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Epigenetic Control of Tumor Suppressor Genes in Lung Cancer

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1. Introduction

Lung cancer is the number one cause of cancer-related deaths worldwide. It claims 1.3 million lives every year (www.who.int/mediacentre) (1). To understand the mechanism of lung carcinogenesis is one of the essential tasks for effective control of lung cancer. Lung cancer and other cancers have long been viewed as “genetic diseases” (2, 3). Particularly, tumor suppressor gene (TSG) deactivation plays a critical role in carcinogenesis.

Historically, majority of the scientific evidence on tumor suppressor gene-related carcinogenesis describes the genetic defects occurring either in the TSGs themselves, such as mutations, or in their environment, such as activation of their inhibitors. Therapeutic applications of such knowledge against cancers have been attempted through reconstituting wild-type TSG products in target cells/tissues by genetic manipulation or biological or chemical molecules, thereby restoring the functions of TSGs and possibly slowing cancer progression.

Ever since the 'two-hit' hypothesis (4) and the first proposal of a potential tumor suppressor gene being involved in the retinoblastomas formation (5), especially after the function of wild-type p53 gene was clearly described (6, 7), the therapeutic research effort has been shifted from optimizing the non-specific radiation and chemotherapy that mainly kill fast-dividing cells to targeting the specific genetic changes. Restoring the function of TSGs has been considered as one of the most promising directions for cancer therapy.

Directly transfecting TSGs into the cancer cells to restore the function of the TSGs and inhibit the tumor growth has become a promising direction to develop novel cancer therapy for the past two decades. Adenoviral and retroviral vectors have been used to deliver TSGs into human tumors by intratumoral or regional administration. The best example is the adenoviral carried wild-type p53 gene used to treat patients with non-small cell lung cancer (NSCLC) by direct intratumoral injection (8). Nonviral gene delivery methods such as cationic liposomes and cationic polymers were also developed for delivering TSGs because of their distinct advantage of lack of immunogenicity (9-11). Our lab has developed a unique cationic liposomal p53 and cationic polymers p53 to treat orthotopic human NSCLC model in mice with intratracheal or aerosol administration, which significantly enhanced the gene delivery efficiency to the airway epithelium and markedly reduced the systemic toxicity, the results showed a great therapeutic potential in the preclinical orthotopic human NSCLC xenografts in mice (12, 13).

However, the overall efficacy of these TSG therapies by direct gene transfection or delivery was limited. One of the important reasons is that most cases of “loss of function” found in tumor tissues are at the mature stage of the carcinogenesis process, at which moment the multiple irreversible genetic defects are established already, therefore, it is very difficult to reverse the carcinogenesis process by restoring or correcting the functions of one or a few TSGs.

More recently, increasing evidence, particularly the “cancer stem cell” model suggests that epigenetic changes, which occur in normal stem or progenitor cells, are the earliest events in cancer initiation (14). Therefore, to catch the early cancer-specific epigenetic changes and reverse them logically becomes a better strategy than those focusing on the irreversible genetic defects.

Multiple research groups have demonstrated that correcting the aberrant methylation in the promoter region of TSGs could inhibit cancer development. Among these studies, we have directly proved the concept in the orthotopic lung cancer models in mice. Briefly, we first proved that azacytidine (Aza), a demethylation agent, could effectively demethylate the hypermethylated promoter of RASSF1a gene (a TSG), at non-cytotoxic concentration range which was a thousand fold lower than its cytotoxic concentration. Then we used intratracheal injection of Aza at a non-cytotoxic dose to treat the airway inoculated NSCLC xenografts in mice, and we found that the demethylation treatment significantly prolonged the life of the tumor-bearing mice, and the locoregional therapy for localized lung cancer in the airway epithelium was significantly superior than the systemic (IV) treatment. Our results demonstrated that the model NSCLC could be inhibited if the TSGs were reactivated by the reversal of the hypermethylation in the promoter regions. In this chapter, we will also discuss the possible application, the advantages and limitation of the current epigenetic methods aimed to enhance cancer therapeutic efficacy by promoting TSGs.

Restoring TSG functions through epigenetic manipulations will be a promising strategy for cancer therapy and prevention, which shifts the focus from treating cells with irreversible genetic lesions to targeting the reversible epigenetic changes. Further work in this field will complement our knowledge of TSG-expression control and enhance our understanding of the carcinogenesis process. This exciting new therapeutic strategy could potentially reduce cancer mortality when applied to populations of individuals at risk.

In this chapter we will briefly summarize the studies describing the aberrant epigenetic alterations in lung cancer and the methods to control carcinogenesis with epigenetic manipulations, particularly, through controlling TSGs. We will also outline advances in the potential use of these epigenetic events for cancer diagnosis, prognosis and targeted epigenetic therapy, and present an experimental study of demethylation therapy at the preclinical level.

2. Epigenetic changes and lung cancer

Epigenetic changes usually are heritable changes in gene expression level without alteration of DNA sequence. Unlike genetic changes, epigenetic changes are reversible. The normal epigenetic process is important for gene expression and genome stability. When this normal process is disrupted, carcinogenesis may start. Therefore, epigenetic changes are considered a key player in the onset and progression of different type of cancers including lung cancer. The most frequently reported epigenetic phenomena are chromatin modifications including post-translational modifications of histones and chromatin modifying complexes, non-

coding RNAs mediated regulations, and particularly DNA methylation. Epigenetic processes are finely tuned, undergo many regulations in response to the environment, and involve almost all the signaling pathways described in the literature. Epigenetics plays a crucial role in the control of nuclear architecture and gene activity and constitutes one of the bases of the biological diversity. In the first part of this chapter, we mainly focus on DNA methylation and its links to TSGs and lung cancer.

2.1 DNA methylation and lung cancer

DNA methylation is the most widely studied epigenetic modification restricted to the DNA motif called CpG dinucleotides, i.e. cytosine followed by guanine residues (15). Enriched in genomic regions known as “CpG islands,” these CpG dinucleotides are typically at least 200 bp and up to several Kb in length with a high GC percentage, and are mainly found near or at the transcription start site within the promoter of about 40% of mammalian genes. CpG islands play a major role in the process of transcriptional regulation, and the ability of a gene to be or not to be transcribed is correlated with the unmethylated and methylated status of a CpG island, respectively, in the presence of the required co-regulators.

Methylation is the only covalent modification of the DNA in mammalian cells and carries out normal physiological functions during embryonic development (16), genomic imprinting (17), and chromosome-X inactivation (18). However, frequent alterations in DNA methylation are observed in cancers such as hypermethylation of CpG islands at tumor suppressor gene (TSG) loci leading to the loss of their expression, genome-wide hypomethylation in the body of genes and in DNA repetitive sequences leading to genomic instability (16), and altered DNA methyltransferases (DNMTs) expression (17).

DNA hypermethylation in the promoter region of TSGs is frequently found in human lung cancer tissues and cell lines. It was proved to be responsible for silencing the TSG and therefore promoting the initiation and development of the lung carcinogenesis. The best-studied example is the case of p16INK4a (CDKN2A); its promoter hypermethylation prevents the negative control exerted by p16INK4a on RB phosphorylation, thereby promoting cell cycle progression. p16INK4a hypermethylation is considered as one of the earliest event in lung tumorigenesis and increases constantly with disease progression (19, 20). An increasing number of other genes have been investigated for their methylation status in lung cancer, including h-cadherin (CDH13) (21), 14-3-3 σ (22), death associated protein kinase 1 (DAPK1) (23), ras association domain family 1 gene (RASSF1a) (24), caspase-8 (25), retinoic acid receptor β -2 (RAR- β), tissue inhibitor of metalloproteinase 3 (TIMP3), o6-methylguanine DNA methyltransferase (MGMT), E-cadherin (ECAD), glutathione s-transferase p1 (GSTP1) (26), FHIT (27, 28), ASC/TMS1, HSRBC, TSLC1, DAL-1, and PTEN (29). Since these genes are involved in a broad range of biological processes, such as cancer cell cycle regulation, proliferation, apoptosis, cell adhesion, mobility, and DNA repair, promoter DNA hypermethylation may be a key event in lung carcinogenesis. Furthermore, genome-wide analyses have suggested that the presence of promoter DNA hypermethylation is probably more extensive than previously thought (30-33). For instance, Shames et al. recently identified 132 genes that are methylated with high penetrance in lung cancer cells (32). More strikingly, Brena et al. reported 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 (30).

