Tumor–stromal cell communication: small vesicles signal big changes

Franz Wendler, Gordon Stamp, Georgios Giamas

Published in
Trends in Cancer

Link to external publisher version
https://doi.org/10.1016/j.trecan.2016.05.007

Forum

Tumor–Stromal Cell Communication: Small Vesicles Signal Big Changes

Q1 Franz Wendler,[1,*] Gordon W. Stamp,[2] and Georgios Giamas[1,*]

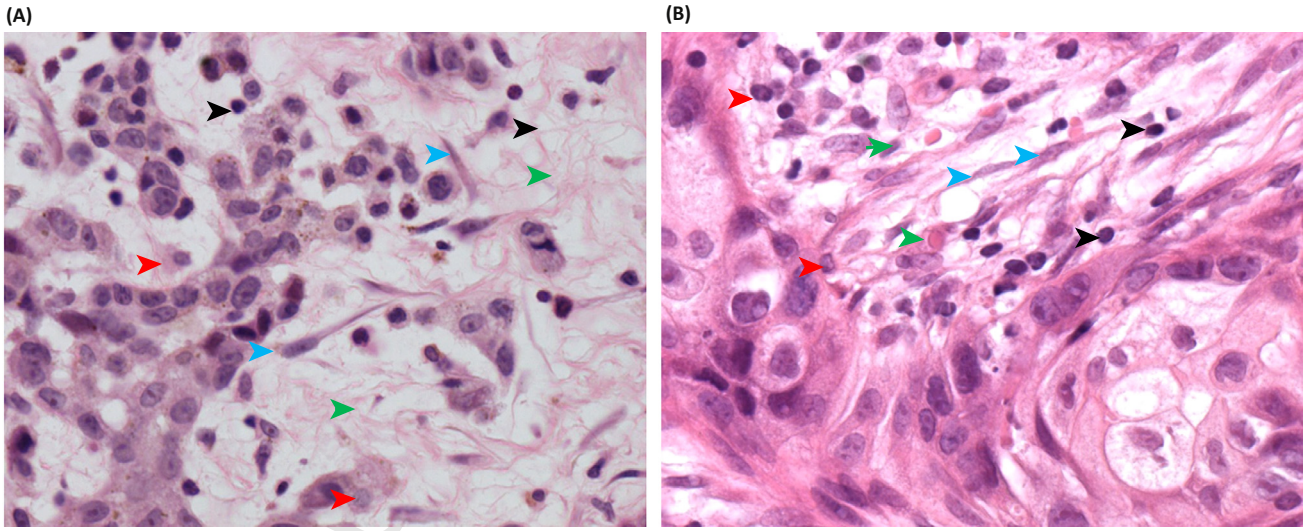
Reciprocal interactions between malignant and stromal cells create a local microenvironment that fosters tumor growth. Extracellular vesicles (EVs) such as exosomes, microvesicles, and large oncosomes are involved in tumor–stroma communication by shuttling signaling cargo and other molecules. Here we 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 lncRNA, tRNA, rRNA, snoRNA, and scaRNA) that depend on the physiology of the cell of origin. As tumors progress, the cargo Q2 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.

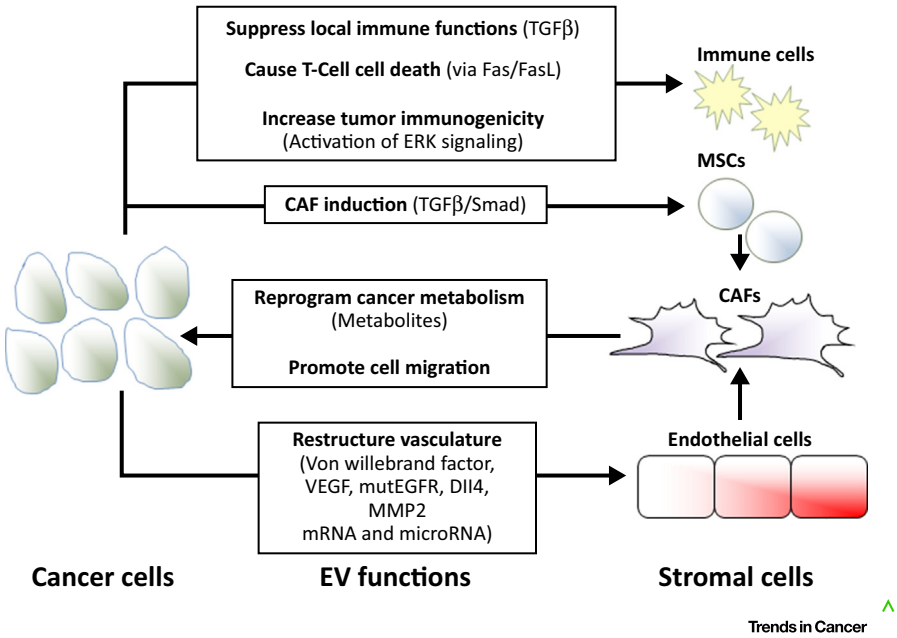
EVs: Recalibrating Local Immune Evasion

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



Trends in Cancer

Figure 1. Extracellular Vesicles (EVs) Participate in Defining the Properties of the Stroma during Malignant Transition. EVs participate in the organization of the microenvironment by inducing cell and matrix transitions, altering metabolism, or acting as chemoattractants for residing cells. (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 (ECM) (green arrows). Early in the process of malignant transition, the stroma tends to be disorganized, much less dense, and less populated by aligned fibroblasts. (B) Transitional cell carcinoma of bladder with desmoplastic response and increase in fibroblastic population. The ECM matures with the laying down of collagen and other components such as elastin, eventually forming very hard tumors (typified also by pancreas and breast ductal cancers). Hematoxylin and eosin stain, magnification, ×400.



