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Commentary

Microexosomes versus exosomes: Shared components but distinct structures

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Just as atmospheric layers surround the earth, all types of living cells are girdled with extracellular substances including matrix and vesicles, which maintain cellular functions. We herein focus on two types of shared-component intercellular carriers, exosomes and microexosomes, specifically their functions based on their discrete structures.

Exosomes, one group of extracellular vesicles, function as a carrier in intercellular transportation typically from tumorigenic cells to neighboring normal cells [1]. The exosomes transport selectively incorporated substances such as proteins, lipids, and ribonucleotides, including microRNA to target cells [2,3] (Fig. 1a). The exosomes have great potential as therapeutic drug-delivery tools owing to their capacity for cell type-specific transportation to target cells [1]. The exosomes are rich in proteins belonging to a membrane protein family, termed tetraspanin [4]. The members of the tetraspanin family have two extracellular loops (EC1 and EC2) and a unique motif, cysteine-cysteine-glycine (CCG), in the larger extracellular loop (EC2) [5]. Since commercially available anti-tetraspanin antibodies recognize the 3-dimensional structure of the CCG-containing region [6], immunoblotting is performed under non-reduced conditions. EC2 can be further divided into a constant region containing conserved helices, and a variable region containing sites for specific protein–protein interactions [7]. Structural analysis of tetraspanin uroplakin indicates close packing of four transmembrane domain helices and an overall rod-shaped structure, which is suitable for the docking of partner proteins, implying that target-cell specificity probably depends on the variety of tetraspanin members located on the plasma membrane of exosomes.

Meanwhile, a well-known exosomal component, tetraspanin CD9, regulates sperm-egg fusion in mice [8–10]. In mammals, membrane protrusions, termed microvilli, on the egg plasma membrane are believed to promote sperm-egg fusion. Since CD9 is involved in this fusion, this protein may organize the formation of microvilli. In fact, *Cd9* deficiency strikingly reduces the number of microvilli on the egg plasma membrane [11]. However, immunoelectron microscopic analysis revealed that CD9 is incorporated into small structures (microexosomes), which are released from the eggs during ovulation, presumably cumulus expansion [11]. Since the microexosomes have no overt lipid bilayers and are small units less than 5 nm in diameter [11,12], they are structurally different from exosomes (Fig. 1b). Notably, the microexosomes restore sperm fusion competency with fusion-incompetent *Cd9*-deficient eggs with impaired microvilli [11], which means that microexosomes, but not microvilli, are essential for the sperm-egg fusion. Otherwise, microexosomes are observed inside the uterus, and contribute to uterine repair after parturition in mice and humans [13].

On the other hand, *Cd9*-deficient macrophages are strongly activated *in vitro* and cause enhanced lung inflammation *in vivo* when they are stimulated with lipopolysaccharide in mice [14]. Furthermore, double deficiency of *Cd9* and *Cd81* causes systemic dysfunction in mice, specifically in lung epithelia and osteoclasts, leading to chronic obstructive pulmonary disease-like symptoms, viz., pulmonary emphysema, weight reduction, osteoporosis, and muscular atrophy [14,15], implying that a loss of microexosomes may weaken homeostasis of normal tissues. These phenomena imply that two types of shared-component intercellular carriers, microexosomes and exosomes, are released from cells, which widely regulate biological and pathological events.

As mentioned above, exosomes structurally differ from microexosomes, because typical lipid bilayers are formed in the exosomes but not in the microexosomes, indicating that their formation processes are expected to differ. The exosomes are

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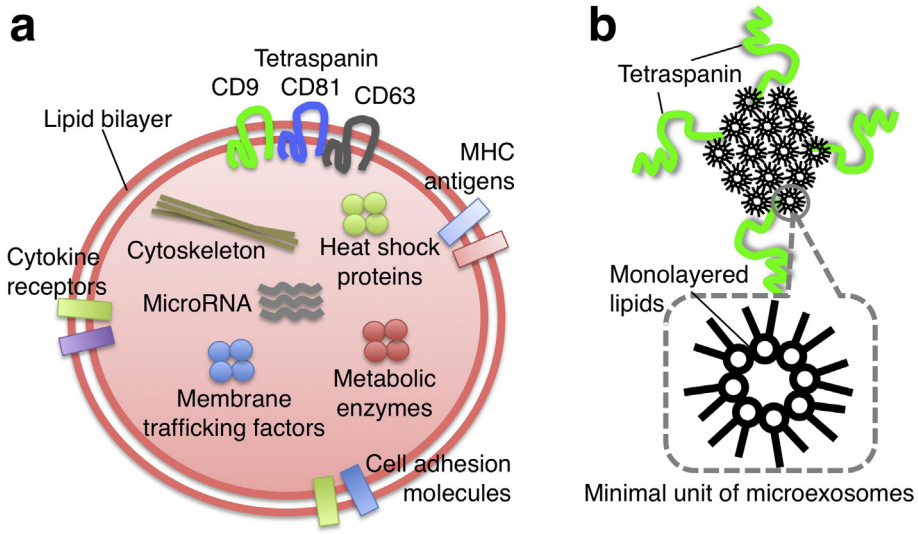

Fig. 1. Distinct pathways of exosomes and microexosomes. Exosomes have typical lipid bilayers and contain cytoplasmic proteins (cytoskeleton, heat shock proteins, metabolic enzymes, and membrane tracking factors) and also carry microRNA (a). The minimal unit of microexosomes is predicted to have monolayered lipids, but not lipid bilayers (b). These units gather and form aggregates. Both structures share the member of tetraspanin family, but structurally differ.

formed as a consequence of fusion of multivesicular late endosomes with the plasma membrane [1,3] (Fig. 2). The initial step in the formation of exosomes is endocytosis, during which the plasma membrane is endocytosed into the cytoplasm to produce endosomes. In turn, small vesicles are formed inside the endosomes by membrane invagination of the endosomes, which are turned into multivesicular bodies. The multivesicular bodies then fuse to the plasma membrane and release the membrane vesicles as exosomes into the external environment.