Due to the spontaneous hydrolytic deamination under physiological conditions, methylated cytosine can be considered as a potent endogenous mutagen for C to T mutations, therefore

DNA hypermethylation can actually predispose to mutational events (14). Although representing only 1% of the bases in the mammalian genome, methylated cytosine might be responsible for as much as 30% of all transition mutations found in human disease such as cancers (34, 35).

The mechanism of the aberrant hypermethylation in the promoter region of TSGs in human non-small-cell lung cancer especially among smoker patients was described as the overexpression of DNA methyltransferases including DNMT1, DNMT3A and DNMT3B. Interestingly, polymorphisms that influence expression of the DNMT3B gene have been connected with increasing risk of lung cancer (36, 37). An adverse consequence of methylation of CpG sites appears to facilitate the binding for benzo[a]pyrene, a carcinogen, found in cigarette smoke, leading to the formation of major DNA damage hotspots in human lung cancer (38, 39). A well-illustrated example of this phenomenon is the occurrence of some hotspot mutations of the p53 tumor suppressor gene in lung tumors (40, 41).

Clinical evidence also showed that TSG promoter methylation is associated with the smoking history of patients with lung cancer. In lung adenocarcinomas and squamous cell carcinomas, the frequency of p16, MGMT, RASSF1, MTHFR, and FHIT promoter methylation was significantly higher among smokers than never-smokers (42-45); but the promoter methylation of other genes such as RASSF2, TNFRSF10C, BHLHB5, and BOLL was higher in never-smoker lung cancer patients than those of smokers (46, 47), suggesting smoking may target specific genes for methylation.

The roles of methylation in lung cancers for early detection, risk assessment, disease progression, and prognosis have also been studied. DNA methylation may serve as a marker for the early detection of lung cancer when found in the sputum of the patient (19, 48). For example, p16INK4a and MGMT promoter methylation could predict the development of squamous cell carcinoma up to three years before clinical diagnosis (49, 50), and RASSF1A, APC, ESR1, ABCB1, MT1G, and HOXC9 genes were found more frequently methylated in stage I lung adenocarcinomas/squamous cell carcinomas than the non-cancerous lesions (51), whereas the prevalence of hDAB2IP, H-cadherin, DAL-1, and FBN2 methylation was associated significantly with advanced stage of lung cancer (52-54). Altogether, these studies highlight promoter methylation as a promising epigenetic approach for early detection and prognosis of NSCLC.

Demethylating drugs have great and promising clinical potential based on their ability to restore the expression of epigenetic silenced TSGs and inhibit tumor cell growth, while inducing manageable short-term side effects at the effective doses (55). The 5-aza-2'-deoxycytidine demethylating agent has been reported to increase the survival of chemotherapy-naïve NSCLC patients, up to 6 years in some cases (56), although the relatively high dose in the treatment could be suspected to contribute partially cytotoxic effect to the final therapeutic outcome. More therapeutic investigations are underway, aiming at combining the demethylating agents with histone deacetylase inhibitors and attempting to integrate epigenetic therapy with more standard therapy.

2.2 Chromatin modifications and lung cancer

Chromatin is formed by basic units called the nucleosome, which is assembled by wrapping approximately 147 bp of genomic DNA around a histone octamere containing two copies of each of the core histones H2A, H2B, H3 and H4. The core histones possess an amino-terminal tail that protrudes outside of the nucleosome, which are subjected to a wide variety of post-translational covalent modifications such as acetylation, methylation,

phosphorylation and ubiquitinylation. Access to the chromatin is thus affected by these modifications and therefore influences almost all DNA-based processes. Consequently, the structure and integrity of the genome and normal patterns of gene expression are potentially affected by the global alterations of histone modification patterns.

The protein complexes that regulate transcription by modifying histones or altering chromatin structure 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. Histone deacetylation mediated by HDACs works synergistically to alter the chromatin condensation status and represses transcription with DNMTs and a group of methylated DNA-binding proteins (57). In general, high HDAC activity is associated with condensed, transcriptionally inactive chromatin.

Altered expression pattern of histone and chromatin modifying enzymes have been found in human tumors, and histone modifications may contribute to tumorigenesis (58, 59). A clinical study including 138 lung cancer patients demonstrated that changes in global levels of histone 3 lysine 4 dimethylation (H3K4diMe), histone 3 lysine 9 acetylation (H3K9Ac), and histone 2A lysine 5 acetylation (H2AK5Ac) are predictive of the clinical outcome of lung cancers. Seligson et al (58) discovered that lower cellular levels of H3K4diMe and H3K18Ac predict significantly poorer survival probabilities for lung cancer patients (60). It has been summarized that the status of acetylation and methylation of specific lysine residues contained within the tails of nucleosome core histones is crucial in regulating chromatin structure and gene expression (61, 62).

In addition to this epigenetic function, certain HDACs also exhibit important cytoplasmatic function by controlling the acetylation status and function of numerous cytoplasmic proteins and transcription factors that may be important in carcinogenesis (63). Moreover, Sasaki et al (64) reported that expressions of HDAC1 correlated with the progression of lung carcinomas. Bartling et al (65) found HDAC3 upregulation in squamous lung cancers compared with non-tumor tissues in lung. Osada et al discovered that in a group of 72 lung cancer patients, the reduction of class II HDAC gene expression was clearly associated with poor prognosis (66). These results suggested that HDAC might be involved in lung cancer occurrence, progression, and prognosis and that inhibition of HDAC activity might be a possible target for lung cancer treatment.

Increasing laboratory evidence has illustrated the therapeutic mechanisms of HDACi: e.g., HDAC inhibitor FK228 suppressed the PI3K/Akt (67) and Src/Raf/MEK/ERK1/2 (68) signaling pathways, resulting in the downregulation of the anti-apoptotic proteins Bcl-2 and Bcl-xL, upregulation of the pro-apoptotic protein Bax, and the induction of time-dependent apoptosis in both adenocarcinoma (69) and small cell carcinoma cells (69, 70). Coincident with inhibition of ERK1/2 and PI3K/AKT survival pathways, the HDAC inhibitor FK228 enhanced JNK and p38MAPK signaling (68), whereas an SIRT1 inhibitor, Sirtinol, impaired activation of Ras/MAPK pathways in response to EGF and insulin-like growth factor-I (71). Furthermore, another HDAC inhibitor, trichostatin A, suppressed the levels of COX-2 mRNA and protein expression, which were correlated with an inhibition in prostaglandin E2 synthesis in lung adenocarcinoma cells (69). 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.

Recently, targeting HDAC activity using inhibitors of HDAC (HDACi) has become a novel and promising anticancer strategy, in particular in the treatment of advanced NSCLC where phase I and II trials have been completed (72). In addition, several HDACi have been shown to increase the cytotoxic effects of radiation in NSCLC by decreasing DNA repair efficiency and promoting cell death (73). HDACi also showed favorable results 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 (74, 75). Ongoing clinical trials are exploring the use of many new HDACi alone or as part of a combination with existing therapeutic modalities such as chemotherapy or radiotherapy (76).

2.3 Micro-RNAs and lung cancer

Micro-RNAs (miRNAs) are small non-coding RNAs with ~22 nucleotides in length (77, 78). miRNAs control a wide range of biological processes including apoptosis, development, proliferation and differentiation (78). High-throughput analyses have highlighted aberrant miRNA expression profiles in an increasing range of human cancer types (79-81) and all these studies suggested that cancer cells express altered miRNAs patterns consisting of both overexpression and downregulation. 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 laboratory and clinical studies demonstrate a deregulation of miRNA expression in lung cancer and highlight them as useful diagnostic, prognostic and therapeutic tools. A growing number of miRNAs has been found aberrantly expressed in lung cancer and our understanding of miRNA expression patterns and functions in normal and lung cancer cells is just starting to emerge. Such miRNAs as miR-21, miR-126, miR-31, miR-519c, Let-7a, miR-133B, miR-15a, miR-16, and miR-183 have been found to regulate lung cancer cell proliferation, migration and invasion by targeting specific molecules, including Crk, EGFL7, VEGF, LATS2, PPP2R2A, HIF-1 α , NIF, MCL-1, Bcl-2, cyclins D1, D2 and E1, and Ezrin (82-89).

