Forum

Tumor–Stromal Cell Communication: Small Vesicles Signal Big Changes

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Reciprocal interactions between malignant and stromal cells create a local microenvironment that fosters tumor growth. Extracellular vesicles (EVs) such as exosomes, microvesicles, and large oncosomes are involved in tumor-stroma communication by shutting signaling cargo and other molecules. Here we discuss how EVs released by cancer or stromal cells impact the proliferation, differentiation, and metabolism of tumors.

EVs Modulate the Tumor Microenvironment

Intercellular communication between cancer and surrounding stromal cells contributes to the creation of a local microenvironment that promotes tumor survival and growth (Figure 1). EVs have come into the limelight as pivotal mediators of this “corrupting” process. Different types of EV can be distinguished based on their subcellular origin. Exosomes are EVs originating in intraluminal vesicles that are released from multivesicular bodies on plasma membrane fusion, while microvesicles and large oncosomes are other classes of EV produced at the plasma membrane by budding or blebbing, respectively. Here they are referred to collectively as EVs, as there is neither a reliable marker nor a method available to distinguish and separate them from each other in conditioned tissue-culture medium. EVs are lipid bilayer transport vesicles containing diverse molecular cargoes (lipids, proteins, DNA, mRNA, metabolites, and a huge number of various noncoding RNAs such as IncRNA, tRNA, rRNA, snoRNA, and scaRNA) that depend on the physiology of the cell of origin. As tumors progress, the cargo released on/in EVs also dynamically changes. This short overview outlines the role of tumor-secreted EVs in governing immune evasion, vascularization, and stromal activation and how this shapes cancer progression.

EVs: Recalibrating Local Immune Evasion

Cargo released by EVs can suppress the function of local immune populations. For instance, it was reported that in head and neck squamous cell carcinoma (HNSCC) cell line PCI-13-derived EVs can upregulate the expression profile of various subsets of T cells, especially activated regulatory T cells (Tregs), by upregulating critical immunoinhibitory protein such as TGFβ, IL-10, and COX-2, as well as CD39, or CD73 and adenosine production [1]. Which EV cargoes cause this suppression remains to be determined. Cancer EVs can also attenuate the cytotoxic function of CD8+ T cells, causing tumor immune escape.

Figure 1, Extracellular Vesicles (EVs) Participate in Defining the Properties of the Stroma during Malignant Transition. EVs participate in the organization of the extracellular matrix (ECM) and matrix transitions, altering metabolism, and creating a more crowded and disorganized stroma. (A) Early malignant melanoma in the dermis. Depicted are examples of fibroblasts (blue arrows), macrophages (red arrows), scattered lymphocytes (black arrows), and a translucent extracellular matrix (green arrows). Early in the process of malignant transition, the stroma tends to be disorganized, much more dense, and populated by fibroblasts. (B) Transitional cell carcinoma of bladder with desmoplastic response and increased fibroblastic population. The ECM matures with the laying down of collagen and other components such as elastin, eventually forming a hard tumor (yellow arrows) that is often found in the pancreas and breast ductal cancers. Hematoxylin and eosin stain, magnification ×400.
Figure 2. Extracellular Vesicle (EV) Functions in the Microenvironment. Depicted are various forms of bidirectional communication between cancer and stromal cells mediated by EVs, and EV cargo involved in this communication.

**Cancer cells**

- **EV functions**
  - Suppress local immune functions (TGFβ)
  - Cause T-Cell death (via Fas/FasL)
  - Increase tumor immunogenicity (Activation of ERK signaling)

**Stromal cells**

- **EV functions**
  - CAF induction (TGFβ/Smad)
  - Reprogram cancer metabolism (Metabolites)
  - Promote cell migration
  - Restructure vasculature (Von willebrand factor, VEGF, mTORC1, PI3K/mTOR, mRNA and microRNA)

**Trends in Cancer**

Experimental evidence shows that cancer-derived EVs can induce the CAF phenotype in various cancer contexts. For example, breast cancer cells secreting TGFβ can differentiate adipose tissue-derived MSCs into α-smooth muscle actin-positive CAFs through the TGFβ–Smad pathway [8]; prostate cancer EVs can induce proangiogenic and invasive CAFs from bone marrow MSCs [9]; and bladder cancer EVs can induce CAFs by promoting EMT of urothelial cells [10]. It has also been shown that EVs derived from blood cancers, such as chronic lymphocytic leukemia, can convert endothelial cells and bone marrow-derived MSCs into CAFs [11].

