

Review

Cell-derived Extracellular Vesicles Open New Perspectives for Cancer Research

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Abstract

This review summarizes the current knowledge about human cell-derived extracellular vesicles (EVs) and their emergence as mediators of a new important mechanism of cell-to-cell communication. A special focus is given on the increasing involvement of tumor cell-derived EVs in cancer. The increased amounts of tumor EVs, when compared with their physiological counterparts have been evidenced in various kinds of cancers. Moreover, these tumor EVs are conveying specific molecular components, proteins, mRNAs and miRNAs, DNA fragments. Some of these compounds actively participate to cancerization processes in the near or remote tumor environments. The cooperation in these tumor “communicasomes” of part of the 98% noncoding genomic DNA with the nano/micro extracellular bullets, long considered as cell “dust”, but in fact precisely reflecting the physiological state of the cells, is really fascinating. The search for cancer biomarkers in various tumor EVs is under intense investigation both “*in vitro*” and in the clinic. Potential use of some of these biomarkers, as biological theranostic tools for diagnosis/prognosis and therapy of many cancers, is a new hopeful thread in cancer research. However, there is still a long way before reaching the promising goal. The first priorities should be to ensure a standardization of EVs purification and nomenclature, to efficiently sort out the different EVs species among their greatly yet uncontrolled heterogeneity and to define a strategy for using the most relevant cancer biomarkers as theranostic tools in the battle to fight cancer.

Keywords Extracellular vesicles, intercellular communication, cancer, tumor diagnosis, antitumoral therapy.

Introduction

Five decades ago, cancer was said to result from an initiation step, followed by a promotion step. The initiation process could have three main origins, genetic, chemical or viral, and many teams worked within these frames, studying, for example, chemical carcinogenesis initiated by benzo (a) pyrene, the main cancer-inducing compound of tobacco smoke. Nowadays, the understanding of what really differentiate cancer cells from normal cells is still a fundamental unanswered question. Some main features of tumor cells such as fast growing, poorly differentiating, inability to die by apoptosis, changes in immunological properties, in metabolic capacities and in angiogenic environment are already recognized. At the molecular level, more and more significant differences between a cancer cell and its

normal homolog are awaited. However, to find the cellular origin of all the biological dysfunctions characteristic for cancer cells, at the genome or at its transcribed (RNA)/translated (protein) levels is still like “searching a needle in a haystack”!

The rather recent introduction of extracellular vesicles (EVs) in the cancer field gave a leading thread to change the volume of the haystack by a factor of about 10^6 (corresponding to the volume ratios of cell/vesicle), which makes the search much less desperate! Moreover, the numerous extracellular vesicles (1), whether originating from the intracellular traffic (exosomes), or by shedding from the plasma membrane (ectosomes/microparticles) or by ultimate embedding of the components of dying cells (apoptotic bodies), represent a heterogeneous continuum of EVs, between 30 nm and 5 μ m in

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diameter. Figure 1 gives an insight of the complexity of eukaryotic cell-derived EVs. Three main EV classes are now generally acknowledged, exosomes, microvesicles and apoptotic bodies. However, two different classes of exosomes are already mentioned and significantly more than three EV subpopulations are morphologically expected (J. Lötvall, private communication). The tissue-specific EV classification, also shown in Figure 1, explains the growing interest of the medical world for EVs. They can be viewed as small "mirrors", reflecting the physiological state – either ill or healthy- of the cells, from which they derive. The exact rules directing the selection of the characteristic cargoes, which they transmit from cell to cell, are not known at the moment, but there seems to be a stringent selection of the molecular components, proteins, mRNAs, microRNAs and DNA fragments incorporated in the various EVs. Due to the 10^6 volume reduction factor between cell and EV, the search for specific tumor signatures should be much easier and worthwhile at the EV level than at the cellular level.

EVs are present in healthy physiological conditions, but also associated with many human diseases. The aim of this short review is to present the current knowledge about cell-derived EVs and to show how these vesicles are entering the field of cancer research.

State of the Art about Extracellular Vesicles

The cell is the basic structural, functional, and biological unit of all known living organisms and is often called the "building block of life". Until about 1980, the cell, considered as a potent micro-factory dealing with all important life processes, was strictly delimited by its plasma membrane; the extracellular medium, although containing active soluble protein ligands, was mostly seen as a waste reservoir of obsolete cellular components. However, starting with the observation of Trams (2), and the works about sheep and human reticulocytes in 1983 (3), the so-called "exosomes" emerged in the biological community and is now experimenting "a nearly explosive growth" (4). EVs are released from almost all cells in the three kingdoms, Archaea, Prokaryotes and Eukaryotes (5). These evolutionary conserved organelles, extending the cell frontier beyond the plasma membrane, might, therefore, be important biological actors. But why do cells release vesicles (6)? Some answer is to be found two years later (7).

