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Ciliary extracellular vesicles: Txt msg orgnlls

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Abstract

Cilia are sensory organelles that protrude from cell surfaces to monitor the surrounding environment. In addition to its role as sensory receiver, the cilium also releases extracellular vesicles (EVs). The release of sub-micron sized EVs is a conserved form of intercellular communication used by all three kingdoms of life. These extracellular organelles play important roles in both short and long range signaling between donor and target cells and may coordinate systemic responses within an organism in normal and diseased states. EV shedding from ciliated cells and EV-cilia interactions are evolutionarily conserved phenomena, yet remarkably little is known about the relationship between the cilia and EVs and the fundamental biology of EVs. Studies in the model organisms *Chlamydomonas* and *C. elegans* have begun to shed light on ciliary EVs. *Chlamydomonas* EVs are shed from tips of flagella and are bioactive. *C. elegans* EVs are shed and released by ciliated sensory neurons in an intraflagellar transport (IFT)-dependent manner. *C. elegans* EVs play a role in modulating animal-to-animal communication, and this EV bioactivity is dependent on EV cargo content. Some ciliary pathologies, or ciliopathies, are associated with abnormal EV shedding or with abnormal cilia-EV interactions, suggest the cilium may be an important organelle as an EV donor or as an EV target. Until the past few decades, both cilia and EVs were ignored as vestigial or cellular junk. As research interest in these two organelles continues to gain momentum, we envision a new field of cell biology emerging. Here, we propose that the cilium is a dedicated organelle for EV biogenesis and EV reception. We will also discuss possible mechanisms by which EVs exert bioactivity and explain how what is learned in model organisms regarding EV biogenesis and function may provide insight to human ciliopathies.

Introduction

"Cilia" is a broad term that includes primary or sensory cilia, motile cilia, and flagella. Most cilia share a similar microtubule-based architecture and are constructed by the evolutionally conserved intraflagellar transport (IFT) machinery (Ishikawa and Marshall, 2011; Rosenbaum and Witman, 2002). Motile cilia propel fluid surrounding tissues or may move the cell itself (Zhou and Roy, 2015). Primary cilia are found on most non-dividing cells in the human body, play central roles in signal transduction and coordination of cellular behaviors, and thus are important for development and health (Zimmerman and Yoder, 2015). Disruption of ciliary formation or function causes a wide spectrum of syndromes

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termed ciliopathies, which show overlapping characteristic symptoms such as cystic kidney disease, retinal degeneration, abnormal neural tube development, polydactyly, and obesity (Pazour and Rosenbaum, 2002).

"Ciliary extracellular vesicles (EVs)" is a comprehensive term we use to refer to ciliary-derived or ciliary-associated EVs. EV is also a broad term referring to any extracellular membrane bound vesicle that includes exosomes derived from multivesicular bodies and ectosomes formed via outward budding of the plasma membrane (Colombo et al., 2014; El Andaloussi et al., 2013; Lo Cicero et al., 2015). Exosomes and ectosomes are tiny: less than 100nm for exosomes and between 100nm – 1um for ectosomes. Cells release EVs containing specific protein, lipid, and nucleic acid-based cargo (Lo Cicero et al., 2015). These cell-specific markers may reveal the source of the EVs, and may be used as biomarkers for normal and diseased states. EV composition and abundance change under different physiological and pathological conditions, therefore understanding the basic process of EV biogenesis, EV cargo selection, and EV-target cell interaction holds exciting significance for diagnostics and therapeutics.

Very recently cilia have been observed as a site for EV release and for EV attachment (Wood and Rosenbaum, 2015). In this review, we discuss this new emerging field of research and offer our perspective on how understanding the relationship between EVs and cilia might advance understanding and treatment of human ciliopathies.

