader with a summary of the research to date in this field and the potential implications for clinicians involved with lung cancer patients. Recent reviews and key primary citations will be listed to enable further reading while ensuring brevity.

EPIGENETICS

Epigenetics in the study of heritable changes in gene expression that occur without changes in DNA sequence. The human DNA sequence and an associated near complete list of genes is known thanks to the success of the Human Genome Project. However, the place and timing of expression of specific gene expression is complex and influenced by epigenetic modification. Thus, epigenetic modifications are heritable during cell division but do not alter the DNA sequence.

Epigenetic mechanisms include **DNA methylation**, as well as post-translational modification of core histone proteins by acetylation, phosphorylation and methylation, often called **histone modification**. These mechanisms contribute to dynamic patterns of gene expression during cell cycling as well as to physiological processes of genomic imprinting and X chromosome inactivation. Although different process, recent data indicate that DNA methylation and histone modifications may be mechanistically linked. The dynamics of genetic and epigenetic silencing are

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complex and have been reviewed recently by Jones and Baylin.[7]

DNA METHYLATION

In mammals, DNA methylation involves covalent modification at the fifth carbon position of cytosine bases that are located 5′- to a guanosine in a CpG dinucleotide (CpG dinucelotides are underrepresented in much of the genome but are found to be concentrated in short regions called CpG islands). Most CpG islands are located in the promoter regions of mammalian genes and are generally unmethylated in normal cells. The mammalian DNA methylation process requires two components, the DNA methyltransferases (DNMTs) and the methyl CpG binding proteins (MBDs)—summarized in recent reviews.[8,9] DNMTs establish and maintain methylation patterns whereas MBDs are thought to be involved in 'reading' methylation marks. Potential mechanisms include spreading of DNA methylation from repetitive sequences into promoter-associated CpG islands secondary to loss of transcriptional activators, gain of methylation secondary to hyperexpression of transcriptional repressors, primary hypermethylation because of hyperexpression of methyltransferases, and interallelic transfer of methylation via gene pairing.[10]

In brief a recently proposed model of DNA methylation and cancer proposes that global hypomethylation in at-risk cells contributes to genomic instability through increased mitotic recombination events, whereas CpG island methylation in cancer cells leads to transcriptional silencing of growth regulatory genes.[8]

DNA hypomethylation in lung cancer

The link between cancer and abnormal methylation has been known since 1983, with the demonstration that cancer genomes are relatively hypomethylated compared with normal counterparts.[11] Hypomethylation in cancer cells is thought to be primarily resulting from loss of methylation from repetitive regions of the genome, with resulting genomic instability.[8] Apart from global genomic demethylation which in many cancers is an early event, gene specific hypomethylation also occurs which can result in functional changes in gene expression.

Studies of oncogene transformed normal human bronchial epithelial (NHBE) cells and clinical lung cancer samples have demonstrated alterations in methylation[12,13] during lung carcinogenesis. The global demethylation is known to result in the derepression of parasitic DNA, loss of imprinting and upregulation of the expression of a number of usually silent genes. Examples of genes altered in these ways include loss of imprinting of the H19, IGF2 and MEST genes. Imprinting is the epigenetic modification in a specific parental chromosome that leads to differential expression of the two alleles in the offspring, thus loss of imprinting is loss of the normal allele specific gene expression which may result in deregulated cell growth. Other genes up-regulated as a result of global hypomethylation include those encoding cancer testes antigens (CTAs) proteins including the MAGE family (recently reviewed by Schrump[9]). 4

DNA hypermethylation in lung cancer

In contrast, site specific hypermethylation of the often unmethylated CpG islands in gene promoters is now the most well characterized epigenetic modification in cancer. It is found in nearly every human cancer type and is associated with transcription silencing of gene expression. The genes silenced by promoter hypermethylation tend to be tumour or growth suppressor genes, and is an alternative to the classic loss of one tumour suppressor gene (TSG) and mutation of the other to satisfy Knudson's two-hit hypothesis. Indeed there are regions of chromosomes where allele loss and hypermethylation may be the predominant method on TSG inactivation, for example, RASSFI1 at chromosome 3p21 and HIC1 are 17p13.3.

It is known that exposure to cigarette smoke induces lung cancer in mice via both genetic and epigenetic pathways.[14] Indeed some of the DNA methylation changes involve the same genes that are altered in human lung cancers.[15] There are now many reports of somatically acquired DNA methylation in the genes involved in lung cancer. We have previously reported on methylation specific PCR (MSP—see below) to examine gene promoter methylation in lung cancer. DNA was examined from 107 resected NSCLC and corresponding normal lung tissue.[16–18] Methylation in the tumour samples was detected in 40% for *RAR*b, 26% for *TIMP-3*, 25% for *p16^{INK4a}*, 21% for *MGMT*, 19% for *DAPK*, 18% for *ECAD*, 8% for *p14 ARF* and 7% for *GSTP1*, whereas it was not seen in the vast majority of the corresponding non-malignant tissues. A total of 82% of the NSCLCs had methylation of at least one of these genes; 37% had one gene methylated, 22% had two genes methylated, 13% had three genes methylated, 8% had four genes methylated and 2% had five genes methylated. Aberrant *p16^{INK4a}* and *FHIT* methylation corresponded with down-regulation[16,19] of gene expression. Thus, we and others have documented examples of epigenetic gene silencing by *de novo* methylation of TSGs in lung cancers, for example, *Rb*, *VHL*, *p16^{INK4a}*, *DAPK*, *GSTP1* and *MGMT*.[16–18] 5

Many other genes are now shown to be methylated to varying degrees in the different subtypes of lung cancer, from primary lung cancers and also lung cancer cell lines. These include genes such as TSLC1;[20] CDH13,[21] hSRBC,[22] SPARC,[23] DBC1.[24] The genes altered by DNA methylation include those involved in cell cycle regulation (e.g. p16), DNA repair (e.g. MGMT), apoptosis (e.g. DAPK, caspase 8, FAS, TRAILR1), RAS signalling (RASSF1A, NORE1A) and invasion (e.g. cadherins, TIMP3, laminin family) with more detailed lists available from recent reviews.[25,26] Some of these pathways affected by epigenetic change are those described as being hallmarks of cancer.[5]

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Detection of DNA methylation

