

Available online at www.sciencedirect.com

ScienceDirect

Biomedical Journal

journal homepage: www.elsevier.com/locate/bj

Review Article

Bring along your friends: Homotypic and heterotypic circulating tumor cell clustering to accelerate metastasis



Nicola Aceto*

Department of Biomedicine, Cancer Metastasis Laboratory, University of Basel and University Hospital Basel, Basel, Switzerland



ARTICLE INFO

Article history:

Received 8 August 2019

Accepted 7 November 2019

Available online 11 February 2020

Keywords:

Circulating tumor cells

Circulating tumor cell clusters

Metastasis

Cell–cell interactions

Liquid biopsy

ABSTRACT

Metastasis formation is a hallmark of invasive cancers and it is achieved through the shedding of circulating tumor cells (CTCs) from the primary site into the blood circulation. There, CTCs are found as single cells or as multicellular clusters, with clusters carrying an elevated ability to survive within the bloodstream and initiate new metastatic lesions at distant sites. Clusters of CTCs include homotypic clusters made of cancer cells only, as well as heterotypic clusters that incorporate stromal or immune cells along with cancer cells. Both homotypic and heterotypic CTC clusters are characterized by a high metastasis-forming capability, high proliferation rate and by distinct molecular features compared to single CTCs, and their presence in the peripheral circulation of cancer patients is generally associated with a poor prognosis. In this short review, we summarize the current literature that describes homotypic and heterotypic CTC clusters, both in the context of their molecular characteristics as well as their value in the clinical setting. While CTC clusters have only recently emerged as key players in the metastatic process and many aspects of their biology remain to be investigated, a detailed understanding of their vulnerabilities may pave the way towards the generation of new metastasis-suppressing agents.

Circulating tumor cells

Circulating tumor cells (CTCs) are cancer cells that have shed from a primary or metastatic tumor lesion and have entered

the blood circulation [1]. The isolation of CTCs from blood specimens has been a major hurdle for a long time, mostly due to the rarity of CTCs in peripheral blood samples and the absence of suitable technologies capable to enrich few CTCs from specimens containing billions of blood cells. More

* Corresponding author. University of Basel and University Hospital Basel, Spitalstrasse 21, 4031 Basel, Switzerland.

E-mail address: Nicola.Aceto@unibas.ch.

Peer review under responsibility of Chang Gung University.

<https://doi.org/10.1016/j.bj.2019.11.002>

2319-4170/© 2019 Chang Gung University. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

recently, however, the development of microfluidic tools applied to the liquid biopsy field has enabled detailed CTC isolation and interrogation [2,3].

These CTC technologies are designed to exploit differences between CTCs and blood cells and can be divided into two major groups, i.e. those that capture CTCs based on the expression of a particular antigen (antigen-dependent) versus those that take advantage of physical parameters such as size and deformability (antigen-independent). One typical example of an antigen-dependent CTC isolation technology is CellSearch [4], based on antibody-mediated CTC recognition through Epithelial cell Adhesion Molecule (EpCAM) and cytokeratin staining (both are expressed by epithelial cells but absent in blood cells), concomitantly with CD45 staining to exclude white blood cells (WBCs). CellSearch has been widely used for CTC enumeration in several studies involving large patient populations, and received FDA approval for the identification of high-risk patients among those with metastatic breast, prostate and colorectal cancer [4–7]. Alternatively, examples for antigen-independent CTC technologies include size-based approaches [8,9], for instance based on narrowing microfluidic channels that allow the passage of blood cells, while due to their larger diameter CTCs are trapped, independently of marker expression [8]. Similarly, micro-scale vortices and inertial focusing of cells also allow CTC enrichment from blood cells, taking advantage of their larger size [9]. While many more CTC isolation technologies are available on the market [2] and others are being produced, CTC-focused investigations have already revealed important features of the metastatic process.

