Categories and Characteristics of Extracellular Vesicles

Subjects: Toxicology

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Extracellular vesicles (EVs) are heterogeneous small membrane structures that originate from plasma membranes. Although most EVs have a diameter of 50–200 nm, larger ones are also observed. Generally, particles up to a diameter of 1000 nm are regarded as EVs. They are typically isolated from the conditioned media of cultured cells. The contents of EVs include proteins, mRNA, microRNA (miRNA), and nucleic acids. Each vesicle performs a specific function in transferring biological material(s) to induce biological processes, such as replication, growth, apoptosis, and necrosis.

Extracellular vesicles

1. Introduction

Extracellular vesicles (EVs) are heterogeneous small membrane structures that originate from plasma membranes. Although most EVs have a diameter of 50–200 nm, larger ones are also observed. Generally, particles up to a diameter of 1000 nm are regarded as EVs [1][2]. They are typically isolated from the conditioned media of cultured cells. The contents of EVs include proteins, mRNA, microRNA (miRNA), and nucleic acids [3]. Each vesicle performs a specific function in transferring biological material(s) to induce biological processes, such as replication, growth, apoptosis, and necrosis [4][5][6]. They are also required for cell-to-cell communication to maintain a normal homeostatic state [7]. EVs can be used as cargo carriers in physiological or pathological conditions and are considered biomarkers representing altered normal physiological states [8]. Based on these characteristics, EVs can be used for diverse purposes, from cosmetic to therapeutic applications. The main advantage of EVs is their limited adverse effects when used for therapeutic or cosmetic purposes, because they are composed of cell-derived materials and because of their potential for targeted cell delivery [9]. In addition, compared to cells, they are easier to store and transport.

The first EV that was identified is involved in transferrin receptor elimination, which plays a role in the maturation of reticulocytes, as reported by Harding et al. in 1983 [10]. The authors demonstrated the release of multi-vascular endosomes from the plasma membrane by exocytosis in rat reticulocytes. EVs can be found in all types of body fluids, such as plasma [11], bile [12], breast milk [13], urine [14], ascites, and cerebrospinal fluid [15]. Thus, these vesicles show potential for revealing abnormal conditions in various organs. EVs from the blood can be used to detect inflammation or an aberrant immune system, whereas those from breast milk can be utilized to diagnose breast conditions [16]. Halvaei et al. reported that EVs can be used for the diagnosis of various cancers using cancer-specific miRNAs [17].

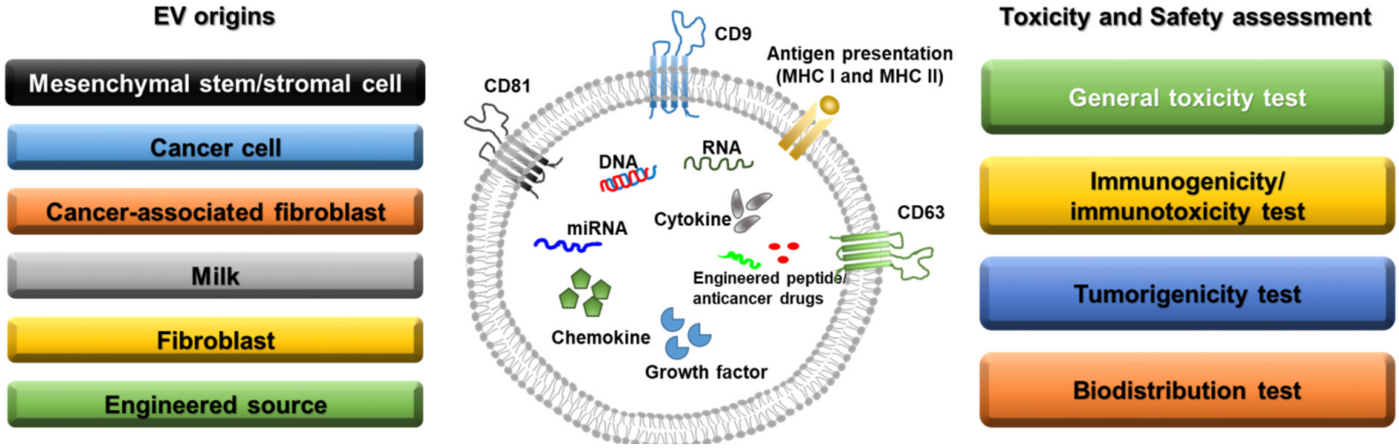

Figure 1. Sources of extracellular vesicles and toxicity/safety assessments. EVs can originate from mesenchymal stem/stromal cells, cancer cells, cancer-associated fibroblasts, milk, normal fibroblasts, and engineered cells. EVs have a lipid bilayer and can contain transmembrane proteins, antigen presentation proteins, DNA, RNA, miRNA, cytokines, chemokines, growth factors, engineered peptides, and anticancer drugs. Before clinical studies of EVs, general toxicity, immunogenicity, tumorigenicity, and biodistribution tests should be performed in preclinical studies depending on the source of the EVs.

