Review

Gergely Milosevits*, János Szebeni and Silke Krol

Exosomes: potential model for complement-stealth delivery systems

Abstract: Exosomes are nature's nanocarriers that transport biological information in humans. Their structural properties, origin and functions are making them interesting objects for the diagnosis of diseases, such as cancer, and also, as innovative tools for drug delivery. The interaction of exosomes with the immune system has been one of the focal points of interest; nevertheless their "stealth" properties helping to avoid adverse immune reactions are still not fully understood. In this review, after giving an overview of recent findings on the role of exosomes in disease pathogenesis and physiological functions, we focused on their interaction with the immune system and possibilities for clinical applications. The potential of exosomes of creating stealth nanoparticles that are better tolerated by the immune system than the presently available synthetic drug delivery systems represent a promising new approach in nanomedicine.

Keywords: complement; drug delivery; exosome; liposome; nanocarrier.

Introduction: nanocarriers of nature

In the field of bio-engineering scientific efforts often mimic nature (1–4), this can be said of liposomes. When Bangham and Horn first visualized phospholipid bilayers under the electron microscope 50 years ago, they thought they reproduced the bilayer membrane of an archetypical cell (5). They could hardly have suspected that two decades later a more elaborate natural design for liposomes would be discovered, in the form of exosomes. Exosomes were first described in the 1980s by Johnstone et al. (5) and were defined as vesicles formed in the endosomal compartments (multivesicular endosomes) which then get secreted into the extracellular space (Figure 1) to serve as nano-rafts carrying biological information between cells. Hence, they play a central role in intercellular communication (5). Exosomes have been found to originate from various types of cells in the body, including stem cells and fully differentiated cells. Their most important feature as compared to the endosomes is that the extracellular leaflet of the plasma membrane is fully preserved as an extracellular part of the exosomes.

Exosomes are defined by a size ranging from 30 to 100 nm (6). Their exact size differs according to their origin. The structure of exosomes depends to the cell type they originate from, as well as on the function they play in intercellular communication (7). Another name which is used interchangeably is microvesicles, which describe 100–1000 nm vesicular structures. But microvesicles are less precisely defined and cover both intra- and extracellular vesicles while exosomes are only the extracellular vesicles (8). In the following sections we will focus on the structure and function of exosomes and on their interplay with the immune system as well as what role their special properties may play in future medical applications.

Structure of exosomes

It follows from the mechanism of exosome formation (Figure 1) that the content of exosomes contains mainly cytosol derived molecules, such as miRNA, mRNA, proteins, peptides, enzymes (9–13) and, as confirmed recently, also dsDNA (14, 15). Details about the content and the composition of exosomes can be found on the web

*Corresponding author: Dr. Gergely Milosevits, 2nd Department of Pediatrics, Semmelweis University, Tüzoltó u. 7-9., Budapest, Hungary, Phone: 36-30-8425722, E-mail: ikkuma@gmail.com; and Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary
János Szebeni: Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary; and SeroScience Ltd, Budapest, Hungary
Silke Krol: Fondazione IRCCS Istituto Neurologico "Carlo Besta", IFOM-IEO-campus, Milan, Italy
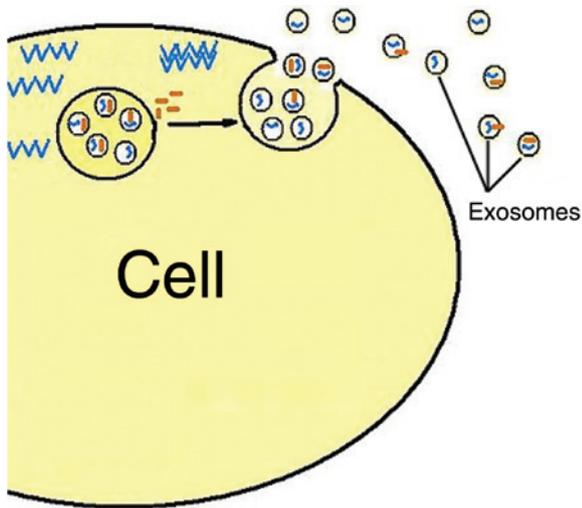
Figure 1: Mechanism of exosome release.

(http://www.exocarta.org/) provided by Mathivanan and Simpson (16). Extensive lists of molecules detected and identified in exosomes and microvesicles can be found in some excellent recently published reviews and research papers (8, 11, 12, 17–19). Figure 2 shows electron-microscopic images of exosomes and microvesicles that differ in content, shape and membrane structure.

The proteins attached to the lipid bilayer of exosomes (arrows) originate from the plasma membrane which is preserved its original orientation. They cover a broad spectrum of immune-modulating and cell recognizing
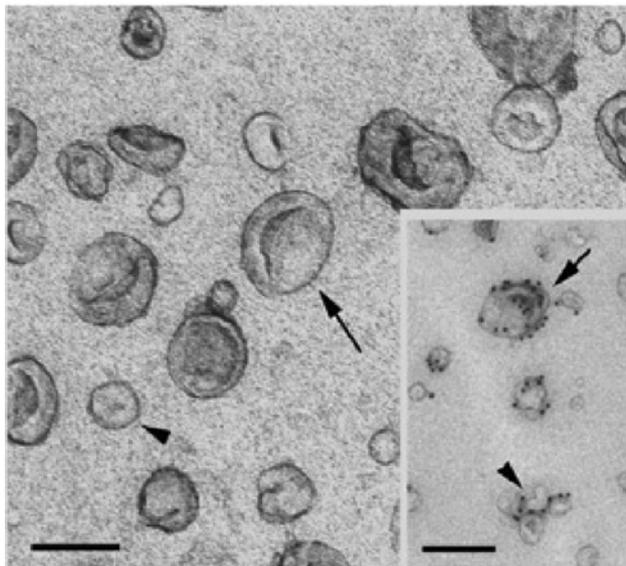

Figure 2: Electron micrographs of dendritic cell derived exosomes and microvesicles. The size bars in the EM images indicate 100 nm. The images were reproduced with permission from (19).

molecules that are either common, ubiquitous proteins or cell-type specific proteins. The former group includes cytoskeletal proteins, such as actin and tubulin, membrane transport and fusion proteins (annexins and Rab proteins), integrins and proteins belonging to the heat-shock family (immune activators such as Hsp70, Hsc70 and Hsp90). The cell-type specific proteins include MHC class-I and class-II proteins, which present antigens, tetraspanins (CD63, CD81, CD82, CD9 and CD86) which are involved in cell-cell contacts and in selective binding to certain target cells (20). As discussed later in detail, the surface exposed proteins have different roles including the targeting of exosomes to specific cells and modulating the immune response via activation or suppression. Additionally it has to be mentioned that the fact that endogenous exosomes are made from fragments of the plasma membrane in the preserved original orientation means that they also inherit the glycome, the glycocalyx from the originating cells with its innate immune tolerance.

