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EXTRACELLULAR VESICLES: CLASSIFICATION, FUNCTIONS AND CLINICAL RELEVANCE

A. V. Oberemko
A. G. Popandopulo
SO «Gusak Institute of Urgent and Recovery Surgery» of the Ukrainian National Academy of Medical Sciences», Donetsk

E-mail: a.oberemko@mail.ru

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This review presents a generalized definition of vesicles as bilayer extracellular organelles of all cellular forms of life: not only eu-, but also prokaryotic. The structure and composition of extracellular vesicles, history of research, nomenclature, their impact on life processes in health and disease are discussed. Moreover, vesicles may be useful as clinical instruments for biomarkers, and they are promising as biotechnological drug. However, many questions in this area are still unresolved and need to be addressed in the future.

The most interesting from the point of view of practical health care represents a direction to study the effect of exosomes and microvesicles in the development and progression of a particular disease, the possibility of adjusting the pathological process by means of extracellular vesicles of a particular type, acting as an active ingredient. Relevant is the further elucidation of the role and importance of exosomes to the surrounding cells, tissues and organs at the molecular level, the prospects for the use of non-cellular vesicles as biomarkers of disease.

Key words: exosomes, microvesicles.

The growing interest in studying extracellular structures, especially exosomes and microvesicles (microparticles), is determined by the understanding their significance for surrounding cells, tissues and organs as cell-to-cell signal transmission mediators. The results of recent cell biology and biochemistry research works give a reason to consider vesicles of 20 nm–1 μm in diameter formed through exocitosis of multivesicular bodies or direct plasmalemma budding, as bilayer extracellular organelles of all cellular forms of life: not only eu-, but also prokaryotic [1].

Classification. Due to a wide diversity of research field disciplines and different classification principles the common solution for cellular vesicles classification is still not obtained [2]. Currently there are two or six types of extracellular eukaryotic vesicles: 1) exosomes; 2) microparticles (microvesicles); 3) membrane particles; 4) apoptotic vesicles (apoptotic bodies); 5) ectosomes; 6) exosome-like vesicles. It was suggested to neglect the last two types in the work [3] because of insufficient evidence of their existence: ectosomes require size clarification, and exosome-like vesicles under transmission electronic microscopy turned out to be damaged or destroyed blisters. Regarding the fact that microcells density and composition are understudied, and allowed diameter range of microparticles includes exosome diameter (30–100 nm), exosomes and microvesicles can be combined into one type. Membrane particles described only for epithelial cells and having the size (50–80 nm, 600 nm) like microparticles (20–1 000 nm) can be regarded as a special case for microparticles and exosomes. Thus only two types of extracellular vesicles can be definitely distinguished: 1) exosomes and microparticles (microvesicles); 2) apoptotic bodies. The last ones are different from the first two for large size (1 000–5 000 nm), heterogeneous morphology, composition (existing histones and genomic DNA fragments) and origin (plasma membrane or endoplasma reticulum).

Exosome and microvesicles research history traces back to the 40's of the last century, when Erwin Chargaff studied the biological significance of thromboplastic protein to understand not only the normal blood clotting process, but also disorders like haemophilia [4]. He discovered that agents, transforming prothrombin into thrombin, are lipoproteins sedimentating in a high field of centrifugal forces (31, 000 g), and remaining in a solution under low centrifugal forces (5,000 g). His experiments showed that decellularised plasma

contains a subcellular factor, which enables blood clotting. After more than 20 years a subcellular plasma fraction was studied and then its composition was identified — small particles (microparticles) with coagulative properties similar to the blood platelet factor-3 and called "thrombocyte trash", because their formation was observed at platelet storage and it seemed that microparticles are platelet desintegration products [5]. Already 70 years have passed since E.Chargaff works till foundation of International Society for Extracellular Vesicles (ICEV) in 2011 [6]. Placelet vesicles have been studied [7], and in 1975 extracellular glomerular microparticles were described [8].

In 1987 the research group led by Rose Johnstone introduced the "exosomes" term (gr. *exo* — outside, off and *soma* — body) to describe extracellular blisters isolated from conditioned cultural medium of sheep's reticulocytes [9]. These vesicles had characteristic properties of reticulocytes plasma membrane (acetylcholinesterase, transferrine receptor etc.) without cytosolic enzymes or their low number that indicated the loss by reticulocytes of their exosome proteins during maturation. The research workers suggested that vesicles externalization (external response process) may be disposal from old membrane proteins during reticulocytes differentiation into erythrocytes. Later exosomes were discovered not only *in vitro*, but also in whole blood of sheep, pigs, rats, rabbits [10] and chickens as well [11], what indicated that exosome generation is a natural phenomenon not only for mammalian red blood cells, and a little later — not only for reticulocytes.

