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Tumor-derived microvesicles: The metastasomes

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ABSTRACT

Metastasis is the leading cause of cancer death, yet it is mechanistically considered a very inefficient process suggesting the presence of some sort of (e.g. systemic) routes for fuelling the process. The pre-metastatic niche formation is described as one such metastasis promoting route. Now, the emerging potentials of tumor-derived microvesicles (TDMVs), not only in formulating the pre-metastatic niche, but also conferring neoplastic phenotypes onto normal cells, has integrated new concepts into the field. Here, we note as an ancillary proposition that, exerting functional disturbances in other sites, TDMVs (we have termed them metastasomes) may aid foundation of the secondary lesions via two seemingly interrelated models: (i) tumor-organ-training (TOTr), training a proper niche for the growth of the disseminated tumor cells; (ii) tumor-organ-targeting (TOTa), contribution to the propagation of the transformed phenotype via direct or indirect (TOTr-mediated disturbed stroma) transformation and/or heightened growth/survival states of the normal resident cells in the secondary organs. Respecting the high content of the RNA molecules (particularly microRNAs) identified in the secretory MVs, they may play crucial parts in such "malignant trait" spreading system. That is, the interactions between tumor tissue-specific RNA signatures, being transferred via metastasomes, and the cell-type/tissue-specific RNA stockrooms in other areas may settle a unique outcome in each organ. Thus, serving as tumor-organ matchmakers, the RNA molecules may also play substantial roles in the seeding and tropism of the process.

Introduction

The term "metastasis" was initially created in 1829 by Jean Claude Recamier and is defined as "the transfer of disease from one organ or part to another not directly connected to it" [1]. Malignant disease can be dated back to Egyptians (1500 BC) and latter to the Greek physicians in the time of Hippocrates (5th century BC) who detected fatal secondary lesions in breast cancer patients. Although such lesions were primarily considered as "independent tumors" arisen from the spread of "toxic humors", with the discovery of cell as the basic unit of organisms, this theory was abandoned and the secondary lesions were supposed to arise from the migration and seeding of tumor cells from primary sites into the others (reviewed in Ref. [2]). Since then, despite the several (e.g. seed-soil) models [3], the mechanism of tumor metastasis has remained enigmatic. In fact, based on the current models, a proposed metastasis founder cell must go through the sequential series of inefficient steps (separation from the primary tumor, intravasation, survival in the circulation, extravasation, and successful colonization in the secondary organs) to found a clinically detectable metastasis [3]. Two questions can be raised here: first, how can metastasis become the main cause of cancer death if it is a very inefficient process? Second, how could the disseminated tumor cells (DTCs) stay dormant even for decades in the eccentric and tumor-suppressive microenvironments of the ectopic regions [4,5] to give then rise to tumor recurrence? Together these conundrums, with the notion that the metastasis founder cell is yet unknown [6], may force a major rethinking of the process. Perhaps, we are missing some parts of the landscape that might be due to our reductionistic views. In this situation, putting all the possibilities together to unravel the matter of metastasis becomes important, as it will provide a base for definition of more effective treatment options and therapeutic strategies.

It is increasingly becoming evident that the tumor "cell" migration from primary site into distant organs is a simplistic view or only a part of the whole panorama of the cancer "disease" dissemination process. Recently, the interplay between primary tumor and secondary organs via circulation has emerged as an essential component of tumor metastasis [6,7]. While cancer cells can

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crosstalk with their surroundings by simple interactions using nucleotides, lipids, small peptides, or proteins [8], a primary tumor may employ more sophisticated mechanisms to achieve efficient interplays with distant areas. One such route which has recently been the focus of attentions is provided by tumor-derived microvesicles (TDMVs), serving as to condition the target organs for metastatic growth [7]. Microvesicle (MV) is a collective term for a broad range of membrane particles including exosomes, shedding vesicles, and apoptotic blebs which are released from and taken up (by fusion, endocytosis, or target-receptor interaction, e.g. via tetraspanins) by almost all cell-types, including cancer cells [9–12]. MVs have been identified in many body fluids, including blood, breast milk, cerebrospinal fluid, saliva, and urine [13–15]. They can be isolated or tracked by ultracentrifugation, sucrose-gradient centrifugation floatation, immune-affinity enrichment procedures, and confocal microscopy [16]. MVs carry various cargos from their mother cells, including lipids, specific proteins and nucleic acids (DNA, mRNAs, and miRNAs) which can be translated into functions in the recipient cells [17–19]. Proteins, mRNA, and miRNA may be sorted into some MVs such as exosomes in a selective and energy-dependent manner [20–22]. Exosomes are a subset of MVs, 40–100 nm in diameter which are formed in specific subcellular compartments called microvesicular bodies (MVBs) [23]. Prostasomes, equivalent to exosomes, improve sperm cell fertilization ability via transferring signaling molecules from prostatic acinar cells to sperms [11]. Remarkably, the tumorigenic factors encased in the circulating TDMVs can be translated into neoplastic phenotypes in the recipient cells [24,25]. These remarks imply that the conditioning of metastatic sites for the growth of cancer (metastatic founder) cells is likely the simple(st) impact that can be attributed to the TDMVs, that is, they may also prompt transformed phenotypes upon the normal recipient cells, hence, foundation of *de novo* tumors in the secondary organs. So with this perspective, here we note as an ancillary proposition that metastasis likely stems from the transfer of "malignant traits" from primary tumors into other sites via TDMVs.