On the other hand, microexosomes are predicted to be directly released from the plasma membrane without an endosomal pathway (Fig. 2). First, selected membrane components, including lipids, are extracted from the plasma membrane, presumably by lipid bilayer deformation [11,16]. In turn, these components are released into the external environment, and concurrently, microvilli are formed on the plasma membrane. From the findings of electron microscopic analysis of the egg plasma membrane [11], tetraspanin is thought to play a role in the process of lipid bilayer deformation. When tetraspanin is absent from host cells, the formation of microexosomes is arrested [11,13,16]. In contrast, we expect that exosomes are formed structurally but the target-cell specificity is disturbed, because the target-cell specificity might depend on the variety of tetraspanin members located on the plasma membrane of exosomes.

A clear understanding of the characteristics and functions of these two types of exosomes holds great potential for elucidating the molecular mechanisms of intercellular transportation- and membrane fusion/membrane repair-related phenomena.

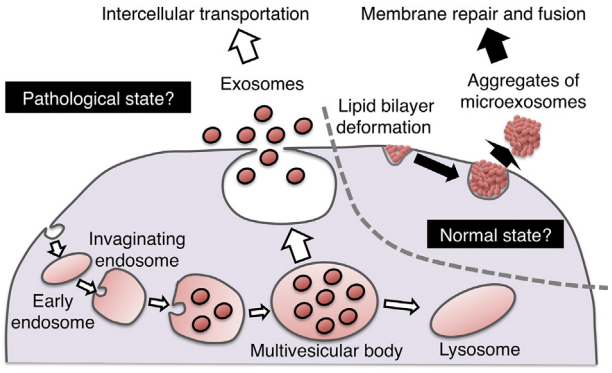

Fig. 2. Distinct formation processes of exosomes and microexosomes. The exosomes are formed as a consequence of fusion of multivesicular late endosomes with the plasma membrane. The initial step in the formation of exosomes is endocytosis. Small vesicles are formed inside the endosomes by membrane invagination of the endosomes, which are turned into multivesicular bodies. The multivesicular bodies then fuse to the plasma membrane and release the membrane vesicles as exosomes. Otherwise, microexosomes are predicted to be directly released from the plasma membrane without an endosomal pathway. First, selected membrane components, including lipids, are extracted from the plasma membrane, presumably by lipid bilayer deformation. In turn, these components are released into the external environment.

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References

[1] Pitt JM, Kroemer G, Zitvogel L. Extracellular vesicles: masters of intercellular communication and potential clinical interventions. J Clin Investig 2016;126:1139–43.
[2] Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654–9.
[3] Desrochers LM, Antonyak MA, Cerione RA. Extracellular vesicles: satellites of information transfer in cancer and stem cell biology. Dev Cell 2016;37:301–9.
[4] Kowal J, Arras G, Colombo M, Jouve M, Morath JP, Primdal-Bengtson B, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci U S A 2016;113:E968–77.
[5] Hemler ME. Tetraspanin proteins promote multiple cancer stages. Nat Rev Canc 2014;14:49–60.
[6] Hasuwa H, Shishido Y, Yamazaki A, Kobayashi T, Yu X, Mekada E. CD9 amino acids critical for upregulation of diphtheria toxin binding. Biochem Biophys Res Commun 2001;289:782–90.
[7] Hemler ME. Targeting of tetraspanin proteins–potential benefits and strategies. Nat Rev Drug Discov 2008;7:747–58.
[8] Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y, Ryu F, et al. Requirement of CD9 on the egg plasma membrane for fertilization. Science 2000;287:321–4.
[9] Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C. Severely reduced female fertility in CD9-deficient mice. Science 2000;287:319–21.
[10] Kaji K, Oda S, Shikano T, Ohnuki T, Uematsu Y, Sakagami J, et al. The gamete fusion process is defective in eggs of Cd9-deficient mice. Nat Genet 2000;24:279–82.

[11] Miyado K, Yoshida K, Yamagata K, Sakakibara K, Okabe M, Wang X, et al. The fusing ability of sperm is bestowed by CD9-containing vesicles released from eggs in mice. Proc Natl Acad Sci U S A 2008;105:12921–6.
[12] Ohnami N, Nakamura A, Miyado M, Sato M, Kawano N, Yoshida K, et al. CD81 and CD9 work independently as extracellular components upon fusion of sperm and oocyte. Biol Open 2012;1:640–7.
[13] Kawano N, Miyado K, Yoshii N, Kanai S, Saito H, Miyado M, et al. Absence of CD9 reduces endometrial VEGF secretion and impairs uterine repair after parturition. Sci Rep 2014;4:4701.
[14] Takeda Y, Suzuki M, Jin Y, Tachibana I. Preventive role of tetraspanin CD9 in systemic inflammation of chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 2015;53:751–60.
[15] Jin Y, Takeda Y, Kondo Y, Tripathi LP, Kang S, Takeshita H, et al. Double deletion of tetraspanins CD9 and CD81 in mice leads to a syndrome resembling accelerated aging. Sci Rep 2018;8:5145.
[16] Iwai M, Hamatani T, Nakamura A, Kawano N, Kanai S, Kang W, et al. Membrane protein CD9 is repositioned and released to enhance uterine function. Lab Invest 2019;99:200–9.