One of the major reasons for the explosion in knowledge regarding tumour methylation is thanks to the rapid technological advances in this area. Frommer *et al*. demonstrated that bisulphite deamination of cytosine and 5-methylcytosine differentially to yield uracil and thymine, respectively, coupled with polymerase chain reaction (PCR) techniques allowed simplified analysis of DNA methylation.[27] These days, the very sensitive bisulphite-based MSP is perhaps the most popular tool for examining DNA methylation patterns (refer Fig. 1). The principles of MSP and other methylation detection techniques, such as restriction digestion by methylation-sensitive enzymes, COBRA, PyroMeth, SnaPmeth, bisulphite SSCP, bisulphite sequencing have been recently reviewed.[28] Some techniques such as restriction landmark genomic scanning (RLGS) and arbitrarily primed PCR (AP-PCR) are genome wide techniques (often methylation-sensitive restriction enzyme-based), for analysing the DNA methylation status of CpG islands[7] (refer Fig. 2). Newer techniques such as array-based epigenomics testing, the high throughput MethyLight or MALDI-TOF mass spectrometry are now increasingly reported.[29]

Characteristics of DNA methylation changes in lung cancer

The profile of aberrant DNA methylation differs between cancers of different cell types.[30] Even in lung cancers, there appears to be heterogeneity in the frequency of DNA methylation changes between lung

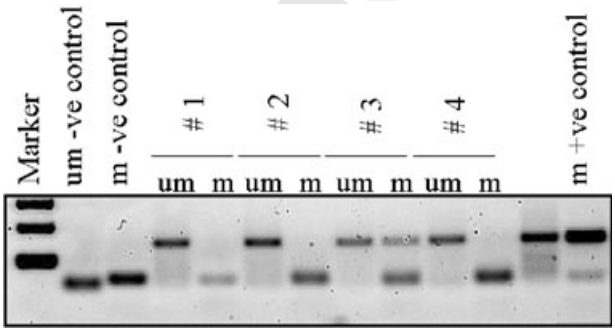

Figure 1 A representative gel showing methylation-sensitive PCR (MSP) of the p16 gene. DNA from four samples were bisulphite modified to convert unmethylated cytosines into uracil. The treated DNA was then amplified with two sets of primers designed to anneal to either regions containing unmethylated cytosines (um) or methylated cytosines (m). In this gel only sample 3 contains methylated cytosines at the p16 promoter region tested.

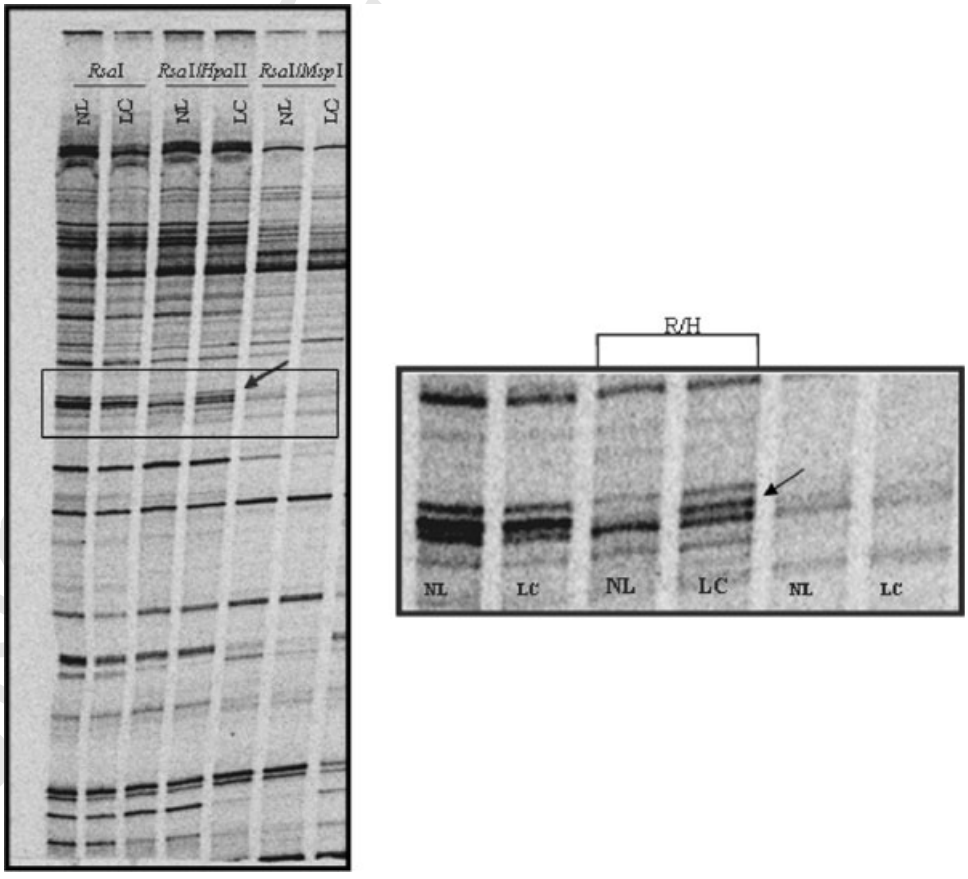

Figure 2 Representative gel showing methylation-sensitive arbitrarily primed PCR (MS-APPCR). DNA was pooled into two groups of five samples, digested with the restriction enzymes *Rsa*I, *Rsa*I/*Hpa*II (methylation-sensitive), *Rsa*I/*Msp*I (methylation insensitive) and amplified with GC-rich primer sets in the presence of radiolabelled ^{33}P-dATP. The PCR products were then resolved on a 5% polyacrylamide gel. The box region is magnified. The arrow indicates a differentially methylated band in the tumour sample as compared with the corresponding normal sample.

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cancer subtypes, for example, NSCLC compared with SCLC.[26,31]

In addition, the study of invasive lung cancers, preneoplasia and normal tissues afford the chance to understand the timing of methylation changes. Like the genetic multistep progression model of lung cancer, it is likely that there are increasing numbers of epigenetic alterations as a predisposed cell progresses towards invasive cancer. One such model suggests that the accumulation of epigenetic change parallels conventional morphological abnormalities that have long been recognized for squamous carcinomas.[13] Indeed, aberrant methylation of the p16 gene has been known for some years to be an early event in lung cancer development.[32] Further support for DNA methylation-induced gene silencing comes from animal models exposed to tobacco carcinogens.[15] Moreover, different carcinogens appear to have specificity for inducing various epigenetic alterations.

Thus, while some DNA methylation events occur early in lung cancer, and multiple gene promoters are aberrantly methylated in invasive lung cancers, the challenge is to translate this knowledge into better epigenetic biomarker-based methods of lung cancer detection and diagnosis, prognostication and therapeutics.