Generation ways. At the same time it was suggested that membrane proteins mechanism of packing into exosomes repeats the way, which was described for transferring receptor conjugated with colloidal gold, namely: endocytosis of specified membrane proteins followed by generation of microvesicular bodies due to further invagination [9, 12]. These multivesicular endosomes release content (exosomes) into the environment through the fusion with plasmalemma and that is currently a classic exosome generation way. The direct exosome and microvesicle generation occurs by budding from the plasma membrane [3].

It is assumed [13] that microparticles develop when asymmetric lipid distribution between internal and external plasma membrane layers gets disturbed. Generally phosphatidylserine is located on the internal monolayer. When cells undergo activation or apoptosis the phosphatidylserine externalization is the earliest indicator of the process. That is why microvesicles were separated into a single group as blisters thrown-in by stressed cells plasma membrane. However, the existence of microvesicles without phosphatidylserine (from epithelial cells) put this definition into question and engaged the researches to introduce a new extracellular vesicles type — "membrane particles". Besides, the name "membrane particles" is not adequate as exosomes with microvesicles are such as well for being of membrane origin. Actually "membrane particles" are microparticles without phosphatidylserine, and microparticles are the same exosomes, but emitted by stressed cells.

The research group led by Rose Johnstone also discovered that most of transmembrane proteins (anion carriers) remain in full in mature red blood cells — that is an indicator of high selectivity of specific protein group recognition mechanism. It is still not investigated how particular proteins and RNA are chosen for externalization.

Interaction with cells. Apoptotic bodies as products of programmed cell death rather quickly undergo phagocytosis [14]. Whereas exosomes can be captured by surrounding cells and transmit stored information that is approved on internalization (capture) of exosomes with a green fluorescent mark [15] presumably provided involving specific receptor molecules of cell surface. As it was shown [16, 17] internalization and exosome functionality can exploit heparan sulfate proteoglycans of cellular surface as endocytosis receptors of variable amount entering connective cells (lipoproteins, growth factors, morphogenes etc.).

Exosome modification by surrounding cells is also possible: particular exosome molecules may be a substrate for cell cytoplasm membrane enzymes of other type different from exosome source type. It was discovered that transferrine receptor of sheep's exosomes served as a substrate for a plasma membrane protease of human granulocytes — this way plasmalemma proteins having completed their task can leave a cell as part of exosomes and undergo lysis [18].

Composition. The concept stating that exosomes and microvesicles can act as paracrine effectors is based on their ability to perform transport of bioactive molecules between cells: inside a particular microenvironment or distantly through inclusion into biological fluids (blood, lymph, amniotic fluid etc.). This way protein and RNA (mRNA, microRNA, ribosomal and other noncoding RNA) transfer [19] towards particular target-cells is provided. It consequently may result in activation, phenotypic modification and reprogramming of cell functions. As it was approved that

exosomes may sometimes include mitochondrial DNA [20] upon cancer cell origin — double-stranded genomic DNA fragments [21, 22], there is a theoretical possibility of a horizontal transporting of genes under their participation — the process of genetic code transfer to other non-descendant organism.

The antigens of a parent cell may serve to identify exosome and budding microvesicles. There is also a range of exosomal markers offered — membrane protein tetraspanins (though they are mainly considered as hemopoietic cell characteristics): CD9, CD63, CD81, CD82, CD89 [23]. Exosomes secreted by antigenpresenting cells like B-lymphocytes and dendrite cells demonstrate molecules of main histocompatibility complex of I and II class [24, 25], by mammary gland cells — milk fat globule membrane proteins [26]. Occurrence of heat shock proteins — hsp70 and hsp90 — is still considered as an exosomal quality [27] despite the fact that these proteins in exosomes were described for the first time in a close noncovalent bond with transferin receptors in maturating reticulocytes [28]. Heat shock proteins locate in cells and under non-stressful conditions help to split peptide bond between aminoacids in abnormal proteins and for newly synthesized proteins to correctly coagulate, besides, it is rather possible that they are characteristic for exosomes from any eukaryotic cells as well as cytoskeleton proteins, e.g. actin and tubulin (for bacteria — their homologs). Considering the fact that exosomes are hardly detached from microvesicles due to particular correspondence of their size, the above mentioned qualities can be present in microvesicles either.