Ciliary proteins are EV cargo

In humans, mutations in the polycystin-encoding genes cause autosomal dominant polycystic kidney disease (ADPKD) (Ong and Harris, 2015) and polycystin ciliary trafficking defects are thought to be an underlying cause of this ciliopathy (Cai et al., 2014). The mammalian polycystins localize to cilia as well as urinary EVs released from renal epithelial cells (Hogan et al., 2009; Pazour et al., 2002; Pisitkun et al., 2004; Yoder et al., 2002). Pisitkun *et al* first observed polycystin-1 in urinary exosomes (Pisitkun et al., 2004). Ward and colleagues then showed polycystin-1 and -2 and the autosomal recessive PKD-gene product fibrocystin colocalized to urinary exosome-like vesicles (Hogan et al., 2009). Polycystin-2 and a peripheral ciliary membrane protein retinitis pigmentosa 2 (RP2), are shed into media in the MDCK canine kidney cell culture (Hurd et al., 2010). The components of exocyst and the ciliary GPCR Smoothened are found in the urinary exosome-like vesicle proteome (Chacon-Heszele et al., 2014). Bioinformatics analysis revealed that some of the known interactors of Smoothened, polycystin-1 and polycystin-2 are present in the urinary exosome-like vesicle proteome (Chacon-Heszele et al., 2014), suggesting signal transduction modules may be contained in EVs.

The embryonic nodal floor releases membrane bound particles called `nodal vesicular parcels' (Tanaka et al., 2005). These nodal vesicular parcels contain Sonic hedgehog and other signaling molecules that are swept by nodal flow to the left side. Hirokawa and colleagues proposed that nodal vesicular parcels interact with immotile cilia for left-right axis determination (Tanaka et al., 2005). This hypothesis, while controversial and

unresolved, was the first to propose that ciliary-associated EVs carry morphogens that influence important aspects of animal development.

In the green algae *Chlamydomonas reinhardtii*, the flagella membrane is continuously shed by releasing flagellar EVs, with a complete turnover of flagellar membrane protein every six hours (Dentler, 2013). A major flagellar membrane protein FMG1, a large glycoprotein (~350 kD), is shed into media in a distinct manner. After crosslinking with lectins or antibodies, FMG1 first aggregates at the flagellar tip, then is transported back in the direction of the cell body and shed into media at the flagellar base in a membrane bound form (Bloodgood et al., 1986). *Chlamydomonas* ciliary ectosomes are shed from the flagellar tip and also contain polycystin-2 (Cao et al., 2015; Wood et al., 2013).

Ciliary EVs are no simply the breaking off of membrane from cilia. Proteins found in ciliary EVs and cilia are identical. *C. elegans* EVs contain the polycystins and CIL-7, a myristoylated EV biogenesis regulator, but not the ciliary kinesin-3 motor KLP-6, components of the IFT machinery, or ciliary beta-tubulin TBB-4 (Maguire et al., 2015; Wang et al., 2014). *Chlamydomonas* ciliary ectosomes contain polycystin-2, FMG1, the flagellar membrane polypeptide SAG1-C65, and vegetative lytic enzyme VLE but not typical ciliary proteins including IFT81, IFT139, alpha tubulin, and axonemal protein Bug22 (Cao et al., 2015; Wood et al., 2013). Combined, these results suggest that there exits an active EV sorting machinery that selects some cargo but not others to be packaged in EVs.

In conclusion, various ciliary proteins are also present on ciliary-derived EVs that are released into the extracellular space. While extensive proteomic analysis of different types of EVs and cilia are available, a systematic look for overlap is lacking. How are cargo targeted to ciliary EVs? What are the functions of the ciliary proteins and other cargo in EVs? Do cilia provide a unique ciliary EV composition to elicit a synchronized response within target cells and tissues? We propose that ciliary EVs acts as vessels with a high concentration of cargo for a robust signal transmission and provide a stable environment to enable long distance travel in time and space.

Not all ciliated cells shed EVs equally: *C. elegans* as a model for ciliary EV biogenesis and function

C. elegans cilia are situated on distal dendritic endings of sensory neurons (Bae and Barr, 2008). The self-fertilizing hermaphrodite has a simple nervous system of 302 neurons of which 60 are ciliated. 32 out of 60 of ciliated sensory neurons in the hermaphrodite, including the amphid, phasmid and inner labial organs are either directly or indirectly exposed to the environment through openings generated by glial cells (Ward et al., 1975). The hermaphrodite releases EVs from six inner labial type 2 (IL2) ciliated sensory neurons (Figure 1A), suggesting that EV shedding is intrinsic property of certain ciliated neurons (Wang et al., 2014).