One of the clearest features of CTCs, based on large clinical studies and confirmed with multiple technologies, is that their presence in the bloodstream of cancer patients (with varying thresholds based on the cancer type) correlates with a poor prognosis, compared to patients in whom CTCs are not found or are found to be below threshold [10]. This aspect is important for patient stratification, but it also allows to access tumor-derived material (cancer cells, in the case of CTCs, but also other tumor derivatives such as cell-free nucleic acids or extracellular vesicles) from a liquid biopsy, allowing minimally-invasive disease monitoring and interrogation [11]. Further, it is well-established that CTCs can be found within the bloodstream as single cells (single CTCs) or as cell clusters (CTC clusters) composed of cancer cells-only or admixed with immune or stromal cells [12]. While the presence of both single and clustered CTCs has been postulated first, then directly observed in surgical specimens already in the 19th century [13–15], their biological properties have only recently been investigated.

Circulating tumor cell clusters

CTC clusters are defined as multicellular CTC aggregates of two or more cells, held together through intercellular junctions. CTC clusters (a.k.a. tumor microemboli) were first postulated in 1858 by Rudolph Virchow, a German physician, anthropologist, politician and social reformer, best known for being the founder of the field of cellular pathology, affirming that metastasis could be explained by the arrest of tumor microemboli in the vasculature [13]. CTC clusters were then

also directly observed and investigated for the first time several decades ago [14–19], often as an incidental finding during autopsy of cancer patients. Generally, CTC clusters appear to be a feature of adenocarcinomas of the breast, colon, lung or stomach, and they have also been found in hepatocellular carcinoma, prostate cancer, choriocarcinoma and renal cell carcinoma, among other cancer types [20–23]. Their presence in the bloodstream of cancer patients has also been associated to a poor clinical outcome in a number of studies and cancer types [24–30].

It is however only recently that the biology underlying clustering of CTCs has been investigated. This has been possible not only by the development of specialized microfluidic tools that enable isolation of clustered CTCs alongside with single CTCs, but also thanks to sophisticated single cell-resolution assays and next-generation sequencing methods. While this field of research is still in its infancy, these recent studies (reviewed below) have highlighted important biological insights featuring clusters of cancer cells only (i.e. homotypic CTC clusters) as well as clusters that comprise non-cancerous cells additionally to cancer cells (heterotypic CTC clusters), suggesting a prominent role for these multicellular aggregates in the metastatic process.

Homotypic CTC clusters

Homotypic CTC clusters typically comprise a minority (1–30%) of the total CTC events found in the peripheral circulation of patients or mouse models, and their abundance is subject to disease stage, as well as size and molecular characteristics of the tumors [24,31–33]. Despite their rarity in comparison to single CTCs, CTC clusters have been shown to be responsible for the formation of the majority of metastatic lesions in animal models, and to carry a substantially elevated metastasis-initiating ability [24,34,35]. The number of detectable CTC clusters increases during disease progression, and their frequency rises as well when drawing blood from upstream locations, e.g. from the tumor draining vessel as opposed to more peripheral sites [32,36], arguing that CTC clusters may be trapped in small capillary networks before reaching the periphery. Similarly to single CTCs, the presence of CTC clusters is also an indicator of a poor prognosis in breast, colon and small-cell lung cancer [24,29,37], and CTC clusters appear to be a better independent survival predictor compared to CTCs alone, although bigger patient cohorts of several cancer types will be needed to confirm this hypothesis.

At the molecular level, homotypic CTC clusters present important features that have been recently identified. The presence of functional cell–cell junctions between cells in a cluster is critical for their existence, and in this context, key players such as plakoglobin, claudin 3 and claudin 4 have been identified and functionally validated [24,38]. CTC clusters have also been shown to express the epithelial cytoskeletal protein keratin 14 (K14), and to be dependent upon K14 expression for the achievement of metastatic dissemination [35]. In a separate study, breast CTC clusters have also been reported to rely on CD44 homophilic interactions to maintain their multicellular structure [39]. Generally, intercellular junctions in CTC clusters appear to have a more profound effect than just

