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REVIEW

Apoptotic extracellular vesicles: mechanisms, applications, and therapeutic potential

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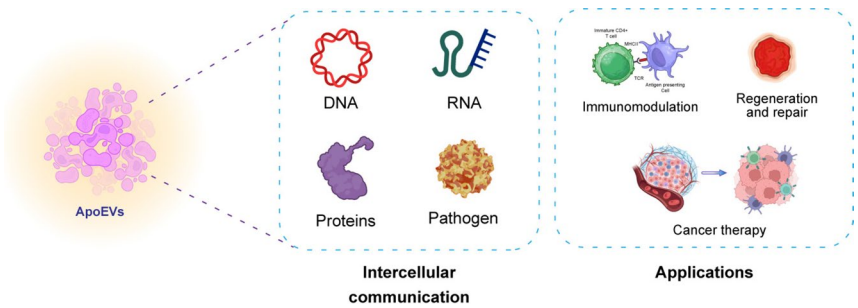
Abstract

Apoptotic extracellular vesicles (ApoEVs) are membrane-bound vesicles released during apoptosis, crucial for intercellular communication by delivering bioactive molecules to recipient cells. These vesicles are increasingly recognized for their potential in tumor therapy, immune modulation, and tissue regeneration. Recent studies reveal that ApoEVs play diverse roles in the medical fields. In tumor therapy, they enhance targeted drug delivery and antitumor immunity. Immune modulation is achieved by presenting antigens to immune cells, fostering specific responses. ApoEVs also aid in tissue regeneration, promoting wound healing and tissue repair. Advances in isolation and engineering techniques have improved the purity and functionality of ApoEVs, enabling their use as therapeutic delivery platforms. ApoEVs hold significant clinical potential by transferring genetic material, proteins, and other bioactive molecules. However, challenges such as standardizing production, ensuring safety, and addressing heterogeneity must be overcome. Future research should optimize isolation methods, elucidate ApoEV mechanisms, and develop strategies to enhance therapeutic efficacy. ApoEVs offer promising applications in cancer treatment, immune regulation, and tissue regeneration. This review summarizes the latest research and potential clinical applications of ApoEVs, highlighting their therapeutic promise and the challenges ahead.

Graphical Abstract



Highlights

- Formation Mechanisms: Exploring how ApoEVs form, focusing on key proteins and signaling pathways.
- Isolation Techniques: Advancements in techniques to improve the purity and yield of ApoEVs.
- Communication Roles: How ApoEVs facilitate the transfer of DNA, RNA, proteins, and pathogens between cells.
- Therapeutic Potential: The use of ApoEVs in cancer therapy, immune modulation, and tissue regeneration.

Keywords Apoptotic extracellular vesicles · Immunomodulation · Regeneration · Wound healing · Targeted delivery

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Introduction

Extracellular vesicles (EVs) represent a diverse array of membrane vesicles, characterized by their heterogeneous origins and the array of soluble intracellular substances they encase. Released by virtually all cell types, EVs facilitate essential intercellular communication across both healthy and diseased states [1–3]. They are traditionally categorized based on size, origin, and molecular contents into three primary types: exosomes (50–100 nm), microvesicles (MVs, 50–1000 nm), and apoptotic extracellular vesicles (ApoEVs, 50–5000 nm) [4, 5]. Recent advancements in nanotechnological analysis have led to the discovery of additional EV types, including migrasomes, which are specifically released during cellular migration to transport unique cytoplasmic components [6, 7]. Despite this classification, distinguishing among these subpopulations remains a complex challenge due to their overlapping sizes and similar structural features [8]. Nevertheless, the role of EVs in regulating key physiological functions, such as metabolism, tissue regeneration, and immune responses, is indisputably critical [9].

Decades of intensive research have focused on exosomes and MVs, exploring their potential in clinical diagnostics and therapies for various diseases [10–13]. These vesicles carry diverse biological cargoes that have shown promise in treating immune disorders [14], cancer [15], neurodegenerative diseases [16], and infectious diseases [17]. Notably, vesicles derived from stem cells have demonstrated significant therapeutic benefits in conditions such as ischemic injuries [18], diabetes, and spinal cord injuries, as well as in autoimmune diseases [19, 20]. Additionally, engineered vesicles, both exosomes and MVs, have been tailored through endogenous and exogenous modifications to enhance their targeting capabilities and cellular communication. These modifications facilitate specific interactions with recipient cells, significantly improving therapeutic efficacy [21]. Despite these advancements, several challenges remain, including the limited availability of source materials, complex manufacturing processes, instability in biological environments, and unintended effects on non-target cells, all of which require urgent resolution [22].

ApoEVs, a subcategory of EVs, originate from cells undergoing apoptosis, setting them apart from exosomes and MVs. Historically, research on ApoEVs has been limited due to their rapid clearance in vivo [23]. During apoptosis, the formation of ApoEVs through cellular fragmentation results in high variability in their number, composition, and size, which complicates the establishment of standardized research methodologies [24]. To date, the characterization of ApoEVs has predominantly involved optical and electron microscopy to examine their morphological properties during derivation [25]. Despite these challenges, the intrinsic properties of ApoEVs allow them to encapsulate substantial cargoes and introduce extensive modifications in recipient cells, suggesting a potent therapeutic potential [26]. Typically, ApoEVs are internalized by macrophages, tumor cells, or parenchymal cells through phagocytosis after release into the extracellular space [27]. The interaction between ApoEVs and recipient cells involves a sophisticated recognition system governed by receptor proteins or specific biochemical traits, especially within the immune system [28]. This precise interaction underscores the potential of ApoEVs as natural delivery vehicles and tools for elucidating disease mechanisms.

As technology advances, our grasp of the biological roles and mechanisms of ApoEVs has significantly deepened. Enhanced centrifugation techniques and cutting-edge technologies have refined the isolation and purification processes of ApoEVs, leading to streamlined procedures, higher sample purity, and improved yields. Such progress has catalyzed further research into these entities. Recent studies have employed various engineering strategies to modify or mimic ApoEVs, significantly improving their diagnostic and therapeutic precision [29–31]. This review aims to comprehensively introduce the critical role of ApoEVs and detail the latest advancements in their detection, isolation, and functional modifications. It highlights the potential of ApoEVs as a novel delivery system, addressing both the ongoing challenges and future research directions. This narrative equips researchers with a detailed reference for understanding and advancing the development and application of ApoEVs.

Biogenesis, release, classification, uptake, and metabolism of ApoEVs

Biogenesis and release

EVs are lipid-membrane-enclosed cellular entities released through meticulously regulated morphological transformations. Exosomes are generated from multivesicular body exocytosis, while MVs emerge from plasma membrane budding [32]. In contrast, ApoEVs form during the final stages of apoptosis, marked by chromatin and nuclear condensation [30]. This process was once thought to be random, but recent research has unveiled that the formation of apoptotic bodies (ApoBDs) involves several well-orchestrated morphological stages [33]. The earliest change, apoptotic membrane blebbing, entails dynamic plasma membrane bleb formation regulated by a series of protein kinases, notably Rho-associated protein kinase 1 (ROCK1) and myosin light chain kinase

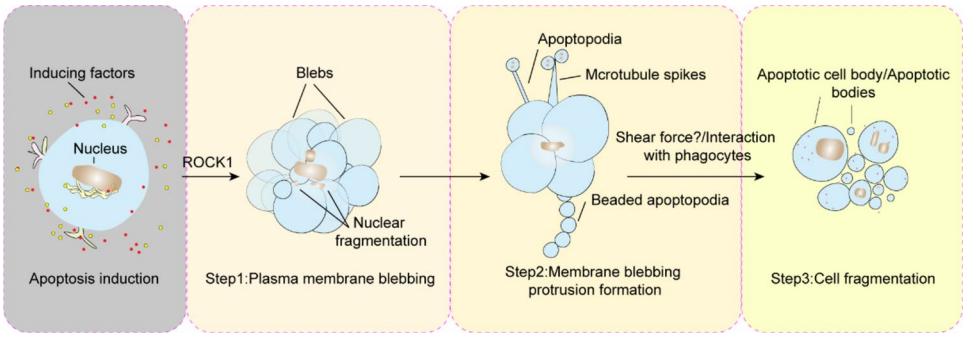

Fig. 1 The formation and release of ApoBDs involve three processes: (I) Plasma membrane blebbing: mediated by caspase-3 protease activation of kinases, including Rho-associated coiled-coil containing protein kinase 1 (ROCK1), LIM domain kinase 1 (LIMK1), and p21-activated kinase 2 (PAK2). (II) Formation of membrane protrusions: the creation of microtubule spikes, beaded apoptopodia, or apoptopodia via caspase-3/7-activated pannexin 1 (PANX1) channel and vesicular trafficking. (III) Shear forces or caspase-assisted cleavage to release ApoBDs