State of the art of extracellular membrane vesicles has been recently reviewed (8, 9), in parallel with the standardization of their preparation (10-12). Most works until 2011 concerned exosomes in biology and microparticles in medicine. However, no consensus

is yet obtained about either EVs isolation (11) or their nomenclature (13). Therefore, the current use of the generic term of "extracellular vesicles" is now suggested for all cell-derived EVs, whatever their biogenesis (13). Three databases summarize the currently known components of extracellular vesicles (4, 14-16).

The first evidenced exosome function was to remove obsolete transferrin receptors from reticulocytes (3). The cell-derived microvesicles observed in the eukaryotic microorganism *Dictyostelium discoideum* (17) had both a physiological- and a multidrug (MDR) detoxifying function. Such a cell-derived EVs mediated mechanism was later suggested as a general new MDR mechanism (18). The non-pathogenic microorganism *Dictyostelium discoideum*, already known as a model for human disease (19), has indeed also many assets as a model for the study of eukaryotic EVs (20).

Another exosome key function was related to the observations that B lymphocytes secrete antigen-presenting vesicles (21) and that exosomes from mature dendritic cells induce antigen-specific effector immune responses (22). The exosome preponderant role in regulating immune properties is now well established (23, 24).

Another very important exosome function has been evidenced recently: the exosome-mediated transfer of mRNAs and microRNAs, which are functional when transferred in recipient cells (25, 26) and also the transfer of genomic DNA (27). This promotes a novel mechanism of genetic exchange between cells.

Altogether, EVs are now suggested to be important mediators in intercellular communications (28-31). These bioactive vesicular organelles, carrying a wide range of macromolecular components, contribute to the maintenance of physiological homeostasis, by regulation of gene expression, activation of cell signaling, distribution of catalytic activities and removal of cellular trash (9).

Importantly, EVs, containing multiple functional molecules (32), are not only cell physiological mediators of intercellular communications in healthy conditions. They also reflect various cellular disease conditions (8, 9, 33-36), and especially in the field of cancer, as reviewed below.

Emergence of Extracellular Vesicles in Cancer Research

The increasingly important field of EVs related to cancer during the last years is shown by an overview in PubMed of the numbers of papers published yearly

