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Extracellular Vesicles as Intercellular Communication Messengers Involved in Human Skin Health and Disease

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Abstract

Cell-derived extracellular vesicles (EVs) are newly uncovered messengers for intercellular communication. They are released by almost every cell type in the three domains of life, i.e. Archea, Bacteria and Eukarya. They are known to mediate important biological functions and to be increasingly involved in cell physiology and in many human diseases, especially cancers. The aim of this review is threefold: (1) to stress the outstanding characteristics of Extracellular Vesicles (EVs) as Intercellular Communication Messengers; (2) to focus on some interesting papers searched in PubMed, specifically dealing with EV involvement in skin physiology and in a few important skin diseases treated in dermatology; (3) to mention the potential of EVs as future theranostic agents for diagnosis, prognosis and therapy of some major skin illnesses, such as Melanoma and Merkel Cell Carcinoma - an ongoing goal for achieving skin-devoted liquid biopsy and future individual patient precision medicine. However, many challenging points about EV research must still be resolved before reaching this promising perspective.

Introduction

Cell slowly emerged as the basic component of all living organisms between the seventeenth and the nineteenth century during which cell theory was established. Many cellular components and cell functions were progressively deciphered. It was initially believed that the entire cell was highly protected by its plasma membrane. However, after some pioneering works starting about five decades ago, a new major cell function appeared, greatly extending the cell power beyond its membrane. Almost all cells of the three domains of life, including human cells, are able to release extracellular nano- or microvesicles with a diameter between 30 nm and 5 µm, mediating important biological functions like intercellular messengers involved in human health and disease[1-3].

The aim of this review is, in the first place, to briefly summarize the present knowledge about the cell-derived EVs and some of their outstanding characteristics; second, to focus on dermatological topics by means of a PubMed advanced search, namely on the EV involvement both in skin normal physiology and in some major skin diseases; and third, to analyze some promising perspective for using EVs as theranostic agents, for diagnosis, prognosis after treatment or even therapy. Only some selected references are reported in order to give a current overview of this interesting topic to dermatologists not yet familiar with EVs.

Outstanding Characteristics of Extracellular Vesicles

EV research is increasing[4] worldwide, and is becoming an important topic in both biology and medicine. An International Society devoted to the EV studies (ISEV) has been founded in 2012, together with an EV dedicated Journal (JEV).

Human cells release a wide panel of extracellular vesicles with a diameter ranging from 30 nm to 5 µm.The EV continum has been classified into three main categories, following their respective biosynthesis. Exosomes (EXs), the smallest EVs (diameter between 30 nm and 140 nm) are formed intracellularly as intraluminal vesicles along the endocytic pathway until the latest multivesicular bodies (MVBs). MVBs either fuse with lysosomes for further degradation of their obsolete contents, or some of them fuse with the plasma membrane for further releasing exosomes. Microvesicles (MVs) are directly shed from the plasma membrane as small "bubbles" containing cellular components. Apoptotic Bodies (ABs) are issued from apoptotic dying cells, which package their important cellular contents into bigger "bubbles", also fusing with the plasma membrane and extracellularly delivering their saved cellular contents by means of opening blebs. The EV cargos contain proteins, lipids, RNAs (mRNAs, miRNAs et long non-coding RNAs), DNAs and metabolites. Their contents are specific but reminiscent of the cells from which they originate. However, EXs, MVs and ABs suffer from somewhat overlapping sizes and mostly from current lack of specific biomarkers, precluding any clear EV subset identification. Up to now, exosomes have been the most studied EVs.

First considered as mere garbage containers, EVs have now been acknowledged, for about one decade, as a new powerful means of intercellular communication[5,6], mediating important biological functions[7]. Relative to normal cells, EV release is increased in tumor cells; besides, EVs are involved in mediating tumor progression through near-or distant intercellular communications[8,9]. The EV-transported miRNAs have a major influence in intercellular communication by their capacity of modifying the genetic expression of the EV-recipient cells, as demonstrated by a novel mechanism of exosome-mediated genetic exchange between cells[10]. For a more complete EV description, refer to[2].