Impact on surrounding cells, the functions and clinical relevance. The impact of exosomes and microvesicles on many biological processes, taking place under laboratory and living organism conditions, are currently studied. It is established that with their participation the management of vital functions of cells, tissues and organs is provided. Transferring regulatory molecules by exosomes is important for proliferation and differentiation of progenitor cells. For example, exosome executed transition of microRNA-122 between neighboring cells is essential (along with influencing cell metabolism and other functions) for slowing cell growth [29]. Exosomal microRNA generated form muscular threadworm tubes suppressed the synthesis of Sirt1 protein in myoblasts that contributed their differentiation into myocytes [30], and macrophage microvesicles induced the monocytes differentiation into tissue-specific macrophages [31].

It was shown that secretion of immunomodulatory placenta protein modifying maternal immunity is carried out by exosomes [32]. The microRNA transition is assisted by milk exosomes that is important for a baby immune system development [33]. Exosomes are also likely to take part in transferring interfering RNA (microRNA, short type RNA acting on *piwi*-type etc.) between animal kingdoms: floral microRNA was isolated from mice blood held on rice diet and then it was integrated into liver cells resulting in decreasing of low density proteins in plasma [34]. It was suggested that exosomes and telocyte gap junctions (particular type of very long stromal stem cells) are a part of a unique primitive nervous system [35] and this fact is rather controversial because exosome generation is characteristic not only for telocytes. Obviously it would be more correctly to say about exosomal transportation as a universal communication medium between cells.

For pathological processes development submicrone extracellular vesicles are of the same value as for normal organism functioning. Tumor exosomes launch malignant transformation of stem cells [36], suppress immune cells functions [37], may cause DNA damage by active oxygen forms and autophagia of surrounding cells [38], providing the generation of so called "cancer niches" and cancer progression. Virus afflicted cell exosomes may facilitate viral expansion by direct distribution of the virus [39] or defective genome products, for example as CD4+ of human lymphomaT-cells (*Hut*-78 line), which expressed functionally defective HIV-1 without releasing viral particles into the environment (*F*12 clone), and which induced activation and tolerance for HIV-1-replication of non-stimulated primary CD4+ T-human lymphocytes [40]. It was investigated that cardiomyocyte hypertrophy also runs under participation of exosomes transferring hypertrophy inductors (microRNA-21*) from cardiac fibroblasts to cardiomyocytes [41]. According to these data it may be suggested that exosomes as information carriers play a substantial role in the development of any disease.

As it was mentioned it can be concluded that exosomal impact on surrounding cells is determined by their content which is in its turn determined by source cells, and transportation is its main function.

The perspectives for clinical application of small vesicles (up to 1, 000 nm) having cellular origin are rather extensive. Their ability to cross blood-brain barrier gives an opportunity to cure brain damages and neurodegenerative diseases. As far as the positive effect of cell therapy using multipotent mesenchymal stromal

cells and hematopoietic stem cells (MSC and HSC correspondingly) may be significantly determined by paracrine impact of exosomes and microvesicles, they have potential for common application with stem cells. There are also data published, confirming that MSC exosomes of a human cord blood due to hepatocyte protection weaken the consequence of liver cirrhosis [42] and healthy influence liver cells *in vivo* [43It is also possible to integrate stem cells after previous cell culture with particular set of exosomes and microvesicles for cell commitment (to point for a particular development path).

Monocyte exosomes due to tumor-sensitive protein Tsg101 (*tumor susceptibility gene 101*) which brings to cancerogenesis in case of shortage [44], can be used for cancer therapy, as well as exosomes of cells not affected by cancer due to membrane proteins of Wnt intracellular signaling pathway, regulating cellular differentiation and malignant tumor development [45]. Despite the established positive impact of MSC on neoangiogenesis, there is evidence that exosomes of these cells suppress tumor angiogenesis as it was shown by the example of mammal gland cancer *in vitro* and *in vivo* at subcutaneous injection to inbred mice of cancer cells mixture and MSC exosomes. But it is still uncertain at what cost MSC exosomes provide such an effect, partially due to decreasing the expression of VEGF endothelial growth factor in tumor cells [46].

In general exosomes of correctly functioning cells may be applied for repairing cells of the same type but with lost function. It was shown that endothelial cell exosomes stimulate cell migration and angiogenesis while exosomes from endothelial cells without microRNA-214 are not able to perform such an action [47].

Exosome utilization strategies for clinical purposes may include inflammation and immune response regulation: single injection of macrophage exosomes brought to decreasing thermal abnormally high sensitivity for pain stimulus (hyperalgesia) on the inflammatory pain model of mice [48].