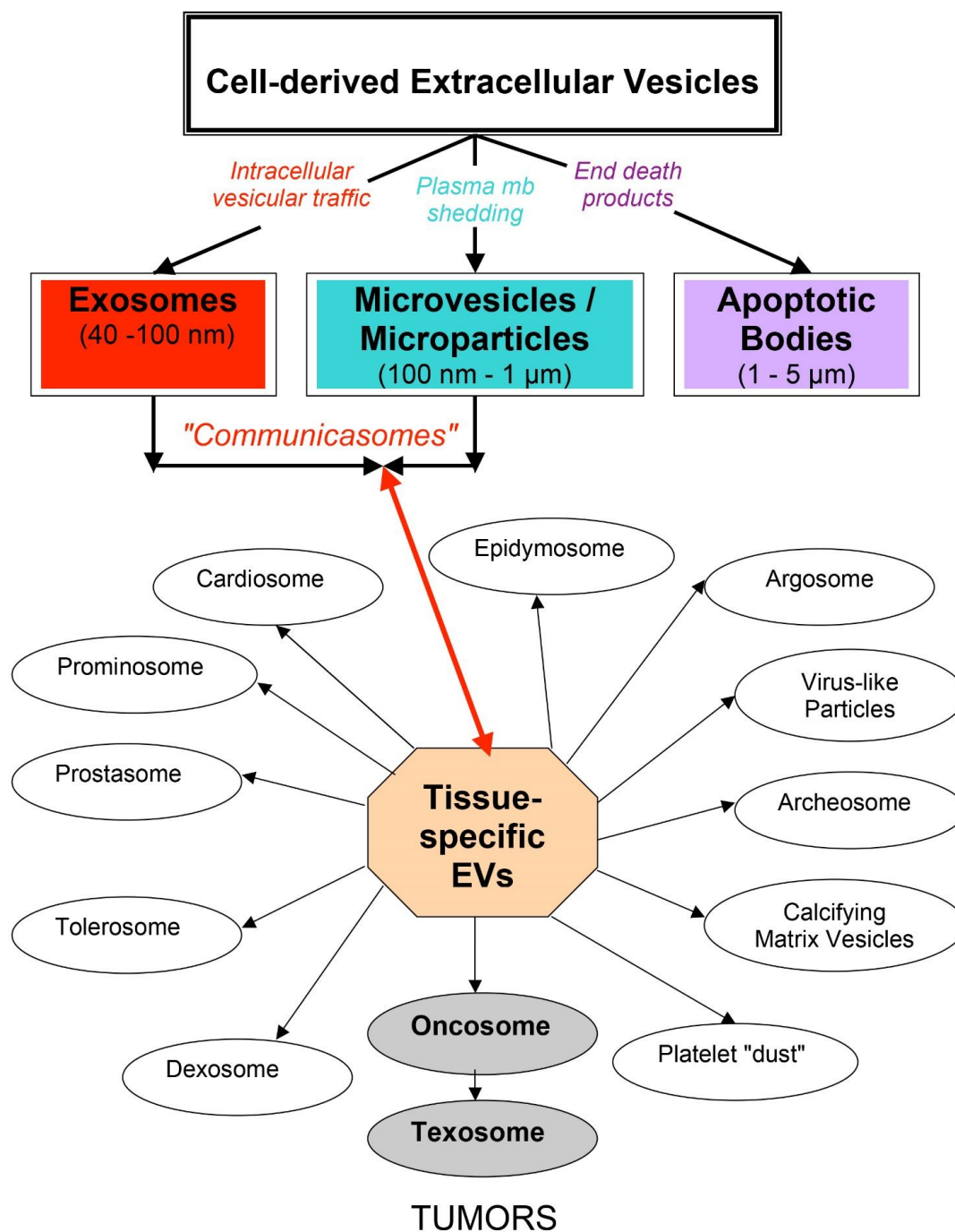


Figure 1. Complexity of eucaryotic EVs. Following their respective biogenesis, three main classes of cell-derived EVs are now recognized : exosomes, microvesicles/microparticles and apoptotic bodies. A tissue-specific EV classification is also shown to point out their interest for the medical field.

on this topic (fig. 2). EVs have already invaded the research field of various human cancers, such as (non-exhaustive list) bladder- (37), brain-(38-40), breast- (41-43), colon- (44, 45) and colorectal-(46, 47), head and neck-(48), hepatocellular- (49, 50), lung- (51), ovarian- (52), and prostate- (46, 47, 53,

54) cancers, gliomas (55), glioblastomas (56) and mesotheliomas (57), nasopharyngeal-(58) and renal-(59) carcinomas, melanomas (60), myelomas (61), lymphomas (62) and chronolymphocytic leukaemia (63). Table 1 gives a survey of EVs properties observed for various human cancers.

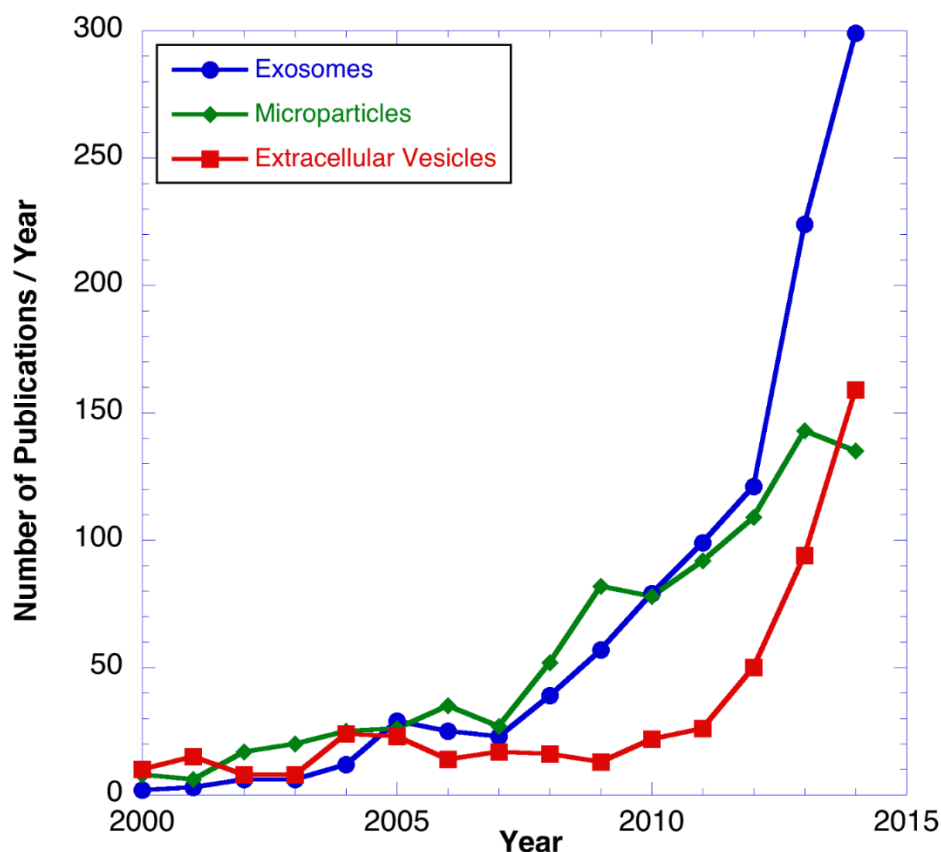


Figure 2. Overview of the numbers of papers published yearly in PubMed on EVs (exosomes/microparticles/ EVs) related to Cancer.

Presence and Characterization of Tumor Cell-derived EVs

Tumor EVs (oncosomes) are associated with various cancers and cancer cells (8, 64-67), often in increased amounts as compared to the equivalent normal cells (64, 68, 69). A higher EVs concentration in the plasma of cancer patients than in the healthy controls is a general observation (70, 71). Healthy individuals have about 10^{11} microvesicles (MVs) per ml serum, while cancer patients have approximately 10-fold more and these tumor-derived MVs transport cargoes with cancer-specific signatures (72). An interesting description of EVs from plasma of healthy subjects, based on cryo-electron microscopy, is given in Brisson et al, 2014. It brings novel insights on EVs from normal plasma and provides a reference for further studies of EVs in disease situations (73).