2. Mesenchymal Stem/Stromal Cell-Derived EVs

Mesenchymal stem cells (MSCs) have been widely investigated as therapeutic options for various diseases, including graft versus host disease [18] and cardiac [19], neurological [20], and orthopedic [21] disorders. MSCs mainly reduce inflammation, enhance progenitor cell proliferation, improve tissue repair, and decrease infection. According to the U.S. Food and Drug Administration, over 35,000 clinical trials have been conducted in the USA, France, and Canada on cell-based therapies [22]. However, despite the potency of MSCs, numerous side effects, such as tumorigenesis and immunogenicity, have been reported in preclinical and clinical trials [23]. In addition, there are some limitations to the generation and storage of MSCs intended for use as therapeutics [24]. To maintain the efficacy of MSCs and overcome these drawbacks, MSC-derived EVs have received attention as therapeutic agents that can be used for renal protection and to manage various disorders, including cardiac dysfunction, myocardial infarction, stroke, hepatic fibrosis, and vascular proliferative diseases [25][26][27][28][29][30]. In particular, MSC-derived EVs are composed of factors such as cytokines, growth factors, RNA, and miRNAs, which originate from MSCs and thus exert similar effects to those of MSCs [31].

The effects of MSC-derived EVs in cancer cell biology are controversial [32]. Many groups have reported that MSC-derived EVs increase cancer proliferation, invasion, and metastasis. Bone marrow MSC-derived EVs were reported to stimulate the hedgehog signaling pathway in the growth of osteosarcoma and gastric cancer [33], whereas adipocyte MSC-derived EVs promoted breast cancer cell growth via activation of the Hippo signaling pathway [34]. However, adipose MSC-derived EVs inhibited prostate cancer growth by delivering miR-145 [35].

MSC-derived EVs of different origins show different effects in various diseases with divergent mechanisms. Further information is provided in **Table 1**.

Table 1. MSC-derived EVs of different origins with different effects in various diseases.

EV Origin	Target Disease	Mechanisms & Characteristics	Animals Used	Ref. No.
Bone marrow-derived mesenchymal stem cells	Wound healing	Promoting M2 polarization of macrophages miR-223 wound healing by transferring EV-derived microRNA	6–8 weeks old female C57BL/6 J mice	[28]
Mesenchymal stem cells	Alzheimer's disease	Evaluating mouse cognitive deficits Stimulating neurogenesis in the subventricular zone Alleviating beta amyloid 1−42-induced cognitive impairment	7–8-week-old C57BL/6 mice	[36]
Adipose tissue-derived mesenchymal stem/stromal cells	Cisplatin-induced acute kidney injury	Protection of animals from death due to cisplatin-induced acute kidney injury	6-week-old male Sprague Dawley rats	[37]
Bone marrow-derived mesenchymal stem cells	Pilocarpine-induced status epilepticus	Neuroprotective and anti-inflammatory effects Increasing normal hippocampal neurogenesis and cognitive and memory function	6–8-week-old C57BL/6 mice	[38]
Mesenchymal stromal cells	A newborn rat model of bronchopulmonary dysplasia (BPD) induced by 14 days of neonatal hyperoxia exposure (85% O_2)	Protecting from apoptosis, inhibiting inflammation, and increasing angiogenesis Preventing the disruption of alveolar growth, increasing small blood vessel number, and inhibiting right heart hypertrophy at P14, P21, and P56	Newborn rats	[39]
Embryonic mesenchymal stem cells	Critical-sized osteochondral defects (1.5 mm diameter and 1.0 mm depth)	Complete restoration of cartilage and subchondral bone	8-week-old female Sprague Dawley rats	[40]
Umbilical cord mesenchymal stem cells	Perinatal brain injury (hypoxic-ischemic and inflammatory with lipopolysaccharide)	Inhibiting the production of pro-inflammatory molecules and preventing microgliosis in rats with perinatal brain injury	2-day-old Wistar rat pups	[41]

