

## Biogenesis and Functions of Exosomes and Extracellular Vesicles

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### Abstract

Research on extracellular vesicles (EVs) is a new and emerging field that is rapidly growing. Many features of these structures still need to be described and discovered. This concerns their biogenesis, their release and cellular entrance mechanisms, as well as their functions, particularly *in vivo*. Hence our knowledge on EV is constantly evolving and sometimes changing. In our review we summarize the most important facts of our current knowledge about extracellular vesicles and described some of the assumed functions in the context of cancer and HIV infection.

**Key words** Exosomes, Extracellular vesicles, Biomarker, HIV, ESCRT

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### 1 Introduction

In recent years the function of extracellular vesicles (EVs) attracted increasing interest, particularly in cancer and viral research. Described to harbor and deliver a diverse repertoire of functional molecules to recipient cells, including genetic information, EVs seemingly constitute a new layer of complexity in multicellular organisms, which has been recognized only a few years ago. Based on the latest research, EVs have been described, for example, to support tumor growth, formation of metastasis, and immune evasion and stimulate HIV viral replication. Seminal work was published in 2008 when Skog et al. reported that glioblastoma cells secrete EVs containing mRNA transcripts harboring tumor-specific mutations [1]. Importantly, circulating EVs derived from glioblastoma patients also contained mutated mRNA transcripts encoding the EGFRvIII which were not detectable in healthy individuals [1]. In 2009 Muratori et al. published that HIV-infected cells shed large amounts of EV for reasons that were not clear at that time [2]. Meanwhile, EVs are associated with many more diseases and conditions and even parasites seem to release EV. Based on these findings, it is anticipated that circulating EVs

have a far greater importance in living organisms than previously thought and EV research is expected to increase significantly. In this chapter, we will concentrate on the current knowledge of EVs in general and reflect on some aspects of these novel factors in cancer and HIV infection.

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## 2 Extracellular Vesicles

Intercellular communication represents an event of vital importance for multicellular organisms. It has been believed for decades that this process is solely mediated by the secretion of single soluble factors secreted into the extracellular space. However, the discovery that EVs contain a multitude of factors including signaling molecules, enzymes, and miRNA added a new layer of complexity to our understanding of intercellular communication. EVs are small and defined spherical structures limited by a lipid bilayer which are secreted into extracellular space [3]. Latest research identified EVs as autocrine stimulators as well as short- and long-distance messengers, which are taken up and processed by recipient cells to elicit various cellular responses. In addition, different types of EVs have been described, which differ with respect to their subcellular origin, their biophysical and/or biochemical properties, their receptor composition, and possibly their content (Table 1).

In addition to the EV types described in Table 1, Muratori and coworkers described a new type of EVs that are shed in clusters, or microvesicle clusters (MC), and do not originate, like typical exosomes, from multivesicular bodies (MVBs) or the plasma membrane, as, for example, microvesicles [2] (see below). These clusters were found to be released not only by HIV-infected T cells *in vitro* and *in vivo* but also after classical stimulation of T cells, for example, by PHA/PMA.

Current research focuses mainly on the investigation of two types of EVs, exosomes, and microvesicles. The term exosomes was coined by Trams et al. who described the release of EVs with 5'-nucleotidase activity from various normal and neoplastic cell lines [4]. These EVs had an average diameter of 500–1000 nm and were accompanied by a second vesicle population with a diameter of ~40 nm [4]. Subsequently it had been reported that reticulocytes actively secrete microvesicles of ~50–100 nm in diameter, mediated by fusion events of multivesicular endosomes with the cellular plasma membrane [5, 6]. These microvesicles were referred to as exosomes. In recent years exosomes have been extensively investigated and many biological functions were unraveled and have been attributed to these vesicles [7–11]. However, since exosomes are difficult to discriminate and/or purify from EV of other origin, many EV-induced biological functions reported in the