Keratinocytes-derived Extracellular Vesicles during Human Skin Senescence

The skin is an important organ of the human body, accounting for 16% of its weight[11], with a protective function for the whole body against a harmful environment. It is constituted of an upper part, the epidermis, separated from the deeper dermis by a basal membrane. Keratinocytes represent about 90% of the cells in the epidermis, beside melanocytes. The dermis structure is more complex with fibroblasts as main cells, but also nerves and blood vessels. Keratinocytes, like almost all living cells, are releasing EVs of the three main types: exosomes, microvesicles and apoptotic bodies[2]. However these EVs are difficult to discriminate, due to their overlapping properties in sizes and cargo compositions in lipids and proteins. Two recent pioneering works of Than et al.[12, 13] brought a new insight on EVs by a thorough compared study of the respective miRNAs compositions of each class of keratinocytes-derived EVs during the keratinocyte physiological senescence processes[12]. They found that the abundant but different miRNAs panels might be an efficient tool to discriminate each main EV subpopulation. Moreover, they compared the keratinocytes-derived EVs, with regard to their miRNAs, from a human keratinocyte cell line, and from primary patient keratinocytes[13]. They could thus show that the observed miRNAs panels kept a defined stamp of the cells from which they originate. Thus, the EV miRNA cargo composition, which is now obtained by using modern biological tools, might be a convenient means to specifically characterize an EV subpopulation, with regard to its cell origin and its biosynthesis process.

Beside this very interesting approach on keratinocytes-derived EVs, other more classical works have already focused on exosomes as biomarkers in dermatology[14], and as a novel pathway for regulating development and diseases of the skin[11]. The present review intends to report some of the most recent EV developments in dermatology.

Intercellular EV Messengers Functions during Physiological Skin Health

Senescence is an important concern for physiology of the normal skin. Skin aging is closely associated with changes in dermal fibroblasts, but the effects of senescent fibroblasts-derived EVs on epidermal homeostasis remained to be elucidated. Recently, Choi et al.[15] showed that the human dermis was highly involved through their fibroblasts-derived EVs in controlling the differentiation of keratinocytes in the epidermis. Human epidermal keratinocytes were treated by EVs secreted from untreated young or senescent human dermal fibroblasts (HDFs). Compared to young HDFs, senescent HDFs produced much higher EV amounts, due to an increased neutral sphingomyelinase (nSMase) activity and altered lysosomal activity. They less supported keratinocyte differentiation and its barrier function, but increased proinflammatory cytokine IL-6 levels. A new EV-mediated intercellular communication, through the basal membrane between the dermis fibroblasts and the epidermis keratinocytes, was thus evidenced.

A comparable query about EV involvement during cellular human skin aging was investigated first *in vivo* by

transmission electron microscopy of skin sections, then by focusing on an EV-miRNA cross-talk from fibroblasts to keratinocytes in monolayers and in 3D skin models[16]. They were able to isolate small EVs (sEVs) from the human skin and to strongly suggest the existence of EVs in the interstitium of the skin. Moreover, they showed that sEV-packaged miRNAs, such as miR-39, were transferred *in vitro* from fibroblasts to keratinocytes, confirming the role of sEV-miRNAs cross-talk between dermal and epidermal cells, beside the known influence of soluble factors to influence the human skin homeostasis.

By using a two cell line co-culture, another kind of reverse EV-mediated intercellular communication was found between α− or X-irradiated (HaCaT) keratinocytes and unirradiated (WS1) fibroblasts[17]. The authors found that after 1h irradiation, four miRNAs, namely miR-19, miR-27a, miR-27b and miR-141, that were reported as involved in cell migration, were up-regulated in irradiated HaCaT cells. These cells secreted exosome-encapsulated miR-27a, which turned out to be a major mediator of intercellular communication between irradiated HaCaT cells and unirradiated WS1 cells, leading to oxidative stress and inhibited cell migration in WS1 cells.

