Review

Harnessing extracellular vesicles for biomarker discovery in psoriatic disease

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ABSTRACT

Extracellular vesicles (EVs) have emerged as potent mediators of intercellular communication. These nano-sized structures encapsulate bioactive molecules that reflect the pathophysiological state of the originating cell, offering potential as both biomarkers and therapeutic targets. Mass spectrometry (MS) techniques have facilitated in-depth profiling of the EV proteome, unveiling valuable insights into disease mechanisms and progression. Specifically, the protein composition of EVs is indicative of the cell of origin, and those derived from diseased patients have shown distinct proteomic signatures compared to healthy individuals. Given the role of EV in cell-to-cell communication and immune modulation, studying their proteomic content can provide insights into disease pathogenesis and aid in developing new diagnostic strategies to manage conditions such as psoriatic arthritis. Identifying EV protein biomarkers could potentially lead to the development of non-invasive diagnostic and prognostic tests, aiding early detection and monitoring of disease progression.

Introduction and background

EVs are a heterogeneous group of cell-derived membranous structures, including exosomes, microvesicles and apoptotic bodies, which are present in nearly all biological fluids and involved in various physiological and pathological processes (3, 10).

Extracellular vesicles in biological processes

EVs had initially been thought to function primarily in the disposal of cell waste, but they have since been shown to play a role in a broad range of biological processes such as immune responses, angiogenesis, coagulation, and the spread of pathogens, amongst others (3, 8, 11). EVs also play a crucial role in intercellular communication. They influence a spectrum of biological activities (1, 3, 12) and they have been associated with the pathogenesis of several diseases (7, 8, 13, 14). The cargo of EVs is diverse and includes proteins, lipids, and nucleic acids that are reflective of the cell of origin (8, 9, 15).

EVs derived from patients with diseases are distinct from those of their healthy counterparts, underscoring the potential of EV proteomic biomarkers in clinical settings (7-9). For example, EVs have been implicated in the progression of cancer, where they can modulate the tumour microenvironment, promote angiogenesis, and contribute to metastasis (16, 17). Interestingly, tumour exosomes colonise specific organs and exosomal integrins can predict organ-specific metastases (16).

Biogenesis and regulation of EV formation

EVs are released by a myriad of cell types and are found in several body fluids (3). They contain a diverse range of bioactive molecules, such as proteins, lipids, and nucleic acids (1, 2). EV biogenesis is tightly regulated and involves complex molecular machinery. EV formation requires cytoskeleton rearrangement and changes in lipid distribution in the plasma membrane (7). The cargo of EVs is influenced by the cell of origin. It can mediate or modulate a host of cell responses and activities, including susceptibility to infection, signal transduction, and immune regulation (8, 18, 19).

EVs as emerging biomarkers in disease

The study of extracellular vesicles represents a rapidly evolving field that

could reshape our understanding of cellular communication and disease mechanisms. A diverse range of extracellular vesicles contribute to various physiological and pathological processes. However, their biogenesis, cargo packaging and functional role remain poorly characterised. Given the increasing recognition of their role in intercellular communication, notably in the context of inflammatory diseases, EVs and their unique proteomic cargo might be valuable biomarkers for disease diagnosis and monitoring of disease progression (2, 20).

Types of extracellular vesicles

Exosomes

EVs are categorised into three major types: exosomes, microvesicles (MVs), and apoptotic bodies (3). Exosomes range from 30 to 100 nm in diameter (1, 21). They originate from the endosomal compartment, where they form multivesicular endosomes via the inward budding of endosomes. The exosomes are released when fused with the plasma membrane, releasing the exosomes into the extracellular space (1). Exosomes were initially thought to function in the removal of cellular waste, but they have since been suggested to play a more complex role in cellular communication (7, 17). Exosomes carry various biomolecules that have been implicated in numerous biological processes, such as the modulation of the immune response, tissue regeneration, and even tumour progression (17, 21).

Microvesicles

Microvesicles are larger than exosomes, with 100 to 1000 nm diameters. Unlike exosomes, they are formed by direct outward budding and fission of the plasma membrane in a process referred to as blebbing (22). The cargo of MVs is diverse and is reflective of the cell of origin (8, 9).

