UDC 575/616
DOI https://doi.org/10.32782/wba.2023.2.12

BIOLOGICAL SIGNIFICANCE OF EXOSOMES AND MICROPARTICLES

Botond Géza KÁLMÁN *– Adjunct faculty,*
Department of Business and Management,
Kodolányi János University of Applied Sciences (Hungary),
kalman.botond.geza@kodolanyi.hu, ORCID ID: 0000-0001-8031-8016
Victoria Frants *– Graduate student,*
Department of Entomology and Biodiversity Preservation,
Uzhhorod National University (Ukraine),
franzvika96@gmail.com, ORCID ID: 0000-0002-2604-6344

The development of cell and molecular biology is accelerating along with the development of technology. Microvesicles are one of the focal points of ongoing research in this area.

Extracellular vesicles (exosomes, microvesicles) are recently discovered participants in intercellular communication, whose role has been proven in many physiological and pathological conditions. These 100–1000 nm membrane-surrounded particles originating from a wide variety of cells play a role in communication between cells, in the shrinkage of certain tumors, in the formation of metastases, in the formation of blood vessels, and also play a primary role in the regeneration of tissues.

Exosomes were discovered nearly fifty years ago. Today, thanks to their versatile use, they have become important elements of everyday science. Their main function is to contribute to genetic variability. This happens primarily in the way that they are able to transfer genetic material between different species and even between plants and animals. Experiments on the practical use of exosomes in tumor therapy, the treatment of autoimmune diseases, or the cure of infectious diseases are still ongoing.

A large amount of microvesicles circulate in body fluids, which play an important role in intercellular communication and in the detoxification and regeneration processes of cells. Scientists are actively researching the role that exosomes may play in signaling between cells; it is assumed that exosomes, being able to unite with distant cells and empty their contents into them, are thus able to influence the processes taking place in distant cells. Clarifying the exact role of exosomes may open many new possibilities in the future, from their use as biomarkers to their use as therapeutic targets.

This study summarizes the most basic knowledge on the biological significance of exosomes and microparticles.

Key words: exosomes, microvesicles, an autoimmune disease, biomarkers, cellular and molecular biology.

Introduction. Research into exosomes goes back more than thirty years. In their article [1], Théry et al briefly describe the historical background of exosomes research. The beginning is dated to 1981, when Tramsa et al.'s article on

this topic was published [2]. Although the term exosome has been used before, for example by Mishra and Tatum in 1973 [3], the 1981 definition refers to microvesicles produced by cells of a cancer cell line and exhibiting 5'-nucleotidase activity. In 1987, Johnstone already uses the term for small membrane vesicles that are produced and secreted into the intercellular space by eukaryotic cells (reticulocyte culture) [4]. They arise from multivesicular endosomes, and then merge with the plasma membrane and reach the extracellular space. This development from the inside out is called "inward/reverse budding" by Yang and tsai [5]. In 1989, Peters and Mayer demonstrated the fusion of late endosomes and the cell membrane in cytotoxic T cells [6]. In 1996, it was possible to trace the formation of multivesicular endosomes in B cells transformed by the Epstein-Barr virus, and then a more thorough study of exosomes began. Thanks to this, the results were soon confirmed for dendritic cells as well.

Material and method. The purpose of my present work is to present the biological significance of exosomes and microparticles. Since a significant amount of material has been collected during more than 30 years of research, I have chosen the review and processing of the literature as my method. In the introduction, I presented a brief history of the research so far, and in the following, I will review the nomenclature, biogenesis and composition of microvesicles. Next, I will summarize the knowledge gathered so far about the functions of exosomes, and then I will describe in more detail the results of the more frequent areas of research (oncology, circulatory diseases, neurodegenerative diseases, kidney diseases); finally, the relationship between common infections and exosomes. I will summarize the latest results and test methods, and then the future prospects; and in the summary I summarize the biological significance of exosomes and microparticles.

Literature results. The names of the particles are not yet completely uniform in the literature. The following division is based on Beyer and Pisetsky's 2009 classification (taken from: Gy. Nagy and tsai's presentation-10.28.2010) [7] sizes (Table 1):

Table 1. **Classification of microvesicles based on diameter size (adapted from Nagy et al. [7])**

Name	Measurement range (diameter)
exosome	10–100 sqm
ectosome / microparticle	100–1000 sq m
apoptotic body	1000–4000 sq m

Knowing the above division, in cases where it is not specifically one group or another, but microvesicles in general, I will use the names exosomes and summary microvesicles in the following.

Another possibility for categorization is the morphological classification based on electron microscopy, which Yang and Tsai use in their article [5]. They differentiate:

– cup-shaped and

– saucer-shaped exosomes.

Schorey et al. [8] categorize exosomes based on the mother cell rather than the mechanism of production; for hemopoietic and non-hemopoietic origins.

Edit Buzás also deals with measurement and detection issues in her lecture [9] and proposes the use of differential detergent lysis for the separation of impurities caused by protein complexes.

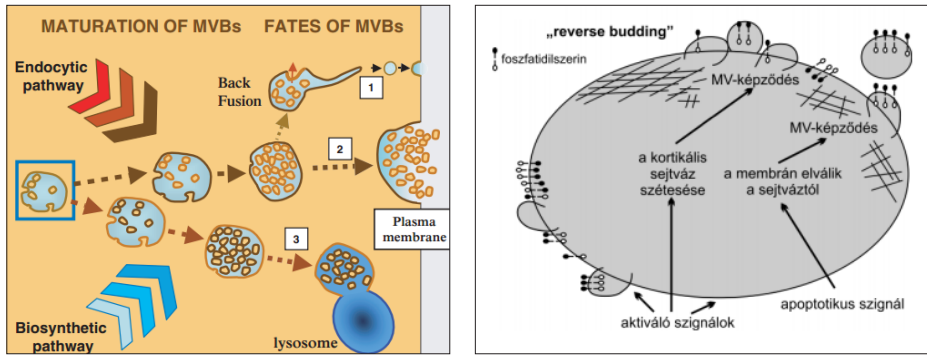
Now let's briefly review the biogenesis of exosomes! All eukaryotic cells produce similar microvesicles [10]. Their formation usually takes place during cell activation, although they are also continuously produced in certain cells (epithelial cells, immature dendritic cells). They can originate in two ways [10] (Fig. 1).

Those between 30–100 nm (exosomes) are multivesicular bodies branching from the endosome-lysosome pathway (Fig. 2) and leave the cell by exocytosis (this pathway was first described by Pan and Johnstone in reticulocytes in 1983).

Those between 100-1000 nm (microparticles/ectosomes) are created by reverse splicing as a result of an activation signal or an increase in the intracellular Ca^{2+} concentration.

Although there are still areas to be described in the exploration of biogenesis, primarily the mechanisms of the selection of substances entering the vesicles.

In contrast to generation, the description of the composition and functions of exosomes is quite detailed (see the ExoCarta topic later). Exosomes also carry proteins, lipids and nucleic acids (Fig. 3).



a) b)

Fig. 1. **The two ways of the formation of exosomes (Sources: a) van Niel et al, b) Pap and tsai [10])**

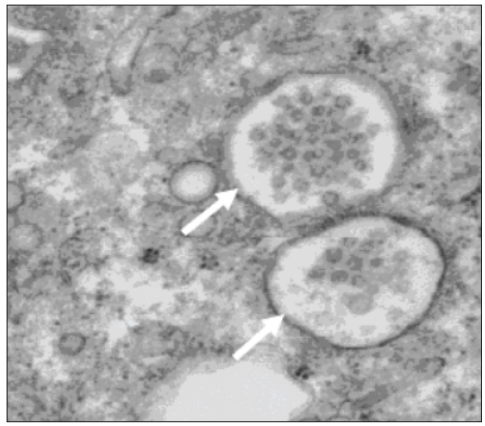


Fig. 2. **TEM image of multivesicular bodies (indicated by arrows) from a kidney (Prepared by: Leileata Russo) (Source: Noerholm [25])**

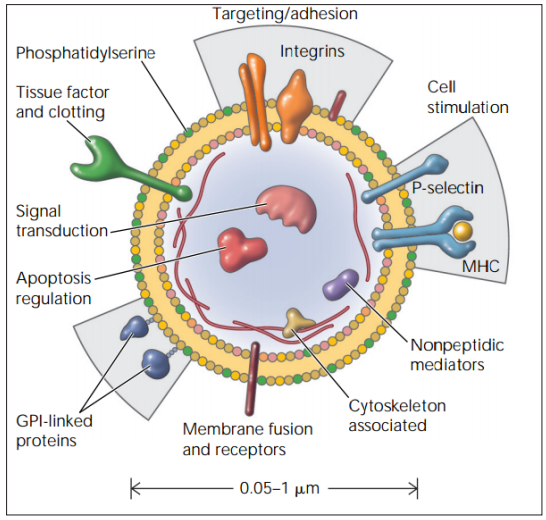


Fig. 3. **Structure and functional components of microvesicles (Source: Hugel et al.)**

Proteins include:

– cytoskeletal and adhesion proteins (annexin, CD9, CD11, CD18, lactadherin),

– apoptotic proteins (thioredoxin peroxidase II, galectin),

– heat shock proteins (Hsp60, Hsp90),

– antigen-presenting proteins (MHC I, MHC II),

– enzymes (peroxidases, kinases, enolases).