(MLCK) [34]. Activated by caspase-3-mediated proteolysis, ROCK1 phosphorylates the MLC of myosin II, promoting membrane blebbing through increased actomyosin contraction. While the molecular mechanisms governing MLCK activation in apoptosis are not fully elucidated, MLCK inhibition is known to hinder membrane blebbing. Furthermore, caspase-activated LIM domain kinase 1 (LIMK1) facilitates apoptotic membrane blebbing by activating cofilin, an actin-binding protein [35]. Another essential kinase, p21-activated kinase 2 (PAK2), is activated via caspase-3 cleavage and subsequent myristoylation, targeting it to the cell membrane where it regulates cytoskeletal dynamics and activates the stress-associated c-Jun N-terminal kinase (JNK) signaling pathway. This pathway supports membrane blebbing during apoptosis, with PAK2 interacting with various proteins to ensure the orderly release of ApoEVs [36, 37].

After plasma membrane blebbing, certain cells like neurons and epithelial cells can produce structures such as microtubule spikes, apoptopodia, and beaded apoptopodia. These structures aid in the formation of ApoEVs either alongside or independent of membrane blebbing. Microtubule spikes, for instance, assist in separating membrane blebs into individual ApoEVs by connecting to the spikes [38]. In apoptotic THP-1 cells and primary human neutrophils, beaded apoptopodia—membrane protrusions can generate ApoEVs more efficiently (approximately 10–20 ApoEVs per strand) than microtubule spikes or apoptopodia, suggesting a unique and rapid formation mechanism [39]. Remarkably, beaded apoptopodia and the generation of ApoEVs are also seen in genetically altered monocytes incapable of forming membrane blebs, indicating that membrane blebbing is not essential for ApoEV formation [40].

However, the detailed mechanisms governing the separation of individual ApoBDs from the apoptotic cell body are not yet fully understood. Shear forces generated by media flow or interactions with neighboring phagocytes might contribute to this process [27]. Some studies have identified caspases as key facilitators in the release of ApoBDs into the extracellular space. These enzymes aid in the disintegration of cellular structures, promoting ApoBDs formation through their proteolytic activity [41]. Additionally, there may be an unknown intrinsic abscission force that assists in the separation of individual ApoBDs from the cellular protrusion [40] (Fig. 1).

Classification of ApoEVs

Initial research indicated that apoptotic cells generate ApoBDs with diameters exceeding 1 μm. However, recent studies have revealed that apoptotic cells release a variety of vesicles of different sizes, collectively referred to as apoptotic ApoEVs. As the final products of programmed cell death, ApoEVs exhibit diverse morphological and biochemical characteristics, which form the basis for their classification [42]. Currently, there is no unified standard for classifying ApoEVs in the academic community. In this review, we summarize and categorize ApoEVs based on the latest research from different perspectives. According to their cellular origin, ApoEVs can be classified into monocyte-derived ApoEVs, T cell-derived ApoEVs, tumor cell-derived ApoEVs, influenza virus-infected cell-derived ApoEVs, and stem cell-derived ApoEVs. These ApoEVs from various cellular origins play distinct regulatory and therapeutic roles in different diseases and physiological processes [30, 43–45].

Advancements in detection techniques have identified smaller vesicles derived from apoptotic cells [46]. These vesicles, along with ApoBDs, have been classified based on their sizes into apoptotic MVs (ApoMVs, 200–1000 nm) and apoptotic exosomes (ApoExos) [47]. The recent discovery of ApoExos highlights the difficulty in distinguishing exosomes from healthy or apoptotic cells due to their common marker

proteins and high heterogeneity [48]. Recent studies have shown that different subtypes of apoptotic vesicles, including ApoBDs (1–5 μm) and apoptotic small extracellular vesicles (ApoSEVs, < 1 μm), derived from bone marrow mesenchymal stem cells (BMMSCs), exhibit distinct effects on cell proliferation, migration, and differentiation. ApoSEVs significantly enhance these cellular processes and promote macrophage polarization to specific phenotypes, contrasting with the inhibitory effects observed with ApoBDs [49]. This finding highlights the importance of discriminating among ApoEVs of different sizes in reagent research to derive valid conclusions.

Additionally, ApoEVs can be classified into nuclear-enriched ApoEVs and cytoplasm-enriched ApoEVs based on their contents [50]. Nuclear-enriched ApoEVs are abundant in nuclear materials, such as DNA, nuclear proteins, and other nucleus-associated components. These ApoEVs potentially carry genetic information and functional proteins, thereby influencing gene expression and cell cycle control [51]. In contrast, cytoplasm-enriched ApoEVs contain cytoplasmic proteins, lipids, and organelles, including mitochondria and the endoplasmic reticulum. These vesicles are laden with metabolic enzymes, energy molecules, oxidants, repair proteins, signaling molecules, and second messengers, all of which are crucial for cellular metabolism, energy production, and signal transduction [52].

Uptake

During the early stages of apoptosis, dying cells emit various 'find-me' and 'eat-me' signals to facilitate the swift recruitment of macrophages or non-professional phagocytes [53]. These signals ensure the subsequent recognition and engulfment by phagocytes. Traditional 'find-me' signals include soluble factors such as nucleotides (ATP and UTP), lysophosphatidylcholine (LPC), sphingosine-1-phosphate (S1P), and chemokines (CX3CL1, MCP-1, and IL-18) [52, 54–58]. These molecules create a chemotactic gradient that directs phagocytes to the site of apoptosis, promoting the efficient and immunologically silent clearance of dying cells. This process is crucial for preventing secondary necrosis, inflammation, and autoimmune responses. Additionally, these mediators are involved in ApoEVs-mediated recruitment of phagocytes, suggesting that ApoEVs possess similar chemoattractive properties [59]. Notably, different sizes or origins of ApoEVs selectively recruit distinct phagocytes both in vitro and in vivo. For example, Berda-Haddad et al. found that only ApoBDs promote neutrophil migration, while ApoMVs and ApoExos do not [60]. Thus, it can be concluded that different populations of ApoEVs may play more specific roles in the clearance of dying cells, their disassembled products, or even the ApoEVs themselves.

During the 'eat-me' stage, phagocytes recognize and engulf ApoEVs through specific membrane receptors. One such receptor is phosphatidylserine (PS), which translocates from the inner leaflet of the plasma membrane to the outer leaflet during early apoptosis [61]. Interestingly, some cancer cells also express abnormal levels of PS on their outer membranes, potentially acting as a transducing molecule in cell differentiation and vesicle formation [62]. Other potent 'eat-me' signals include oxidized forms of PS (PSox) [63], calreticulin (CRT) [64], annexin I [65], and tubby and tubby-like protein 1 (Tulp1) [66], which enhance the recognition and uptake of apoptotic cells by phagocytes. Phagocytes recognize PS through various receptors, such as Phosphatidylserine receptors (PSRs) on their surface, which bind directly to PS and aid in the clearance of ApoEVs [67]. Additionally, integrins, particularly αvβ3 and αvβ5, as well as TAM receptors (Tyro3, Axl, Mer) [68], can also recognize PS, facilitating the recognition and engulfment of apoptotic cells. This specific recognition and engulfment process between ApoEVs and phagocytes offers a novel method for targeted delivery of cargoes to phagocytes for therapeutic purposes, especially targeting immune cells (Fig. 2).