The *C. elegans* male has 383 neurons, sharing 60 ciliated sensory neurons with the hermaphrodite and also possessing 48 male-specific ciliated sensory neurons devoted to sexual behaviors (O'Hagan et al., 2014; Sulston et al., 1980). *C. elegans* males shed and

release EVs from 27 ciliated extracellular vesicle releasing neurons (EVNs) including six shared IL2 neurons and 21 male-specific polycystin-expressing EVNs in the head (four CEM neurons) and tail (16 ray B-type RnB neurons and one hook B-type HOB neuron) (Figure 1A)(Wang et al., 2014). In these male-specific EVNs, the polycystins *lov-1* and *pkd-2* are required for male sex drive, response to mate contact, and vulva location (Barr et al., 2001; Barr and Sternberg, 1999; Barrios et al., 2008). The *C. elegans* cilium is also a source of bioactive polycystin-containing EVs: the *C. elegans* polycystins homologs LOV-1 and PKD-2 are shed into environment from male-specific EVNs (Wang et al., 2014). That *C. elegans* and mammalian polycystins localize to cilia and EVs, and act in a sensory capacity indicates remarkable ancient functions.

In addition to their EV shedding ability, the 27 ciliated EVNs display several unique ultrastructural characteristics. Most notably, the tips of EVN cilia protrude into the environment from sensory organs (sensilla). This is in contrast to the chemosensory amphid and phasmid cilia that are protected within channels or pores that have access to the surrounding environment. An EVN cilium including the distal-most dendritic ending is surrounded by an extracellular lumen formed by two glial cells, a sheath cell and a socket cell (Figure 1B). In the cephalic sensillum that houses the cephalic male (CEM) EVN, glial cell bodies are close to the CEM cell body, and send their processes along the CEM dendrite to wrap the neuron with a tight junction at the distal-most dendritic ending (Figure 1C). The CEM neuron shares the extracellular lumen with CEP neuron present in both males and hermaphrodites, but only cilium of CEM enters the cuticular pore and is directly exposed to the environment (Figure 1C). The glial encased portion of the distal dendrite is slightly larger than the rest of dendrite and may correspond to the periciliary compartment of the amphid neurons, which is dedicated to exocytosis and endocytosis (Kaplan et al., 2012).

High resolution reconstruction of the adult hermaphrodite anterior sensilla found no EVs in the amphid channel and cephalic lumen and a few EVs in the inner labial lumen, the latter housing the IL2 EVNs (Doroquez et al., 2014). Electron tomography of the anterior sensilla in the male nose reveals 62 to 259 EVs in cephalic lumen surrounding the CEM cilium (Wang et al., 2014). EVs in the lumen surrounding the CEM cilia may be the source of the GFP-labeled EVs visibly released outside of the worm. In the amphid channel lumen that surrounds 10 cilia, we observed fewer than ten EVs. The position of the EVs found in amphid lumen corresponds to distal regions of amphid channel cilia whereas EVs are found in the cephalic lumen are situated along the length of the entire CEM cilium, including the periciliary compartment (Wang et al., 2014). We observed one EV budding from or fusing with the ciliary membrane in the CEM ciliary base area (Figure 2A), which suggests that EVs may be shed from this region. However, we could not resolve the distal most ciliary tip and cannot eliminate the possibility that EVs are also shed from this site, as in EV shedding from *Chlamydomonas* flagellar tips (Figure 2A). These results suggest that EVs in the male cephalic and amphid channel lumen are quantitatively and qualitatively different, and are consistent with specific ciliated cell types possessing the ability to shed and release ciliary EVs.

The kinesin-3 protein KLP-6 and myristoylated coil-coil protein CIL-7 are exclusively expressed in the 27 EVNs and regulate ciliary EV biogenesis (Maguire et al., 2015; Wang et

al., 2014). These 100–150nm sized EVs can be visualized via GFP-labeled EV cargo, which includes PKD-2 and LOV-1 shed and released from male-specific EVNs and CIL-7 from all 27 EVNs. The ability to visualize GFP-tagged EVs combined with the powerful *C. elegans* molecular genetic toolkit enable study of EV biogenesis, shedding, release, and signaling in a living animal.

