Editorial

Development of predictive liquid biomarkers for response to treatment in small cell lung cancer

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Comment on: Carter L, Rothwell DG, Mesquita B, *et al.* Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. Nat Med 2017;23:114-9.

Abstract: Small cell lung cancer (SCLC), despite being initially chemosensitive, behaves aggressively and tends to progress rapidly after or during first-line chemotherapy. Predictive indicators of response to specific treatment for SCLC have not yet been established. Carter *et al.* had reported that they established a genetic classifier to predict whether SCLCs were "chemosensitive (sensitive relapse)" or "chemorefractory (refractory relapse)". They used whole genome amplification products of native circulating tumor cell (CTC) from patients to develop this classifier. These CTC classifiers could accurately identify patients with SCLC as "sensitive relapse" or "refractory relapse" to first-line chemotherapy. Although this study represented a remarkable step forward in biomarker research in SCLC, classifiers obtained in the same fashion at disease progression could not predict the response to further treatment. This may imply that the inherent genetic background for the initial response to first-line chemotherapy differs from that for newly acquired resistance to treatment. In order to improve our understanding of the biological backgrounds of SCLC, extensive research into concepts such as cancer stem cells, epithelial mesenchymal transition/ mesenchymal epithelial transition, and circulating tumor microemboli might be necessary.

Keywords: Circulating tumor cell; liquid biopsy; small cell lung cancer; tumor biomarker

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Small cell lung cancer (SCLC) is different from non-small cell lung cancer (NSCLC) in that it is more malignant and aggressive; it progresses rapidly and metastasizes by the time of diagnosis. Even though SCLCs are initially highly responsive to first-line chemotherapy, most patients relapse within a few months to a year after the initial therapy (1). The response to first-line chemotherapy and the length of the interval after the last dose of the first-line chemotherapy can predict the subsequent clinical outcome of the second line chemotherapy (2-5). Based on these factors, relapsed SCLCs are classified into "sensitive relapse" and "refractory relapse". Patients who respond to first-line chemotherapy and then relapse after 2–3 months are considered "sensitive relapse," whereas patients whose disease progressed during the first-line chemotherapy or whose tumors recurred within 2–3 months after the first-line chemotherapy are considered "refractory relapse."

Because of the disseminated nature of SCLC, surgical resection or serial biopsies are seldom indicated. The genomic profile and background of SCLC are not as well established as that of many other cancers due to lack of surgical or biopsy specimens. Nevertheless, SCLC was reported recently to show high levels of genetic alterations, including in RB transcriptional co-repressor 1 (*RB1*) and tumor protein p53 (*TP53*); indeed, lung cancer is the second most frequent type of cancer associated with genetic mutations (6-9). Despite recent progress, very few genetic molecular biomarkers are available to predict the clinical outcome of SCLC (10).

Circulating tumor cells (CTCs) and circulating tumor

DNA (ctDNA) are the most well-established blood-borne biomarkers for tumors. They are considered tumor-derived cells and DNA which are shed into the bloodstream; they are collected through a process called "liquid biopsy" and studied as reliable alternatives for conventional biopsies. They provide detailed molecular data useful for clinical management of patients with lung cancer, and analysis of these biomarkers may be useful for selecting treatment methods, for tumor monitoring, and for studying resistance mechanisms. In particular, the companion diagnostic test to detect a mutation (T790M) that provides resistance against serum epidermal growth factor receptor (EGFR) was approved by the U.S. Food and Drug Administration; it provides the clinical indication for the use of third-generation EGFR-tyrosine kinase inhibitor to treat patients with advanced NSCLC. On the other hand, many devices have been developed recently to detect and capture CTCs. They are broadly classified as label-dependent and label-independent, according to the methods used for detecting CTCs. In label-dependent assays, CTCs are separated from other blood cells using cell surface markers such as epithelial cell adhesion molecule (EpCAM) and CD45 (11). The label-independent assays rely on biophysical differences between CTCs and blood cells (11). The CellSearch® is a well-validated assay to detect EpCAM expressing CTCs. The presence of CTCs detected using this assay is associated with poor prognosis in patients with some cancers, and might be useful for monitoring patients with metastatic breast, colorectal, or prostate cancers (12-14).

Regarding SCLCs, the detection rate of CTCs by the CellSearch® assay has been reported to be relatively high; the majority of patients (67–86%) had 2 or more CTCs per 7.5 mL of blood (15-20). However, the prognostic impact of CTCs and their association with metastases in patients with SCLC remains unknown. Changes in the number of CTCs before and after chemotherapy might indicate the patients' survival and treatment response (15-20).

As mentioned above, despite recent progress in research on genetic molecular alterations, sufficient data to predict the clinical outcome of SCLC is not available. As an excellent alternative for primary tumor materials, liquid biopsy can be used. It is a feasible, repeatable, and less invasive procedure, and the specimens obtained have been used for molecular analysis to understand the biology of SCLCs and the mechanisms behind the treatment response. However, there are two major limitations for the application of CTCs in genetic molecular analysis. One is the challenge behind separating and extracting single CTCs from a mixture with other contaminating blood cells without losing small amounts of CTCs. The other is the comprehensive investigation of molecular traits of individual CTCs. A recent technological advance, the DEPArray™ technology (Silicon Biosystems S.p.A.) can automatically prepare a suspension of isolated single CTCs, already sorted and enriched by the CellSearch® assay in order to perform the whole genome amplification (WGA) (21).