Metabolism

The phagocytosis of ApoEVs by phagocytes is a critical process for the removal of dying cells and the maintenance of tissue homeostasis [25]. Once ingested, ApoEVs undergo a series of metabolic processes within the phagocyte to ensure efficient degradation and prevent the release of potentially harmful intracellular components. Inside the cell, ApoEVs fuse with the plasma membrane to form phagosomes. This process involves actin cytoskeleton rearrangements driven by signaling pathways activated by receptors such as integrins and scavenger receptors [69]. Following the formation of the early phagosome, Rab5 is activated by guanine nucleotide exchange factors (GEFs) like Rabex-5, which facilitate the exchange of GDP for GTP on Rab5 [70, 71]. Activated Rab5 anchors to the phagosomal membrane and recruits various effector proteins, including early endosome antigen 1 (EEA1) [72]. With Rab5 and EEA1 in place, the nascent phagosome is primed for fusion with early endosomes. This fusion is mediated by a complex interplay of proteins, including SNAREs (soluble NSF attachment protein receptors) such as syntaxin 13, syntaxin 7, Vti1b, and VAMP8. These SNARE proteins form a complex that brings the membranes of the phagosome and early endosome into close proximity, allowing them to merge. This fusion

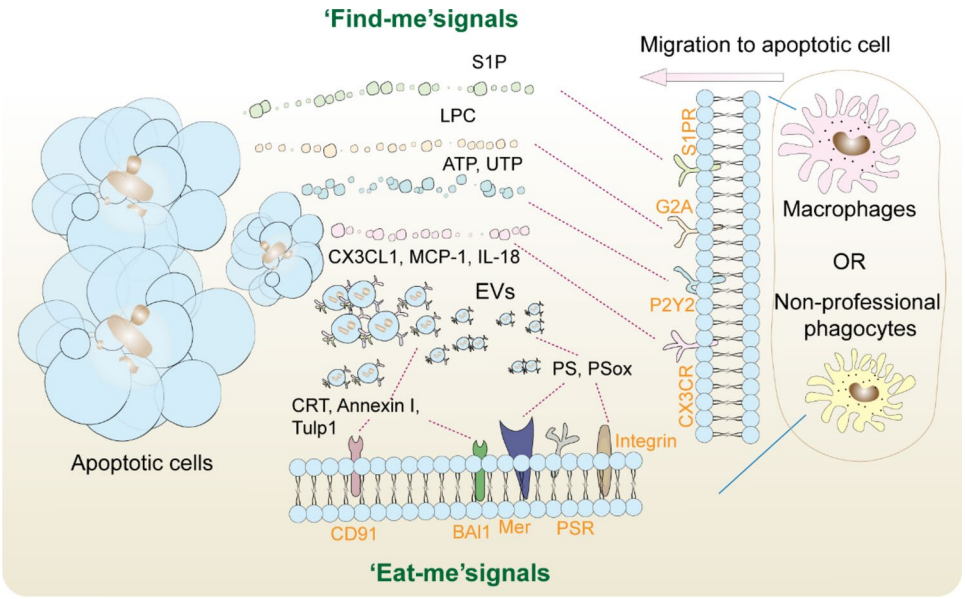

Fig. 2 The regulation of phagocytosis of apoptotic cells involves a myriad of molecules. Factors, phagocytic receptors, and apoptotic ligands interact to coordinate the phagocytosis of apoptotic bodies. These 'find-me' and 'eat-me' signals facilitate the rapid recruitment of macrophages or non-professional phagocytes to the site of apoptosis and mediate the subsequent recognition and engulfment by phagocytes

also helps lower the pH within the phagosome, creating the acidic environment necessary for degradation [73–75].

Within the acidic environment of the phagolysosome, lysosomal enzymes become active, including various cathepsins (such as cathepsin B, D, and L) and DNase II. Cathepsins are proteases that cleave proteins into smaller peptides and amino acids, while DNase II degrades DNA, preventing intact DNA fragments from escaping and triggering an immune response [76, 77]. The degradation of the ApoBD's contents generates numerous small molecules, which the phagocytes can recycle. Amino acids from protein degradation are transported out of the phagolysosome and used for new protein synthesis or as energy sources [78]. Similarly, lipids are broken down by lysosomal lipases and can be reused in membrane synthesis or oxidized to produce energy [79]. Not all degradation products are recycled within the phagocytes, some byproducts need to be expelled from the cell to maintain intracellular balance. This expulsion occurs through exocytosis, where the phagolysosome membrane fuses with the plasma membrane, releasing its contents into the extracellular space. This process ensures that any potentially harmful substances are safely removed from the cell [69, 80]. Beyond the physical degradation of ApoBDs, phagocytes play a pivotal role in modulating the immune response. Engulfment of apoptotic cells often leads to the secretion of anti-inflammatory cytokines such as TGF-β and IL-10. These cytokines facilitate the resolution of inflammation and promote tissue repair, thereby ensuring that the removal of apoptotic cells does not trigger an unnecessary immune response. This process, known as efferocytosis (the engulfment of apoptotic cells), is crucial for maintaining tissue homeostasis and preventing chronic inflammation [81, 82].

Isolation of ApoEVs

To further elucidate the functional importance of ApoEVs and enhance their modification and application, it is crucial to isolate highly pure ApoEVs. This will improve the accuracy of downstream analyses and the effectiveness of their applications. Without proper isolation, it is challenging to draw accurate conclusions about the roles of ApoEVs in specific contexts [83]. Continuous advancements in instrumentation have gradually optimized the techniques for isolating, purifying, and characterizing ApoEVs. Various methods can be employed to isolate ApoEVs, but the most common techniques include differential centrifugation (with or without filtration), density gradient centrifugation, and fluorescence-activated cell sorting (FACS). Here, we summarize the advantages and disadvantages of these reported techniques for isolating ApoEVs, as well as their respective application fields.

Differential centrifugation

Differential centrifugation is the most widely used technique for isolating ApoEVs and is generally considered the gold standard in this field [69]. This method separates vesicles based on their size and density through multiple rounds of low-temperature centrifugation, with increasing centrifugal force and time in each round. Typically, the process begins with a centrifugal force of 300–500 × g to remove residual cells and debris, followed by 1000–3000 × g to pellet ApoBDs [44, 61, 84]. This technique can achieve a purity of isolated ApoBDs between 84 and 98%, as confirmed by flow cytometry analysis [85]. The primary advantage of differential centrifugation is its high efficiency, allowing the entire

process to be completed within an hour, thus minimizing the lysis of ApoBDs during separation. However, despite being a standard technique, many variations exist in practice. For instance, some studies pellet ApoBDs by centrifuging at 16,000 × g for 30 min after removing whole cells and debris at 800 × g [86]. Due to the high centrifugal force, ApoBDs and smaller EVs such as MVs may co-sediment, so the resulting data should be interpreted with caution. In another approach, researchers isolate ApoBDs at 3000 × g for 30 min after centrifuging at 800 × g, followed by 16,000 × g to pellet ApoSEVs [49]. Since this method relies purely on density to separate vesicles, it often results in a heterogeneous population of vesicles and cannot isolate ApoBDs from complex samples like tissues or biological fluids. Interestingly, some studies suggest that different types of centrifuge rotors also affect the yield and type of isolated vesicles. Specifically, swinging bucket rotors can yield more vesicles, which are generally larger than those isolated using fixed-angle rotors [87]. However, this conclusion has mainly been discussed in the context of exosome isolation, and its impact on ApoEVs isolation has yet to be fully explored.