Growing evidence indicates that miRNA expression profiles confer important clues for clinical diagnosis and prognosis of human lung cancer. MicroRNA microarray analyses have identified profiles which could discriminate lung cancers from noncancerous lung tissues, as well as molecular signatures that differ according to tumor histology (80, 90). Interestingly, recent identification of Has-miR-205 has suggested it to be a highly specific marker for squamous carcinoma (91), therefore a clinical diagnostic assay based on miR-205 expression levels could aid the differential diagnosis of NSCLCs. Since miRNAs are more stable than mRNA and more tissue specific than DNA, their measurement could provide a novel and promising non-invasive approach to discriminate between normal and cancer patient samples. Studies found that aberrant miRNA expression could be used as a marker for the diagnosis of NSCLC in sputum specimen (92) and miRNA expression in peripheral blood or in serum correlated well with its expression in the tumor sample (93).

In terms of prognostic value, several miRNAs are reported to be associated with the clinical outcome of lung cancer. For instance, clinical study indicated that overexpression of mature miR-21 in the tissue and sputum samples could be an independent negative prognostic

factor for overall survival in NSCLC patients (94). Detection of miR-21 expression in sputum as a non-invasive approach for the diagnosis of lung cancer confers better sensitivity than sputum cytology (92). Clinical studies also showed reduced let-7 (81) and miR-34 (95) expression or enhanced miR-146b (96) and miR155 (80) expression with short survival or a high probability of relapse in patients with NSCLC. In patients with NSCLC, a five-miRNA signature including miR-221, let-7a, miR-137, miR-372, and miR-182 was identified and validated as an independent predictor of cancer relapse and survival (97). Remarkably, this signature is valuable even after patient stratification by stage or histology. In addition, expression levels of miR-486, miR-30d, miR-1, and miR-499 in serum could be used to predict survival for patients with NSCLC (98). Overexpression of miR-155 correlates with a poor prognosis when all clinical variables are considered together (80). Since miRNAs are upstream regulators of gene expression, they may be more powerful prognostic markers than their downstream target genes. For example, miR-146b alone was found to have a predictive accuracy for prognosis in ~78% of patients with lung squamous cell carcinoma (96), better than the overall predictive accuracy of 68% for a 50-gene signature (99).

The potential for using miRNAs in lung cancer therapy is now being explored. Let-7 overexpression confers radio-sensitivity to lung cancer cells (100). miR-128b LOH, a direct regulator of EGFR, correlates with clinical response and survival following gefitinib treatment (101). miR-221, miR-222 and miR-17-92 sensitize lung cancer cells to cytotoxic agents (102-104). 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 clinic.

Accumulative scientific evidence suggests that cancer, particularly lung cancer, is not only a genetic disease (2, 3), but also an epigenetic disease. Laboratory and clinical studies clearly demonstrate that many aberrant epigenetic events occurring before the genetic changes are responsible for the cancer initiation and progression. It has been confirmed that disruption of the normal epigenetic processes promotes lung carcinogenesis and lung tumor growth through complicated mechanisms involving TSGs silencing and oncogene activation. Majority of these disruptions are found to be the consequence of exposure to environmental carcinogens, particularly from cigarette smoking causing heritable epigenetic changes. The management of aberrant epigenetic states as a way to target early lung cancer development or lung tumor progression is therefore a logical therapeutic approach.

In the future, to develop new anti-tumor agents, such as DNMTi or HDACi, and the specific treatment strategies including tumor-targeted drug delivery system and specific administration routes, and to avoid the non-specific toxicity of anti-cancer drugs, will be of particular interest. Indeed, the side effects of these epigenetic compounds may have unscheduled consequences in terms of gene expression, in that they may display growth-promoting effects on tumor cells. As more critical miRNAs are found and the expression of many of them reduced in lung cancer cells, targeting miRNA is becoming a promising strategy in terms of cancer treatment. Administration of synthetic oligonucleotides that mimic endogenous miRNAs might be used to treat specific tumor types if an effective delivery system can be developed. Moreover, targeting oncogenic miRNAs through administration of anti-sense oligonucleotides, called anti-miRNA oligonucleotides (AMO) is coming into focus, given that the use of antagomirs, which are AMOs conjugated with cholesterol, has emerged as an efficient approach to inhibit miRNA activity (105). Further

studies to uncover the potential usefulness of chromatin modifying drugs in restoring the loss of expression of tumor suppressor miRNAs are underway. There is no doubt that a more comprehensive dissection of the cellular and molecular pathways controlled by epigenetic processes will provide new insights into cancer related mechanisms and will highlight promising fields for the development of novel therapies to fight lung cancer.

3. A preclinical study to use demethylating agent to treat orthotopic human lung cancer xenografts

Lung cancer has remained as the number one cause of cancer-related deaths worldwide for decades (106). Traditional methods are mainly non-specific cytotoxic radiation therapy and chemotherapy; their non-specificity and therefore, life-threatening toxicity determined their limitation in the application. There is an urgent need to develop more sensitive diagnosis and more effective therapeutic methods to save lung cancer patients. About 90% of lung cancer cases are the end result of cumulative aberrant epigenetic changes and genetic damage to the respiratory epithelium chronically exposed to environmental, particularly tobacco carcinogens (107-109). One of the well accepted mechanisms of carcinogenesis in lung cancer is the aberrant methylation of CpG islands in the promoter regions of tumor suppressor genes (TSGs) leading to underexpression or absence of the proteins of those genes thus propagating tumorigenesis (110).

It has also been proved that the promoter hypermethylation down regulated TSGs can be reversed by DNA-methyltransferase inhibitors (DMTI) like azacytidine (Aza), Aza-2'-deoxycytidine, and Zebularine (11, 111, 112). These agents have been in clinical treatment for NSCLC patients by systemic administration with high doses and limited efficacy.

Inhalation of carcinogens, mainly as a result of tobacco exposure, causes a field cancerization effect thereby placing the entire bronchial epithelium at risk of developing bronchogenic carcinoma. Any strategies that aim at decreasing the incidence of lung cancer or decreasing the incidence of a second primary in a patient with a history of lung cancer would have to have an effect on the entire bronchial epithelium. In the case of a pharmacologic agent, this would be possible and feasible by inhalation of aerosolized solution of the drug. DMTI agents like Aza have the potential to reexpress tumor suppressor genes, which might lead to reversal of premalignant changes, slow the carcinogenesis process, and eventually decrease the incidence of bronchogenic carcinoma (113). Systemic administration of these drugs has been explored in advanced NSCLC patients but not pursued because of significant systemic toxicity (114).

In this part we present a study with two objectives: 1. To prove that whether reversing the hypermethylation in promoter region of the TSGs can make a positive contribution to the therapeutic outcome of lung cancer. 2. To prove whether the airway administration more effective than systemic administration to treat airway localized advanced bronchial premalignancy or endobronchial lung cancer. In this study, we used intratracheal injection of Aza to treat the orthotopic lung cancer models in mice, the low dose (non-cytotoxic dose) we used mainly brought demethylating effect of Aza and avoided its cytotoxic effect.

3.1 Experimental design and methods

In order to prove whether demethylation on the hypermethylated promoters of TSGs will contribute therapeutic efficacy in the preclinical level, we tested the demethylation function of a typical demethylation agent azacytidine (Aza) in vitro and in vivo. Particularly, we

used intratracheal injection of Aza to treat orthotopic lung cancer xenografts in the efficacy study. The detailed experimental studies are presented below.

We selected three different human non-small cell lung cancer cell lines for the in vitro and in vivo studies: a squamous cell carcinoma cell line H226, a bronchioalveolar carcinoma cell line H358, and a metastatic large cell carcinoma H460 from pleural effusion. All cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured per ATCC's protocols.

3.1.1 In vitro studies

In order to distinguish the cytotoxic effect and demethylation effect of Aza, we used MTT assay to determine the growth inhibition range of Aza and methylation-specific PCR with the samples treated by a set concentrations of Aza to detect the minimal concentration for the effective demethylation at the promoter region of TSGs. MTT assay used was literature method (115), briefly, ~5,000 cells in 0.135 μ L RPMI 1640/well were seeded in 96-well plates. After 24 h of culture, Aza at various concentrations was added to the cells. Three days later, the cells were stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lysed. The absorbance of each well was measured in a microplate reader at 570 nm. The percent growth inhibition of the cells was calculated as the absorbance of treated cells normalized to no treatment cells.