Tumor EVs contain lipids and proteins; they may also contain RNAs, gDNAs (74-76) and probably metabolites, which can be transferred between cells. The tumor-specific cargoes seem to be highly regulated, but the mechanisms of regulation are still under study. These cargoes, different from those of

analogous physiological EVs, may possess common hallmarks to various tumors and may also have some specific characteristics for a given tumor. The characterization of all these tumor EVs components is under intense investigation, with the hope of evidencing useful cancer biomarkers (77).

Tumor signatures were first searched at the protein and genomics levels (69). Proteomics allowed the finding of a great number of proteins carried by EVs (4). But in two different breast cancer cell lines, MCF-7 and MDA-MB 231, the cell-derived EVs revealed different protein profiles. From the database search, 59 proteins were identified in MCF-7 EVs and 88 proteins in MDA-MB 231 EVs, with 27 proteins common between the two exosome-like vesicle types (42). The search for well-conserved EV marker proteins irrespective of the tumors origin, will help delineating the more significant proteins (78). Indeed, in cancer research, it is interesting to point out some specific proteins as effectors in the cancer processes. Such effectors are especially searched in tumor EVs circulating in body fluids (12, 79, 80).

Up to now, a few tens of major potentially active

tumor EVs proteins have been discovered. They include membrane receptors, such as Epithelial Growth Factor Receptor (EGFR) (79), transforming growth factor (TGF-beta), protein families of signaling pathways (66), oncogenic proteins, such as HER2-related or up-regulated proteins (81), involved in cellular malignancy, and Survivin, one of the key members of apoptosis inhibitors (82).

The next tumor signatures search was at the RNA level. In 2007, Valadi et al. evidenced that EVs are carrying mRNAs and microRNAs (miRNAs) (26) and that, when transferred to recipient cells, these nucleic acids were functional. This opened a new investigation field in cancer biology (77, 79, 83, 84). Some EVs-transported miRNAs representing tumor signatures (oncomirs), such as miR-520g have emerged (38) and dysregulations of miRNAs in cancer have been widely observed. Non-coding RNAs, including retrotransposons were also shown in glioma EVs (85).

The most recently discovered macromolecular components in some tumor EVs are genomic DNAs (77, 86-88), mutated and non-mutated oncogenes (38, 64, 85, 89). EVs were designed as "vehicles that spread cancer genes" (89). Lee et al. found that rat epithelial cell transformed by the human H-ras oncogene leads to an increase in production of small, exosomal-like extracellular vesicles by viable cancer cells. These EVs contained chromatin-associated double-stranded DNA fragments covering the entire host genome, including full-length H-ras (86). Following the earlier observation of Ronquist, that human prostasomes contain chromosomal DNA (87), Lazaro-Ibanez et al. evidenced various gDNA contents, eventually with specific mutations, in the subpopulations of prostate cancer EVs: apoptotic bodies, microvesicles and exosomes (70).

Among the "omics" measurements about tumor EVs composition, lipidomics and metabolomics are up-to now much less involved than proteomics, transcriptomics and genomics, as shown in EVpedia (4). The EVs-shuttled cancer biomarkers evidenced in pre-clinical and clinical studies of various cancers have been summarized recently (67).

Functions of Tumor Cell-derived EVs

EVs general properties are to transport extracellularly cell-selected macromolecular compounds, which are thus protected from enzymatic degradation. Moreover, EVs mediate the transfer of some of these compounds to specific recipient cells, where they remain functional and may change the fate of these cells. The same properties are shared by tumor EVs, which are thus able to promote cancer growth and metastasis, especially through body fluids. Table 2

reports the common biological functions of human tumor EVs.

In 2010, a workshop about the emerging concepts of cell-cell communication in the tumor microenvironment summarized the current knowledge and identified the many research priorities in the field (72). Identifying the various components of tumor EVs was a pre-requisite for underlining the main tumor-specific components for cancer diagnosis purposes, but the interest of tumor EVs became quite outstanding, when their effector activities in tumor progression became established (77, 79, 84, 90-96). Many EVs-mediated functions are at work for preventing or promoting cancer via cell-to-cell communication and the roles in cancer of the so-called "small but mighty"(97) "communicasomes" (31) are under intense investigation.

As noticed earlier for the multidrug transporter (98), EVs are "double-edged swords": they can protect cells, from which they are released from toxic bacterial products (9), but they can also mediate a new multidrug resistance mechanism, preventing the healing activity of antitumoral chemotherapy (18, 99). Following different infections, they can induce reactive immuno properties from the cellular "immunity sentinels", but some EVs can also disseminate the infection agents, such as bacteria, viruses and prions (100) among normal cells, thus promoting infections, hence the question "are EVs protective or pathogenic?"(101). This duality also occurs for tumor EVs, although their preponderant functions seem to improve the tumor "wellness". The yin-yang in cancer biology of microvesicles (exosomes), inholding both oncogenes and tumor suppressors, has been recently discussed (102).