Apoptotic bodies

Apoptotic bodies are the largest among the EVs, with diameters ranging from 1 to 5 μm. They are released from cells undergoing programmed cell death and contain fragments of the dying cell, including cell organelles and DNA (23, 24). They play a role in antigen presentation, where they present fragments to phagocytes for efficient clearance of the dying cells and to prevent the induction of an immune response (23, 25). They can be recognised and engulfed by phagocytes, preventing the release of potentially harmful cellular components into the extracellular environment (23, 24, 26). The presence of uncleared apoptotic cells has been linked to inflammation, autoimmunity and cancer (23).

Oncosomes and migrasomes

In addition to these three well-known types of EVs, recent studies have identified other types of vesicles, such as large oncosomes (1–10 μm in diameter) produced by cancer cells (27), and migrasomes (1-3 μm in diameter), which are generated by retraction fibres during cell migration (28). Migrasomes are suggested to function in intercellular communication and the removal of damaged mitochondria from the contractile fibres (29).

Biogenesis of extracellular vesicles

The biogenesis of EVs involves complex cellular processes, each category undergoing a distinct mechanism (3, 7, 15). Exosomes originate within the endosomal network. The early endosome matures into a multivesicular body (MVB) via inward budding of the endosomal membrane, forming intraluminal vesicles (ILVs). These ILVs become exosomes when the MVB fuses with the plasma membrane and is released into the extracellular space (1).

Role of ESCRT machinery

The endosomal sorting complex required for transport (ESCRT) machinery, which comprises four protein complexes (ESCRT-0, -I, -II, and -III) and associated proteins, is instrumental in the biogenesis of exosomes (30). The cargo packing of exosomes is mediated by ubiquitination, where protein ubiquitination is a signal for packaging into exosomes for secretion (30, 31). Some exosomes are also formed in an ESCRT-independent manner, relying on lipids such as ceramides (7, 17).

Microvesicle biogenesis

Microvesicles, unlike exosomes, are generated by direct outward budding and fission of the plasma membrane. The biogenesis of MVs involves the redistribution of phospholipids across the bilayer, which is regulated by enzymes such as flippases, floppases, and scramblases. During this process, the cytoskeleton also undergoes rearrangement (21, 32).

Apoptotic body formation and clearance

Apoptotic bodies are formed during the late stages of apoptosis. The cell undergoes various morphological changes, such as cell shrinkage, chromatin condensation, and nuclear fragmentation. Eventually, the plasma membrane starts to bleb and gives rise to large vesicles containing cellular debris, including organelles and nuclear fragments, known as apoptotic bodies (23, 25).

Cargo sorting and packaging into EVs

This cargo packaging into EVs is tightly regulated and is suggested to influence the function of EVs (33). The mechanisms underlying cargo sorting into EVs are complex and not completely understood. Several pathways have been proposed, including the ESCRT machinery, lipid-dependent sorting, and tetraspanin-enriched microdomains (15). Additionally, specific miRNAs and RNAs seem to be selectively packaged into EVs through mechanisms that utilise RNA-binding proteins. Here, the heterogeneous nuclear ribonucleoproteins (hnRNPs) have been identified as key players in RNA sorting into exosomes (34, 35).

The cargo sorting and packaging into EVs is a highly regulated process that determines the molecular content and potentially the function of EVs. However, more research is needed to fully understand the underlying mechanisms that determine cargo packing, how they influence the biological state of other cells and their implications for health and disease (7). Cellular conditions and stimuli can also influence the packaging of cargo into EVs. For example, cell

stress, such as hypoxia, can alter the cargo composition of EVs, potentially affecting their biological functions (36).

Function and role of EVs in cell-cell communication

EVs have gained significant research interest for their role in intercellular communication (1, 2). These nano-sized structures have a unique capacity to mirror the pathophysiological state of the originating cell and have been suggested to function as potent agents in cellular crosstalk via their encapsulated bioactive molecules, which allows them to modulate physiological processes (3).