We selected RASSF1a as the first candidate TSG to prove the concept. Because it was found silenced by promoter hypermethylation in 32.6% of NSCLC patients (116). The human NSCLC cells H226 were treated with Aza at 0.1, 1, and 10 ng/ml. On day 4 after the treatment, the cells were harvested. About 8×10^4 cells were used to detect the methylation status of the RASSF1a promoter using EZ DNA Methylation-Direct Kit™ (Zymo Research Corp., Orange, CA, USA), according to the manufacturer's instructions. The bisulfate-converted DNA was then used as a template for methylation-specific PCR reactions using primers specific for either the modified methylated or modified unmethylated promoter sequences of the RASSF1a genes. The primers used have been described previously by others (117). The sequences are: methylated: 5'-GGG TTT TGC GAG AGC GCG-3' (forward) and 5'-GCT AAC AAA CGC GAA CCG-3' (reverse); unmethylated: 5'-GGT TTT GTG AGA GTG TGT TTA G-3' (forward), and 5'-CAC TAA CAA ACA CAA ACC AAA C-3' (reverse). Briefly, PCR reactions contained 1-4 microliters of bisulfate-converted DNA, purified as above, 300 ng each of forward and reverse primers, 45 microliters of Platinum® PCR SuperMix (Invitrogen), and distilled water to a final reaction volume of 50 microliters. PCR amplification conditions were as described in the literature (117), unless otherwise noted. The PCR products were separated on 2% agarose gels containing ethidium bromide and were visualized under UV illumination.

3.1.2 Animal studies

The studies were begun from testing acute toxicity and finding the therapeutic dose. CD-1 mice (Harlan) were used to evaluate and compare the acute toxicities of Aza by the intratracheal or intravenous routes. Two groups of mice (5-8 mice/group) were treated with 90 mg/kg of Aza via intravenous injection (IV) or intratracheal injection (IT), respectively. The dose of 90 mg/kg is the maximum tolerated dose (MTD) of IV Aza in mice. The IV and IT injection methods were described previously (12, 118). Briefly, for the IT the mice were anesthetized with intraperitoneal injection of 30-50 mg/kg of Nembutal or isoflurane

inhalation, fixed on the small animal fixing board. The mouth of mouse was open with a forceps; the drug solution or cell suspension was carefully injected into the trachea through mouth via a 22-gage feeding needle attached to a 1 ml syringe. The injection volume did not exceed 100 μ l/mouse. If necessary, a “tube” type of light inserting into the mouth can be used to help locate the trachea.

For myelotoxicity assessment, blood (100 μ l/mouse, 5 mice/group) was drawn from the tail vein before treatment (at day 0) and on days 4, 7, 14, and 28 after treatment. Red blood cells (RBC) were removed from the blood samples using RBC lysis buffer (eBioscience San Diego, CA) as per the manufacturer’s protocol. White blood cells (WBC) were collected and counted with a hemacytometer under a microscope. Blood samples from mice without treatment were used as controls. In the organ toxicity studies, groups of mice given IT of Lactated Ringer’s Solution or without treatment were used as vehicle and normal controls, respectively. Creatinine levels, and liver function tests were determined at Antech Diagnosis (Lake Success, NY). Organ pathological examination was performed at different time points. Briefly, 5 mice in each group were euthanized on day 4, 7, 14, and 28 days after administration of the drug. Blood was drawn from the caudal vena cava, and lungs, livers and kidneys were resected and fixed with 10% buffered Formalin. The fixed tissues were processed with standard procedure for H&E staining. The toxicity levels were determined by giving toxicity grade to each tissue sample. The grading based on the general pathology guidelines was 0 to 4, they reflect a percentage of damaged tissue of 0, <10 (mild), 10-30 (moderate), 30-60 (severe), >60 (life threaten), respectively.

To mimic human NSCLC, we developed a mouse model by intratracheal inoculation of human lung cancer cells in nude mice (12). Briefly, the nude mice (in this particular example, male and female NCRNU-M-F nude mice, 6-7 weeks old, purchased from Taconic Farms, Germantown, NY) were given tumor inoculation with $2\sim 5 \times 10^6$ cells/mouse by IT described above. In this model, we found that the cancer cells initially attached on the airway epithelium of the mice and survived (from day 0 to 10), and then they formed micro nodules in the airway (from day 7 to 21), and finally the tumors invaded lung tissue (from day 14 to 35). The tumors mainly remain in the lung during the rest of lifetime of the mice, and the animal on average die on day 45 to 70 from the lung tumor burden (13). This model mimics the human NSCLCs that develop on the airway epithelium before they invade the lung parenchyma, and it is one of human lung cancer relevant models to evaluate lung cancer therapeutics by different administration routes, particularly by airway administration.

In the antitumor efficacy test, we used a relative low dose of Aza with intratracheal injection to avoid the cytotoxic effect and emphasize the demethylation effect of Aza. Ten days after the intratracheal tumor inoculation, the nude mice were randomly divided into 3 groups of 5 mice each in each test, and were treated with daily intravenous injection (IV) of Aza at 6.25 mg/kg/day $\times$ 6 doses or intratracheal injection (IT) of Aza at 2.5 mg/kg/every other day $\times$ 3 doses. The IT used the same method of the tumor inoculation described above. These optimal doses for the therapeutic study were determined in prior dose ranging studies. A group of mice without treatment was used as control. Survival was used as the major endpoint to evaluate efficacy.

The same Aza formulation was used for the IT and IV treatment. It was made by dissolving azacytidine powder (Sigma, St Louis, MO) in Lactated Ringer’s Injection (Hospira, Inc., Lake Forest, IL) and passing the solution through 0.22 μ m filter immediately prior to use.

3.1.3 Statistical analysis

Differences among different groups were analyzed by two-side Log Rank Assay. A difference was considered statistically significant when $p < 0.05$.

3.2 Results

3.2.1 Growth inhibition and demethylation function of Aza in human NSCLC cell lines

Aza has the both functions. In order to know whether Aza’s demethylation effect can function at a non-toxic concentration, we measured its cell growth inhibition and demethylation function in the selected human NSCLC cell lines. We found that Aza inhibited the growth of the NSCLC cells in a dose dependent manner as shown in Figure 1. The 50% inhibitory concentrations (IC₅₀) of Aza were 0.6, 3.4, and 4.9 $\mu\text{g/ml}$ in H226, H358, and H460 cells, respectively. In this study, Aza at a concentration below 0.6 $\mu\text{g/ml}$ did not cause significant growth inhibition in all tested cell lines.

Fig. 1. Growth inhibition of Aza on human NSCLC cell lines. H226, H358, and H460 cells were treated with (5-fold) increasing concentrations of Aza. The percentage of growth inhibition was measured with MTT Assay. The data for each cell line are mean $\pm$ standard deviation obtained from 3 independent experiments.

The demethylation function of Aza were detect by a methylation-specific PCR method in the H226 NSCLC cell line at a very low concentration range (0.1 ~ 10 ng/ml). As shown in Figure 2, the unmethylated band (#5) of the promoter of RASSF1a gene was found in the H226 cells at the lowest concentration of 0.1 ng/ml , which is 6000-fold lower than the IC₅₀ of Aza in the same cell line. This indicates that when directly exposing lung cancer cells to Aza, Aza can function as effective demethylation agent at an extremely low concentration without causing any direct cytotoxicity.

3.2.2 Intratracheal administration of Aza results in significantly reduced toxicity

Myelosuppression is the dose-limiting toxicity of intravenously administered Aza when used clinically. In this study, we compared the myelotoxicity of IT and IV Aza at the same

dose, 90 mg/kg that is MTD when using IV administration. IT Aza produced significantly less myelotoxicity than IV Aza at the MTD of IV Aza. As shown in Figure 3, IV Aza significantly reduced the total WBC by > 68% on day 4 and 7 ($p < 0.004$) and >38% on day 14 ($p < 0.006$), the WBC count recovered to about 90% of the normal level on day 28. While the only detectable WBC reduction in IT Aza treated mice was about 13% on day 7 ($p < 0.01$). The recovery was faster (on day 14) and complete (>97% of the normal level, $p > 0.5$) compared with IV Aza (Figure 3).