Below is a short review of the many tumor EVs-mediated functions known to accompany cancer progression. Tumor EVs are involved in the transfer of oncogenes and of miRNAs regulating gene expression of recipient cells. Tumor EVs transfer genomic or proteic oncogenic components to near- and / or remote-recipient cells via body fluids. These EVs-transported oncogenes can either promote the tumor by spreading the cancer genes (89) or they can prevent the tumor by keeping or disseminating tumor suppressive oncogenes, like p-53 or PTEN (for Phosphatase TENsin homolog) (103). EVs and tumor EVs are also mediating a fine efficient tuning of immunity (23, 24, 67, 80, 93, 96, 104-108), leading to immuno-surveillance, and either immuno-suppression or immuno-tolerance of the tumor cells. Moreover, tumor EVs are conditioning the tumor's environment and the surrounding angiogenesis (94, 104, 109, 110) for an improved tumor growth.

Table 1. Properties of Tumor EVs observed for various Human Cancers

Cancer types (cell lines/ cancer patients)	Properties of Tumor EVs		Ref.
	Function	Mechanism	
Bladder cancer (T24)	Inhibition of apoptosis	Activation of Akt and ERK	(37)
Brain cancer	Transport of transformation related molecules	EGFRvIII, PTEN mRNAs, microRNAs (miR-520g), nc RNAs and DNAs	(38-40)
Breast cancer (MCF-7, MDA-MB 231; HMEC B42 and B42 cl. 16)	Mediation of drug resistance and control of exosome release	Compared proteomics MicroRNA delivery	(41-43)
Colon cancer (Mutant KRAS) (LIM1863 organoids)	Transport of tumor-promoting proteins involved in tumor progression	KRAS, EGFR, SRC family kinases, and integrins	(44, 45)
Colorectal cancer (SW480 and SW620)	Role in cancer progression, metastasis and multidrug resistance	MET, S100A8, S100A9, TNC; EFNB2, JAG1, SRC, TNK1; CAV1, FLOT1, FLOT2, PROM1	(46, 47)
Head and neck cancer (HNC)	Role in HNC biology and carcinoma progression	Galectin-9 and/or LMP1, EVB-miRNAs	(48)
Hepatocellular cancer (HCC) (HCC and liver cirrhosis patients, healthy controls)	Correlation MVs levels / HCC classification. Promotion of HCC growth and spread	Transfer of a 1,198 bp ultraconserved noncoding RNA TUC339	(49, 50)
Lung cancer	Pivotal role in tumor progression and metastasis	Transfer of several proangiopoietic factors in stroma cells	(51)
Ovarian cancer (SKOV3)	Cell-to-cell communication	Implication of exosomes and cells proteins	(52)
Prostate cancer (PCa)	Role in malignant cell growth and proliferation.	Novel biomarkers for PCa	(46, 47, 53, 54)
Glioblastomas multiform (GBM) (cell lines and patients)	Role in glioblastoma growth and invasion	EV-transported miR-1 Hypoxia-regulated mRNAs and proteins	(56)
Melanomas (primary and metastatic)	Exosome-specific melanoma signature. Metastasis by EVs "educating" bone marrow progenitors	Rab27A- dependent exosome production MET	(60)
Malignant Mesotheliomas (MM) (mice- in vivo-)	Improved survival of tumour bearing mice	DC exosomes immunotherapy	(57)
Multiple Myelomas (MM.1S and U266 MM)	EVs relative protein abundances	Not studied	(61)
Epstein-Barr virus (EVB) Nasopharyngeal carcinomas (NPC) (NPC patients)	Apoptosis in Th1 lymphocytes, Improvement of anti- NPC immunotherapy	Galectin-9 /Tim-3 ligand	(58)
Renal carcinomas (ACHN and Jurkat T)	Apoptosis of Jurkat T cells and tumor immune evasion	Fas ligand	(59)
B-Lymphomas (cell lines)	EVs Heterogeneity	Magnetic bead- isolation	(62)
Chronolymphocytic leukaemia (CLL) (CLL blood plasma)	Prosurvival microenvi-ronment for CLL cells	EVs transported specific proteins	(63)