Are ciliary EVs exosomes, ectosomes, or both?

Determining the identity of an EV is no small task. Exosomes are generated by fusion of multivesicular bodies (MVBs) with the plasma membrane and subsequent release of MVB intraluminal vesicles (Colombo et al., 2014; El Andaloussi et al., 2013). Ectosomes are formed via outward budding of the plasma membrane, although mechanisms controlling formation of ectosomes are not well understood. Exosomes contain certain cargo that may be used as identifiers. However, ectosomes may also carry exosomal markers. Exosomes and ectosomes are shed into bodily fluids and proteomic analysis cannot determine with certainty the nature of the vesicle. To diagnose whether an EV is an ectosomes or exosome, one would ideally visualize release of the EV in real time. Given their small size, this is a great technical challenge.

In *Chlamydomonas*, EV budding from the flagellar tip can be directly observed by differential intereference microscopy (Wood et al., 2013). As *Chlamydomonas* flagella are devoid of MVBs, these ciliary EVs are clearly ectosomes (Wood and Rosenbaum, 2015). In *C. elegans*, ciliary EV identification as exosome or ectosome is not so obvious. GFP-labeled EVs are shed and released from ciliated EVNs, but the site of release (cilia base or cilia tip) cannot be resolved using standard fluorescence-based microscopy. Super-resolution microscopy might address this issue. Using transmission electron microscopy and electron tomography, we observed one EV connected to the ciliary base membrane with a long stalk, indicating that this EV was either budding off of or fusing with the membrane (Wang et al., 2014). We did not observe MVB structures spanning the CEM neuron from the distal dendrite to the ciliary tip. Moreover the MVB components STAM-1 (ESCRT-0), MVB-12 (ESCRT-1) and ALIX-1 (required for endosomal intralumenal vesicle formation) are not required for PKD-2: :GFP EV shedding or release (Wang et al., 2014). Combined, these results suggest but do not prove that *C. elegans* EVs are ectosomes.

EVs are associated with mammalian cilia. The stem-cell marker prominin-1 labels primary cilia and EVs associated with cilia tips of mouse neuroepithelial cells (Dubreuil et al., 2007). Intriguingly, prominin-1 labeled EVs are observed surrounding short cilia. Authors propose that ciliary membrane budding may be a mechanism to control ciliary length, which varies depending on the neuroepithelial stage of development and cell cycle. EVs bind to primary cilia of mouse embryonic fibroblasts (Pampliega et al., 2013). EVs surround cholangiocyte primary cilia in the autosomal recessive PKD mouse model whereas a single EV was occasionally observed attached to a wild-type cilium (Woollard et al., 2007). In three cases, it is not known whether cilia were shedding outward bound EVs or receiving inward bound EVs.

Polycystin-containing exosome-like vesicles isolated from mammalian urine interact with primary cilia of kidney and biliary epithelial cells (Hogan et al., 2009). These EVs are termed exosome-like vesicles because they express exosome markers and display exosome morphology. Transmission electron microscopy and immunogold labeling of polycystin-1 showed polycystin-1 on intraluminal vesicles in rat cholangioctyes MVBs. While these data suggest an MVB origin, Ward and colleagues conclude that budding from the apical membrane or cilia cannot be eliminated (Hogan et al., 2009). While unambiguous data supporting a universal origin of ciliary EVs is lacking, we propose that ciliated cells may use multiple mechanisms to shed EVs (Figure 2A). Cilia may shed EVs at the ciliary base via budding as ectosomes or via fusion of MVBs and release of interluminal vesicles as exosomes. Cilia may also shed EVs as ectosomes at the ciliary tip, as demonstrated in *Chlamydomonas* by Rosenbaum and colleagues (Wood et al., 2013). These ciliary EVs may have different functions depending on their origins and cargo composition.

