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Chapter

Introductory Chapter: An Overview to the Extracellular Vesicles

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1. Extracellular vesicles

Cells are basic structural, functional, and biological units delimited by a plasma membrane that contains all the molecules necessaries for living. Cells could be a complete organism, such as yeast or bacterium, or form part of a multicellular organism which is specialized, such as neurons or adipocytes. The communication between cells is very important to react to the environment and to initiate signaling cascades in all the organisms, from bacteria to eukaryotes. This communication involves the secretion of proteins to the extracellular environment through direct secretion (classical secretion pathways) or can be mediated by the secretion of extracellular vesicles (EVs). This communication allows cells sending and receiving messages about the inside and the outside environment. Different strategies are applied for cellular crosstalk. Current studies have emerged as EVs as an important mechanism of cell communication [1, 2]. The secretion of EVs is a well conserved process through all the organisms, from bacteria to mammals [3].

The term extracellular vesicle includes a heterogeneous group of membrane vesicles with diverse origins, sizes, and shapes. EVs are being identified in almost all the cells, from prokaryotic to eukaryotic, and not only in healthy conditions but also associated with many diseases [4]. The composition, origin, and functions are the focus of attention of researchers and clinicians. The number of papers published yearly in Pubmed related to EVs has increased exponentially during the last 10 years (**Figure 1**). Despite the enormous amount of data published and because of that, there are many new questions unanswered. We still cannot claim the complete understanding of the function of these vesicles in the cell.

The release of EVs to the media allows the cell sending and receiving messages without direct interaction. The content of EVs could participate in controlling important processes, such as growth and differentiation, pathogenesis or metabolic processes. The diversity and complexity of EVs are enormous. Each cell can produce different types of EVs, varying the biogenesis process and the content. The term of EVs is a generic term for all types of vesicles. There are different types of EVs attending to their function, size, and content [5]. Three EV types are mainly accepted: exosomes (30–90 nm), microvesicles (100 up to 1 μm), and apoptotic bodies. The EVs are formed by lipid bilayer with integrated proteins. This capsule protects the inside content from the proteases and nucleases that can affect their content. EVs transport lipids, proteins, growth factors, and RNA. In summary, the EVs are able to transfer genetic material and proteins that can contribute to change the receiving cell. Although, the main components are the same, different EVs from different cells or different EVs from the same cell can possess different contents and characteristics.

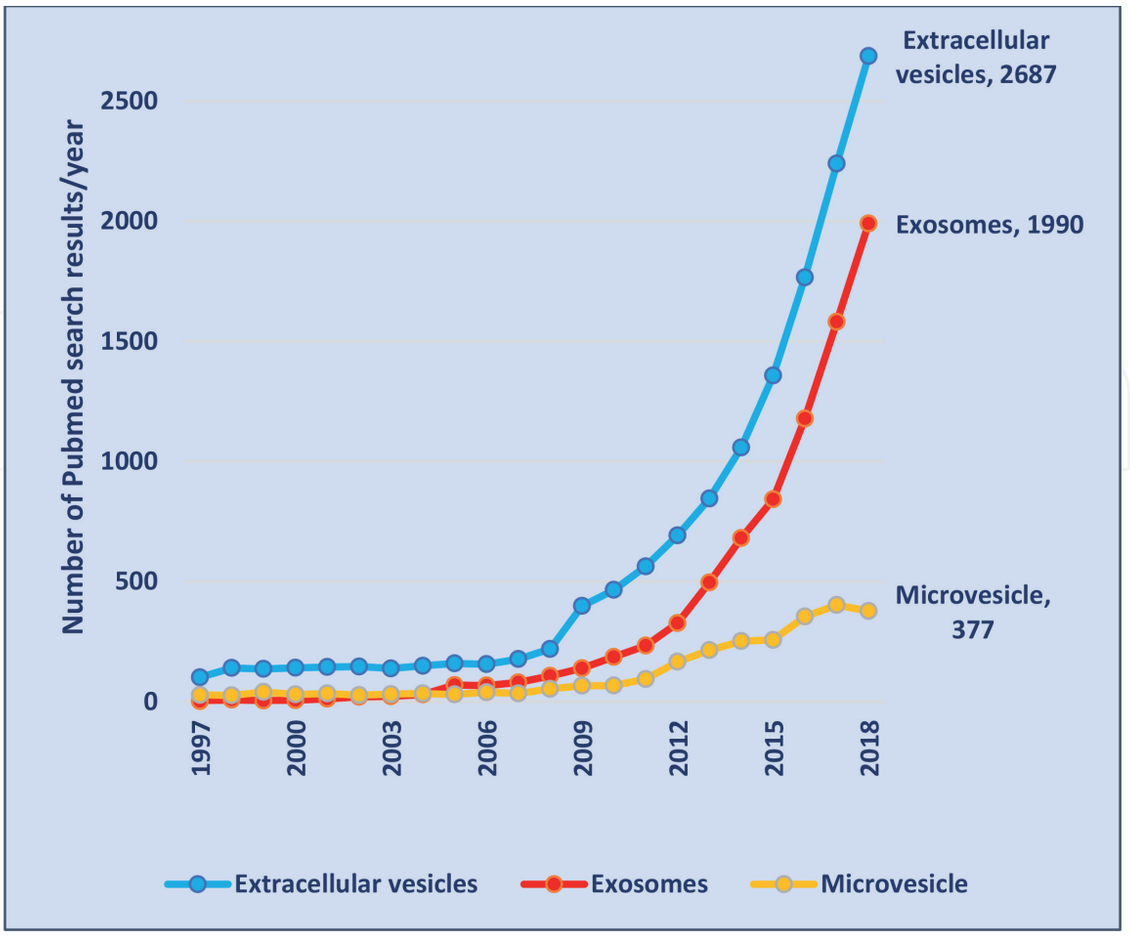

Figure 1.
Evolution of the number of papers published in PubMed over the past 21 years (1997–2018).