It is important to note that, despite their endogenous origin, we typically do not find ergastoplasmic, mitochondrial, or nuclear proteins among them. Atay

and tsai in 2011 already approx. 300 exosomal proteins have been described that play a role in regulating the microenvironment of the donor cell [11]. And Wang and Tsai report a total of more than 1,000 such proteins in their article [12].

Lipids, the most important components are phosphatidylcholine, phosphatidylserine and sphingomyelin. The important role of exosomal sphingolipids in the development of Alzheimer's disease was described this year by Yuyama and tsai [13].

Among nucleic acids, mRNA and microsomal RNA (miRNA) occur. These are collectively called exosomally delivered RNA (esRNA). miRNA is a non-coding RNA with a length of 19-24 nucleotides that 3'binds to the end of the mRNA in the target cell and inhibits the synthesis of proteins (so-called post-transcriptional silence). This inhibitory effect affects about 30 % of the protein-coding genes of human cells [14]. Tumor markers, viruses, and even *M. tuberculosis* and *T. gondii* can be detected in the exosomes produced by pathologically changed cells, depending on the nature of the change [8]. Exosomes thus play a role both in the cell-to-cell spread of infections (HIV) and in the immune response.

As a result of their complex composition, many functions of exosomes have been described, and the number of known functions is currently growing. I describe them based on the classification of Buzás [9] and He and Hannon [14]:

Alternative protein secretion pathway

Since exosomes can be considered secret packages that contain substances covered with a lipid layer, including proteins, they represent a different pathway for protein secretion than traditional pathways. This pathway is also suitable for removing dangerous molecules from the cell [9].

Intercellular communication

This area is of increased interest [1], because this intercellular communication mechanism is significantly different from the traditional ones (when the cell produces a protein that binds to the receptor of the neighboring cell and transmits the information, or the transmembrane channels – gap junction – pass through the information). The traditional substances of chemical message transmission (neurotransmitters, hormones, etc.) enter the intercellular space on their own, while microvesicles contain the substances in a "packaged" form [10]. This protects the transmitters from intercellular degradation and "extends" the spatial functions of the producing cell. Matkó classifies the intercellular information flow as follows [15]:

direct cell-cell contacts:

– immunological synapse (15–40 nm intercellular gap),

– nanotube communication (50–100 micron intercellular distance),

indirect intercellular communication:

– cytokine network (auto- or paracrine regulation via receptors),

– microvesicles, microparicles, exosomes.

In contrast to the traditional 1 receptor-1 signal molecule mechanism, microvesicles are also capable of targeting molecular combinations (proteins, nucleic acids, lipids) through their internal molecular content and materials integrated into their membrane, triggering complex stimuli and the simultaneous initiation of stimulatory or inhibitory cascades. Therefore, microvesicles have a pleiotropic effect, i.e. vesicles from a given cell can interact with several cells, i.e. common pathways for different functions [10].

Antigen presentation (and the related biodiagnostic marker role [9].

Raposo et al. started researching the topic in 1996 [16]. It was then described that the antigen-presenting cells (they examined B-lymphoblasts, but macrophages also have this ability) are rich in MHC-II type proteins, so-called they contain a late endocytotic compartment and also secrete MHC-II proteins. It was shown that the secreted MHC-II is packaged in exosomes and is excreted from the cell, and that exosomes can also play a role in antigen presentation in vivo. Their research continued in two directions. On the one hand, we managed to learn a lot about the role of exosomes in tumor immunity, and on the other hand, about their immunosuppressive effect. The two phenomena are partly related to each other, because some tumor cells secrete exosomal substances that weaken the immune system by inducing the inhibition or apoptosis of T-cells (helper and killer) or myeloid suppression. The relationship between tumors and exosomes is the broadest front of exosome research, so I will cover this topic separately after explaining the functions.

Exosomes also play a role in the pathogenesis of autoimmune diseases (e.g. rheumatoid arthritis). This effect is partly the result of complement components and activator molecules detectable on the surface of their membrane, and partly the result of citrullinated and nuclear proteins detectable in exosomes. The relationship between exosomes and autoimmune diseases was summarized by Mária Szente-Pásztói in her doctoral dissertation in 2009 [17].

Exosome research in pregnancy is also of an immunological nature [18], and the Institute of Genetics, Cell and Immunobiology of the Semmelweis University also conducts studies in this direction [10]. The essence of these researches is that, although the fetus is a foreign substance in the mother's body from the point of view of maternal immunity, it does not trigger a rejection reaction. Exosomes also play a role in communication between mother and fetus [18]. Exosomes can be found in the serum of all pregnant women from the 28th to the 30th day of pregnancy. week, but in those pregnancies that end in premature birth, their activity is less pronounced, which proves that immunological factors also play a role in premature birth.

Lässer et al also investigated breast milk exosomes and determined their role in the development of the newborn's immune system [19]. Exosomes from saliva, blood and breast milk were first detected and proved to contain mRNA. It was

proven that esRNA remains functional even after being taken up by macrophages. The uptake of breast milk exosomes is also possible under the acidic conditions of the alimentary canal [20]. Thus, esRNA of maternal origin may play a role in the development of the infant's immune system. Redman and tsai (2011) suggested that changes in not only the number but also the size of exosomes may play an important role in the development of preeclampsia of pregnancy [21], through their proinflammatory, antiangiogenic and procoagulant effects.

RNA transfer (new cell properties).

Many substances are transported in exosomes. So are nucleic acids. Nucleic acids (mRNA and miRNA) of exosomes are functional even after entering the target cell (appearance of new proteins). Because of their importance, the two exosomal RNAs were named exosomal shuttle RNA (esRNA) by Valadi et al [22]. It appears, for example, that RNA transfer may also play a role in chemotherapy resistance of tumors.

Transfer of infectious agents between cells [22].

Ever since Beatty et al. described and investigated the bacilli Calmette-Guérin hiding in apparently healthy cells, the exosomal connection of the hiding ability of infectious agents has been brought to light [23]. During immuno- and fluorescence examination of living cells, the authors found Mycobacterium components associated with the organelles of apparently healthy cells. According to this, Mycobacterium are able to hide from the immune system by merging with the lysosomal compartment of macrophages and thus presenting themselves to the immune system as their own. Complementing this with the known immunomodulatory effect of the bacterial cell wall, it becomes clear how Mycobacterium can spread from the focus of infection with the help of exosomes and infect the surrounding cells unnoticed. Later, other "hiding" ones were also detected (e.g. the causes of slow virus type infections, such as prions or HIV) (Fig. 4).

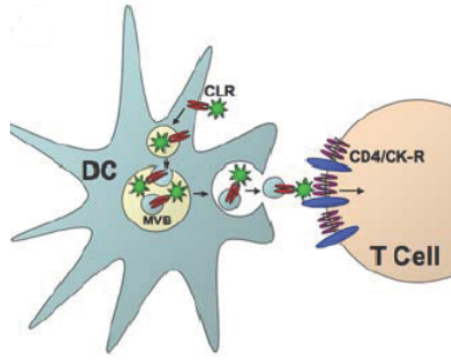


Fig. 4. **Mechanism of HIV transfection via exosomes (Source: Matkó J. 2011.12.01)**

As a result of this line of research, the so-called "Trojan exosome" hypothesis [24], which provides a new explanation for the spread of retroviruses. Retroviruses from the receptor and the so-called He explains his infectivity independent of the Env protein and his ability to "hide" from the otherwise healthy immune system by the fact that retroviruses use the exosome biogenesis and pathway already present in cells for both infection and hiding. The current importance of the theory is given by its application in AIDS research [15] and Figure 4: The HIV virus is ingested by the cells of the immune system and reaches the surface of the (helper) T-cell as exosomes, and then fuses with its cell membrane to enter the T-cell and destroy the helper cell. Oncogenic receptors can also be transferred from one cell to another via similar pathways [9], which can play a role in the formation and spread of tumors.