Table 2. Properties and Common Biological Functions of Human Tumor EVs

Common Biological Functions Related to Cell-to-Cell Communication	General Characteristics of Tumor EVs		References
Horizontal Transfer Of Bioactive Molecules (Proteins, RNAs and/ or DNAs), Modifying the Fate of Recipient Cells	Heterogeneity. Increased tumor EV amounts compared to amounts of control non-tumoral EVs.		(8, 64-72)
	Search for Cancer Specific Biomarkers (Proteins, RNAs and DNAs)		(38, 64, 74-77, 85, 89)
	Proteins		(4, 16)
	Unique proteomic content.	- see (69), tables 1 and 2-	(69)
	Conserved EV marker proteins.	CD9 and CD81.	(78)
	Cancer effectors in body fluids.	- see (79), table 1-	(12, 79, 80)
	Active tumor proteins.	EGFR, TGF-beta, signaling pathways proteins, HER2-related proteins, and Survivin.	(66, 79, 81, 82)
	RNAs		(4, 16)
	EVs transported mRNAs and microRNAs (miRNAs).	Intercellular genetic exchange.	(26)
	Oncomirs, Non-coding RNAs, Retrotransposons.	miR-520g. Dysregulations in cancer biology.	(38, 77, 79, 83-85)
	DNAs		
	Heterogeneous chromosomal DNA in EVs subpopulations.	Tumor cell entire genome +/- mutations.	(70, 77, 86-88)
EV-transported oncogenes.	Spreading of cancer genes.	(38, 64, 85, 89)	
Mediation of Tumors Immuno-surveillance	Fine efficient tuning of immunity, mediated by EVs in association with the normal immune cells.	Immuno-suppression or immuno-tolerance of the tumor cells.	(23, 24, 67, 80, 93, 96, 104-108, 156)
Modulation of the Tumor Microenvironment (angiogenesis)	Effectors in tumor progression - see (84), table 1-	Reprogramming of stroma cells, supporting tumor growth and metastasis.	(31, 72, 77, 79, 84, 90-97, 104, 109, 110)
“Yin-Yang” Opposed Influences	Protection against toxics.	EV-mediated elimination from cells.	(9)
	Propagation of multidrug resistance (MDR).	Transfer of MDR proteins.	(99, 111-113)
	Spreading of cancer genes and tumor suppressive oncogenes.	Transfer of H-and K-RAS, EBV, Myc, p-53, PTEN.	(89, 102, 103)

Altogether, tumor EVs mediate both tumor progression and metastasis. Lastly, tumor EVs mediate a new multidrug resistance against chemotherapeutic drugs (99, 111-113), which impair their efficiency also at the benefit of the tumor.

Briefly, as stated by Muralidharan-Chari et al. (84), tumor-derived microvesicles orchestrate the modulation of the tumor microenvironment to support tumor growth and survival and govern angiogenesis by horizontal transfer of VEGF and miRNAs, that impact endothelial cells. By horizontal transfer of oncogenic receptors and other paracrine signals, they promote the acquisition of aggressive phenotype. Tumor EVs also control the evasion of immune surveillance by fusion with immune cells, which alter the immune response. EVs protease cargo facilitates matrix degradation and joint deposition of paracrine signals help tumor invasion and metastasis. Moreover, by ensuring chemotherapeutic drug efflux from cells, tumor EVs mediate multi-drug resistance. Thus, tumor-derived microvesicles, which can affect a variety of cellular events, are key actors of the cancer processes with a great impact on tumor progression and metastasis.

Extracellular Vesicles and Cancer Diagnosis /Prognosis

Table 3 shows an overview of the future use of EVs for theranostics (Diagnostic/Prognostic and Therapy) of human cancers. As long as EVs are derived from healthy cells, they convey and transmit healthy signals to other cells, thus maintaining physiological homeostasis and the right ratios of the important biological functions, such as growth, differentiation and apoptotic death. Some EVs carry cell-to-cell immunity mediators, which keep the many existing undetectable microtumors under control. One can figure out that above a threshold of tumor cell-derived EVs, harbouring and transferring tumor messages, the natural immuno-surveillance is overwhelmed and tumor EVs are then actively working for the own tumor benefit, progressively changing the tumor microenvironment (stroma modification, neo-angiogenesis, immunotolerance) and disseminating tumor and metastasis agents among healthy cells.

It is important to use some already characterized tumor EVs compounds, to try and figure out efficient early diagnosis and therapeutic tools, with the hope of changing the "cancer war" issue. An early diagnosis is often a real challenge for increasing the chance of curing cancer. After treatment, the prognosis of cancer evolution is also dependent upon efficient cancer biomarkers. As stated by Kislinger (114) analysis of tumor EVs open new avenues for

biomarker discovery. Many tumor EVs components have already been identified as potential cancer biomarkers for diagnosis of various specific cancers (50, 77, 79, 93, 115-118). Pre-clinical and clinical EV-shuttled biomarkers have been recently recapitulated -see table 1 in (67)-. This table illustrates " The fusion of two worlds: Non-coding RNAs and extracellular vesicles" in the clinics (119). Many EV-shuttled biomarkers are miRNAs (67), but most of them are specific of a given cancer, which is also the case for other tumor EVs biomarkers. This means that, actually, the search for an EVs diagnosis/prognostic tool is specifically targeted to each kind of cancers (50, 116, 120). No universal EVs tumor-specific biomarker has yet appeared. Moreover, although quite promising for the future (67, 77, 93, 115-117, 121), the elaboration of statistically significant cancer specific EVs biomarkers is still in its infancy. The same analysis is valid for the promising use of EV-shuttled noncoding RNAs as potential cancer biomarkers (122-126).