2. Study of EVs

EVs can be released and interact with the target cell through different mechanisms. Currently, there are several databases that serve as resource for EVs research: Vesiclepedia [6, 7], EVpedia [8–10], ExoCarta [11–14], and EVmiRNA [15]. These databases include data from different organisms, type of vesicle, and content type (protein, mRNA, miRNA, and lipid). The composition of each kind of EV derives from the cell and purpose of the vesicle realized to the media. As such, these vesicles became an important marker for diseases, including cancer, infectious diseases, renal diseases, and diabetes [16–20]. In order to achieve this, EVs must be characterized and analyze their confidence to be candidates for those used by humans. The isolation and purification of different sets of EVs in a single cell is complicated.

Even though several studies isolate EVs from all kinds of fluids, including blood, urine, saliva, tears, semen, and cerebrospinal fluid, there is not a consensus to determine the best isolation technique to purify EVs. Several approximations have been published to isolate and analyze EVs from different sources [5, 21–23]. Differential centrifugation and ultracentrifugation are necessary steps at the beginning of the process to avoid sample contamination with non-EVs contaminants that can confound the analysis (ex. cellular debris, protein aggregates or lipoproteins). After the centrifugation steps, there are several techniques that can be used to isolate and analyze the EVs [24, 25]. Several common techniques are listed below:

- Differential ultracentrifugation
- Density gradient ultracentrifugation

- Size exclusion chromatography
- Filtration
- Immunological separation
- Commercial precipitation kits
- Microfluidic chip

The methods summarized above are the most common. However, there are more variations of these methods used to purify EVs. All the methods have different advantages and disadvantages. Because of the diversity of the EVs, there is not a unique method to purify them. The selection of the method and the adaptation to the particular scenario is vital for the success of the isolation. After the EVs isolation, several procedures can be performed to characterize, quantify, and validate the correct purification of these organelles [5, 26–30]. The most common methods are listed below:

- Transmission electron microscopy (TEM)
- Atomic force microscopy (AFM)
- Nanoparticle tracking analysis (NTA)
- Dynamic light scattering (DLS)
- Single-particle tracking (SPT)
- Protein identification by mass spectrometry (MS)
- Western blot
- ELISA
- Flow cytometry
- RNA analysis
- *In vitro* functional analyses
- *In vivo* functional analyses
- Lipidomic analysis

3. The role of the extracellular vesicles in human health

Extracellular vesicles are released by many different cell types and organisms. The role of the EVs is mediated by the signals transmitted through them by the protein, lipids, and nucleic acids that are part of the EVs cargo. The release of the EVs to the environment allows the emitting cell to send messages to sites close or even far from the origin. These vesicles are able to transport biological information through

the system with cell targeting properties, making them as great candidates for drug delivery systems [31–33]. EVs are naturally secreted by human cells and they are not strange for the system, avoiding the negative response by the immune cells. The use of EVs as drug delivery systems is focused on improving their ability to reach the target recipient cells and deliver the content, controlling the purity of EV preparation, and analyzing the best administration routes. The challenges are prolonging their circulating and improving targeting. The use of EVs as drug delivery systems is being studied for several disorders, including cancer, infectious diseases, brain disorders, liver diseases, and among others [34–37]. However, this field is still in the early stage of development with great potential for future applications, but also with big challenges to attempt [38].

More applications for EVs have been described. The specific cargo of the EVs makes them useful in the discovery of biomarkers for clinical diagnosis. Because EVs are secreted from almost all cells, they are found in various body fluids, making them easy to collect and analyze and playing a critical role in diagnosis of several conditions, such as cancer, Alzheimer's, epilepsy, and liver diseases [37, 39–42]. EVs participate in pathogenesis and can also be used as diagnosis or vaccines [33, 35, 43]. Last novel applications for EVs include using their signaling properties to repair injured muscle or use them as biomarkers for male infertility or pregnancy-related disorders [44–46].

4. Conclusions

This first chapter presents a big picture of the EVs research and application as a fast-growing field. There are many EVs studies trying to demonstrate their potential critical role in cell communication and their use in many different biomedical applications. The diversity of biogenesis mechanisms and their cargo content outstand many challenges and questions to be addressed. Advances in standardized methods for purification and analysis are necessary. The increasing knowledge of the different cargo compositions and secretion mechanisms to differentiate the healthy cells and the sick cells need to be clarified to use them as biomarkers, vaccines or therapy. The combination of knowledge and experts deriving from all the fields together with the progress on characterization methods is contributing to use EVs in the biomedical field.

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