DNA methylation—lung cancer diagnostics

In many cancers DNA methylation changes can be detected in corresponding body fluids. This includes lung cancer, where MSP detection of methylation reveals detectable methylation of various promoters in sputum, bronchial lavages, peripehral blood, and where tumour and serum DNA methylation patterns are closely related.[25] Specific genes affected include p16 (sputum, bronchial lavage, pleural lavage), MGMT (sputum, bronchial lavage), RARb and DAPK (bronchial lavage) and hMLH1 (sputum)—summarized by Miyamoto.[33] Thus, DNA methylation is a promising tool for early diagnosis from body fluids, including peripheral blood which is known to contain circulating tumour free DNA. So far, most studies have been relatively small case control studies and assays not standardized leaving questions as to varying sensitivity and specificity. Nonetheless, prospective longitudinal studies are being performed and the use of a panel of putative DNA methylation markers for detecting early lung cancer remains a definite future diagnostic possibility, especially with rapidly changing technologies. Like all tumour biomarkers, however, appropriate study designs and independent validation of robust tests are needed to ensure their translation to the clinic.

Risk-prediction is another aspect of the early detection paradigm, where accurate prediction of lung cancer risks afford the chance for primary prevention (smoking cessation) and secondary prevention (e.g. chemoprevention) and well and disease screening (e.g. optimization of helical low-dose CT screening strategies). For instance, a recent study showed that concomitant methylation of multiple gene (MGMT, ras effector homologue 1, DAPK and PAX5a) promoters in sputum is strongly associated with lung cancer risk.[34] Again, properly designed large scale population-based studies are required.

DNA methylation—lung cancer prognostics

Lung and other cancer outcomes are only partially predicted by gross tumour characteristics such as pathological subtype or TNM stage, and patient characteristics such as performance status. Apart from these conventional prognostic factors, increasing evidence indicate that molecular characterization may provide refined information as to outcome and thus guide the choice of therapies. Molecular predictive factors include genetic and epigenetic factors, some of which may be able to predict relative response to specific therapies and other toxicities from drugs. In addition, there may also be prognostic factors which are related to the biology of the tumour such as growth rate and metastatic behaviour. Much interest has been centred on genetic profiling through gene expression chips, but epigenetic or epigenomic factors are very likely to have a complementary role in the future. For instance, methylation-dependent transcriptional silencing of 14-3-3sigma, a major G2-M checkpoint control gene, appears to be an independent prognostic factor for survival in NSCLC patients receiving platinum-based chemotherapy which can be detected in the serum, thus obviating the need for tumour tissue analysis.[35]

DNA methylation—lung cancer therapeutics

Inhibition of DNA methylation as anticancer therapy

In foetal development epigenetic control of gene expression is a dynamic process, attesting to the potential for reversal of DNA methylation. The demethylating action of 5-azacytidine was first described in 1980,[36] and regained scientific interest when the therapeutic effect of low doses of the deoxy derivative 5-aza-2′-deoxycitidine was reported in haematological malignancies.[37,38] Nucleoside analogues of cytidine incorporate into DNA and specifically inhibit DNMTs resulting in hypomethylation and reactivation of previously silenced hypermethylated genes, for example, the P15/INK4B gene in patients with myelodysplasia.[39] The drugs are cytotoxic, causing myelosuppression in high doses. Instability of the cytosine analogues in aqueous solution limits their clinical application. A newer orally active compound (zebularine) with similar actions to 5-aza-2′-deoxycitidine is capable of restoring imprinting patterns in tumour cells by demethylation[40] and, in association with reactivation of p16, suppresses the growth of human bladder cancer xenografts.[41] Other agents under current investigation for their demethylating activity include procaine,[42] hydrallazine,[43] epigallocathecin-3-gallate (green tea polyphenol),[44] and s-adenosyl methionine inhibitors.[42]

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Experimentally, transcriptional reactivation by the nucleoside analogues is neither complete nor universal[45] suggesting that other silencing mechanisms may need to be addressed to achieve a clinical outcome in cancer patients. DNA methylation by methyltransferases leads to interactions with methylated DNA binding proteins, histone modification, and chromatin restructuring and each of these elements of epigenetic silencing could potentially be targeted therapeutically, as already described for histone deacetylase inhibitors (HDACi). However, the observation that HDACi can restore expression of unmethylated genes but not genes with methylated promoters attests to the dominance of DNA methylation in transcriptional repression,[46] and therefore its importance as a therapeutic target. Synergism between low-dose demethylating nucleoside analogues and histone deacetylases (HDACs) in lifting gene silencing[47–49] leads to an expectation of improved clinical responses from combination therapy directed at cancer-related epigenetic aberrations. Other possible avenues of therapeutic exploration include methyltransferase inhibition independent of binding to the replication fork of DNA, disruption of DNA methyl binding proteins, and interference with methylation and phosphorylation modification of histones.

Demethylating agents in lung cancer

Preclinical

In vitro and preclinical studies indicate that restoring the function of genes silenced by methylation in lung cancer could produce an antitumour effect by inducing growth arrest,[48] apoptosis,[50] inhibition of angiogenesis,[51] or modulation of immunological recognition and targeting.[52] In murine models of NNK-induced lung tumours 5-aza-2′-deoxycitidine reduced tumour incidence, both alone[53] and in combination with HDACi,[49] indicating chemopreventive potential for demethylating agents.

Clinical

Lung cancer patients included in clinical phase 1/2 studies of nucleoside analogue demethylating agents in solid tumour patients with extensive disease have not generally shown objective disease responses[25] but increased foetal haemoglobin synthesis during treatment indicates that gene reactivation by demethylation is probably occurring in these patients.[54] A phase 1/2 clinical trial of single agent 5-aza-2′-deoxycitidine was associated with 5 years survival in one of 15 non-small cell carcinoma patients,[55] but no clinical responses were observed in the lung cancer patients included on a phase 1 study of this agent in combination with cisplatin.[56]

In the light of interactions outlined above, the combination of low-dose demethylating agents and HDACi is a rational path to follow in clinical trials. Recent investigations have further defined the dependency of chromatin mediated gene repression on DNA methylation, through induction of signature histone modifications representing transcriptional-activation marks and loss of silencing marks.[57,58] Clinical trials of this approach are now underway in lung cancer patients, for example, the phase 1 trial of 5-aza-2′-deoxycitidine in combination with depsipeptide.[59]

Future directions in therapeutic targeting of DNA methylation

Most currently known demethylating agents act globally. Therefore, they may also theoretically disrupt essential methylation or augment hypomethylation of retrotransposons and repetitive DNA which have in turn been linked to chromosomal instability and nuclear disorganization.[60,61] This consideration is particularly relevant to the future application of methylation inhibiting strategies in preventive settings, such as high-risk smokers. However, the clinical toxicities of demethylating agents have not supported concerns over the possibility of large scale gene activation. This is perhaps because transcription in normal cells is not commonly controlled by CpG promoter methylation and, where this is the case, reactivation is not easily achieved by demethylation alone, for example, X chromosome inactivation is unaffected by 5-azacytidine.[62]

Future directions for clinical therapies targeting epigenetic pathways in lung cancer are to overcome limitations of nucleoside analogue demethylating agents imposed by formulation instability, to determine optimal dosing schedules to sustain demethylation effects while minimizing myelosuppressive and other side-effects, to define the clinical potential of non-nucleoside methylation inhibitors such as -epigallocatechin-3-gallate, and selenium compounds, and to evaluate novel demethylating agents such as the psammaplins, small molecule methyltransferase inhibitors and oligonucleotides.