Typically, the diameter of ApoBDs is defined as 1–5 μm [40]. To further purify ApoBDs beyond differential centrifugation, researchers have incorporated additional filtration steps. By passing the supernatant containing ApoBDs obtained from the initial centrifugation (300 × g) through filters of specific sizes, vesicles within the 1–5 μm range can be collected [88]. This quick and straightforward filtration step removes larger cell debris and other impurities, enhancing the purity of isolated ApoBDs. Selecting the appropriate pore size is crucial for isolating ApoBDs within a desired size range, which is essential for downstream applications. However, the key to this method lies in choosing the correct filter pore size and adjusting the subsequent centrifugal force. Using small pore size filters (e.g., 1.2 μm) and high-speed centrifugation (e.g., 100,000 × g) can result in a mixture of small ApoBDs, MVs, and exosomes [89]. Filtering with a single pore size can lead to the loss of ApoBDs that are too large or too small, and some ApoBDs or proteins may non-specifically adhere to the filter material, reducing yield [90]. Additionally, strong filtration may cause artificial fragmentation or lysis of cells or vesicles, so filter choice should be made cautiously. Although size is a useful criterion for characterizing EVs, relying solely on this parameter to separate ApoEVs may not always be appropriate. Recent studies have shown that some human cell line-derived ApoBDs can be as large as 8–10 μm, exceeding the typical 1–5 μm range and falling into the category of larger vesicles like large oncosomes [51]. Additionally, human T-cell lines undergoing primary necrosis and murine macrophages undergoing pyroptosis induced by plant defensin NaD1 or lipopolysaccharide (LPS) and nigericin also generate vesicles similar in size to ApoBDs [91–93]. Therefore, in addition to physical characteristics such as size, additional biological characteristics should be considered to accurately identify and isolate ApoBDs (Fig. 3).

Ultracentrifugation

Low-speed (< 20,000 × g) differential centrifugation typically isolates larger ApoEVs (> 100 nm). Increasing evidence suggests that ApoEVs of different sizes exhibit significant differences in their internal cargoes and downstream functional regulation [84]. Consequently, researchers are focusing on the efficient and accurate isolation of ApoExos. An ultracentrifugation method, adapted from exosome isolation techniques, has been proposed for separating ApoExos. This method involves an initial centrifugation at 300–500 × g for 10 min to remove whole cells and cell debris, followed by centrifugation at 2000 × g for 10 min to eliminate larger debris. Finally, centrifugation at 120,000 × g for 1–2 h separates ApoBDs and smaller EVs [94]. This approach more effectively isolates and purifies ApoExos, reducing contamination by impurities. However, completely avoiding exosome contamination remains challenging, and ultracentrifugation is costly and time-consuming. Extending ultracentrifugation time can increase the yield of exosomal RNA and protein, but excessively long ultracentrifugation may lead to protein aggregation within exosomal particles [95]. Additionally, ultracentrifugation can cause co-aggregation of proteins and lipids with EVs, interfering with the purity of size-based vesicle separation [96, 97]. These phenomena have not yet been fully elucidated in the context of ApoEVs isolation. Thus, while ultracentrifugation is a viable option for separating smaller ApoEVs, careful consideration is required to distinguish between exosomes and ApoExos in functional studies (Fig. 3).

Density gradient centrifugation

Density gradient centrifugation separates vesicles from contaminants based on their buoyant density [98]. This method involves creating a continuous density gradient in a centrifuge tube and layering the sample on top. The tube is then centrifuged at high speed (100,000 × g) overnight (16–18 h) to separate the components. Exosomes, with a buoyant density of 1.13–1.19 g/mL, are typically collected from the upper layers of the gradient [99]. This method yields purer products compared to ultracentrifugation alone and can be used to refine crude vesicle concentrates prepared by ultrafiltration, ultracentrifugation, or other methods [100]. However, due to the significant heterogeneity of ApoEVs, there is no consensus on their buoyant density. Therefore, this separation method is not widely used for isolating ApoEVs.

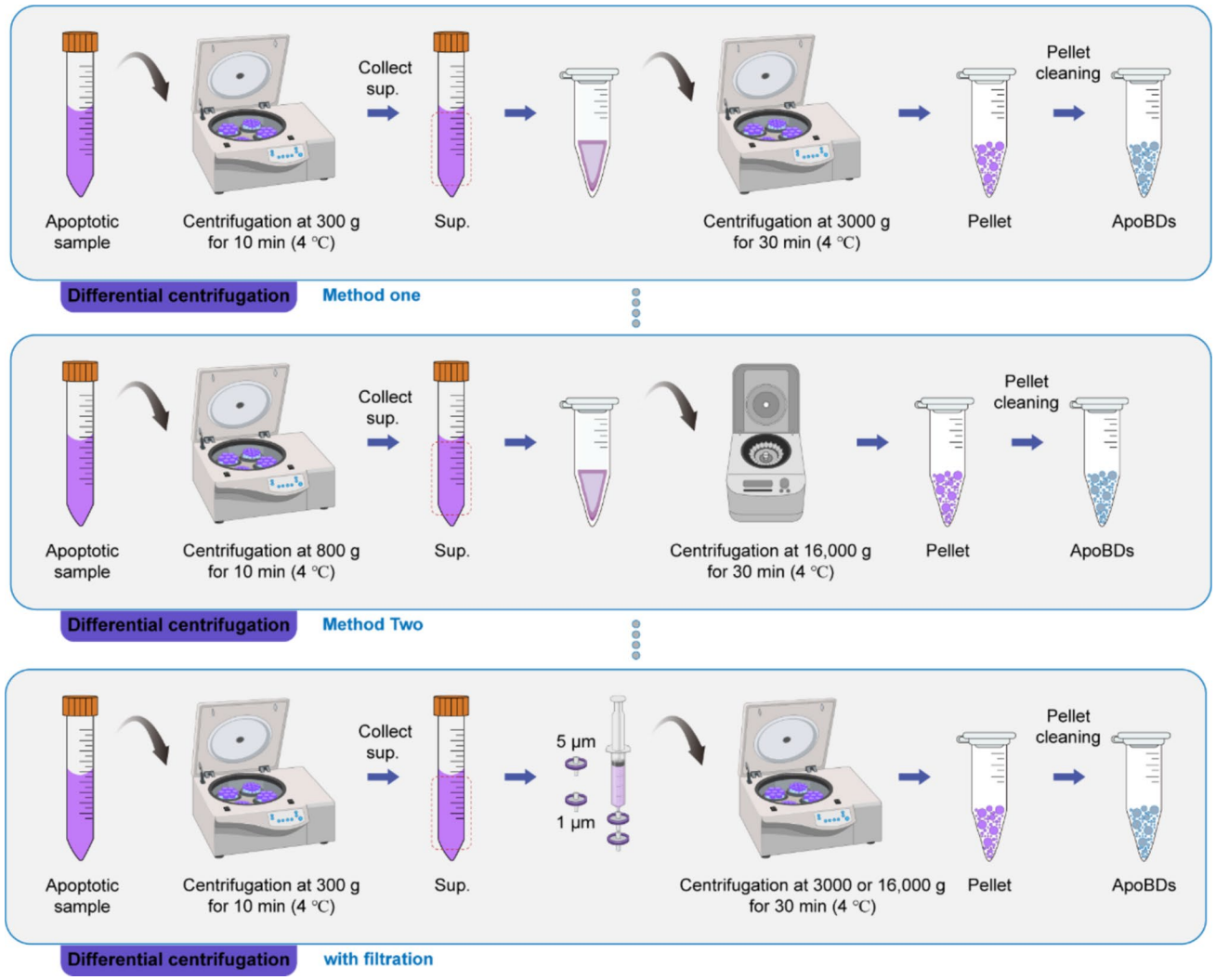

Fig. 3 Approaches for ApoBD isolation. Differential centrifugation is the current gold standard method for isolating ApoEVs from whole cells and smaller EVs based on density. Although there are slight variations between methods, differential centrifugation remains the preferred approach

Theoretically, density gradient centrifugation can separate ApoEVs of different densities in the same tube, resulting in purer ApoEVs compared to differential and ultracentrifugation. It is important to note that this method cannot separate high-density lipoprotein (HDL) particles from EVs in plasma and serum samples, making it less suitable for these samples [101]. Additionally, configuring the density gradient precisely and extracting vesicles from different layers can lead to vesicle loss [102] (Fig. 4).