cell–cell junction: in a recent study, the ability of cancer cells to form clusters was shown to impact on DNA methylation dynamics, and lead to the hypomethylation of binding sites for stemness- and proliferation-related transcription factors such as OCT4, SOX2, NANOG and SIN3A, among others [38]. Dissociation of CTC clusters into single cells, either via cell–cell junction disruption or pharmacological treatment, leads to re-methylation of these critical transcription factor binding sites and suppression of their metastasis-seeding ability [38]. Interestingly, the link between cell–cell junctions and DNA methylation dynamics represents an analogy with classic stem cell biology, whereby cell–cell junction activity has been shown to safeguard pluripotency and to be required for a complete reprogramming of somatic cells into stem cells, with cell–cell junction disruption in human embryonic stem cells leading to the loss of their stemness activity [40–42].

CTC clusters appear to be advantaged during the metastatic process because of two main reasons. While their multicellular nature impacts directly on those molecular features that promote stemness and metastasis, CTC clusters are also intrinsically carrying a physical advantage mainly represented by their larger size compared to single cells. Despite the evidence that clusters may be able to traverse capillary-sized vessels *in vitro* [43], when labeling CTCs with a fluorescent dye and assessing circulation time *in vivo* flow cytometry imaging, it was found that the circulation half-life of CTC clusters is between 6 and 10 min, while single CTCs circulate for 25–30 min, i.e. clusters are cleared from the circulation at least three times faster than single cells [24]. This argues in favor of a model whereby CTC clusters are trapped in capillary beds due to their larger size, and reach distant vital organs through physical entrapment. This physical advantage of CTC clusters to get trapped in distant organs, coupled with their increased metastasis-seeding ability conferred by active cell–cell junctions, make CTC clusters highly equipped precursors of metastasis.

Together, recent studies have suggested a prominent role for homotypic CTC clusters in the metastatic process, as well as they have started to identify their biology and potential opportunities to intervene pharmaceutically to counteract their action, possibly leading to new anti-metastasis therapies. However, several studies have shown that CTCs may not only cluster among themselves, but also aggregate with non-cancerous cells, i.e. form heterotypic CTC clusters.

Heterotypic CTC clusters

Heterotypic CTC clusters are cellular aggregates of cancer cells with non-malignant stromal or immune cells. These non-malignant cells comprise white blood cells, fibroblasts, endothelial cells and platelets, and have been shown to contribute to the metastatic potential of CTCs in various ways. For instance, recent findings have highlighted that CTCs can be found within the bloodstream of patients and mouse models in association with immune cells, which in the majority of the cases belong to the myeloid lineage [36,44]. When myeloid cells at various stages of differentiation (myeloid-derived suppressor cells to neutrophils) adhere to CTCs, their main effect is to increase CTCs' metastatic potential by promoting proliferation

[36,44]. In CTC-neutrophil clusters, this is mediated by a cytokine-receptor crosstalk involving IL-1 β and IL6, whose depletion is sufficient to abrogate neutrophil-induced proliferation in CTCs, resulting in a reduced metastatic ability [36]. CTC-neutrophil clusters are kept together through VCAM-1-dependent intercellular junctions, and accordingly, CRISPR-based depletion of VCAM-1 in xenografts prevents CTC-neutrophil clusters formation [36]. Interestingly, the first interaction between CTCs and neutrophils does not seem to occur within the bloodstream, but at the level of the primary tumor, where tumor-infiltrating neutrophils detach from the primary cancer site together with cancer cells and enter the bloodstream already in the form of CTC-neutrophil clusters [36]. Neutrophil recruitment to the primary tumor is a well-described phenomenon, influenced by a variety of released factors, but also by the genetic makeup of tumor cells themselves [36,45–47]. Given this, we speculate that heterotypic CTC clusters may be likely to comprise various cell types in different cancers, depending on what is the most abundant infiltrating immune or stromal cells in a given tumor.

