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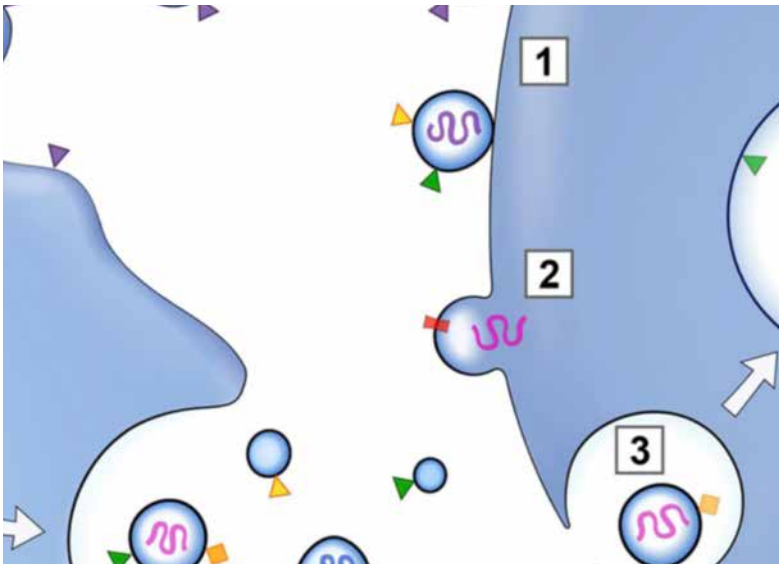
# EXTRACELLULAR VESICLES, THE RECENTLY DISCOVERED ELEMENTS OF INTER-CELLULAR COMMUNICATION

Cells are considered as the elementary building unit of complex, multicellular organisms. The size and morphology of human cells show large variations: typical blood cells are round with a diameter of 10 to 20 µm, whereas the cells of skeletal muscles may be up to 20 cm long and the processes of certain nerve cells can extend over one meter. Typical cells possess various subcellular organelles, specialized for well-defined functions, such as storage and transcription of the genetic material in the nucleus, energy production by the mitochondria, protein synthesis by ribosomes, storage of signalling molecules in the endoplasmic reticulum or storage of nutrients in lipid droplets or releasable products in secretory granules, just to mention a few vital functions.

Extracellular vesicles (EVs) differ in two major properties from typical cells: their size is significantly smaller, and generally they do not contain any internal organelle. EVs are produced from cells by different mechanisms and divided in different categories (György et al 2011, Raposo et al 2013, Colombo et al, 2014). Exosomes are the smallest EVs, their diameter being less than 100 nm (Fig.1.).




Formation of different types of EVs and their effects on target cells. From: Raposo and Stoorvogel, J. Cell Biol, 2013

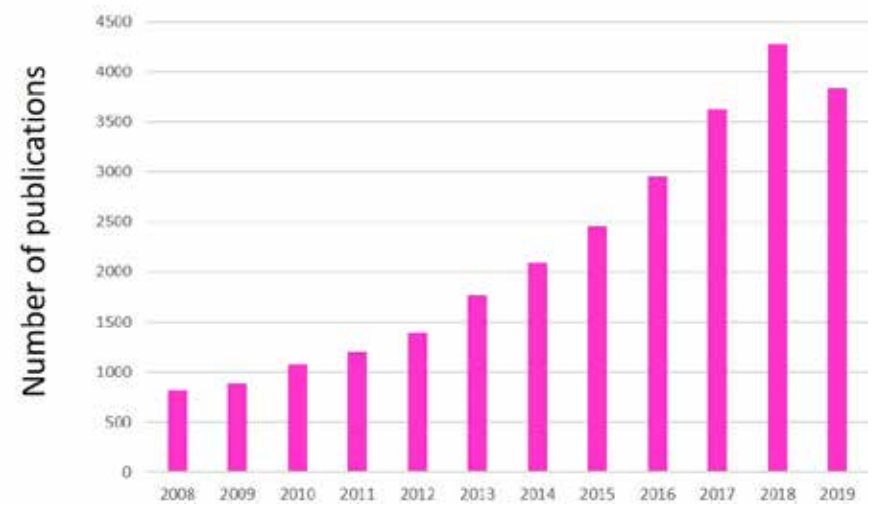
They are formed inside the cells and stored in membrane-surrounded organelles, the multivesicular endosomes (MVE), which can be clearly distinguished by electron microscopy. Exosomes are released following the fusion of the membrane of MVE with the cell membrane and carry their content to the extracellular space. The other typical group of EVs is referred to under different names, such as microvesicles, microparticles, ectosomes. They are formed by budding from the surface of the cell membrane. Their size can vary from 50 nm up to 1 µm.

Dying cells release larger vesicles, typically with a diameter above 1 µm, called apoptotic bodies. However, released EVs represent a continuous range rather than discrete groups and on the basis of the size alone it is not possible to distinguish the different types of EVs (Kowal et al 2016).