Proof of connection between administration of therapeutic agents and a tumour response directly dependent on demethylation-induced re-expression of critical genes is still awaited. Further development of clinically useful tests of demethylating activity is warranted to improve on surrogate indicators such as foetal Hb and demethylation of imprinted genes. Measurement of 5-methylcytosine DNA levels with high performance capillary electrophoresis could provide a quantitative indicator of molecular effect in blood and other clinical samples.[63]

Methods of achieving gene-specific demethylation rather than global demethylation need to be explored, as do novel methods of targeting epigenetic modifications. Because synthetic transcription factors and short interfering RNAs targeting promoter regions are able to induce DNA methylation to affect specific gene expression,[64,65] approaches targeting demethylation to specific regions may be able to overcome problems arising from non-specific gene derepression.

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HISTONE MODIFICATION IN LUNG CANCER

Function of HDACs

The packaging of DNA affects its transcription.[66] Chromosomes are organized into coiled chromatin fibres, which are themselves made up of units (nucleosomes) comprising a central core of histone proteins around which DNA is coiled.[67] This condensed structure renders the DNA transcriptionally inactive by reducing interaction between the DNA and proteins involved in DNA transcription and replication. In contrast, chromatin structure can be reversibly remodelled through uncoiling, in order to allow access to DNA-binding factors and activate transcription. A major remodelling process is histone acetylation, in which histone acetyltransferases (HATs) add acetyl groups to lysine residues on the histone N-terminal tails which protrude from the nucleosome complex.[68] Histone acetylation leads to a loss of positive charge, reduced affinity between histones and DNA, and consequently uncoiling to allow access to RNA polymerase and transcription factors.[67] HATs are therefore transcriptional coactivators.

Conversely, HDACs remove acetyl groups, and act as transcriptional repressors. Histone deacetylation is mediated by DNA methylation through binding of repressor proteins in methylated CpGs in DNA. Hence post-translational modification of histones by acetylation and deacetylation represents a major mechanism of transcription regulation at the chromatin level, thereby influencing gene expression.[66] At least 18 human HDAC enzymes have been identified.[69] The classical HDAC family consists of Class I (HDAC1, 2, 3 and 8) and Class II (HDAC4, 5, 6, 7, 9 and 10) HDACs, with HDAC11 showing overlapping features. Class I HDACs are localized to the nucleus, whereas Class II HDACs traffic to and from the nucleus.[66] HDACs are involved in many biological processes, including regulation of cell cycling, cell proliferation, differentiation, cell death, DNA replication and mitosis, and the development of cancer.[69,70]

Role of HDAC in cancer

The biological role of HDAC in human cancers has recently been reviewed.[69,71] Imbalance of acetylation and deacetylation has been implicated in the pathogenesis of haematological and solid tumours.[71] To explore the effects of HDAC aberrations in cancer, HDAC and HDACi have been studied in a variety of cancer cell lines, because immortalized cell lines provide useful *ex vivo* models of cancer biology for testing of anticancer agents. Studies of the pathogenesis of leukaemia have provided much of the information about the role of HDAC in cancer.[71] In acute promyelocytic leukaemia, there is arrest of leukaemic cells in the proliferative phase, preventing mature differentiation of cells.[71] This is because of the presence of aberrant retinoic acid receptor fusion proteins which associate excessively with HDAC complexes, leading to transcriptional silencing. With the addition of pharmacological levels of retinoic acid treatment, there is a release of HDAC complexes, allowing transcriptional activation and myeloid differentiation.[71] Other examples of translocation products in haematological tumours include transcriptional repressor LAZ3/BCL6 in non-Hodgkin's lymphoma and the AML1-ETO fusion protein in acute myeloid leukaemia.[71] In these diseases, HDACs are also recruited excessively, leading to transcriptional repression and downstream effects that favour oncogenesis.

HDAC overexpression has been found in solid tumours including breast, gastric, oesophageal and colon cancers, and variably linked with prognosis.[69] The potential mechanisms of action of HDAC have been explored in various tumour cell lines. Histone acetylation status would be expected to correlate with the level of gene expression in cancer cells. In a study of gastric cancer cell lines, genomic fragments within histones were analysed to identify differential gene expression with various deacetylation status.[72] The level of histone acetylation in the gastric cancer cells correlated with the level of gene expression of a number of cancer-related genes, including several involved in cell cycling. This was further demonstrated in a study of resected oesophageal squamous cell cancers in which the expression of metastasis-associated protein (MTA1) and acetylation of histone 4 were determined using immunohistochemistry.[73] Deacetylation of histone 4 correlated with overexpression of MTA1, which predicted tumour invasion of the oesophageal wall, lymph node metastasis and poorer prognosis. Histone acetylation and deacetylation have been studied in relation to other pathways involved in oncogenesis. Apoptosis leads to programmed cell death and an antitumour effect. A study of a breast cancer cell line showed that epidermal growth factor induces the NF-κB-mediated association of HDAC1 with the death receptor 5 gene, which is one of the receptors for the tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL).[74] The observed deacetylation leads to an antiapoptotic and oncogenic effect, and therefore provides rationale the use of HDACi in the treatment of cancer. Finally, a tangible link between HDAC overexpression and tumour pathways was also recently shown in a study of colon cancer, in which resected specimens showed increased expression of HDAC2.[75] HDAC2 prevented apoptosis in the colon cancer cell line HT-29 and was a requisite for cell survival. Furthermore, these authors demonstrated in mice that loss of the TSG, adenomatous polyposis coli (APC), induced HDAC2 expression through the Wnt and c-Myc pathways. Thus, the results from studies of haematological and solid tumours suggests that blocking the inappropriate transcriptional repression of HDACs would be a promising strategy for antitumour therapy.

