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Genetic and epigenetic changes in lung carcinoma and their clinical implications

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Lung cancer is the leading cause of cancer deaths worldwide. Recent advance in targeted therapy for lung cancer patients with epidermal growth factor receptor (*EGFR*) mutations has demonstrated a promising development toward personalized therapy for lung cancer patients. The development of lung cancer is a complex process, involving a series of genetic and epigenetic changes. Tobacco smoke is the predominant etiologic risk factor for lung cancer. However, some lung cancers, especially adenocarcinomas, arise in patients who have never smoked, suggesting the importance of host genetic/epigenetic susceptibility in the occurrence and development of lung cancer. Understanding of these genetic and epigenetic changes will further aid in the biomarker-driven personalized therapy for lung cancer patients. In this review, we summarize the genetic and epigenetic alterations observed in lung cancers, including chromosomal loss of heterozygosity, tumor-suppressor gene mutation, gene methylation, histone modification, and microRNA expression changes. Clinical and preclinical studies have implied specific genetic/epigenetic changes for clinical application in lung cancer patients. However, more efforts are required in validation of the identified molecular markers in lung cancer patients for early detections, assessment for treatment response, and survival predictions.
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Lung cancer is the leading cause of cancer deaths in both men and women worldwide, with over a million deaths annually.[1] Histopathologically, lung cancer is classified as adenocarcinoma, squamous carcinoma, large cell carcinoma, small cell carcinoma, and other subtypes that are less frequently diagnosed.[2] The first three types of lung cancer collectively accounts for 85% of lung cancers.

Tobacco smoke is the predominant etiologic risk factor for lung cancer.[3] Carcinogens present in tobacco smoke or their intermediate metabolites might bind covalently to DNA at certain specific sites, forming bulky adducts and leading to gene mutations. However, a fraction of lung cancers, especially adenocarcinomas, arise in patients who have never smoked, indicating that host susceptibility is a factor in lung cancer carcinogenesis. It is still not fully understood whether the specific host susceptibility, alone or together with the environmental factors including tobacco smoking, has a unique or a synergetic role in the carcinogenesis of lung cancer. In lung cancer, heritable genetic changes can occur at chromosomal level with bulky loss, gain or translocation of chromosomes. At molecular level, the changes may be mutations in specific genes such as single-nucleotide polymorphism (SNP) or deletion. In addition to gene structural changes, reversible changes in gene expression that may be independent of changes in the primary DNA sequence can occur. These so-called epigenetic changes include DNA methylation, histone modifications, and abnormal expression of non-coding RNAs including microRNAs (miRNAs). These genetic and epigenetic alternations interact at all stages of cancer development, working together to promote cancer progression.[4]

In this review, we summarize the characteristic genetic and epigenetic changes observed in lung cancers and the implication of these changes in patients for early detection, survival prediction, and treatment responses.

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Genetic changes

Chromosomal Aberration—Loss of Heterozygosity

Somatic alterations in cellular DNA are common in almost all human cancers, including lung cancers. By analysis of 371 lung adenocarcinomas using dense SNP arrays, Weir *et al*[5] found that 26 of 39 autosomal chromosome arms showed consistent large-scale copy-number gain or loss and 31 focal alterations, including 24 amplifications and 7 homozygous deletions. Previous studies suggested that fractional allelic losses located primarily on chromosomes 1p, 2p, 2q, 3p, 6q, 7p, 9p, 12p, 16p, 17p, 17q, 19p, and 21q occur more frequently in lung carcinomas than in adjacent normal tissues,[6–17] indicating the existence of tumor-suppressor genes or potential candidates, such as *HLJ1* at 1p31, *FHIT* at 3p14, *RASSF1A*, *FUS1*, *LIMD1, SEMA3B*, and *SEMA3F* at 3p21, *p16* at 9p21, and *p53* at 17p13.

Genetic changes similar to those found in lung cancers can be detected in the non-malignant bronchial epithelium of current and former smokers, and such changes may persist for many years after smoking cessation. Wistuba *et al*[9] reported that biopsy specimens from smokers with either normal or preneoplastic lung tissue showed loss of heterozygosity (LOH) at multiple chromosomal sites, a phenomenon frequently observed in carcinoma *in situ* and invasive cancer. However, no genetic alterations were detected in non-smokers.[9] Recently, Yendamuri *et al*[18] reported increased deletions of the 3p22.1 and 10q22.3 regions in the bronchial epithelium in the lung harboring the tumor and the tumor itself compared with the contralateral normal bronchial epithelium in 122 squamous cell carcinoma/adenocarcinoma patients. These results indicated that the detection of genetic changes, such as LOH, might identify individuals at high risk for developing lung cancer.