Regulation of EV biogenesis, shedding, and release

Intraflagellar transport (IFT) is an evolutionarily conserved process required for the ciliary formation and maintenance in organisms as diverse as algae, worms, mice, and men (Rosenbaum and Witman, 2002). The IFT train is composed of IFT-A and IFT-B multiprotein complexes and transported by anterograde kinesin-2 from ciliary base to tip and retrograde dynein motor from the tip to base. The IFT machinery may also have a role beyond ciliary construction and maintenance (Baldari and Rosenbaum, 2010). Some clues from *C. elegans* and *Chlamydomonas* suggest that the IFT machinery may be required for EV biogenesis, EV shedding, EV release, and/or cilia-EV interactions.

Chlamydomonas sheds ciliary ectosomes that contain a lytic enzyme that digests the mother cell wall and is required for post-mitotic hatching of daughters (Wood et al., 2013). Flagella-less *ift88*-null mutants cannot hatch and addition of ciliary ectosomes isolated from wild-type cells induced hatching. These results indicate that an intact flagellum is required for the production of functional EVs. The role of the IFT machinery in *Chlamydomonas* ciliary shedding has yet to be explored.

In *C. elegans* EVNs, IFT-A and IFT-B components, IFT kinesin-2 and dynein motors, and the EVN-specific ciliary kinesin-3 KLP-6 are required for the release of PKD-2: :GFP-labeled EVs (Wang et al., 2014). In these mutant backgrounds, the quantity of environmentally released EVs is significantly reduced (Wang et al., 2014) and PKD-2: :GFP accumulates in the ciliary base region, resulting in a similar ciliary localization defective or Cil phenotype (Bae et al., 2008; Bae et al., 2006; Peden and Barr, 2005; Qin et al., 2005). Whether PKD-2: :GFP accumulates in the distal dendrite or cephalic lumen cannot be resolved by light microscopy. Transmission electron microscopy and electron tomography showed that *klp-6* mutant males accumulate a large number of luminal EVs and possess a lumen doubled in volume compared to wild-type (Figure 1C) (Wang et al., 2014). The excessive accumulation of luminal EVs indicates that *klp-6* is either a negative regulator of EV biogenesis and shedding or a positive regulator of EV environmental release. In EVN cilia, *klp-6* also modulates IFT (Morsci and Barr, 2011). The similar Cil phenotype of *klp-6*

and IFT mutants suggest a similar mechanism of action. Future ultrastructural analysis of IFT mutants will reveal what role the IFT machinery plays in EV biology.

How might IFT contribute to EV biogenesis, shedding, release, and/or signaling? In *Chlamydomonas*, polystyrene microspheres adhere to and are moved bidirectionally along the external flagellar surface (Bloodgood, 1988; Bloodgood, 1995). IFT drives flagellar gliding motility and the extraflagellar transport of the major flagellar surface glycoprotein protein FMG1-B (Shih et al., 2013). When an anti-FMG1-B antibody is attached to beads, beads and IFT trains move in similar speeds. In *Ctenophores*, or comb jellies, individual cells are transported up the external ciliary surface, independent of ciliary beating, to build the statolith, a gravity sensing organ (Noda and Tamm, 2014). In a similar scenario, IFT and KLP-6 inside the cilium may propel EVs along the outside ciliary surface. In this model, EVs and cilia express unidentified surface proteins that couple the EV to the cilium, enabling internal ciliary motors to propel EVs along the ciliary surface (Maguire et al., 2015).

Targeting cargo to ciliary EVs

Understanding how cargo is directed to EVs has important therapeutic implications (Gyorgy et al., 2015). Cargo sorting to *C. elegans* ciliary EVs appears to be selective – not all ciliary proteins get packaged into EVs (Wang et al., 2014). Additionally, EV cargo displays cell type specificity. The myristoylated coil-coil protein CIL-7 localizes to ciliary EVs released by all 27 EVNs (Maguire et al., 2015), while the polycystins PKD-2 and LOV-1 are released in EVs by the male-specific EVNs and not the shared IL2 EVNs (Wang et al., 2014). Conversely, we identified EV cargo released from the IL2 but not male-specific EVNs (Wang et al., under review). We hypothesize that EVs have at least two types of cargo, one for structure (for example, CIL-7) and the other for function (for example, the polycystins).

