THE DISCOVERY OF EXTRACELLULAR VESICLES (EVs): TRACING THE JOURNEY FROM INCEPTION TO PRESENT DAY

BECKMAN COULTER Life Sciences

1. UNVEILING THE SECRETS OF BLOOD CLOTTING: THE EARLY DAYS OF EV RESEARCH

1945

Chargaff: During his work to establish a centrifugation protocol for separating clotting factors from cells, Chargaff observed that "the addition of the high-speed sediment to the supernatant plasma brought about a very considerable shortening of the clotting time."

1946

Chargaff and West: In their study, Chargaff and West identified a particulate fraction that "sedimented at 31,000 x *g* and had high clotting potential." They suggested that this fraction likely included "a variety of minute breakdown products of blood corpuscles" in addition to the thromboplastic agent.

1967

Wolf: Wolf's work involved the identification of platelet dusts, which he described as a "material in minute particulate form, sedimentable by high-speed centrifugation and originating from platelets but distinguishable from intact platelets." This study also provided the first electron microscopy images of EVs.

1971

Crawford: Crawford's research demonstrated that EVs contain lipids and carry cargo such as ATP and contractile proteins.

1974

Nunez: Nunez's study focused on the presence of small extracellular vesicles in the bat thyroid gland during arousal from hibernation. The authors observed multivesicular bodies (MVBs) near the apical membrane and proposed that the fusion of the MVB's outer membrane with the apical plasma membrane could lead to the release of the vesicles into the luminal space.

1971–1979

Aaronson and others: Studies conducted by Aaronson and colleagues on various organisms indicated that vesicular structures extruded from cells were not unique to mammals. For example, they observed vesicles budding from cells and isolated them through centrifugation in *Ochromonas danica*, a flagellated alga. EVs were also shown to be released by *Candida tropicalis, Corynebacterium, Acinetobacter, Escherichia coli,* and other species.

2. UNRAVELING THE EV ENIGMA: ACCUMULATING EVIDENCE, UNCERTAIN ROLE

1956–1975

Multiple Researchers: During this period, researchers actively searched for "virus-like particles" that could potentially cause diseases, including infections and cancer. However, it was noted that labeling structures with the morphological characteristics of naturally occurring vesicles from multivesicular bodies and microvesicles associated with epithelial cells as "virus-like" was unwarranted.

1983

Johnstone and Stahl: Their work demonstrated the release of intraluminal vesicles from cells, using reticulocyte maturation as a model. They defined these vesicles as exosomes, which were released from the lumen of multivesicular bodies upon fusion with the plasma membrane. This discovery unveiled the exosome secretion pathway.

1986

Gawrisch: Gawrisch's study revealed that the lateral diffusion of lipids and proteins in vesicle membranes differed between extracellular vesicles (EVs) and red blood cells (RBCs). The diffusion was higher in EVs, likely due to their lower protein content and random lipid composition compared to RBC membranes.

1989

Johnstone: This study demonstrated that exosomes released from reticulocytes retained enzymatic activity.

1990

Smalley: Smalley's research highlighted the production of extracellular vesicles by *Porphyromonas gingivalis* and showed their interaction with human polymorphonuclear leukocytes.

1991

Johnstone: Based on the internalization and shedding of EV components at different times, it was suggested that exosomes served as a major route for externalizing obsolete membrane proteins. This finding challenged the perception of EV release as solely a waste disposal mechanism.

1993

Vidal & Stahl: Their work led to a better understanding of vesicular trafficking, including the components of EVs.

1996

Raposo: Raposo's study revealed that EVs derived from immune cells had the ability to present antigens. This expanded the understanding of the utility of EVs, particularly in the development of therapeutic strategies, such as in cancer treatment (as demonstrated by Zitvogel in 1998).

1998

Escola: Escola's study confirmed the presence of tetraspanins as components of EVs.