Certain chromosomal changes are related to lung cancer's metastasis. Wrage *et al*[19] reported that the loss of 4q, especially 4q12–q23, in primary lung cancer was significantly associated with bone marrow metastasis. In addition, the same loss was also found to be common in brain metastases from lung cancer patients. Therefore, a specific pattern of genomic changes, such as 4q deletion, might drive an early hematogenous dissemination of lung cancer cells.

The LOH or other chromosomal abnormalities observed in lung cancers could be associated with patient survival. Bepler *et al*[20] observed an association between LOH at 11p15.5 and poor survival in 180 lung cancer patients with adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Marsit *et al*[12] reported that LOH at 9p13 was a significant predictor of improved survival, whereas the homozygous deletion was associated with poor survival in 100 squamous cell carcinoma/adenocarcinoma patients undergoing surgical resection. Tseng *et al*[21] linked LOH at 1p36.23 with smoking, squamous carcinoma, and late-stage disease. Furthermore, LOH at q37.3 and 6p21–p22 were significantly associated with poor prognosis in squamous cell carcinoma/adenocarcinoma patients, using both univariate and multivariate Cox regression analyses. These markers can potentially be used for early lung cancer detection and prognosis and for potentially identifying novel clones of new tumor-suppressor genes that might contribute to carcinogenesis of squamous cell carcinoma/adenocarcinoma when their functions were lost. These findings need to be examined further in larger studies to confirm the association of LOH with patient survival.

p53 Mutation

Mutations in the *p53* tumor-suppressor gene are one of the most frequent changes identified in human tumor cells. The common mutations, usually occurring in the DNA-binding domains of *p53* gene, lead to the formation of the mutant forms with altered amino-acid sequences that lack DNA-binding activity.

It has been reported that 40–60% of lung cancers are associated with mutations in the *p53* gene,[22,23] and smoking is a primary factor inducing *p53* gene mutation. Evidence indicated that these mutations were more commonly observed in tobacco-associated lung cancer (26–71%) than in lung cancer of never-smokers (10–47%).[24–30] A significant dose–response relation between tobacco smoking and *p53* gene mutations in lung cancer has been reported. In addition to the mutation frequency, the *p53* mutational signatures, that is, the ratio of transitions, transversions and deletions, and the mutational spectrum, such as the distribution of mutations along the gene, are distinct in lung cancers between smokers and never-smokers. Tobacco-associated lung cancer is characterized by a high frequency of G to T transversion with a pronounced coding strand bias of 93%, whereas lung cancer in never-smokers shows a higher proportion of G to C transversions and G to A transitions at CpG dinucleotides,[23] suggesting that G to T transversion is a molecular signature of mutagenesis by distinct exogenous factors such as tobacco smoking.

p53 protein expression probably is a prognostic factor for survival in lung cancer patients. In a meta-analysis of 74 eligible papers dealing with *p53* assessment in lung cancer, the combined hazard ratios indicated that an abnormal *p53* status had an unfavorable effect on survival at each clinical stage of squamous cell carcinoma/adenocarcinoma.[31] Recently, in a total of 131 cases of primary lung adenocarcinoma, both univariate and multivariate analyses showed that overexpression of *p53* protein was an independent prognostic factor in node-negative lung adenocarcinoma.[32] In another 266

lung cancer patients, patients with stage I disease and *p53* mutation had a hazard ratio of 1.79 (95% confidence interval, 1.04–3.10) for overall survival compared with patients with the wild-type *p53* gene.[33] However, the role of *p53* as a prognostic factor for survival in lung cancer is still controversial. Kosaka *et al*[34] found that *p53* mutations were not an independent prognostic factor in their cohort of patients with lung adenocarcinoma. Similarly, Lim *et al*[35] also reported that *p53* mutations did not have a survival effect in 88 lung cancer patients with squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. The controversy is probably due to the different methods or antibodies used to detect *p53* gene mutation or protein expression, or the variation in stage classification of lung cancer patients. Therefore, further clinical studies are required to determine whether *p53* gene mutation can be used as a predictive marker for lung cancer patients.