CIL-7 and the kinesin-3 KLP-6 are both expressed in all 27 EVNs, localize to cilia, regulate EV biogenesis, and are required for polycystin-mediated male mating behaviors (Maguire et al., 2015). However, CIL-7 but not KLP-6 is EV cargo (Maguire et al., 2015). The myristoylation site in CIL-7 is necessary for CIL-7 function and localization to EVs but not cilia (Maguire et al., 2015). N-myristoylation is used by proteins for membrane anchoring and for ciliary localization of proteins in *Trypanosome* flagella, *C. elegans* sensory neurons, mammalian photoreceptors, and retinal pigment epithelial cells (Evans et al., 2010; Maric et al., 2010; Ramulu and Nathans, 2001; Wright et al., 2011). Myristoylation targets proteins to EVs in Jurkat T-cells (Shen et al., 2011). In the *cpk* mouse model of PKD, the *cpk* mutation lies in the Cystin gene, which encodes a myristoylated cilia- and EV-associated protein (Hogan et al., 2009; Tao et al., 2009). The Cystin myristoylation signal is necessary for ciliary targeting in inner medullary collecting duct cells (Tao et al., 2009), and perhaps EVs. We conclude that, *in C. elegans*, myristoylation provides a cis-acting motif for EV targeting *in vivo*. As ciliary EVs and their cargo are identified and characterized, perhaps “EV zip codes” or “EV localization signals” similar to nuclear localization signals will be discovered.

Ciliary EV signaling and bioactivity

EVs mediate broad range of intercellular communication by carrying bioactive proteins, lipids and nucleic acids (El Andaloussi et al., 2013). The function of ciliary EV is largely unknown, here we will speculate based on limited data. The cilium may shed and release EVs as a rapid way to modulate membrane composition, adjust protein levels, and downregulate signaling molecules. This might be important for the ciliary sensory function, as signaling molecules are directly related to sensitivity and sensory adaptation. In *Chlamydomonas*, the major flagellar glycoprotein FMG1 is constitutively shed while the SAG1 protein is shed only during signaling events but not in resting gametes (Bloodgood et al., 1986; Cao et al., 2015), supporting this idea.

In *C. elegans*, the ciliary kinesin-3 KLP-6 and myristoylated coiled-coil protein CIL-7 are specifically expressed in the 27 EVNs, regulate EV release, and are required male mating behavior. *klp-6* and *cil-7* mutant males accumulate excessive amounts of EV in the glial-surrounded lumen of male-specific sensory organs (Figure 1C)(Maguire et al., 2015; Wang et al., 2014). In invertebrate and specialized sensory organs of higher animals, sensory neurons are ciliated and either exposed to the environment or associated with extracellular matrix, which may be important for sensory transduction (Andres et al., 2014; Cook et al., 2008; McGlashan et al., 2006). In this context, EVs may contribute to the physiological functioning of *C. elegans* male-specific sensory organs.

In addition to cilia-dependent functions, ciliary EVs are bioactive and can act non-autonomously. *Chlamydomonas* flagella release EVs containing an enzyme to digest the mother cell wall and free daughter cells (Wood et al., 2013). In *C. elegans*, isolated EVs from wild-type cause a change in male locomotion and trigger male tail chasing behavior, whereas EVs isolated from a *klp-6* mutant and lacking PKD-2: :GFP do not elicit male tail chasing (Wang et al., 2014). These results show that EV cargo content is essential for EV bioactivity. This is a radically new function for EVs, which are generally thought influence intercellular communication within an animal, and a first demonstration that EVs are a way to communicate between individuals and influence the behavior of other animals.

In addition to triggering short-term signaling events between animals (a matter of minutes), one intriguing possibility is that ciliary EVs may transfer small RNAs or cilia may receive EVs containing small RNAs to influence long-term signaling processes. This would be a means for communication between tissues to coordinate developmental timing (Benkovics and Timmermans, 2014), between animals of the same species as a form of quorum sensing (Sarkies and Miska, 2013), or between pathogen and host to co-opt the host's immune response (Buck et al., 2014).