Mechanisms of EV-mediated communication

It has been recognised that EVs contribute to the propagation of inflammatory signals significantly (37, 38). By carrying a range of molecules, including pro-inflammatory cytokines, autoantigens, and miRNAs, they can modulate the activity of immune cells, thereby influencing the local tissue microenvironment (7, 39, 40). The role of EVs in intercellular communication positions them as key mediators in both health and disease, making their bioactive cargo potential biomarkers for diagnosing disease.

Influence of EV cargo on recipient cells

The release and uptake of EVs are critical steps in the process of intercellular communication mediated by these vesicles (3, 7). Once released, EVs can interact with recipient cells in various ways. EV uptake involves specific interactions between EVs and their target cells that are mediated by specific ligand-receptor interactions on the surface of EVs and recipient cells (7, 41, 42). They can bind to the surface of recipient cells and stimulate cell signalling via surface receptors, or they can be internalised by cells through several mechanisms including endocytosis, phagocytosis, or micropinocytosis (41, 43). The internalisation process is often cell-type specific and can be influenced by the state of the recipient cell and the microenvironment (7, 44). Following internalisation, EVs' cargo can be released into the cytosol and have functional effects on the recipient cell.

To conclude, EVs play diverse roles in cell-cell communication, contributing to both normal physiological processes and disease pathogenesis. However, our understanding of these processes remains incomplete, and ongoing research is set to unravel the complexity of EV-mediated cell-cell communication further.

EVs in inflammation and autoimmune responses

EVs play a role in inflammatory and autoimmune pathways. Their capacity to transfer bioactive molecules, especially those promoting inflammation, makes them potential contributors to the progression of inflammatory diseases and autoimmune responses. EVs have been demonstrated to play a role in perpetuating and initiating immune responses (7-9).

Contribution to inflammatory pathways

Inflammatory cytokines, autoantigens and miRNAs, carried by EVs, can modulate the activity of immune cells, further influencing the local tissue microenvironment and potentially exacerbating inflammatory responses (7, 39, 40).

EVs as biomarkers in autoimmune and inflammatory conditions

It has been suggested that EVs influence the development and progression of autoimmune diseases. The bioactive molecules encapsulated by EVs are implicated in intercellular communication and immune modulation. EV-derived biomarkers are valuable in the detection of liver autoimmune disease. IL2, IL8 and Interferon-gamma are increased in EVs isolated from cholangiocarcinoma patients (45). miRNA from lymphocyte-derived EVs have been demonstrated to exacerbate inflammation and contribute to the progression of Hashimoto's Thyroiditis (46).

EV-mediated disease progression

EVs have emerged as a valuable source of biomarkers for the early detection of pancreatic cancer, where they provide insights into early disease and have been implicated in initiating metastasis. The EV release machinery is more active in the pancreatic cancer cell line PANC-1. The inhibition of vesicle transport in PANC-1 cells reduced cell viability and produced alterations in tissue structure that contribute to native pancreatic tissue architecture (47). These findings underscore the contribution of EVs to disease progression and highlight their impact.

Role of EVs in rheumatoid arthritis

It has been demonstrated that EVs play a role in the pathogenesis of rheumatoid arthritis (RA), where they have been shown to influence immune cell activity and tissue homeostasis (48-53). EVs have also been demonstrated to play a role in immune signalling and cartilage repair in cell culture models of RA (53). EVs released by dendritic cells, neutrophils, and myeloid-derived suppressor cells have been shown to influence inflammatory responses in the joint environment, which contributes to disease progression in RA (48, 54-56). EVs derived from the synovium of RA patients have also been shown to inhibit Treg differentiation and they contain enzymes that promote tissue matrix degradation. MSC-derived EVs have been demonstrated to be effective in the treatment of RA (48, 57, 58).

Psoriasis and psoriatic arthritis

Clinical features and pathophysiology of psoriasis

Psoriasis (PsO) is a chronic, immune-mediated skin disorder. Its hallmark is the hyperproliferation of keratinocytes, which, accompanied by the infiltration of immune cells into the skin, leads to the formation of erythematous, scaly plaques (59). The pathophysiology of PsO is characterised by periods of flares and remission, with the severity and manifestation of symptoms varying wildly among patients (60).