Along these lines, in a context-dependent fashion, heterotypic CTC clusters have also been found to contain non-immune stromal cells. For instance, in lung cancer mouse models, CTCs have been found in association to fibroblasts, and this interaction also increased the metastatic ability of CTCs themselves [48]. Fibroblast depletion resulted in a decreased tumor growth rate and reduced metastasis, indicating that the interaction between cancer cells and fibroblasts may support the metastatic spread of cancer [48]. Further, other cell types have been found to promote the metastatic ability of cancer cells. These include cancer-endothelial cells co-culture models used for subsequent transplantation in mice and shown to promote tumor growth rate and metastasis [49], as well as cancer cells-platelet co-cultures, also found to increase metastatic ability upon transplantation in mice in a TGF β 1-dependent fashion [50]. The interaction between CTCs and platelets has been postulated also in patient samples in various other studies, mostly by detecting the expression of platelet markers (e.g. ITGA2B, ITGB3, SELP, SPARC) from total RNA extracts of CTCs (both single and clustered) [24,36,51]. Together, the data on heterotypic interactions of CTCs with immune and non-immune stromal cells provides evidence for multiple types of possible interactions, many of which result in CTCs “bringing their own soil” and increasing their likelihood to efficiently metastasize. While this field of research is still at an early stage and the literature is not extensive, future work will be key to classify the frequency and composition of heterotypic CTC clusters in various cancer types and along disease progression, together with a broader understanding of their implications for the metastatic process.

Outlook and conclusions

While the identification of clustered CTCs traces back to the 19th century, recent technologies have enabled their molecular and functional interrogation, leading to the identification of important aspects of the metastatic process [Table 1]. Nonetheless, several aspects that relate to CTC clusters biology are yet to be investigated, and new, unconventional

Table 1 Examples of recent investigations of CTC clusters biology. The table summarizes recent literature on homotypic and heterotypic CTC clusters, as well as it indicates some of the major take-home messages of these selected studies.

CTC cluster type	Major findings	References
Homotypic	Physical entrapment of CTC clusters in capillary beds	[13,43]
	Correlation of CTC clusters with bad prognosis	[24–27,29,30,37]
	Elevated metastatic ability of CTC clusters	[24,35]
	Molecular cell–cell adhesion mechanisms supporting CTC clusters	[24,35,38,39]
Heterotypic	Epigenetic features and vulnerabilities of CTC clusters	[38]
	CTC-myeloid cell clusters are endowed with a high metastatic ability	[36,44]
	IL-1 β and IL6 are critical cytokines for CTC-neutrophil crosstalk	[36]
	CTCs interact with additional stromal cells, such as fibroblasts and platelets	[48,50]

experimental approaches will be needed to resolve important biological questions.

Among these, future studies will be needed to conclusively determine whether the generation of clustered CTCs is a stochastic (passive) process or an active phenomenon that is influenced by specific tumor-intrinsic or microenvironmental cues. Should clusters be shed in an active fashion, the identification of those factors that promote CTC clusters formation may lead to the ability to prevent their intravasation, possibly leading to a reduced metastatic spread. Secondly, it is also poorly understood what is the degree of mutational heterogeneity within cells of individual clusters in patients. While this is likely to be largely dependent on the cluster size (i.e. bigger clusters are more likely to be mutationally heterogeneous than smaller clusters), should CTC clusters be able to carry more than one tumor sub-clone at a time, this would support a scenario whereby individual CTC clusters can directly seed multiclonal metastasis. Understanding this aspect of CTC cluster biology would be important to better comprehend the dynamics that underlie mutational heterogeneity along the metastatic cascade. Lastly, it will be important to investigate circulation dynamics of CTC clusters in patients. Based on mouse models, CTC clusters appear to be shed early and to be significantly more abundant when the blood is taken prior to passage through a capillary network as opposed to more peripheral locations, indicating that tumors might shed many more CTC clusters than originally anticipated [36]. Being able to draw blood from up-stream locations (e.g. tumor draining vessel) in patients may highlight highly-unexpected patterns of CTC composition, and eventually enable CTC cluster detection and isolation even in those patients with small, localized disease.

