Aberrant Epigenetic Regulation: A Central Contributor to Lung Carcinogenesis and a New Therapeutic Target

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OVERVIEW

Carcinogenesis is driven by a combination of genetic and epigenetic abnormalities. Aberrancies in gene promoter methylation patterns and histone acetylation are associated with silencing of tumor suppressor genes in lung cancer and other solid tumors. Identification of key epigenetic modifications has been shown to be prognostic in early-stage non-small cell lung cancer. Previous clinical trials aimed at modifying the epigenome with single-agent demethylating agents or histone deacetylase inhibitors given at maximally tolerated doses have provided disappointing results. A recent clinical trial using a combination of a demethylating agent and a histone deacetylase inhibitor at “epigenetically targeted” doses concomitantly has shown promising results, including a patient with a complete objective response. Biomarkers associated with this clinical trial suggest that patients who undergo robust demethylation, as detected in the peripheral blood after a month on treatment, identifies those who gain the most benefit from this novel treatment strategy. Based on observations of unusually durable responses to subsequent therapy after administration of combined epigenetic therapy, epigenetic therapy may also play a role in “priming” patients to better respond to standard cytotoxic therapy or immunotherapy. This manuscript will review the data on the role of epigenetics in lung cancer and the history of epigenetic treatments in lung cancer spanning over the last 40 years.

Genetic abnormalities have been associated with malignancies for decades, and defining key driver mutations in subsets of patients with lung cancer has become a primary mode of classifying this disease and optimizing treatment. Mutations that inactivate tumor-suppressor genes or activate oncogenes are linked with carcinogenesis.1 Mutations in genes or aberrations of chromosomes are not the sole cause of malignancy; epigenetic changes also play a central role in carcinogenesis.2 Epigenetic changes reflect patterns of altered gene expression that are not caused by direct changes in the DNA sequence but rather by alterations in chromatin and other associated factors that modify the ability of genes to be transcribed.2 Primary levels of epigenetic regulation include modifications of histones as well as changes in the patterns of DNA methylation.2 Through these epigenetic changes, tumor-suppressor genes can be “silenced,” allowing for dysregulated cell growth.2

HISTONE MODIFICATION

The transcriptional state of chromatin depends on the interplay of a complex set of post-translational modifications to histone lysine tails including acetylation, methylation, and phosphorylation, referred to globally as the “histone code.”3 Modifications of specific residues on histones are recognized by secondary binding proteins which affect chromatin conformation and transcription.2 Histone modifications in the regions of specific gene promoters are of particular importance in modifying the expression of those genes.3 Acetylation of lysine residues on the NH2-terminal tails of histones neutralizes the positive charge of the histone tail and decreases histone affinity for negatively charged DNA.4 As a consequence, it is thought that histone acetylation leads to changes of nucleosomal conformation that allow DNA to become more accessible to transcription factors, as illustrated in Fig. 1. In general, acetylation of lysine residues on histone tails is associated with transcriptionally active chromatin (euchromatin), whereas deacetylated histones are associated with transcriptionally inactive chromatin (heterochromatin).2 Histone acetylation, which opens the DNA for transcription, is primarily controlled by histone acetyltransferases (HATs), whereas signaling to more tightly pack the DNA and decreasing transcription factor accessibility, is managed through histone deacetylases (HDACs).5

DNA METHYLATION

Histone modification is just one method of altering DNA transcription patterns. At a fundamental level, genes may be suppressed based on direct changes in the methylation pattern of their promoters as demonstrated in Fig. 1.5 Many gene promoters contain a high frequency of CG

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CURRENT EXPERIENCE WITH GENE SILENCING IN LUNG CANCER

Cancer-specific DNA methylation has been implicated in controlling multiple facets of lung tumorigenesis. Genes often aberrantly methylated and epigenetically silenced in non-small cell lung cancer (NSCLC) include RASSF1A, CDH13, MGMT, hMLH1, hMLH2, DAPK, and beta-Catenin. The CDKN2A gene encoding p16 is more frequently methylated in squamous cell carcinoma, whereas the APC and CDH13 genes are more commonly methylated in adenocarcinomas.

PROGNOSTIC IMPLICATIONS OF GENE SILENCING IN LUNG CANCER

Brock and colleagues evaluated the detection of tumor-specific methylation changes as a predictive biomarker for recurrence in patients with early-stage lung cancer by using a retrospective nested case control study of 167 patients with stage I NSCLC. Fifty-one patients with stage I NSCLC who had rapid disease recurrence after definitive surgical resection were compared with 116 control patients who had not had a relapse within 40 months of follow-up. The primary tumor, regional lymph nodes, and mediastinal lymph nodes were assessed for aberrant methylation. Presence of defined patterns of tumor suppressor gene promoter methylation, particularly in combinations, in either tumor or in lymph nodes predicted up to a 15-fold increased risk of recurrence. Combinations of four genes, CDKN2A (p16), RASSF1A, CDH13 (H-cadherin), and APC, were identified as strong predictors of early recurrence. The presence of methylation in at least two of these four genes in paired primary tumors and mediastinal lymph nodes identified a subset of patients at markedly increased risk of early recurrence (median time to recurrence 9, months vs. 25 months; p ≤ 0.04).