Physiological functions of exosomes

The first exosomes that were documented originated from circulating blood cells, particularly reticulocytes (5). Since then the importance of this phenomenon has been confirmed, further adding to the understanding of red blood cell differentiation (21). Platelets also shed exosomes, some of which may inhibit aggregation and act against thrombosis (22). White blood cell derived exosomes have several functions, the most complex one is the modulation of the immune system. These cell derived nano-vesicles mediate antigen presentation, which is one of the basic mechanisms of adaptive immunity and as such, has been a focal point of interest in immunology research (23). Recent publications revealed very complex roles of exosomes in immune modulation (24). Tracking of exosome release from immune cells was achieved by Soo et al., using antibody connected magnetic beads for visualizing the dynamics of this process (25). Exosome-mediated immune responses in tumor patients (26) represents a special new field that will be discussed below in detail.

Role in disease pathogenesis

Exosomes play a central role in the manifestation and progression of several diseases, as well as in drug resistance,

therefore mapping their functions in intercellular communication is essential in understanding the pathomechanism of these medical problems.

Several recent publications discuss the function of exosomes in oncogenesis, tumor cell exchanges and metastatic activities of tumors. Exosomes can transfer oncogenic materials which affect organization of tumor cells and the progression of a tumor. Intercellular communication can happen through the delivery of genetic information in the form of microRNA that is delivered from tumor cells to normal or pathological cells (27). Exosomes are pivotal in shielding tumor cells from the immune system, and at the same time induce inflammatory and angiogenetic responses helping tumor cell adhesion and metastatic growth (28).

In infections by different pathogens (viral, bacterial, fungal and parasite) exosomes play a key role in several areas. The pathomechanism of oncogenesis after specific viral infections, such as Epstein-Barr virus, has been recently connected to viral RNA carried by exosomes, creating an intercellular pathway for genetic information passage, leading to tumor formation (29). In HIV infection replication of the virus is helped by the presence of exosomes derived from HIV expressing cells (30). Exosomal delivery of virus components and proteins are essential for disease progression in human T-lymphotropic virus type 1 infections (31). The pathomechanism of parasite infections in some cases involves exosomes, where the vesicles can induce adhesion of the pathogen (32).

In neurologic disorders exosomes can be important factors. Exosomal communication between microglia and neurons of the brain is a way for the central nervous system to modulate the pathology of amyotrophic lateral sclerosis, which is the most common and most aggressive form of adult motor neuron degeneration (33). Exosomes can be a reason behind drug resistance in neurological diseases and cancer, an example of this can be found in the treatment of multiple myeloma (34). Exosomes have been identified as mediators of neuroinflammation after injuries of the central nervous system and have been implemented as potential therapeutic agents (35).

In the field of internal medicine the investigations of exosomal pathways present a new perspective on the pathomechanism of several diseases. A recent study found that exosomes may affect glycemic control in diabetes via the adiponectin pathway (36). It has also been observed that exosomes released by pancreatic cancer cells may play a role in the induction of diabetes associated with this type of malignancy (37). Exosomes coming from adipocytes can induce liver pathology as they deregulate hepatocytes, leading to obesity-related liver disease (38).

Exosomes and the immune system

Exosomes are frequently exposed to the immune system both in health and disease and developed or expose mechanisms to avoid recognition, or they modulate the immune system to induce immune tolerance. The interaction of exosomes with the immune system depends on their origin; on antigens derived from diseased cell (e.g., tumor or virus-infected cell), and on immune modulating molecules enclosed in the vesicular container. As to how much exosomes are able to interfere with the immune system was described in detail in a review by Thery (8).

Evidence for the appearance of microvesicular structures and exosomes in the early fetal development was provided for exosomes containing FasL ligand (39). This molecule is involved in many immune modulating activities, such as self-tolerance in T cells towards fetal tissue during gestation, progression of autoimmunity, clonal deletion of activated T cells, B-cell regulation and the establishment of immune privilege in certain organs, such as brain, ovary, testis, pregnant uterus, placenta and eye (40). Two mechanism are proposed as to how the FasL ligand induces immune tolerance: i) apoptosis in the relevant antigen-specific lymphocytes that respond to the administered antigen (41, 42) and ii) the uptake of FasL induced apoptotic cells by antigen-presenting cells (such as dendritic cells) and, in consequence, modulation of the activation of regulatory cells (43). The same mechanism was shown to be hijacked by tumors to induce immune tolerance (39, 44, 45).

Additionally, immune modulation can be gained by release of small (40 nm) exosome-like vesicular structures presenting MHC class II with antigens called "tolerosomes" (46). Another mechanisms for exosomes to modulate the immune system is by delivering microRNA enclosed in the exosomes into the cytosol of other cells.

Despite being effective modulators of immune response, exosomes are not recognized by the immune system as being foreign. Thus, they are not immunogenic and are not known to induce nonspecific innate immune responses, such as complement (C) activation or C activation-related pseudoallergy (CARPA), which is an immune barrier to the therapeutic use of liposomes and many other nanoparticle based i.v. drugs (47). Amazingly, there is no visible immune attack against exosomes when they get into the circulation. The explanation probably lies in the fact that exosomes are derived from the membranes of self-cells, against which the body develops specific and nonspecific tolerance.

The immunity of exosomes from immune destruction is due to an "inheritance" of surface molecules from their original cell that protects them from recognition. As to the

Table 1: Molecules on exosomes allowing escape of immune recognition.

Molecules	Physiologic function	Literature
Complement modulators		
CD46	Membrane cofactor protein; control of C3	41
CD55	Decay-accelerating factor control of C3	42, 43
CD59	Control of MAC (membrane attack complex inducing pore formation) via C9	41, 42
CK2	Phosphorylates C9 and prevents lysis	44
Specific tolerance inducers		
Human leucocyte antigen (HLA)-G class I molecule	Induces immune tolerance to tumors	45
MHC class I and II +antigens	Induce immune tolerance to tumors	46
FasL	Induce apoptosis in activated T cells	44

question, which surface molecules might provide this property, Table 1 may give the answer, that is further visualized by Figure 3. It shows the molecules present on the surface of exosomes, which include 4 C inhibitors, each of which is known to be effective inhibitor of C activation. In addition, the surface of exosomes contain three molecule types that effectively induce specific tolerance.