HDAC expression in lung cancer

Several studies have examined HDAC expression in lung cancer resection specimens. In a study of 102 non-small cell lung cancer resection specimens, the mRNA and protein expression of HDAC1 was

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increased in patients with Stages III and IV lung cancer, compared with Stage I or II.[76] There was no difference in mRNA expression between tumour and adjacent non-tumour lung tissue. In a study of 72 non-small cell lung cancer resection specimens, the mRNA expression of HDAC1 to 8 and 10 was correlated with prognosis.[77] Low expression of Class II HDACs, in particular HDAC10, showed association with poorer prognosis after surgery. The protein expression of HDAC3 was found to be up-regulated in squamous cell lung cancer specimens using antibody arrays and confirmed by immunoblot analysis.[78] Collectively, it can be postulated from these preliminary studies that HDACs repress critical gene pathways involved in protecting against lung cancer and therefore HDAC activity may promote tumorigenesis. In contrast, in chronic obstructive pulmonary disease, which is another example of a smoking-induced lung disease, there is a reduction in lung HDAC anti-inflammatory activity that correlates with disease progression.[79] Only one study has examined the role of genetic variation in HDAC genes in lung cancer. There was no association of polymorphisms in the HDAC3, 4 and 5 genes and the risk of lung cancer in a study of 432 lung cancer patients and 432 controls in Korea.[80]

HDAC inhibitors

Given the potentially pathogenic role of HDAC and deacetylation in cancer, HDACi have emerged as novel anticancer agents.[71] HDACi are compounds that bind to the active zinc site of the catalytic domain of HDAC.[69] Several classes of HDACi exist, including hydroxamate, cyclic peptide, aliphatic acid and benzamide classes.[69] Examples of HDACi include trichostatin A, sodium butyrate, suberoylanilide hydroxamic acid (SAHA), FK228 and valproate. The exact mechanism of action of HDACi remains unclear.[69] It is known that HDACi induce growth arrest of cells, terminal differentiation, caspase-dependent apoptosis and cell death. However, normal cells appear to be less sensitive to the effects of HDACi than transformed cells.[69] Furthermore, inhibition of HDAC may increase or decrease overall gene expression, depending on the genes targeted. Binding of HDACi to other non-histone regulatory proteins also influences the alteration of transcriptional activity.

Effect of HDACi on lung cancer cell lines

Initial studies in lung cancer cell lines suggested that HDACi have inhibitory effects on cell cycling and promote apoptosis.[9] Subsequent studies have further investigated the effect of specific HDACi on chromatin structure and phenotypes related to cell growth, with the aim of confirming that HDACi have biological effects in cells of lung origin. In a study of normal lung fibroblasts, the HDACi trichostatin A derepressed the activity of telomerase, whereas there was no effect in non-small cell lung cancer cell lines.[81] In the A549 lung carcinoma cell line, treatment with the HDACi sodium butyrate resulted in global histone hyperacetylation, chromatin decondensation and intense acetylation patterns at the periphery of the nucleus.[82] Treatment of the H69 small cell lung cancer cell line with trichostatin A showed a chromatin decondensation pattern of nuclear texture.[83] Together, these studies of chromatin remodelling indicate that HDACi have biological effects in lung cancer cell lines.

The role of pathways that interact with HDACs has been investigated with HDACi, to identify pathways that influence the responsiveness of lung cancer cells to HDACi. FK228, an HDACi, was tested in the A549 lung adenocarcinoma cell line and PC14, a lung adenocarcinoma cell line that is relatively more resistant to HDACi.[84] In the A549 non-small cell lung cancer cells, FK228 inhibited the Akt-mediated signalling pathway in the A549 cells but not the resistant PC14 cells. In another study of A549 cells, the HDACi trichostatin A was pro-apoptotic, as indicated by the down-regulation of the antiapoptotic Bcl-2 protein, up-regulation of the pro-apoptotic Bax protein and activation of caspases.[85] In a study of the small cell lung cancer cell lines, H69 and H526, the HDACi FR901228 induced caspase-dependent apoptosis through the mitochondrial pathway, rather than the death receptor pathway.[86] Cyclooxygenase 2 (COX2) mRNA expression was also decreased with trichostatin A treatment, implicating involvement of the cyclooxygenase pathway in mediating the anticancer effect of trichostatin A. Hence these results show that are complex, interrelated mechanisms of action of individual HDACi on both deacetylation and non-histone pathways, and these should be studied in detail in order to understand lung cancer responsiveness to HDACi treatment.

Combination therapy of HDACi and other agents

The effects of HDACi have been limited to a certain extent by their activation of pathways that tend to antagonize the antitumour effect. For example, non-small cell lung cancer cell lines are relatively resistant to the HDACi SAHA, despite SAHA having adequate HDACi activity.[87] The reason may be that SAHA stimulates the activity of the transcription factor NF-κB which is antiapoptotic and therefore further increases cell survival. This has lead to the testing of combinations of HDACi with other proven or putative antitumour agents. The combination of the HDACi, SAHA, with the chemotherapeutic agent, gemcitabine, was tested in a study of non-small cell lung cancer cell lines.[88] SAHA with gemcitabine was found to enhance apoptosis in the tumour cells, possibly through the attenuation of SAHA-induced NF-κB activation by gemcitabine. In another study focusing on NF-κB, H322 and H460 lung cancer cell lines were treated with trichostatin A and calphostin C (a protein kinase C inhibitor).[89] Using this combination, 90–96% of cells underwent apoptosis, and there was an associated reduction in induced NF-κB activation. Another HDACi, N-acetyldinaline (CI-994), was tested in the

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A549 and LX-1 (squamous cell lung cancer) cell lines.[90] N-acetyldinaline treatment of the cells produced a concentration-dependent inhibition of cell survival, an effect which was synergistic with gemcitabine or docetaxal treatment. Also in A549 cells, the combination of TNF-related apoptosis-inducing ligand (TRAIL) and either SAHA, trichostatin A or sodium butyrate substantially increased apoptosis in the cell line.[91] Finally four non-small cell lung cancer cell lines were treated with SAHA and bortezomib, a proteosome inhibitor.[92] The combination enhanced reactive oxygen species generation and apoptosis, leading to greater cell death than either agent singly. Therefore, the use of adjunctive therapy improves the antitumour efficacy of HDACi, overcoming some of the limitations caused by activation of growth pathways by HDACi.

