Commentary

A novel role of exosomes in the vaccination approach

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Comment on: Montaner-Tarbes S, Borrás FE, Montoya M, *et al.* Serum-derived exosomes from non-viremic animals previously exposed to the porcine respiratory and reproductive virus contain antigenic viral proteins. Vet Res 2016;47:59.

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Exosomes are the type of extracellular vesicles (EVs) derived from inward budding of the inner endosomal membrane to form multivesicular bodies, followed by the fusion of multivesicular bodies with the plasma membrane (1). These vesicles range in size from 30 to 150 nm. Composed of a lipid bilayer, they contain both transmembrane and cytosolic proteins and enclose miRNAs, mRNAs, long non-coding RNAs and DNA (2). Cells use these vesicles to communicate with both adjacent and distant cells. Exosomes have biological activities *in vivo* and exert significant roles in various pathological conditions such as cancer, autoimmune diseases, infectious and neurodegenerative diseases (3). The presence of exosomes in body fluids, along with the plasticity of exosomal content in response to various physiological stimuli and pathological states render exosomes an ideal biomarker for disease state. Exosomes secrete from immune cells and their immunomodulatory functions are very well characterized (4,5). The finding that EVs released by B-cell lines carry MHC class-II, co-stimulatory and adhesion molecules suggested that such vesicles could directly stimulate CD4 T-cell clones (6). This idea received further support with the demonstration that vaccination of mice with tumor peptide-pulsed dendritic cells (DCs) exosomes primes tumor-specific CTLs and suppresses tumor growth in a T-cell dependent manner (7). Similarly, EVs pulsed with peptides from Epstein-Barr virus, cytomegalovirus and influenza virus have been shown to directly trigger *in vitro* IFNγ secretion by a small percentage of human peripheral CD8 T cells, and probably memory T cells (8). Exosome-based cancer immunotherapy is an attractive approach against cancer as tumor derived exosomes carrying tumor associated antigens are reported to recruit the immune responses (9).

In the context of immune responses against pathogens, exosomes also carries pathogen antigens and known to evoke immune responses. *Mycobacterium bovis* BCG-infected macrophages release EVs containing mycobacterial antigen that, in the presence of DCs, promote T-cell immunity in mice (10). Certain viral antigens are also targeted into the exosome pathway. T cells segregate the HIV Gag protein into plasma membrane-derived EVs (11), and CMV-infected endothelial cells release EVs containing CMV gB protein that stimulate memory CD4 T cells in the presence of APCs (12). In a recent issue of *Veterinary Research*, Montaner-Tarbes *et al.* described the presence of porcine respiratory and reproductive syndrome virus (PRRSV) antigens in serum derived exosomes isolated from both viremic (V) and non-viremic (NV) pigs (13). Moreover, immune sera from pigs previously exposed to PRRSV specifically reacted against exosomes purified from NV pig sera. The presence of antigenic viral protein in serum-derived exosomes free of virus, suggested the possibility of these exosome derived viral antigens as a novel vaccine approach against PRRSV. They first isolated the exosomes by size exclusion chromatography and characterized the exosomes from serum of naive control (CN) pigs (PRRSV negative), V animals (PRRSV RNA positive and seropositive) and NV animals (PRRSV RNA negative and seropositive). The phenotypical characterization of exosomes was based on the classical exosome markers, CD63 and CD81. Nanoparticle tracking analysis (NTA) and cryo-electron microscopy revealed

the size and concentration of exosomes. Exosomal protein characterization by liquid chromatography and mass spectrometry identified the unique pattern of PRRSV proteins associated with the exosomes. Both the NV and V group of animal exosomes contained the peptides from major envelope glycoproteins GP5-Tm:pFc (a fusion protein of GP5 with no transmembrane domain and pig fragment crystallizable portion), from envelope glycoprotein GP3, NSP2 and partial ORF2b. Apart from these common peptide sequences, more interestingly, the exosomes from NV animals showed peptides from RNA-dependent RNA polymerase and nucleocapsid protein N. The proteomic analysis also identified for the first time the porcine proteins contained within the exosomes, which were related to exosome composition and function. Importantly, this will facilitate the future studies between host and pathogens in PRRSV and other animal diseases.

Furthermore, the authors also tested the antigenic properties of exosome derived viral proteins and demonstrated that immune sera from pigs previously exposed to PRRSV, specifically reacted to the exosomes from NV animals. This exosomal protein mediated antigenic activity was very similar to the antigenic activity contained in the commercially available vaccine (Porcilis PRRSV vaccine, Intervet, Boxmeer, The Netherlands). Though the immunogenic properties of pathogen derived exosomal antigens have been tested in several preclinical models and diseases of parasitic and viral origin (14,15), the circulation of viral antigens through the exosomes in the serum of the host with no pathogen load detected in peripheral circulation (NV) suggested the importance of this study for a novel vaccine approach.

Since the physical and chemical characteristics of many EVs, including exosomes as well as their biogenesis pathways, resemble those of retroviruses (16), the authors used the polyethylene-glycol (PEG) precipitation and size exclusion chromatography process to scale up the exosome isolation based on retrovirus isolation method (17). The scaling up process did not affect the immunological property of exosomes. Thus, collectively the authors demonstrated the isolation, characterization, antigenicity and scaling-up process of serum-derived exosomes from pigs previously infected with PRRSV and warranted the further exploration of this study as a novel vaccination approach to eradicate PRRSV. This approach will overcome the current limitations of current conventional vaccines against PRRSV.

However, it should be noted that there are certain areas remain to be explored. It is tempting to speculate that why the viral RNA dependent RNA polymerase and nucleocapsid protein are circulating in the serum derived exosomes from NV animals without the ongoing viral replication. Whether these viral proteins in exosomes will act as immune modulators to establish the viral infection by immune evasion mechanisms (18) or the proteins like RNA dependent RNA polymerase and nucleocapsid will aid in enhancement of viral RNA replication in immunized animals already infected with PRRSV still remains elusive. Recently, Li *et al.* found that the viral RNA dependent RNA polymerase and helicase increase the virulence of the atypical highly pathogenic HP-PRRSV emerging in China (19), but information regarding mechanisms by which they could contribute to pathogenicity remains unknown. Similarly, the nucleocapsid protein interacts with different cellular factors of the host to facilitate virus infection. The N protein and three non-structural (Nsps) PRRSV proteins have been identified as playing an important role in type I IFN suppression and modulation of the NF-κB pathway as it is translocated to the nucleus during early stages of infection (20). Continuous studies to evaluate these mechanisms will provide more specific insights to understand the encapsidation of viral proteins in exosomes and their role as a novel vaccine approach.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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