Editorial Commentary

DNA methylation profiling in early lung adenocarcinoma to predict response to immunotherapy

Charles De Smet

Group of Genetics and Epigenetics, de Duve Institute, Université catholique de Louvain, Brussels, Belgium

Correspondence to: Charles De Smet. de Duve Institute, 75 Avenue Hippocrate, 1200 Brussels, Belgium. Email: Charles.desmet@uclouvain.be.

Comment on: Guidry K, Vasudevaraja V, Labbe K, *et al*. DNA methylation profiling identifies subgroups of lung adenocarcinoma with distinct immune cell composition, DNA methylation age, and clinical outcome. Clin Cancer Res 2022;28:3824-35.

Keywords: Lung adenocarcinoma; DNA methylation; immunotherapy

Submitted Feb 23, 2023. Accepted for publication Mar 24, 2023. Published online Mar 29, 2023.
doi: 10.21037/tlcr-23-96
View this article at: https://dx.doi.org/10.21037/tlcr-23-96

The management of patients with lung cancer has changed significantly in recent decades, and this is largely due to the advent of new treatment options (1). Among these, immunotherapeutic approaches seem particularly promising (2). These strategies are based on the principle that, while tumors can be recognized and destroyed by immune T cells, they mobilize a variety of escape mechanisms that allow them to resume development without immune constraint. As an escape mechanism, some tumors turn up immune checkpoint pathways that put the breaks on T cell activities. One therapeutic option is to inhibit the action of checkpoint proteins, with the hope of unleashing T cell responses and enabling tumor rejection. Immune checkpoint inhibitors (ICI) have shown clinical efficacy in lung cancer, and are now considered as a first-line treatment for patients with advanced non-small cell lung cancer (NSCLC), often in combination with chemo- or radiotherapy.

Although clinical results have generated enthusiasm among physicians, there is substantial concern about the significant proportion of patients that do not respond to ICI treatments. Clearly, there is an urgent need to develop reliable diagnostic tools to select the patients that will benefit from ICI treatments. In this line, the presence of immune cells infiltrating the tumor, particularly $CD8^+$ cytotoxic T lymphocytes, appears to be a valid indicator to predict better chances of response to ICI (3). It is considered indeed that such "hot" tumors comprise immunogenic cancer cells and invading T lymphocytes, but that the latter became paralyzed when immune checkpoints were activated. Accordingly, ICI treatments would more likely resuscitate immune responses in hot tumors rather than in non-infiltrated "cold" tumors (4). There are multiple ways by which the immune component of tumors can be assessed, for instance by immunohistochemical detection of T-cell markers, or by gene expression analyses to identify immune-cell signatures (5). In a recent study published in *Clinical Cancer Research*, Guidry and colleagues assessed the value of analyzing DNA methylation profiles to gauge the immune composition in lung adenocarcinoma (LUAD), the most common type of NSCLC (6).

DNA methylation, which consist in the addition of a methyl group on cytosines in CpG dinucleotides, is an epigenetic mechanism that establishes long-term repression of defined genes. Specific patterns of DNA methylation, which are set during development of the embryo and fetus, are later maintained with a fairly high level of fidelity in differentiated cells (7). Every cell type in adult individuals is therefore characterized by a fixed profile of genomic methylation (8). Taking this principle into account, an analytical tool (MethylCIBERSORT) was developed that scans global genomic methylation data in search of cell-type specific methylation signatures, and estimates thereby the abundances of individual cells types in a mixed cell population (9). The procedure is an adaptation of the originally RNAseq-applied deconvolution method Cell type Identification by Estimating Relative Subsets of known RNA Transcripts (CIBERSORT) (10). An advantage of DNA methylation-based screening procedures lies on the robustness of DNA, and hence the

fact that they can be easily applied to both fresh frozen or formalin-fixed paraffin-embedded tissue samples. Evidence that MethylCIBERSORT can be used to classify immunologically hot and cold tumors was previously provided in head and neck squamous carcinoma, where immunohistochemical analyses demonstrated that most *in silico* defined hot tumors were indeed infiltrated by immune cells (9). In their study, Guidry and colleagues applied MethylCIBERSORT to a cohort of 88 resected early-stage LUAD samples from which they generated methylomic data using the Infinium methylation assay, a procedure that allows determination of the methylation status of several hundred thousands CpGs distributed throughout the genome. This enabled classification of immunologically hot and cold tumors, which appeared to be present in equal amounts among LUAD samples. Comparison of the two immunological phenotypes revealed no difference in overall survival, an observation that was in disagreement with previous studies where hot tumors correlated with a better prognosis (11,12). Further examination of possible correlations between immune cell composition of LUAD samples and clinical parameters revealed that patients with a smoking history displayed increased immune cell infiltration within their tumor.