Other factors should be considered in designing trials of HDACi with chemotherapy. The timing of the administration of HDACi in relation to the chemotherapy is likely to be a critical factor. In a study of the A549 lung cancer cell line, SAHA or sodium butyrate HDACi agents were added to the cell culture the day before, the day of or the day after the chemotherapy agent, which in this study was the topoisomerase I inhibitor, camptothecin.[93] The HDACi was most effective in reducing cell survival when added after camptothecin, when camptothecin had already caused G2-M arrest of tumour cells. The timing of other lung cancer therapy may also enhance the efficacy of HDACi. HDACi may act as a radiosensitizer, as shown in breast cancer cell line studies, by increasing mitotic cell death in irradiated cells,[94] although this effect has not yet been shown in lung cancer cell lines.[90]

HDACi in lung cancer clinical trials

Phase 1/2 trials in solid tumours and haematological malignancies have shown that HDACi are well tolerated.[71] There have been two small clinical trials of HDACi in lung cancer. The oral HDACi CI-994 was studied in a phase 1 trial in 30 patients with advanced solid tumours, of whom four had non-small cell lung cancer.[95] Analysis of all tumour subtypes (lung and non-lung) showed that this oral agent was well tolerated when administered with paclitaxel and carboplatin, and the fold increase of lymphocyte histone 3 acetylation was associated with better response rate. In a phase 2 study of 47 patients with chemotherapy-refractory lung cancer (mostly Stage IV), i.v. pivaloyloxymethyl butyrate was well tolerated and demonstrated some anticancer activity.[96] Further trials of combination therapy of HDACi with other chemotherapy agents are in progress. Thus, HDACi has emerged as a promising new modality in the treatment of lung cancer, and more detailed studies are needed.

SUMMARY

Thus, increasing evidence paints the scenario whereby epigenetic alterations such as DNA methylation and histone modifications (which may interact) contribute to the molecular pathogenesis of lung cancer cells, in addition to the more widely known genetic mutations found in all types of lung cancer. Epigenetic alterations are relatively frequent, affect multiple genes and in some cases occur early during lung carcinogenesis. Furthermore, epigenetic changes unlike genetic changes are potentially reversible, leading to an intense study of potential therapeutic translation. For these reasons, much research is focussed on developing the diagnostic, prognostic and therapeutic potential of these changes. Efforts such the Human Epigenome Consortium (HEC) and the proposed Human Epigenome Project should go a long way to advancing our knowledge, helping us to understand why (to date) epigenetics is only rarely implicated in hereditary cancer and thus hopefully lead to more effective treatments in our fight against lung cancer.

REFERENCES

1 Jemal A, Murray T, Ward E *et al.* Cancer statistics, 2005. *CA Cancer J. Clin.* 2005; **55**: 10–30.
2 Ezzati M, Lopez AD. Estimates of global mortality attributable to smoking in 2000. *Lancet* 2003; **362**: 847–52.
3 Zhang H, Cai B. The impact of tobacco on lung health in China. *Respirology* 2003; **8**: 17–21.
4 Danaei G, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors. *Lancet* 2005; **366**: 1784–93.
5 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57–70.
6 Karpinets TV, Foy BD. Tumorigenesis: the adaptation of mammalian cells to sustained stress environment by epigenetic alterations and succeeding matched mutations. *Carcinogenesis* 2005; **26**: 1323–34.
7 Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.* 2002; **3**: 415–28.
8 Robertson KD. DNA methylation and human disease. *Nat. Rev. Genet* 2005; **6**: 597–610.
9 Schrump DS, Nguyen DM. Targeting the epigenome for the treatment and prevention of lung cancer. *Semin. Oncol.* 2005; **32**: 488–502.
10 Tycko B. Epigenetic gene silencing in cancer. *J. Clin. Invest.* 2000; **105**: 401–7.
11 Feinberg AP, Tycko B. The history of cancer epigenetics. *Nat. Rev. Cancer* 2004; **4**: 143–53.
12 Vertino PM, Spillare EA, Harris CC, Baylin SB. Altered chromosomal methylation patterns accompany oncogene-induced transformation of human bronchial epithelial cells. *Cancer Res.* 1993; **53**: 1684–9.
13 Herman JG. Epigenetics in lung cancer: focus on progression and early lesions. *Chest* 2004; **125**: 119S–22S.
14 Hutt JA, Vuillemenot BR, Barr EB *et al.* Life-span inhalation exposure to mainstream cigarette smoke induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways. *Carcinogenesis* 2005; **26**: 1999–2009.
15 Belinsky SA. Silencing of genes by promoter hypermethylation: key event in rodent and human lung cancer. *Carcinogenesis* 2005; **26**: 1481–7.