Lo Cicero *et al.*[18] discovered another important physiological intercellular communication in the epidermis between normal human keratinocytes (NHK) and melanocytes. NHK-derived exosomes carrying selected miRNAs were targeted to melanocytes to modulate their pigmentation by means of a miRNA-induced alteration of gene expression and enzyme activity, regulating the melanin synthesis. In order to define the miRNA influence, they analysed the miRNA composition of Black, Caucasian and irradiated Caucasian NHK exosomes. More than 30 miRNAs were differentially expressed between Caucasian and Black NHK exosomes. But after ultraviolet B exposure, known to modify the exosome miRNA profile, only one miRNA, hsa-miR-3196, was differentially expressed between irradiated and non-irradiated Caucasian NHK exosomes. Although this miRNA shows at least 50 targets predicted by ***in silico*** analysis, its role in melanogenesis was thus revealed for the first time.

Functions of Intercellular EV Messengers in Some Non-Tumoral Skin Diseases

As seen above, skin cell-derived EVs can be major contributors to intercellular communications during important physiological processes, such as skin pigmentation and senescence. They can even transfer irradiation stress information to other unirradiated skin cells. However, as already known for HIV, EV release in the presence of viruses are “double-edged swords”, which can also propagate diseases[19]. Furthermore, the implication of the human skin microbiome in inflammatory skin diseases has recently been established[20]. Inasmuch, as all cells release EVs “even through the wall”[21], it is now worthwhile to question the literature about the potential involvement of skin-homed bacteria and yeast-derived EVs in some usual non-tumoral inflammatory skin diseases.

Atopic Dermatitis / Eczema

Atopic Dermatitis (AD) is a skin disease induced either by aeroallergens, such as house dust mites and pollens, or by microbes, through IgE-mediated mechanisms. It is characterized by lesions with histological alterations such as epidermal thickening and dermis infiltration by eosinophils and mast cells. *Staphylococcus aureus (S. aureus*) appears to play a great role, because it colonizes almost all lesional skins among AD patients. Hong et al.[22] addressed the influence of *S. aureus* EVs on the pathogenesis of AD. They had previously discovered for the first time, that the Gram-positive *S. aureus* bacteria shed spherical EVs with a diameter of 20-100 nm from their membrane. A comparative proteomic analysis showed that the EV protein cargo differed from the bacterial proteins, and contained some pathogen components. In the cited work, they first studied the influence of *S. aureus*-derived EVs *in vitro* on mouse dermal fibroblasts and they focused on the generation of an atopic dermatitis mouse model, in order to check the influence of *S. aureus*-derived EVs *in vivo* on the induction of skin inflammation. They thus observed the hallmarks of AD, namely epidermal thickening and the infiltration of dermis by inflammatory cells. Moreover, they looked for the presence of *S. aureus* EVs in the skin lesions of atopic dermatitis patients. They found out that EVs isolated from the skin lavage fluids of three AD patients contained *S. aureus* EV-specific proteins on the one hand, and *S. aureus* EVs-specific antibodies within the serum of a cohort of AD patients on the other hand. This work provides evidence for the importance of *S. aureus* EVs in the pathogenesis of AD.

A comparable approach was taken with *Malassezia,* the most abundant fungal skin inhabitant of humans[23]. *Malassezia sympodialis (M. sympodialis)* is a dominant commensal fungi in the human skin microbiome, also associated with atopic eczema (AE). *M. sympodialis* release EVs that are carriers of small RNAs and allergens, and can induce inflammatory cytokine responses. These EVs represent an heterogeneous population with different densities and with a size (171 +- 12 nm; or 245+-10.9 nm) function of the culture conditions. The first proteomic characterisation of these EVs, compared with the whole proteome of *M. sympodialis* cells, identified 2439 proteins among which 110 were enriched in EVs. Six of the cell allergens were also found in EVs, among which two, Mala s1 and s7, were even enriched in EVs. Moreover, in this study, it was shown that the *M. sympodialis* EVs were actively internalised by endocytosis by both human primary keratinocytes and monocytes.