**Table 1**  
**Types and characteristics of EVs**

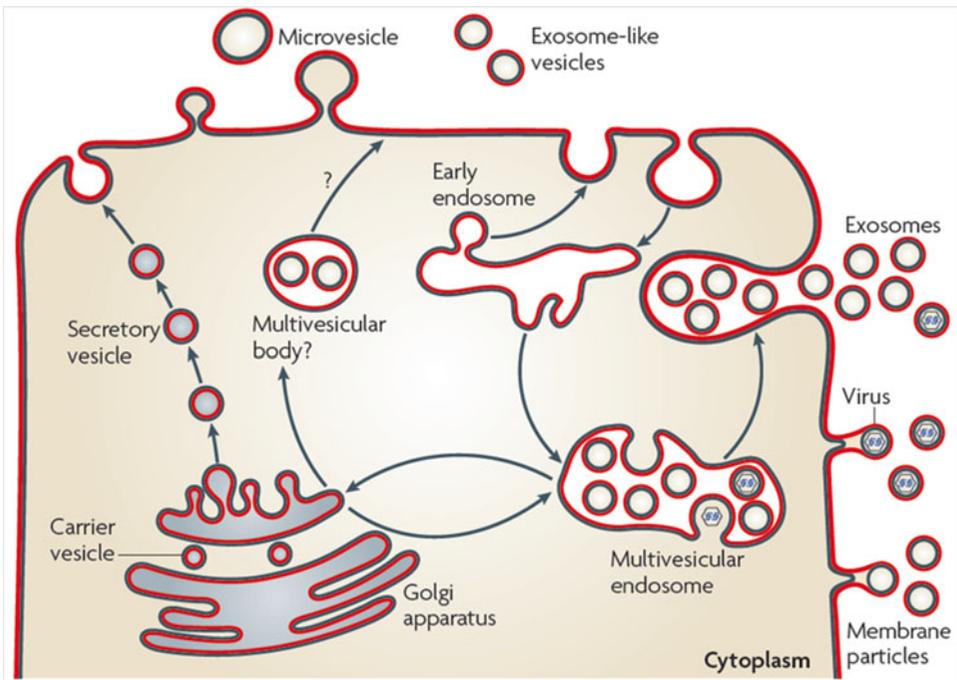
<b>Feature</b>	<b>Exosomes</b>	<b>Microvesicles</b>	<b>Ectosomes</b>	<b>Membrane particles</b>	<b>Exosome-like vesicles</b>	<b>Apoptotic vesicles</b>
Size	50–100 nm	100–1000 nm	50–200 nm	50–80 nm	20–50 nm	50–500 nm
Density in sucrose	1.13–1.19 g/ml	ND	ND	1.04–1.07 g/ml	1.1 g/ml	1.16–1.28 g/ml
Appearance by electron microscopy	Cup shape	Irregular shape and electron-dense	Bilamellar round structures	Round	Irregular shape	Heterogeneous
Sedimentation	100,000 ×g	10,000 ×g	160,000–200,000 ×g	100,000–200,000 ×g	175,000 ×g	1200 ×g, 10,000 ×g or 100,000 ×g
Lipid composition	Enriched in cholesterol, sphingomyelin, and ceramide, contain lipid rafts, expose phosphatidylserine	Expose phosphatidylserine	Enriched in cholesterol and diacylglycerol, expose phosphatidylserine	ND	Do not contain lipid rafts	ND
Main protein markers	Tetraspanins (CD63, CD9), Alix, and TSG101	Integrins, selectins, and CD40 ligand	CRI and proteolytic enzymes, no CD63	CD133, no CD63	TNFR1	Histones
Intracellular origin	Internal compartments (endosomes)	Plasma membrane	Plasma membrane	Plasma membrane	Internal compartments?	ND

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literature are not necessarily induced by exosomes alone. Nevertheless, EVs that originate from MVB and are released after fusion of the MVB with the plasma membrane are considered as exosomes.