A more detailed description of some areas follows:

Tumors and exosomes

Mikkel Noerholm [25] lists the following known (marked with !) and suspected (marked with ?) exosome functions in relation to tumors in his November 2011 presentation (Fig. 5):

– endocrine signaling between adjacent tumor cells (?),

– autocrine signaling between distant tumor cells (?),

– stimulate tumor angioneogenesis and tumor stroma regenerative processes(!),

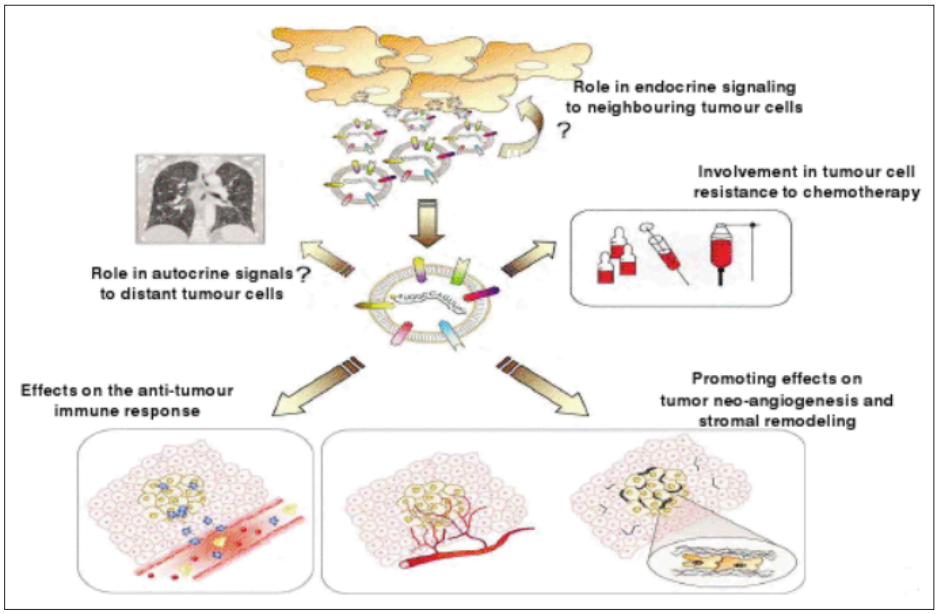


Fig. 5. **Known and suspected exosomal functions related to tumors (Source: Noerholm [25])**

– they affect anti-tumor immune processes (!),

– they play a role in the development of tumor cell chemoresistance (!).

Yang and Robbins (5) summarize the role of tumor cell-derived exosomes:

Antitumor effects

– Immunogenic effect – as a result of carried tumor antigens.

– Induction of tumor cell apoptosis – Bcl-2 and Bax genes trigger the metabolic cascades that lead to tumor cell death.

– Neoplasmogenic role (Fig. 6).

Tumor cells produce exosomes, the composition of which is characteristic of the cell that secretes them, and they play the following role in the progression of the neoplasm [26]:

Immunosuppressive effect – inhibition of T cells, by influencing myeloid cell differentiation and through their antigens;

Enhancement of tumor invasion and metastasis formation – with angiogenic factors (tetraspanin);

Stroma-enhancing effect (on fibroblasts) – with metalloproteinases antiapoptotic and oncogenic effect – with RNA and protein transport.

Development of chemoresistance.

Valadi and tsai [22] showed that RNA in exosomes remains functional even after delivery. This may play a role in chemotherapy resistance of tumors.

Although the exact "result" of the processes has not yet been described, it seems that the tumor stage and the patient's immune status determine whether the neoplasmogenic or the opposite processes dominate.

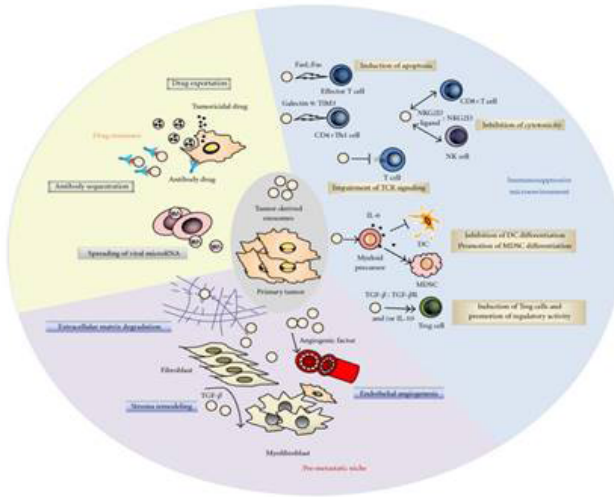


Fig. 6. **Neoplasmogenic effects of tumor cell exosomes (Source: Yang and Robbins [5])**

Regarding immunomodulation, Karlsson et al developed the so-called tolerosome theory [27]. Tolerosomes are approx. Exosome-like vesicles with a diameter of 40 nm, which carry MHC-II protein complexes. Intestinal epithelial cells produce and secrete them into the intestinal lumen. After oral antigen administration, they can also be detected in the plasma soon after. Based on their structure, they are highly effective antigen presenters, so they play an important role in tolerance to orally introduced antigens, i.e. in inhibiting intense immune reactions against such antigens. Researchers assume that exosomes play a similar role in the immunosuppressive effect of tumors.

Another mechanism of the anti-immune effect is the so-called Fas tumor-counterattack theory from Andreola et al. [28]. The Fas (also known as FasL) ligand is a type II transmembrane protein belonging to the tumor necrosis factor (TNF) family. Its trimerized form acts most actively. By binding to its T-cell receptor, it weakens the immune system through the apoptosis of the target cell and helps the tumor to hide from the immune system and to progress. It reaches the extracellular space in the form of an exosome. In particular, many active aquired Fas ligands are expressed by certain types of tumors (e.g. melanoma, breast cancer, colon cancer, esophageal cancer), as reported by O'Connell et al. in their article [29]. It has also been shown that the degree of Fas expression observed in ovarian, breast, liver and breast cancer as well as melanoma is inversely proportional to a good prognosis (Fig. 7).

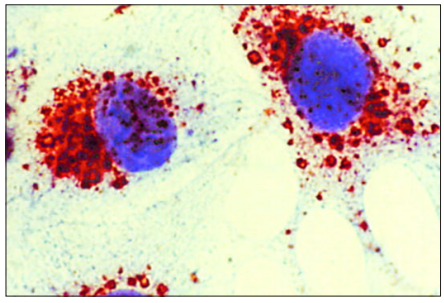


Fig. 7. **FasL expression in human melanoma cells detected by immunocytochemistry (Source: Andreola et al [28])**

In connection with the research results related to exosomes, new directions for tumor therapy are being outlined. One direction is the idea of an anti-cancer vaccine, which has already been raised by several research groups. Results, considerations and issues related to this are described in Clayton and Mason's 2009 article [30]. Raposo and tsai [16] and then Zitvogel and tsai [31] showed that exosomal markers from antigen-presenting cells, e.g. heat shock protein number 70 (Hsp70) increases the migration and cytolytic activity of natural killer T cells.

The 2012 article by Hartmann et al also deals with the issue of tumor vaccination [32]. One of the reasons for the low effectiveness of the traditional vaccination method is that many non-cell surface antigens (exosomes) also play an important role in the case of tumors, AIDS and other diseases. The adenovirus vector transduction procedure proposed by the authors facilitates the presentation of exosomal antigens. The experiment was carried out with tumor cells, but the results seem to be applicable to viral and parasitic diseases, so research continues in all directions: *T. gondii, M. bovis, S. pneumoniae* [33], SARS and adenoviruses [34] are also research areas, such as autoimmune arthritis and so-called late hypersensitivity [35]. We can also be diagnostic and therapeutic helpers in many other diseases (heart attack, arteriosclerosis). Their use in relation to infarction is described in the article by Lai Ruenn Chai et al [36].