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UNCORRECTED PROOF

16 Zochbauer-Muller S, Fong KM, Maitra A *et al.* 5′-CpG island methylation of the FHIT gene is correlated with loss of gene expression in lung and breast cancer. *Cancer Res.* 2001; **61**: 3581–5.
17 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.
18 Virmani AK, Rathi A, Zochbauer-Muller S *et al.* Promoter methylation and silencing of the retinoic acid receptor-beta gene in lung carcinomas. *J. Natl. Cancer Inst.* 2000; **92**: 1303–7.
19 Geradts J, Fong KM, Zimmerman PV, Maynard R, Minna JD. Correlation of abnormal RB, p16ink4a, and p53 expression with 3p loss of heterozygosity, other genetic abnormalities, and clinical features in 103 primary non-small cell lung cancers. *Clin. Cancer Res.* 1999; **5**: 791–800.
20 Heller G, Fong KM, Girard L *et al.* Expression and methylation pattern of TSLC1 cascade genes in lung carcinomas. *Oncogene* 2006; **25**: 959–68.
21 Kim JS, Han J, Shim YM, Park J, Kim DH. Aberrant methylation of H-cadherin (CDH13) promoter is associated with tumor progression in primary nonsmall cell lung carcinoma. *Cancer* 2005; **104**: 1825–33.
22 Zochbauer-Muller S, Fong KM, Geradts J *et al.* Expression of the candidate tumor suppressor gene hSRBC is frequently lost in primary lung cancers with and without DNA methylation. *Oncogene* 2005; **24**: 6249–55.
23 Suzuki M, Hao C, Takahashi T *et al.* Aberrant methylation of SPARC in human lung cancers. *Br. J. Cancer* 2005; **92**: 942–8.
24 Izumi H, Inoue J, Yokoi S *et al.* Frequent silencing of DBC1 is by genetic or epigenetic mechanisms in non-small cell lung cancers. *Hum. Mol. Genet* 2005; **14**: 997–1007.
25 Digel W, Lubbert M. DNA methylation disturbances as novel therapeutic target in lung cancer: preclinical and clinical results. *Crit. Rev. Oncol. Hematol.* 2005; **55**: 1–11.
26 Belinsky SA. Gene-promoter hypermethylation as a biomarker in lung cancer. *Nat. Rev. Cancer* 2004; **4**: 707–17.
27 Frommer M, McDonald LE, Millar DS *et al.* A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. *Proc. Natl. Acad. Sci. USA* 1992; **89**: 1827–31.
28 Laird PW. The power and the promise of DNA methylation markers. *Nat. Rev. Cancer* 2003; **3**: 253–66.
29 Fukasawa M, Kimura M, Morita S *et al.* Microarray analysis of promoter methylation in lung cancers. *J. Hum. Genet* 2006; **00**: 000–000.
30 Paz MF, Fraga MF, Avila S *et al.* A systematic profile of DNA methylation in human cancer cell lines. *Cancer Res.* 2003; **63**: 1114–21.
31 Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. *Cancer Res.* 2001; **61**: 3225–9.
32 Belinsky SA, Nikula KJ, Palmisano WA *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.
33 Miyamoto K, Ushijima T. Diagnostic and therapeutic applications of epigenetics. *Jpn. J. Clin. Oncol.* 2005; **35**: 293–301.
34 Belinsky SA, Klinge DM, Dekker JD *et al.* Gene promoter methylation in plasma and sputum increases with lung cancer risk. *Clin. Cancer Res.* 2005; **11**: 6505–11.
35 Ramirez JL, Rosell R, Taron M *et al.* 14-3-3sigma methylation in pretreatment serum circulating DNA of cisplatin-plus-gemcitabine-treated advanced non-small-cell lung cancer patients predicts survival: the Spanish Lung Cancer Group. *J. Clin. Oncol.* 2005; **23**: 9105–12.
36 Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. *Cell* 1980; **20**: 85–93.
37 Kantarjian HM, O'Brien S, Cortes J *et al.* Results of decitabine (5-aza-2′deoxycytidine) therapy in 130 patients with chronic myelogenous leukemia. *Cancer* 2003; **98**: 522–8.
38 Issa JP, Garcia-Manero G, Giles FJ *et al.* Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2′-deoxycytidine (decitabine) in hematopoietic malignancies. *Blood* 2004; **103**: 1635–40.
39 Daskalakis M, Nguyen TT, Nguyen C *et al.* Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2′-deoxycytidine (decitabine) treatment. *Blood* 2002; **100**: 2957–64.
40 Barletta JM, Rainier S, Feinberg AP. Reversal of loss of imprinting in tumor cells by 5-aza-2′-deoxycytidine. *Cancer Res.* 1997; **57**: 48–50.
41 Cheng JC, Matsen CB, Gonzales FA *et al.* Inhibition of DNA methylation and reactivation of silenced genes by zebularine. *J. Natl. Cancer Inst* 2003; **95**: 399–409.
42 Villar-Garea A, Fraga MF, Espada J, Esteller M. Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. *Cancer Res.* 2003; **63**: 4984–9.
43 Segura-Pacheco B, Trejo-Becerril C, Perez-Cardenas E *et al.* Reactivation of tumor suppressor genes by the cardiovascular drugs hydralazine and procainamide and their potential use in cancer therapy. *Clin. Cancer Res.* 2003; **9**: 1596–603.
44 Fang MZ, Wang Y, Ai N *et al.* Tea polyphenol (-) -epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* 2003; **63**: 7563–70.
45 Costello JF, Fruhwald MC, Smiraglia DJ *et al.* Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. *Nat. Genet.* 2000; **24**: 132–8.
46 Suzuki H, Gabrielson E, Chen W *et al.* A genomic screen for genes upregulated by demethylation and histone deacetylase inhibition in human colorectal cancer. *Nat. Genet.* 2002; **31**: 141–9.
47 Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat. Genet.* 1999; **21**: 103–7.
48 Boivin AJ, Momparler LF, Hurtubise A, Momparler RL. Antineoplastic action of 5-aza-2′-deoxycytidine and phenylbutyrate on human lung carcinoma cells. *Anticancer Drugs* 2002; **13**: 869–74.
49 Belinsky SA, Klinge DM, Stidley CA *et al.* Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. *Cancer Res.* 2003; **63**: 7089–93.
50 Weiser TS, Guo ZS, Ohnmacht GA *et al.* Sequential 5-Aza-2′-deoxycytidine-Depsipeptide FR901228 Treat-