3. UNLOCKING THE FUNCTIONAL SECRETS OF EVs: DECIPHERING THEIR ROLE IN CELLULAR PROCESSES

The beginning of the 21st century marked a turning point in understanding the role of extracellular vesicles (EVs) in both physiological processes and diseases. Scientists began to investigate the content of EVs using different approaches, including proteomics, lipidomics, genomics, and biochemistry. These studies shed light on the potential roles of EVs in different contexts.

This research focused on immune cells, inspired researchers in the field of immune therapies against cancer, as demonstrated by the work of Chaput (2003) and Zitvogel (2005). Ratajczak (2006) made a breakthrough when demonstrating that cell-derived microvesicles could reprogram other cells through the horizontal transfer of mRNA and protein delivery, a discovery highlighted the significant impact EVs could have on cellular behavior.

Furthermore, it became evident that EVs could play crucial roles in supporting healing processes, such as in the case of infections (Colino, 2007) or trauma (Bruno, 2009). For example, compelling evidence suggests that EVs have multiple roles in regulating the immune response (Figure 1).

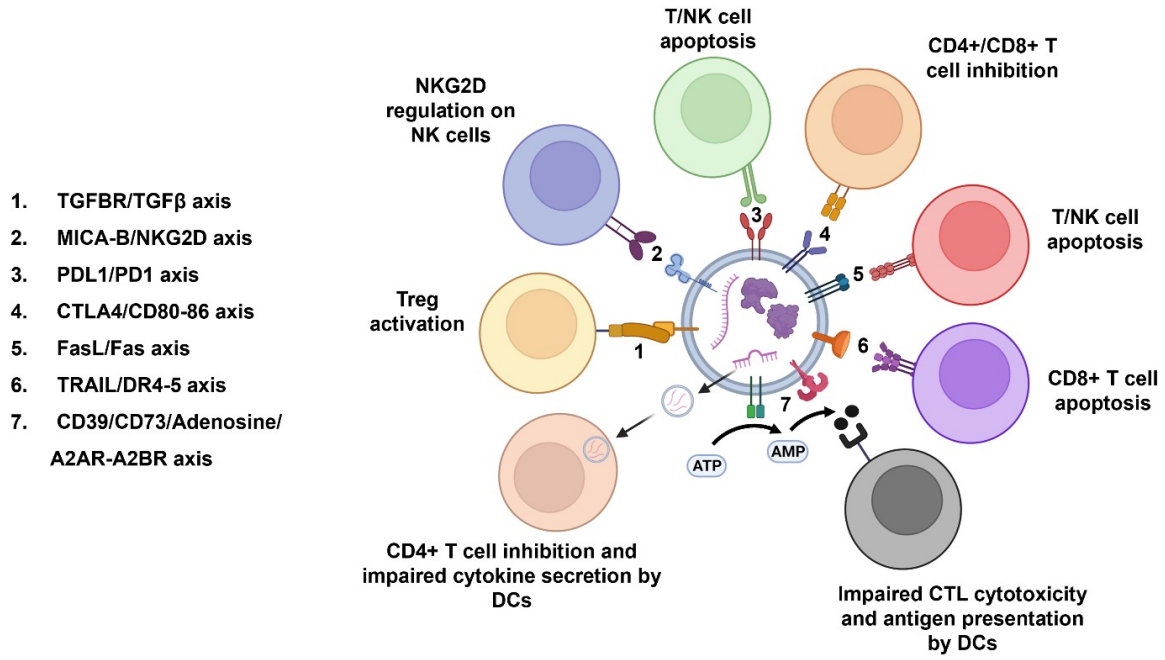

Figure 1: Regulatory Functions of EVs. The central EVs express specific markers on their surface and interact with various immune cells, including T cells, Natural Killer cells (NK), and dendritic cells (DC). Regulatory T cells (Treg), cytotoxic T lymphocytes (CTL), adenosine triphosphate (ATP), and adenosine monophosphate (AMP) are involved in these interactions. Please note that the size of the extracellular vesicle in the figure has been intentionally exaggerated for illustrative purposes.

