Epigenetics of lung cancer

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Lung cancer is the leading cause of cancer-related mortality in the United States. Epigenetic alterations, including DNA methylation, histone modifications, and noncoding RNA expression, have been reported widely in the literature to play a major role in the genesis of lung cancer. The goal of this review is to summarize the common epigenetic changes associated with lung cancer to give some clarity to its etiology, and to provide an overview of the potential translational applications of these changes, including applications for early detection, diagnosis, prognostication, and therapeutics. (Translational Research 2015;165:74–90)

Abbreviations: 5-meC = 5-methylcytosine; BMI-1 = B-cell-specific Moloney murine leukemia virus integration site 1; DNMT = DNA methyltransferase; EZH2 = enhancer of zeste homologue 2; HDAC = histone deacetylase; HOTAIR = Hox transcript antisense intergenic RNA; lncRNA = long noncoding RNA; MALAT1 = metastasis-associated lung adenocarcinoma transcript 1; mRNA = messenger RNA; NNK = nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NSCLC = nonsmall cell lung cancer; PcG = polycomb group gene; PRC = polycomb repressive complex; SCLC = small cell lung cancer

Despite large-scale reductions in cigarette consumption during the past several decades, lung cancer remains the leading cause of cancer-related mortality in the United States and is the second leading cause of death overall, after heart disease.[1] Although lung cancer rates have declined steadily among men since the 1980s and seem to have plateaued among women, there still remain an estimated 228,190 new cases and 159,480 deaths each year.[1] This high mortality rate is driven by the high incidence of this disease coupled with its dismal 5-year survival rate of only 17%.[1]

The vast majority of lung cancer is can be characterized as small cell (neuroendocrine) carcinoma (SCLC) or nonsmall cell carcinoma (NSCLC), which includes, broadly, squamous cell carcinoma, adenocarcinoma, and large cell carcinoma subtypes.[2] NSCLC is by far the more common of the types, accounting for approximately 85% of all lung cancer cases.[3] Although these histologies share a common organ of origin and some molecular attributes, they also exhibit unique molecular traits and represent distinct diseases, and it has become clear that it is important to make the distinction between the histologies for the purpose of treating the disease.

Although smoking remains the major risk factor for all histologies (especially small cell and squamous cell carcinoma), it is important to note that only some 10% of smokers will ultimately develop lung cancer.[4] Moreover, not all patients with lung cancer have a smoking history; there are other risk factors for the disease. Adenocarcinoma, which accounts for

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approximately 37% of all lung cancers in the United States, is the most common form among nonsmokers.[5] Globally, an estimated 15% of men and 53% of women with lung cancer are never-smokers.[6] Other risk factors for the disease include radon, asbestos, and environmental/occupational exposure to polycyclic aromatic hydrocarbons and other pollutants.[2] However, as with smoking, not all who are exposed to these environmental factors go on to develop lung cancer.

The carcinogenic process is driven by the accumulation of genetic and epigenetic alterations that result in dysregulation of key oncogenes, tumor suppressor genes, and DNA repair/housekeeping genes. The probability of incurring these pathologically important events is dependent primarily on the individual exposome in conjunction with interpersonal phenotypic variability. Although genetic heterogeneity accounts for some of the variable risk, it does not explain this phenomenon *in toto*.[7] *Epi*genetic variability, including DNA methylation, histone modifications, and noncoding RNA expression, also contribute to the phenotypic makeup of an individual (eg, xenobiotic metabolism, DNA repair capacity, immunity, and so on) and, accordingly, risk of malignancy.

This underscores the importance of enhancing our knowledge of lung cancer epigenetics (in addition to genetics) to comprehend more fully the pathogenesis of this disease. Moreover, continued expansion of our understanding of various epigenetic events involved with different types of lung cancer expands the potential battery of diagnostic and prognostic biomarkers available to clinicians, as well as introduces new avenues for the discovery of novel therapeutic targets. The primary objective of this review is to summarize the common epigenetic events that are associated with lung cancer and provide an overview of the potential translational applications of these events for the management of this disease.

EPIGENETICS AND ETIOLOGY

Tumorigenesis. Lung cancer involves an accumulation of genetic and epigenetic events in the respiratory epithelium.[8] Although somatic genetic aberrations, such as mutations and copy number alterations, play a well-known role in oncogenesis, epigenetic alterations are, in fact, more frequent than somatic mutations in lung cancer.[9]

Tumor suppressor gene inactivation through promoter methylation, often referred to as hypermethylation, is a hallmark of lung cancer and tends to occur as an early event in the carcinogenic process.[10,11] Promoter methylation of specific tumor suppressor genes, along with the overall number of hypermethylated genes, increases with neoplastic progression from hyperplasia to adenocarcinoma.[12,13] Promoter methylation can couple with mutation or deletion events to inactivate a tumor suppressor gene (ie, a different inactivation event for each allele). This is because inactivation of 1 allele for dominantly acting suppressor gene loci is generally insufficient to lead to clonal selection, because the protein can still be produced from 1 normal allele. However, there is also evidence that, at some gene loci, both copies do not necessarily need to be inactivated to impact the cell adversely; rather, partial inactivation of 1 allele, giving rise to haploinsufficiency (ie, 1 wild-type allele is insufficient to provide full functionality) can contribute to carcinogenesis.[14] In these cases, inactivation of 1 allele by promoter methylation would be sufficient for clonal selection.

Many of the tumor suppressor genes that are hypermethylated in lung cancer are also frequently hypermethylated in other types of solid tumors.[6] Some are specific, although many are not, similar to what is observed for somatic mutations. In premalignant and malignant states, promoter methylation is commonly observed in genes involved with crucial functions, including cell cycle control, proliferation, apoptosis, cellular adhesion, motility, and DNA repair.

Some of the most oft-studied genes in the context of promoter methylation in lung cancer include *p16INK4a*, *RASSF1A*, *APC*, *RARβ*, *CDH1*, *CDH13*, *DAPK*, *FHIT*, and *MGMT*. Although *p16INK4a* is hypermethylated, mutated, or deleted frequently in NSCLC, with estimates for the prevalence of alteration of this gene around 60%, *p14arf*, which is also encoded on the *CDKN2A* gene, is inactivated much less commonly (~8%–30% of NSCLC).[15,16] Moreover, although a common event in NSCLC, *p16INK4a* is disrupted in less than 10% of SCLC.[15] In addition, *RASSF1A* is deleted or hypermethylated in 30%–40% of NSCLC and 70%–100% of SCLC,[15] *FHIT* is deleted or hypermethylated in 40%–70% of NSCLC and 50%–80% of SCLC,[15] and *TSLC1* is hypermethylated in an estimated 85% of NSCLC.[15] A more extensive list of known, commonly hypermethylated genes in lung cancer is provided in Table I.[16-68]

Hypermethylation of *CDKN2A* may occur early in the genesis of some lung cancers, having been identified in premalignant lesions.[69] Promoter methylation of *RASSF1A*, *APC*, *ESR1*, *ABCB1*, *MT1G*, and *HOXC9* have been associated with stage I NSCLC,[70] suggesting they too may occur relatively early during the development of the cancer. CpG island methylation of homeobox-associated genes is also common in stage I lung cancer, appearing in nearly all early-stage tumors.[71] Conversely, other commonly hypermethylated genes, such as *hDAB2IP*, *H-Cadherin*, *DAL-1*, and *FBN2*, have been associated with advanced-stage