The authors did not stop at the MethylCIBERSORT analysis of cell composition, but decided to dive deeper into the DNA methylation data with the goal of identifying variations that could help them better characterize LUAD subgroups. The point here is that variations of DNA methylation in tumors not only depend on the composition of infiltrating cells, but also reflect epigenetic alterations that affect the genome of cancer cells. It is well documented indeed that many cancerous cells show profound alterations in their DNA methylation pattern (13). Both gains and losses of methylation are observed in different parts of the tumor genomes, and there is evidence that these changes contribute to malignant progression, notably by contributing to the repression of tumor suppressor genes or the activation of oncogenes, respectively (14,15). In other instances, DNA methylation changes do not immediately modify the transcription status, but lock genes that are already repressed into an irreversibly silent state. This may modify the way cancer cells respond to signaling cues (16). The mechanisms underlying DNA methylation changes in cancer cells are still partially understood, but involve imbalances of methylation and demethylation activities, which can result from mutations in epigenetic regulators, or be associated with hypoxia, inflammation, and aging (17-20). More recently, infiltration of immune cells in the tumor was found to correlate with DNA methylation changes in the cancer cells, often within genes that are involved in immune escape mechanisms (21). In summary, defining altered DNA methylation patterns in cancer cells provides information not only on their current gene expression program, but also on what they have been through, and how they will respond to environmental challenges.

Another aspect of DNA methylation is its natural evolution during the individual's lifetime. Several studies have indeed identified specific CpGs that exhibit age-related changes in the different tissues (22,23). Such changes can be computed to provide a DNA methylation age (DNAm age) that estimates the "biological age" of tissues. DNAm ages match remarkably well with the chronological age of the donors (24). There are however between-person variations in the biological aging process, which can be influenced by genetic background, lifestyle, or disease. Not surprisingly, application of the DNAm age calculation to tumor tissues revealed significant acceleration of biological aging in most samples (24,25).

In their study, Guidry and colleagues applied the Horvath's DNAm age calculation procedure (based on the analysis of 353 CpGs) to all of their 88 LUAD samples, and observed discrepancies between the biological age of the tumors and the chronological age of the corresponding patients (at the time of resection). Surprisingly, tumors with both accelerated and decelerated aging were observed. Tumor aging could however not be significantly correlated with either overall survival or infiltration by $CD8^+$ T cells (6). The authors then went back to the complete DNA methylation data they had generated, and performed an unsupervised clustering analysis with the goal of identifying LUAD tumor subgroups that would display specific patterns of DNA methylation changes. The procedure identified 6 distinct subgroups. To determine whether this classification could inform the immune phenotype of the samples, the authors used their MethylCIBERSORT data to calculate the proportion of hot tumors in each subgroup. Interestingly, one subgroup of tumors (#4, 20 samples) was composed of 100% hot tumors, whereas two others (#1, 11 samples, and #2, 19 samples) contained 100% cold tumors. The other three subgroups were composed of a mixture of hot and cold tumors. Importantly, the authors performed immunohistochemical analyses on representative tumor samples of the different subgroups, which confirmed that infiltration of $CD8^+$ T cells was lower in subgroups #1 and #2, and higher in subgroup #4 (6). These results therefore

support the idea that DNA methylation profiles can be used to determine the immune phenotype of LUAD tumors.