A recent study by Carter *et al.* reported a copy-number aberration (CNA)-based SCLC CTC classifier, comprising 16 different CNA profiles, to identify genetic features that could distinguish "sensitive relapse" from "refractory relapse" (22). In this study, blood samples were collected and enumeration of CTCs was performed using the CellSearch® assay, and stored CTCs that were obtained in a previous study were used. Individual SCLC CTCs from the CTC-enriched suspension were extracted using the DEPArray™ system, and the WGA products obtained from them were used for the CNA analysis to develop the SCLC CTC classifiers. They found that 83.3% of cases were correctly classified as "refractory relapse" or "sensitive relapse," based on the patient's own CTC-based CNA classifier. In addition, the progression-free survival (PFS) of patients was calculated with the baseline CNA classifier. The PFS of patients with "sensitive relapse" was significantly longer than that of patients with "refractory relapse." Such analyses are called retrospective-prospective analyses, and require further prospective investigation to confirm the results. However, this result suggested that their baseline CNA classifier might be capable of accurately predicting the response for first-line treatment as well as the clinical outcome of SCLCs.

Furthermore, the authors used the same CNA classifiers on the serially collected CTC samples of the corresponding patients to analyze the acquired genetic alterations. Notably, the CTC CNA classifier of patients with initially "sensitive relapse" did not become a "refractory relapse" CNA profile at disease progression, indicating that the CNA profile classified the disease as chemosensitive at the disease progression. No chromosomal changes were detected. This result suggested that the genetic background for the initial response to chemotherapy differs from that for acquired chemoresistance.

These contradictions offer some scope for speculation. As mentioned previously, most epithelium-derived CTCs in the bloodstream are thought to express EpCAM on their cell surface; however, CTCs have also been detected in

cancers that do not express markers of epithelial origin (23). In addition, the expression levels of this cell surface marker vary in tumor cells during epithelial-mesenchymal transition (EMT) (23). During EMT, the epithelial tumor cells lose their epithelial features and are subsequently converted into mesenchymal cells; they then migrate into surrounding connective tissues and blood vessels. In contrast, tumor cells in metastatic lesions generally exhibit an epithelial appearance; this suggests that a reversed version of EMT may occur in these metastatic sites. Once tumor cells reach their destination organs, they lose their mesenchymal aspects and regain their epithelial features, and this process is called mesenchymal-to-epithelial transition (MET). During these processes, cell surface markers such as EpCAM are thought to be lost to some extent and their expression levels may vary and become heterogeneous (23). CTCs in the bloodstream may have different characteristics after EMT, and some CTCs may lose their epithelial markers. Carter L. *et al.* employed EpCAM-dependent CellSearch® assay to detect SCLC CTCs, and this might be why they did not detect epithelial marker-negative CTCs (22,24).

Similar to single CTCs, tumor cell clusters in the peripheral blood, named circulating tumor microemboli, have been reported recently (25). These tumor cell clusters had a higher metastatic potential than single tumor cells (26,27). Apoptotic and proliferating cells were seldom seen in these CTC clusters, indicating that these cells have a survival advantage against cytotoxic chemotherapy and against anoikis, which is a form of programmed cell death that occurs in cells that lose contact with the surrounding extracellular matrix (20,28). These cell clusters had also lost the epithelial cell markers, and expressed mesenchymal markers. Therefore, epithelial-marker dependent CTC detection assays, including the CellSearch® assay, might have missed these cell clusters (16,20).

Furthermore, SCLCs are known to be malignant and aggressive, and the occurrence of rapid relapses after highly effective chemotherapy suggests that SCLCs may contain cancer stem cell (CSC) components (29). As part of CTCs, CSCs may play a crucial role in tumor biology, including tumor heterogeneity, resistance to chemotherapy and radiotherapy, recurrence, and metastasis. Importantly, EMT may induce stem cell characteristics; in fact, some CTCs possess CSC characteristics, and are called, "circulating cancer stem cells" (28-32). These cells may share their origin with CSCs. Therefore, a thorough understanding of CSCs, including circulating cancer stem cells and EMT/MET, would be necessary for understanding the biological features of SCLCs.

As mentioned previously, recent rapid advances in liquid biopsy has made it possible to use ctDNA in the clinical setting to facilitate clinical decision-making. This is undoubtedly an extremely sensitive method to detect the cancer burden, even if they are small. However, ctDNA is not suitable for analyzing proteins or for functional and morphological analysis of cancer cells. CTCs allow structural evaluation of the cancer phenotype, permit *in vivo* and *in vitro* assays, make molecular characterization of the disease possible, and enable immunocytochemical labeling techniques, even though their inherent heterogeneity makes it difficult to detect their presence. They can therefore be used as complementary tools with ctDNA (11). Observing a single aspect of tumor biology might deny us full comprehension, and so, deep and extensive research on liquid biopsies, using multiple modalities, is required.

