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Review

Outsmart tumor exosomes to steal the cancer initiating cell its niche

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ABSTRACT

Exosomes are small vesicles that derive from endosomes and are delivered by many cells, including tumor cells that are a particular rich source of exosomes. Exosomes are suggested to be the most potent intercellular communicators. Being recovered in all body fluids, they can communicate with neighboring as well as distant cells. The latter was first described for dendritic cell exosomes that can initiate T cell activation. However, tumor exosomes (TEX) may impede this crosstalk. Besides with hematopoietic cells, TEX communicate with the tumor cell itself, but also with host stroma cells and endothelial cells. This crosstalk received much attention as there is strong evidence that TEX account for angiogenesis and premetastatic niche formation, which may proceed directly via binding and uptake of TEX by cells in the premetastatic organ or indirectly via TEX being taken up by hematopoietic progenitors in the bone marrow (BM), which mature toward lineages with immunosuppressive features or are forced toward premature release from the BM and homing into premetastatic organs. Knowing these deleterious activities of TEX, it becomes demanding to search for modes of therapeutic interference. I here introduce our hypothesis that metastasis formation may be hampered by tailored exosomes that outsmart TEX. The essential prerequisites are an in depth knowledge on TEX binding, uptake, binding-initiated signal transduction and uptake-promoted target cell reprogramming.

1. Introduction

1.1. Metastasis and cancer initiating cells

Metastasis frequently are the corner stone for curative therapy despite considerable progress in surgery, radiation and cytotoxic drug therapy [1,2]. Metastasis formation is the result of a cascade of events that primary tumor cells pass through by changing their phenotype and by their cross-talk with the host environment. In epithelial tumors the metastatic cascade is initiated through a process called epithelial to mesenchymal transition (EMT), followed by individual tumor cells separating from the primary tumor mass to intravasate, extravasate and finally to settle and grow in distant organs [3,4].

The propensity to metastasize most likely relies on so called cancer initiating cells (CIC), a small subpopulation suggested accounting for primary tumor growth as well as metastatic spread [5]. CIC are long lived, slowly progressing through the cell cycle, radiation and drug resistant and use similar signaling pathways that guide the fate of embryonic and adult stem cells (ESC, ASC) [6], sharing with ESC over-expression of Oct4, Nanog, and c-Myc [7] and the signaling pathways Notch, Wnt and Hedgehog, important in shaping structure, cell fate and identity [8]. There is also compelling evidence for joint altered epigenetic regulation in ASC and CIC. Thus, polycomb genes, which play a role in transcriptional repression through histone modifications, associate with the promoter and regulatory regions of target genes in ASC as well as CIC [9]. Furthermore, the miRNA profile of tumor cells differs significantly from that of non-transformed cells with upregulation of several miRNA that support tumor growth, collectively called oncoMirs [10], which may act by targeting tumor suppressors [11]. Instead, tumor suppressor miRNA, e.g. let-7, miR-15a and others that suppress MET and Bcl2 are downregulated [12,13]. We recently experienced that a non-metastatic variant of a highly metastatic rat tumor line

Abbreviations: ADAM, A disintegrin and metalloproteinase; AML, actute myeloid leukemia; ASC, adult stem cells; BM, bone marrow; BMC, BM cell; CAF, cancer-associated fibroblast; CIC, cancer initiating cells; CML, chronic myeloid leukemia; CTL, cytotoxic T cells; DC, dendritic cell; ECM, extracellular matrix; EMT, epithelial mesenchymal transition; ESC, embryonic stem cell; ESCRT, endosomal sorting complex required for transport; HCV, hepatitis C virus; HSP, heat shock protein; LIC, leukemia-initiating cell; MDSC, myeloid-derived suppressor cells; MMP, matrix metalloproteinase; MSC, mesenchymal stem cells; Mϕ, macrophage; TEX, tumor exosomes; Th, helper T cells; Treg, regulatory T cells.

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expressed the tumor suppressors let-7b, let-7d, let-7e and miR-101 at a high level, but oncoMir miR-494 and Pten-regulating miR-21, known for promoting metastasis [14] and apoptosis-regulating miR-24-1 [15] were highly expressed in the metastasizing line [16].

1.2. Metastasis and the niche

The fate of ESC and ASC is determined by their position and is minutely regulated by few adjacent cells creating a defined environment, the niche [17]. CIC, too, appear to require a niche, possibly during oncogenesis and during settlement and growth in distant organs [18]. The latter is called the pre-metastatic niche, because it is initiated by the primary tumor before metastasizing cell arrival [19]. SC niches, composed of epithelial and mesenchymal cells and extracellular substrates, are important for maintenance of stemness [20] and function as an extrinsic regulatory system, which maintains and governs the location, adhesiveness, retention, homing, mobilization, quiescence/activation, symmetric/asymmetric division and differentiation [18,20]. The main contributors, Wnt, Hedgehog, Notch, TGFβ, several tyrosine kinase receptors all use similar intracellular signaling pathways like the Ras-Raf-MAPK and PI3K-Akt pathway [21].