Another direction of research is aimed at the more specific use of existing drugs with fewer side effects through the artificial production and modification of microparticles (nanomedicine) [37], which, through its specificity, enhances the effect of cytostatics (cytostatics "packaged" in exosomes can be delivered to tumor cells in a targeted manner) and enables makes chemotherapy-resistant cancer cells end their resistance. In this way, the dose of cytostatics (e.g. cisplatin, doxorubicin, paclitaxel) can be reduced, thus reducing their systemic toxicity. With the possibility of specific treatment, the basis of personalized medicine (theranostics) was born. The essence of the trend is that it does not use epidemiological data and statistics based on the examination of a large number of patients, but rather examines the individual effect of the applicable drugs in the given patient and selects the ideal therapy based on this. The use of personalized medicine has already started in our country: on July 22, 2010, the Hungarian Society of Personalized Medicine (www.mszmt.hu) was established.

After tumors, let's move on to the topic of circulatory diseases [36]. Cardiovascular diseases are important targets for experimental stem cell treatment. Although, in principle, the transplanted stem cells differentiate and replace the damaged tissues. But they seem to reduce tissue damage and stimulate regeneration through the secretions they produce. The authors investigate the role of exosomes in the treatment of cardiovascular diseases (mainly myocardial infarction). Mesenchymal stem cells secrete in two ways (paracrine secretion – Fig. 8):

– traditional way: delivering soluble proteins to the extracellular space, which exert their effect by binding to the receptors of the target cell;

– with exosomes: their "packaged" proteins and nucleic acids are much more stable and protected; and they exert their effect not only on cell surface receptors, but also by reaching the target cell. Due to their complex "inner content", they are also capable of complex effects. Parolini et al showed that the exosome assembly activity and efficiency of cells depends on several

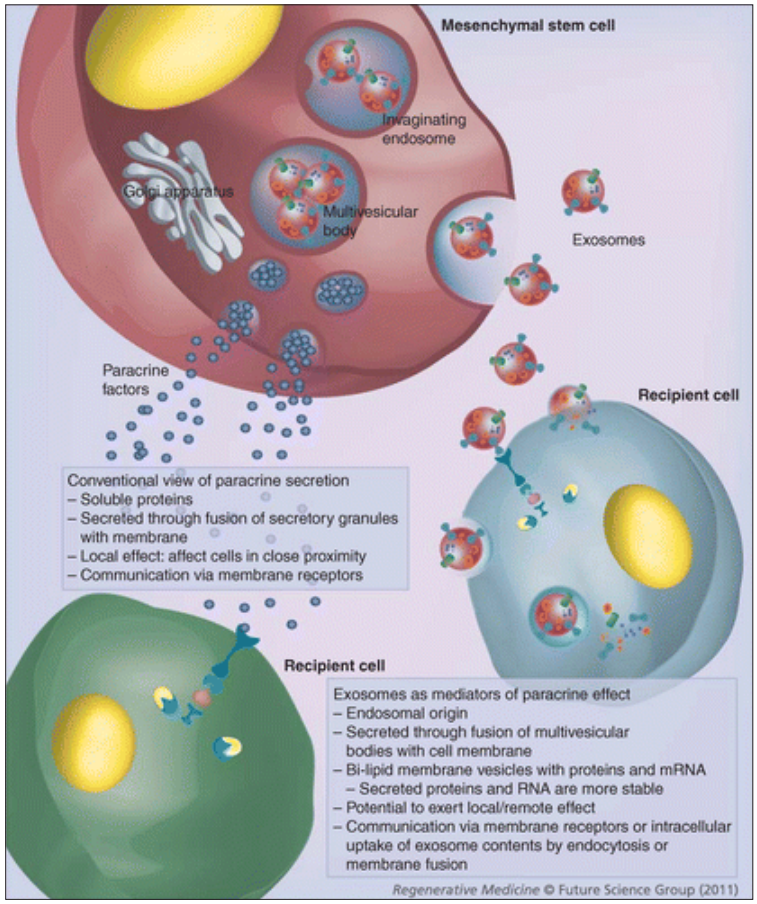


Fig. 8. **Paracrine mechanisms of mesenchymal stem cells (http://www.futuremedicine.com/action/showPopup?citid=citart1&id=f1&doi=10.2217%2Frme.11.35 - status as of 02/09/2012)**

microenvironmental conditions, including tissue pH value (20). The therapeutic efficiency of stem cell secretions therefore correlates well with the fact already described by Schrader in 1985 that ischemic heart cells are characterized by acidic conditions (38). Exosomes are ideal candidates for therapeutic use. They are mainly photogenic because, paradoxically, the artificial enhancement of blood and oxygen supply to ischemic myocardial tissue leads to the exacerbation of ischemia-induced cell insults. Among exosomal proteins, the role of enzymes is particularly important in this case, as they break down ischemic metabolic products, thus correcting the cell-damaging cascade processes. The enthusiasm caused by the positive results of the animal experiments was unfortunately dampened by the fact that the stem cells often do not integrate into the heart

muscle tissue or are not able to survive there. These problems can be solved by subcellular exosomes, which are capable of exerting a complex local effect without reactions occurring against the cells.

In addition to infarction, the role of exosomes in atherosclerosis, diabetes or hypertension has been suggested. The white blood cell-endothelial adhesion effect of microparticles is a key initial step in the development of atherosclerosis [39]. An important new result is the 2011 discovery by Vickers et al. of the relationship between the presence of HDL-miRNA complexes and familial hypercholesterolemia [40].

The relationship between neurodegenerative diseases and exosomes can be summarized as follows: I mentioned earlier that the role of exosomal sphingolipids in the development of Alzheimer's disease was described this year by Yuyama and tsai [13]. Exosomes related to Alzheimer's disease were also investigated by Street and his research group [41]. They investigated whether the cerebrospinal fluid also contains exosomes and, if so, the variability of exosomal proteins related to individuals. With their method based on ultracentrifugation, they managed to detect two characteristic exosome markers (Flotillin-1 and TSG101), which was also confirmed by transmission electron microscopy. Since the tests were carried out on a more concentrated sample than the physiological one, the goal was to develop a method for analyzing samples with a physiological concentration. Another goal is the development of detection methods, because with the current ones it is not possible to extract exosomes from biological fluids without contamination with non-exosomal proteins. One of the hallmarks of Alzheimer's disease is the extracellular accumulation of tau protein. Its role is similarly neurodegradative as that of alpha-synuclein in Parkinson's disease or prions in Kreutzfeld-Jacobs disease. The presence and accumulation of all three groups of substances indicates the presence and role of exosomes in the development of neurodegenerative pathologies.

The relationship between kidney diseases and exosomes deserves attention because the damage to the kidneys is often irreparable and the patient eventually needs dialysis or a kidney transplant. Balkom et al. [42] investigated the exosomal relationship between kidney physiology and diseases. They managed to show that the absence or mutation of the Tamm-Horsfall protein (uromodulin), which is also found in exosomes, can be the cause of kidney diseases. Unfortunately, the diagnostic rapid test is still waiting to be developed, but encouraging initial steps are reported in the differential diagnosis of kidney diseases (polycystic kidney, Nieman-Pick disease, cystinosis, Gitelman and Bartter syndrome). Urinary-derived exosomes can also be used to detect liver damage, as these are also associated with secondary renal manifestations. They also play a role in diagnosing hypertension and monitoring drug therapy, as well as comparing the effectiveness of drugs for a given patient (personalized medicine).

Finally, a few words about another topic: common upper respiratory tract infections. Lässer and his research team, already mentioned in relation to pregnancy [19], examined exosomes from nasal secretions in another experiment [43]. They were the first to reveal the RNA content of the exosomes of this secretion and its significance in the communication taking place in the nose, as well as the role of esRNA biomarker in upper respiratory diseases.

Based on the research so far, development continues at an ever-faster pace, so in 2011 many new results were achieved. Let's start with exosomal RNA research! Kosaka and Ochiya summarized the knowledge gathered on microRNA secretion [43]. MicroRNA is a biological fine-tuner, its deregulation leads to diseases, including cancer. It can be found in the circulation of both healthy and sick people, namely – since the circulation also contains RNase enzyme – in microvesicles, apoptotic bodies or linked to nucleic acid-binding proteins, this was demonstrated by Kwak et al. in 2011 (44). Because of the intervention in gene regulation, it became the focus of research on the development and diagnosis of cancer. In 1978, Stroun et al. described the in vitro modifying effect of extracellular RNA on DNA synthesis, and the possibility of this in vivo also arose [45]. The relationship between serum RNA, tumor mass and therapeutic sensitivity was described by Wieczorek et al. in 1987 [46], and Garzon et al. in 2010 that deregulation of miRNA leads to the development of cancer and changes in normal tissues, thus the miRNA diagnostic came into being, prognostic and therapeutic use in oncology [47]. The authors proved the communication significance of secreted miRNA in the transfer of information between cancer cells and with their microenvironment (Fig. 9).