The role of *p53* as a chemosensitivity predictive factor in lung cancer has also been studied. In a phase III intergroup trial that randomly assigned 482 patients with completely resected stage IB and II squamous cell carcinoma/adenocarcinoma either to receive four cycles of adjuvant cisplatin plus vinorelbine or to be observed without further intervention, Tsao *et al*[36] found untreated *p53*-positive patients had significantly shorter overall survival than did patients with *p53*-negative tumors (hazard ratio 1.89; 95% confidence interval, 1.07–3.34; $P=0.03$). However, these *p53*-positive patients also showed a significantly greater survival benefit from adjuvant chemotherapy (hazard ratio 0.54; $P=0.02$) compared with patients with *p53*-negative tumors (hazard ratio 1.40; $P=0.26$; interaction $P=0.02$). The results suggested that *p53* protein overexpression is a significant prognostic marker of shortened survival as well as a predictive marker for patients who might benefit from adjuvant chemotherapy after surgical resection. Similarly, a retrospective study of 55 patients with adenocarcinoma/squamous cell carcinoma also suggested that *FHIT−/p53+* status might be a biological variable affecting the efficacy of carboplatin/gemcitabine treatment in patients.[37]

EGFR Mutation

The epidermal growth factor receptor (*EGFR*) family comprises *c-erbB-1* (*EGFR*, *HER-1*), *c-erbB-2* (*HER-2/neu*), *c-erbB-3*, and *erbB-4*, located in the cellular membrane. On binding of ligands such as transforming growth factor α and epidermal growth factor (*EGF*), the receptors form homodimers or heterodimers with other family members, resulting in autophosphorylation of key tyrosine residues in the receptor cytoplasmic domain and in further activation of downstream signaling events, including *PI3K/Akt/mTOR*, *Ras-Raf-MEK-ERK*, and *JAKs-STATs*, that trigger anti-apoptosis, cell proliferation, angiogenesis, tumor invasion, and metastasis.[38]

Higher frequencies of mutations in the *EGFR* tyrosine kinase domain are found in adenocarcinomas from Asian patients (25–50%) in comparison with those in North American and Western European patients (10%).[39] The most common *EGFR* mutations observed in lung cancer are small in-frame deletions in exon 19 and point mutations that result in substitution of arginine for leucine at amino acid 858 (L858R) in exon 21, which account for 50% and 40% of total *EGFR* mutations in lung cancers with exclusion of small cell carcinoma, respectively. Both point mutations in exon 18 and in-frame insertions and point mutations in exon 20 account for 5% of *EGFR* mutations in lung cancer with exclusion of small cell carcinoma.[40] These mutations induce oncogenic transformation *in vitro* and *in vivo* through constitutive activation of *EGFR*. It was reported that *EGFR* mutations, including those involving exons 18, 19, and 20 and L858R, can transform fibroblasts and lung epithelial cells in the absence of exogenous *EGF* with constitutive autophosphorylation of *EGFR*, *Shc* phosphorylation, or *STAT* pathway activation.[41] Furthermore, transgenic mice with inducible expression of *EGFR* exon 19 deletion mutants or the L858R mutation in type II pneumocytes developed lung adenocarcinoma after sustained *EGFR* mutant expression, confirming their oncogenic potential.[42]

Transformation by most *EGFR* mutants led to dramatic tumor regression by small molecular tyrosine kinase inhibitors, such as erlotinib and gefitinib.[41–43] This is consistent with the results from clinical trials, demonstrating an underlying association between mutations in the *EGFR* tyrosine kinase domain and tyrosine kinase inhibitors responsive lung carcinomas. A retrospective review of studies using tyrosine kinase inhibitors treatment found an average response rate of 77% (range, 30–100%) in mutation-positive cases, with most series reporting response rates >60%, compared with 10% in mutation-negative cases (range, 0–33%).[39] Furthermore, with tyrosine kinase inhibitors treatment, patients with *EGFR* mutation-positive tumors showed improved survival, with a median survival of up to 30 months compared with patients without *EGFR* mutations.[39] In addition to *EGFR* mutation, lung cancer patients with high *EGFR* gene copy numbers in the tumor, as detected by fluorescent *in situ* hybridization (FISH), also showed a higher response rate and better survival in patients treated with erlotinib.[44] However, *EGFR* FISH-positive status predicted worse survival in untreated patients.[44] This suggests that patients *EGFR* copy-number change may also be benefit from tyrosine kinase inhibitors treatment.