Clinical manifestations of psoriatic arthritis

In close association with PsO, psoriatic arthritis (PsA) presents as a multifaceted disease that substantially impacts quality of life (61). PsA encompasses

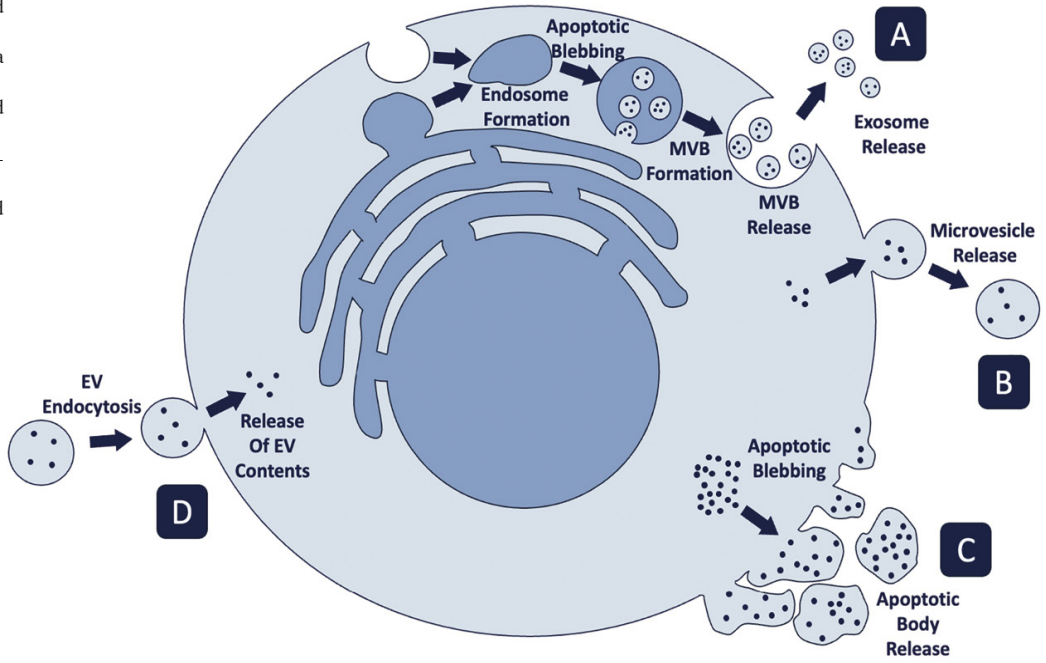

Fig. 1. The formation, release and uptake of extracellular vesicles.
A: The release of exosomes from a multivesicular body.
B: Microvesicle formation and release.
C: Apoptotic blebbing and the release of apoptotic bodies.
D: Microvesicle endocytosis and the release of its contents.

a spectrum of clinical manifestations, from peripheral arthritis and enthesitis to dactylitis and axial disease (62-65). Most patients also experience psoriatic skin and nail lesions (66) and most will have cutaneous features before the onset of arthritis (61, 64, 66-68).

Link between PsO and PsA

PsO and PsA are chronic inflammatory diseases with common genetic and immune-mediated components affecting the skin and musculoskeletal system, respectively. Both conditions exhibit a complex aetiology that remains to be fully understood. PsO and PsA impose a substantial health burden on populations worldwide. Both conditions share common genetic and immune-mediated components, underlining their close association and significant impact on affected individuals. Given the close association between PsO and PsA, there is significant interest in understanding the underlying molecular differences between the two conditions. Understanding these differences will enable the identification of individuals with PsO who are likely to develop PsA (59, 66). The variability in symptoms among patients and the lack of a molecular diagnostic assay emphasises the need for a deeper molecular understanding (60).