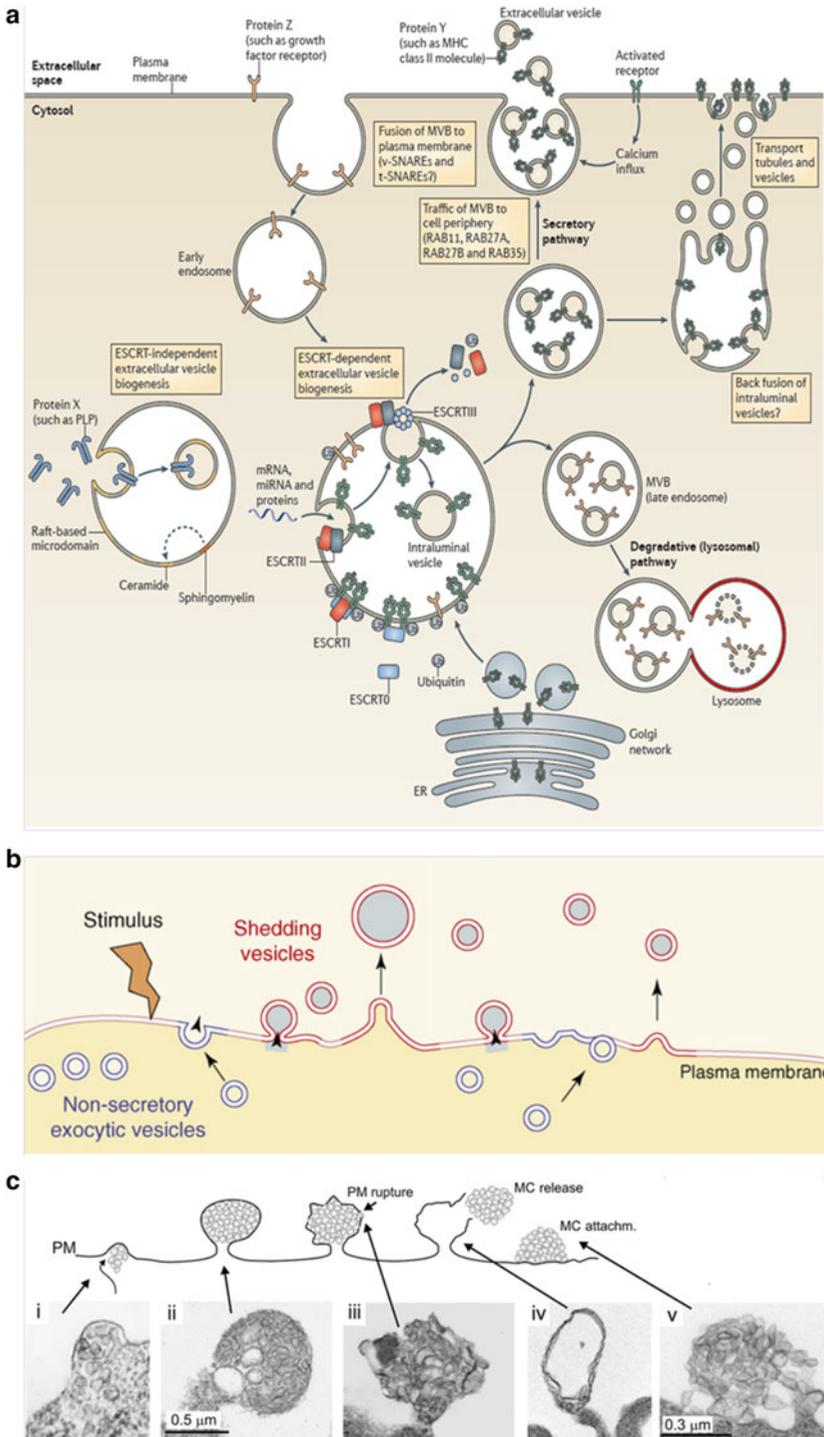
### 3 Biogenesis of EVs

Various types of EVs have been identified and the same cell can produce multiple species of secreted vesicles. These different types of vesicles are generated at distinct subcellular locations and exhibit common as well as distinct key features (Fig. 1 and Table 1). With respect to their biogenesis, mainly three types of EVs have been investigated, namely, exosomes, microvesicles (Fig. 1), and microvesicle clusters (MC). Hallmarks of exosome biogenesis are first an endocytic event at the plasma membrane [5, 6], and, after the maturation of early endosomes to the late endosomes [12], the



