Editorial Commentary

Prognostic value of epigenomic profiling in lung cancer

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Background: epigenetics landscape and lung cancer

Among all types of lung cancers, non-small cell lung cancer (NSCLC) is the most common cause of cancer-associated death. Conventional therapy of NSCLC includes radiation, surgery, and cis-platin therapy. However, other approaches of treatment, including epigenetic therapy, have been proposed (1-4). Cancer epigenetics is very complex, primarily because gene silencing by epigenetics varies in different tumor types (5-9). There are very few examples of immunotherapy in solid tumors and the article by Duruisseaux *et al.* (10) on epigenetic prediction of program death receptor (PD-1) treatment on lung cancer is a remarkable achievement in identifying population responsive to treatment. The article provides the groundwork for investigation in other tumor types.

Both genetics and epigenetics contribute to cancer development and multicellular organisms cannot develop and function without the interaction between the epigenome and genome (11). While genetics provides a repertoire of the genetic potential of an organism, epigenetics regulate gene expression and dictates how much of this genetic potential is actualized. Chromatin comprises both DNA and protein scaffold and the basic structure of chromatin, nucleosome, consists of two copies of each of the four histone proteins, namely H2A, H2B, H3, and H4, in the form of an octamer with DNA wrapped around it to neutralize the basic charge of the DNA providing stability to the genome. Histone H1 is a linker and functions as a scaffolding protein. Histone proteins respond to the microenvironment and post-translational modifications such as acetylation, butylation, phosphorylation, ubiquitination, and methylation, which can modulate non-covalent interactions between DNA and histones. MicroRNAs (miRNAs) and non-coding RNAs (small interfering RNAs, small nucleolar RNAs, piwi-interacting RNAs) are essential components of epigenetics which mediate gene expression. miRNAs are a class of non-coding RNAs that act post-transcriptionally and affect gene expression. miRNAs have been reported to contribute in inducing resistance to anticancer drugs, for example, resistance to 5-fluorouracil is mediated by miR-21, miR-27a/b, and miR-155, to docetaxel by miR-98, miR-192, miR-194, miR-200b, miR-212, and miR-424 (12). Mechanistically, the expression of miRNAs can be regulated by DNA methylation and histone modifications. More specifically, expression of those miRNAs which inactivate tumor suppressor genes can be reversed by treatment with demethylating or acetylating agents (epigenetic drugs). Thus, oncogenic miRNAs (oncomiR) can be targeted by epigenetics and cell proliferation due to cancer development can be reduced or stopped.

Drug resistance in lung cancer and a need to develop new approaches

Aberrant epigenetic changes play an important role in lung cancer initiation, development and progression, therefore drugs targeting epigenetic components are being evaluated for the treatment of this cancer type (3,4). Both DNA methyl transferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi) were administered sequentially followed by the induction of a favorable response to immunotherapy. The main player in this therapy was cytoplasmic double stranded RNA-mediated interferon response. The involvement of endogenous retrovirus transcription was

also demonstrated (13). In order to explore other avenues of therapy, one group of investigators applied a combination of radiation therapy with epigenetic inhibitor suberoylanilide hydroximic acid (SAHA) and successfully inhibited epithelial-mesenchymal transition and stemness of NSCLC cells (14). In another example of alternative therapy, Taguchi *et al.* proposed meta-analysis of reprogrammed NSCLC to identify targets for epigenetic therapy (15). Duruisseaux *et al.* suggested integration of tissue-derived epigenetic biomarkers and epigenetics drugs in clinical trial design to prevent drug resistance and improve lung cancer patient outcomes (16,17).

Armas-López *et al.* reported mesenchyme homeobox2-GLI1 transcription axis and involvement of H3K27AC and H3K4me3 in drug resistance (17). Above examples indicate that different approaches are now available for epigenetic therapy of lung cancer and epigenetic disruptions could present reliable biomarkers for lung cancer risk assessment, early detection, disease stratification, and follow up of survival.

Although successful drugs and therapies have been produced for the treatment of cancer, the development of resistance remains a critical challenge owing to the fact that mechanisms contributing to resistance remain largely unknown (18,19). There is a need to develop new approaches to treat lung cancer because drug resistance develops in some of the patients who had chemotherapy. In the recent years, drugs developed by Pharmacogenomics have improved survival of lung cancer patients, but all patients do not have those mutations where actionable chemotherapy can be designed. For those patients who have metastasis, immunotherapy, especially immune checkpoint receptor-based therapy, seems reasonable because PD-1 or its ligand (PD-L1) based therapies have been successful in treating the disease. PD-1 based therapy has been applied as a first line of therapy in the case of NSCLC and three drugs targeting PD-1 (nivolumab, pembrolizumab, and atezolizumab) have been approved (10,16). This receptor is located on the T or B cells and its ligand is present on cancer cells. Binding of the ligand with the receptor leads to immune suppression resulting in inhibition of the tumor growth.