Altogether, while not yet extensive, the literature on homotypic and heterotypic CTC clusters clearly suggests their propensity to act as major drivers of the metastatic process. Targeting or preventing the formation of CTC clusters may lead to a significant delay in metastasis formation independently of primary tumor shrinkage, yet, given the rather long latency between primary tumor formation and the occurrence of metastasis, innovative clinical trial designs will be needed to measure the efficacy of new anti-cluster drugs in patients (i.e., within reasonable timeframes). New studies will also be required to gain further insights into the biology and vulnerabilities of CTC clusters in several cancer types, always considering blood circulation dynamics as a function of the tumor location, as a

better understanding of these phenomena may lead to novel anti-metastasis therapies.

Conflicts of Interest

N.A. is listed as inventor in patent applications related to CTC clusters, and is a paid consultant for companies with an interest in liquid biopsy.

Acknowledgements

Research in the Aceto lab is supported by the European Research Council (678834), the European Union (801159-B2B), the Swiss National Science Foundation (PP0P3-163938), the Swiss Cancer League (KFS-3811-02-2016 and KLS-4222-08-2017), the Basel Cancer League (KLbB-4173-03-2017), the two Cantons of Basel through the ETH Zurich (PMB-01-16), and the University of Basel.

REFERENCES

- [1] Mohme M, Riethdorf S, Pantel K. Circulating and disseminated tumour cells - mechanisms of immune surveillance and escape. *Nat Rev Clin Oncol* 2017;14:155–67.
- [2] Cho H, Kim J, Song H, Sohn KY, Jeon M, Han KH. Microfluidic technologies for circulating tumor cell isolation. *Analyst* 2018;143:2936–70.
- [3] Ferreira MM, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. *Mol Oncol* 2016;10:374–94.
- [4] Riethdorf S, Fritsche H, Muller V, Rau T, Schindlbeck C, Rack B, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. *Clin Cancer Res : Off J Am Assoc Cancer Res* 2007;13:920–8.
- [5] Hayes DF, Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Miller MC, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res : Off J Am Assoc Cancer Res* 2006;12:4218–24.
- [6] Hardingham JE, Grover P, Winter M, Hewett PJ, Price TJ, Thierry B. Detection and clinical significance of circulating tumor cells in colorectal cancer—20 Years of progress. *Mol Med* 2015;21:S25–31.

