Extracellular vesicles: eat glutamine and spit acidic bubbles

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Extracellular vesicles: eat glutamine and spit acidic bubbles

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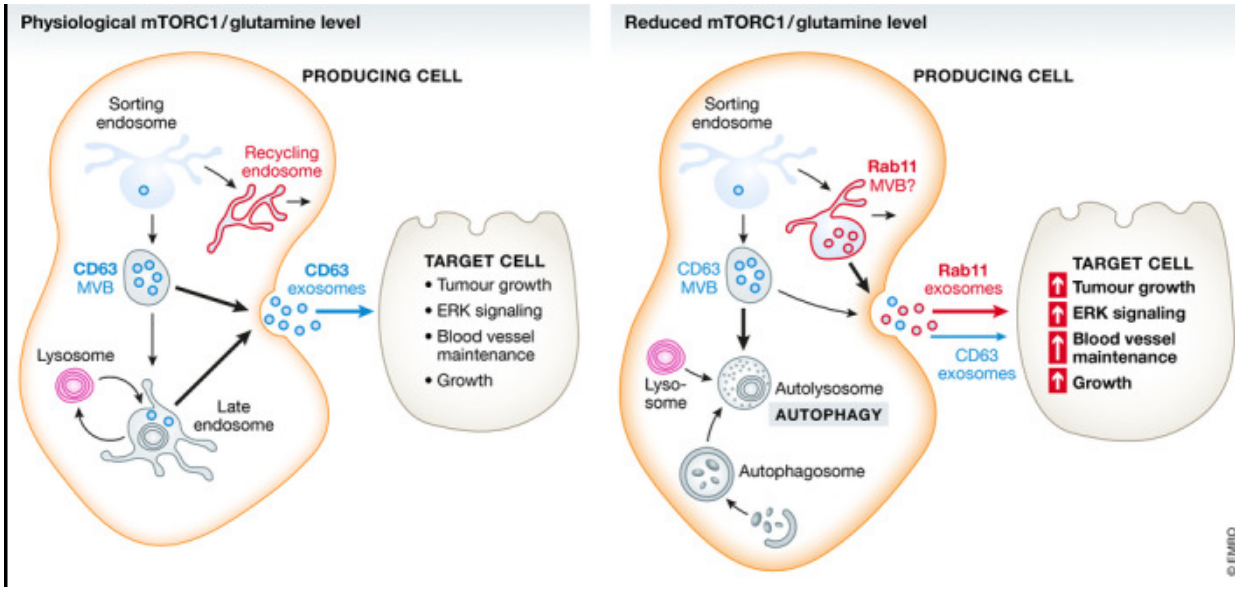
Figure:
(Inspired from the last figure of the Fan et al EMBO J 2020 paper)



Abstract
Extracellular vesicles mediate transfer of diverse molecular content to target cells in order to induce phenotypic changes, which has put them under the spotlight as likely major players in cell-to-cell communication. However, extracellular vesicle heterogeneity in terms of intracellular origin has only recently been recognized as a potential determinant of their activity. Recent work by Fan et al (2020) illustrates how lack of external resources that affect cellular homeostasis and signaling can also modulate EV biogenesis, by inducing the production of a novel subpopulation of exosomes enriched in Rab11a with context-dependent roles in Drosophila gland physiology and cancer cell aggressiveness.

Text

All cells release in their environment not only simple secreted proteins, but also more complex structures called extracellular vesicles (EVs) (Van Niel 2018). EVs are composed of cytosolic components enclosed in a lipid-bilayer coming from either the plasma membrane or endocytic compartments of the cell. Plasma membrane-derived EVs are called ectosomes (or microvesicles or microparticles), whereas EVs formed as intraluminal vesicles (ILVs) inside late multivesicular endosomes, and released upon fusion of these compartments with the plasma membrane, are called exosomes (Witwer 2019). However, it remains very difficult to analyze separately EVs coming from one or the other sub-cellular location, once they are released outside: the smallest EVs budding of the plasma membrane share most biophysical properties

(size, density) with exosomes (Mathieu 2019). Therefore, most studies relying on regular EV isolation techniques, even if they use the term “exosomes” in fact analyze a very heterogeneous mixture of ectosomes and exosomes.
The field has started recognizing this issue around 5 years ago, when we proposed that the knowledge on EVs and their heterogeneity was similarly positioned as was, back in the 1950s, the knowledge on immune cells: at that time, only red and white blood cells (the latter including only phagocytes and lymphocytes) were known as different immune cell subtypes…. Consequently, EV research is now fast evolving to better decipher the specificities, in terms of composition and of function, of different EV subtypes, especially by improving the separation techniques (Jeppesen 2019; Zhang 2018). However, the only way to really discriminate EVs coming from different subcellular locations is to identify their specific mechanisms of biogenesis and to live track their secretion (Verweij 2018): therefore, cell biology studies are crucial today for the advancement of the field.