Fig. 2. The demethylation function of Aza in the NSCLC cells. H226 human NSCLC cells (8×10^4 cells) were treated with Aza at 0.1, 1, and 10 ng/ml. The methylation status of the RASSF1a promoter was detected by methylation-specific PCR method using the EZ DNA Methylation-Direct Kit. The pair of bands from 1 to 3 are samples of water, methylated DNA control, and unmethylated DNA control; from 4 to 7 are samples of H226 cells treated with Aza at 0, 0.1, 1, and 10 ng/ml, respectively. The letters “U” and “M” represent unmethylated and methylated detection, respectively.

Fig. 3. IT administration of Aza is 5-fold less myelosuppression compared to IV Aza. Aza was administered IT (round dots) or IV (triangle dots) at a dose of 90 mg/kg. Control mice were not given any treatment (square dots). Blood was drawn on day 0, 4, 7, 14, and 28 after treatment. WBC was counted after removal of red blood cells. The data of each group (5-8 mice each) are mean $\pm$ standard deviation.

To know whether the IT Aza will cause the locoregional toxicity or systemic toxicity in other organs, an organ toxicity study in a scope of acute toxicity of IT Aza were performed and the IV Aza was used as comparison. At the MTD, the results of serum liver function tests and serum creatinine measurements were normal for all mice and there were no differences among the groups of IT Aza, IV Aza, and no treatment (data not shown). On histopathological evaluation, no liver or kidney toxicities were identified in any treatment group (data not shown). By lung histological evaluation, moderate pulmonary toxicity was observed in all 5 animals in the IT Aza group on day 7 but not at the other time points. Of note is that the IT dose used in these experiments is 12-fold higher dose than the optimized total dose used in the therapeutic experiments. The lung toxicity was described as moderate pneumonitis, characterized by type II pneumocyte hypertrophy, neutrophilic infiltration, and lymphohistiocytic inflammation (Figure 4, photograph 2). As stated, no pulmonary

Fig. 4. IT Aza at the therapeutic dose does not produce pulmonary toxicity. ICR mice were intratracheally injected with 90 mg/kg of Aza, 2.5 mg/kg qod x 3 of Aza, or the equal volume of vehicle (Lactated Ringer's Injection). The lungs of mice were resected on day 4, 7, and 14 after injection. Standard H & E staining of lung tissues was used to assess pulmonary toxicity. Photographs 1 to 6 are the lungs from mice receiving 90 mg/kg of IT Aza (1~3) or the same volume of IT vehicle (4~6). Photograph 7 is the lung from mice receiving the therapeutic dose of IT Aza (2.5 mg/kg, qod x 3) on day 7 post the final injection. Photograph 8 is the lung from untreated mice.

toxicity was observed in animals treated with IT Aza on days 4 or 14. At the optimal therapeutic dose (2.5 mg/kg, qod x 3), IT Aza did not cause lung toxicity (Figure 4, photograph 7) and any other toxicity (data not shown). The pulmonary toxicity grades are listed in Table 1. These results indicate that the pulmonary toxicity caused by IT Aza at supratherapeutic doses (the MTD of IV Aza) is moderate (photograph 2) and reversible within 2 weeks (photograph 3). IT Aza at the therapeutic dose, IT vehicle, and IV Aza (data not shown) did not cause detectable pulmonary toxicity.

Day	4	7	14	28
IT Aza (90 mg/kg)	0*	2*	0*	0
IT Aza (2.5 mg/kg x 3)	0	0*	0	0
IT Vehicle	0*	0*	0*	0
IV Aza (90 mg/kg)	0	0	0	0
No Treatment	0	0*	0	0

* histopathological photographs are shown in Figure 3.

Table 1. Toxicity grade of lungs of the mice treated with IT Aza

3.2.3 Intratracheal administration of Aza significantly prolonged the survival of mice bearing orthotopic human NSCLC xenografts

To evaluate the efficacy of IT Aza in clinically relevant NSCLC models, we inoculated the human NSCLC cell lines H266, H358, and H460 into the lungs of nude mice via the trachea. These models mimic closely orthotopic human NSCLC. In mice, small mucosal tumor nodules are evident at 1-3 weeks after the inoculation of tumor cells. In the absence of any intervention, the mice succumb to the tumor in 6-10 weeks. The survival curve in these models closely correlates with the tumor burden (13), and can be utilized as an endpoint for the evaluation of treatment efficacy.

Treatments were initiated on day 10 post tumor inoculation. The survival observed in mice treated with IT Aza was compared to that in mice treated with IV Aza and untreated tumor-bearing mice. Animals in each treatment group were given multiple injections; these doses and schedules were optimized in a preliminary study (data not shown). The total dose was 7.5 mg/kg for IT Aza (2.5 mg/kg, qod x 3) and 37.5 mg/kg for IV Aza (6.25 mg/kg, qd x 6). Both dose levels are significantly lower than the corresponding MTD's. Results are shown in Figure 5. IV Aza had limited efficacy against three lung cancer models at the optimal therapeutic dose: the median survival increased by 10% in H226 model (72 vs. 67), , and 22% in the H358 model (73 vs. 60 days, p > 0.05) and 60% in the H460 model (80 vs. 50 days, p < 0.01), whereas IT Aza demonstrated significantly increased efficacy: the median survival increased by 107% (139 vs. 67), 63%, (98 vs. 60 days, p < 0.006), and 142% (121 vs. 50 days, p < 0.002) in the H226, H358, and H460 model, respectively. The increased lifespan (%ILS) (119) of IT Aza treated mice bearing H226, H358, or H460 lung tumors was 3.2- to 8.6-fold higher than that of IV Aza treated mice (96.2% vs. 11.2%; 75.8% vs. 21.5%; 131.3% vs. 40.7%). The efficacy of each treatment is summarized in Table 2.

Fig. 5. Intratracheal administration of low dose Aza significantly prolongs survival of mice bearing orthotopic human NSCLC xenografts. Mice intratracheally inoculated with H226 (left), H358 (middle), or H460 (right) human NSCLC cell lines were treated with IT Aza (thick line) or IV Aza (dash line) on day 10 at a dose of 2.5 mg/kg qod x 3 for IT and 6.25 mg/kg daily x 6 for IV. The control was a group of untreated mice (thin line).

		No treatment	IV	IT	IT vs. V	P value
H226	Median Survival (Range)	67 (50~84)	72 (55~101)	139 (89~178)	1.93	0.016
	%ILS	0	11.2	96.2	8.59	
H358	Median Survival (Range)	60 (46~75)	73 (54~93)	98 (75~146)	1.34	0.018
	%ILS	0	21.5	75.8	3.52	
H460	Median Survival (Range)	50 (42~75)	80 (58~100)	121 (75~183)	1.51	0.005
	%ILS	0	40.7	131.3	3.23	

Table 2. Efficacy summary

3.3 Discussion

Azacytidine (Aza) is approved by the Food and Drug Administration for the treatment of myelodysplastic syndromes (11), a preleukemic condition, and has potential for the treatment of other cancers and premalignant conditions as a result of a cytotoxic effect, a DNA demethylating effect, or both. As a cytotoxic agent in proliferating cells, Aza can disrupt RNA metabolism, DNA synthesis, and protein synthesis. Particularly, Aza is incorporated into DNA and inhibits DNA methyltransferases and causes hypomethylation of replicating DNA (120, 121) which can result in re-expression of tumor suppressor genes silenced by hypermethylation. From 1973 to 1977, there were at least 9 clinical studies in solid tumor patients with intravenous Aza, which included 78 lung cancer patients (122). The therapeutic efficacy observed was limited, possibly due to two major reasons: First, the studies were performed in advanced lung cancer patients where reversal of hypermethylation per se may not be sufficient to have a therapeutic impact; second, all the studies were done using systemic administration or a sub-optimal dose schedule, which limits the use of these agents as a result of systemic toxicity. The studies presented here

were designed to provide the foundation for the potential use of a regional demethylation strategy for malignant or premalignant conditions of the bronchial epithelium in which DNA hypermethylation plays an important role. We used Aza as a model compound and tested its toxicity and antitumor efficacy by direct delivery in the respiratory airways via the trachea in models of endobronchial human NSCLC. Our studies demonstrate that IT Aza produced a 5-fold reduced myelosuppression (as assessed by WBC nadir) than IV Aza at a dose equivalent to the IV MTD and 3-fold higher antitumor efficacy (as assessed by %ILS) at a dose 5-fold lower than that of IV Aza, the end-result being a 75-fold increased therapeutic index. These results justify continuing exploring regional demethylating therapy for the treatment of malignant or premalignant conditions of the lungs that are easily accessible through the airways, including advanced premalignancy, bronchioalveolar carcinoma, and small parenchymal metastatic disease.