As mentioned before, another immune interacting aspect of exosomes that should not be underestimated is the fact that the exosome lipid membrane derives from endogenous cell membranes and the outer leaflet of the plasma membrane is also the outer leaflet of the exosomes. With this orientation they inherit the immune tolerance induced by the glycocalix of the cells. In several works the importance of the glycome for innate immunity (48, 49) and the recognition of different or foreign glycan patterns
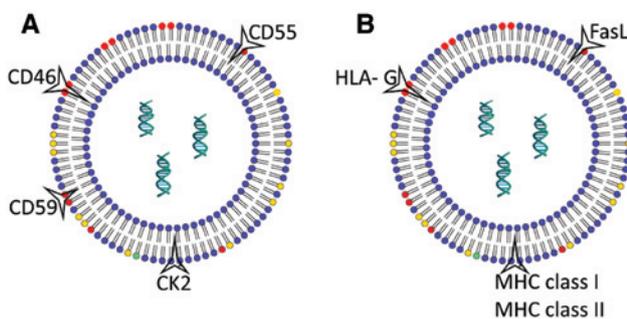
Figure 3: Exosomes with complement system modulator (A) and other immune-tolerance promoting molecules (B).

by the immune system (49–51) were emphasized. That the disguise of the immune system is most effective if the glycome is similar is supported by the observation about the immune evasion of HIV-1 and their glycome similarity with microvesicles. HIV-1 and microvesicles from T cells share a common glycome, arguing for a common origin (48).

Potential of exosomes for clinical applications

There are several approaches that make use of the specificity and function of exosomes in the intercellular communication of tumors. An area of increasing interest in the field of oncology is cancer immunotherapy, where exosomes have been shown to have a therapeutic potential (52, 53). Exosomes containing antigens of specific tumors can induce an immune response against cancer cells containing their antigen spectrum. These acellular structures can activate cytotoxic T cells which leads to apoptosis of the tumor cells they are directed against. Creating vaccines based on specificity of tumor-derived exosomes which contain the biomarkers necessary to target tumor cells can be a realistic option for immunotherapy (54–58). One possibility was outlined in a recently published article using exosomes derived from dendritic cells to induce antitumor immunity (59). The tumor derived exosomes present a first therapeutic vaccine with antigen-presenting dendritic cell derived exosomes approved in 2010 by the name of Provenge® for the personalized treatment of metastatic prostate cancer (60). So far it is the only FDA approved exosomes based cancer immune therapy.

The discussion about the medical benefit of Provenge® was controversial. The best benefit for the patients was found for low PSA (prostate specific antigen) levels. In a 512 patients double-blind, placebo-controlled, multicenter phase 3 trial study a general improved survival in the treated patients was found to be 4.1 months with no effect on the tumour progression or decrease PSA levels (61). This improved survival was the reason for approval by the FDA and in 2013 also by EMA (European Medical Agency) and seems to be closely related to an activated immune system. There is still a critical discussion about the usefulness of PSA levels as prognostic markers in prostate cancer and if the high price of the treatment is justified but the approach to use dendritic cell derived exosomes was followed up in other clinical trials for the standalone treatment or combination therapy for other types of cancer (62).

Another option for a cancer therapy involves the general removal of exosomes from the circulation (6). This idea stems from the fact that exosomes play an important role in the pathomechanism of tumor progression and can also interfere with anti-tumor chemotherapy and lead to drug resistance. But one of the drawbacks is that the approaches used so far remove all exosomes, also the ones released from healthy cells and involved in cell-cell communications leading to adverse effects. A possible solution for this problem might be the selective removal of tumor-derived exosomes. With increasing knowledge about the mechanisms of immune escape and immune modulation of cancer derived exosomes it may be possible to identify molecules only present on the surface of these exosomes.

Exosomes for diagnosis and follow-up of medical conditions

Understanding the function of exosomes in the pathomechanism of diseases is an important step towards creating diagnostic strategies based on detecting disease-specific exosomes. Structural properties of these nanocarriers can be harnessed as biomarkers for various diseases, which can be detected in several ways. This strategy has been recently implemented in the following cases.

Diagnosis, risk assessments and follow-up examinations of laryngeal squamous cell carcinoma can be achieved by using exosomal biomarkers specific to the tumor cells (63). In patients with colorectal carcinoma, exosomes that contain tumor-specific antigens can be extracted from ascetic fluid or the plasma. These entities may be used as a fast and effective diagnostic tool (64, 65). In pancreatic cancer, the protein and microRNA content of tumor-specific exosomes can be analyzed in order to increase the effectiveness of diagnosis and complement available diagnostic strategies (66). Exosomes that contain different cargos and receptors, like epidermal growth factor receptor, in their structure can be used in the diagnosis of lung cancer (67, 68).

Exosomes and their microRNA contents are important factors in the pathomechanism of *asthma bronchiale* and other pulmonary conditions, where exhaled breath can be a source of exosome extraction. This has a potential to create an easy way of quantifying disease specific biomarkers for the follow-up controls of pulmonary diseases and also a possible diagnostic tool (69).

A recent review gives a concise overview of the clinical applications of exosomes (70). Another appealing application of exosomes is their use as a stealth drug delivery system which we will discuss in the next paragraph.

Exosomes as immune-tolerated delivery systems

Mapping tumor-specific exosomes in the body is important for diagnosis, but using their structural and "stealth" properties is a promising solution for creating novel highly specific immune-tolerated therapeutic systems. As mentioned before this approach failed if using synthetic liposomal delivery systems which can cause immune activation to an extent that can be fatal for the patient (71, 72). Creating a stable formulation that mimics the structure and function of exosomes is a complex task and has to be the result of careful consideration of previous trials and errors in the field of nanomedicine (73, 74).

Tumor cell derived exosomes that are synthesized based on the tumor cell's antibody spectrum also present a novel delivery solution. Hereby exosomes loaded with different agents (such as diagnostic particles or drugs) specifically bind to the target cells creating a therapeutic accumulation in a specific area. Modeling of the exosomal structure has been already proven to work on a large-scale basis (75). In a recent publication the potential use of exosomes combined with staphylococcal enterotoxin B in the therapy of pancreatic cancer was described (76). Exosomes were extracted from the tumor and based on their specific antigen biomarker could reach tumor sites, where the enterotoxin was unloaded and induced apoptosis of tumor cells.

There is an important feature of tumor-derived exosomes that needs to be kept in mind and needs close monitoring during their use for treatment and that is their involvement in preparing a niche for circulating cancer cells in the proccess of metastasis formation in distant tissues (77–80). While this property is of minor importance for cancer immune therapy it can be important for drug delivery as the equipment of molecules on the exosomes surface can affect auto-immunity and binding to unknown distant organs (81, 82). But Jung et al. also showed that for the pre-metastatic niche and cancer formation some soluble factors and circulating cancer cells are required (78).

There are also several medical fields, other than oncology, where the potential therapeutic use of exosomes may be possible. A recently published article in the field of cardiology pinpoints microRNA containing exosomes as the mediators of cardiac muscle regeneration after ischemic stress. This phenomenon can be used in direct therapy,

if these particles can be engineered ex vivo before being given to the patients directly (83).