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Footnote

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References

1. Hansen HH. Management of small-cell cancer of the lung. Lancet 1992;339:846-9.
2. Ardizzoni A, Hansen H, Dombernowsky P, et al. Topotecan, a new active drug in the second-line treatment of small-cell lung cancer: a phase II study in patients with refractory and sensitive disease. The European Organization for Research and Treatment of Cancer Early Clinical Studies Group and New Drug Development Office, and the Lung Cancer Cooperative Group. J Clin Oncol 1997;15:2090-6.
3. Giaccone G, Donadio M, Bonardi G, et al. Teniposide in the treatment of small-cell lung cancer: the influence of prior chemotherapy. J Clin Oncol 1988;6:1264-70.
4. Kim YH, Goto K, Yoh K, et al. Performance status and sensitivity to first-line chemotherapy are significant prognostic factors in patients with recurrent small cell lung cancer receiving second-line chemotherapy. Cancer 2008;113:2518-23.
5. Postmus PE, Berendsen HH, van Zandwijk N, et al. Retreatment with the induction regimen in small cell lung cancer relapsing after an initial response to short term chemotherapy. Eur J Cancer Clin Oncol 1987;23:1409-11.
6. Rudin, CM, Durinck S, Stawiski EW, et al. Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. Nat Genet 2012;44:1111-6.
7. Peifer M, Fernández-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012;44:1104-10.
8. George J, Lim JS, Jang SJ, et al. Comprehensive genomic profiles of small cell lung cancer. Nature 2015;524:47-53.
9. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499:214-8.
10. Dowlati A, Lipka MB, McColl K, et al. Clinical correlation of extensive-stage small-cell lung cancer genomics. Ann. Oncol 2016;27:642-7.
11. Calabuig-Fariñas S, Jantus-Lewintre E, Herreros-Pomares A, et al. Circulating tumor cells versus circulating tumor DNA in lung cancer-which one will win? Transl Lung Cancer Res 2016;5:466-82.
12. Cristofanilli M, Hayes DF, Budd GT, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005;23:1420-30.
13. de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 2008;14:6302-9.
14. Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:3213-21.
15. Tanaka F, Yoneda K, Kondo N, et al. Circulating tumor cell as a diagnostic marker in primary lung cancer. Clin Cancer Res 2009;15:6980-6.
16. Hou JM, Greystoke A, Lancashire L, et al. Evaluation of circulating tumor cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy. Am J Pathol 2009;175:808-16.
17. Wu C, Hao H, Li L, et al. Preliminary investigation of the clinical significance of detecting circulating tumor cells enriched from lung cancer patients. J Thorac Oncol 2009;4:30-6.
18. Hiltermann TJ, Pore MM, van den Berg A, et al. Circulating tumor cells in small-cell lung cancer: a predictive and prognostic factor. Ann Oncol 2012;23:2937-42.
19. Naito T, Tanaka F, Ono A, et al. Prognostic impact of circulating tumor cells in patients with small cell lung cancer. J Thorac Oncol 2012;7:512-9.
20. Hou JM, Krebs MG, Lancashire L, et al. Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. J Clin Oncol 2012;30:525-32.
21. Polzer B, Medoro G, Pasch S, et al. Molecular profiling of single circulating tumor cells with diagnostic intention. EMBO Mol Med 2014;6:1371-86.
22. Carter L, Rothwell DG, Mesquita B, et al. Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. Nat Med 2017;23:114-9.
23. Aceto N, Toner M, Maheswaran S, et al. En Route to Metastasis: Circulating Tumor Cell Clusters and Epithelial-to-Mesenchymal Transition. Trends in Cancer 2016;2:159-60.
24. de Wit S, van Dalum G, Lenferink AT, et al. The detection of EpCAM(+) and EpCAM(-) circulating tumor cells. Sci Rep 2015;5:12270.
25. Stott SL, Hsu CH, Tsukrov DI, et al. Isolation of circulating tumor cells using a microvortex- generating

herringbone-chip. Proc Natl Acad Sci U S A 2010;107:18392-7.
26. Fidler IJ. Metastasis: Quantitative analysis of distribution and fate of tumor embolilabeled with 125 I-5-iodo-2'-deoxyuridine. J Natl Cancer Inst 1970;45:773-82.
27. Liotta LA, Saidel MG, Kleinerman J. The significance of hematogenous tumor cell clumps in the metastatic process. Cancer Res 1976;36:889-94.
28. Frisch SM, Screaton RA. Anoikis mechanisms. Current Opinion in Cell Biology 2001;13:555-62.
29. Codony-Servat J, Verlicchi A, Rosell R. Cancer stem cells in small cell lung cancer. Transl Lung Cancer Res 2016;5:16-25.
30. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 2009;9:265-73.
31. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704-15.
32. Scatena R, Bottoni P, Giardina B. Circulating tumour cells and cancer stem cells: a role for proteomics in defining the interrelationships between function, phenotype and differentiation with potential clinical applications. Biochim Biophys Acta 2013;1835:129-43.

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