Q4 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.

tolerance or 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 can also influ-ence the tumor phenotype. For example, EVs from activated $CD8^+$ T cells can increase tumor immunogenicity by activat-ing ERK and NF-κB signaling through TNF-related signaling leading ultimately to the

Q3 upregulation of MMP9 [2] (Figure 2).

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, EVs 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 oppos-ing mechanisms that promote or inhibit vascular branching have been proposed [3,4]. High-level expression of Wnt5A in melanoma cells can also induce the release of EVs containing immune regula-tory and proangiogenic proteins, including IL-6, VEGF, and MMP2 [5]. Beyond vas-cular signaling, cancer EVs also modify endothelial tube formation under hypoxic conditions through miRNA cargo [6]. EVs are also involved in the modulation of lymphangiogenesis, as MDCK cells over-expressing podoplanin (PDPN) undergo epithelial–mesenchymal transition (EMT) and stimulate the release of positive EVs that significantly stimulated both the length of tubes and the number of closed capillary-like structures, thus promoting lymphatic vessel formation [7].

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.

Experimental evidence shows that cancer-derived EVs can induce the CAF phenotype in various cancer contexts. For example: breast cancer cells EV carrying 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 of cancer and stromal EVs was observed; fibroblast-derived EVs are taken up by cancer cells, loaded with Wnt11 protein, and then released into the tumor where Wnt11 activates autocrine Wnt planar cell-polarity signaling at the leading edge of cancer cells, promoting cell migration [12]. Stromal EVs can also have profound effects on protection from drug treatment and MSC-derived EVs were shown to induce development of drug resistance in 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 intermedi-ates that can strikingly reprogram the

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 the type and stage of cancer. Second, EVs can potentially be engineered for targeted intervention, including stimulation of immune responses or for 'trapping' of disseminated cancer cells. Finally, during cancer treatment EVs may switch their composition, which can be used for 'real-time' monitoring of therapeutic efficiency. These undertakings are facilitated by the fact that EVs are easily accessible from body fluids. However, many questions remain unanswered, including a better understanding of the biology of EV biogenesis and EV-specific uptake by recipient cells. In addition, spatial and 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.

Acknowledgments

This work was supported by Action Against Cancer. The authors apologize to all those whose work they could not cite because of space restraints.

[1]University of Sussex, School of Life Sciences, John Maynard Smith Building, Falmer, Brighton BN1 9QG, UK
[2]Division of Diabetes, Endocrinology, and Metabolism, Imperial College Hammersmith Campus, Du Cane Road, London W12 0NN, UK

*Correspondence: f.wendler@sussex.ac.uk (F. Wendler) and g.giamas@sussex.ac.uk (G. Giamas).

http://dx.doi.org/10.1016/j.trecan.2016.05.007

References

1. Muller, L. *et al.* (2016) Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets. *Sci. Rep.* 6, 20254
2. Cai, Z. *et al.* (2012) Activated T cell exosomes promote tumor invasion via Fas signaling pathway. *J. Immunol.* 188, 5954–5961
3. Sharghi-Namini, S. *et al.* (2014) Dll4-containing exosomes induce capillary sprout retraction in a 3D microenvironment. *Sci. Rep.* 4, 4031
4. Sheldon, H. *et al.* (2010) New mechanism for Notch signaling to endothelium at a distance by Delta-like 4 incorporation into exosomes. *Blood* 116, 2385–2394
5. Ekstrom, E.J. *et al.* (2014) WNT5A induces release of exosomes containing pro-angiogenic and immunosuppressive factors from malignant melanoma cells. *Mol. Cancer* 13, 88
6. Umezu, T. *et al.* (2014) Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. *Blood* 124, 3748–3757
7. Carrasco-Ramirez, P. *et al.* (2016) Podoplanin is a component of extracellular vesicles that reprograms cell-derived exosomal proteins and modulates lymphatic vessel formation. *Oncotarget* 7, 16070–16089
8. Cho, J.A. *et al.* (2012) Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells. *Int. J. Oncol.* 40, 130–138
9. Chowdhury, R. *et al.* (2015) Cancer exosomes trigger mesenchymal stem cell differentiation into pro-angiogenic and pro-invasive myofibroblasts. *Oncotarget* 6, 715–731
10. Franzen, C.A. *et al.* (2015) Urothelial cells undergo epithelial-to-mesenchymal transition after exposure to muscle invasive bladder cancer exosomes. *Oncogenesis* 4, e163
11. Paggetti, J. *et al.* (2015) Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. *Blood* 126, 1106–1117
12. Luga, V. *et al.* (2012) Exosomes mediate stromal mobilization of autocrine Wnt–PCP signaling in breast cancer cell migration. *Cell* 151, 1542–1556
13. Ji, R. *et al.* (2015) Exosomes derived from human mesenchymal stem cells confer drug resistance in gastric cancer. *Cell Cycle* 14, 2473–2483
14. Zhao, H. *et al.* (2016) Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. *Elife* Published online February 27, 2016. http://dx.doi.org/10.7554/eLife.10250