- [7] Kuske A, Gorges TM, Tennstedt P, Tiebel AK, Pompe R, Preisser F, et al. Improved detection of circulating tumor cells in non-metastatic high-risk prostate cancer patients. *Sci Rep* 2016;6:39736.
- [8] Chudziak J, Burt DJ, Mohan S, Rothwell DG, Mesquita B, Antonello J, et al. Clinical evaluation of a novel microfluidic device for epitope-independent enrichment of circulating tumour cells in patients with small cell lung cancer. *Analyst* 2016;141:669–78.
- [9] Sollier E, Go DE, Che J, Gossett DR, O'Byrne S, Weaver WM, et al. Size-selective collection of circulating tumor cells using Vortex technology. *Lab Chip* 2014;14:63–77.
- [10] Pantel K, Alix-Panabieres C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. *Nat Rev Clin Oncol* 2019;16:409–24.
- [11] Cristofanilli M, Pierga JY, Reuben J, Rademaker A, Davis AA, Peeters DJ, et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): international expert consensus paper. *Crit Rev Oncol-Hematol* 2019;134:39–45.
- [12] Aceto N, Toner M, Maheswaran S, Haber DA. En route to metastasis: circulating tumor cell clusters and epithelial-to-mesenchymal transition. *Trends Cancer* 2015;1:44–52.
- [13] Virchow R. Cellular pathologie. *Nutr Rev* 1858;47:23–5.
- [14] Ashworth TR. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. *Aust Med* 1869:146–9.
- [15] Paget S. The distribution of secondary growth in cancer of the breast. *Lancet* 1889;133:571–3.
- [16] Zeidman I, Buss JM. Transpulmonary passage of tumor cell emboli. *Cancer Res* 1952 Oct;12:731–3.
- [17] Fidler IJ. The relationship of embolic homogeneity, number, size and viability to the incidence of experimental metastasis. *Eur J Cancer* 1973;9:223–7.
- [18] Liotta LA, Saidel MG, Kleinerman J. The significance of hematogenous tumor cell clumps in the metastatic process. *Cancer Res* 1976;36:889–94.
- [19] Molnar B, Ladanyi A, Tanko L, Sreter L, Tulassay Z. Circulating tumor cell clusters in the peripheral blood of colorectal cancer patients. *Clin Cancer Res: Off J Am Assoc Cancer Res* 2001;7:4080–5.
- [20] Chan CK, Hutcheon MA, Hyland RH, Smith GJ, Patterson BJ, Matthay RA. Pulmonary tumor embolism: a critical review of clinical, imaging, and hemodynamic features. *J Thorac Imaging* 1987;2:4–14.
- [21] King MB, Harmon KR. Unusual forms of pulmonary embolism. *Clin Chest Med* 1994;15:561–80.
- [22] Roberts KE, Hamele-Bena D, Saqi A, Stein CA, Cole RP. Pulmonary tumor embolism: a review of the literature. *Am J Med* 2003;115:228–32.
- [23] Tsoi DT, Rowsell C, McGregor C, Kelly CM, Verma S, Pritchard KI. Disseminated tumor embolism from breast cancer leading to multiorgan failure. *J Clin Oncol: Off J Am Soc Clin Oncol* 2010;28:e180–3.
- [24] Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* 2014;158:1110–22.
- [25] Murlidhar V, Reddy RM, Fouladdel S, Zhao L, Ishikawa MK, Grabauskiene S, et al. Poor prognosis indicated by venous circulating tumor cell clusters in early-stage lung cancers. *Cancer Res* 2017;77:5194–206.
- [26] Jansson S, Bendahl PO, Larsson AM, Aaltonen KE, Ryden L. Prognostic impact of circulating tumor cell apoptosis and clusters in serial blood samples from patients with metastatic breast cancer in a prospective observational cohort. *BMC Canc* 2016;16:433.
- [27] Brandt B, Junker R, Griwatz C, Heidl S, Brinkmann O, Semjonow A, et al. Isolation of prostate-derived single cells and cell clusters from human peripheral blood. *Cancer Res* 1996;56:4556–61.
- [28] Giuliano M, Shaikh A, Lo HC, Arpino G, De Placido S, Zhang XH, et al. Perspective on circulating tumor cell clusters: why it takes a village to metastasize. *Cancer Res* 2018;78:845–52.
- [29] Hou JM, Krebs MG, Lancashire L, Sloane R, Backen A, Swain RK, 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: Off J Am Soc Clin Oncol* 2012;30:525–32.
- [30] Wang C, Mu Z, Chervoneva I, Austin L, Ye Z, Rossi G, et al. Longitudinally collected CTCs and CTC-clusters and clinical outcomes of metastatic breast cancer. *Breast Canc Res Treat* 2017;161:83–94.
- [31] Cho EH, Wendel M, Luttgen M, Yoshioka C, Marrinucci D, Lazar D, et al. Characterization of circulating tumor cell aggregates identified in patients with epithelial tumors. *Phys Biol* 2012;9:016001.
- [32] Suo Y, Xie C, Zhu X, Fan Z, Yang Z, He H, et al. Proportion of circulating tumor cell clusters increases during cancer metastasis. *Cytometry* 2017;91:250–3.
- [33] Vetter M, Landin J, Szczerba BM, Castro-Giner F, Gkoutela S, Donato C, et al. Denosumab treatment is associated with the absence of circulating tumor cells in patients with breast cancer. *Breast Cancer Res* 2018;20:141.
- [34] Cheung KJ, Ewald AJ. A collective route to metastasis: seeding by tumor cell clusters. *Science* 2016;352:167–9.
- [35] Cheung KJ, Padmanaban V, Silvestri V, Schipper K, Cohen JD, Fairchild AN, et al. Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. *Proc Natl Acad Sci USA* 2016;113:E854–63.
- [36] Szczerba BM, Castro-Giner F, Vetter M, Krol I, Gkoutela S, Landin J, et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* 2019;566:553–7.
- [37] Zhang D, Zhao L, Zhou P, Ma H, Huang F, Jin M, et al. Circulating tumor microemboli (CTM) and vimentin+ circulating tumor cells (CTCs) detected by a size-based platform predict worse prognosis in advanced colorectal cancer patients during chemotherapy. *Cancer Cell Int* 2017;17:6.
- [38] Gkoutela S, Castro-Giner F, Szczerba BM, Vetter M, Landin J, Scherrer R, et al. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. *Cell* 2019;176:98–112.e14.
- [39] Liu X, Taftaf R, Kawaguchi M, Chang YF, Chen W, Entenberg D, et al. Homophilic CD44 interactions mediate tumor cell aggregation and polyclonal metastasis in patient-derived breast cancer models. *Cancer Discov* 2019;9:96–113.
- [40] Li L, Bennett SA, Wang L. Role of E-cadherin and other cell adhesion molecules in survival and differentiation of human pluripotent stem cells. *Cell Adhes Migrat* 2012;6:59–70.
- [41] Li L, Wang S, Jezierski A, Moalim-Nour L, Mohib K, Parks RJ, et al. A unique interplay between Rap1 and E-cadherin in the endocytic pathway regulates self-renewal of human embryonic stem cells. *Stem Cells* 2010;28:247–57.
- [42] Pieters T, van Roy F. Role of cell-cell adhesion complexes in embryonic stem cell biology. *J Cell Sci* 2014;127:2603–13.
- [43] Au SH, Storey BD, Moore JC, Tang Q, Chen YL, Javaid S, et al. Clusters of circulating tumor cells traverse capillary-sized vessels. *Proc Natl Acad Sci USA* 2016;113:4947–52.
- [44] Sprouse ML, Welte T, Boral D, Liu HN, Yin W, Vishnoi M, et al. PMN-MDSs enhance CTC metastatic properties through reciprocal interactions via ROS/Notch/Nodal signaling. *Int J Mol Sci* 2019;20:1916.