Another important field were the immune modulating properties of exosomes were explored for therapeutic applications are dendritic cell-derived Fas L contain and/or antigen presenting exosomes for inflammatory diseases such as arthritis (84).

There are several ongoing preclinical studies on the therapeutic uses of exosomes, which show the potential for clinical use in the treatment of several medical conditions (85). While dendritic cell-derived exosomes were mainly used to induce immune response other cell type released exosomes were used for their potential as a stealth drug delivery system. Lai et al. reviewed e.g. the utility of mesenchymal stem cell-derived exosomes as a vehicle for drug delivery (86). In this review Lai and his co-worker identified the loading of the exosomes without disturbing their unique stealth properties as the major obstacle for the use as drug delivery system mainly for interfering RNA. The loading can be done during exosomes biogenesis in the cells or afterwards by electroporation or incubation with lipofectamine. Both techniques are likely to change the membrane of the exosomes as lipofectamine® is known to decrease cell viability as a positively charged polymer by nanoporation of the plasma membrane and depolarization of the mitochondrial membrane (for a review see e.g. the effect of surface properties on nanoparticle-cell interactions (87). As the interaction between membrane and polycation is electrostatic it is unlikely that the effect is reversible and a resealing will take place.

On the other hand electroporation was successfully investigated for some types of siRNA while for other type of RNA such as mRNA, miRNA or shRNA the approach was unsuccessful. A third strategy which was tested was the overexpression of RNA by the exosomes producing cells which allows even the incorporation of fully functional miRNA and mRNA (88).

That exosome loading with drug molecules or therapeutic proteins, peptides, and hormones is feasible is clearly indicated by the negative example of the
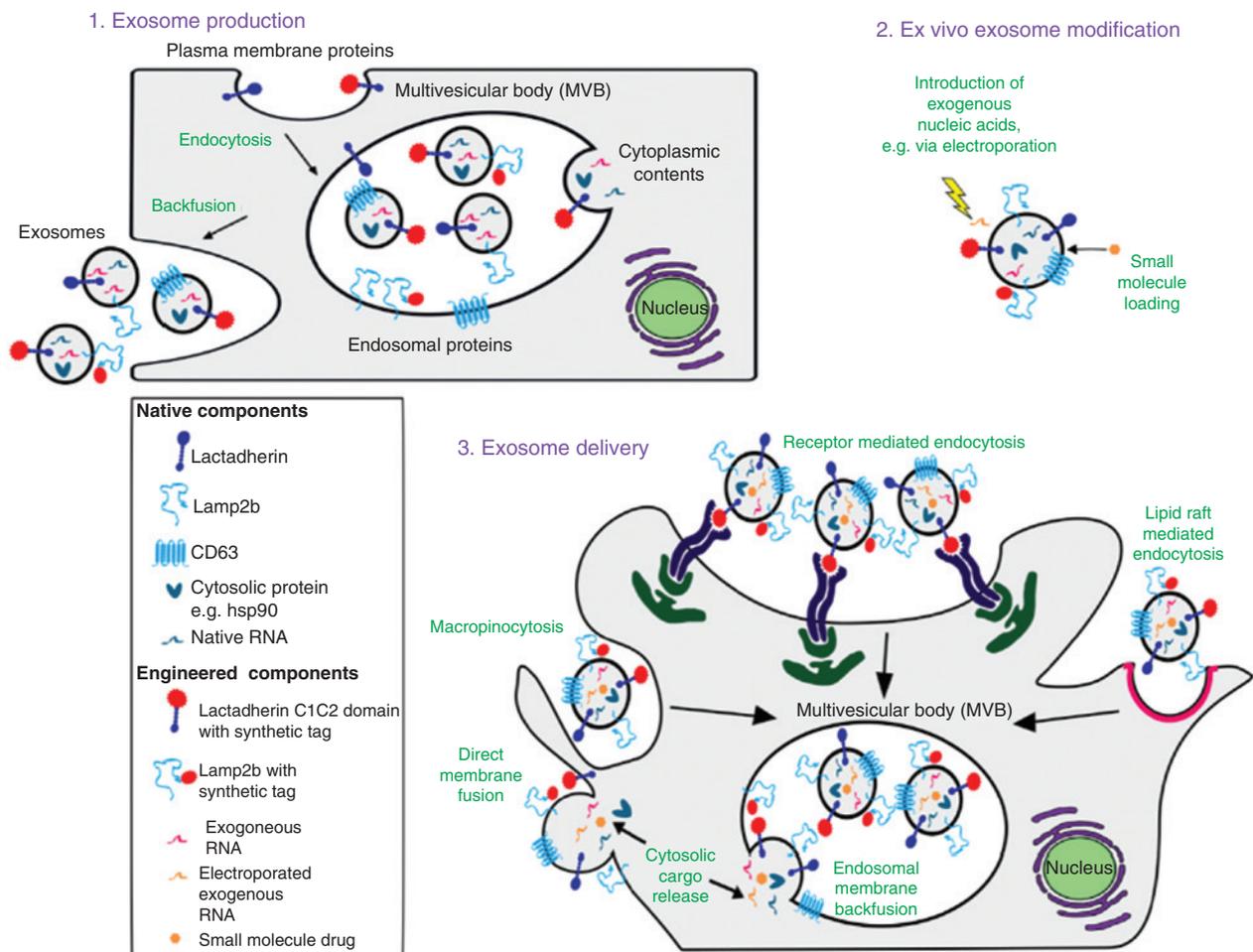

Figure 4: Exosomes based drug delivery (reproduced from the work of Marcus and Leonard) (88) (Permission has been granted by the authors).

Table 2: Exosomes based drug delivery [reproduced from Marcus and Leonard 2013 (88)] (Permission has been granted by the authors).

Exosome source (H=human cell)	Recipient cell type	Cargo delivered	Functional consequences
Immunosuppressive effects			
(H) EBV transformed B cells	Monocyte-derived DC	Viral miRNA	Down-regulation of antiviral response
Serum of pregnant human patients	(H) Jurkat T cells	FasL	Suppression of CD3 signaling and IL-2 production
Murine BMDC overexpressing IL-10	Murine T cells	Antigen, presented on MHCII	Suppression T cell proliferation
Immunostimulatory effects			
Murine BMDC	Murine CD8 and CD4 T cells (in vitro and in vivo)	Antigen, presented on MHC	Induction of T cell proliferation
CD28 stimulated CD3 T cells (H)	Unstimulated CD3 T cells (H)	Unidentified	T cell activation, induction of proliferation and cytokine production when co-delivered with IL-2
Murine BMDC	Murine BMDC (allogeneic)	Antigen	Transfer of foreign antigen, followed by foreign antigen presentation to and activation of T cells
PMBC derived DC incubated with HIV	Jurkat T cell line expressing CCR5	HIV viral particles	Delivery of functional HIV viral particles encapsulated in exosomes, leading to HIV infection of recipient cells

pathogenic exosome-loading of prion (89), or other disease-related proteins (90). An overview summarizing the progress made so far for exosomes as a drug delivery system mainly for therapeutic RNA in cancer therapy was published by Johnsen et al. (91) and even for a broader application by Marcus and Leonard, which is visualized in Figure 4 (88) and Table 2 (88).