A recent article published in EMBO J clearly illustrates the power of such cell biology approaches (Fan, Kroeger…Goberdhan, EMBO J 2020). In this work, the authors now demonstrate an additional complexity of EVs formed intracellularly in endosomes, by showing that exosomes can contain the usual late endosomal compartments such as the tetraspanin CD63 and the Rab7 intracellular trafficking GTPase, but also molecular actors of earlier endosomal compartment such as Rab11a that decorates recycling endosomes. The study reveals the existence of likely two subpopulations of exosomes that are respectively enriched in different proteins (CD63 and Rab11a). Importantly, generation and secretion of the Rab11a-containing exosomes is regulated by availability of nutrients, especially glutamine which regulates the activity of the amino-acid sensor mTORC1 (mechanistic target of rapamycin complex 1). The direct or indirect modulation of sensing of external resources results in a switch in the secretion of Rab11a (and Caveolin 1) at the expense of CD63-positive exosomes.

How thus can Rab11a, that controls the last step of slow recycling, end up in ILVs that are rather generated in later endosomes? Using state-of-the-art imaging technologies, the authors show that Rab11a-exosomes are secreted in vivo in an intact organism, the Drosophila male accessory gland. These Rab11a-exosomes likely originate from an endosomal non-acidic compartment that contain both CD63 and Rab11 in secondary cells. This co-localization is intriguing as Rab11 and CD63 are rather present in distinct compartments in HCT116 colorectal cancer cells, as shown in Fan et al. But secondary cells are a specialized secretory cell type whose secretome contributes to ejaculate composition and reproductive outcome (Hopkins 2019). These compartments that contain Rab11 but also Rab6 and CD63 (Prince 2019) and are regulated by the ESCRT machinery (Fan 2020) have the features of secretory lysosome-related organelles (LROs), such as melanosomes (Delevoye 2019). The work of Fan et al clearly illustrates how, as in mammalian specialized cells, functional adaptations of the endo-lysosomal system would allow the generation of Lysosome-related organelles in Drosophila to fulfill specific functions. By doing so, specialized cells would release a new subpopulation of exosomes with a specific composition and hence specific functions.

The endosomal pathway is composed of a continuum of interconnected structures whose maturation from early sorting endosomes into recycling endosomes or late endosomes involves a complex coordination of inward budding (intraluminal vesicle formation) /outward budding (tubulations) and fusion/fission cycles. This interconnection explains why

manipulation of sorting mechanisms or maturation process of one compartment likely affects afferent compartments, as illustrated by the unexpected co-localisation of Rab11, Rab7 and Rab5 in a single MVB of secondary cells when expressing a dominant mutant of Rab5.
Nevertheless, in mammalian cancer cells, Fan et al show that Rab11 and CD63 are rather present in distinct compartments. The most advanced imaging techniques are unable to detect ILV formation in Tfn-positive recycling compartments (Hoffman 2020). How, then, would recycling compartments contribute to exosome release, and their components be incorporated into ILVs? So far, Rab11 (Savina 2005) and other regulators of recycling endosomes such as Munc13-4 (Messenger 2018) have been proposed to modulate exosome secretion from MVB by potentiating MVB for fusion with the plasma membrane. The presence of Rab11a within exosomes adds an extra level of complexity in the generation of ILVs in particular under starvation. In their study, Fan et al revealed that Glutamine deprivation enhances uptake of Tfn and promotes CD63 degradation. This is in line with a role of mTORC1 in the maintenance of the canonical endocytic recycling pathway against lysosomal delivery in an ESCRT-0 dependent manner (Dauner 2017). Under starvation, late endosomal compartments, enriched in CD63, would then be consumed by fusion with autophagosome to produce amphisomes and autolysosomes, at the expense of their secretion to release exosomes. Yet, inhibition of the mechanisms supporting endosomal recycling by inactivation of mTORC1 may yield to another phenomenon that remains to be investigated: the inward budding of Rab11 microdomains, forming ILVs and future exosomes at the expense of outward budding allowing generation of recycling endosomes. This working hypothesis would support the observed switch between CD63 and Rab11a subpopulations of exosomes in favor of the newly identified one.

A crucial input of this study is to comfort the idea that peculiar conditions, such as starvation, would modulate the biogenesis pathway of exosomes and hence their action. Indeed, the observation of Fan et al is also functionally relevant, since the authors show that these Rab11a-exosomes promote tumor growth in vitro and in vivo, possibly through signaling through the EGFR receptor AREG, in a human cancer cell line. In this work, glutamine deprivation is consistent with a situation of starvation encountered in the poorly vascularized central parts of solid tumors. This new level of exosome production could be also important in other pathologies where exosomes have been involved in their etiology. For instance, in pathological conditions where PI3P generation is altered in neurons, and consequently the entire endosomal-lysosomal pathway modified, cells start secreting a novel type of exosomes enriched in undigested amyloid (Miranda 2018).

All in all, the work of Fan et al thus provides an additional evidence that even endosome-derived exosomes are not a single homogenous entity, and highlights the need to consider the possible effect of environmental signals when analyzing EV or exosome secretion. Indeed, if hypoxia has been shown several years ago to change the nature of EV/exosome secretion (Kucharzewska 2013), although never with clear distinct evaluation of exosome versus ectosome secretion, many internal or external stimuli can also do so. This work thus feeds the groundwork that will allow the EV field to evolve as the immune cells field evolved: many subpopulations with distinct functions that adapt to their environment to provide the adequate intercellular message.

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Conflict of Interest

The authors declare that they have no conflict of interest