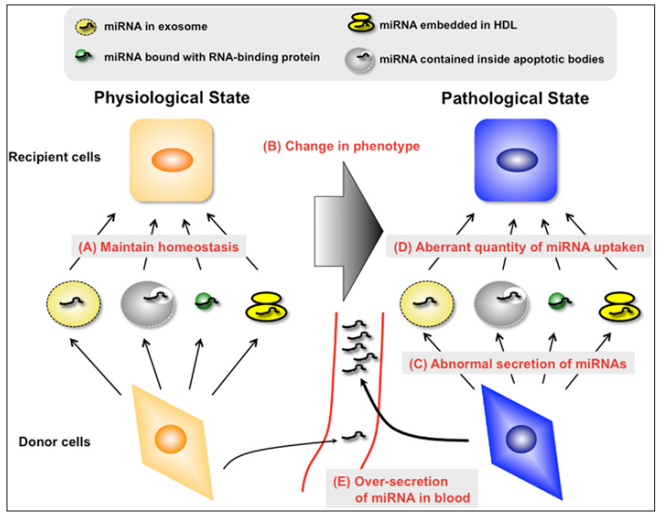


Fig. 9. **The effect of miRNA secretion of healthy and pathological cells on the recipient cell (Source: Kosaka and Ochiya [43])**

A new discovery (Lim and tsai, 2011) is that the intercellular transport of miRNA can also take place via gap junctions [48]. The mechanism that selects the miRNA entering the exosomes and binds it to proteins still needs to be described and clarified, but there are many indications that the Ago2 protein plays an important role in the process [49].

Hood et al (2011) describe that miRNA plays a role in melanoma's ability to break through the immune barrier of lymph nodes and metastasize to distant organs through genetic modification of the target organ [50].

It has been known for nearly a decade that RNA transport takes place in plants and nematodes as well, through transmembrane channels formed by SID-1 proteins; and also, that the membrane of human cells also contains proteins similar to SID-1. From the report of Molnar et al. [51], we can conclude that the intercellular transport of RNA is characteristic of all multicellular organisms.

It was a real surprise, however, when Zhang and Tsai reported in 2011 that exogenous plant-derived miRNA was detected in animal tissues and serum [52]. MIR168a from rice, which has a very strong regulatory effect, probably entered the animal body through nutrition, since esRNA can withstand the acidic pH of the alimentary canal, as Lasser et al. showed at about the same time [19]. In addition, MIR168a has already been found in the serum of the healthy Chinese population. In the examined sera, Zhang and tsai detected nearly 30 similar miRNAs. They also managed to prove that the materials of the exosomes are taken up by the cells of the small intestine and passed on to the cells of the body. Exogenous MIR168a inhibits hepatocyte LDL uptake, thereby increasing serum LDL levels. The authors came to the conclusion that the exosomal RNAs taken up with plant food – with their regulatory effects – are just as essential nutrients as e.g. the vitamins. The article is the first report that there can be a direct interaction between plant and human cells at the molecular level.

Gan and Gould research the process of development of retroviruses, with particular reference to the HIV virus [53]. The relationship between viral development and exosomes is being investigated. They found that neither the HIV protease nor the lack of p6 glycosaminoglycan sequences characteristic of the virus inhibits viral development, as previously assumed. However, surprisingly, the virus's own Gag-Pol protein has a developmental inhibitory effect. The inhibitory signal for the development of HIV is therefore found in the virus itself. Nabhan and tsai report [54] that some developing viruses take advantage of the cell's TSG101 protein and the endosomal sorting mechanism (ESCRT) so that virus particles are transferred to exosomes instead of physiological particles, thus spreading from cell to cell.

One of the latest immunological publications discusses the role of exosomes in the development of atypical allergic rhinitis [55]. The pathological role of CD8+ type T cells, which are activated by exosomes produced in mucosal

cells and containing microbial substances indicative of previous infection, has been confirmed.

In their article published this year, Choi and Tsai report on the protein networks created by exosomes [56] and Figure 10. Exosomes have been shown to possess a number of cluster-forming proteins that are similar to proteins in other subcellular networks.

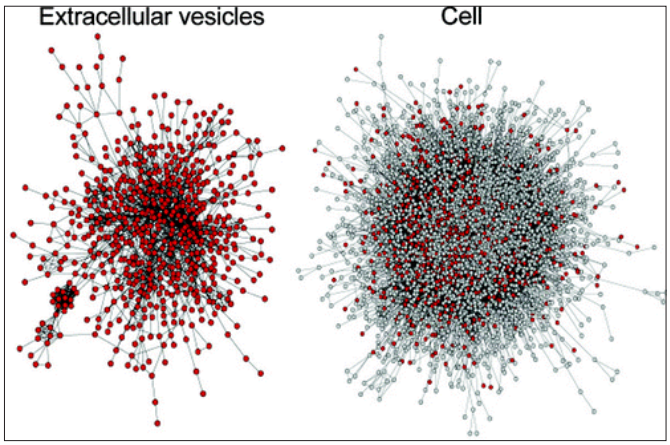


Fig. 10. **Network of exosomal proteins (left) and the cell covered by them (right) (Source: choi et al. [56])**

It was experimentally proven that the protein interactions in the cell have an influence on the biogenesis of exosomes and the sorting of their proteins.

Of course, new discoveries are also due to new techniques. Let's look at some of them!

On April 7, 2011, Global Newswire reported on the new technology developed by Caris Life Sciences [57]. The procedure is a so-called a blood-based test that is based on the role of circulating microvesicles and is just as effective in the diagnosis of colon, breast, lung and prostate cancer as other, much more complicated procedures. The essence of the test is the "capture" of circulating microvesicles with the help of specific antibodies, followed by the analysis, examination, and classification of the captured vesicles (Fig. 11, 12) [58]. The exact details of the technology are not known as they are under patent protection.

The test fits perfectly into the toolset of Theranostics, a new trend that uses nanodiagnostic and nanotherapeutic procedures primarily used in tumor treatment [37].

The procedure allows for diagnostic, therapeutic and theranostic conclusions based on the different miRNA profiles of microvesicle populations

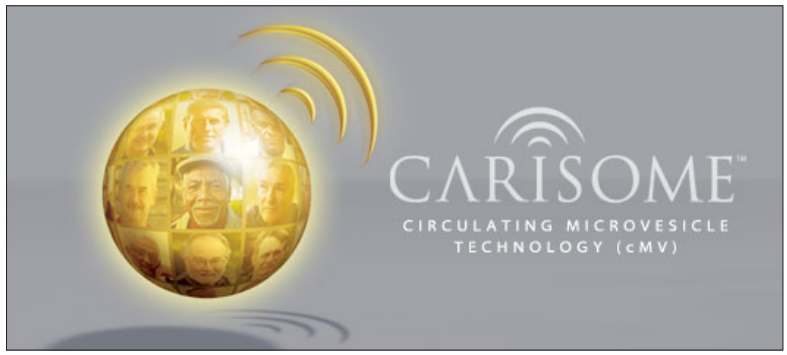


Fig. 11. **Logo of Carisome technology**
(Source: Caris Life Science [58])

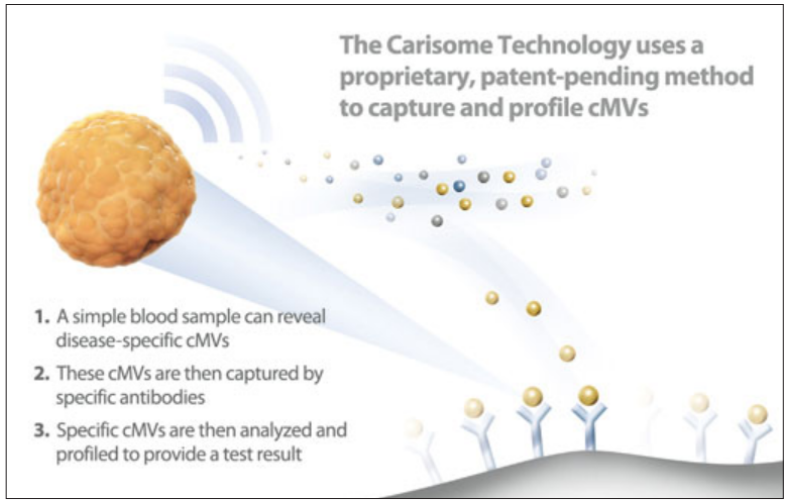


Fig. 12. **The essence of the Carisome process**
(Source: Caris Life Science [58])

detectable in the blood circulation. In this case, the researchers used the biomarker function of exosomes.