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ment Induces Apoptosis Preferentially in Cancer Cells and Facilitates Their Recognition by Cytolytic T Lymphocytes Specific for NY-ESO-1. *J. Immunother.* 2001; **24**: 151–61.
51 Miki K, Shimizu E, Yano S, Tani K, Sone S. Demethylation by 5-aza-2′-deoxycytidine (5-azadC) of p16INK4A gene results in downregulation of vascular endothelial growth factor expression in human lung cancer cell lines. *Oncol. Res.* 2000; **12**: 335–42.
52 Olsson L, Behnke O, Sorensen HR. Modulatory effects of 5-azacytidine, phorbol ester, and retinoic acid on the malignant phenotype of human lung cancer cells. *Int. J. Cancer* 1985; **35**: 189–98.
53 Lantry LE, Zhang Z, Crist KA *et al.* 5-Aza-2′-deoxycytidine is chemopreventive in a 4-(methylnitrosamino) -1-(3-pyridyl) -1-butanone-induced primary mouse lung tumor model. *Carcinogenesis* 1999; **20**: 343–6.
54 Carr BI, Rahbar S, Doroshow JH *et al.* Fetal hemoglobin gene activation in a phase II study of 5,6-dihydro-5-azacytidine for bronchogenic carcinoma. *Cancer Res.* 1987; **47**: 4199–201.
55 Momparler RL, Bouffard DY, Momparler LF, Dionne J, Belanger K, Ayoub J. Pilot phase I–II study on 5-aza-2′-deoxycytidine (Decitabine) in patients with metastatic lung cancer. *Anticancer Drugs* 1997; **8**: 358–68.
56 Schwartsmann G, Schunemann H, Gorini CN *et al.* A phase I trial of cisplatin plus decitabine, a new DNA-hypomethylating agent, in patients with advanced solid tumors and a follow-up early phase II evaluation in patients with inoperable non-small cell lung cancer. *Invest. New Drugs* 2000; **18**: 83–91.
57 Fahrner JA, Eguchi S, Herman JG, Baylin SB. Dependence of histone modifications and gene expression on DNA hypermethylation in cancer. *Cancer Res.* 2002; **62**: 7213–18.
58 Kondo Y, Shen L, Issa JP. Critical role of histone methylation in tumor suppressor gene silencing in colorectal cancer. *Mol. Cell Biol.* 2003; **23**: 206–15.
59 Schrump DS, Nguyen DM, Kunst TE *et al.* Phase I study of sequential deoxyazacytidine/depsipeptide infusion in patients with malignancies involving lungs or pleura. *Clin. Lung Cancer* 2002; **4**: 186–92.
60 Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* 2003; **300**: 455.
61 Espada J, Ballestar E, Fraga MF *et al.* Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J. Biol. Chem.* 2004; **279**: 37175–84.
62 Wolf SF, Migeon BR. Studies of X chromosome DNA methylation in normal human cells. *Nature* 1982; **295**: 667–71.
63 Fraga MF, Uriol E, Borja Diego L *et al.* High-performance capillary electrophoretic method for the quantification of 5-methyl 2′-deoxycytidine in genomic DNA: application to plant, animal and human cancer tissues. *Electrophoresis* 2002; **23**: 1677–81.
64 Kawasaki H, Taira K. Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. *Nature* 2004; **431**: 211–17.
65 Morris KV, Chan SW, Jacobsen SE, Looney DJ. Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* 2004; **305**: 1289–92.
66 de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* 2003; **370**: 737–49.
67 Strachan T, Read AP. *Human Molecular Genetics*, 3rd edn. Garland Science, New York, 2004.
68 Yang XJ, Gregoire S. Class II histone deacetylases: from sequence to function, regulation, and clinical implication. *Mol. Cell Biol.* 2005; **25**: 2873–84.
69 Dokmanovic M, Marks PA. Prospects: histone deacetylase inhibitors. *J. Cell Biochem.* 2005; **96**: 293–304.
70 Sengupta N, Seto E. Regulation of histone deacetylase activities. *J. Cell Biochem.* 2004; **93**: 57–67.
71 Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat. Rev. Cancer* 2006; **6**: 38–51.
72 Kaneda R, Toyota M, Yamashita Y *et al.* High-throughput screening of genome fragments bound to differentially acetylated histones. *Genes Cells* 2004; **9**: 1167–74.
73 Toh Y, Ohga T, Endo K *et al.* Expression of the metastasis-associated MTA1 protein and its relationship to deacetylation of the histone H4 in esophageal squamous cell carcinomas. *Int. J. Cancer* 2004; **110**: 362–7.
74 Shetty S, Graham BA, Brown JG *et al.* Transcription factor NF-kappaB differentially regulates death receptor 5 expression involving histone deacetylase 1. *Mol. Cell Biol.* 2005; **25**: 5404–16.
75 Zhu P, Martin E, Mengwasser J, Schlag P, Janssen KP, Gottlicher M. Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis. *Cancer Cell* 2004; **5**: 455–63.
76 Sasaki H, Moriyama S, Nakashima Y *et al.* Histone deacetylase 1 mRNA expression in lung cancer. *Lung Cancer* 2004; **46**: 171–8.
77 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.
78 Bartling B, Hofmann HS, Boettger T *et al.* Comparative application of antibody and gene array for expression profiling in human squamous cell lung carcinoma. *Lung Cancer* 2005; **49**: 145–54.
79 Ito K, Ito M, Elliott WM *et al.* Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N. Engl. J. Med.* 2005; **352**: 1967–76.
80 Park JM, Lee GY, Choi JE *et al.* No association between polymorphisms in the histone deacetylase genes and the risk of lung cancer. *Cancer Epidemiol. Biomarkers Prev.* 2005; **14**: 1841–3.
81 Mukhopadhyay NK, Gordon GJ, Maulik G *et al.* Histone deacetylation is directly involved in desilencing the expression of the catalytic subunit of telomerase in normal lung fibroblast. *J. Cell Mol. Med.* 2005; **9**: 662–9.
82 Bartova E, Pachernik J, Harnicarova A *et al.* Nuclear levels and patterns of histone H3 modification and HP1 proteins after inhibition of histone deacetylases. *J. Cell Sci.* 2005; **118**: 5035–46.
83 El-Khoury V, Gomez D, Liautaud-Roger F, Trussardi-Regnier A, Dufer J. Effects of the histone deacetylase inhibitor trichostatin A on nuclear texture and c-jun gene expression in drug-sensitive and drug-resistant

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human H69 lung carcinoma cells. *Cytometry A* 2004; **62**: 109–17.
84 Kodani M, Igishi T, Matsumoto S *et al.* Suppression of phosphatidylinositol 3-kinase/Akt signaling pathway is a determinant of the sensitivity to a novel histone deacetylase inhibitor, FK228, in lung adenocarcinoma cells. *Oncol. Rep.* 2005; **13**: 477–83.
85 Choi YH. Induction of apoptosis by trichostatin A, a histone deacetylase inhibitor, is associated with inhibition of cyclooxygenase-2 activity in human non-small cell lung cancer cells. *Int. J. Oncol.* 2005; **27**: 473–9.
86 Doi S, Soda H, Oka M *et al.* The histone deacetylase inhibitor FR901228 induces caspase-dependent apoptosis via the mitochondrial pathway in small cell lung cancer cells. *Mol. Cancer Ther.* 2004; **3**: 1397–402.
87 Mayo MW, Denlinger CE, Broad RM *et al.* Ineffectiveness of histone deacetylase inhibitors to induce apoptosis involves the transcriptional activation of NF-kappa B through the Akt pathway. *J. Biol. Chem.* 2003; **278**: 18980–9.
88 Rundall BK, Denlinger CE, Jones DR. Suberoylanilide hydroxamic acid combined with gemcitabine enhances apoptosis in non-small cell lung cancer. *Surgery* 2005; **138**: 360–7.
89 Maxhimer JB, Reddy RM, Zuo J, Cole GW, Schrump DS, Nguyen DM. Induction of apoptosis of lung and esophageal cancer cells treated with the combination of histone deacetylase inhibitor (trichostatin A) and protein kinase C inhibitor (calphostin C). *J. Thorac. Cardiovasc. Surg.* 2005; **129**: 53–63.
90 Loprevite M, Tiseo M, Grossi F *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.
91 Sonnemann J, Gange J, Kumar KS, Muller C, Bader P, Beck JF. Histone deacetylase inhibitors interact synergistically with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to induce apoptosis in carcinoma cell lines. *Invest. New Drugs* 2005; **23**: 99–109.
92 Denlinger CE, Rundall BK, Jones DR. Proteasome inhibition sensitizes non-small cell lung cancer to histone deacetylase inhibitor-induced apoptosis through the generation of reactive oxygen species. *J. Thorac Cardiovasc. Surg.* 2004; **128**: 740–8.
93 Bevins RL, Zimmer SG. It's about time: scheduling alters effect of histone deacetylase inhibitors on camptothecin-treated cells. *Cancer Res.* 2005; **65**: 6957–66.
94 Nome RV, Bratland A, Harman G, Fodstad O, Andersson Y, Ree AH. Cell cycle checkpoint signaling in-volved in histone deacetylase inhibition and radiation-induced cell death. *Mol. Cancer Ther.* 2005; **4**: 1231–8.
95 Pauer LR, Olivares J, Cunningham C *et al.* Phase I study of oral CI-994 in combination with carboplatin and paclitaxel in the treatment of patients with advanced solid tumors. *Cancer Invest.* 2004; **22**: 886–96.
96 Reid T, Valone F, Lipera W *et al.* Phase II trial of the histone deacetylase inhibitor pivaloyloxymethyl butyrate (Pivanex, AN-9) in advanced non-small cell lung cancer. *Lung Cancer* 2004; **45**: 381–6.

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