The hypothesis of metastasome

We speculate that TDMVs carrying malignant traits, we call them metastasomes, promote a series of cell biological actions in other sites throughout the body of cancer patients that finally lead to the development of secondary lesions in vulnerable areas. Now, describing the concept of metastasome we propose two mechanistic models for tumor metastasis: first, "tumor-organ-training (TOTr)", modulation of the microenvironment of the target sites to serve as hospitable hosts for the alien DTCs, formation of pre-metastatic niche; second, "tumor-organ-targeting (TOTa)", contribution to the propagation of the transformed phenotypes upon normal resident cells, thereby foundation of *de novo* and "second primary" tumors in the target organs.

The metastasomes

A growing body of evidence shows that many cancer-related cell-biological and clinical events are associated with the accelerated rates of MVs secretion from cancer cells [7]. It is noteworthy that the apical-basal polarity and careful arrangement within the underlying basement membrane and neighboring cells are principles of epithelial cells and warrants their normal and stable homeostasis [26] (note that about 80% of the life-threatening cancers – carcinomas – arise from the epithelial tissues [3]). A consequence of such organization is the oriented and controlled trafficking of the transport vesicles by epithelial cells (e.g. exocytosis/secretion of prostasomes/MVs by the prostatic/breast acinar cells into their lumens) [27,28]. The exocyst complex which is located at the adherens junctions (AJs) in the epithelial cells also plays crucial role in the polarized secretion of the exosomes, so that, mutation of its components results in the cytoplasmic accumulation of the transport vesicles [29]. It can be anticipated that loss of tissue architecture, cell polarity, and AJs in carcinomas may give rise to intrusions in the amounts, contents, and direction of secretion of the MVs by epithelial cells. Accordingly, an active and constitutive shedding of the GFP-labeled MVs from the MDAMB231 and U87 human cancer cells, but not from the normal NIH 3T3 cells, have been explored [25]. The oncogenic mutations in some cancer genes such as EGFR and K-ras may also promote secretion of the exosomes with invasive potentials from cancer cells [30,31]. Besides, hyperactivation of the Rho-dependent signaling pathway promotes tumor development via fuelling of the MV formation from cancer cells [32]. From a clinical standpoint also, in the plasma samples of the patients with colorectal (CRC), lung, melanoma, ovarian, and prostate cancers higher levels of prostasomes/exosomes, compared to those of the normal controls, have been detected [33–37]. The high levels of plasma exosomes have been associated with poorly differentiated tumors and shorter disease overall survival in CRC [34] and the degree of malignancy in ovarian cancer [37]. Even high levels of circulating HER2-bearing exosomes in the HER2-positive breast cancer patients may play some mechanistic roles in the development of resistance to HER2-targeted therapy [38].

Furthermore, as tumor progression proceeds, its microenvironment becomes acidified which, as a hallmark of tumor malignancy, enhances not only the release of MVs, but also their uptake efficiencies by cancer [39] and likely the surrounding stromal cells. This scenario, which is attributable to the higher membrane sphingolipids (sphingomyelin/ganglioside GM3) in TDMVs generated in the acidic environment [39], can establish a self-amplifying positive feedback loop between cancer and the "surrounding" stromal cells. For instance, activated tumor associated macrophages (TAMs) release miRNAs within exosomes that promote the invasive potentials of breast cancer cells [40]. Indeed, TDMVs can facilitate tumor growth and progression by modulating the immune system, i.e. by inhibiting tumor-suppressing T cells and hindering or educating the differentiation of bone marrow derived cells (BMDCs) [7,41–44]. TDMVs (exosomes) can also trigger differentiation of the surrounding fibroblasts to myofibroblasts which support tumor progression and metastasis [45]. It can be anticipated that circulating TDMVs are not the products of carcinoma cells only; indeed, they represent the interplays between a heterogeneous population of cells within the tumor mass, "tumor organ" (Fig. 1). The same tumor yet contains various subclones of neoplastic cells [46], each likely with distinct MV-release capacity or releases MVs with diverse invasive potentials.

The major breakthrough in understanding the role of MVs in cancer is provided by the studies uncovering proinvasive and cell cycle-related factors, and oncogenic proteins, DNAs, mRNAs, and miRNAs within TDMVs, enabling them to confer malignant traits onto normal cells or boost the tumorigenic potential of cancer cells [17,22,24,25,30,33,47–49]. Consistent with the potential relevance of this notion, synergic treatment of endothelial cells by human squamous carcinoma cell (A431)-derived MVs along with an inhibitor of MV-uptake (Diannexin) has led to reduction of their tumor promoting effects in the xenograft tumor in mice [50]. Therefore, it can be theorized that, equivalent to the so-called "Trojan horse mechanism" of infection (MV-mediated spread of certain infections, e.g. HIV or prions) [20], TDMVs may act as efficient delivery vehicles that aid tumor spread by paracrine diffusion of "malignant traits" throughout the body of cancer patients, thus, corresponding to oncosomes (carrying oncogenic factors) [30] we coined them "metastasomes".