The procedure is suitable for detecting certain cancers and some other complex diseases. Its complexity is enhanced by the fact that it uses all three size ranges of circulating microvesicles (exosomes – ectosomes – apoptotic bodies).

Almost two years before the appearance of the Carisome method, NanoSight [59] reported on an instrument (Fig. 13) and a procedure that allows for multifaceted analysis of nanoparticles (size, concentration, aggregation) with one instrument.

The fast and direct procedure (Nanoparticle Tracking Analysis – NTA) detects, measures and counts nanoparticles in a soluble state (Fig 14).

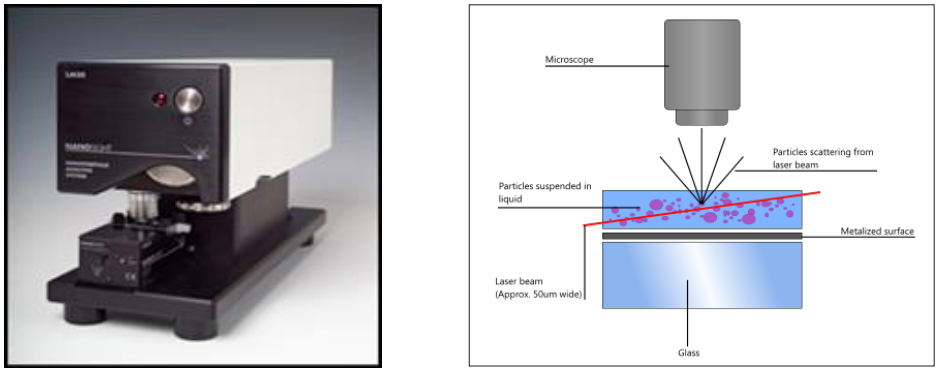


Fig. 13. **Nanosight's instrument, the LM20, and its operation diagram (Source: http://www.nanosight.com/technology/nanosights-technology – 2 02/09/2012 status)**

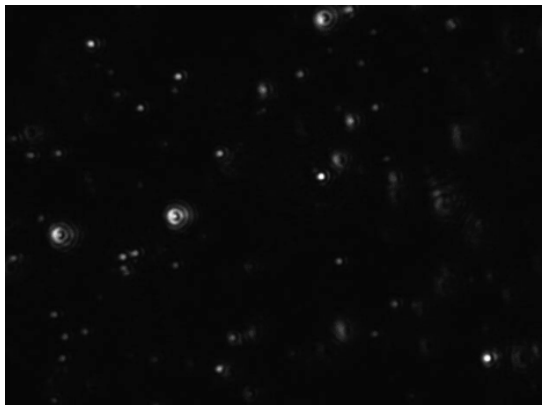


Fig. 14. **Video frame to illustrate NTA (Source: http://www.nanosight.com/technology/nanosights-technology – 2 02/09/2012 status**

The smallest detectable size is 10–20 nm, which can be detected even at a concentration of 10^{7}–10^{9} particles/ml. The procedure is based on fluorescence, which enables visual validation in a unique way: the movement of the particles, reminiscent of Brownian motion, can be recorded on video and archived for later use.

The advantage of the procedure is that it is cheap, fast, easy to use and high-performance, the disadvantage is that it always requires a diluted sample and, since it is a new procedure, the limits of its reliability range require further testing. However, it is still used today for the additional validation of already introduced measurement procedures, and the NTA can also be calibrated with their help.

A similar procedure, also based on Brownian motion, is Dynamic light scattering (DLS) from Malvern [60]. With this, even particles smaller than 1 nm can be detected. The procedure has already been calibrated and cooperates with the ISO 13321 and 21 CFR Part 11 standards. The conventional 90° instrument has been replaced by two types of Non Invasive Back Scatter: Zetasizer Nano S and Nano ZS, which already operate at an angle of 173°.

Mikkel Noerholm, director responsible for European operations of Exosome Diagnostics, also reported on his company's latest research results in the aforementioned November 2011 presentation [25]. Their technology examines the RNA content of exosomes extracted from body fluids (they call it exoRNA, but it is the same as the nucleic acid called esRNA by Valadi et al.) [22]. The Exosome Biofluid Molecular Diagnostic Procedure developed for this purpose is used to detect prostate cancer, glioblastoma multiforme and malignant melanoma. The procedure was trademarked in 2008 by Harvard/ Massachusetts General Hospital.

Cataloging of the components of exosomes is currently underway, based on published and unpublished results [61]. This catalog is ExoCarta (www. exocarta.org). During the printing time of Mathivanan et al.'s article (61), so much changed that 19.12.2011. Since version 3.2 is currently available and can be downloaded, browsed and searched with more than fourteen thousand data. The data are contained in five text files (Table 2):

Table 2. **ExoCarta's downloadable databases (Source: http://exocarta.org/download - as of February 10, 2012)**

ExoCarta Download - 3.2 (Release date: 19 December 2011)	
Protein/mRNA data	ExoCarta_protein_mRNA_details_3.2.txt
miRNA data	ExoCarta_miRNA_details_3.2.txt
Lipid data	ExoCarta_lipid_details_3.2.txt
Exosome study details	ExoCarta_experiment_details_3.2.txt
Gene details	ExoCarta_gene_details_3.2.txt

In addition to being a freely usable database, it also creates an opportunity for a forum, through which researchers can jointly search for solutions to their questions and share their results. The biological interaction database BioGRID (can be used as a supplement to ExoCarta.

The challenges of the future

Research on exosomes is multidirectional. Biogenesis is being researched, the components are being explored in more and more detail, and the role of microvesicles is emerging in connection with more and more new diseases. In

biogenesis, the mechanisms for the selection of substances entering the vesicles still need to be clarified.

Based on the above results, one of the future possibilities of using exosomes is vaccine production. With their application, it will be possible to produce a vaccine that is cell-free, induces the immune system, and thus prevents tumor cells from hiding from the defense system.

The future of secretory RNA research has three directions: on the one hand, the complete typing of miRNA has begun, and on the other hand, the detailed exploration of the secretion mechanism; and the third direction is the description of the functions of secretory RNA and the examination of its precise relationship with physiological and pathological conditions – goal: prevention, diagnosis and treatment of diseases related to secretory RNA.

Micro- and nanovesicles that can be detected in the circulation play an increasingly important role as disease markers, so more and more procedures are being developed for their detection and measurement. These (electron microscopy, flow cytometry, specific ligands or antibodies) were described in his presentation by dr. Paul Harrison [62]. The procedures are currently being refined and standardized, because they provide important diagnostic and prognostic tools.

Summary. Exosomes were first described more than 30 years ago. The biological role of these particles, produced in cells and then secreted, bounded by a lipid membrane, seems to extend to almost all areas of the living world. They have been described in plants, animals and humans. It has been shown that different species and even biological countries (in taxonomy: regnum), e.g. it is also possible to transfer exosomes between plants and animals. This also increases the significant genetic variability created by mutations and recombinations through the transfer of nucleic acids. The process is practically an alternative path of evolution. It can also be seen as the "fast lane" that has existed since the time of single-celled earth history, in addition to the traditionally slow "evolution highway". Just like on the evolutionary high street, positive (resistance) and negative (apoptosis) changes can occur here as well. Changes at the cellular level in higher organisms can result in disease or even healing.

Therefore, the importance of exosomes is increasing in several areas, e.g. in cancer research, viral (e.g. HIV, HSV) or prion infections (Jacobs-Kreutzfeld) or Alzheimer's disease. These determine the main research directions, but of course further investigations are also underway. The main practical research areas are:

– isolation and characterization methods (detection, measurement, analysis),

– normal physiological role of exosomes, communication between cells,

– infection processes (HIV, prions, Trypanosoma),

– exosomal RNA (and tumors),

– use of exosomes as a therapeutic carrier (e.g. autoimmune vaccination).

In the three decades that have passed since their discovery, exosomes have become an independent scientific subfield, whose practitioners are already specializing and researching one area of expertise (detection, diagnostic use, therapy). For years, multi-section conferences have been held to summarize exosome research, and more and more research results reach the phase of practical application.

БІОЛОГІЧНЕ ЗНАЧЕННЯ ЕКЗОСОМ І МІКРОЧАСТИНОК

Ботонд Геза Калман, *Університет прикладних наук Яноша Кодолані (Угорщина),*
Вікторія Франц, *Ужгородський національний університет (Україна)*

Разом з розвитком технологій прискорюється розвиток клітинної та молекулярної біології. Мікровезикули є одним із фокусів поточних досліджень у цій галузі.