All EVs are surrounded by a membrane constituted of double lipid layer, similar to the cell membrane. EVs contain various ingredients issued from the parent cell, although the composition of

EVs generated by the same cells under different environmental conditions can vary significantly. Detection of various nucleic acids (DNA, RNA, miRNA, non-coding RNA, etc.) in EVs was a ground breaking observation (Valadi et al) that influenced our views on EVs a lot. The composition of different EVs was extensively investigated by the different "omics" technics (proteomics, lipidomics, metabolomics, etc), but no unambiguous characteristics or markers could be identified for distinction of exosomes from microvesicles or apoptotic bodies. Therefore, the guidelines issued by the International Society for Extracellular Vesicles (ISEV) suggest to use the general term EV for all vesicles, unless the biogenesis of the vesicles is verified by specific cell biology techniques.

EVs were not discovered in the last decade. Such structures were seen in many electron microscopic images 40 to 50 years ago but they were regarded as non-functional cell debris. What has changed in the last decade that is on one hand the observation that EVs can play very important biological (both physiological and pathological) roles, and on the other hand, that they are generated practically by every cell. These discoveries initiated a plethora of investigations in all kinds of organisms from bacteria to plants and mammals. The "hotness" of the topic is well represented by the exponential increase of the number of publications in the last decade (Fig.2.) and the fact that the Journal of Extracellular Vesicles (JEV) published by ISEV became in 5 years one of the highest ranked journals in the entire field of cell biology.

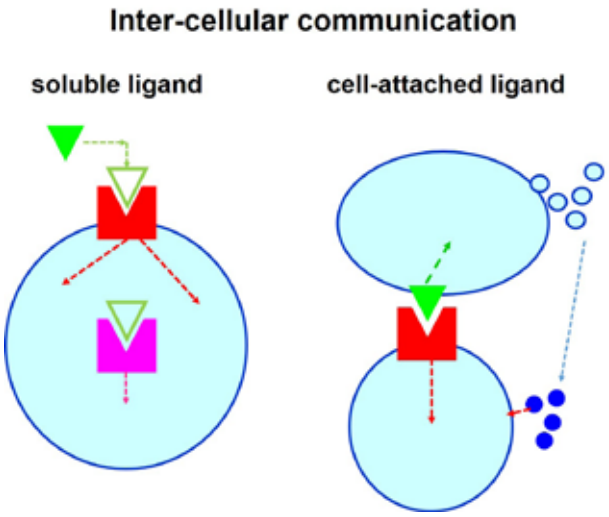



**Annual number of publications on EVs. Extracted from PubMed by Ferenc Kolonics MD.**

The main biological role of EVs is probably inter-cellular co mmunication. Several ways of cell-to-cell communication have been revealed earlier (Fig. 3.). Soluble molecules released from one cell can affect the function of another cell upon binding to a receptor either on the cell surface or in case of lipid-soluble molecules, in the cell interior.

The parent and the target cells can be in close proximity as in case of neurotransmission, the typical synaptic communication between nerve cells. The regulation is considered as paracrine, if the soluble molecule released from specific cell(s) can freely diffuse to and act on the neighbouring cells, as it happens with various cytokines. Other soluble molecules, like the hormones, are released into the blood stream and distributed in the whole body. In addition to soluble mediators, specific cell surface molecules were also discovered as communication signals, in this case the signal being spatially restricted. A typical case is the important immunological function of antigen presentation, when a piece of the foreign molecule embedded in the body's own macromolecules is presented by the antigen presenting cell to the T lymphocyte which recognizes the foreigner by its specific receptors and initiates the reaction which leads eventually to elimination of the particle carrying the foreign molecules. Similarly, specific cell surface molecules direct the grows of nerve cell processes in the developing brain or decide the survival or death of bone-eating cells. The line of examples is long.

Extracellular vesicles are released by one cell and can act on another cell, nearby or at considerable distance (Fig.3.). They are carried by the blood stream and EVs issued by the most hidden, non-motile cell types such as brain cells, bone cells, or cells residing in joints, etc. can be detected in blood samples.




Different types of inter-cellular communication.

EVs are not restricted to blood, they are present in all body fluids, such as urine, saliva or even sweat. EVs can act on the target cell via specific receptors or they can be engulfed and their contents, among other, the nucleic acids can affect the function of the target cell (Fig. 1.).

One of the first well-documented functions of EVs was their role in antigen presentation. EVs were both able to transfer foreign proteins to antigen presenting cells and thereby initiate the processing as well as transfer the presentable processed molecules to T-cells. Horizontal transfer of receptors has also been demonstrated, although in these cases EVs contributed to pathological processes by transferring mutated oncogenic receptors or receptors allowing the uptake of infectious agents such as HIV viruses, or transfer of resistance against malaria-drugs between erythrocytes. Horizontal transfer of enzymes was shown to be required for synthesis of various mediator molecules and transfer of trophic factors, mostly from various stem cells was found to support regeneration. Occasionally cells also use EVs to liberate themselves from potentially dangerous components such as complement complexes or non-functional molecules as the transferrin receptors in mature erythrocytes (Yanez-Mo et al 2015).