Acne vulgaris

Acne vulgaris is a chronic inflammatory disease accompanied by scarring and hyperpigmentation, that can affect the quality of life, especially of young people. In addition to genetic and various other factors, the skin commensal Gram-positive bacterium *Propionibacterium acnes* has been widely suspected to contribute to the development of acne by inducing inflammatory events. A recent study demonstrates in a reconstituted human skin model[24] and for the first time, that *P. acnes* constitutively produces EVs inducing typical acne-like phenotypes, such as increased secretion of inflammatory cytokines and dysregulated epidermal differentiation in human keratinocytes. The *P. acnes*-derived EVs increased the expression of proinflammatory cytokines in human epidermis, evoking rapid and strong cellular responses, compared with the ones of *P. acnes* extracts at the same protein concentration; *P. acnes* EVs seemed to be internalized into keratinocytes through clathrin-dependent endocytosis. They stimulated both human epidermal keratinocytes and myeloid cells. However, *P. acnes* EV treatment of human monocytic cell line THP-1 intensively increased IL-6, IL-8, tumor necrosis factor α, and IL-1β protein levels in a dose dependent manner, whereas only IL-8 increased in *P. acnes* EV-treated human primary keratinocytes (NHEKs). This suggests that immune-related cells might be more sensitive to *P. acnes* EV stimulation than epidermal keratinocytes. The demonstrated complex influence of *P. acnes* EVs on acne development needs to be further elucidated for a future possible EV-based therapy of this skin disease.

The bacteria-derived EVs have a quite specific influence on human keratinocytes, as seen with the two skin commensal Gram-positive bacteria, *S. aureus* and *P. acnes.* A third Gram-positive bacterium, *Streptococcus pneumonia,* which normally colonizes the respiratory tract, also released pneumococcal MVs exhibiting an heterogeneous nature and average particle sizes of 130-160 nm. These fluorescently labelled EVs were slowly uptaken by human keratinocytes (HaCaT) cells, but without impairing their viability or exhibiting any cytotoxic influence. By contrast, they were rapidly internalised by immune cells and altered their cytokine release[25], showing that the modification of immunity is an important function of foreign cell-derived EVs.

Psoriasis

Recently, Wang et al.[26] reviewed the various observations, relating chronic inflammatory skin diseases and skin tumors to exosomes. Though it is rather difficult to already find any convincing "common thread" about the exosome influence on all the studied diseases, it is interesting to catch a global insight of the current dermatological situation, especially about psoriasis. This skin disease is a chronic skin inflammation belonging to the so-called "autoimmune diseases". EVs were already involved in immunity more than two decades ago. Now, fine-tuning immunity during human health, by mediating complex intercellular communications between immune and non-immune cells, remains one of the most important EV deciphered biological functions. Robbins et al.[27], after a review of the EV-mediated mechanisms at work for normal immunity homeostasis, investigated the EV regulation of chronic inflammatory and immune processes.

Psoriasis, which is one of the most widespread chronic inflammatory diseases of the skin, has been much studied; however and according to many previous observations, the taking into account of EVs in this disease is rather recent. This is the case for Caveolin-1, assessed as pathophysiological factor and target in psoriasis[28]. Caveolae are characteristic plasma membrane invaginations with a typical size of 50-100 nm, found in many different types of cells. Caveolin-1 (Cav-1) protein is their principal structural component. Low expression of Cav-1 is typical in psoriatic lesions and overexpression of Cav-1 leads to a reduction of inflammation and suppression of epidermal hyperproliferation. At the same time, the interfacial layers of the white adipose tissue adjacent to psoriatic lesions demonstrate much higher stiffness. The level of Cav-1 expression was found to be correlated to the clinical severity of psoriasis. Human psoriatic skin has a specific microRNA profile, with miR-21, miR-31, miR-146a and miR-203 strongly upregulated, possibly interacting with Cav-1. Now, a common vesicular transport of miRs and Cav-1 is suggested as a long range communication mechanism in the psoriatic skin.