Позаклітинні везикули (екзосоми, мікровезикули) є нещодавно відкритими учасниками міжклітинної комунікації, роль яких доведена у багатьох фізіологічних і патологічних станах. Ці оточені мембраною частинки розміром 100–1000 нм, що походять із широкого спектру клітин, відіграють важливу роль у спілкуванні між клітинами, у зменшенні певних пухлин, у формуванні метастазів, у формуванні кровоносних судин, а також відіграють основну роль в регенерації тканин.

Екзосоми були відкриті майже п'ятдесят років тому. Сьогодні, завдяки різнобічному використанню, вони стали важливими елементами повсякденної науки. Їх основна функція полягає в тому, щоб сприяти генетичній мінливості. Це відбувається насамперед через те, що вони здатні передавати генетичний матеріал між різними видами і навіть між рослинами та тваринами. Експерименти з практичного використання екзосом в терапії пухлин, лікуванні аутоімунних захворювань або лікуванні інфекційних захворювань все ще тривають.

У рідинах організму циркулює велика кількість мікровезикул, які відіграють важливу роль у міжклітинній комунікації та в процесах детоксикації та регенерації клітин. Вчені активно досліджують роль, яку можуть відігравати екзосоми в передачі сигналів між клітинами; передбачається, що екзосоми, будучи здатними з'єднуватися з віддаленими клітинами і виливати в них свій вміст, тим самим здатні впливати на процеси, що відбуваються в далеких клітинах. З'ясування точної ролі екзосом може відкрити багато нових можливостей у майбутньому, від їх використання як біомаркерів до використання як терапевтичних мішеней.

Дана стаття підсумовує найосновніші дослідження мікровезикул та їх значення в клітинній і молекулярній біології.

Ключові слова: екзосоми, мікровезикули, аутоімунні захворювання, біомаркери, клітинна та молекулярної біології.

REFERENCES

1. Clotilde Théry, Matias Ostrowski & Elodie Segura. (2009). Membrane vesicles as conveyors of immune responses. *Nat. Rev. Immunol*., 9, 581-593. doi:10.1038/nri2567.
2. Eberhard G. Trams a, Carl J. Lauter a, Jr. Norman Salem a, Ursula Heine b (2981). Exfoliation of membrane ecto-enzymes in the form of micro-vesicles Biochim. *Biophys. Acta,* Vol. 645, Issue 1, 63-70.
3. Mishra N.C., Tatum E.L. (1973). Non-Mendelian inheritance of DNA-induced inositol independence in Neurospora. *Proc. Natl. Acad. Sci.,* 70(12), 3875-9.
4. Johnstone, R.M., Adam, M., Hammond, J.R., Orr, L. and Turbide, C. (1987). Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J. Biol. Chem,* 262, 9412–9420.
5. Yang C, Robbins P.D. (2011). The roles of tumor-derived exosomes in cancer pathogenesis. *Clin Dev Immunol,* 842849.
6. Peters, C., and Mayer, A. (1998). Ca2+/calmodulin signals the completion of docking and triggers a late step of vacuole fusion. *Nature,* 396, 575–580.
7. György Nagy, Bence György, Zsuzsanna Baka, Károly Módos, Éva Pállinger, Krisztina Pálóczi, Mária Pásztói, Petra Misják, Mária Deli, Áron Sipos, Anikó Szalai, István Voszka, Anna Polgár, Kálmán Tóth, Mária Csete, Steffen Gay, András Falus, Ágnes Kittel, Buzás Edit: Microparticles and the vasculature (DROP3 lecture, 28.10.2012. URL: www.rheumatology.hu/.../E11-Nagy-Gyorgy-Microparticulumok.ppt
8. Jeffrey S. Schorey, Sanchita Bhatnagar: Exosome Function: From Tumor Immunology to Pathogen Biology. *Traffic*, 9, 871-881. doi: 10.1111/j.1600-0854.2008.00734.x
9. Buzás Edit (2011). The extracellular vesicular compartment. Huzella Tivadar memorial lecture. URL: https://docs.google.com/viewer?a=v&q=cache:iO8bg6boy1kJ:semmelweis-egyetem.hu/hirek/files/2011/11/Buz%25C3%25A1s-Edit-Huzella-el%25C5%2591ad%25C3%25A1s.pdf+nanoparticle+ tracking+analysis&hl=hu&gl=hu&pid=bl&srcid=ADGEEShgLT1ITJrBIZ1YjqANhTtAJ7qs92UdnYFifLew4wiKYIjUjWuc6uJtwgreKwHx_N8mt 5CsVWYaeGWI9VaRAj69G9PZj0jcf9sWyVgy7oqEFyyF0w2Cqkt_sR94C_xvzRFuipRA&sig=AHIEtbSfFAzpaM5kavMxvp4qSipkwdpbsQ
10. Erna Pap, Éva Pállinger, András Falus (2009). How do cells talk? Or micro-vesicles as newly recognized actors of communication between cells. *Magy Tud.,* 12, 1488–1495.
11. Atay, S., Gercel-Taylor, C., Kesimer, M., and Taylor, D. D. (2011). Morphologic and proteomic characterization of exosomes released by cultured extravillous trophoblast cells. *Exp. Cell. Res.,* 317, 1192–1202.

12. Wang, Z., Hill, S., Luther, J.M., Hachey, D.L. and Schey, K.L. (2012). Proteomic analysis of urine exosomes by multidimensional protein identification technology (MudPIT). *Proteomics*, 12, 329–338. doi: 10.1002/pmic.201100477).
13. Yuyama K, Sun H, Mitsutake S, et al. (2012). Sphingolipid-modulated exosome secretion promotes the clearance of amyloid-β by microglia. *J. Biol. Chem.,* Feb 2.
14. He L, Hannon G.J. (2004). MicroRNAs: small RNAs with a big role in gene regulation. *Nat. Rev. Genet.,* 5, 522–531.
15. Matkó J. (2011). Communication between the cells of the natural and adaptive immune systems Lecture at ELTE. 2011.12.01.
16. Raposo G., Nijman H.W., Stoorvogel W., Liejendekker R., Harding C.V., Melief C.J., Geuze H.J. (1996). B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.,* 183, 1161–1172.
17. Mária Szente-Pásztói (2009). Investigation of the role of regulatory and effector factors in the pathomechanism of autoimmune diseases, Doctoral thesis – Semmelweis University Molecular Medical Sciences Doctoral School, Budapest. URL: phd.sote.hu/mwp/phd_live/vedes/export/szentepaztoimaria. m.pdf
18. Douglas D. Taylor, Sibel Akyol, and Cicek Gercel-Taylor (2006). Pregnancy-Associated Exosomes and Their Modulation of T Cell Signaling. *J. Immunol.*, 176, 1534–1542.
19. Cecilia Lässer, Vesta Seyed Alikhani, Karin Ekström, Maria Eldh, Patricia Torregrosa Paredes, Apostolos Bossios, Margareta Sjöstrand, Susanne Gabrielsson, Jan Lötvall and Hadi Valadi (2011). Human saliva, plasma and breast milk exosomes contain RNA: uptake by macrophages. *J. Transl. Med*., 9, 9. doi: 10.1186/1479-5876-9-9
20. Parolini I, Federici C, Raggi C et al. (2009). Microenvironmental pH is a key factor for exosome traffic in tumor cells. *J. Biol. Chem,* 284, 34211–34222.
21. Redman C.W., Tannetta D.S., Dragovic R.A., et al. (2012). Does size matter? Placental debris and the pathophysiology of pre-eclampsia. *Placenta,* Feb., 48–54.
22. Valadi H., Ekstrom K., Bossios A., Sjostrand M., Lee J.J., Lotvall J.O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell. Biol.,* 9, 654–659.
23. Beatty, W.L., Rhoades, E.R., Ullrich, H.-J., Chatterjee, D., Heuser, J.E. and Russell, D.G. (2000). Trafficking and Release of Mycobacterial Lipids from Infected Macrophages. *Traffic*, 1, 235–247. doi: 10.1034/j.1600-0854.2000.010306.x
24. Stephen J. Gould*, Amy M. Booth*, and James EK Hildreth: The Trojan exosome hypothesis (2003). *Proc. Natl. Acad. Sci.,* Vol. 100, no. 19, 10592–10597.

