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Clusters of circulating tumor cells: A biophysical and technological perspective

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Abstract

The vast majority of cancer associated deaths result from metastasis, yet the behaviors of its most potent cellular driver, circulating tumor cell clusters, are only beginning to be revealed. This review highlights recent advances to our understanding of tumor cell clusters with emphasis on biophysics and enabling technologies. A number of strategies for isolating these clusters from patient blood have enabled studies aimed at unraveling the importance of intercellular adhesions in cluster metastasis. Furthermore, due to their metastatic potency, the utility of circulating tumor cell clusters for cancer diagnosis, drug screening, precision oncology and as targets of antimetastatic therapeutics are currently being explored. The continued development of tools for isolating and understanding circulating tumor cell clusters will enhance our fundamental understanding of the metastatic process and may be instrumental in devising new strategies to suppress and eliminate metastasis.

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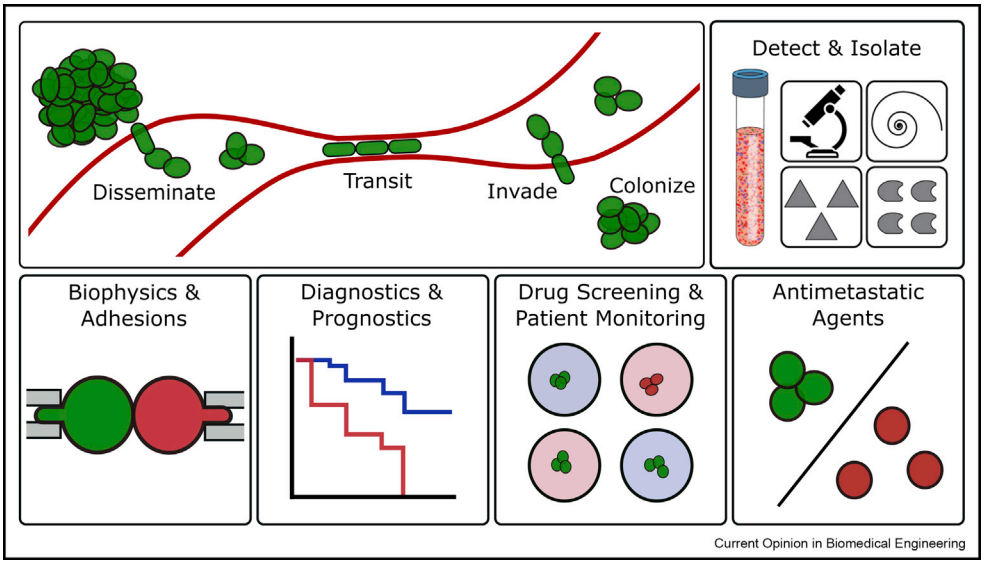
Abbreviations

CTC, Circulating tumor cell; DLD, Deterministic lateral displacement; K14, Keratin 14; RBC, Red blood cell.

Ninety percent of cancer associated deaths are a result of metastasis. The conventional study of the metastatic process focused on individual circulating tumor cells (CTCs), which disseminate from primary tumors to colonize distant organs [1]. However, evidence [2] suggests that individual CTCs may not be the only drivers of metastasis. Instead, multicellular aggregates of CTCs (CTC clusters) appear to play a large role in metastasis and their potential utility in the clinic has begun to be explored (Fig. 1). Clues to the greater potency of CTC clusters in establishing tumors were first discovered over four decades ago in experiments where cancer cells were injected into the circulation of mice to study their abilities to generate metastases. Fidler et al. [3] found that when cancer cells were aggregated into clusters before injection, they established several-fold more tumors than equal numbers of individual cancer cells, a finding that was later replicated by other groups [4,5]. Further work exploring the biology and behavior of cancer cell aggregates was hampered for many years due to the lack of available technologies for the efficient isolation of CTC clusters from blood and their subsequent analyses.

Emergent technologies [6–10] have reinvigorated this research, leading to the efficient isolation of CTCs from patient blood [10–14] and to numerous surprising discoveries [2] such as reports that these clusters are responsible for seeding ~50–97% of metastatic tumors in mouse models [15,16]. Alarmingly, studies also revealed that the isolation of one or more CTC clusters in patient blood at any given time point correlated with significantly worse survival rates in patients with prostate [15], breast [15], colorectal [17] and small-cell lung [18] cancers. It is quite likely that CTC clusters play a far greater role in the metastatic process than previously believed.

Figure 1



Topics covered in this review. Schematic of formation, transit, invasion and colonization of distant organs by circulating tumor cell clusters (top left). Microfluidic strategies for isolating CTC clusters from blood which may be useful for: biophysical studies, diagnostics and prognostics, drug screening and the development of antimetastatic agents.

Much of the biology and biophysical characteristics of CTC clusters however, remains poorly understood. Here, we review recent advancements in the field of CTC clusters with an emphasis on enabling technologies and strategies that have the potential to accelerate the study and potential clinical utility of clusters (Fig. 1). Recent literature elucidating their cellular characteristics and role in metastatic progression will be highlighted, with a focus on the importance of cellular biophysics. We will then review tools and techniques for isolating these rare cellular aggregates from blood. Finally, we will discuss their utility as diagnostic or prognostic markers and ultimately their potential as targets of anti-metastatic interventions.

Intercellular Adhesions and Biophysics of Metastasis

Studies have begun to reveal the extent to which clusters contribute to the formation of new tumors in patients with cancer. Metastatic tumors were traditionally believed to be established by the invasion and proliferation of individual CTCs into distant organs. Three recent studies [15,16,19] have challenged this assumption by exploring the abilities of CTC clusters to transit through the circulation to reach distant organs and to establish secondary tumors. A common element among these studies is the important biophysical role that intercellular adhesions, a defining feature of multicellular aggregates, play in their metastatic potentials.

Perhaps the most prominent rationale for the belief that CTC clusters were incapable of seeding metastatic tumors was that these clusters, which sometimes contain over one hundred cells [14], are incapable of transiting through capillaries of 5–10 μm in diameter, and therefore immediately arrest in circulation leading to the rupture of vessel walls [1,20,21]. Using microfluidic constrictions that modelled the microcirculation, we demonstrated that clusters containing over 20 cells could travel through capillary-sized constrictions under physiological pressures by reorganizing into chains of single cells [19]. This behavior was then recapitulated in true blood vessels by injecting clusters into the circulation of zebrafish, which have vascular geometries and physiology similar to the human microcirculation. Importantly, it was the strengths of intercellular adhesions within clusters that dictated whether clusters were capable of rearranging into intact chains at constrictions. Computational simulations demonstrated that weakening or strengthening the adhesive energies among cells in CTC clusters to levels outside the range found in typical cancer clusters resulted in dissociation of clusters into single cells or complete occlusion, respectively. These findings have dissuaded us from using the traditional nomenclature of “circulating tumor microemboli” when referring to clusters since these aggregates are often no more likely to occlude capillaries than individual CTCs and therefore the label of “embolism” is ill-suited.

After transiting through the circulation to reach distant organs, CTC clusters need to proliferate to establish macrometastases and secondary tumors. Aceto et al. [15] and Cheung et al. [16] explored the colonization ability of clusters through the use of fluorescently tagged cancer cells injected into immunodeficient mice in various configurations. Both groups found that CTC clusters were not only capable of establishing