Do abnormal cilia-EV interactions contribute to ciliopathies?

Ciliary EVs have specific cargo that include surface-associated proteins and receptors (Bloodgood, 1995; Cao et al., 2015; Wang et al., 2010; Wood et al., 2013). These EV surface proteins may be important for EV-target cell interactions (Figure 2B). Fractionated *Chlamydomonas* EVs bind only to flagella and rarely to the cell body, suggesting that

flagellar EVs bind to a specific region of the single celled algae (Cao et al., 2015). We speculate that the polycystins (or another ciliary receptor) may mediate an interaction between cilia and EVs, allowing a homotypic interaction between polycystin-positive cilia and polycystin-positive EVs (Figure 2B). Indeed, rapid interaction between exosome-like vesicles and primary cilia has been reported. A defect in ciliary EVs (Figure 2C) or a defect in the cilium (Figure 2D) would result in abnormal EV-cilia interactions and a potentially pathogenic cellular response.

There is experimental evidence for this hypothesis. The fibrocystin protein, which is encoded by causal gene of ARPKD, pkhd1, is required for the rapid interaction between mammalian primary cilia and EVs (Hogan et al., 2009; Masyuk et al., 2010). In the ARPKD patient or the mouse model (scenario in Figure 2D), EVs are found attached to cilia, but not all cilia (Hogan et al., 2009), consistent with selective EV targeting and attachment.

Somlo and colleagues made a surprising discovery that simultaneous inactivation of the polycystins and cilia resulted in a decrease in PKD severity compared with polycystin-only knockout (Ma et al., 2013). Authors propose that the polycystins act as inhibitory signals that modulate an unidentified pathway and that requires intact cilia to function. A tantalizing but untested possibility is that polycystin inactivation results in both abnormal ciliary EVs and abnormal ciliary signaling, resulting in severe pathology (scenerios shown in Figure 2C and 2D). The latter was previously demonstrated while the former only recently: EVs isolated from ADPKD patients display different proteins than unaffected individuals and hence the proposal that urinary exosomes contain biomarkers for ADPKD (Hogan et al., 2015). By removing the cilium in a Pkd1 or Pkd2 mutant, abnormal EV-cilia interactions and signaling are abrograted, thereby suppressing cystogenesis. Future studies on the cilia-dependent cyst-activating pathway should reveal whether or not EVs play a role in ADPKD or other ciliopathies.

One critical and perhaps overlooked aspect of the cilium is its dynamic nature: the protein composition changes constantly, the membrane is renewed, cilia length is changing…even the entire cilium can be shed and regenerated! The finding that the cilium shed EVs adds another layer of complexity to this essential organelle. A deeper understanding of the dynamic regulation of cilia may provide insight for intervention or ultimately a cure for ciliopathies. EVs are promising diagnostic tool and have great therapeutic potential to deliver cell specific treatments (Lo Cicero et al., 2015). Urinary EVs may eventually be used in diagnosis and monitoring ADPKD (Hogan et al., 2015). Many exciting new areas in this emerging new field of ciliary EVs await exploration. The best is yet to come.

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Abbreviations

ADPKD	autosomal dominant polycystic kidney disease
CEM	cephalic male neuron
EV	extracellular vesicle
EVN	extracellular vesicle releasing neuron
IFT	intraflagellar transport
IL2	inner labial type 2 neuron
MVB	multivesicular body