25. In November 2011, Mikkel Noerholm, director of European operations at Exosome Diagnostics, reported on his company's latest research results in his presentation entitled Microvesicles for Non-Invasive Molecular Diagnostics held at the boimarker conference in Munich. URL: http://www.google.hu/url?sa=t&rct=j&q=biofluid%20molecular%20diagnostics&source=web&cd=6&sqi=2&ved=0CGcQFjAF&url=http%3A%2F%2Fwww.m4.de%2Fuploads%2Fmedia%2FS1-4-Noerholm_Exosome_Diagnostics.pdf&ei=BtEzT63yDMGk-gaRpYWkAg&usg=AFQjCNE1isshsPNJj_1_a33nvqNUHCSuZQ
26. Iero M., Valenti R., Huber V., Filipazzi P., Parmiani G., Fais S., Rivoltini L. (2008). Tumour-released exosomes and their implications in cancer immunity. *Cell Death Differ,* 15(1), 80-8. Epub 2007 Oct 12.)
27. Malin Karlsson, Samuel Lundin, Ulf Dahlgren, Helena Kahu, Inger Pettersson, Esbjörn Telemo (2001). "Tolerosomes" are produced by intestinal epithelial cells, *Eur. J. Immunol,* 31(10), 2892-2900.
28. Andreola G., Rivoltini L., Castelli C., Huber V., Perego P., Deho P., Squarcina P., Accornero P., Lozupone F., Lugini L., Stringaro A., Molinari A., Arancia G., Gentile M., Parmiani G. et al. (2002). Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. *J. Exp. Med.,* 195, 1303–1316.
29. O'Connell J., Bennett M.W., O'Sullivan G.C., Collins J.K., Shanahan F. (1999). The Fas counterattack: cancer as a site of immune privilege. *Immunol. Today,* 20(1), 46–52.
30. Clayton A., Mason, M.D. (2009). Exosomes in tumor immunity. *Curr. Oncol.,* 16(3), 46-9.
31. Zitvogel L., Regnault A., Lozier A., Wolfers J., Flament C., Tenza D., Ricciardi-Castagnoli P., Raposo G., Amigorena S. (1998). Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat. Med.,* 4(5), 594–600.
32. Hartman Z.C., Wei J., Glass O.K., et al. (2011). Increasing vaccine potency through exosome antigen targeting. *Vaccine,* 29(50), 9361-7.
34. Kuate S., Cinatl J., Doerr H.W., Uberla K. (2007). Exosomal vaccines containing the S protein of the SARS coronavirus induce high levels of neutralizing antibodies. *Virology,* 362, 26–37.
35. Kim S.H., Bianco N.R., Shufesky W.J., Morelli A.E., Robbins P.D. (2007). MHC class II+ exosomes in plasma suppress inflammation in an antigen-specific and Fas ligand/Fas-dependent manner. *J. Immunol.* 179, 2235–2241.
36. Ruenn Chai Lai, Tian Sheng Chen and Sai Kiang Lim (2011). Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease. *Regen. Med.,* 6(4), 481-92.

37. Sumer B., Gao J. (2008). Theranostic nanomedicine for cancer. *Nanomedicine (Lond),* 3(2), 137-40.
38. Schrader J. (1985). Mechanisms of ischemic injury in the heart. *Basic Res. Cardiol.,* 80(Suppl. 2), 135–139.
39. Luciano C.P. Azevedo, Marcelo A. Pedro, Francisco R.M. Laurindo. (2007). Circulating microparticles as therapeutic targets in cardiovascular diseases. *Recent. Pat. Cardiovasc. Drug. Discov.,* 2(1), 41–51.
40. Vickers, K.C., Palmisano, B.T., Shoucri, B.M., Shamburek, R.D., Remaley, A.T. (2011). MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat. Cell Biol.,* 13, 423–433.
41. Street J.M., Barran P.E., Mackay C.L., et al. (2012). Identification and proteomic profiling of exosomes in human cerebrospinal fluid. *J. Transl. Med.,* 10(1), 5.
42. Bas W.M. van Balkom, Trairak Pisitkun, Marianne C. Verhaar and Mark A. Knepper (2011). Exosomes and the kidney: prospects for diagnosis and therapy of renal diseases. *Kidney Int.,* 80, 1138–1145. doi: 10.1038/ki.2011.292.
43. Kosaka N., Ochiya T. (2011). Unraveling the Mystery of Cancer by Secretory microRNA: Horizontal microRNA Transfer between Living Cells. Front. Genet., 97.
44. Kwak, P.B., Iwasaki, S., and Tomari, Y. (2011). The microRNA pathway and cancer. *Cancer Sci.,* 101, 2309–2315.
45. Stroun, M., Anker, P., Beljanski, M., Henri, J., Lederrey, C., Ojha, M., and Maurice, P.A. (1978). Presence of RNA in the nucleoprotein complex spontaneously released by human lymphocytes and frog auricles in culture. *Cancer Res*., 38, 3546–3554.
46. Wieczorek, A.J., Sitaramam, V., Machleidt, W., Rhyner, K., Perruchoud, A.P., Block, L.H. (1987). Diagnostic and prognostic value of RNA-proteolipid in sera of patients with malignant disorders following therapy: first clinical evaluation of a novel tumor marker. *Cancer Res*., 47, 6407–6412.
47. Garzon, R., Marcucci, G., and Croce, C.M. (2010). Targeting microRNAs in cancer: rationale, strategies and challenges. *Nat. Rev. Drug. Discov*., 9, 775–789.
48. Lim, P.K., Bliss, S.A., Patel, S.A., Taborga, M., Dave, M.A., Gregory, L.A., Greco, S.J., Bryan, M., Patel, P.S., Rameshwar, P. (2011). Gap junction-mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. *Cancer Res.,* 71, 1550–1560.
49. Arroyo, J.D., Chevillet, J.R., Kroh, E.M., Ruf, I.K., Pritchard, C.C., Gibson, D.F., Mitchell, P.S., Bennett, C.F., Pogosova-Agadjanyan, E.L., Stirewalt, D.L., Tait, J.F., Tewari, M. (2011). Argonaute2 complexes carry a

population of circulating microRNAs independent of vesicles in human plasma. *Proc. Natl. Acad. Sci.,* 108, 5003–5008.
50. Hood, J.L., San, R.S., Wickline, S.A. (2011). Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. *Cancer Res.,* 71, 3792–3801.
51. Molnar, A., Melnyk, C.W., Bassett, A., Hardcastle, T.J., Dunn, R., and Baulcombe, D.C. (2010). Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science,* 328, 872–875.
52. Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., Li, J., Bian, Z., Liang, X., Cai, X. ., Yin, Y., Wang, C., Zhang, T., Zhu, D., Zhang, D., Xu, J., Chen, Q., Ba, Y., Liu, J., Wang, Q. ., Chen, J., Wang, J., Wang, M., Zhang, Q., Zhang, J., Zen, K., and Zhang, CY (2011). Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Res*. PMID: 21931358.
53. Gan X., Gould S.J. (2012). HIV Pol Inhibits HIV Budding and Mediates the Severe Budding Defect of Gag-Pol. *PLoS One,* 7(1), e29421.
54. Nabhan J.F., Hu R., Oh R.S., Cohen S.N., Lu Q. (2012). Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. *Proc. Natl. Acad. Sci.,* Feb 6.
55. Lässer C., O'Neil S.E., Ekerljung L., Ekström K., Sjöstrand M., Lötvall J. (2011). RNA-containing exosomes in human nasal secretions. *Am. J. Rhinol. Allergy,* 25(2), 89-93. Epub 2010 Dec 17.
56. Choi D.S., Yang J.S., Choi E.J., et al. (2012). The protein interaction network of extracellular vesicles derived from human colorectal cancer cells. *J. Proteome Res.,* 11(2), 1144-51.
57. http://www.globenewswire.com/newsroom/news.html?d=218181
58. http://www.carislifesciences.com/blood-based-diagnostics
59. http://www.nanosight.com/technology/nanosights-technology
60. http://www.malvern.com/LabEng/technology/dynamic_light_scattering/dynamic_light_scattering.htm
61. Mathivanan S., Fahner C.J., Reid G.E., et al. (2012). ExoCarta 2012: database of exosomal proteins, RNA and lipids. *Nucleic. Acids. Res.,* 40, D1241-4.
62. Dr. Paul Harrison (2010). How to detect and measure microparticles (Presentation abstract, 2010-06-11. URL: http://www.multiwebcast.com/eha/ 2010/15th/4889/paul.harrison.how.to.detect.and.measure.microparticles.html.