Resistance to tyrosine kinase inhibitors over time has been reported in patients who had advanced lung carcinomas with *EGFR* exon 19 deletions or

L858R mutations and who initially responded to the *EGFR* tyrosine kinase inhibitors treatment. A major mechanism of resistance to tyrosine kinase inhibitors is secondary resistance mutations. A threonine-to-methionine substitution at position 790 (T790M) in exon 20 of the *EGFR* tyrosine kinase domain has been observed in primary[45] and secondary[46,47] tyrosine kinase inhibitors-resistant lung carcinomas. T790M mutation confers tyrosine kinase inhibitors resistance by either activating wild-type *EGFR* or increasing the ATP affinity of the oncogenic L858R mutation.[48] Other secondary resistance mutations including D761Y, L747S, and T854A have been reported but seem to be rare.[49] Another possible mechanism of tyrosine kinase inhibitors secondary resistance is that some other pathway might be activated to bypass the *EGFR* pathway, such as *HER3*-dependent activation of *PI3K* caused by *MET* amplification,[50] which was detected in about 20% of lung carcinomas from patients who acquired resistance to gefitinib or erlotinib.[50,51] Furthermore, in half of the lung carcinomas with *MET* amplification, T790M mutation was also found.[50,51] An *in vitro* study of a multi-kinase inhibitor (XL880) with potent activity against *MET* reported inhibited growth of the NCI-H820 lung cancer cell line, which harbors *MET* amplification in addition to a drug-sensitive *EGFR* mutation and the drug-resistant T790M change.[51] These results suggest that a *MET* inhibitor might be potentially used to treat lung cancer patients with acquired resistance to gefitinib or erlotinib. In addition, many strategies to inhibit downstream *EGFR* signaling are also being evaluated as potential targets for lung cancer therapy. Of these, the *Ras-Raf-MEK-MAPK*, *PI3K-Akt-mTOR*, and phospholipase *C-PKC* pathways have been most intensively studied alone or in combination with *EGFR*-targeting agents for lung cancers.[52] However, these drugs are still undergoing phase I or II clinical trial to determine their toxicity and effectiveness. In combination with more than one target inhibitors might overcome the drug resistance of the single-target therapy and improve patient survival.

EGFRvIII, an in-frame deletion of exons 2–7 from the *EGFR* extracellular domain commonly observed in gliomas, was initially reported to be present in 16% of squamous cell carcinoma.[53] Tissue-specific expression of *EGFRvIII* in the murine lung may lead to the *EGFRvIII*-dependent development of adenocarcinomas.[54] However, a later analysis showed that this mutation was present in only a small fraction (5%) of squamous carcinomas, and was not found in 123 cases of human lung adenocarcinoma,[54] suggesting a limited role of *EGFRvIII* in lung cancer.

Another *EGFR* family member, *HER2*, was expressed at a higher level in about 40–60% primary lung cancers including squamous cell carcinomas, adenocarcinomas, and large cell carcinomas compared with normal lungs[55,56] and was correlated with poor clinical prognostic indicators such as advanced clinical stage.[56] *ErbB2* kinase domain mutations, mostly in the form of in-frame insertions in exon 20 and rare mis-sense substitutions, were found in 1–10% of lung adenocarcinomas[57–59] and mutually exclusive with mutations in *EGFR* and *K-ras* in the same tumor.[57] Like *EGFR* mutations, *HER2* mutations have similar associations with female sex, non-smoking status, and Asian ethnic background in patients with adenocarcinoma.[57] Furthermore, insertion mutations in exon 20 of *ErbB2* also enhanced the tyrosine kinase activity of *ErbB2* and resulted in resistance to *EGFR* tyrosine kinase inhibitor-targeted therapy.[60]

The overexpression and hyperactivation of another two *ErbB* family members *ErbB3* and *ErbB4* have been studied previously.[61–63] However, mutations in the *ErbB3* and *ErbB4* kinase domain are rare in lung cancers,[64–66] suggesting that they might be of limited value for molecular-targeted therapy.