**Fig. 1** Overview of multiple EV species and their subcellular origin. Vesicle trafficking between various subcellular compartments represents a fundamental cellular mechanism mediated through carrier vesicles which remain intracellular. On the other hand, cells generate vesicles destined for secretion. Various types of secreted vesicles exist, and they may differ in respect of their subcellular origin, their biogenesis pathway, their cargo uploading mechanisms, and their molecular composition. In addition they can differ in size and various other biophysical and biochemical characteristics. In general, one and the same cell can generate and secrete multiple EV species like vesicles, exosomes, membrane particles, or exosome-like vesicles. [Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology, They et al. [53], copyright 2009]

formation of intraluminal vesicles (ILVs) by inward budding of the endosomal membrane, which gives rise to multivesicular bodies (MVBs) [13] (Fig. 2a). The process of MVB biogenesis is mediated by at least two distinct pathways and involves the sorting of various molecules into ILVs. The first pathway leading to MVB formation requires the *endosomal sorting complex required for transport* (ESCRT). This multimolecular machinery is consistent of ESCRT0, ESCRTI, ESCRTII, and ESCRTIII and is recruited to the endosomal membrane where the individual steps of ILV biogenesis are orchestrated [14]. This involves the recognition of ubiquitinated cargo proteins by ESCRT0, ESCRTI, and ESCRTII and the invagination of the late endosomal membrane mediated by ESCRTI and ESCRTII, a process facilitated through curvature-inducing factors [14, 15] (Fig. 2a). Recruitment of ESCRTIII to the site of membrane invagination occurs through binding to ESCRTII and leads to the deubiquitination of cargo proteins, the promotion of vesicle abscission, and thereby to the generation of ILVs [15, 16]. The second pathway of MVB formation is independent of the ESCRT machinery and is based on the specific lipid composition of the endosomal membrane. Raft-based microdomains are present on the limiting plasma membrane of endosomal compartments and contain high amounts of sphingolipids which represent substrates for the neutral sphingomyelinase2 (nSMase2) [17–20]. At the endosomal membrane, nSMase2 is able to convert sphingolipids to ceramide which in turn induces coalescence of microdomains into larger structures thereby promoting domain-induced budding and formation of ILVs [18]. Following the formation of MVBs, they are either destined for the degradative or the secretory pathways, which are both governed by Rab GTPases [17]. While Rab7 can mediate the degradation through the fusion of MVBs with lysosomal compartments [17], several other Rab proteins like Rab5b, Rab9a, RAB27a, RAB27b, and Rab35 were reported to be crucial for intracellular MVB trafficking and secretion events [21, 22]. The final release of ILVs occurs upon MVB fusion with the cellular plasma membrane, a process which is yet not well investigated but probably mediated, at least in part, by *soluble N-ethylmaleimide-sensitive factor attachment protein receptors* (SNAREs), like the vesicle-associated membrane protein (VAMP) TI-VAMP/VAMP7 [23]. Once the ILVs are secreted, they are termed exosomes. Until today they represent the only known type of EVs of endosomal origin (Fig. 2a). The capacity to secrete exosomes differs from cell type to cell type and can occur on a constitutive or inducible basis. For example, dendritic cells (DCs) [24] and macrophages [25] secrete EVs on a constitutive bases, while mast cells [26] or T cells [27] have to be activated. In addition, the release of exosomes has been described for DCs [28, 29] and for B cells [30] upon interactions with T cells. In tumor



**Fig. 2** Biogenesis and secretion of exosomes, microvesicles, and microvesicle clusters. **(a)** Exosomes, 40–100 nm in diameter, are generated by formation of intraluminal vesicles (ILVs) in an ESCRT- or sphingomyelinase-dependent manner into an endosomal compartment or MVB. These structures can enter either the degradative or the secretory route. MVBs destined for exosome generation follow the secretory

cells genotoxic stress leads to an increased activation of p53 transcription factors and, among other changes, to an enhanced p53 and tumor suppressor-activated pathway 6 (TSAP6) expression, which mediates augmented exosome secretion [31, 32].