TARGETING THE EPIGENOME

DNA Methyltransferase Inhibitors

DNA methyltransferase (DNMT) inhibitors have been under investigation for over 40 years. The most widely used DNA methyltransferase inhibitor is 5-azacitidine. This compound is a cytidine analog that, after incorporation into DNA, functions as a suicide inhibitor of DNA methyltransferases. 5-azacitidine is not a specific inhibitor of DNA methyltransferases. As a result of its ribonucleoside structure, most of the compound becomes incorporated into RNA and thereby interferes with protein translation. This reduced specificity has been addressed by the development of a deoxyribonucleoside analog, decitabine, which is more exclusively incorporated into DNA and causes more efficient inhibition of DNA methyltransferases.

DNA Methyltransferase Inhibitors in Lung Cancer

5-azacitidine has been studied in several phase I/II trials in solid tumors. Between 1972 and 1977, seven clinical studies of patients with mixed solid tumors (sample size between eight and 177) treated with 5-azacitidine were performed that included a total of 103 patients with lung cancer. A variety of doses and regimens (including intravenous or subcutaneous routes of administration) were studied, with overall modest or no benefit. Of the 103 patients with lung cancer treated on all seven trials, five had objective responses (4.8%) lasting between 1 and 3 months.

Since the 1980s, eight clinical phase I/II studies of patients with solid tumors treated with decitabine have been performed. Over 200 patients with solid tumors have been treated with single-agent decitabine and only two documented transient objective responses have been reported.

Histone Deacetylase Inhibitors

Histone deacetylases (HDACs) are critically important in the regulation of gene expression and are being actively studied as target-specific anticancer drugs in many clinical contexts. HDACs deacetylate histones, which, in general, leads to repression of gene transcription. In addition to differential subcellular localization and distinct tissue expression patterns, different HDACs have been shown to associate with
distinct transcription regulatory complexes. Thus, different HDAC family members may preferentially target different sets of promoters in controlling gene expression. Some of the transcriptional repressors recruit heterochromatin-like complexes that cause gene silencing that is mediated via specific repression pathways. Many of these appear to involve HDAC and heterochromatin proteins that convert the gene region into a heterochromatic environment.

Several chemical groups of HDAC inhibitors have been identified, including: (1) hydroxamates, (2) aliphatic acids, (3) cyclic peptides, and (4) benzamides. The first generation of HDAC inhibitors included compounds such as trichostatin A, valproic acid, sodium phenylbutyrate, and romidepsin. Most of the first-generation HDAC inhibitors have not been useful clinically because of issues with stability and potency. Romidepsin is the only first-generation agent that is approved for use as an HDAC inhibitor.

Multiple subsequent generation HDAC inhibitors are now in clinical development. The second-generation HDAC inhibitors fit into two categories: hydroxamates and benzamides. All of these are available in oral formulations, in contrast to the first-generation agents, which were generally intravenous agents that often required prolonged infusions. The main difference, biologically, between the two second-generation groups is that hydroxamates are pan-HDAC inhibitors, whereas benzamides target some or all of the class I
Histone Acetylase Inhibitors in Lung Cancer

Over 20 clinical trials of single-agent HDAC inhibitors have been reported in the literature with a total enrollment of over 650 patients. Twenty-one patients with NSCLC have been treated. Of all these trials, only one partial response was documented. Thirty-three patients were reported to have stabilization of disease—generally less than 4 months in duration. Vorinostat was the most widely studied with a total of 25 patients treated between phase I and II clinical trials. Half of these patients experienced 3 to 4 months of disease stabilization. The next largest single-agent cohort was treated with romidepsin. Nineteen patients were treated and one-half had short-term disease stabilization.

Preclinical Use of the Combination of DNMT and HDAC Inhibitors

The use of HDAC inhibitors, and in particular DNMT inhibitors, for patients with solid tumors has been impeded by substantial toxicity at maximally tolerated and cytotoxic doses. An observation by Cameron and colleagues changed the utility of these agents in solid tumors. This study showed that the methylation pattern of colon cancer cell lines that were treated with a combination of DNMT and HDAC inhibitors was able to be modulated with combinatorial low-dose treatment. The effects were not just additive; they were synergistic.

The combination of DNA methyltransferase and HDAC inhibitors has been assessed in a lung cancer animal model. Decitabine and phenylbutyrate were studied in the preventative setting in mice. Mice treated with decitabine had 30% fewer tobacco-carcinogen–induced lung cancers. Mice treated with decitabine and phenylbutyrate had over 50% fewer tobacco-carcinogen–induced lung cancers. These data in preclinical models are encouraging for the use of these classes of agents in patients.