K-ras Mutation

K-ras is one of the three human ras oncogenes (*K-ras*, *H-ras*, *and N-ras*) that regulate cellular proliferation by playing a role in the transduction of signals across cellular membranes. Mutations of the *ras* proteins contribute to the development of cancer. The mutations in *K-ras* occurs in 4–30% of squamous cell carcinomas/adenocarcinomas,[30,67–71] with a relatively higher mutation frequency of 12–57% in adenocarcinomas, compared with 2–9% in squamous and other types of lung cancers.[30,67,69] Mutant *K-ras* was associated with poor overall survival of lung cancer patients.[35]

The percentage of *K-ras* mutations present in lung adenocarcinomas from smokers is markedly higher than reported for non-smokers with lung adenocarcinoma (10–43 *vs* 0–8%), unlike *EGFR* tyrosine kinase domain mutations primarily occurring in lung adenocarcinomas of non-smokers.[25,69,72–76] Most *K-ras* mutations in lung adenocarcinomas from smokers are characteristic G to T transversions, a typical mutation type induced by benzo(a)pyrene diolepoxide *in vitro*.[77] This mutation occurs at the first two bases of codon 12 (normally GGT, which codes for glycine), resulting in the mutant codons TGT (cysteine) or GTT (valine),[67,72–74] whereas never-smokers were significantly more likely than former or current smokers to have a transition mutation (G–>A).[78] In addition, the *K-ras* codon 12 mutation pattern in lung adenocarcinomas from smokers is distinct from that in gastrointestinal malignancies,[79] suggesting that *K-ras* codon 12 may be a specific target of the mutagenic activity of tobacco smoke.

The *EGFR* tyrosine kinase domain and *K-ras* mutations are mutually exclusive, in that no tumors examined had mutations in both genes. The exclusivity indicates that, in smokers, tobacco carcinogens might specifically induce *ras* signaling pathways through mutations in *K-ras*, whereas in

never-smokers the unidentified carcinogens might selectively target the *EGFR* pathway through mutations in *EGFR*. Because of the exclusivity of *EGFR* and *K-ras* mutations, researchers have studied the possible role of *K-ras* mutation as an indicator of *EGFR* tyrosine kinase inhibitors' sensitivity. In a study of advanced lung adenocarcinomas treated with *EGFR* tyrosine kinase inhibitors, *K-ras* mutation with or without increased *EGFR* copy number suggested disease progression.[80]

LKB1 Mutation

The genetic alterations of the *LKB1* gene (also known as *STK11*), which has been implicated in the regulation of multiple biological processes and functions as a tumor-suppressor gene,[81] occurs more commonly in lung adenocarcinomas and large cell carcinomas than in squamous cell carcinomas and small cell carcinomas.[82–85] In a recent study, Matsumoto *et al*[86] found that *LKB1* mutations were significantly more frequent in lung cancer cell lines with *K-ras* mutations than in those without. More importantly, *LKB1* mutations were found in 8% of male smokers with lung cancer but in none of the 64 female smokers and/or non-smokers,[86] indicating that lung cancer *LKB1* genetic alterations possibly correlate with smoking in men.

ALK Rearrangement

A small inversion within chromosome 2p, resulting in the formation of a fusion gene comprising portions of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*) gene, has recently been identified in lung squamous cell carcinomas/adenocarcinomas.[87] Intronic sequences downstream of exons 13, 20, and 6 of *EML4* are fused to intron 19 of *ALK* to generate variants 1, 2, and 3 of *EML4–ALK*, respectively.[87,88] This fusion tyrosine kinase showed transforming potential both *in vivo* and *in vitro*. In lung carcinomas with *ALK* gene rearrangements, *ALK* protein was expressed and phosphorylated, leading to the activation of multiple signaling pathways that contribute to cell survival and transformation, such as *Akt* and *Erk1/2.* The *ALK* inhibitor *TAE684* could completely abolish these phosphorylations and suppress cell growth.[89]

In lung cancer patients, the *EML4–ALK* fusion was detected in 2–7% of tumors, with a higher percentage in adenocarcinomas and a complete absence in carcinomas of other types.[87,90–95] In addition, *ALK* rearrangement was associated with younger age and never-smoker status, similar to findings for *EGFR* mutation.[91,93,94] However, *EML4–ALK* fusion was mutually exclusive with *EGFR* or *K-ras* mutation.[87,92,94,96] Therefore, *ALK* inhibitors may provide a means to control lung adenocarcinomas in patients with genomic *ALK* rearrangements, for whom effective treatments are rarely available.