A typical effect of different types of EVs is enhancement of blood coagulation, mainly through lipid components enriched in the EV membrane such as phosphatidylserine. Tumour cells often produce an abundant amount of EVs and that could contribute to the increased coagulation observed in many oncological cases.

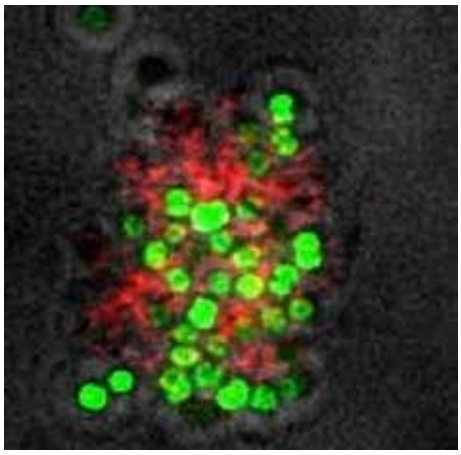
EVs offer a great potential for diagnostic purposes. As EVs released from all cells are present in blood, and as most (if not all) EVs contain nucleic acids, samples from practically all cells, even those localized at remote and hidden places become available. In fact, the currently often used term “liquid biopsy” refers in many cases to diagnosis based on material separated from circulating EVs. Obtaining a blood sample is clearly much easier and less burden for the patient than getting a tissue sample even from relatively accessible organs. An exosome-based urine test has been approved by the US Food and Drug Administration for early diagnosis of prostate cancer. Many other tests are under development.

Certainly many ideas emerged for application of EVs in human therapy. On one hand attempts are made to exploit the effects of EVs themselves, mainly of stem cell derived EVs in boosting tissue regeneration. On the other hand, EVs are applied as vehicles of targeted drug delivery.

Although the therapeutic potential offered by EVs seems to be unlimited, lack of well-controlled and comprehensive data on mobility and organ distribution of administered EVs presents a realistic obstacle at the moment.

Our own group investigates the function and regulation of neutrophilic granulocytes, which represent the most abundant population of white blood cells. Neutrophils are especially active in protection against bacteria, thanks to their motility and capacity to engulf and eliminate foreign particles. Elimination involves production of reactive oxygen metabolites and liberation of antibacterial peptides as well as degrading enzymes. Insufficient number of neutrophils or deficient function of any of the mentioned processes result in serious recurrent infections, indicating the importance of the physiological functioning of these cells.

Neutrophilic granulocytes also generate EVs. Our group has shown that the property of the EVs varies depending on the conditions prevailing at the time of their release (Timar et al 2013, Lőrincz et al 2015). Under resting conditions, a small amount of EV is released spontaneously and until now we were not able to influence this constitutive EV production in any way. These EVs have mostly anti-inflammatory effects, keeping other cells at rest as well. In contrast, opsonized particles such as bacteria or yeast, initiate the intensive production of EVs which are able to impair the growth of bacteria. Interestingly, the antibacterial effect of neutrophil-derived EVs is completely different from the mechanism the cells apply: there is no engulfment and no production of toxic oxygen metabolites. Instead, antibacterial EVs form large aggregates with bacteria, although they do not seem to fuse with them (Fig. 4.). Shielding the bacteria from the nutrients may be an important factor in diminishing bacterial growth. The antibacterial EVs have pro-inflammatory, activating effect on neighbouring cells. Neutrophils are short-lived cells and during their spontaneous death they produce yet another type of EVs, with properties differing both from spontaneously formed and from antibacterial EVs.



**Aggregation of green-stained bacteria and red-stained EVs in thin slice of confocal microscopic imaging. Image made by Csaba Timár MD, PhD.**

Most recently we were able to identify the specific receptors in the plasma membrane of neutrophils which trigger the biogenesis of antibacterial EVs (Lőrincz et al.2020). We also determined part of the intracellular signalling process that leads eventually to release of antibacterial EVs (Lőrincz et al. 2019).

Taken together, we suggest that EVs are "custom-made", their composition and properties depending on environmental cues. Biogenesis of the different EV populations proceeds on separate, distinguishable pathways within the same cell, opening the possibility of targeted modulation of EV production.

Extracellular vesicles are thus recently recognized regulatory agents, which offer vast potential both for medical diagnosis and therapy. It was interesting to observe the development of this new research field, where experimental cell biology proceeds parallel to clinical observations and trials, and the innovative thinking was present from the very beginning. It took only a few years for start-up companies to appear and offer their products for investigation or separation of EVs, or their application in medical diagnosis.

And less than a decade passed between the original observation of nucleic acids in EVs and the publication of the first prospective clinical study on a prostate cancer diagnostic test based on combinatorial analysis of genes detected in urinary exosomes. No doubt, many exiting results will follow in the future.

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