Many recent technological tools are now available to elaborate new cancer diagnostic tools based on a given cargo component of the cancer cell-derived EVs. However, given the great number of characteristic components of the oncogenic cell dysfunctions, making the best choice is rather cumbersome. Moreover, achieving all the necessary steps from the bench to the clinic takes years! Hopefully, for the cancer patients, they do not have to wait for a full explanation of the tumoral process, but they can already benefit from some interesting observations.

This is illustrated by the PTEN story, which began about two decades ago. It was noticed that the PTEN gene, which was expressed in many various normal cells, depicts a greatly reduced expression and/or different mutations in a great number of various kinds of tumor cells. Quite recently, it was observed that cancer cells get rid of PTEN protein into the cargo of cancer cell-derived EVs (103), partly explaining the observed reduced PTEN expression in cancer cells. Moreover, it gave an interesting tool to work out the biological functions of EVs related to prostate cancer (103).

It was observed that transporting PTEN protein through exosomes was only a property of cells from DU-145 prostate cancer cell line, whereas normal prostate epithelium cells, although also expressing PTEN, never used exosomes to lower the PTEN protein amount. Inside exosomes, PTEN phosphatase was active to phosphorylate the protein (127), which remained thus protected from enzymatic degradation and functional inside the exosomes. These PTEN-protein containing exosomes from DU 145 cells were

also able to transfer their tumor suppressor cargo to PTEN knockdown cells, DU145Kd, and to induce active PTEN expression, leading to reduced proliferation and apoptosis induction of the tumor cells. The PTEN protein was not merely eliminated from the cancer cells through exosomes, but it remained functionally active to disseminate the oncogenic suppression properties to other cells in the near or remote environment. Other tumor suppressor proteins did not depict the same behavior as the PTEN protein and this turned out to be independent of the presence of the other tumor suppressor p53 in tumor cells.

Although the mechanisms underlying the exosomes-mediated oncogenic PTEN activity is far from being understood, the exosomal PTEN protein appeared as a good candidate to help diagnosis and prognostic of severe forms of prostate cancer before and after prostatectomy. Thirty patients and eight healthy volunteers were enrolled in a clinical study for compared exosomal PTEN and PSA measurements, and it turned out that PTEN protein was present in all the thirty patients' exosomes- and absent in the eight control serum exosomes. Moreover, the amount of PTEN protein was well correlated with the Gleason score measuring the grade of the diseases, which was not the case for exosomal PSA. After confirmation of these results with a higher amount of patients, measurement of exosomal PTEN protein could depict a higher selectivity than PSA blood tests, which were unfortunately not performed in this study. The joined measurements of PSA blood tests, Gleason scores and exosomal PTEN protein measurements might help to narrow the true prostate cancer diagnostic and prognostic, when compared with the current PSA blood tests, suffering from too many false positive or false negative assessments. Even a therapeutic purpose through PTEN protein-bearing exosomes directly injected in a prostate advanced recurrent- or bone metastatic- tumor is now considered in a human phase II clinical study (103).

The PTEN story is indeed a good illustration of the joined efforts of fundamental biological and medical research aimed to benefit the patients as fast as possible, and pointing in parallel important mechanisms of the intercellular dispersion of the oncogenic processes, mediated by the cancer cell-derived EVs.

Extracellular Vesicles and Cancer Therapy

EVs are not only a potential wealth for elaborating cancer diagnostic tools, but they might also be used as therapeutic agents for antitumoral therapy (80, 93, 128). However, this approach has to be deeply

worked out before achieving an efficient clinical practice. Given the many EVs recognized functions, different EVs-mediated antitumoral targets can be designed, as briefly discussed below.

One of the earliest observed EV functional activities was the capacity of the dendritic cells exosomes (dexosomes) to convey immune properties to the human immunity watchers, such as specialized lymphocytes and macrophages (21). It is understandable that one of the first therapeutic goal was aimed to use dexosomes for elaborating antitumoral vaccines (129-133). However, EVs modeling of immune properties turned out to be quite complex, as both physiological and tumoral cells might release vesicles with immune information, depending on their own story (inflammation, tumor progression, etc). Although regulation of immune responses by EVs has now been thoroughly investigated (24), the promising immunotherapeutic potential of EVs, as therapeutic agents and / or targets (57, 107, 134) is still under study.

Another important EVs characteristic is linked to their (phospho)lipidic bilayer (135), which enables them to fuse or interact with the analogous cell plasma membranes and to behave as perfect "Trojan horses" for delivering their protected cargoes into the recipient cells. Therefore, EVs, and especially exosomes, are remarkably interesting biological organelles (136, 137), qualified as "the ideal nanovectors for drug delivery" (138). In contrast to these "natural delivery systems", synthetic (phospho) lipid vesicles, or liposomes, have been employed as drug carriers for decades, resulting in several approved liposomal nanomedicines used in the clinic (139). The similarities and differences between EVs and liposomes are discussed in that review, and EVs are highly attractive as potentially better alternatives (140). Knowing EVs capacity for transmitting miRNAs with functional gene expression properties (25, 26, 141), it will probably be possible, in the future, to engineer EVs for gene therapy (57, 65, 141-145), by delivering some important regulating miRNAs (146, 147), siRNAs (148) or tumor suppressor, such as PTEN (103).