But the first step in using the exosomes, both for diagnosis and for therapeutic applications is the isolation of the intact exosomes from body fluids such as urine (92), blood, semen (93) and others (9, 11, 94) which will be described with detection and characterization methods in the next paragraph.

Exosome isolation, detection, and characterization

In order to be able to explore not only the diagnostic value of exosomes derived from blood but also to produce and load exosomes from exogenous sources the process of isolating exosomes from surrounding biomaterial (such as plasma or interstitial fluid) is essential in being able to coherently investigate their structure and functions.

The extraction process can be achieved using several methods. Ultracentrifugation onto a sucrose cushion is a simple way of separating the vesicles. Ultracentrifugation can be followed by filtering the exosomes through a porous membrane. A recently published report shows another easy and efficient way of separating exosome populations from a sample that is called ExoQuick™ (95–98). Reproducability of isolation methods is an important factor in exosome research and recent approaches, such as an effective semi-automated nanoparticle tracking analysis, have shown developments in this field (99).

Dynamic light scatter measurements, flow cytometric analysis, microspectroscopy and electron microscopy examination of exosome containing samples is an option for the characterization of specific exosomes. Other imaging technologies that have also been implemented for such use include micro nuclear magnetic resonance, and X-ray scattering (99–102). Finding the origin of a population of exosomes extracted from a sample can be achieved by analyzing the specific marker antigens on the surface of the exosome. A recent approach has been to use a micro-fluidic exosome analysis platform that enables identification using disease-specific markers (103).

Summary and outlook

In the past 10 years exosomes raised high expectations for a better understanding of long distance cell-cell

communication, development and progress of several severe diseases such as cancer and neurodegeneration due to protein oligomers and aggregates. Now it is commonly accepted that exosomes are involved in intercellular cell transport of proteins, peptides, RNAs and dsDNA. The molecules which allow the exosomes to evade immune recognition are identified and they are either immune suppressive or immune modulating by interaction with different complements.

In order to adapt the immune-stealth properties for beneficial use such as drug or gene delivery several techniques were explored either to produce autologous exosomes for cancer immune therapy but more interestingly to produce in large scale drug- or RNA-loaded exosomes from stem cells. While the main focus has been on identifying the immune modulators so far little attention has been given to the glycome. If exosomes are produced by exogenous cells, the glycome may not be similar and immune recognition may occur. The fact that synthetic liposomes cause immune response and CARPA can also be related to the mismatch of the glycome or the absence of it which induces the strong immune stimulation.

References

1. Allan B. Closer to nature: new biomaterials and tissue engineering in ophthalmology. Brit J Ophthalmol 1999;83:1235–40.
2. Samsudin R. Stem cell and tissue engineering – the challenge of imitating nature. Malaysian J Med Sci: MJMS 2003;10:1–3.
3. Simpson DG, Bowlin GL. Tissue-engineering scaffolds: can we re-engineer mother nature? Expert Rev Med Devices 2006;3: 9–15.
4. Bangham AD, Standish MM, Miller N. Cation permeability of phospholipid model membranes: effect of narcotics. Nature 1965;208:1295–7.
5. Johnstone RM, Bianchini A, Teng K. Reticulocyte maturation and exosome release: transferrin receptor containing exosomes shows multiple plasma membrane functions. Blood 1989;74:1844–51.
6. Marleau AM, Chen C-S, Joyce JA, Tullis RH. Exosome removal as a therapeutic adjuvant in cancer. J Trans Med 2012;10:134.
7. Camussi G, Deregibus MC, Bruno S, Grange C, Fonsato V, Tetta C. Exosome/microvesicle-mediated epigenetic reprogramming of cells. Am J Cancer Res 2011;1:98–110.
8. Thery C. Exosomes: secreted vesicles and intercellular communications. F1000 Biol Reports 2011;3:15.
9. Keller S, Sanderson MP, Stoeck A, Altevogt P. Exosomes: from biogenesis and secretion to biological function. Immunol Lett 2006;107:102–8.
10. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013;200:373–83.
11. Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics 2009;6:267–83.
12. Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta 2012;1820:940–8.
13. Zhao L, Liu W, Xiao J, Cao B. The role of exosomes and "exosomal shuttle microRNA" in tumorigenesis and drug resistance. Cancer Lett 2014;356:339–46.
14. Kahlert C, Melo SA, Protopopov A, Tang J, Seth S, Koch M, et al. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. J Biol Chem 2014;289:3869–75.
15. Thakur BK, Zhang H, Becker A, Matei I, Huang Y, Costa-Silva B, et al. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res 2014;24:766–9.
16. Mathivanan S, Simpson RJ. ExoCarta: a compendium of exosomal proteins and RNA. Proteomics 2009;9:4997–5000.
17. Grant R, Ansa-Addo E, Stratton D, Antwi-Baffour S, Jorfi S, Kholia S, et al. A filtration-based protocol to isolate human plasma membrane-derived vesicles and exosomes from blood plasma. J Immunol Method 2011;371:143–51.
18. Kalra H, Adda CG, Liem M, Ang CS, Mechler A, Simpson RJ, et al. Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma. Proteomics 2013;13:3354–64.
19. Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Current protocols in cell biology/editorial board, Juan S. Bonifacino [et al]. 2006;Chapter 3:Unit 3 22.
20. Anand PK. Exosomal membrane molecules are potent immune response modulators. Commun Int Biol 2010;3:405–8.
21. Blanc L, De Gassart A, Geminard C, Bette-Bobillo P, Vidal M. Exosome release by reticulocytes–an integral part of the red blood cell differentiation system. Blood Cells, Mole Diseases 2005;35:21–6.
22. Srikanthan S, Li W, Silverstein RL, McIntyre TM. Exosome poly-ubiquitin inhibits platelet activation, downregulates CD36 and inhibits pro-atherothombotic cellular functions. J Thromb Haemostasis: JTH 2014;12:1906–17.
23. Testa JS, Apcher GS, Comber JD, Eisenlohr LC. Exosome-driven antigen transfer for MHC class II presentation facilitated by the receptor binding activity of influenza hemagglutinin. J Immunol 2010;185:6608–16.
24. Bobrie A, Colombo M, Raposo G, Thery C. Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 2011;12:1659–68.
25. Soo CY, Song Y, Zheng Y, Campbell EC, Riches AC, Gunn-Moore F, et al. Nanoparticle tracking analysis monitors microvesicle and exosome secretion from immune cells. Immunol 2012;136: 192–7.
26. Schorey JS, Bhatnagar S. Exosome function: from tumor immunology to pathogen biology. Traffic 2008;9:871–81.