It was suggested to use bacterial exosomes, so called "external membrane vesicules", to manufacture anti-infective cell-free vaccines [49].

Moreover blood and urinary exosomes can be considered as biomarkers for various diseases and serve for diagnostics [50], because they are molecular content carriers (e.g. proteins and RNA) that indicate to pathological and physiological status of source cells. Besides exosomal RNA is protected from ribonuclease impact unlike intracellular RNA and blood plasma RNA [51]. Using exosomes can be an alternative for invasive diagnostics, for example, to provide data obtaining without making biopsy at cancer [52]. It is also discussed to use blood plasma exosomes as status biomarkers of multiple sclerosis [53], cardiovascular risk [54], liver damages [55] and a range of other diseases.

Thus, studying submicrone vesicles of cell origin is promising and important for healthcare. Despite the remarkable progress for the last two decades in understanding the structure, functions and clinical value of exosomes and microparticles, the fundamental molecular mechanisms of vesicles generation of particular composition and capturing them by target-cells is still unclear as well as the criteria for identification of various vesicles types, biological value of extracellular vesicles for a living organism. These questions give a wide field for new researches and still challenging is the name for research line introduced by Rose Johnstone — "Alice's Adventures in Wonderland" [56].

The most interesting in the context of practical healthcare is studying exosomes and microvesicles impact on the development and progression of a given disease, a possibility to correct pathologic behavior using particular extracellular vesicles as biotechnological remedy. It is also relevant to study the further role and value of exosomes for surrounding cells, tissues and organs at the molecular level, of application perspectives and authenticity of extracellular vesicles as disease progress biomarkers.

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ПОЗАКЛІТИННІ ВЕЗИКУЛИ: КЛАСИФІКАЦІЯ, ФУНКЦІЇ ТА КЛІНІЧНА ЗНАЧУЩІСТЬ

А. В. Оберемко, А. Г. Попандопуло

ДУ «Інститут невідкладної і відновлювальної хірургії ім. В. К. Гусака НАМН України», Донецьк

E-mail: a.oberemko@mail.ru

Наведено узагальнене визначення везикул як двомембранних екстраклітинних органоїдів, що є характерними для всіх клітинних форм життя: не лише ев-, але й прокаріотичних. Подано також історію вивчення напряму, номенклатуру, структуру і склад позаклітинних везикул, показано їх вплив на життєві процеси в нормі та за патології. Ґрунтуючись на властивостях екзосом і мікровезикул, розглядається можливість корегування перебігу низки хвороб та їх діагностики. Велика кількість невирішених питань цієї галузі залишає за собою широке поле для нових досліджень.

Найбільш перспективними з погляду практичної охорони здоров'я є напрями з вивчення впливу екзосом і мікровезикул на розвиток тієї чи іншої хвороби, можливість корегування перебігу патологічного процесу за допомогою позаклітинних везикул певного типу, що виступають як біологічно активні речовини. Актуальним є також подальше з'ясування ролі і значущості екзосом для прилеглих клітин, тканин і органів на молекулярному рівні, перспективи використання позаклітинних везикул як біомаркерів захворювань.

Ключові слова: екзосоми, мікровезикули.

ВНЕКЛЕТОЧНЫЕ ВЕЗИКУЛЫ: КЛАССИФИКАЦИЯ, ФУНКЦИИ И КЛИНИЧЕСКАЯ ЗНАЧИМОСТЬ

А. В. Оберемко, А. Г. Попандопуло

ГУ «Институт неотложной и восстановительной хирургии им. В. К. Гусака НАМН Украины», Донецк

E-mail: a.oberemko@mail.ru

Приведено обобщенное определение везикул как двумембранных экстраклеточных органоидов, характерных для всех клеточных форм жизни: не только эу-, но и прокариотических. Представлены также история изучения направления, номенклатура, структура и состав внеклеточных везикул, показано их влияние на жизненные процессы в норме и при патологии. Основываясь на свойствах экзосом и микровезикул, рассматривается возможность корректировки течения ряда заболеваний и их диагностики.

Наиболее перспективными с точки зрения практического здравоохранения представляются направления по изучению влияния экзосом и микровезикул на развитие и прогрессирование той или иной болезни, возможность корректировки течения патологического процесса при помощи внеклеточных везикул определенного типа, выступающих в качестве биологически активных веществ. Актуальным является также дальнейшее выяснение роли и значимости экзосом для окружающих клеток, тканей и органов на молекулярном уровне, перспективы использования внеклеточных везикул в качестве биомаркеров заболеваний.

Ключевые слова: экзосомы, микровезикулы.