Epigenetic changes

Methylation

Aberrant methylation of cytosine at the promoter regions of genes is one of the major mechanisms of the downregulation or upregulation of genes in lung cancers. An increasing number of genes have been intensively investigated for their methylation status in lung cancers, including *p16*, *RASSF1A*, *APC*, *RARβ-2*, *CDH1*, *CDH13*, *DAPK*, *MGMT*, *ASC/TMS1*, *FHIT*, *hSRBC*, *TSLC1*, *DAL-1*, and *PTEN*.[97] These genes are involved in cancer cell-cycle regulation, proliferation, apoptosis, cell adhesion, mobility, and DNA repair. Aberrant DNA methylation provides another mechanism for the inactivation of tumor-suppressor genes along with the genetic mechanisms to promote lung cancer occurrence and progression.

Methylation of genes has been shown to be associated with the smoking history of patients with lung cancer. In lung adenocarcinomas/squamous cell carcinomas, the frequency of *p16*, *MGMT*, *RASSF1*, *MTHFR*, and *FHIT* promoter methylation was significantly higher among smokers than never-smokers.[98–101] On the other hand, methylation in certain genes, such as *RASSF2*, *TNFRSF10C*, *BHLHB5*, and *BOLL*,[102,103] was higher in lung cancers from never-smokers than those from smokers, suggesting smoking may target specific genes for methylation.

Given the aberrant gene methylation observed in lung cancers, researchers have studied the roles of methylation in lung cancers for early detection, risk assessment, disease progression, and prognosis. The *RASSF1A*, *APC*, *ESR1*, *ABCB1*, *MT1G*, and *HOXC9* genes were more frequently methylated in stage I lung adenocarcinomas/squamous cell carcinomas than the non-cancerous lesions,[104] whereas the prevalence of *hDAB2IP*, *H-cadherin*, *DAL-1*, and *FBN2* methylation was associated significantly with advanced stage of lung cancer,[105–107] indicating that these genes might be involved in different phases of lung cancer progression.

Gene methylation has also been studied for the roles of genes as prognostic markers. Hypermethylation of *RASSF1A*, *PTEN*, *DAPK*, *p16*, *Wif-1*, *CXCL12*, *DLEC1*, *MLH1*, *H-cadherin*, *APC*, *RUNX3*, *H-cadherin*, *SPARC*, and *DAL-1* was significantly associated with poor prognosis in patients with surgically resected lung adenocarcinomas/squamous cell carcinomas.[101,106,108–113] In addition, methylation of *14-3-3 sigma* in pretreatment serum DNA was found to be an independent prognostic factor for survival in patients with lung adenocarcinoma, squamous cell carcinoma, and large cell carcinoma who were treated with platinum-based chemotherapy.[114]

Previous researchers have reported that methylation, in addition to occurring in tumor tissues, can also be detected in blood samples or in exfoliated material of the aero-digestive tract epithelium from lung cancer patients, such as the methylation of *RASSF1A*,[115,116] *p16*, *H-cadherin*,[117] *MAGE A1*, and *MAGE B2*.[118] Moreover, it became evident that methylation of certain genes is also detectable in cancer-free smokers. Therefore, gene methylation might be used as a surrogate marker for screening in high-risk populations. Belinsky *et al*[119] found that methylation of *p16*, *PAX5-b*, *MGMT*, *DAPK*, *GATA5*, and *RASSF1A* in sputum collected within 18 months of lung cancer diagnosis was associated with a >50% increased lung cancer risk. Furthermore, the concomitant methylation of three or more of the above six genes was associated with a 6.5-fold increased risk, with values of 64% for both sensitivity and specificity.