27. Hannafon BN, Ding WQ. Intercellular Communication by Exosome-Derived microRNAs in Cancer. Int J Mole Sci 2013;14:14240–69.

28. Roma-Rodrigues C, Fernandes AR, Baptista PV. Exosome in tumour microenvironment: overview of the crosstalk between normal and cancer cells. BioMed Res Int 2014;2014:179486.

29. Ahmed W, Philip PS, Tariq S, Khan G. Epstein-Barr virus-encoded small RNAs (EBERs) are present in fractions related to exosomes released by EBV-transformed cells. PloS one 2014;9:e99163.

30. Arenaccio C, Chiozzini C, Columba-Cabezas S, Manfredi F, Federico M. Cell activation and HIV-1 replication in unstimulated CD4+ T lymphocytes ingesting exosomes from cells expressing defective HIV-1. Retrovirology 2014;11:46.

31. Jaworski E, Narayanan A, Van Duyne R, Shabbeer-Meyering S, Iordanskiy S, Saifuddin M, et al. Human T-lymphotropic virus type 1-infected cells secrete exosomes that contain Tax protein. J Biol Chem 2014;289:22284–305.

32. Neves RF, Fernandes AC, Meyer-Fernandes JR, Souto-Padron T. Trypanosoma cruzi-secreted vesicles have acid and alkaline phosphatase activities capable of increasing parasite adhesion and infection. Parasitol Res 2014;113:2961–72.

33. Brites D, Vaz AR. Microglia centered pathogenesis in ALS: insights in cell interconnectivity. Frontiers in Cell Neurosci 2014;8:117.

34. Wang J, Hendrix A, Hernot S, Lemaire M, De Bruyne E, Van Valckenborgh E, et al. Bone marrow stromal cell-derived exosomes as communicators in drug resistance in multiple myeloma cells. Blood 2014;124:555–66.

35. de Rivero Vaccari JP, Brand F, 3rd, Adamczak S, Lee SW, Barcena JP, Wang MY, et al. Exosome-mediated inflammasome signaling after central nervous system injury. J Neurochem 2015 Jan 27. [Epub ahead of print].

36. Santovito D, De Nardis V, Marcantonio P, Mandolini C, Paganelli C, Vitale E, et al. Plasma exosome microRNA profiling unravels a new potential modulator of adiponectin pathway in diabetes: effect of glycemic control. J Clin Endocrinol Metabol 2014;99:E1681–5.

37. Korc M. Pancreatic cancer-associated diabetes is an "Exosomopathy". Clin Cancer Res. 2015;21:1508–10.

38. Koeck ES, Iordanskaia T, Sevilla S, Ferrante SC, Hubal MJ, Freishtat RJ, et al. Adipocyte exosomes induce transforming growth factor beta pathway dysregulation in hepatocytes: a novel paradigm for obesity-related liver disease. J Surg Res 2014;192:268–75.

39. Stenqvist AC, Nagaeva O, Baranov V, Mincheva-Nilsson L. Exosomes secreted by human placenta carry functional Fas ligand and TRAIL molecules and convey apoptosis in activated immune cells, suggesting exosome-mediated immune privilege of the fetus. J Immunol 2013;191:5515–23.

40. Greil R, Egle A, Villunger A. On the role and significance of Fas (Apo-1/CD95) ligand (FasL) expression in immune privileged tissues and cancer cells using multiple myeloma as a model. Leukemia and Lymphoma 1998;31:477–90.

41. Hamad AR, Arcara K, Uddin S, Donner T. The potential of Fas ligand (apoptosis-inducing molecule) as an unconventional therapeutic target in type 1 diabetes. Frontiers in Immunol 2012;3:196.

42. Whiteside TL. Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumour-derived exosomes). Biochem Soc Trans 2013;41:245–51.

43. Green DR, Ferguson TA. The role of Fas ligand in immune privilege. Nat Rev Mol Cell Biol 2001;2:917–24.

44. Motz GT, Santoro SP, Wang LP, Garrabrant T, Lastra RR, Hagemann IS, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. Nature Med 2014;20:607–15.

45. Zhang HG, Su X, Liu D, Liu W, Yang P, Wang Z, et al. Induction of specific T cell tolerance by Fas ligand-expressing antigen-presenting cells. J Immunol 1999;162:1423–30.

46. Karlsson M, Lundin S, Dahlgren U, Kahu H, Pettersson I, Telemo E. "Tolerosomes" are produced by intestinal epithelial cells. Eur J Immunol 2001;31:2892–900.

47. Szebeni J. The interaction of liposomes with the complement system. Crit Rev Ther Drug 1998;15:57–88.

48. Krishnamoorthy L, Bess JW, Jr., Preston AB, Nagashima K, Mahal LK. HIV-1 and microvesicles from T cells share a common glycome, arguing for a common origin. Nature Chem Biol 2009;5:244–50.

49. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol 2009;9:338–52.

50. Gabius HJ, Wu AM. The emerging functionality of endogenous lectins: A primer to the concept and a case study on galectins including medical implications. Chang Gung Med J 2006;29: 37–62.

51. Davicino RC, Elicabe RJ, Di Genaro MS, Rabinovich GA. Coupling pathogen recognition to innate immunity through glycan-dependent mechanisms. Int Immunopharmacol 2011;11: 1457–63.

52. Gehrmann U, Naslund TI, Hiltbrunner S, Larssen P, Gabrielsson S. Harnessing the exosome-induced immune response for cancer immunotherapy. Semin Cancer Biol 2014;28:58–67.

53. Altevogt P, Bretz NP, Ridinger J, Utikal J, Umansky V. Novel insights into exosome-induced, tumor-associated inflammation and immunomodulation. Semin Cancer Biol 2014;28:51–7.

54. Hao S, Liu Y, Yuan J, Zhang X, He T, Wu X, et al. Novel exosome-targeted CD4+ T cell vaccine counteracting CD4+25+ regulatory T cell-mediated immune suppression and stimulating efficient central memory CD8+ CTL responses. J Immunol 2007;179: 2731–40.

55. Hao S, Moyana T, Xiang J. Review: cancer immunotherapy by exosome-based vaccines. Cancer Biother Radio 2007;22: 692–703.

56. Rountree RB, Mandl SJ, Nachtwey JM, Dalpozzo K, Do L, Lombardo JR, et al. Exosome targeting of tumor antigens expressed by cancer vaccines can improve antigen immunogenicity and therapeutic efficacy. Cancer Res 2011;71:5235–44.

57. Yao Y, Chen L, Wei W, Deng X, Ma L, Hao S. Tumor cell-derived exosome-targeted dendritic cells stimulate stronger CD8+ CTL responses and antitumor immunities. Biochem Biophys Res Commun 2013;436:60–5.