Role of EVs in the pathogenesis of PsO and PsA

It has been recognised that EVs play a pivotal role in the pathogenesis of inflammatory and autoimmune diseases due to their contribution to the propagation of inflammatory signals (37, 38). The identification of EV-derived protein biomarkers could potentially lead to the development of non-invasive diagnostic and prognostic tests, opening avenues for early detection and meticulous monitoring of disease progression.

miRNA biomarkers in EVs for PsO and PsA differentiation

Two recent studies investigated the micro-RNA (miRNA) content isolated from plasma EVs (69) and serum EVs (70) to identify biomarkers for PsA in PsO patients. The study by Lättekivi *et al*. identified no significant differences in miRNA levels between PsO and PsA EV samples (with FDR correction for multiple testing $p<0.05$). However, one miRNA was significantly reduced in PsA EV samples compared to healthy controls (has-miR-10b-5p), and 12 miRNAs were significantly differentially expressed between PsO and healthy control EV samples (with FDR correction for multiple testing $p<0.05$) (70). Plasma EV miRNAs revealed potential biomarkers to distinguish PsO from PsA. The study found that nine miRNAs were upregulated in PsA patient EV samples (has-miR-23a-3p, -379-5p, -98-5p, 29a-3p, 27b-3p, 27a-3p, 26a-5p, 146a-5p, has-let-7e-5p) and ten miRNAs were downregulated in PsA patient EV samples (has-miR-92a-3p, -139-3p, -92b-3p, -486-5p, -1180-3p, -3158-3p, -4732-3p, -203a, has-let-7b-5p, -7b-3p) compared to PsO patient EV samples in the discovery cohort (n=29 individuals) (69). The downregulation of two miRNAs in PsA EV samples (let-7b-5p and miR-30e-5p) could further be confirmed in the validation cohort (n=57 individuals) (69). One limitation of the above-named studies is the small study populations, and due to differences in sample matrix and EV extraction procedures, these studies are not directly comparable. These findings suggest that the miRNA contents of EVs may be distinct between PsO and PsA populations.

Proteomic profiles of EVs in psoriatic diseases

EVs derived from patients with PsO and PsA may also harbour distinct proteomic profiles compared to those from healthy individuals (7-9). Changes in the protein content of EVs might exacerbate the inflammation attributed to the onset of psoriatic disease. Char-

acterising the EV proteome might uncover a role of EVs in the development and progression of PsA, which can be exploited for diagnostic assays, therapeutic intervention and disease management.

Mass spectrometry for EV proteomics

Sample preparation and methodological considerations

Mass spectrometry (MS) has proven invaluable in proteomics, facilitating in-depth profiling of complex protein mixtures such as those found within EVs (71-73). MS-based proteomic analysis involves the isolation and purification of EVs, followed by protein extraction, digestion, and peptide separation (74). Rigorous and standardised methodologies become imperative to ensure accuracy and reproducibility in the results obtained (75).

Applications of MS-based proteomics for EVs: potential of MS in EV biomarker discovery

The high sensitivity and capability to identify and quantify proteins within intricate samples like EVs make MS a suitable technique for characterising the EV proteome (5, 6). Through MS-based techniques, the in-depth profiling of the extracellular vesicle (EV) proteome is possible, providing insights that may accelerate the discovery of potential disease biomarkers (71-73). The use of MS in the proteomic analysis of extracellular vesicles offers a promising avenue for both the identification of disease-associated proteomic signatures and the development of MS-based diagnostic assays, which could massively improve disease management.

Challenges in mass spectrometry-based proteomics of EVs

Challenges in EV isolation

A significant challenge in EV proteomics is the isolation of the vesicles. Standard techniques to isolate EVs include ultracentrifugation, density gradient centrifugation, size exclusion chromatography, and precipitation (2). Each method has demonstrated variable efficacy, with distinct differences in purity and yield. A significant concern is that different methods may isolate varying subsets of vesicles, which could influence comparative analyses between studies.

The experimental design can also influence the co-isolation of non-EV components. Such co-isolates, including protein aggregates, lipoproteins, and even viruses, can inadvertently influence subsequent proteomic analyses, leading to potential misinterpretations (73, 76). This aspect underscores the necessity of rigorous and standardised methods in sample preparation to ensure accuracy and reproducibility, as the introduction of contaminants can significantly skew proteomic data (75).

Implications of EV heterogeneity

Additionally, the inherent heterogeneity of EVs poses its own set of challenges. Given the diversity in their size, origin, composition, and function, EVs can showcase varied protein content and proteomic profiles (77). Such variability can be significant, even among samples representing the same disease state. Consequently, pinpointing consistently altered proteins becomes a challenging endeavour (78).

