Editorial

Circulating tumor cells in lung cancer: cluster circulating tumor cells as hybrid epithelial-mesenchymal transition/mesenchymal-epithelial transition (E/M)

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Provenance: This is an invited Editorial commissioned by the Section Editor Ji-Gang Wang (Department of Pathology, The Affiliated Hospital of Qingdao University, Qingdao University, Qingdao, China).

Comment on: Lindsay CR, Faugeroux V, Michiels S, *et al.* A prospective examination of circulating tumor cell profiles in non-small-cell lung cancer molecular subgroups. Ann Oncol 2017;28:1523-31.

Submitted Aug 24, 2017. Accepted for publication Sep 05, 2017.

doi: 10.21037/jtd.2017.09.63

View this article at: http://dx.doi.org/10.21037/jtd.2017.09.63

Metastasis is the most frequent cause of death among lung cancer patients (1), as such it is crucial to understand the mechanism of cancer metastasis to control the disease. Almost all metastases rise from isolated tumor cells (ITCs) which are dislodged from the original cancer lesion. Among patients with lung cancer, the regions where ITCs, which are reported as prognostic indicator, are detected vary in the pleural space (2), the lymph nodes (3), the parenchyma of the lung (4), the surgical margin of the lung (5), the bone marrow (6), the circulating peripheral blood (7) and so on. The circulating tumor cells (CTCs), which are one of the ITCs, is suitable for clinical-pathological investigation because the grade of intervention to extract CTCs is very little. Like as other ITCs, CTCs are dislodged from the original cancer lesion so that the CTCs are speculated to keep the same molecular characteristics as the original cancer lesion. As the molecular characteristics of the lung cancer cell attributes to tumor histology (8,9) and the status of epithelial-mesenchymal transition (EMT) of cancer cells in the cancer lesion (10-12), the same attribution of the molecular characteristics to CTCs is speculated.

Based on above research question, Lindsay *et al.*, among patients with lung cancer, investigated CTCs extracted using CellSearch® method, which extracts EpCAM positive (+) CD45 negative (–) single cells from the peripheral circulating blood, and revealed (13) that (I) the occurrence of CTC >5 was an indicator of poor prognosis; (II) the EMT status of CTC by way of vimentin was not an indicator of prognosis and (III) the positive relationship between vimentin status and molecular status was shown in the status of EGFR but in KRAS nor in ALK. Since the sub classification according to molecular status associates with pathological diagnoses (8,9) and the prognosis of patients (14), it is speculated that the molecular sub classification also associates with the EMT status of CTCs. However, the results from the Lindsay report are aside. The reason why the results fell in as shown in Lindsay report is that the target CTC selected was EpCAM (+) single CTC. If the Lindsay study employed another CTC selection method which can extract not only single CTCs but also cluster CTCs, the results might be different. This is because the EMT status of CTC changes time by time and is different between single CTC and cluster CTCs (15) (*Figure 1*).

The CTCs, which is one aspect ("snap-shot") of metastasis and dislodged from the original cancer lesion, varies in single CTC and cluster CTCs. The status of single or cluster depends on the EMT status which attribute to the potential of tumorigenesis, i.e., cluster CTCs pose greater potential of metastasis as 50 times as single CTC (16) (*Figure 1*). In days when the concept of EMT had been on developing, it is reported that the cluster cancer cells pose greater potential of cancer stem cell than single cancer cell (17,18). This may be because, as shown in *Figure 1*, the cluster cancer cells associate with hybrid (transitional) EMT/mesenchymal-epithelial transition (E/M) (15). The hybrid E/M CTCs are dislodged from the cancer lesion with cluster morphology