58. Gu X, Erb U, Buchler MW, Zoller M. Improved vaccine efficacy of tumor exosome compared to tumor lysate loaded dendritic cells in mice. Int J Cancer 2015;136:E74–84.

59. Bu N, Wu H, Sun B, Zhang G, Zhan S, Zhang R, et al. Exosome-loaded dendritic cells elicit tumor-specific CD8+ cytotoxic T cells in patients with glioma. J Neuro-oncology 2011;104:659–67.

60. Tan A, De La Peña H, Seifalian AM. The application of exosomes as a nanoscale cancer vaccine. Int J Nanomed 2010;5:889–900.

61. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411–22.

62. Vacchelli E, Vitale I, Eggermont A, Fridman WH, Fucikova J, Cremer I, et al. Trial watch: Dendritic cell-based interventions for cancer therapy. Oncoimmunology 2013;2:e25771.

63. Wang J, Zhou Y, Lu J, Sun Y, Xiao H, Liu M, et al. Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma. Med Oncol 2014;31:148.

64. Arscott WT, Camphausen KA. Exosome characterization from ascitic fluid holds promise for identifying markers of colorectal cancer. Biomarkers in Med 2011;5:821–2.

65. Silva J, Garcia V, Rodriguez M, Compte M, Cisneros E, Veguillas P, et al. Analysis of exosome release and its prognostic value in human colorectal cancer. Genes, Chromosomes and Cancer 2012;51:409–18.

66. Madhavan B, Yue S, Galli U, Rana S, Gross W, Muller M, et al. Combined evaluation of a panel of protein and mirna serum exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. Int J Cancer 2015;136:2616–27.

67. Yamashita T, Kamada H, Kanasaki S, Maeda Y, Nagano K, Abe Y, et al. Epidermal growth factor receptor localized to exosome membranes as a possible biomarker for lung cancer diagnosis. Die Pharmazie 2013;68:969–73.

68. Rodriguez M, Silva J, Lopez-Alfonso A, Lopez-Muniz MB, Pena C, Dominguez G, et al. Different exosome cargo from plasma/bronchoalveolar lavage in non-small-cell lung cancer. Genes Chromosomes Cancer 2014;53:713–24.

69. Fujita Y, Yoshioka Y, Ito S, Araya J, Kuwano K, Ochiya T. Intercellular communication by extracellular vesicles and their microRNAs in asthma. Clin Ther 2014;36:873–81.

70. Lässer C. Exosomes in diagnostic and therapeutic applications: biomarker, vaccine and RNA interference delivery vehicle. Expert Opin Biol Ther 2015;15:103–17.

71. Szebeni J, Muggia F, Gabizon A, Barenholz Y. Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: prediction and prevention. Adv Drug Delivery Rev 2011;63:1020–30.

72. Szebeni J, Bedocs P, Rozsnyay Z, Weiszhar Z, Urbanics R, Rosivall L, et al. Liposome-induced complement activation and related cardiopulmonary distress in pigs: factors promoting reactogenicity of Doxil and AmBisome. Nanomed: Nanotechnol, Biol Med 2012;8:176–84.

73. Barenholz Y. Doxil(R)–the first FDA-approved nano-drug: lessons learned. J Control Release 2012;160:117–34.

74. van der Meel R, Fens MH, Vader P, van Solinge WW, Eniola-Adefeso O, Schiffelers RM. Extracellular vesicles as drug delivery systems: Lessons from the liposome field. J Control Release 2014;195:72–85.

75. Jo W, Kim J, Yoon J, Jeong D, Cho S, Jeong H, et al. Large-scale generation of cell-derived nanovesicles. Nanoscale 2014;6:12056–64.

76. Mahmoodzadeh Hosseini H, Ali Imani Fooladi A, Soleimanirad J, Reza Nourani M, Mahdavi M. Exosome/staphylococcal enterotoxin B, an anti tumor compound against pancreatic cancer. J BUON 2014;19:440–8.

77. Azmi AS, Bao B, Sarkar FH. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. Cancer Metastasis Rev 2013;32:623–42.

78. Jung T, Castellana D, Klingbeil P, Cuesta Hernandez I, Vitacolonna M, Orlicky DJ, et al. CD44v6 dependence of premetastatic niche preparation by exosomes. Neoplasia 2009;11:1093–105.

79. Kahlert C, Kalluri R. Exosomes in tumor microenvironment influence cancer progression and metastasis. J Mole Med 2013;91:431–7.

80. Yang C, Robbins PD. The roles of tumor-derived exosomes in cancer pathogenesis. Clin Develop Immunol 2011;2011:842849.

81. Filipazzi P, Burdek M, Villa A, Rivoltini L, Huber V. Recent advances on the role of tumor exosomes in immunosuppression and disease progression. Seminars in Cancer Biol 2012;22:342–9.

82. Pilzer D, Gasser O, Moskovich O, Schifferli JA, Fishelson Z. Emission of membrane vesicles: roles in complement resistance, immunity and cancer. Springer Seminar Immun 2005;27:375–87.

83. Ibrahim AG, Cheng K, Marban E. Exosomes as critical agents of cardiac regeneration triggered by cell therapy. Stem Cell Reports 2014;2:606–19.

84. Kim SH, Bianco N, Menon R, Lechman ER, Shufesky WJ, Morelli AE, et al. Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. Mol Ther 2006;13:289–300.

85. Akyurekli C, Le Y, Richardson RB, Fergusson D, Tay J, Allan DS. A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles. Stem Cell Rev 2015;11:150–60.

86. Lai RC, Yeo RW, Tan KH, Lim SK. Exosomes for drug delivery – a novel application for the mesenchymal stem cell. Biotechnol Adv 2013;31:543–51.

87. Verma A, Stellacci F. Effect of surface properties on nanoparticle-cell interactions. Small 2010;6:12–21.

88. Marcus ME, Leonard JN. FedExosomes: engineering therapeutic biological nanoparticles that truly deliver. Pharmaceuticals (Basel, Switzerland) 2013;6:659–80.

89. Fevrier B, Vilette D, Archer F, Loew D, Faigle W, Vidal M, et al. Cells release prions in association with exosomes. Proc Natl Acad Sci USA 2004;101:9683–8.

90. Bellingham SA, Guo BB, Coleman BM, Hill AF. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? Frontiers in Physiol 2012;3:124.

91. Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles-Endogenous nanocarriers for targeted cancer therapy. Biochim Biophys Acta 2014;1846:75–87.

92. Moon PG, You S, Lee JE, Hwang D, Baek MC. Urinary exosomes and proteomics. Mass Spectrom Rev 2011;30:1185–202.

93. Ronquist GK, Larsson A, Stavreus-Evers A, Ronquist G. Prostasomes are heterogeneous regarding size and appearance but affiliated to one DNA-containing exosome family. Prostate 2012;72:1736–45.