Historically in 2013, on the basis of studies done with PD-1/PD-L1 and azacytidine, Wrangle and colleagues hypothesized that the combination of epigenetic therapy and PD-1 pathway blockade might produce a synergistic anti-tumor response (20). Schiffmann *et al.* have elegantly compiled updates about epigenetic therapies in combination with immunotherapies in lung cancer (21). Additionally, the current status of immunotherapies for advanced lung cancer can be found at https://www.cancer.gov/news-events/cancer-currents-blog/2018/pembrolizumab-lung-cancer-first-line.

In the present study authors have emphasized that the successful treatment of NSCLC patients with any of these three drugs ranges from 30% to 44%, therefor new biomarkers are needed which could predict the prognostic outcome of immunotherapy in NSCLC patients. Investigators of this article selected epigenetic profiling (degree of DNA methylation, referred as EPIMMUNE in this article) in biospecimens of NSCLC patients by following the approaches used by other investigators for (1) glioblastoma and (2) cancers of the unknown primary origin. In this study, the unmethylated status of the regulatory T cell transcription factor fork head box P1 was used as a prediction biomarker of the response to therapy. Duruisseaux *et al.* followed mutational load and PD-L1 expression but a direct correlation of response to treatment could not be observed with PD-L1 expression and mutational load (10). That observation also supported the idea of epigenetic regulation as an alternative mechanism of prediction to response to conventional therapy.

Issue of heterogeneity and prospectively collected samples

Biospecimens were collected from multiple institutes located in France, Spain, and Italy and heterogeneity of the population remains a point of discussion. Further, the population under study is Caucasian, therefore, implication of the findings reported in this article to other racial and ethnic groups is not certain yet. It will be worth using the same approach in other populations and conducting a prospective study with multiple samples collected temporally and analyzed for the EPIMMUNE characteristics. Chances of variation due to methylation array analysis are less because all the analysis of samples collected from different institutes was done in a central place located in the Bellvige Biomedical Research Institute.

The inclusion criteria for participation in this study was the presence of stage IV NSCLC and previously removed tumor samples from the participants.

Different cell lineages and methylation marks

In this article, whole exome sequencing was performed to get genomic information and evaluating its association with EPIMMUNE profiling (10). During the evaluation

of enriched CG dinucleotide (CpG) sites, non-responder CpGs were removed based on their threshold. Methylation profiling was determined for different cell lineages (myeloid, lymphoid, endothelial or mesenchyme) to avoid any lineage-associated methylation marks and conducting Wilcoxon signed rank test was very appropriate.

Integration of data from other epigenetic marks

Four major components of epigenetic regulation are: DNA methylation, histone modifications, non-coding RNAs, and chromatin accessibility. There is a coordination in all these four components. Therefore, measuring all these changes in same samples will reduce variation and improve intervention and therapy in non-responsive individuals as has been suggested in the literature (22).

Challenges and potential solutions

Therapeutic resistance is a major problem in clinical oncology (18). One of the challenges in epigenetic therapy is how to select cancer patients that may benefit from epigenetic therapy, either alone or in combination, at the greatest extent and how to measure the response to therapy quantitatively. Accumulation of genetic and epigenetic alterations contributes to the self-renewal and drug-resistant capacity of cancer stem cells (CSCs) mainly due to their multipotent properties (23). Differentiation by inflammation, oncogenes, or similar approaches might present challenges to therapies exclusively targeting CSCs. However, targeting CSCs and modulating the tumor microenvironment by epigenetic approaches to augment immune attacks on cancer could be a strategy worth investigating.

One intrinsic problem with epigenetic therapy is that epigenetic processes are needed for normal development also, therefore, a rigorous understanding of the effect of inhibitory functions of epigenetic drugs is needed to design rational therapies capable of inhibiting oncogenic properties and either none or minimally affecting normal functions. Despite all the hurdles discussed above, the potential for epigenetic drugs in the treatment of resistant cancers is high and clinical trials have just begun to evaluate the area (https://clinicaltrials.gov/). Epigenetics is independent of the sequence events that physically affect the condensing of chromatin and gene expression. Involvement of epigenetic regulators in cancer heterogeneity and drug resistance has opened the possibility for the development of new drugs/approaches aimed at epigenetic component targets. Aberrations in epigenetic components, especially methylation and miRNAs, may involve in the development of drug resistance in targeted therapy and a combined therapy with epigenetic drugs has been proposed (24). Chemoresistance in cancer is a significant unmet clinical obstacle which is being addressed by different approaches including epigenetic approaches and immunotherapy, as discussed (10). Novel therapeutic approaches are nonsensible to overcome the disease. The new idea in cancer treatment is dual epigenetic therapy in combination with immune checkpoint blockade to finally making tumors responding to therapy. There is a need to develop a comprehensive panel of treatment predicting factors to improve stratification of patients for targeted (and cytotoxic) therapies so that ineffective treatment could be eliminated. Furthermore, a thorough investigation of the molecular mechanisms of resistance could indicate the use of anticancer drugs, alone or in combination, in an effective way and help predict the response to therapy accurately. Finally, the article by Duruisseaux *et al.* contributes significantly in our understanding or applying epigenetic approaches to predict treatment response.

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