DNA methylation is mediated by *DNA methyltransferases* (*DNMTs*), which transfer a methyl group to the 5′-position of cytosine. Three members of the *DNMT* family (*DNMT1*, *DNMT2*, *DNMT3a, 3b*) have been cloned in mammals. *DNMT1*, *DNMT3a*, and *DNMT3b* proteins are highly expressed in lung carcinoma, particularly in smokers.[120] Furthermore, their overexpression correlated with hypermethylation in the tumor-suppressor gene promoters.[120,121] This is consistent with the observation *in vivo* that the tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), attenuated *DNMT1* degradation and enhanced *DNMT1* nuclear accumulation and hypermethylation of the promoters of tumor-suppressor genes. In lung carcinomas, *DNMT1* and *DNMT3b* overexpression was significantly associated with poor prognosis.[120–122]

Histone Modification

In addition to gene promoter methylation, histone modification is another epigenetic control of gene transcription. Recent findings have confirmed that histones are not merely simple 'DNA-packaging' proteins but rather dynamic regulators of gene activity that undergo many post-translational chemical modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. The status of acetylation and methylation of specific lysine residues contained within the tails of nucleosomal core histones is crucial in regulating chromatin structure and gene expression.[123]

Changes in global levels of several histone modifications are predictive of the clinical outcome of lung cancers. By immunohistochemistry and a recursive partitioning analysis, 138 patients with stage I and II lung adenocarcinomas/squamous cell carcinomas/large cell carcinomas were classified into seven distinct prognostic groups based on TNM stage, histology, and histone modifications: histone 3 lysine 4 dimethylation (H3K4diMe), histone 3 lysine 9 acetylation (H3K9Ac), and histone 2A lysine 5 acetylation (H2AK5Ac).[124] In a recent study, Seligson *et al*[125] found that lower cellular levels of histone H3 lysine 4 dimethylation (H3K4me2) and H3K18 acetylation (H3K18ac) predict significantly poorer survival probabilities for lung cancer patients.

Histone deacetylation is mediated by *histone deacetylases* (*HDAC*s), which work synergically to alter the chromatin condensation status and repress transcription with *DNMTs* and a family of methylated DNA-binding proteins.[126] In general, high *HDAC* activity is associated with condensed, transcriptionally inactive chromatin. In addition to this epigenetic function, it is now recognized that certain *HDAC*s also exhibit important cytoplasmatic function by controlling the acetylation status and function of numerous cytoplasmic proteins and transcription factors.[127] Several authors have examined *HDAC* expression in lung cancer specimens. Sasaki *et al*[128] reported that the mRNA and protein expressions of *HDAC1* correlated with the progression of lung carcinomas, although there was no difference in mRNA expression between tumor and adjacent non-tumor lung tissue. However, Bartling *et al*[129] found that *HDAC3* was upregulated in squamous lung cancers compared with non-tumorous lung tissues. In a group of 72 patients with lung adenocarcinoma/squamous cell carcinoma/large cell carcinoma, mRNA expression levels of *HDAC* class I (*HDACs 1–3* and *8*) and class II (*HDACs 4–7*, *9*, and *10*) genes in cancer tissues were measured using real-time RT–PCR. Reduced expression of each class II *HDAC* gene was significantly associated with poor prognosis with *HDAC10* as the strongest predictor for poor prognosis.[130] 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. Previous studies found that *HDAC* inhibitor suppressed the *PI3K/Akt*[131] and *Src/Raf/MEK/ERK1/2*[132] 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[133] and small cell carcinoma cells.[133,134] Coincident with inhibition of *ERK1/2* and *PI3K/AKT* survival pathways, the *HDAC* inhibitor *FK228* enhanced *JNK* and *p38MAPK* signaling,[132] whereas an *SIRT1* inhibitor, Sirtinol, impaired activation of *Ras/MAPK* pathways in response to *EGF* and insulin-like growth factor-I.[135] Furthermore, another *HDAC* inhibitor, trichostatin A, suppressed the levels of *COX-2* mRNA and protein expression, which were correlated with an inhibition in prostaglandin E_2 synthesis in lung adenocarcinoma cells.[133] However, *HDAC* inhibitors do not always function in inducing apoptosis of lung cancer cells despite their ability to effectively inhibit deacetylase activity. *HDAC* inhibitors could stimulate *NF-κB*, resulting in expression of *NF-κB*-dependent genes such as *IL-8*, *Bcl-XL*, and

MMP-9, which cause failure to induce apoptosis in lung carcinoma cells.[136] Therefore, members of the *HDAC* family have a potential role as lung cancer treatment targets.