FACS

FACS is another method for isolating ApoEVs, offering higher purity than differential centrifugation [44]. The purity of FACS-isolated ApoEVs can reach up to 99%, meeting the stringent requirements for scientific research and applications demanding high purity. This method is based on the size of apoptotic cells and ApoEVs, the exposure of PS, and the activation of the PANX1 channel [103]. Fluorescently labeled Annexin V binds to exposed PS, and the nucleic acid dye TO-PRO-3, selectively absorbed through the caspase-activated PANX1 channel, isolates specific cell types of ApoEVs, including those from human peripheral blood mononuclear cells, human Jurkat T cells, and human umbilical vein endothelial cells [50, 51, 103]. FACS can qualitatively and quantitatively separate ApoEVs from viable cells, apoptotic cells, and necrotic cells based on size and granularity. Pre-sorting centrifugation at 3000 × g for 6 min to collect 84% pure ApoEVs helps reduce the number of small EVs in the sample [44, 85]. The greatest advantage of FACS-based isolation is achieving higher purity of ApoEVs (about 99%) and the ability to isolate specific cell types of

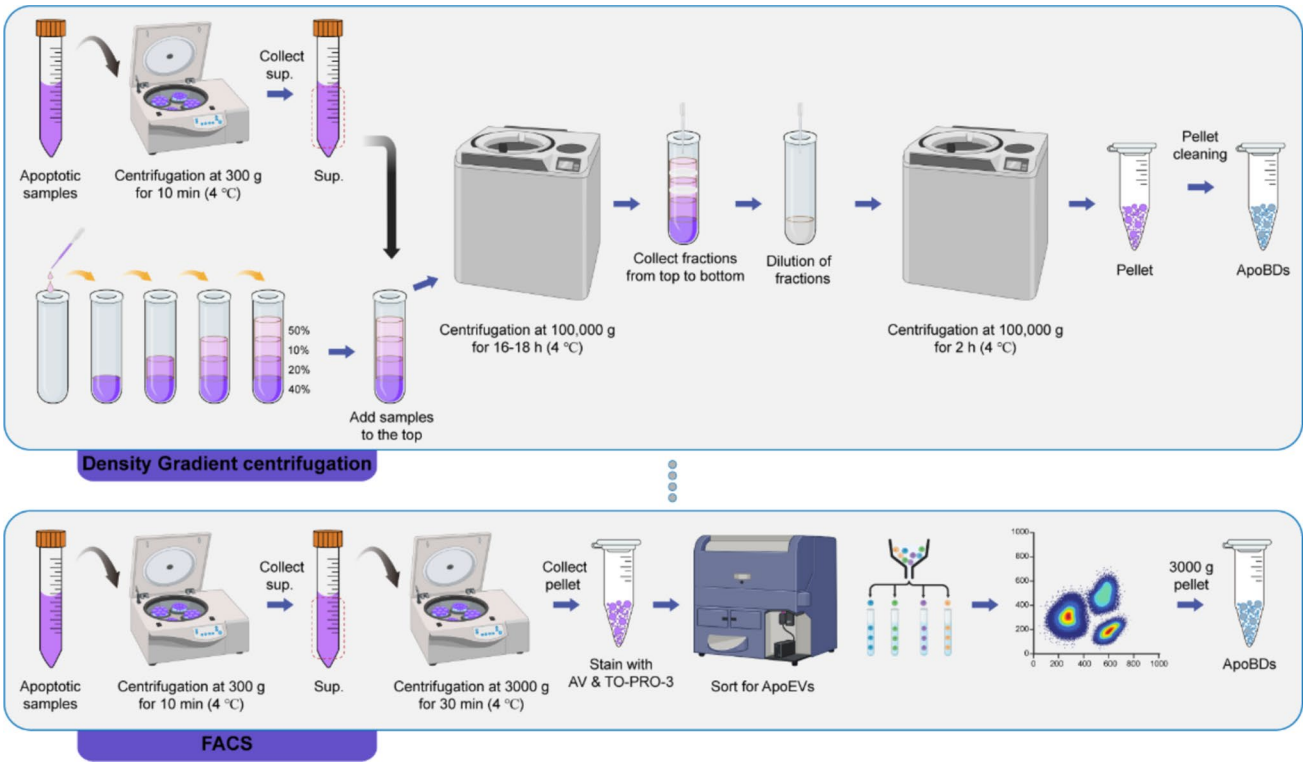

Fig. 4 Approaches for ApoBD isolation. Density Gradient Centrifugation and Fluorescence-activated cell sorting (FACS) can isolate ApoEVs with relatively higher purity, with FACS achieving the highest purity. However, both methods are time-consuming

ApoEVs from tissue samples, body fluids, or blood-derived samples. However, the speed and efficiency of obtaining ApoEVs limit its application range. Therefore, combining exosome extraction techniques with other unreported methods for isolating apoptotic vesicles, such as size exclusion chromatography, microfluidic technology, immunoaffinity isolation, and laser capture methods, also deserves exploration in future research (Fig. 4).

Characterization of ApoEVs

Although differential centrifugation, filtration, density gradient centrifugation, and FACS are commonly used methods for isolating ApoEVs [51, 85], confirming successful induction of apoptosis and characterizing ApoEVs remain critical steps. Without these quality control measures, it is difficult to confidently assess the role of ApoEVs in physiological or pathological contexts. In EV-related research, fundamental characterization involves detecting EV-specific markers. ApoEVs possess numerous markers during formation, including exposed PS [104], DNA [105], nuclear proteins [106], CRT [107], calnexin [108], and Bip/GRP78 [109]. However, many of these markers are not exclusively expressed by ApoEVs. For instance, PS can also be detected on other vesicles such as MVs and necrotic bodies [110–112]. Additionally, ApoBDs derived from different cell types may expose varying levels of PS [51]. Interestingly, a recent study demonstrated that ApoBDs do not express typical exosome markers but show increased expression of GM130 and tubulin. Furthermore, some classic exosome markers, such as CD9, CD81, and TSG101, are not expressed in ApoExos [113]. This may pave the way for new standards in the identification and isolation of ApoExos. Since ApoBDs are generated exclusively by apoptotic cells, it is reasonable to assume that apoptosis-related proteins could serve as valuable potential markers for ApoEVs [114–116].

In addition, various conventional and advanced techniques are used to quantify and characterize ApoEVs. Reported techniques include immunofluorescence [117], Western blotting (WB) [118], scanning/transmission electron microscopy (SEM, TEM) [119], nanoparticle tracking analysis (NTA) [117], dynamic light scattering (DLS) [120], mass spectrometry [121], flow cytometry [122], atomic force microscopy (AFM) [123], and fine-tuned fluid systems [119]. In practical research, researchers typically combine multiple characterization methods depending on the research objectives to analyze ApoEVs, including their size, shape, distribution, composition, and communication