Whether CIC require a niche has not been unequivocally answered. First, the niche for ASC provides a regulatory system, which may well function to prevent tumorigenesis by controlling ASC, but could also promote CIC proliferation [18]. Beyond this, CIC may be reprogrammed by exposing them to an embryonic microenvironment, which concept has received experimental support for malignant melanoma [22]. A preformed niche also can support CIC survival and homing [23], demonstrated for neural and colorectal CIC [24,25]. Important contributors to the CIC niche are cancer-associated fibroblasts (CAF), contributing to extracellular matrix (ECM) remodeling by provision of HGF, IL6, PDGFβ, prostaglandins and proteases [24,26] as well as by miRNA, where miR-31, which targets the chromatin remodeling homeobox gene SATB2 is strikingly downregulated [27]. In addition, exchange of miRNA between CIC and their niche plays an important role. Several miRNA implicated in cell proliferation were shown to become transferred from mammary cancer stroma cell into tumor cells [28]. Other important players are mesenchymal stem cells (MSC) [29] where nestin1+ MSC together with CAF and Mϕ are particularly important for leukemia-IC [30]. Furthermore, tumor cell-derived IL1 induces PGE2 secretion by MSC, which operates in an autocrine manner to promote cytokine secretion that induce β-catenin signaling and CIC formation in adjacent tumor cells [31]. Further evidences for CIC requiring a niche is the finding that EMT is promoted by the tumor stroma. Myofibroblasts secret TGFβ and by back-signaling force CIC into EMT [32]. Additional inflammatory mediators delivered by the stroma, like TNFα and IL6, sustain TGFβ production, where IL6 attracts MSC to produce CIC supportive CXCL7 [33]. Thus, CIC shape their own environment by recruiting and activating specific cell types that support their maintenance. Additionally, CIC may even differentiate into niche cells, demonstrated for glioblastoma CIC [34].

Of particular interest with respect to the CIC niche is the study of Kaplan et al. [19] describing the formation of a pre-metastatic niche in organs, where tumor cells are likely to settle and grow, in advance to tumor cell arrival. It was suggested that resident fibroblasts become stimulated by tumor-derived growth factors to secrete fibronectin that promotes attachment of hematopoietic progenitors expressing VEGFR1 and VLA-4. In addition, stromal fibroblasts frequently express high amounts of CXCL12 that attract CXCR-4 expressing hematopoietic progenitors and CIC [35]. CAF also secrete CXCL12, attracting tumor cells as well as endothelial cell progenitors [36], demonstrated in several malignancies [37]. Via HGF expressing MSC, c-Met also becomes involved in angiogenesis [38] and β-catenin, one of the key players in metastasis [38], translocates to the nucleus upon activation, e.g. via Wnt signaling, and interacts with the Tcf/Lef complex turning on cell cycle related genes like *cyclin D1* and *c-Myc* [39]. β-catenin localization in the nucleus is further supported by integrin-linked kinase activation via matrix-bound β1 integrins and costimulatory signals like HGF, EGF, TGFβ from the environment [40].

Taken together, CIC/metastasizing tumor cells depend on short ranged and long distance intercellular cross-talks, suggested to be the prime activity of exosomes [41–43]. Notably, many of the described contributors are widespread and it is difficult to imagine, particularly for long-distance communications, how selectivity is achieved. In view of these unexplained features and based on our finding that exosomes from a metastasizing rat pancreatic tumor line are essentially required to allow a non-metastasizing variant to settle in lymph nodes and lung [44], we proposed exosomes as the central actor. After a brief introduction on tumor exosomes (TEX), I will discuss how they communicate with surrounding and distant tissues and finally comment on exosomes as potential therapeutics.

2. Exosomes

Exosomes are small 30–100 nm vesicles, which derive from the fusion of the intraluminal vesicles of multivesicular bodies (MVB) with the plasma membrane [41]. The molecular composition of exosomes reflects their origin from intraluminal vesicles. Besides a common set of membrane and cytosolic molecules, which includes tetraspanins, exosomes harbor subsets of proteins, such as adhesion molecules, molecules associated with vesicle transport, cytoskeletal proteins, signal transduction molecules, enzymes and others that are linked to cell type-specific functions. They also contain selected mRNA and miRNA [45,46].

2.1. Exosome generation and composition

It is well known that the relative abundance of proteins, mRNA and miRNAs differs between exosomes and donor cells. This implies active sorting into MVB. Protein sorting depends on mono-ubiquitinylation and the endosomal sorting complex required for transport (ESCRT). Besides, protein recruitment into tetraspanin networks and other internalization prone detergent resistant membrane domains can also be decisive, where raft microdomains enriched in sphingolipids, which form ceramide, play an important role [47–49]. Notably, recruitment via tetraspanin-enriched or raft microdomains is accompanied by exosomal recovery of protein complexes rather than singular molecules, that may have an impact on exosome targeting and the crosstalk with target structures [50]. mRNA recruitment may be guided by a zip code in the 3′-UTR [51]. miRNA recruitment is facilitated by physical and functional coupling of RISCs (RNA-induced silencing complexes) to components of the sorting complex. GW182 containing GW bodies, sorted into MVB, promote continuous assembly/disassembly of membrane-associated miRNA-loaded RISC [52,53]. The release of miRNA being actively controlled through a ceramide-dependent machinery associated with exosome secretion [54] points toward a contribution of tetraspanins known to interact with gangliosides [55].

According to their origin from endosomes/MVB, the exosomal protein profile is rich in molecules located in membrane domains prone for internalization as well as in molecules engaged in fission, scission and vesicular transport [46,56,57], where tetraspanins are frequently used to differentiate exosomes from other extracellular vesicles [46,58]. Notably, protein complexes as formed during internalization are maintained and recovered in exosomes. Thus, during stress-induced internalization the tetraspanin