94. Keller S, Ridinger J, Rupp AK, Janssen JW, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. J Translat Med 2011;9:86.

95. Taylor DD, Zacharias W, Gercel-Taylor C. Exosome isolation for proteomic analyses and RNA profiling. Method Molecul Biol 2011;728:235–46.

96. Zhu L, Qu XH, Sun YL, Qian YM, Zhao XH. Novel method for extracting exosomes of hepatocellular carcinoma cells. World J Gastroenterol: WJG 2014;20:6651–7.

97. Caradec J, Kharmate G, Hosseini-Beheshti E, Adomat H, Gleave M, Guns E. Reproducibility and efficiency of serum-derived exosome extraction methods. Clin Biochem 2014;47:1286–92.

98. Kanchi Ravi R, Khosroheidari M, DiStefano JK. A modified precipitation method to isolate urinary exosomes. J Vis Exp 2015;95:51158.

99. Mehdiani A, Maier A, Pinto A, Barth M, Akhyari P, Lichtenberg A. An innovative method for exosome quantification and size measurement. J Vis Exp 2015;95:50974.

100. van der Pol E, Hoekstra AG, Sturk A, Otto C, van Leeuwen TG, Nieuwland R. Optical and non-optical methods for detection and characterization of microparticles and exosomes. J Thromb and Haemostasis: JTH 2010;8:2596–607.

101. van der Pol E, Coumans F, Varga Z, Krumrey M, Nieuwland R. Innovation in detection of microparticles and exosomes. J Thromb and Haemostasis: JTH 2013;11 (Suppl 1):36–45.

102. Varga Z, Yuana Y, Grootemaat AE, van der Pol E, Gollwitzer C, Krumrey M, et al. Towards traceable size determination of extracellular vesicles. J Extracellular Vesicles 2014;3.

103. He M, Crow J, Roth M, Zeng Y, Godwin AK. Integrated immuno-isolation and protein analysis of circulating exosomes using microfluidic technology. Lab on a chip 2014;14:3773–80.

Bionotes

Gergely Milosevits
2nd Department of Pediatrics, Semmelweis University, Tüzoltó u. 7-9., Budapest, Hungary,
ikkuma@gmail.com;
and Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary

Since graduating from Semmelweis University in Budapest, Dr. Gergely Milosevits has been working as a medical doctor at the University's II Department of Pediatrics and also as a research fellow in the laboratory of Professor János Szebeni at the Nanomedicine Research and Education Center in Budapest, Hungary. He teaches both Hungarian and international medical students in practical pediatric classes. He is especially interested in flow cytometry, liposomes, exosomes and CARPA.

János Szebeni
Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary; and SeroScience Ltd, Budapest, Hungary

Janos Szebeni, MD, PhD, D, DSc, Med. Habil., immunologist, director of the Nanomedicine Research and Education Center at Semmelweis University in Budapest and full professor at Miskolc University, Department of Nanobiotechnology and the Regenerative Medicine, Institute of Theoretical Medicine, in Miskolc, Hungary. He is also guest professor at the Faculty of Pharmaceutical Sciences and NanoScience Center, University of Copenhagen and has teaching appointments at the Institute of Pathophysiology at Semmelweis University. He teaches biology, immune biology, immunology and nanomedicine. He obtained his MD in 1978 at Semmelweis University and then held various scientific positions in Hungary and abroad, including the Institute of Hematology in Budapest, Christchurch University in New Zealand, ETH in Zurich, University of Arizona in Tucson, Harvard University, in Boston, National Cancer Institute at NIH and the Walter Reed Army Institute of Research, in Bethesda, Maryland, USA. From 1985 until 2006 he lived in the USA, where he became citizen. In 2005 he won a Hungarian Scientific Achievement Award "Szentgyörgyi", and in 2006 he became chief of the Nanomedicine Department at the newly established Nanotechnology Institute of the Bay Zoltan Foundation for Applied Research, in Miskolc. His research over 30 years on various themes in hematology, membrane biology and immunology resulted in more than 100 scientific papers, a dozen book chapters, three patents, a book entitled "The Complement System: Novel Roles in Health and Disease" (Kluwer Academic Press, 2004) and a topical issue of *Critical Reviews in Therapeutic Drug Carrier Systems*, which he edited.

Three fields stand out in his career where he has been most active: liposomes, artificial blood and the complement system. Among others, he described the protective role of cholesterol against heme distribution into biomembranes and he has been a main promoter of the CARPA (complement activation-related pseudoallergy) concept highlighting the causal role of complement activation in numerous drug-induced hypersensitivity (anaphylactic) reactions, including those caused by liposomal and micellar drugs. Along with numerous social commitments in Hungary and abroad, he is a founder and scientific director of an immune toxicity CRO in Hungary (SeroScience Ltd).

Silke Krol
Fondazione IRCCS Istituto Neurologico
"Carlo Besta", IFOM-IEO-campus, Milan, Italy

Since 2011 Silke Krol has been with the 2009 funded Center of Nanotechnology@Fondazione I.R.C.C.S. Istituto Neurologico "Carlo Besta" in Milan, Italy. She is now studying the transport mechanisms for differently functionalized gold nanoparticles across the blood-brain barrier and how this is influenced by blood derived proteins. Moreover different novel metallic and non-metallic delivery systems for various other diseases (cardiovascular, prion disease, epilepsy, glioma, lymphomas, viral diseases) were designed for projects funded by Italian and European foundations. Her group develops multifunctional polymer/nanogold based drug or drug delivery systems as well as diagnostic tools for medical applications. Moreover, the multilayer-nanocoating is used for encapsulation and immune protection of living cells like, e.g. pancreatic islets. She has several pending patents for possible future drugs for prion disease and cancer treatment, viral diseases, and cancer diagnostics.

She occasionally lectures as a contract professor for "Nano-medicine" at the University of Udine and Trieste since 2008 and as guest lecturer for "Nanotoxicology". In 2009 she worked as an expert consultant for the United Nations and serves as external expert reviewer for National projects in France, Italy, Georgia and Greece. Recently she was announced as project technical advisor for three EU-FP7 projects. She is member of the advisory board of the CLINAM-Foundation of the journal *Euro-Nanotox-Letters* and the international advisory committee of the International scientific spring conference in Islamabad, Pakistan. She is an associate editor of **Frontiers in Nanobiotechnology** and an adjunct faculty member at the Pakistan Institute of Engineering and Applied Science. Recently she became a consultant and Member of General Scientific Advisory Board at *Midatech* Pharma PLC. She serves as an external expert reviewer for national projects in France, Italy, and Greece. She frequently reviews for **Nanoscale, Nanomedicine, Nanoletters,** and others.