The biogenesis of microvesicles differs considerably from that of exosomes; however, much less is known about the cellular processes leading to their generation. The formation of microvesicles occurs at the cellular plasma membrane [33]. Prior to their shedding, cytoplasmic protrusions are generated by the cell, which undergoes fission events, and finally microvesicles pinch off the cellular membrane [34]. The mechanisms underlying these shedding events are not well elucidated yet; however, microdomain-induced budding processes seem to be involved in these secretion events [35]. Despite the fact that microvesicles can be generated by resting cells, stimulation events leading, e.g., to increased intracellular calcium levels result in cellular membrane remodeling and an enhanced microvesicle secretion [34, 36]. Furthermore it has been reported that microvesicle secretion can be stimulated using phorbol esters [37] (Fig. 2b).

Microvesicle clusters (MC) have been described first by Muratori et al. in 2009. In this study, it was reported that stimulation of an HIV Nef-inducible Jurkat cell line, or stimulation of Jurkat cells with PHA or PMA, leads to the formation and secretion of MC. In contrast to exosomes or microvesicles, MC were composed of a large number of individual microvesicles (60–80 nm) and had a size of ~5–800 nm. The secretion process of MC, however, differed considerably from the aforementioned mechanisms. The budding event seemed to be initiated by the recruitment of small vesicles from the cytoplasm to the cellular membrane, which

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**Fig. 2** (continued) pathway, translocate to the cellular periphery where they fuse with the plasma membrane, and release their ILVs into the extracellular space. Once secreted, ILVs are termed exosomes. MVBs that enter the degradative pathway fuse with lysosomes where their cargo is degraded, a process of critical importance for the attenuation of signaling events. [Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology, Robbins and Morelli [17], copyright 2014.] **(b)** Microvesicles, 100–1000 nm in diameter, are generated at the plasma membrane in a constitutive manner or upon stimuli. Nonsecretory exocytic vesicles (*blue*) seem to release their vesicular content at the sites of microvesicle generation thereby contributing to the microvesicle biogenesis. In addition, membrane-remodeling events take place leading to the generation of a plasma membrane composition distinct from surrounding areas, but similar to those of exocytosed vesicles (*red*). In the final stage, these areas bud off from the plasma membrane giving rise to secreted microvesicles. The mechanisms underlying the proposed membrane-remodeling or membrane-sorting events remain to be elucidated. **(c)** Microvesicle clusters are 5–800 nm in diameter consisting of smaller vesicle and tubules of about 60–80 nm in diameter. Prior to their secretion, microvesicles accumulate beneath the cell membrane where they bulge the plasma membrane until it ruptures releasing the microvesicle clusters. They remain coherent and attach as vesicle aggregate to the cell surface of bystander cells. *PM* plasma membrane, *MC attachm.* microvesicle cluster attachment

subsequently bulged into a ball-like structure and finally ruptured to release MC into the extracellular space [2]. After their secretion, these clusters remained stable and attached as whole complexes to the surface of bystander cells [2]. Furthermore it was reported that despite the unconventional release mechanism, the identified microvesicles share a number of biophysical and biochemical characteristics with exosomes. For example, they floated in a sucrose gradient at a density of 1.13–1.19 g/ml and contained high amounts of CD63. Further evidence was provided that the described secretion process may be ERK1/2 dependent; however, the underlying molecular mechanisms leading to the biogenesis and secretion of microvesicle clusters remained unclear [2] (Fig. 2c).

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## 4 Molecular Composition of EVs

During their biogenesis and prior to their secretion, various molecules are uploaded into the lumen of EVs. These molecules include various types of proteins like major histocompatibility complex (MHC) class I and II molecules, costimulatory molecules, tetraspanins, proteases, cytokines, growth factors, and death ligands. In addition, EVs can also contain genetic information like mRNA and miRNA molecules and also active enzymes. Even the presence of retrotransposon elements has been reported [38]. Despite this diverse repertoire, the molecular profile of EVs can be generally divided into two groups. The first group includes proteins relevant for the individual EV biogenesis pathways and for EV secretion [39]. These factors are found in EVs across various cell types and include, e.g., TSG101, Alix, or Rab proteins. The second group involves molecules that are specifically uploaded into vesicles by certain cell types thereby assigning EVs a characteristic cell-type fingerprint [39]. These factors involve, e.g., cytokines; surface receptors, like B cell or T cell; or signaling molecules and enzymes.