Lung cancers develop in the epithelium in direct contact with the airways because carcinogens reach the lungs through inhalation. Bronchial premalignancy, carcinoma in situ, small primary or metastatic tumors, and some cases of BAC are theoretically more accessible via the endobronchial space than through the bloodstream. Aerosol approaches to the treatment or prevention of these conditions are therefore a more logical therapeutic strategy than systemic treatment. However, in the present studies we used intratracheal administration rather than aerosol administration because the purpose was proof of concept and administration of drugs by aerosolization to mice is inefficient. The major difference between these two types of drug administration (IT vs. aerosol) would be a higher distribution of the drug to the alveolar space with aerosol administration. We are currently conducting studies to validate the results presented here using the clinically available formulation of Aza administered by aerosolization to mice.

Our toxicity studies demonstrate, as expected, that Aza given IT results in a 5-fold reduced myelosuppression, which is the dose-limiting toxicity of IV Aza. Most importantly, IT Aza at 90 mg/kg only caused moderate pulmonary inflammation on day 7 after IT administration. It was encouraging to see that there was no evidence of lung inflammation on day 14 post IT Aza, even when the dose used for IT was as high as the maximum tolerated dose using the IV route. In the efficacy experiments, the optimal total IT dose used was 12 fold lower, a dose did not cause any pulmonary toxicity. To confirm this, we are currently performing more refined lung toxicity studies in the context of our current therapeutic experiments using aerosolized administration.

In the efficacy experiments, the efficacy (%ILS) of IT Aza at a 5-fold lower dose was 3.2- to 8.6-fold superior to that of IV Aza in mice with endobronchial H226, H358, or H460 tumors. These results indicate that the regional administration route into the airways is more efficient than the intravenous route for the treatment of endobronchial tumors. The main therapeutic potential of airway-administered Aza is secondary prevention of NSCLC due to the field cancerization effect of inhaled carcinogens via tobacco smoke. The proposed mechanism would be hypomethylation of CpG islands of the promoter regions of tumor suppressor genes thereby inhibiting development of dysplasia and progression of dysplasia to cancer. In these studies, we used a mouse model of endobronchial tumors but not dysplasia. We are currently developing an animal model of lung premalignancy in mice by exposing them to tobacco carcinogens directly into the upper airways. We plan to test the ability of aerosolized Aza in reversing tumor suppressor gene hypermethylation in this model.

In these studies, the efficacy endpoint was survival secondary to antitumor effect in models of malignancy. In the anticipated clinical scenario, the intermediate efficacy endpoint would be changes in hypermethylation patterns or effective gene reexpression. In this study, the optimal therapeutic dose was 12-fold lower than the maximum tolerated dose (MTD). This finding suggests that this strategy may have a large therapeutic window and that the risk of acute or chronic side effects might be very low if these agents were used at optimal doses rather than MTD. Therefore, determining optimal doses based on pharmacodynamic assessments in patients enrolled in clinical studies with this new therapeutic strategy are essential to minimize the potential side effects. Particularly, the potential carcinogenicity of this approach could become an important limitation if benefit was demonstrated but required chronic administration of unnecessarily high doses of these agents. Therefore, in the context of our initial Phase I clinical study of inhaled Aza we intend to monitor methylation patterns as well as gene reexpression in the target tissue pre and post-therapy to establish an optimal dose based on target effects rather than a maximum tolerated dose.

In vitro, we have proved that Aza can effectively demethylate the hypermethylation in the promoter of tumor suppressor gene at a non-toxic concentration. In vivo, we found that IT Aza are effective against experimental lung cancer by prolonging the life of the mice bearing orthotopic lung tumors without causing any detectable systemic or locoregional toxicity. Here the functions of both the epigenetic effect and the locoregional administration played an important role. We believe that the lung-specific epigenetic treatment with Aza has great potential to reduce the tumor burden by reversing the hypermethylation in the promoters of the tumor suppressor genes and therefore reactivating the silenced genes. This is an important project to be further studied.

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5. References

- [1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011 Mar-Apr;61(2):69-90.
- [2] Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010 Jan;31(1):27-36.
- [3] Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004 Aug;10(8):789-99.
- [4] Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 1971 Apr;68(4):820-3.
- [5] Benedict WF, Murphree AL, Banerjee A, Spina CA, Sparkes MC, Sparkes RS. Patient with 13 chromosome deletion: evidence that the retinoblastoma gene is a recessive cancer gene. *Science* 1983 Feb 25;219(4587):973-5.
- [6] Baker SJ, Fearon ER, Nigro JM, *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 1989 Apr 14;244(4901):217-21.
- [7] Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* 1990 Aug 24;249(4971):912-5.

- [8] Roth JA, Nguyen D, Lawrence DD, *et al.* Retrovirus-mediated wild-type p53 gene transfer to tumors of patients with lung cancer. *Nat Med* 1996 Sep;2(9):985-91.
- [9] Kim DS, Kim MJ, Lee JY, Kim YZ, Kim EJ, Park JY. Aberrant methylation of E-cadherin and H-cadherin genes in nonsmall cell lung cancer and its relation to clinicopathologic features. *Cancer* 2007 Dec 15;110(12):2785-92.
- [10] Seng TJ, Currey N, Cooper WA, *et al.* DLEC1 and MLH1 promoter methylation are associated with poor prognosis in non-small cell lung carcinoma. *British Journal of Cancer* 2008 Jul 22;99(2):375-82.
- [11] Kaminskas E, Farrell AT, Wang YC, Sridhara R, Pazdur R. FDA drug approval summary: azacitidine (5-azacytidine, Vidaza) for injectable suspension. *Oncologist* 2005 Mar;10(3):176-82.
- [12] Zou Y, Zong G, Ling YH, *et al.* Effective treatment of early endobronchial cancer with regional administration of liposome-p53 complexes. *J Natl Cancer Inst* 1998 Aug 5;90(15):1130-7.
- [13] Zou Y, Zong G, Ling YH, Perez-Soler R. Development of cationic liposome formulations for intratracheal gene therapy of early lung cancer. *Cancer Gene Ther* 2000 May;7(5):683-96.
- [14] Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. *Nature Reviews Genetics* 2006 Jan;7(1):21-33.
- [15] Weber M, Hellmann I, Stadler MB, *et al.* Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* 2007 Apr;39(4):457-66.
- [16] Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 1992 Jun 12;69(6):915-26.
- [17] Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. *Nature* 1993 Nov 25;366(6453):362-5.
- [18] Heard E, Clerc P, Avner P. X-chromosome inactivation in mammals. *Annu Rev Genet* 1997;31:571-610.
- [19] Belinsky SA, Palmisano WA, Gilliland FD, *et al.* Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. *Cancer Res* 2002 Apr 15;62(8):2370-7.
- [20] 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 U S A* 1998 Sep 29;95(20):11891-6.
- [21] Sato M, Mori Y, Sakurada A, Fujimura S, Horii A. The H-cadherin (CDH13) gene is inactivated in human lung cancer. *Hum Genet* 1998 Jul;103(1):96-101.
- [22] Osada H, Tatematsu Y, Yatabe Y, *et al.* Frequent and histological type-specific inactivation of 14-3-3sigma in human lung cancers. *Oncogene* 2002 Apr 4;21(15):2418-24.
- [23] Tang X, Khuri FR, Lee JJ, *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 Sep 20;92(18):1511-6.
- [24] 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 Jul;25(3):315-9.