- [45] Ramasamy S, Saez B, Mukhopadhyay S, Ding D, Ahmed AM, Chen X, et al. Tle1 tumor suppressor negatively regulates inflammation in vivo and modulates NF-kappaB inflammatory pathway. *Proc Natl Acad Sci USA* 2016;113:1871–6.
- [46] Fridlender ZG, Albelda SM. Tumor-associated neutrophils: friend or foe? *Carcinogenesis* 2012;33:949–55.
- [47] Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 2009;16:183–94.
- [48] Duda DG, Duyverman AM, Kohno M, Snuderl M, Steller EJ, Fukumura D, et al. Malignant cells facilitate lung metastasis by bringing their own soil. *Proc Natl Acad Sci USA* 2010;107:21677–82.
- [49] Upreti M, Jamshidi-Parsian A, Koonce NA, Webber JS, Sharma SK, Asea AA, et al. Tumor-endothelial cell three-dimensional spheroids: new aspects to enhance radiation and drug therapeutics. *Transl Oncol* 2011;4:365–76.
- [50] Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell* 2011;20:576–90.
- [51] Ting DT, Wittner BS, Ligorio M, Vincent Jordan N, Shah AM, Miyamoto DT, et al. Single-cell RNA sequencing identifies extracellular matrix gene expression by pancreatic circulating tumor cells. *Cell Rep* 2014;8:1905–18.