Apart from this classification, the selective sorting of molecules into EVs is commonly observed. For example, an accumulation of specific factors was observed, while others were barely detectable despite being present in parental cells [40–44]. Little is known about these selective shuttling mechanisms. However, ubiquitylation may represent a sorting signal, targeting proteins to late endosomes, where they are captured and transported into ILVs through the ESCRT machinery [17] (Fig. 2). In addition, it has been reported that plasma membrane anchor tags such as myristoylation, prenylation, and palmitoylation WHICH can target proteins to the site of vesicle budding and into EVs [45]. Hence, in addition to ubiquitylation, posttranslational lipid modifications seem to play a role in shuttling of proteins into EVs. Furthermore, CD43, a transmembrane sialoglycoprotein, has been implicated in

mediating the selective protein upload into EVs [45, 46]. For example, in breast cancer cells, CD43 interacts with DICER which is uploaded into EVs [46]. Upon CD43 silencing, however, DICER levels significantly decreased in breast cancer EVs, while they increased in the nucleus and in the cytoplasm [46].

MicroRNAs on the other hand seem to be specifically uploaded into EVs, at least in part through shuttling sequences. It has been reported that sumoylated heterogeneous nuclear ribonucleoprotein (hnRNP) A2B1 specifically binds to miRNAs containing these shuttling motifs, leading to their uploading into ILVs [47]. In addition, posttranscriptional modifications of miRNAs through nontemplate nucleotide additions mediate their uploading into EVs [48]. Accordingly, it has been reported that 3'-end uridylylated miRNAs appear to be enriched in EVs, while 3'-end adenylated miRNAs seem to be enriched in their parental cells [48]. Furthermore, the cellular expression level of individual miRNAs and their cognate target mRNA transcripts seem to influence the miRNA shuttling into EVs. In general, individual miRNAs are enriched in EVs in case of a high cellular miRNA/target mRNA expression ratio (i.e. high cellular expression of individual miRNAs and low cellular expression of their cognate target mRNA transcripts) [49]. Conversely, a low cellular miRNA/target mRNA expression ratio led to a decreased shuttling of miRNAs into EVs [49]. The detailed molecular mechanisms, however, remain to be elucidated.

Apart from these studies investigating specific EV uploading mechanisms, it has been suggested recently that the molecular cargo of EVs is not only functional in recipient cells but also in the vesicles itself. Hence, not only mature miRNAs but also pre-miRNA transcripts are present in EVs of breast cancer cells along with key components of the miRNA biogenesis machinery, i.e. DICER, TRBP, and AGO2 [46]. Remarkably, especially DICER and TRBP were functional in EVs, and the coordinated interaction of all factors mediated the cell-independent miRNA maturation with gene-silencing activity in recipient cells [46]. Importantly, these processes were observed *in vivo* using circulating EVs from breast cancer patients [46].

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## 5 EVs in Physiological and Pathophysiological Conditions

In physiological and pathophysiological conditions, EVs act as multimolecular messengers by an autocrine and paracrine manner and proximal or distal from their site of origin. Long-distance EV transfer is very likely as EVs have been detected in various bodily fluids including peripheral blood, cerebrospinal fluid, urine, and saliva [50]. They are able to extravasate from the blood stream into various tissues like lungs or bones [51]. In addition, they seem to

be able to pass the blood-brain barrier and to enter the brain, mediating gene silencing in neurons, microglia, and oligodendrocytes [52]. Basically, the interaction of EVs with recipient cells is mediated by:

1. A direct binding of EV lipids and/or transmembrane proteins with cellular surface proteins and/or receptors.
2. Membrane fusion events and integration of EV membrane-bound factors and lipids into the cellular membrane.
3. A cellular uptake of EVs by macropinocytosis and a subsequent fusion with other endosomal structures.
4. A cellular uptake and release of their molecular cargo into the cellular interior [53].