- [25] Hopkins-Donaldson S, Ziegler A, Kurtz S, *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 Mar;10(3):356-64.
- [26] 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 Jan 1;61(1):249-55.
- [27] Lin R-K, Hsu H-S, Chang J-W, Chen C-Y, Chen J-T, Wang Y-C. Alteration of DNA methyltransferases contributes to 5'CpG methylation and poor prognosis in lung cancer. *Lung Cancer* 2007 Feb;55(2):205-13.
- [28] Kim H, Kwon YM, Kim JS, *et al.* Elevated mRNA levels of DNA methyltransferase-1 as an independent prognostic factor in primary nonsmall cell lung cancer. *Cancer* 2006 Sep 1;107(5):1042-9.
- [29] Heller G, Zielinski CC, Zochbauer-Muller S. Lung cancer: from single-gene methylation to methylome profiling. *Cancer Metastasis Rev* 2010 Mar;29(1):95-107.
- [30] Brena RM, Morrison C, Liyanarachchi S, *et al.* Aberrant DNA methylation of OLIG1, a novel prognostic factor in non-small cell lung cancer. *PLoS Med* 2007 Mar 27;4(3):e108.
- [31] Rauch TA, Zhong X, Wu X, *et al.* High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer. *Proc Natl Acad Sci U S A* 2008 Jan 8;105(1):252-7.
- [32] Shames DS, Girard L, Gao B, *et al.* A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. *PLoS Med* 2006 Dec;3(12):e486.
- [33] 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 Apr 19;26(18):2621-34.
- [34] Tornaletti S, Pfeifer GP. Complete and tissue-independent methylation of CpG sites in the p53 gene: implications for mutations in human cancers. *Oncogene* 1995 Apr 20;10(8):1493-9.
- [35] Jones PA, Rideout WM, 3rd, Shen JC, Spruck CH, Tsai YC. Methylation, mutation and cancer. *Bioessays* 1992 Jan;14(1):33-6.
- [36] Lee SJ, Jeon HS, Jang JS, *et al.* DNMT3B polymorphisms and risk of primary lung cancer. *Carcinogenesis* 2005 Feb;26(2):403-9.
- [37] 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 Sep 1;62(17):4992-5.
- [38] Smith LE, Denissenko MF, Bennett WP, *et al.* Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. *J Natl Cancer Inst* 2000 May 17;92(10):803-11.
- [39] 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 Oct 1;61(19):7110-7.
- [40] 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 U S A* 1997 Apr 15;94(8):3893-8.

- [41] 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 Jun;14(6):4225-32.
- [42] Kim H, Kwon YM, Kim JS, *et al.* Tumor-specific methylation in bronchial lavage for the early detection of non-small-cell lung cancer. *J Clin Oncol* 2004 Jun 15;22(12):2363-70.
- [43] Liu Y, Lan Q, Siegfried JM, Luketich JD, Keohavong P. Aberrant promoter methylation of p16 and MGMT genes in lung tumors from smoking and never-smoking lung cancer patients. *Neoplasia* 2006 Jan;8(1):46-51.
- [44] Vaissiere T, Hung RJ, Zaridze D, *et al.* Quantitative analysis of DNA methylation profiles in lung cancer identifies aberrant DNA methylation of specific genes and its association with gender and cancer risk factors. *Cancer Res* 2009 Jan 1;69(1):243-52.
- [45] Buckingham L, Penfield Faber L, Kim A, *et al.* PTEN, RASSF1 and DAPK site-specific hypermethylation and outcome in surgically treated stage I and II nonsmall cell lung cancer patients. *Int J Cancer* 2010 Apr 1;126(7):1630-9.
- [46] Kaira K, Sunaga N, Tomizawa Y, *et al.* Epigenetic inactivation of the RAS-effector gene RASSF2 in lung cancers. *Int J Oncol* 2007 Jul;31(1):169-73.
- [47] Tessema M, Yu YY, Stidley CA, *et al.* Concomitant promoter methylation of multiple genes in lung adenocarcinomas from current, former and never smokers. *Carcinogenesis* 2009 Jul;30(7):1132-8.
- [48] 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 Nov;50(2):199-209.
- [49] Zochbauer-Muller S, Lam S, Toyooka S, *et al.* Aberrant methylation of multiple genes in the upper aerodigestive tract epithelium of heavy smokers. *Int J Cancer* 2003 Nov 20;107(4):612-6.
- [50] Palmisano WA, Divine KK, Saccomanno G, *et al.* Predicting lung cancer by detecting aberrant promoter methylation in sputum. *Cancer Research* 2000 Nov 1;60(21):5954-8.
- [51] Lin Q, Geng J, Ma K, *et al.* RASSF1A, APC, ESR1, ABCB1 and HOXC9, but not p16INK4A, DAPK1, PTEN and MT1G genes were frequently methylated in the stage I non-small cell lung cancer in China. *J Cancer Res Clin Oncol* 2009 Dec;135(12):1675-84.
- [52] Yano M, Toyooka S, Tsukuda K, *et al.* Aberrant promoter methylation of human DAB2 interactive protein (hDAB2IP) gene in lung cancers. *Int J Cancer* 2005 Jan 1;113(1):59-66.
- [53] Kikuchi S, Yamada D, Fukami T, *et al.* Promoter methylation of DAL-1/4.1B predicts poor prognosis in non-small cell lung cancer. *Clin Cancer Res* 2005 Apr 15;11(8):2954-61.
- [54] Chen H, Suzuki M, Nakamura Y, *et al.* Aberrant methylation of FBN2 in human non-small cell lung cancer. *Lung Cancer* 2005 Oct;50(1):43-9.
- [55] Issa J-PJ. DNA methylation as a therapeutic target in cancer. *Clinical Cancer Research* 2007 Mar 15;13(6):1634-7.

- [56] 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.
- [57] Kopelovich L, Crowell JA, Fay JR. The epigenome as a target for cancer chemoprevention. *Journal of the National Cancer Institute* 2003 Dec 3;95(23):1747-57.
- [58] Seligson DB, Horvath S, McBrien MA, *et al.* Global levels of histone modifications predict prognosis in different cancers. *Am J Pathol* 2009 May;174(5):1619-28.
- [59] Fraga MF, Ballestar E, Villar-Garea A, *et al.* Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet* 2005 Apr;37(4):391-400.
- [60] Barlesi F, Giaccone G, Gallegos-Ruiz MI, *et al.* Global histone modifications predict prognosis of resected non small-cell lung cancer. *J Clin Oncol* 2007 Oct 1;25(28):4358-64.
- [61] LaVoie HA. Epigenetic control of ovarian function: the emerging role of histone modifications. *Mol Cell Endocrinol* 2005 Nov 24;243(1-2):12-8.
- [62] Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007 Apr;8(4):286-98.
- [63] Witt O, Deubzer HE, Milde T, Oehme I. HDAC family: What are the cancer relevant targets? *Cancer Lett* 2009 May 8;277(1):8-21.
- [64] Sasaki H, Moriyama S, Nakashima Y, *et al.* Histone deacetylase 1 mRNA expression in lung cancer. *Lung Cancer* 2004 Nov;46(2):171-8.
- [65] 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 Aug;49(2):145-54.
- [66] 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 Oct 20;112(1):26-32.
- [67] 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 Mar;13(3):477-83.
- [68] Yu XD, Wang SY, Chen GA, *et al.* Apoptosis induced by depsipeptide FK228 coincides with inhibition of survival signaling in lung cancer cells. *Cancer J* 2007 Mar-Apr;13(2):105-13.
- [69] 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 Aug;27(2):473-9.
- [70] 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 Nov;3(11):1397-402.
- [71] Ota H, Tokunaga E, Chang K, *et al.* Sirt1 inhibitor, Sirtinol, induces senescence-like growth arrest with attenuated Ras-MAPK signaling in human cancer cells. *Oncogene* 2006 Jan 12;25(2):176-85.
- [72] 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 Oct;68(1):29-36.