EV Origin	Target Disease	Mechanisms & Characteristics	Animals Used	Ref. No.
		Decreasing TNF-α and IL-1β expression in injured brains		
Umbilical Cord mesenchymal stem cells	CCl_4-induced liver injury	Suppressing the development of liver tumors Inhibiting oxidative stress in liver tumors Reducing oxidative stress and inhibiting apoptosis in liver fibrosis	4–5-week-old female BALB/c mice	[42]
Mesenchymal stromal cells	Cavernous nerve injury (CNI)	Enhancing smooth muscle content and neuronal nitric oxide synthase (nNOS) in the corpus cavernosum Improving erectile function after CNI Increasing penile nNOS expression and alleviating cell apoptosis	10-week-old male Sprague Dawley rats	[43]
Mesenchymal stem cells	Traumatic Brain Injury (TBI) with a 20 mm cylindrical impactor hemorrhaged over 12.5 min using a Masterflex pump	Lowering Neurological Severity Score (NSS) ($p < 0.05$) during the first five days post-injury Faster full neurological recovery	35–45 kg female Yorkshire swine	[44]
Mesenchymal stem cells	UV-irradiated skin	Attenuating UV-induced histological injury and inflammatory response in mouse skin Preventing cell proliferation and collagen deposition in UV-irradiated mouse skin Increasing antioxidant activity	newborn and adult Kunming mice	[45]

onditions, depending on whether they will or will not be used for cancer treatment. Cancer-derived EVs can be detected in all bodily fluids, such as the blood, saliva, urine, and bile [14][46][47]. Based on this characteristic, many scientists have attempted to develop cancer-derived EVs as noninvasive biomarkers for diagnosing cancer in early stages of disease [48]. Specifically, cancer-derived EVs contain various biomarkers, such as miR-17, miR-19a, miR-21, miR-126/miR-141, miR-146, and miR-409, which have a range of effects on tumor growth and can be used for cancer diagnosis and prognosis [49][50][51][52].

The extracellular matrix, cancer-associated fibroblasts, inflammatory immune cells, and tumor-associated vasculature are components of the tumor microenvironment, which can be a major source of tumor-derived EVs [53][54]. Cancer-associated fibroblasts are among the major sources of tumor EVs with different effects before and after chemotherapy [55]. In particular, following chemotherapies, EVs derived from cancer-associated fibroblasts were shown to promote the chemoresistance and proliferation of colorectal and breast cancers [56][57]. EVs from tumors under hypoxic conditions enhanced angiogenesis and metastasis by modulating the microenvironment [58].

Because tumor-derived EVs contain important components, including nucleic acids and oncogenic proteins, they can be used as biomarkers for diagnosis, prognosis, therapeutic response prediction, and targeted therapy [4].

4. EVs as Anticancer Drug Delivery Agents

Jang et al. reported that EV-delivered doxorubicin had a greater effect on reducing tumor size than administration of pure doxorubicin in a colon adenocarcinoma xenograft model [59]. Furthermore, the use of an α_v integrin-specific iRGD peptide with EVs to deliver doxorubicin showed promising anticancer effects in an α_v integrin-positive breast cancer model [60]. Following the investigation of paclitaxel using an EV delivery system in a tumor xenograft model, Kim et al. reported its anticancer effects in vitro and in vivo [61]. Another group reported that EV-encapsulated paclitaxel directly targeted cancer stem cells that exhibited anticancer drug resistance [62]. EVs loaded with the antitumor drugs withaferin A or celastrol were administered to a human lung cancer xenograft mouse model, in which they showed anticancer effects [63][64]. Engineered EVs with superparamagnetic-conjugated transferrin have been shown to target tumor cells and reduce tumor growth in vivo [65]. In addition, an engineered anti-epidermal growth factor receptor nanobody fused with the EV anchor signal peptide glycosylphosphatidylinositol showed direct activity against tumor cells positive for epidermal growth factor receptor-positive tumor cells [66].

Because of their stability in biological fluids, EVs can escape from lung clearance and cross the blood-brain barrier [67][68], thus easily reaching tumors in various organs such as the liver, brain, and breast. Based on these characteristics, EVs can be used for cancer-targeting therapies.

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