These uptake mechanisms are not mutually exclusive but reflect complementary processes allowing the transfer of proteins, mRNAs, and miRNAs to specific locations in recipient cells [54–56]. Hence, EVs can exert pleiotropic functions on multiple biological processes. For example, under physiological conditions EVs may contribute to blood coagulation [57], wound healing [58], or the regulation of immune responses against the fetus during pregnancy [59].

However, EVs also play major roles under pathophysiological conditions like in autoimmune [60] and inflammatory diseases [61], infectious diseases [62], or cancer [62]. Especially in cancer various and partly contradicting roles have been attributed to EVs. In general, cancer cells are able to stimulate themselves and/or modulate their microenvironment to favor tumor growth and progression and to suppress immune reactions [63]. Recently, various studies point at the relevance of EVs in mediating these effects. For example, it has been reported by Al-Nedawi et al. that glioma cells harboring the EGFRvIII mutation upload this mutated protein into EVs and transfer it to cancer cells lacking the EGFRvIII receptor. As a consequence, transforming signaling pathways like MAPK and Akt pathways have been activated in recipient cells and an altered EGFRvIII regulated gene expression was detectable, which finally led to morphological transformations and to an increased anchorage-independent growth capacity [55]. Peinado et al. demonstrated that melanoma exosomes are able to promote the metastatic phenotype of primary tumors through the education of bone marrow progenitor cells. This process is mediated by the exosomal transfer of the receptor tyrosine kinase MET to bone marrow progenitor cells. Furthermore, melanoma exosomes induced vascular leakiness at pre-metastatic sites and altered bone marrow progenitor cells toward a provasculogenic phenotype [51]. In addition to their crucial contribution to cancer growth and progression tumor, EVs are also involved in suppressing immune reactions. Accordingly, malignant melanoma, for example, exploits a mechanism referred

to as tumor counterattack. Andreola et al. reported that melanoma cells are able to express and shuttle membrane-bound FasL into MVBs, while this death ligand was not detectable on the melanoma cell surface [64]. Upon secretion these EVs could induce Fas-mediated T cell apoptosis in a paracrine manner [64], while the secreting melanoma cells could escape an autocrine FasL-vesicle-induced cell death [65, 66].

On the other hand, EVs secreted by immune cells also play a role in pathophysiological conditions. Similar to EVs from cancer cells, multiple features have been attributed to immune cell EVs. This involves their role as mediators of the immune responses. In this context most studies focused on the analysis of DC EVs [56, 67–69]. Initially it was shown by Raposo et al. that EVs derived from B cells carry MHC class II molecules that can induce antigen-specific CD4<sup>+</sup> T cell responses *in vitro*, although the efficiency of antigen presentation was estimated to be inferior as compared to B cells [70]. Zitvogel et al. then demonstrated that DCs also secrete EVs which harbor functional MHC class I, MHC class II, and T cell costimulatory molecules. In this study it was also reported that EVs derived from mouse DCs pulsed with tumor peptides caused tumor growth arrest and tumor eradication in 40–60% when injected in murine tumor models. These effects were described to be mediated by T cells [24]. In a subsequent study, Wolfers et al. extended these findings to tumor cell-derived EVs and demonstrated that they harbor tumor antigens. Upon injection of tumor EVs in a murine tumor model, significant antitumor effects were reported. However, tumor EVs could not directly induce CD8<sup>+</sup> T cell activation *in vitro* despite the presence of MHC class I. Instead, CD8<sup>+</sup> T cell activation was only observed when tumor EVs were first loaded onto DCs. The injection of tumor EV loaded DCs into murine tumor models and then led to a significant tumor growth delay and a curing rate of 33% [8].