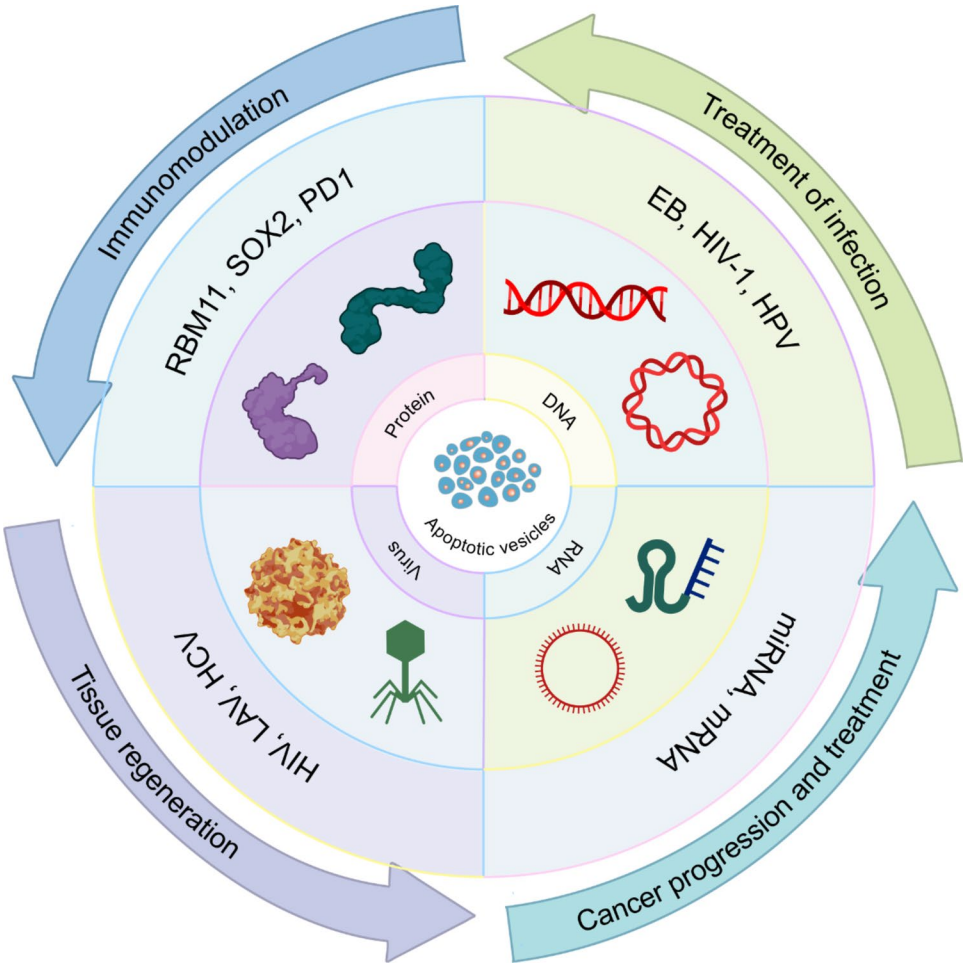

Fig. 5 Intercellular communication of ApoEVs. ApoEVs facilitate the delivery of DNA, RNA, proteins, and viruses to recipient cells, playing vital roles in tumor progression and therapy, immunomodulation, tissue regeneration, and managing infections

pathways. With the continuous advancement of science and technology, new characterization methods based on electrical, optical, and sensing technologies may continue to emerge, aiding in the research of ApoEVs.

Intercellular communication of ApoEVs

It is well known that EVs encapsulate various biologically active molecules, such as nucleotides, proteins, and lipids [124, 125], which act as critical messages in cell-to-cell communication [126]. Similarly, ApoEVs are generated from dying cells and contain a variety of cargoes, including phospholipid membranes, fragmented organelles, and various biofunctional molecules such as membrane-bound or intracellular proteins, RNA, and DNA [105, 127]. Compared to exosomes and MVs, ApoEVs are primarily enriched with DNA, ribosomal RNA, nuclear proteins, and other nuclear-associated substances, whereas exosomes and MVs are mainly rich in cytoplasmic proteins and small RNAs, such as siRNA [84, 128]. Moreover, larger ApoBDs have been found to contain a greater variety of proteins, lipids, RNA, and DNA molecules, potentially exerting broader effects on downstream or recipient cells [129, 130]. The type of cell and the manner of its disintegration significantly impact the variety and quantity of cargoes within ApoEVs [40]. These crucial molecular signals play a significant role in communication with surrounding or even distant cells, serving as important 'transporters' of signals (Fig. 5).

Intercellular communication through transportation of DNA

DNA is driven into apoptotic membrane blebs and subsequently into ApoEVs during the initial phase of apoptotic cell disassembly by actin-myosin contraction [131, 132]. This DNA can be transferred from apoptotic cells to nearby cells through a process called 'apoptotic transformation' [133]. For example, ApoBDs from apoptotic Burkitt lymphoma cells can transfer Epstein-Barr virus (EBV) genes, such as EBNA1 and EBER, to recipient human fetal fibroblasts, macrophages, or bovine aortic endothelial cells, where the EBV DNA can integrate into the genomes of these cells. These genes can be stably expressed in recipient cells,

promoting malignant transformation and increasing the risk of tumor formation. However, it is important to note that this study relied on an in vitro co-culture system and did not rule out potential factors other than ApoBDs that might interfere with the conclusions. Nonetheless, this phenomenon suggests that ApoBDs can mediate the transfer of DNA between cells [134]. Some puzzling issues remain, such as the fact that nuclear DNA is cleaved during apoptosis by nucleases like caspase-activated DNase and endonuclease G, and the cargoes within ApoBDs would be degraded by lysosomes upon entering recipient cells [135–137]. Therefore, whether DNA packaged into ApoEVs can subsequently express functional RNA or proteins should be further investigated in future studies. Similarly, ApoEVs may face similar challenges in the transfer of RNA and proteins (Table 1).

Intercellular communication through transportation of RNA

Studies have shown that ApoBDs contain the highest levels of miRNAs compared to other types of EVs [138]. Although mRNA is largely degraded during apoptosis, miRNAs remain relatively stable [139]. Therefore, miRNAs are believed to serve as important functional molecules, regulating a wide range of physiological and pathological states in recipient cells. Current research on ApoEVs-mediated RNA transfer focuses on stem cells [140–142], immune cells [143, 144], and endothelial cells [145], which play crucial roles in immune regulation and tissue regeneration [146]. For example, studies have shown that ApoBDs produced by LPS-stimulated macrophages can transfer miRNA-221/222 to lung epithelial cells, promoting their proliferation by targeting the CDKN1B-Cyclin D3/CDK4 pathway [144]. Similarly, ApoBDs from endothelial cells transfer miRNA-126 to vascular cells, increasing the production of chemokine (C-X-C motif) ligand 12 (CXCL12) in endothelial cells, thereby maintaining plaque stability through the recruitment of progenitor cells [147]. These examples illustrate that ApoBDs can transfer various miRNAs to recipient cells, thereby affecting their functions.

Interestingly, recent studies have reported significant differences in RNA cargoes between different subtypes of ApoEVs. Endothelial cell ApoBDs are enriched in ribosomal RNA, while ApoExos mainly contain non-ribosomal non-coding RNA [148]. Moreover, the functional differences in RNA cargoes among different ApoEVs subtypes are also notable. For instance, differential genes in ApoSEVs from BMMSCs are significantly enriched in AGE-RAGE signaling pathway in diabetic complications, whereas differential genes in ApoBDs are enriched in multiple inflammation-related pathways [49]. Overall, the shuttling of RNAs carried by ApoEVs among cells demonstrates the functional diversity of ApoEVs (Table 1).

Intercellular communication through transportation of proteins

ApoEVs can transfer not only nucleic acid molecules but also proteins to recipient cells in various environments [149]. These proteins mainly include membrane surface molecules and receptors, intracellular signaling factors, functional proteins, and active enzymes. For example, ApoEVs derived from pluripotent stem cells can transfer the transcription factor SOX2 to skin MSCs. SOX2 can activate the Hippo signaling pathway in recipient cells, accelerating skin wound healing [150]. Additionally, ApoEVs derived from tumor cells can transfer aldehyde dehydrogenase 1A1 (ALDH 1A1) to normal tumor cells, promoting metastasis and stemness of lung adenocarcinoma [151]. Interestingly, large EVs (LEVs) released by apoptotic T cells carry functional catalytic subunits of the proteasome, PSMB9 and PSMB10, which can promote the release of LEVs from apoptotic T cells. This indicates that proteins loaded into ApoEVs not only function within recipient cells but also play crucial roles in regulating ApoEVs themselves [116]. These protein-transferring ApoEVs mainly originate from tumor cells [152], stem cells [150, 153], immune cells [45], and some other somatic cells [154]. They play multiple regulatory roles in injury repair, tissue regeneration, immune regulation, and tumor progression. It is worth noting that how these proteins are retained in ApoEVs during apoptosis, how they are encapsulated into ApoEVs, and how they escape lysosomal degradation in recipient cells to exert their functions remain unknown challenges (Table 1).