During an autoimmune disease, there is a cross-talk between the diseased tissue and the immune system. Tori et al.[29] addressed the extracellular MiR signature at the level of the $CD4^+$ T cell subsets (TH-1, TH-17 and Tregulatory (Treg)) during psoriasis. The *in vitro* identified EV-associated MiR signature was increased in serum of patients with psoriasis and returned to healthy levels after effective treatment with etanercept, a biological drug targeting the TNF pathway and suppressing inflammation. MiRNAs can regulate psoriasis by many different mechanisms, either directly on the tissue concerned by the disease, or on the immune cells fighting it. They can also target the cytokines involved in the immune response, either directly or most frequently indirectly by specifically modulating the "activators" or the "suppressors" of these cytokines[30]. Many such dual associations between miRNAs and cytokines have been reviewed for being at work in psoriasis (cf. Table 1 in[30]): miR-21 and TNFα; miR-31 and IL-1β; miR21 and miR-200a with IL-23; miR-340, miR-210, miR-21, miR-200a and miR-146a with IL-17; and miR-210 with IL-10. At the beginning, miRNAs were supposed to

be mainly associated with the argonaute protein in blood. However the observation of miRNAs as part of the EV cargos gives an interesting insight of more protected miRNAs able to act both in proximate and distant situations, following mechanisms that remain to be fully elucidated.

Another recent and more clinical approach, tried for the first time[31], searched to differentiate between patients suffering from different severities of psoriasis and healthy controls, on the basis of circulating exosomes in the blood. However, neither the concentration, nor the size of the exosomes were significantly different between the groups. Then, the exosome content of different cytokines involved in the immune response against psoriasis inflammation was measured in 81 psoriatic patients (28 mild psoriatic patients and 53 patients with moderate-to-severe psoriasis). Neither the concentrations of the exosome-associated interleukins IL-1β, IL-2, IL-6, IL-10, nor the tumour necrosis factor alpha (TNF-α) were discriminating. However IL-17A exosome levels were significantly higher in patients with moderate to severe psoriasis when compared with those with mild psoriasis (2,3 pg/ml vs 11,8 pg/ml). Moreover, IL-17 exosomal component might represent a promising biomarker for the prognosis of psoriatic patients after systemic treatment. However, this interesting observation for the future liquid biopsy of psoriasis is still controversial and has first to be validated.

Intercellular EV Messengers Functions during Tumoral Skin Diseases

In addition to being sometimes a mere detector of an unknown hidden cancer through a non-tumoral skin disease, the skin can also experience direct cancerisation. Although EVs have already been considered in oncology as important intercellular messengers involved in tumor development, in modification of tumor environment and in preparation of distant tumor metastasis, the consideration of EVs in skin tumors is more recent and worth further investigation.

Epidermolysis-Bullosa-Associated Squamous Cell Carcinoma

Patients with recessive dystrophic epidermolysis bullosa (RDEB) carry loss-of-function mutations in COL7A1, which is essential for maintaining the integrity of the basement membrane in the skin. Owing to repeated cycles of wounding, infection and chronic inflammation, patients with RDEB develop highly aggressive Squamous Cell Carcinoma (SCC)[32]. The authors investigated the feasibility of utilizing tumor-derived EVs as liquid biopsy for the detection of a recently described tumor marker gene Ct-SLCO1B3 (also known as Ct-OATP1B3 mRNA). The transcripts were specifically detected in all patients-derived RDEB-SCC cell lines investigated, whereas Ct-OATP1B3 expression was detected neither in non-tumoral RDEB keratinocytes, nor in human normal keratinocyte cell lines. The accompanying data highlight the feasibility of using this biomarker as a minimally invasive method in the detection of RDEB-SCC.