Thus, for antitumoral therapy, EVs are potentially outstanding for regulating immune properties to fight cancer progression and metastasis and for biological drug delivery, without cell adverse reactions against chemical "magic bullets" for gene therapy. However, EVs as "double-edged swords" are not only beneficial components in the fight against cancer (138); they can also convey and transmit oncogenes, such as H-Ras (77), promote cell-to-cell spread of infectious agents (149); they can also propagate resistance against antitumoral chemotherapy among

Table 3. Tumor EVs for future Theranostics (Diagnosis (D) / Prognosis (P) / Therapy (T)) of Human Cancers

Donor Cells/EVs	D / P / T	EVs Cancer Biomarkers	References
Tumor EVs (exosomes/microvesicles) (Various cancer cell lines and biofluids)	D	Diagnosis of various cancers in peripheral blood and other biofluids	(50, 77, 79, 93, 115-118)
Hepatocellular- Urogenital cancers Glioblastomas	D, P	Diagnosis/ prognostic tool targeted to a specific cancer	(50, 116, 120)
Brain cancer Bladder cancer	D, P	Sequencing tumor EVs RNAs	(123,124,126)
Prostate and Castration- resistant cancers	D, P	EV-shuttled noncoding RNAs (nc RNA), miRNA (miR-1290 and miR-375), and PTEN protein.	(103, 122,125,127)
Melanoma and nonsmall- cell lung cancer (phase I trials)	T	Evaluation of antitumoral vaccines Manipulation of antigen secretion	(129-133)
Malignant mesothelia		EVS as therapeutic agents and / or targets for immunotherapy	(57, 107, 134)
Biological therapeutic vehicles and novel drug targets for cancer therapy	T	"Ideal nanovectors" for drug delivery, potentially better than liposomes for nanomedicine. Assets of EVs immunotherapy compared to mesenchymal stem cell (MCS) therapy. Targeting "bad" EVs transmitting oncogenes or MDR, or targeting their cell release.	(136, 137) (99, 112)
Exosomes nanotheranostic platform	T	EVs engineering for delivery of regulating miRNAs, siRNAs or PTEN for gene therapy	(57, 65, 141- 144, 146, 147, 150)

sensitive cells (99, 112). These bad EVs influences have to be suppressed, either by directly targeting the corresponding EVs (150, 151) or by targeting the mechanism of their cell release. Indeed, as stated by Suntres et al., "much work remains to be done to ensure the safe and effective use of exosomes for therapeutic applications" (80, 138), but EVs potentialities for both antitumoral diagnosis/prognosis and therapy are really promising.

Conclusion

Every scientific observation about cancer cell-derived extracellular vesicles is interesting to increase the general knowledge, but the whole clarification of such a complex biological dysfunction, as expressed in cancer, will probably still await for a long time!—Beside increasing involvement of tumor EVs in cancer research, another quite new field dealing with cancer stem cells and their derived EVs also opens promising perspectives

for both understanding and treating cancers (152, 153).

A number of technical points remain to be urgently clarified, such as the EVs nomenclature and the standardized protocols for their preparation. The scale change from cell to cell-derived EVs opens new promising perspectives for cancer research from the bench to the clinics. However, the complexity of EVs heterogeneity and functions have to be taken into account. One of the most urgent challenges is to set up methods to characterize separately each kind of EVs in order to precisely define their individual cargoes and functions (154). EVs-adapted flow cytometry (155) should be helpful for that purpose, with the aim of sorting out each kind of EVs for further specific characterization.

In the future, the important challenge for cancer research would be to accumulate all the EVs (1, 12) derived from a given cancer cell type and to sort them in different categories for comparison with the corresponding vesicles of their normal homologous cells. This might give an insight of what is really important to govern the many cancer cell behaviours, with regard to the main known biological functions and this would also strengthen the potentialities of EVs for theranostics of human cancers. The increasing knowledge of the specialized cargoes composition of cancer cells-derived EVs would open a field of new possibilities for early cancer diagnosis, together with new potential therapeutic tools and, therefore, a much higher hope of efficiently managing to cure this deadly illness so intimately associated with life.

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The abbreviations :

EGFR	Epithelial Growth Factor Receptor;
EVs	Extracellular vesicles;
gDNA	genomic DNA;
mRNAs	messengers RNAs;
miRNAs	micro RNAs;
ncRNAs	non-coding RNAs;
siRNAs	silencing RNAs;
MVs	Microvesicles;
MDR	Multiple drug resistance;
PSA	Prostate serum antigen;
PTEN	Phosphatase TENsin homolog;
TGF	Transforming growth factor.

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