While MS holds significant promise in characterising the role of EVs in psoriatic disease, the challenges of isolation underscore the need for standardised methodologies and diligent validation.

The heterogeneity of EVs represents a challenge for MS-based proteomics. EVs are characterised by differences in their size, origin, composition, and function, all of which have implications on their protein content and consequent proteomic profiles (77). This vast diversity in EV properties reflects the myriad of cells and physiological states from which they originate and their various roles in intercellular communication and pathophysiological processes.

Variability and reproducibility issues in EV proteomic studies

Reproducibility in the isolation of EVs is critical. The heterogeneity of EVs and differences in the protein content between different EV subtypes can result in pronounced variations in the proteomic profiles of EVs, even when derived from similar biological sources or conditions. This can lead to significant variability between samples from the same disease state. Consequently, this can impact the identification of consistently altered proteins, which is crucial for biomarker discovery and understanding pathogenic mechanisms (78). The challenge posed by the heterogeneity of EVs is further exacerbated when considering the co-isolation of non-vesicular components, such as protein aggregates and other biomolecules, which can confound proteomic analysis (75).

The protocols used for the isolation and purification of EVs, protein extraction, digestion, and subsequent peptide separation play a pivotal role in the quality of the data obtained (74). Due to the intrinsic heterogeneity of EVs, combined with the potential co-isolation of non-vesicular components, there is an increased risk of introducing variability into the samples (75). This variability between samples can potentially mask genuine biological differences or exaggerate minor ones, making it difficult to discern authentic proteomic signatures from mere artefacts or random fluctuations (78).

Multiple isolation methods should be considered to address issues of variability and reproducibility in the isolation of EVs for identifying protein biomarkers. The incorporation of multiple purification methods would reduce the co-isolation of non-vesicular components. Techniques such as electron microscopy and flow cytometry could be employed to validate the successful isolation of EVs. The sequential use of different techniques has been demonstrated to reduce the amount of co-isolated entities (79).

Conclusion and perspectives: the promise and potential of EV proteomics in disease diagnosis and prognosis

EVs are mediators of intercellular communication in a variety of physiological processes and diseases (3). Their distinct proteomic cargo holds immense promise for enhancing our understanding of disease mechanisms, particularly in conditions like PsA and PsO (2).

EV proteomics for disease diagnosis and prognosis

The proteomic content of EVs reflects their parent cells' physiological and pathological states, allowing for the potential identification of disease biomarkers and insights into pathophysiology. The in-depth profiling of the EV proteome can expedite the discovery of therapeutic targets and biomarkers, thereby potentially improving disease management (71, 73).

Potential for biomarker discovery and therapeutic targets in psoriatic diseases

In the specific context of psoriatic diseases, the characterisation of the proteomic content of EVs offers a novel avenue to gain insights into the pathogenesis and progression of these conditions. As demonstrated, the comparative proteomic analysis of EVs derived from individuals with PsO and PsA can reveal molecular markers that predict disease development and progression. Such findings can pave the way for developing non-invasive diagnostic and prognostic tests, enhancing early detection and improving disease management.

EV proteomics represents an exciting frontier in inflammatory disease research. Characterising the EV proteome could unveil novel strategies for understanding, diagnosing, and potentially treating inflammatory diseases.

Future directions: the role of EV proteomics in understanding pathophysiology

EVs and their proteomic content have emerged as a significant area of interest in studying many inflammatory conditions. Their role in cell-to-cell communication and immune modulation underscores their potential as valuable tools for gaining insights into disease pathogenesis (2, 3). Given the roles of EVs in these fundamental biological processes, studying the proteomic content of EVs can provide a deeper understanding of the underlying mechanisms influencing the development and progression of diseases like PsA. The proteomic content of EVs has been found to reflect the physiological and pathological states of the parent cells, suggesting a direct link between the cellular environment and the molecular cargo of these vesicles (3). Identifying EV protein biomarkers could pave the way for developing non-invasive diagnostic and prognostic tests. Such tools are invaluable for early detection, monitoring disease progression, and predicting disease outcomes based on EV proteomic profiles.

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