Merkel Cell Carcinoma

Merkel cell carcinoma (MCC) is a rare skin cancer that usually appears as a pink/red, rapidly growing skin nodule on UV-exposed areas, and is associated with shorter disease-free and overall survival than melanoma[33]. The Merkel cell is a mechanoreceptor cell located in the basal epidermal layer of the skin. Merkel cell polyomavirus (MCPyV) seems to be the major causal factor for MCC because approximately 80% of all MCCs are positive for viral DNAs. Konstantinell et al.[34] reviewed the results of microRNA studies in MCCs and discussed the potential application of microRNAs as biomarkers for the diagnosis, progression and prognosis, and treatment of MCC. Approximately 10% of secreted miRNAs are encapsulated in extracellular vesicles, whereas 90% are secreted in a vesicle-free state as complexes with proteins. MiRNAs can interfere with numerous cellular processes, including cell proliferation, differentiation, development, apoptosis, angiogenesis, metabolism, and immune responses. Previous studies found that eight miRNAs were upregulated in MCC, while three were downregulated compared to non-MCC cutaneous tumors and normal skin. Then, the authors applied next-generation sequencing to examine the microRNAome in exosomes purified from two MCPyV-negative and two MCPyV-positive cell lines. They confirmed the presence of miR-30a, miR-125b, miR-183, miR-190b and miR-375 in exosomes, but the viral MCV-miR-M1 was not detected in any of their samples. Levels of miR-30a and miR-34 were increased in MCPyV-positive MCCs compared to MCPyV-negative ones; hence, these miRNAs may be applied to distinguish between virus-positive and virus-negative MCCs; higher levels of miR-150 were also associated with a worse prognosis of MCC, showing the interest of these easy affordable laboratory tests based on MCC-specific miRNA biomarkers.

Cutaneous Melanoma

Melanoma represents only 10% of all skin malignancies, but is one of the most aggressive cancers[33], with a high propensity to metastasize and a bad prognosis, when discovered in advanced stages. It would be worth its own review, which is out of scope of the present paper. Therefore, the main results of some recent pertinent references will only be summarized in Table 1, in order to give a mere preview of this important topic.

Conclusion

The cell theory came slowly to birth during two centuries. Now, things are going faster; thus, over only two decades,