Stromal cells also secrete EVs that reprogram the environment and cancer cells. In breast cancer, a complex bidirectional interaction between tumor and stromal EVs was observed; fibroblast-derived EVs are taken up by cancer cells, loaded with Wnt11 protein, and then released into the tumour where Wnt11 activates autocrine Wnt/planar cell polarity signaling, stimulating the leading edge of cancer cells, promoting cell migration [12]. Stromal EVs can also have profound effects on protection from drug treatment. MSC-derived EVs were shown to induce development of drug-resistant gastric cancer cells in vivo and ex vivo by activating the CaMK–Raf–MEK–ERK pathway [13]. Finally, metabolomic analyses of CAF-derived EVs revealed that they carry metabolites such as amino acids, lipids, and tricarboxylic acid (TCA) cycle intermediates that can strikingly reprogram the...

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**EVs: Reshaping the Tumor and Lymphatic Vasculature**

The content of tumor cell-derived EVs was shown to include von Willebrand factor and VEGF, mutated EGFR, and other factors that promote the proliferation, migration, and maturation of vascular endothelial cells and, therefore, can contribute to restructuring the tumor vasculature. By apparent contrast, CAFs can also release vascular inhibitors such as Delta-like 4 (Dll4) (a Notch signaling inhibitory ligand). Together with endothelially derived EVs that also transport Dll4, they modulate neoangiogenesis, although how they participate in tip cell versus stalk cell determination remains elusive, as opposing mechanisms that promote or inhibit vascula branching have been proposed [3,4]. High-level expression of Wnt5A in melanoma cells also induces the release of EVs containing immortal regula-tory and proangiogenic proteins, including IL-6, VEGF, and MMP2 [5]. Beyond vascular signaling, cancer EVs also modify endothelial tube formation under hypoxic conditions through miRNACargo [6]. EVs are also involved in the modulation of lymphangiogenesis, as MDCK cells overexpressing podoplanin (PDPN) undergo epithelial–mesenchymal transition (EMT) and stimulate the release of positive EVs that significantly stimulate the length, tubulization, and the number of capillary-like structures, thus promoting lymphangiogenesis [7].

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**EVs: Modifying Cancer Cell–Fibroblast Interactions**

Cancer-associated fibroblasts (CAFs) are phenotypically different fibroblasts, characterized by increased proliferation rate, migratory properties, and heightened deposition of extracellular matrix (ECM), that are abundant in the stroma of many solid tumors. CAFs can derive from normal resident fibroblasts, transdifferentiation of mesenchymal stem cells (MSCs), or EMT of cancer cells, processes regulated by TGFβ, PDGF, FGF2, and other factors and molecules including miRNAs.

49 tolerance triggering T-cell death via the Fas–FasL pathway. While the above examples demonstrate that tumor-derived EVs can downregulate the immune response, it appears that EVs from activated immune cells also have a regulatory role in the tumor microenvironment. For example, EVs from activated DCs can reduce tumor immunogenicity by inhibiting ERK-K and NFκB signaling through TNF-related signaling leading ultimately to the upregulation of MMP9 [2] (Figure 2).

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metabolism of cancer cells; for instance, by affecting the generation of energy in the acceptor cancer cells [14].

Concluding Remarks
These few examples provide an outline of the much bigger spectrum of EVs as modulators of the tumor environment. As our knowledge of the biology of EVs increases, so do opportunities to use this knowledge to design better diagnostic tools and targeted therapies. First, the composition of EVs holds important clues about hypoxia and cancer. Second, EVs can potentially be engineered for targeted intervention, including stimulating immune responses or for “trapping” of disseminated cancer cells. Finally, during cancer treatment EVs may switch their composition or behaviour to produce a more therapeutic efficiency. These undertakings are facilitated by the fact that EVs are easily accessible from body fluids. However, many questions remain about the biology of EV biogenesis and how EV-specific uptake by recipient cells is regulated. Temporal regulation underlying tumor development may also affect EV composition, a fact often ignored in tissue-culture settings or mouse EV-injection experiments.

In conclusion, the studies briefly described in this review make a sounding case for the involvement of EVs in many stages of cancer development and progression. Hence, the quest to intercept and exploit EV-mediated cellular communication has just begun.

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