Editorial

Vps4A-mediated tumor suppression upon exosome modulation?

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Submitted Apr 04, 2016. Accepted for publication Apr 09, 2016.
doi: 10.21037/atm.2016.04.18
View this article at: http://dx.doi.org/10.21037/atm.2016.04.18

In cancer, communication between tumor cells and components of its surrounding microenvironment is critical for tumor growth, progression and metastatic potential (1). Components of the tumor microenvironment include extracellular matrix structures, fibroblasts (myofibroblasts), immune-reactive and inflammatory cells, and endothelial cells. Importantly, therapeutic opportunities may be opened up by combined targeting of tumor cells and their microenvironment (2). In this regard, better understanding of the cellular mechanisms mediating the communication between tumor cells and their microenvironment is urgently required.

Extracellular vesicles, e.g., exosomes, together with their protein and nucleic acid cargo enable an intriguing form of cell communication in paracrine and endocrine fashions and, accordingly, considerable research effort is currently focused on the detailed role of exosomes in shaping the tumor microenvironment (2). A recent publication highlights an interesting finding of a novel candidate tumor suppressor protein, Vps4A, to influence exosomal functions involving the loading and delivery of microRNA cargo (3).

Extracellular vesicles can be divided into three main classes: exosomes (20–100 nm in size), microvesicles (100–1,000 nm in size) and apoptotic bodies (1–5 μm in size). These vesicles differ among themselves not only by size, but also by origin and composition (4). Microvesicles are formed through outward budding of the plasma membrane and intracellular space. In contrast, exosomes are actively packed in intracellular endosomes, which progress to multivesicular bodies as a consequence of inwards budding of the plasma membrane, and then are targeted to either lysosomes or are released to the extracellular space through fusion with the plasma membrane. Extracellular release of exosomes can then lead to endocytosis by other cells and cargo molecules become effective inside these recipient cells (5).

The composition of the exosomes displays enrichment for specific proteins, lipids and RNAs, while other macromolecules appear absent. This indicates the presence of a regulatory mechanism controlling the sorting of cargo into exosomes (6). So far, 4,563 different proteins, 1,639 different mRNAs, and 764 different microRNAs (miRNAs) have been identified in exosomes originating from various tissues (7).

MicroRNAs are 21–23 nucleotide long RNAs that act as important regulators of gene expression (8). Mature miRNAs associate with Argonaute (Ago) protein and together form the RNA-induced silencing complex (RISC), a ribonucleoprotein complex effecting posttranscriptional gene silencing. Complementary base pairing of the miRNA with target messenger RNA serves as a guide for the Ago protein and directs degradation, destabilization or translational repression (9). In mammals, more than 60% of protein-coding genes are believed to be under the control of miRNAs (10). Functional studies suggest that almost every cellular process investigated to date is under miRNA influence. Aberrant miRNA expression contributes to a range of human pathologies, including cancer (11,12). The selective packaging of macromolecules into exosomes is a topic of major interest. The presence of non-random miRNAs in tumor cell-derived exosomes raises the possibility of miRNA-mediated gene regulation in proximal recipient cells to modulate the local microenvironment and in distant recipient cells to possibly help the formation of a metastatic niche (13).

The protein Vps4A is a part of the Endosomal Sorting Complexes Required for Transport (ESCRT) machinery, which consists of five different protein complexes: ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III and Vps4 itself (14). Protein and nucleic acid sorting to exosomes can be ESCRT-

dependent or ESCRT-independent (using tetraspanins or employing a lipid-dependent mechanism) (5).