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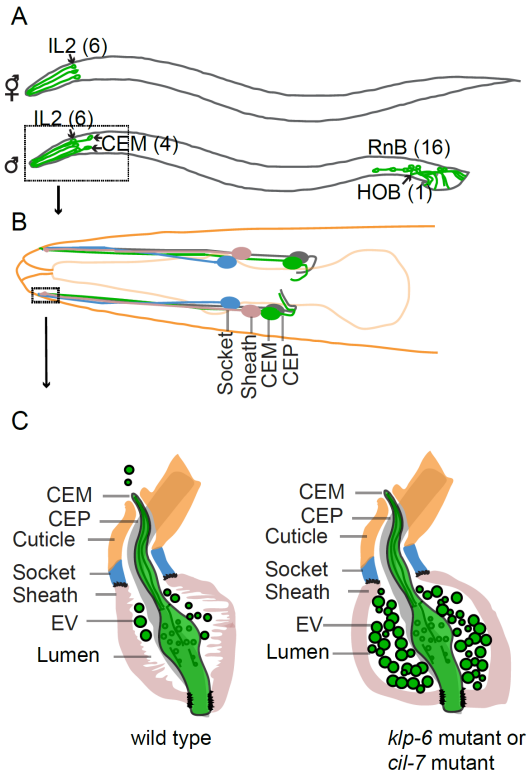

Figure 1.

C. elegans EV releasing neurons (EVNs). *C. elegans* cilia are located on distal dendritic endings of sensory neurons. There are 60 and 108 sensory ciliated neurons in *C. elegans* hermaphrodite and male respectively, however only six IL2 neurons in hermaphrodites and 27 neurons in males shed and release EVs into environment. (A) Names and anatomical position of the EVNs in the hermaphrodite and male. The number of each EVN type is in parenthesis. (B) EVN sensory organs display common ultrastructure features. Each EVN has a sister neuron, their cilia and ciliary bases are isolated in a lumen formed by two glial cells, the sheath cell and the socket cell. The lumen is continuous with a cuticular pore, from which the EVN cilium protrudes into environment directly while the sister cilium does not. Only the male specific cephalic sensory organ components are shown. (C) A model of the cephalic sensory organ based on electron tomograph (reproduced from (Wang et al., 2014). The cephalic sensillum contains CEM and CEP cilia, CEM-derived EVs, and the lumen formed by sheath and socket cell. The CEM cilium sheds EVs into the lumen that may be released through the cuticular pore to environment. In a *klp-6* (an EVN specific ciliary kinesin) or a *cil-7* (a myristoylated coiled-coil protein) mutant, EVs accumulate in the cephalic lumen as diagnosed by transmission electron microscopy and PKD-2: :GFP EVs are not released outside (Maguire et al., 2015; Wang et al., 2014). Mutation in either *klp-6* or *cil-7* disrupts EVN-mediated sensory functions.

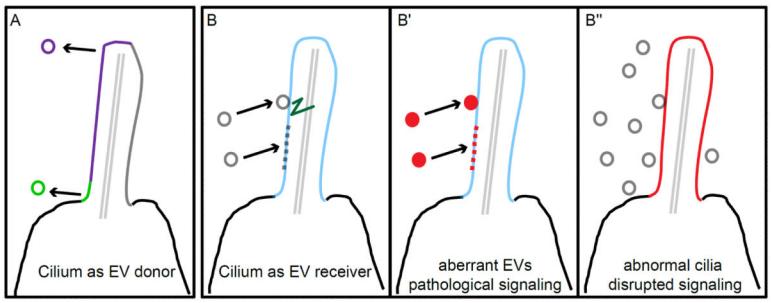

Figure 2.

Model depicting the cilium as an EV donor (A) and EV acceptor (B). Cilia are highly compartmentalized organelles with an microtubule axonemal skeleton (gray lines) (Blacque and Sanders, 2014). The ciliary membrane has different domains that are enriched with certain proteins. The IFT machinery and other ciliary complexes contribute to ciliary compartmentalization. The IFT machinery is required for some aspect of EV biogenesis (Wang et al., 2014). Therefore, we propose that (A) ciliary compartmentalization might be used for sorting of ciliary EV cargoes. EVs maybe released by different ciliary regions including the base (green) or from the tip of ciliary membrane (purple). (B) Cilia are sensory organelles that may interact with EVs (gray bubbles). EVs may originate from nearby neighbors or from cells at long distances. The EV may interacts the ciliary membrane (blue) or may fuse with the ciliary membrane (gray dashes) to promote signal transduction (green arrow). In a disease state, (B') aberrant EVs (red) may trigger a pathological signal or (B") abnormal cilia (red) may fail to transduce an EV-induced signal.