Rationale for Clinical Development of Combination Epigenetic Therapy in Lung Cancer

As briefly summarized above, a wide array of data supports the importance of epigenetic alterations in the pathogenesis of lung cancer. Since the initial development of demethylating agents, we have learned that cytotoxic doses are not required to achieve epigenetic changes, allowing for reduced dosing which increases patient tolerability of demethylating treatment. We also now have second-generation HDAC inhibitors that are more potent and can be given orally rather than through prolonged daily infusions. In vitro, investigators have observed that a combination of a DNA methyltransferase inhibitor with inhibitors of histone deacetylase activity synergistically induce re-expression of tumor suppressors silenced by DNA methylation in cancer. These observations led to a study assessing double epigenetic blockade in NSCLC.

Double Epigenetic Blockade in Lung Cancer

A phase I/II clinical trial has been published by our group assessing the efficacy of double epigenetic blockade with a combination of 5-azacitidine and entinostat in advanced, refractory NSCLC. This clinical trial enrolled 45 patients who had at least one line of previous treatment that had failed in their lung cancer. The treatment was well-tolerated with mainly grade 1 and 2 toxicities, notably including skin site reactions from the azacitidine injections, nausea, vomiting, and constipation. Two durable objective responses were reported including one complete response. Two patients had stabilization of their disease for over 1 year. The overall disease control rate at 12 weeks was over 25%. Median survival in the entire cohort was 6.4 months (95% CI 3.8, 9.2), comparing favorably with existing therapeutic options.

Among the key aspects of this trial were the biologic correlates. One of the patients with an objective response had a complete response that lasted over 1 year. Epigenetic analysis of her original tumor found that it was densely methylated at multiple key gene promoters. A biopsy done at the time of eventual disease progression documented a second primary lung cancer clone that contained a Kras mutation. No evidence of her epigenetically driven cancer was found at the time of her death. An additional key correlate was generated from samples of peripheral blood drawn serially from patients enrolled on the study. Demethylation of the same four key lung cancer genes defined as predictors of recurrence in early-stage disease were analyzed in DNA isolated from the peripheral blood of patients on study. Demethylation of these key targets over the first month of therapy correlated with statistically significant improvements in progression-free (p = 0.034) and overall survival (p = 0.035). One drawback of this biomarker is that it requires patients to receive therapy for a month to identify this demethylation signature. Identification of a reliable biomarker which identifies patients who may respond to this treatment, before initiation of therapy, would be of great utility and is a focus of ongoing research. A primary strategy in the pursuit of better lung cancer treatment is the identification of subpopulations of patients who harbor targetable drivers that control the growth of their cancer. Relative to genetic aberrations, driver epigenetic changes are more difficult to identify given the almost ubiquitous presence of epigenetic modifications in cancer. One key may be to identify those patients whose tumor growth is primarily controlled by these epigenetic abnormalities as opposed to bystander changes.
Epigenetic Therapy As a Primer for Subsequent Therapy

Given the widespread presence of epigenetic abnormalities in cancer and the global roles of epigenetic regulation defining patterns of gene expression, reversal of epigenetic changes is likely to affect multiple cellular processes that may not lead directly to cancer cell death. One of those processes may be chemotherapy drug resistance. During the clinical trial of double epigenetic blockade, we observed that several patients had durable responses to their immediate next line of therapy. Among these were two patients who had responses to a single-agent standard cytotoxic chemotherapy that lasted over 4 years as fifth-line therapy or beyond. Both of these patients had progressive disease on epigenetic therapy. We hypothesize that the epigenetic therapy primed their cancer for response to subsequent treatment. In addition, multiple patients treated with combined epigenetic blockade followed by targeted immunotherapy by using antibodies directed against the critical immune checkpoint regulators PD-1 and PD-L1 have had durable responses to these agents. Ongoing preclinical analyses are investigating the potential mechanisms responsible for these effects, and future clinical trials are being planned to further evaluate these observations.

CONCLUSION

Epigenetic alterations are strongly associated with lung cancer development. Combined epigenetic therapy with azacitidine and entinostat, inhibitors of DNA methylation and histone deacetylation, respectively, offers a unique and durable approach to managing this challenging disease in a subset of patients. This therapy is well-tolerated, and objective responses were observed. Trials are currently in progress to further define potential biomarkers for this therapy as well as to assess the utility of epigenetic therapy as a primer for other cancer treatments such as chemotherapy and immunotherapy. Epigenetic therapy offers a promising and novel strategy in the fight against lung cancer that may complement other anticancer approaches including standard cytotoxic and immunotherapeutic agents.

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Relationships marked “U” are uncompensated.

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