MicroRNAs

miRNAs are a family of small RNA molecules (~22 nt) that regulate specific gene expression post-transcriptionally. Abnormal expression of miRNAs are believed to be involved in the initiation and progression of human cancer.[137] Such miRNAs as *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-1α*, *NIRF*, *MCL-1*, *BCL2L2*, *cyclins D1, D2 and E1*, and *Ezrin*.[138–146]

Abnormally expressed miRNAs may have potential as markers in lung cancer diagnosis, treatment response, and prognosis. *Has-miR-205*, which suppresses epithelial-to-mesenchymal transition, has been identified as a highly specific marker for squamous carcinoma of the lung.[147,148] A well-studied miRNA in lung cancer is *miR-21*. In a group of 48 lung adenocarcinomas/squamous cell carcinomas, overexpression of mature *miR-21* was found in 52% of cases compared with their corresponding non-cancerous tissues. During the follow-up period, mature *miR-21* was upregulated in 16 (55%) of 29 patients who had relapse and in 15 (65%) of 23 patients who died. Mature *miR-21* overexpression correlated with overall patient survival ($P=0.027$), suggesting that overexpression of mature *miR-21* could be an independent negative prognostic factor for overall survival in patients with lung adenocarcinoma/squamous cell carcinoma patients.[149] In a recent study, *miR-21* expression in sputum specimens was significantly higher in patients with lung adenocarcinoma/squamous cell carcinoma (76.32 ± 9.79) than in cancer-free individuals (62.24 ± 3.82) ($P<0.0001$). Furthermore, detection of *miR-21* expression had 70% sensitivity and 100% specificity for the diagnosis of lung cancer, compared with 48% sensitivity and 100% specificity for sputum cytology, suggesting that measurement of altered miRNA expression in sputum could be a useful non-invasive approach for the diagnosis of lung cancer.[150] Studies also showed that lower *let-7*[151] and *miR-34*[152] expression predicted short survival or a high probability of relapse in patients with lung adenocarcinomas/squamous cell carcinomas/large cell carcinomas, whereas high *miR-146b*[153] and *miR155*[154] expression correlated with poor survival. Through analysis of miRNA expression in patients with lung adenocarcinoma/squamous cell carcinoma patients, 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.[155] 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 lung adenocarcinoma/squamous cell carcinoma.[156]

Compared with gene expression-based predictive classifier, certain miRNA signatures were more informative in predicting survival. For example, *miR-146b* alone was found to have a predictive accuracy for prognosis in ~78% of patients with lung squamous cell carcinoma,[153] better than the overall predictive accuracy of 68% for a 50-gene signature.[157] This is probably due to miRNAs being upstream regulators of gene expression with hundreds of downstream targets, suggesting that miRNAs may have more powerful prediction abilities than their target genes.

Conclusions

The development of lung cancer is a complex process, involving a series of genetic and epigenetic changes. Smoking directly induces the gene mutation, while methylation, HDACs, and miRNAs might indirectly affect carcinogenesis via modulating gene mutation and expression. A better understanding of how these factors participate in lung cancer development and progression would provide us with powerful tools for lung cancer prevention, diagnosis, and treatment by identifying practical molecular markers correlated with clinical parameters. Furthermore, understanding the molecular characteristics of lung cancers would aid in targeted therapy development, as in the example of EGFR inhibitors including monoclonal antibodies (eg, cetuximab) and small-molecule tyrosine kinase inhibitors (eg, erlotinib, gefitinib), which were developed and applied clinically based on our knowledge of EGFR mutation in lung cancer patients.[158] To date, clinical utilization has been approved for very few biomarkers. Tremendous efforts are required to translate the findings generated from basic research to clinical application in lung cancers. Further validation of these potential biomarkers should involve careful selection of the markers, larger sample sizes with long-term follow-up, and multi-center studies. In addition, molecular testing for multiple biomarkers may generate higher sensitivity or specificity for clinical application than a single marker. Similarly, multi-target therapies that interfere with more than one pathway might be more effective than single-target agents in treating the deadly disease of lung cancer.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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