On the function and heterogeneity of extracellular vesicles

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Both, prokaryotic and eukaryotic cells contain the ability to communicate with cells in their environment. Classically, intercellular cell communication was thought to be mainly mediated by direct cell-cell contacts and by soluble factors including cytokines, growth factors and hormones. In 1996 Raposo and colleagues showed that intercellular communication can also be mediated by vesicles; specifically they reported that B lymphoblastoid cells release MHC class II carrying vesicles which are able to induce antigen-specific MHC class II-restricted T cell responses (1). Since then, an exponentially increasing amount of studies demonstrates the importance of extracellular vesicle (EV) mediated intercellular signaling in a huge variety of different cellular systems and organisms ranging from bacteria and yeast to plants and humans. It became evident that EV-mediated intercellular communication is essentially involved in many physiological and pathophysiological processes (2). Thus, EV-mediated intercellular communication provides a third, very complex mode of intercellular communication. Unraveling the basic principles of this novel intercellular communication system and gaining insight in its complexity will for sure have large impacts on the overall understanding of biological processes and certainly will pave the way for novel diagnostic and therapeutic approaches (2-4).

EVs carry a number of different molecules. Initially, together with lipids several proteins were identified being expressed on EVs like major histocompatibility complex (MHC) class II molecules and tetraspanins (1,5,6). In 2001 the first proteomic approach was reported by Théry and colleagues, and a number of different proteins were discovered, several of them being connected to the EVs' functional properties (7). In 2006 RNAs have been detected in EV fractions, and it has been shown that EVs shuttle functional RNA molecules between cells (8,9). These findings increased the interest in EV research and massively boosted the field. Although some publications may raise the impression that EVs mainly act as vehicles for miRNAs, they should rather be seen as extracellular cell organelles, which composed by a collection of specific lipids, proteins and RNAs specifically transmit a special combination of signals from signal sending to the signal receiving cells (10). More recently, single stranded and double stranded DNA have also been identified in EV fractions (11-13). However, although EV-associated DNAs have been associated with the progression of certain diseases, e.g., cancer, their function remains elusive, yet.

In addition and as nicely reviewed by Fonseca and colleagues, recently, a collection of metabolic proteins have been identified in EV fractions which are able to control metabolic activities of target cells and tissues (14). Affecting metabolic processes provides another level of complexity to the EV mediated intercellular signaling. Learning more and more about the processes of how EVs can influence their target cells, we have also to bear in mind that different EV entities exist. It is important to consider that eukaryotic cells release a variety of different vesicle types. The most prominent EV types are the exosomes, microvesicles and apoptotic bodies (15). Exosomes are defined as derivatives of the endosomal system and correspond to intraluminal vesicles formed during endosome maturation by inward bodying of

the outer endosomal membrane, the limiting membrane. Previously, it was assumed that the intraluminal vesicles and all their cargo molecules are destined for the degradation in lysosomes. However upon studying transferrin trafficking in reticulocytes it was discovered that a proportion of the endosomes containing intraluminal vesicles, the so called multivesicular bodies or multivesicular endosomes, can fuse with the plasma membrane to release their intracellular vesicles, commonly having sizes between 70 and 150 nm, into their extracellular environment (15-17). In contrast, microvesicles are formed by the outward bodying of the plasma membrane. Microvesicles are reported to have sizes between 100 nm and 1 µm (15). Apoptotic bodies arise by the fragmentation of apoptotic cells, their reported sizes range from 500 nm to several µm (15). However, upon analyzing apoptotic cells, the formation of vesicles in the same size range than exosomes can be observed. Up to now, neither markers have been qualified allowing a clear discrimination between exosomes, microvesicles, apoptotic bodies and other EV types, nor are well established technologies available allowing purification of certain EV subtypes, yet. Conventionally, different vesicle types are enriched in parallel resulting in very heterogeneous vesicle fractions. Accordingly, to use an appropriate terminology, members of the International Society of Extracellular Vesicles (ISEV) recommended to decipher vesicles in experimental enriched vesicle fractions as EVs rather than as exosomes, microvesicles or apoptotic bodies etc. (15,18). The issue of heterogeneity is further complicated by the finding that different pathways have been identified controlling the intraluminal vesicle formation in the endosomal compartment. Specifically, intraluminal vesicles can be formed in an ESCRT-dependent or an ESCRT-independent manner (19). Regarding the ESCRT-dependent intraluminal vesicle formation, different pathways have been defined as well. For now, ubiquitin- and sumoylation-dependent pathways can be discriminated (20). The complexity is further multiplied due to the fact that EVs always contain a set of molecules reflecting their cellular origin. Thus, different cell types release different variants of given EV subtypes. Furthermore, the alteration of environmental parameters regularly results in changes in the EVs' molecular signatures.

So far, the question of heterogeneity in given EV samples is rarely addressed at the experimental level, as mentioned before, mainly due to the lack of qualified technical platforms. Very recently, two studies made use of bead-capturing methods to separate and characterize different EV subtypes (21,22). Despite characterizing the molecular content of the different EV subpopulations, it will be important to analyze their biological functions independently. In the future we need to address the issue of heterogeneity in more detail. It will be important to learn whether all EVs taking part in intercellular communication contain RNAs/DNAs or whether nucleic acids are transported by specific EV subtypes. If it comes to the EVs' function it will also be essential to learn whether there are subtypes specialized on certain functions, e.g., to mediate metabolic activities, to mediate intercellular signaling pathways or to transport waste out of given cells, or whether all functional properties are unified in identifiable subtypes. For sure, novel technological platforms allowing single EV analysis and novel purification techniques enabling enrichment of distinct EV subpopulations that can be analyzed at a functional level will help to increase our understanding of EV mediated intercellular signaling massively. I am convinced that an improved biological understanding of EVs will affect the field of life sciences tremendously.

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Footnote

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