Based on these and other findings, phase I clinical trials were carried out which recently have been completed [71, 72]. In one of these studies, DC EVs have been loaded with melanoma-associated antigen (MAGE) A3 peptides and were used for the vaccination of patients bearing MAGE-A3<sup>+</sup> advanced melanomas [72]. The vaccination of 15 melanoma patients finally revealed one individual with an objective response, one with a minor response, and two patients with disease stabilizations. These observations were partly associated with tumor regressions. On the cellular level however, no MAGE-A3-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses could be determined, but, instead, an enhanced natural killer (NK) cell effector function was reported for eight patients. These surprising results were then further investigated and it has been shown by Viaud et al. that DC EVs can promote the proliferation and activation of NK cells in a natural killer group 2 member D (NKG2D) and interleukin-15 receptor  $\alpha$  (IL-15R $\alpha$ )-dependent

manner. This suggested a mechanistic explanation on how DC EVs can induce tumor regression *in vivo* [73]. In addition it was recently reported that mouse DC EVs, independently of antigen presentation events, can directly induce apoptosis in various tumor cell lines in a caspase-dependent manner. This process was described to be mediated by tumor necrosis factor (TNF), FasL, and/or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expressed in the correct orientation on the DC EV surface [74].

While numerous studies have concentrated on the role of EV in cancer, rather little is known about the role of EV in HIV infection. Similar as in the cancer field, there are no confirmed data on the relative concentration of HIV-specific EV in circulation nor where they originate. The first report came in 2009, when Muratori and colleagues reported that HIV-infected PBMC *in vivo* and *in vitro* secrete large amounts of MC in a Nef-dependent manner. At that time, the results were hampered by the fact that there was no obvious function of these vesicles, as there was little known about vesicle functions in general. In 2013 the same group published the molecular mechanism by which Nef induces the release of vesicles [75]. Nef seemed to target and associate with proteins of an integrin-associated signaling complex that included ADAM10 and ADAM17 proteases. The Nef-induced vesicles uploaded these ADAM proteases and could stimulate the release of TNF in target cells. Supporting these findings, vesicles purified from the plasma of HIV-infected individuals, but not of healthy controls, contained both ADAM proteases and Nef. As TNF is the most important activator of HIV replication *in vivo*, these results provide a first logical explanation of why these vesicles are relevant in HIV infection and pathogenesis. Supporting this conclusion, the group of M. Federico has shown that HIV-/Nef-induced vesicles can stimulate resting T cells to replicate HIV in an ADAM17-dependent manner [76, 77]. This suggested that HIV-/Nef-induced vesicles are potentially critical to enable HIV replication in the predominantly resting T cell compartment.

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## 6 EVs as Novel Biomarkers

Based on their molecular cargo, which appears to be altered in pathophysiological conditions, EVs have been suggested as biomarkers for diagnostic purposes. Accordingly, it has been reported that a protein signature present in circulating exosomes of melanoma patients was identified which could be linked to distinct clinical tumor stages. Skog et al. demonstrated that circulating EVs derived from glioblastoma patients contained mRNA transcripts reflecting mutations typically found in these tumors. Hence, the authors reported that EGFRvIII transcripts were detectable in 28% of the glioblastoma patients analyzed. Furthermore they deter-

mined the presence of mutated mRNA transcripts in two patients in which the mutation was not detectable in the primary tumor sample thereby confirming the general genetic heterogeneity of tumor cell populations. In addition, protein signatures, mutated mRNA transcripts, and aberrant miRNA expression patterns have been used to characterize various tumor types. Taylor et al. demonstrated that miRNAs are readily detectable in circulating EVs derived from ovarian cancer patients using the miRNA microarray technology. The authors identified a set of eight miRNAs which was found to be significantly enriched in ovarian cancer patients compared to individuals with benign disease, while this miRNA signature was not detectable in circulating EVs derived from healthy individuals [78]. Furthermore, Ogata-Kawata et al. reported the identification of a signature consisting of seven miRNAs derived from circulating EVs of colorectal cancer patients. Compared to healthy individuals, this signature was significantly enriched in primary colorectal cancer patients. Upon surgical resection, however, these miRNAs were again significantly downregulated [79].

Taken together, these studies demonstrate the enormous potential of circulating EVs as easy accessible novel biomarkers for cancer and possibly other diseases. It is likely that they can also be used for the monitoring of disease progression or for the evaluation of therapy responses. In addition it is conceivable that circulating EVs from sensitive physiologic sensing systems like the immune system, but not from the limited number of residual cancer cells, may reflect another promising surrogate biomarker source for the detection of residual cancer cells and for the clinical classification, surveillance, and therapy once the molecular determinants have been identified.

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