The study by Wei *et al*. (3) shows that Vps4A can act as tumor suppressor in hepatocellular carcinoma (HCC), possibly through regulation of exosomal miRNA sorting. According to the study, Vps4 is significantly down-regulated in primary human HCCs and, furthermore, low Vps4 expression is correlated with hepatitis B viral infection, increased tumor size, reduced tumor capsule integrity, and regional lymph node metastasis. Incubation of HCC cells (SMMC-7721) with self–derived exosomes caused a notable increase in cell growth, migration and invasion. Accordingly, ectopic expression of Vps4 in SMMC-7721 cells represses their growth, colony formation, migration and invasion. Of interest, transfection of HCC cells toward overexpression of Vps4A repressed the tumor growth of these cells in subcutaneous murine xenograft experiments. The authors propose that Vsp4a acts by weakening the cell response to exosomes. Furthermore, they demonstrate that Vps4A facilitates the secretion into exosomes of oncogenic miRNAs (miR-27b-3p and miR-92a-3p) and the cellular retention of tumor suppressor miRNas (miR-193-39, miR-320a, miR-132-3p). Additionally, incubation of Vps4A-transfected HCC cells with exosomes originating from control HCC cells showed cellular accumulation of tumor suppressor miRNAs (miR-122-5p, miR-33a-5p, miR-34a-5p, miR-193a-3p, miR-16-5p and miR-29b-3p). We note that miR-16-5p, which is found upregulated after transfection of Vps4a expressing cells with control HCC cell exosomes, is reported to regulate Vps4A expression itself (15). This finding may suggest the existence of a feedback loop leading to downregulation of Vps4A upon incubation with control cell exosomes. In this light, it is not clear what would be the outcome of prolonged incubation of Vps4A overexpressing cells with control cell exosomes. Therefore, we feel that it would be interesting to revisit this experiment and address both short-term and long-term effects of exosomal incubation.

Previous studies have shown that early ESCRT complexes, ESCRT-I and ESCRT-II, are involved in cargo sorting while ESCRT-III complex together with Vps4A is necessary for scission of the membrane neck that connects the bud to the parental membrane during exosome biogenesis (14,16). The study by Wei *et al*. (3) raises therefore the intriguing possibility that Vps4A may have an additional role in the earlier events of cargo sorting to exosomes. Clearly, more work is necessary to fully clarify the role of Vps4A complex in exosome biogenesis.

The key finding of Wei *et al*. is the discovery that Vps4A acts as HCC tumor suppressor. At the same time, the detailed mechanism of Vps4A tumor suppressive activity is not fully elucidated, particularly its effect on selective packaging of microRNAs in tumor exosomes. It would be highly interesting to deduce how Vps4A allows secretion of oncogenic miRNAs and selective retention or uptake of tumor suppressive ones.

The detailed mechanism of miRNA packing into exosomes is still unknown. According to the earlier studies, subsets of miRNAs containing the EXOmotif (GGAG) sequence are loaded into exosomes by binding to the heterogeneous ribonucleoprotein A2B1 (hnRNPA2B1) (5). However, the oncogenic microRNAs described by Wei *et al*., as found to be specifically enriched in the exosomes, do not contain the EXOmotif. This suggests that there are alternative mechanisms for miRNA sorting into exosomes. Further, Wei *et al*. point out that in humans there are two paralogs of Vps4, namely Vps4A and Vps4B. Vps4B is also found downregulated in human HCC. It would be interesting to see whether Vps4B acts as tumor suppressor as well and if it also has an impact on miRNA sorting.

In conclusion, considering that exosomes are important for shaping the tumor microenvironment and tumor progression toward metastatic spread, it would be highly desirable to understand the exact role of Vps4A in exosome formation and how Vps4A directs miRNA sorting to the exosomes. Since exosomes not only have a substantial impact on tumor development but also promise to serve as targets in tumor therapy, it would be of considerable significance to describe the mechanism of Vps4A downregulation in human HCC and to assess if restoration of Vps4A expression could be used in tumor therapy.

Acknowledgements

Funding: Work of the authors is funded by the German Cancer Aid (project 109886). I. Akrap is supported by a fellowship of Tübingen University. Both I. Akrap and A. Thavamani are members of the International Max Planck Research School Tübingen ("From Molecules to Organisms").

Footnote

Provenance: This is a Guest Editorial commissioned by Guest Editor Haitao Zhao, MD, PhD (Department of Liver Surgery, Peking Union Medical College Hospital, Chinese

Academy of Medical Sciences and Peking Union Medical College, Beijing, China).
Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Wei JX, Lv LH, Wan YL, *et al*. Vps4A functions as a tumor suppressor by regulating the secretion and uptake of exosomal microRNAs in human hepatoma cells. Hepatology 2015;61:1284-94.

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Cite this article as: Akrap I, Thavamani A, Nordheim A. Vps4A-mediated tumor suppression upon exosome modulation? Ann Transl Med 2016;4(9):180. doi: 10.21037/atm.2016.04.18

