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Review

Intercellular Transfer of Cancer Drug Resistance Traits by Extracellular Vesicles

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Extracellular vesicles (EVs) are nanosized particles (100–1000 nm) enclosed by a phospholipid bilayer that have been described as important mediators of intercellular communication. The role of EVs in oncobiology has been extensively studied, including their contribution to the horizontal transfer of drug resistance from drug-resistant to drug-sensitive cancer cells. This review focuses on the EVs cargo responsible for this intercellular transfer of drug resistance; namely, drug-efflux pumps, miRNAs, long noncoding RNAs (lncRNAs), and other mediators. Additionally, the known molecular mechanisms and features of this transfer are discussed. This is an emerging area of research and we highlight topics that need to be further studied to fully understand and counteract the intercellular transfer of drug resistance mediated by EVs.

Trends

EVs shed by drug-resistant (donor) cells contribute to the dissemination of CDR by transferring their cargo to drug-sensitive (recipient) cells.

The cargo of the drug-resistant EVs may be selectively packaged and may include drug-efflux pumps, miRNAs, or lncRNAs.

The drug-efflux pumps transferred by EVs to drug-sensitive cells are functional in the recipient cells.

Drug-efflux pumps carried by EVs may be responsible for the sequestration of drugs in those EVs.

EVs may protect miRNAs from the action of RNase.

Evidence for the Transfer of a Cancer Drug Resistance Phenotype by EVs

Intercellular communication represents a highly complex network that influences the malignant potential of cancer cells [1]. Classically, this network has been described as involving direct secretion of molecules from cells or direct cell–cell interaction. Nevertheless, in the past 25 years another fundamental mechanism has emerged that is based on the shedding of EVs (Box 1) by some cells (the donor cells) and their incorporation by others (the recipient cells) [2].

The biogenesis, function, and cargo selection of EVs in normal physiology, as well as under pathological conditions, has received much attention over the past few years. Despite their recognized importance in autoimmune malignancies, neurodegeneration and, infection, so far the best understood role of EVs in disease is in the field of tumor biology, namely in tumor progression and aggressiveness [3]. Various studies have already described the signaling pathways involved in the role of EVs in: 'educating' the prometastatic behavior of progenitor bone marrow cells [4]; cancer cell protrusive activity, motility, and metastasis [5]; angiogenesis-modulating stimuli [6]; and cancer immune escape and progression [7].

Importantly, EVs have also been described as a noteworthy vehicle of dissemination of cancer drug resistance (CDR). The role of EVs in sustaining CDR networking has been associated with mechanisms such as promotion of immune escape, angiogenesis and metastasis, epithelial-to-mesenchymal transition, and fibroblast-like cell formation [8]. EVs may be shed from drug-resistant (donor) cells, transferring mediators of drug resistance to the drug-sensitive (recipient) cells, which then may acquire the CDR phenotype (Figure 1). In the past few years, several players associated with drug resistance in cancer cells have been identified as being transferred by EVs and, moreover, to be mediators of the dissemination of this phenotype (Table 1). These mediators include drug-efflux pumps, **miRNAs** (see Glossary), and **lncRNAs** (Table 1). In

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Box 1. EVs: Exosomes and Microvesicles

The term EVs collectively refers to several types of vesicle, such as microvesicles, exosomes, and apoptotic bodies, with different sizes, origin, and molecular cargo (which may be used as markers). The classification of the different types of vesicles remains a debatable issue, but currently there is a set of characteristics that is almost consensual. Exosomes seem to be smaller (30–100 nm) and to have an endocytic biogenesis. Microvesicles represent a larger and more heterogeneous population (100–1000 nm) and originate from cellular membrane budding (Figure 1) [76]. Currently available methods do not allow pure isolation of exosomes from microvesicles, therefore hindering the proper characterization of each type of vesicle individually. As a result, in the past few years the scientific community has been stressing the need that all published results in this field include a detailed description of the isolation protocols used and that authors refer to 'EVs' as a whole [37,77].

addition to the studies of their transfer to drug-sensitive cells, other reports have described the enrichment of some of these mediators (namely lncRNAs) in EVs, as well as an increase of their expression in the cells of origin, following treatment with chemotherapeutic agents [9,10]. Moreover, the involvement of EVs in the sequestration of drugs due to the presence of drug-efflux pumps in those EVs has also been described [11,12]. Additionally, tumor acidity was shown to increase the release of EVs by tumor cells [13] and drugs may be trapped within the highly acidic EVs released by tumor cells [14–16]. This sequestration of drugs in tumor-derived EVs decreases