Intercellular communication through transportation of pathogens

Pathogen invasion frequently leads to apoptosis [155], and the pivotal role of ApoEVs in pathogen transmission and dissemination is significant [156]. For instance, ApoBDs derived from T cells infected with human immunodeficiency virus type 1 (HIV-1) can facilitate the transfer of HIV-1 to human kidney-2 (HK2) cells, human renal proximal tubule epithelial cells (HRPTEC), and primary renal tubular cells [157]. Similarly, ApoBDs derived from monocytes infected with influenza A virus (IAV) promote viral dissemination by carrying IAV mRNA, proteins, and viral particles [45]. More importantly, inhibiting the formation of ApoBDs from infected monocytes can control the spread of IAV, offering an exciting direction for post-infection therapy. Due to the variety of pathogens, whether all pathogens can spread

Table 1 ApoEVs derived from different cell types carry different cargoes and perform various functions

Origin of ApoEVs			Cargoes in ApoEVs		Recipient cell	Function of ApoEVs	Ref
Types	**Name**	**ApoEVs types**	**Types**	**Name**			
Lymphoid cell	BL41	ApoBDs	DNA	EBNA, EBER	Fibroblasts, monocytes, or endothelial cells	Changes in growth characteristics and signaling pathways	[134]
Immune cell	HuT78, PBMC	ApoBDs	DNA	HIV-1 DNA	Fibroblasts, DCs	Enhanced antigen presentation and virus transmission	[161]
Cancer cell	Carcinoma cell	ApoBDs	DNA	HPV-16, HPV-18	HPF, Cancer cell	Promoting malignant tumorigenic transformation	[163]
Immune cell	T Cell	ApoBDs	DNA	Y-chromosome	Epithelial cell	Transient expression of exogenous DNA	[242]
Cancer cell	REF	ApoBDs	DNA	H-ras^{V12}, Human c-myc	MEF	Promoting malignant tumorigenic transformation	[105]
Stem cell	ADSCs	ApoBDs	miRNA	miRNA-21-5p	Macrophages	Inducing M2 macrophages polarization	[140]
Stem cell	ADSCs	ApoBDs	miRNA	miRNA-20a-5p	THP-1	Balancing macrophage inflammatory polarization	[141]
Stem cell	MSCs	ApoEVs	miRNA	miRNA-210-3p	ECs	Promote vascularization and would healing	[142]
Stem cell	hBMMSCs	ApoEVs	miRNA	miRNA1324	RAW264.7, hBMMSCs	Promoting osteogenesis and inhibiting osteoclast formation	[219]
Stem cell	mBMMSCs	ApoBDs	miRNA	miRNA-223-3p	Pre-OCs	Inhibiting osteoclast differentiation and alveolar bone resorption	[221]
Stem cell	hBMMSCs	ApoEVs	miRNA	miRNA-4485-3p	MSCs	Promoting bone regeneration	[164]
Somatic cells	/	ApoBDs	miRNA	miRNA-328-3p	BMMSCs	Promoting self-renewal and osteo-/adipo-genic differentiation of BMSCs	[88]
Immune cell	mBMDMs, RAW264.7, THP-1	ApoBDs	miRNA	miRNA-221, miRNA-222	A549	Promoting the proliferation of lung epithelial cells	[144]
Immune cell	RAW264.7	ApoEVs	miRNA	miRNA155	MSCs	Inhibiting osteogenesis and promoting adipogenesis of MSCs	[143]
Immune cell	mBMDMs	ApoBDs	miRNA	miRNA-21-5p	Macrophages	Promoting M2 polarization of macrophages	[243]
FSCT	L-Wnt-3A	ApoBDs	miRNA	miRNA-339-5p	Macrophages	Inducing M2 macrophages polarization	[244]
ECs	HUVECs	ApoBDs	miRNA	miRNA-126	HUVECs, SMCs, mAECs	Promoting the production of CXCL12	[147]
ECs	HUVECs	ApoExos	mRNA	PCSK5	HUVECs	Increasing PCSK5 protein expression	[145]

Table 1 (continued)

Origin of ApoEVs			Cargoes in ApoEVs		Recipient cell	Function of ApoEVs	Ref
Types	**Name**	**ApoEVs types**	**Types**	**Name**			
Stem cell	rBMMSCs	ApoEVs	mRNA	Multiple mRNA	BMSCs	ApoSEVs and ApoBDs exhibit opposing roles in stem cell proliferation, migration and differentiation	[49]
Cancer cell	GBM	ApoEVs	Protein	RBM11	GBM	Promoting the resistance and aggressive migratory phenotype of GBM	[152]
Cancer cell	BEAS2b, LUAD	ApoEVs	Protein	ALDH1A1	LUAD	Activating the NF-κB signaling pathway	[151]
Stem cell	hDPSC	ApoEVs	Protein	TUFM	HUVECS	Promoting angiogenesis	[224]
Stem cell	MSCs	ApoEVs	Protein	DNA repair proteins	BMMSCs	Repairing DNA damage and suppressing premature cellular senescence	[222]
Stem cell	hESCs	ApoEVs	Protein	SOX2	mSMSCs	Promoting SMSC proliferation and migration	[150]
Stem cell	SHED	ApoEVs	Protein	PD1	HUVECs	Modulate the ECs glycolysis	[245]
Stem cell	mBMMSCs	ApoEVs	Protein	Rab7	rBMMSCs	Restoring autolysosomes formation	[153]
Stem cell	Epithelial stem cell	ApoBDs	Protein	Wnt8a	Basal stem cells	Activating Wnt signaling	[162]
ECs	HUVECs, mECs	ApoExos	Protein	20S proteasome	B cell	Inducing autoantibody production	[113]
Blood cell	hRBC	ApoEVs	Protein	CA1	hBMMSCs	Promoting osteogenesis of hBMSCs	[154]
HHCs	KG1a	ApoBDs	Protein	IGF2BP3	SKM1, ML-1	Promoting Ara-C resistant of HHCs	[246]
Somatic cells	/	ApoBDs	Protein	RNF146	BMMSCs	Promoting self-renewal and osteo-/adipo-genic differentiation of BMSCs	[88]
Osteoclast	mOCs	ApoBDs	Protein	RANKL	MC3T3-E1	Promoting osteogenesis	[247]
Hepatocyte	RLW	ApoBDs	Virus	JFH1	hMDMs, LX2 cells	Activating inflammasomes	[165]
Immune cell	THP1	ApoBDs	Virus	IAV	A549	Promoting viral propagation and antiviral immune response	[45]
Immune cell	CD4 T cells	ApoEVs	Virus	HIV	HK2s, HRPTECs	Transfering HIV-1 into tubular cells	[157]

REF Rat embryonic fibroblasts, *MEF* Mouse embryonic fibroblasts, *HPF* Human primary fibroblasts, *HUVECs* Human umbilical vein endothelial cells, *ADSCs* Adipose derived mesenchymal stem cells, *FSCT* Fibroblast-Like Cells, *ECs* Endothelial cells, *Pre-OCs* Pre-osteoclasts, *SMCs* Smooth muscle cells, *mAECs* Mouse aortic endothelial cells, *GBM* Glioblastoma, *hDPSC* Human deciduous pulp stem cell, *TUFM* Transferring mitochondrial Tu translation elongation factor, *hESCs* Human embryonic stem cells, *mSMSCs* Mouse skin mesenchymal stem cells, *SHED* Stem cells from human exfoliated deciduous teeth, *PD1* Programmed cell death 1, *Rab7* Ras-related protein 7, *mECs* Murine aortic endothelial cells, *hRBC* Human red blood cells, *CA1* Carbonic anhydrase 1, *HHCs* Human hematopoietic cell lines, *IGF2BP3* Insulin-like growth factor 2 mRNA-binding protein 3, *Ara-C* Cytosine arabinoside, *mOCs* Mature osteoclasts, *RLW* Huh$^{7.5CYP2E1}$ cells, *hMDMs* Human monocyte-derived macrophages

via ApoEVs is a question that requires extensive research. Furthermore, the selectivity of ApoEVs for recipient cells following infection and the functional impact of ApoEVs within recipient cells need additional investigation (Table 1).

Each day, over 50 billion cells must undergo apoptosis in the human body to maintain tissue homeostasis [158]. ApoEVs are certainly not just 'garbage bags' from dying cells but significant information carriers that need to be cleared [159]. They can also serve as platforms, like other vesicles, to target and deliver molecules to recipient cells, altering their functions [160]. These recipient cells primarily include immune cells [140, 161], endothelial cells [145, 147], tumor cells [152], and stem cells [88, 162]. Therefore, ApoEVs can be preferred carriers for functional intervention in these cells. Utilizing various natural and engineered ApoEVs can regulate downstream target cells, serving as an effective strategy for disease diagnosis and therapy.