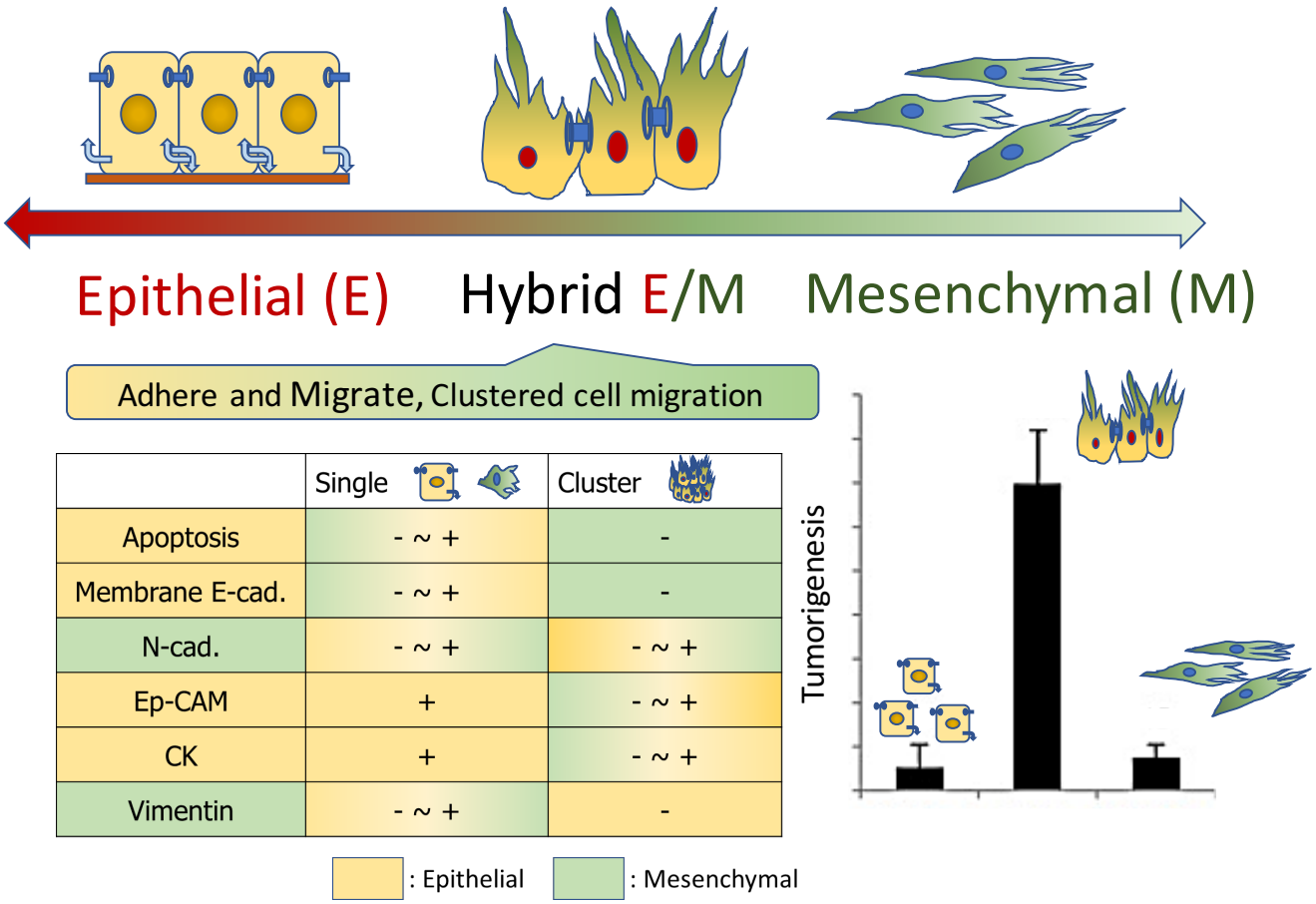

Figure 1 Hybrid epithelial-mesenchymal transition/mesenchymal-epithelial transition (E/M). The epithelial-mesenchymal transition (EMT) status of circulating tumor cell (CTC) changes time by time and is different between single CTC and cluster CTCs. In addition, the hybrid E/M CTCs are dislodged from the cancer lesion with cluster morphology posing abilities of (I) adherence, (II) migration, (III) anti-anoikis and (IV) tumor formation. The status of single or cluster depends on the EMT status which attribute to the potential of tumorigenesis, i.e., cluster CTCs pose greater potential of metastasis than single CTC. Cad., cadherin.

posing abilities of (I) adherence, (II) migration, (III) anti-anoikis and (IV) tumor formation (19). In practice, among lung cancer patients who underwent surgery the detection of cluster CTCs is reported as an indicator of poor prognosis compared to single CTC alone or no detection of CTC (20,21). This phenomenon mentions the cruciality to distinguish cluster CTCs from single CTC. In addition, the pattern of expression of mesenchymal marker is revealed to be deferent at single CTC from at cluster CTCs (22) (*Figure 1*), above all vimentin expression occurred more frequently at single CTC than at cluster CTCs, as such more further insight is needed into the clinical implication of EMT markers at CTCs.

As mentioning so far, the tumorigenesis of CTC associates with morphological characteristics such as single or cluster which is a surrogate of high malignancy. The high potential tumorigenesis of cluster CTCs attributes to the status of hybrid E/M. In the study presented by Lindsay *et al.* (13) employed the CellSearch® as the method of CTC extraction. Because this equipment catches EpCAM positive CTC via magnetic antibody, the method is not good at catching cluster CTCs especially huge cluster CTCs (23). The representative commercially available CTC detection method are listed in *Table 1* based on the ability to extract cluster CTCs (24,25). The methods via gravity for pulmonary vein (PV) blood or the method of size selection for peripheral blood may be recommended to extract cluster CTCs posing the status of hybrid E/M CTC which is crucial to control cancer

Table 1 Commercially available circulating tumor cell detection systems

Method	Selection	Target	Exclusive machine	Cell collection	
				Rate	Clustered cells
CellSearch®	Positive, Antibody	EpCAM(+), CD45(–), CK(+)	Needed (FDA approved)	Excellent	Fair
RosettSep™	Negative, Density	CD45(–)	Not needed	Good; PV-blood Fir; CTC	Excellent
ISET®	Size	Large, Cluster	Needed	Excellent	Excellent
ScreenCell®	Size	Large, Cluster	Not needed	Excellent	Excellent

FDA, Food and Drug Administration; PV, pulmonary vein; CTC, circulating tumor cell.

metastasis.

The summary is follows: (I) CTCs are the "seeds" of metastasis; (II) the cluster CTCs, in comparison to single CTC, pose high potential to make metastasis; (III) the pattern of EMT marker appearance is different between single CTC and cluster CTCs which frequently express vimentin negative; (4) cluster CTCs are hybrid E/M with higher potential of (i) adherence, (ii) migration, (iii) anti-anoikis and (iv) tumor formation compered to single CTC and (v) detection method of CTC varies thus the ability to extract cluster CTCs is different each other and size selection method is recommended for peripheral circulating blood to extract cluster CTCs.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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Cite this article as: Sawabata N. Circulating tumor cells in lung cancer: cluster circulating tumor cells as hybrid epithelial-mesenchymal transition/mesenchymal-epithelial transition (E/M). J Thorac Dis 2017;9(10):3547-3550. doi:10.21037/jtd.2017.09.63