- [73] 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 Aug;18(7):793-800.
- [74] Komatsu N, Kawamata N, Takeuchi S, *et al.* SAHA, a HDAC inhibitor, has profound anti-growth activity against non-small cell lung cancer cells. *Oncol Rep* 2006 Jan;15(1):187-91.
- [75] 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(1):39-48.
- [76] Cang S, Ma Y, Liu D. New clinical developments in histone deacetylase inhibitors for epigenetic therapy of cancer. *J Hematol Oncol* 2009;2:22.
- [77] Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer* 2006 Apr;6(4):259-69.
- [78] Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006 Nov;6(11):857-66.
- [79] Volinia S, Calin GA, Liu CG, *et al.* A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* 2006 Feb 14;103(7):2257-61.
- [80] Yanaihara N, Caplen N, Bowman E, *et al.* Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006 Mar;9(3):189-98.
- [81] Takamizawa J, Konishi H, Yanagisawa K, *et al.* Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 2004 Jun 1;64(11):3753-6.
- [82] Liu B, Peng XC, Zheng XL, Wang J, Qin YW. MiR-126 restoration down-regulate VEGF and inhibit the growth of lung cancer cell lines in vitro and in vivo. *Lung Cancer* 2009 Nov;66(2):169-75.
- [83] Liu X, Sempere LF, Ouyang H, *et al.* MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. *J Clin Invest* 2010 Apr 1;120(4):1298-309.
- [84] Cha ST, Chen PS, Johansson G, *et al.* MicroRNA-519c suppresses hypoxia-inducible factor-1alpha expression and tumor angiogenesis. *Cancer Res* 2010 Apr 1;70(7):2675-85.
- [85] He X, Duan C, Chen J, *et al.* Let-7a elevates p21(WAF1) levels by targeting of NIFB and suppresses the growth of A549 lung cancer cells. *FEBS Lett* 2009 Nov 3;583(21):3501-7.
- [86] Crawford M, Batte K, Yu L, *et al.* MicroRNA 133B targets pro-survival molecules MCL-1 and BCL2L2 in lung cancer. *Biochem Biophys Res Commun* 2009 Oct 23;388(3):483-9.
- [87] Bandi N, Zbinden S, Gugger M, *et al.* miR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer. *Cancer Res* 2009 Jul 1;69(13):5553-9.
- [88] Wang G, Mao W, Zheng S. MicroRNA-183 regulates Ezrin expression in lung cancer cells. *FEBS Lett* 2008 Oct 29;582(25-26):3663-8.
- [89] Sun Y, Bai Y, Zhang F, Wang Y, Guo Y, Guo L. miR-126 inhibits non-small cell lung cancer cells proliferation by targeting EGFL7. *Biochem Biophys Res Commun* 2010 Jan 15;391(3):1483-9.
- [90] Liang Y. An expression meta-analysis of predicted microRNA targets identifies a diagnostic signature for lung cancer. *BMC Med Genomics* 2008;1:61.

- [91] Lebanony D, Benjamin H, Gilad S, *et al.* Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. *J Clin Oncol* 2009 Apr 20;27(12):2030-7.
- [92] Xie Y, Todd NW, Liu Z, *et al.* Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. *Lung Cancer* 2010 Feb;67(2):170-6.
- [93] Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin Lung Cancer* 2009 Jan;10(1):42-6.
- [94] Markou A, Tsaroucha EG, Kaklamanis L, Fotinou M, Georgoulas 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 Oct;54(10):1696-704.
- [95] Gallardo E, Navarro A, Vinolas N, *et al.* miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis* 2009 Nov;30(11):1903-9.
- [96] Raponi M, Dossey L, Jatkoe T, *et al.* MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. *Cancer Res* 2009 Jul 15;69(14):5776-83.
- [97] Yu SL, Chen HY, Chang GC, *et al.* MicroRNA signature predicts survival and relapse in lung cancer. *Cancer Cell* 2008 Jan;13(1):48-57.
- [98] Hu Z, Chen X, Zhao Y, *et al.* Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. *J Clin Oncol* 2010 Apr 1;28(10):1721-6.
- [99] Raponi M, Zhang Y, Yu J, *et al.* Gene expression signatures for predicting prognosis of squamous cell and adenocarcinomas of the lung. *Cancer Res* 2006 Aug 1;66(15):7466-72.
- [100] Weidhaas JB, Babar I, Nallur SM, *et al.* MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. *Cancer Res* 2007 Dec 1;67(23):11111-6.
- [101] Weiss GJ, Bemis LT, Nakajima E, *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 Jun;19(6):1053-9.
- [102] Hayashita Y, Osada H, Tatematsu Y, *et al.* A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 2005 Nov 1;65(21):9628-32.
- [103] Matsubara H, Takeuchi T, Nishikawa E, *et al.* Apoptosis induction by antisense oligonucleotides against miR-17-5p and miR-20a in lung cancers overexpressing miR-17-92. *Oncogene* 2007 Sep 6;26(41):6099-105.
- [104] Garofalo M, Quintavalle C, Di Leva G, *et al.* MicroRNA signatures of TRAIL resistance in human non-small cell lung cancer. *Oncogene* 2008 Jun 19;27(27):3845-55.
- [105] Krutzfeldt J, Rajewsky N, Braich R, *et al.* Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 2005 Dec 1;438(7068):685-9.
- [106] Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA: a Cancer Journal for Clinicians* 2005 Mar-Apr;55(2):74-108.
- [107] Williams MD, Sandler AB. The epidemiology of lung cancer. *Cancer Treatment & Research* 2001;105:31-52.
- [108] Belinsky SA. Gene-promoter hypermethylation as a biomarker in lung cancer. *Nature Reviews* 2004 Sep;Cancer. 4(9):707-17.

- [109] Zochbauer-Muller S, Minna JD, Gazdar AF. Aberrant DNA methylation in lung cancer: biological and clinical implications. *Oncologist* 2002;7(5):451-7.
- [110] Tsou JA, Hagen JA, Carpenter CL, Laird-Offringa IA. DNA methylation analysis: a powerful new tool for lung cancer diagnosis. *Oncogene* 2002 Aug 12;21(35):5450-61.
- [111] Momparler RL. Epigenetic therapy of cancer with 5-aza-2'-deoxycytidine (decitabine). *Semin Oncol* 2005 Oct;32(5):443-51.
- [112] Marquez VE, Kelley JA, Agbaria R, *et al.* Zebularine: a unique molecule for an epigenetically based strategy in cancer chemotherapy. *Annals of the New York Academy of Sciences* 2005 Nov;1058:246-54.
- [113] Mufti G, List AF, Gore SD, Ho AY. Myelodysplastic syndrome. *Hematology Am Soc Hematol Educ Program* 2003:176-99.
- [114] 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. *Anti-Cancer Drugs* 1997 Apr;8(4):358-68.
- [115] Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* 1987 Feb 15;47(4):936-42.
- [116] Wang J, Wang B, Chen X, Bi J. The prognostic value of RASSF1A promoter hypermethylation in non-small cell lung carcinoma: a systematic review and meta-analysis. *Carcinogenesis* 2011 Mar;32(3):411-6.
- [117] Wang Y, Yu Z, Wang T, Zhang J, Hong L, Chen L. Identification of epigenetic aberrant promoter methylation of RASSF1A in serum DNA and its clinicopathological significance in lung cancer. *Lung Cancer* 2007 May;56(2):289-94.
- [118] Zou Y, Ling YH, Van NT, Priebe W, Perez-Soler R. Antitumor activity of free and liposome-entrapped annamycin, a lipophilic anthracycline antibiotic with non-cross-resistance properties. *Cancer Res* 1994 Mar 15;54(6):1479-84.
- [119] Zou Y, Yamagishi M, Horikoshi I, Ueno M, Gu X, Perez-Soler R. Enhanced therapeutic effect against liver W256 carcinosarcoma with temperature-sensitive liposomal adriamycin administered into the hepatic artery. *Cancer Research* 1993 Jul 1;53(13):3046-51.
- [120] Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. *Cell* 1980 May;20(1):85-93.
- [121] Juttermann R, Li E, Jaenisch R. Toxicity of 5-aza-2'-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. *Proc Natl Acad Sci U S A* 1994 Dec 6;91(25):11797-801.
- [122] Digel W, Lubbert M. DNA methylation disturbances as novel therapeutic target in lung cancer: preclinical and clinical results. *Critical reviews in oncology/hematology* 2005 Jul;55(1):1-11.

Tumor Suppressor Genes

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Functional evidence obtained from somatic cell fusion studies indicated that a group of genes from normal cells might replace or correct a defective function of cancer cells. Tumorigenesis that could be initiated by two mutations was established by the analysis of hereditary retinoblastoma, which led to the eventual cloning of RB1 gene. The two-hit hypothesis helped isolate many tumor suppressor genes (TSG) since then. More recently, the roles of haploinsufficiency, epigenetic control, and gene dosage effects in some TSGs, such as P53, P16 and PTEN, have been studied extensively. It is now widely recognized that deregulation of growth control is one of the major hallmarks of cancer biological capabilities, and TSGs play critical roles in many cellular activities through signaling transduction networks. This book is an excellent review of current understanding of TSGs, and indicates that the accumulated TSG knowledge has opened a new frontier for cancer therapies.

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