Table 1. Recent Studies on Human Skin Melanoma under the Light of EV Research

Year[Ref]	*In vitro*	*In vivo*	Clinical	Main Results
2016[35]	X	N. A.	X	Melanoma exosomes promote phenotype switching in primary melanocytes. Mitogen-activated protein kinase (MAPK) pathway and exosomal miRNAs let-7i, let-7a & miR-191 are involved in exosome-mediated EMT[a].
2018[36] Review	N.A.	N. A.	N. A.	Exosomes in melanoma have a role in tumor progression, metastasis and impaired immune system activity. Melanosomes and exosomes also influence the organotropism of melanoma metastases.
2019[37]	X	X	N. A.	Melanoma exosomes, through a complex oncogenic molecular reprogramming, induce the formation of a PD-1[a] overexpressing cell sub-population from naive mesenchymal stem cells, inducing rapid tumor progression *in vivo*.
2019[38] Review	N.A.	N. A.	N. A.	MiRNAs, modulating the extracellular matrix and the activity of fibroblasts, endothelial and immune cells, are involved in the formation of the pre-metastatic niche and all stages of melanoma metastasis.
2019[39]	X	N. A.	X	Characterisation of melanoma-originating lymphatic EVs (L-EVs), with 18 immune-modulating proteins, able to promote pre-metastatic niche formation in the sentinel lymph node by inhibiting dendritic cell maturation.
2019[40]	X	X	N. A.	EVs shed by melanoma tumor cells in response to chemotherapy promote the growth of viable tumor cells as a novel route for tumor repopulation and treatment failure in melanoma.
2020[41] Review	N.A.	N. A.	N. A.	Abundant production of EVs and altered microRNAs, non-coding RNAs, histones, and abnormal DNA methylation have been associated with melanoma progression towards metastasis.
2020[42]	X	N. A.	X	A prospective study evaluated the usefulness of circulating exosomal PD-L1[a] in melanoma patients' follow-up after immunotherapy. Exosomal membrane PD-L1[a] was cancer-specificic and easy to detect in plasma.
2020[43]	N.A.	N. A.	X	Plasma exosomes of melanoma patients were separated into melanoma cell-derived exosomes (MTEX) and normal cell-derived exosomes (non-MTEX). MTEX suppress functions of primary immune effector cells.
2020[44]	X	N. A.	X	Optimized isolation of subpopulations of small and large EVs directly from human metastatic melanoma tissue, further separated into high and low-density fractions, characterized and identified by quantitative proteomics.
2020[45]	X	N. A.	X	Compared proteomes and miRNomes of 4 melanoma cell lines whole cell lysates, and EVs isolated after normoxia or hypoxia culture conditions, and EV influence on proliferation, invasion and migration.
2020[33] Review	N.A.	N. A.	N. A.	Clinical Relevance of Liquid Biopsy in Melanoma and Merkel Cell Carcinoma for the early diagnosis, prognosis and follow-up of the disease, via many evaluated biomarkers, such as EVs and miRNAs.
2020[46]	X	N. A.	X	Tracking plasma EV phenotypic changes, using a multiplex EV phenotype analyzer chip (EPAC), enables treatment monitoring in melanoma patients, with control of specific drug resistance-EV profiles.

[a]EMT: epithelial-mesenchymal transition; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1

the antitumoral immunotherapy is reaching the clinics for the treatment of some cancers. Regarding the promising therapeutical use of EVs, there remain many challenges to solve, such as the standardisation of their separation and characterisation, and especially the elucidation of the mechanisms of loading their specific cargo composition. Another remaining big challenge for the EV research field is the current difficulty to associate a given EV subpopulation to a precise biological function. None of the current EV separation methods is sufficiently discriminating among the whole continuum of the many cell-released EVs. Moreover, even with different mechanisms of biosynthesis, the EV properties are overlapping both in sizes and in cargo compositions with regard to lipids and proteins. Therefore, no unique specific EV biomarkers are yet available for further clinical EV applications though they are urgently needed.

The first most important biological function of EVs was their capacity to present antigens to specific recipient cells. Now, the EVs involvement in immunity fine-tuning has been widely deciphered and their therapeutic use in immuno-oncology or against some auto-immune diseases are promising. Another important discovered function of EVs was their mediation of a novel genetic exchange between cells and their overall EV miRNA-mediated epigenetic regulation of gene expression in near- and distant intercellular communications[10]. Furthermore, the recent works of Parker and his team[12, 13] on human keratinocytes-derived EVs during skin senescence are also changing the EVs landscape. A new breakthrough is thus appearing with the possibility of discriminating the EV subpopulations by means of their respective whole miRNAs cargos. Moreover, the authors enumerate an impressive theoretical list of possible cell gene regulations by means of these miRNAs[13]. Despite the fact that all this remains highly speculative, it stresses the high potential of the EV-transported miRNAs to modify many cell pathways of these EV-recipient cells. In parallel, a new method for EV separation into controlled subpopulations, more appropriate for a clinical use than ultracentrifugation, has been elaborated and successfully tested by using *D. discoideum* EVs[47, 48]. Due to the worldwide increase of EV research[4], accompanying new promising

technological developments, EVs are now on the way of becoming recognised as important epigenetic miRNAs conveyors, thus reaching a quite fundamental role in both biology and medicine. The use of the epigenetic EV regulators of gene expression, as theranostic tools against many human diseases, is indeed a fascinating goal, but it might still be a rather long query.

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Author Disclosure Statment

The author declares that no conflict of interest exists in relation to the contents of this article.

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