The study by Guidry *et al.* thus adds to other work suggesting that DNA methylation can be used as a basis for the development of diagnostic tools that characterize the immune profile of tumors, and thus their likely response to ICI. In an ideal clinical setting, it would be preferable to identify a handful of informative CpGs whose methylation status could be easily analyzed by methylation-specific PCR. Interestingly, the author's results suggest that DNA methylation changes associated with immune phenotype reflect not only the cellular composition of the tumor microenvironment, but also epigenetic alterations in the cancer cells that strive to resist immune attacks. It would be interesting to examine the genes that are affected by DNA methylation changes in cancer cells, as this could shed light on the biological mechanisms by which these cells escape the immune system, and hence open new therapeutic perspectives. Another strength of Guidry's study is that it focused on early stage LUAD tumors, and suggests that immune profiling by DNA methylation profiling is applicable to patients who are at an early stage of disease development. This is important because it adds to current efforts to develop tools that would allow earlier diagnosis and management of lung cancer.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Lung Cancer Research*. The article did not undergo external peer review.

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-96/coif). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

References

1. Alexander M, Kim SY, Cheng H. Update 2020: Management of Non-Small Cell Lung Cancer. Lung 2020;198:897-907.
2. Doroshow DB, Sanmamed MF, Hastings K, et al. Immunotherapy in Non-Small Cell Lung Cancer: Facts and Hopes. Clin Cancer Res 2019;25:4592-602.
3. Li F, Li C, Cai X, et al. The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: A systematic review and meta-analysis. EClinicalMedicine 2021;41:101134.
4. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348:56-61.
5. Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. Nat Rev Cancer 2020;20:662-80.
6. Guidry K, Vasudevaraja V, Labbe K, et al. DNA Methylation Profiling Identifies Subgroups of Lung Adenocarcinoma with Distinct Immune Cell Composition, DNA Methylation Age, and Clinical Outcome. Clin Cancer Res 2022;28:3824-35.
7. Ushijima T, Watanabe N, Okochi E, et al. Fidelity of the methylation pattern and its variation in the genome. Genome Res 2003;13:868-74.
8. Schultz MD, He Y, Whitaker JW, et al. Human body epigenome maps reveal noncanonical DNA methylation variation. Nature 2015;523:212-6.
9. Chakravarthy A, Furness A, Joshi K, et al. Pan-cancer deconvolution of tumour composition using DNA methylation. Nat Commun 2018;9:3220.
10. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015;12:453-7.
11. Djenidi F, Adam J, Goubar A, et al. CD8+CD103+ tumor-infiltrating lymphocytes are tumor-specific tissue-resident memory T cells and a prognostic factor for survival in lung cancer patients. J Immunol 2015;194:3475-86.
12. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat

Rev Drug Discov 2019;18:197-218.
13. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer 2011;11:726-34.
14. Esteller M. Epigenetics in cancer. N Engl J Med 2008;358:1148-59.
15. Van Tongelen A, Loriot A, De Smet C. Oncogenic roles of DNA hypomethylation through the activation of cancer-germline genes. Cancer Lett 2017;396:130-7.
16. Gal-Yam EN, Egger G, Iniguez L, et al. Frequent switching of Polycomb repressive marks and DNA hypermethylation in the PC3 prostate cancer cell line. Proc Natl Acad Sci U S A 2008;105:12979-84.
17. Issa JP. Aging and epigenetic drift: a vicious cycle. J Clin Invest 2014;124:24-9.
18. Nishiyama A, Nakanishi M. Navigating the DNA methylation landscape of cancer. Trends Genet 2021;37:1012-27.
19. Niwa T, Ushijima T. Induction of epigenetic alterations by chronic inflammation and its significance on carcinogenesis. Adv Genet 2010;71:41-56.
20. Thienpont B, Steinbacher J, Zhao H, et al. Tumour hypoxia causes DNA hypermethylation by reducing TET activity. Nature 2016;537:63-8.
21. Jung H, Kim HS, Kim JY, et al. DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load. Nat Commun 2019;10:4278.
22. Bocklandt S, Lin W, Sehl ME, et al. Epigenetic predictor of age. PLoS One 2011;6:e14821.
23. Koch CM, Wagner W. Epigenetic-aging-signature to determine age in different tissues. Aging (Albany NY) 2011;3:1018-27.
24. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013;14:R115.
25. Lau CE, Robinson O. DNA methylation age as a biomarker for cancer. Int J Cancer 2021;148:2652-63.

Cite this article as: De Smet C. DNA methylation profiling in early lung adenocarcinoma to predict response to immunotherapy. Transl Lung Cancer Res 2023;12(4):657-660. doi: 10.21037/tlcr-23-96