4. NAVIGATING THE GAPS IN THE FIELD OF EVs

EV or not EV

Currently, there is a faction of scientists within the scientific community who use the term "exosome," while originally these structures were referred to as "microparticles." This discrepancy in nomenclature can lead to confusion, particularly when arbitrary size ranges are assigned to different types of vesicles. To address this issue, it has been suggested that the umbrella term "extracellular vesicles" (EVs) should be used to describe non-replicating structures that are bounded by a lipid bilayer.

The establishment of the International Society for Extracellular Vesicles (ISEV) in 2011 has played a crucial role in fostering consensus on this terminology. The definition of EVs as non-replicating structures delimited by a lipid bilayer has been formalized in the current recommendations outlined in the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines (2018). The official journal of the society, the Journal of Extracellular Vesicles (JEV), further reinforces the accepted terminology and provides a platform for research in the field.

Ensuring reproducibility

A major topic of debate in the field of extracellular vesicles (EVs) revolves around the selection of methods for their isolation and enrichment. Various techniques are available, including ultracentrifugation, ultrafiltration, size-exclusion chromatography, precipitation methods, immunoaffinity-based assays, magneto-immunocapture, microfluidic immunochips, and lipid nanoprobes. The choice of method depends largely on the specific objectives of the study.

In terms of EV characterization, several biophysical approaches have been developed, such as nanoparticle tracking analysis and reverse pulse sensing, which enable the counting and sizing of EVs. Further characterization involves methodologies like proteomics, genomics, lipidomics, and more. While these techniques provide insights into EV populations as a whole, flow cytometry allows for the individual characterization of EVs. Single-EV information is particularly valuable as it unravels the heterogeneity within EV populations. However, ensuring the accuracy and reproducibility of studies is crucial. To address this, various working groups came together in 2011 to establish the International Society for Extracellular Vesicles (www.isev.org), which has played a pivotal role in advancing the EV field.

In 2018, the society published updated guidelines for EV analysis known as MISEV (Minimal Information for Studies of Extracellular Vesicles). These guidelines aim to address controversies and questions surrounding EV research. As stated by Ramirez et al., “a persistent concern in flow cytometry is the reliable distinction between EVs carrying specific protein markers and those that do not, in order to accurately measure the proportion of EVs of a particular type.” Quantitative and qualitative analyses of EV heterogeneity within samples are crucial for comprehensive understanding.

5. CONCLUSIONS

The research on extracellular vesicles (EVs) has evolved into a distinct field, complete with its own dedicated society and scientific conferences. The discovery of EVs has illuminated a previously unknown realm that plays a crucial role in regulating various physiological processes. Further studies in this field hold the potential to unravel the heterogeneity of EVs and elucidate their diverse functions. Moreover, they could pave the way for identifying novel biomarkers of diseases. By monitoring cell-specific EVs, researchers can search for specific biomarkers that are present on or within EVs. This approach offers a non-invasive means of defining disease biomarkers, as it only requires a simple blood draw. Promising results have already been demonstrated in the context of Alzheimer’s disease.

Furthermore, EVs are currently the subject of intense investigation for their potential use as carriers to transport therapeutic compounds to target cells or organs. By engineering EVs loaded with drugs that can specifically target certain cells, such as tumors, researchers hope to overcome challenges associated with conventional therapies. This targeted delivery approach could improve the efficacy of treatments and minimize unwanted side effects. This has important implications, as current therapies are often systemically adminisstered, resulting in suboptimal functionality and potential to cause harm to health tissues

References

Asleh et al. Extracellular vesicle-based liquid biopsy biomarkers and their application in precision immuno-oncology. Biomark Res 11, 99, 2023.

Ramirez et al. Technical challenges of working with extracellular vesicles. Nanoscale 10, 881-906, 2018.

Théry et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J. Extracell Vesicles. 7(1):1535750, 2018.

Couch et al. A brief history of nearly EV-erything – The rise and rise of extracellular vesicles. J. Extracell Vesicles. 10(14):e12144, 2021.

Tao-Ran et al. Extracellular vesicles as an emerging tool for the early detection of Alzheimer’s disease. Mech Aging and Dev. 184:111175, 2019.

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