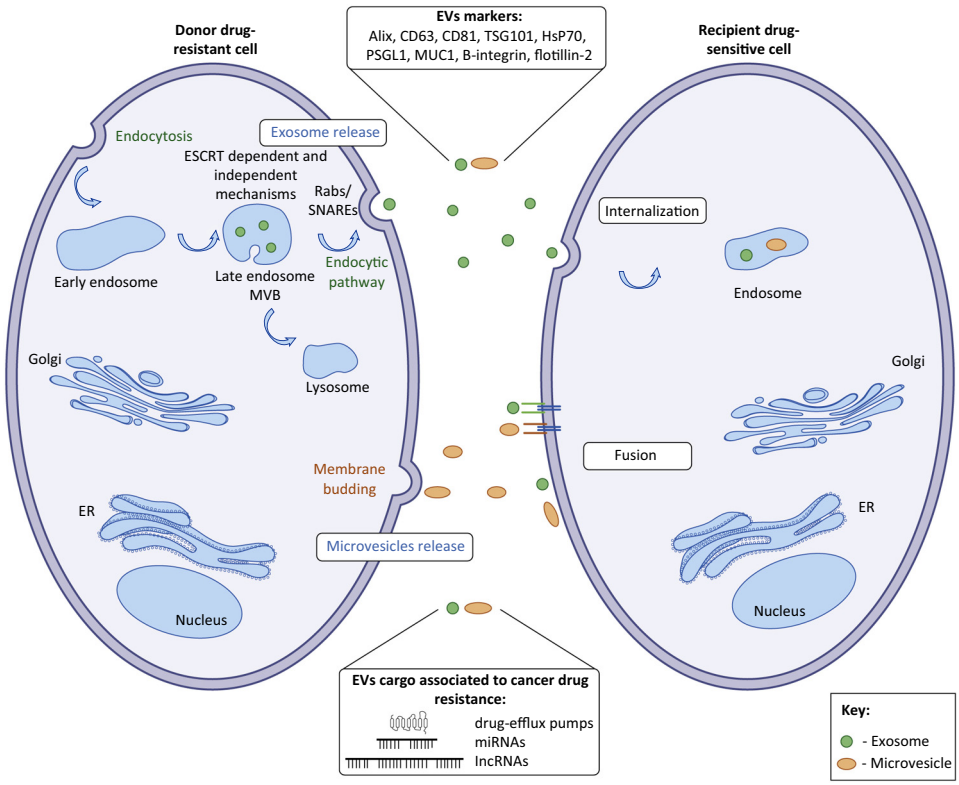

Figure 1. Mechanisms Involved in Intercellular Transfer of Drug Resistance Mediated by Extracellular Vesicles (EVs). Drug-resistant (donor) cells may communicate with drug-sensitive (recipient) cells by the intercellular transfer of various types of EVs, namely, exosomes and microvesicles. Exosomes, of endocytic origin, are formed by inward budding of the multivesicular body membrane. The initial steps of this process are usually controlled by the endosomal sorting complex required for transport (ESCRT). The mechanisms involved in the release of exosomes are also regulated by other protein families, such as Rab GTPases and SNARES. By contrast, microvesicles emerge from outward blebbing of the cellular plasma membrane. Once EVs reach the recipient cell, they may fuse with the plasma membrane or be internalized by the endocytic pathway. EVs may transfer miRNAs, long noncoding RNAs (lncRNAs), proteins (such as drug-efflux pumps), and other key players responsible for drug resistance, which allows the dissemination of cancer drug resistance traits to the recipient cells.

Glossary

Long noncoding RNAs (lncRNAs): a group of noncoding RNAs, more than 200 nucleotides long, that act as important transcriptional and post-transcriptional regulators.

miRNAs: small noncoding RNAs (18–24 nucleotides) that regulate gene expression. Alterations in miRNA profile have been associated with several malignancies such as cancer.

Multidrug resistance (MDR): a phenomenon whereby cancer cells develop cross-resistance to various drugs following exposure to one drug that is structurally and functionally very dissimilar. Overexpression of ABC transporters is one of the major causes of the MDR phenotype observed in cancer cells. The main function of ABC transporters is the efflux of several amphipathic substrates, thereby protecting normal cells from toxic xenobiotics. In a drug resistance context, high levels of these efflux pumps, such as P-gp, ABCG2/breast cancer resistance protein (BRCP), or MRP1/ABCC1, results in the efflux of chemotherapeutic drugs from cancer cells culminating in intracellular sublethal concentrations of these compounds.