Applications of ApoEVs

Although many mechanisms by which ApoEVs interact with various types of recipient cells and exert their functions remain unknown, increasing evidence suggests that ApoEVs can trigger multiple responses in recipient cells, regulating diverse biological functions [24]. Existing reports indicate that these intervened biological processes primarily involve immunomodulation [140], tumorigenesis and progression [105, 163], as well as tissue regeneration and repair [142, 164]. In the future, ApoEVs may attract attention and be reported in more fields. It is noteworthy that the broad biological functions of ApoEVs not only maintain homeostasis but also have the potential to disrupt it [165]. Additionally, the compositional heterogeneity of ApoEVs, their different sources, and varying conditions of formation have not been carefully distinguished in current research. Their clinical applications in diagnosis and therapy require extensive research support. In this section, we will systematically review and analyze the intervention roles of ApoEVs in some important biological functions and the application of engineered ApoEV-based carriers in disease treatment, distinguishing their advantages and disadvantages to lay the foundation for subsequent research.

Applications based on non-engineered ApoEVs

Similar to exosomes, ApoEVs are critical signal carriers for intercellular communication. ApoEVs can deliver essential biomolecules such as DNA, RNA, and proteins to recipient cells, along with intact organelles including mitochondria, ribosomes, and endoplasmic reticulum, as well as notable nuclear fragments [166]. Derived from the sequential disassembly of cells under precise molecular regulation, ApoEVs are far from being mere 'garbage bags.' Instead, they act as 'engineers' in cell function regulation, homeostasis maintenance, and disease progression (Fig. 6) (Table 2) [160].

Diagnosis

As products of dying cells, ApoEVs and their cargoes serve as critical evidence for assessing cell status and disease progression [167]. Currently, the application of ApoEVs as diagnostic and prognostic indicators primarily focuses on tumors [168–171], infection [172], autoimmune diseases [173–175], nervous system injuries, neurodegenerative diseases [176], and cardiovascular diseases [177]. Studies have reported that ApoBDs are enriched in prostatic adenocarcinoma tissues and present in 13% of high-grade prostatic intraepithelial neoplasia but are rarely seen in benign mimics' needle biopsies. This phenomenon provides histological diagnostic evidence for challenging cases of prostate adenocarcinoma [168]. Further studies have found that ApoBDs derived from prostate cancer carry characteristic genomic DNA (gDNA), including MLH1, PTEN, and TP53 genes, which can serve as potential markers for diagnosing prostate cancer and assessing tumor progression [169]. Additionally, the drug sensitivity and toxicity of pancreatic tumor cells can be assessed using high-throughput single-particle impedance cytometry on ApoBDs [170]. Moreover, studies have shown that ApoBDs are significant in diagnosing celiac disease, with a marked increase in Crypt ApoBDs in small intestinal tissues highly correlated with disease severity [173, 174]. Other researchers have found that circulating ApoBDs can be biomarkers of apoptosis in ischemic stroke and neurodegenerative disease patients. High-purity and intact ApoBDs isolated by combining centrifugation and flow cytometry can help clinicians detect cell damage and disease activity in cerebrovascular and neurodegenerative disease patients [176]. Williams et al. further revealed that ApoEVs produced by human cells under varying pathological conditions, such as cholesterol accumulation or endotoxin exposure, harbor distinct molecular subsets, influencing endothelial function through multiple, well-characterized molecular pathways. This suggests that ApoEVs could serve as both novel biomarkers and mediators of endothelial dysfunction [177]. However, ApoEVs cannot yet be used as standalone diagnostic indicators for diseases, as their specificity and accuracy need further observation. It is anticipated that as research on ApoEVs deepens, ApoEVs themselves and their cargoes may become a minimally invasive and accurate diagnostic tool, playing an irreplaceable role in clinical disease diagnosis and prognosis.

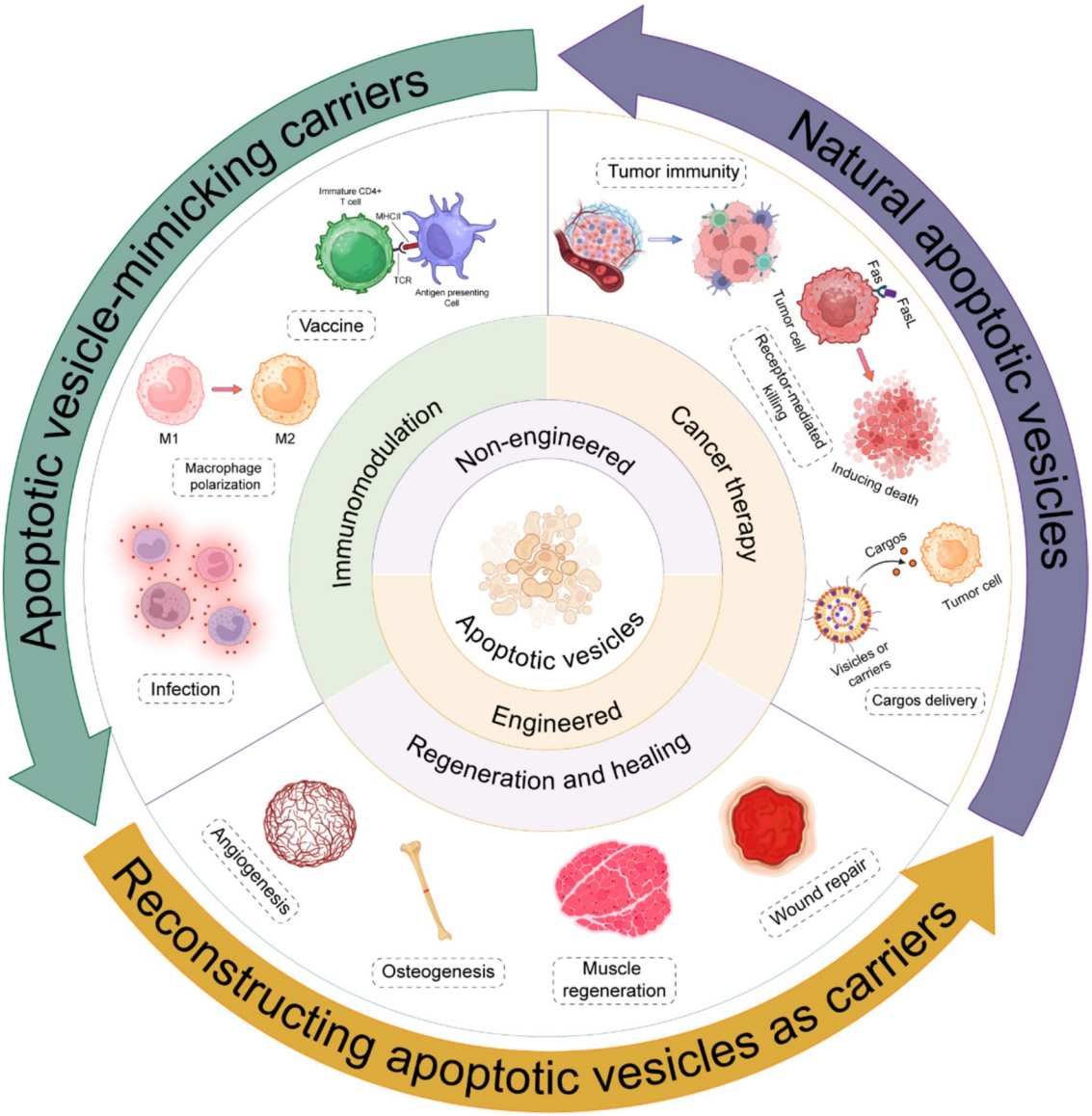

Fig. 6 Applications of ApoEVs. There is a broad scope of research and application for natural and engineered ApoEVs in areas such as immune regulation, tumor therapy, and tissue regeneration

Immunomodulation

ApoEVs play a crucial role in immune modulation by serving as vaccines that present antigens to antigen-presenting cells (APCs), thus activating immune responses [156, 178]. ApoEVs provide unique therapeutic strategies as efficient vaccines for various diseases, including tumors [179–182], infections [183–186], and autoimmune disorders [187]. For example, dendritic cells (DCs) pulsed with allogeneic leukemic ApoBDs have been used as vaccines to treat B-cell chronic lymphocytic leukemia (B-CLL), progressing to clinical trials [180]. Schaible et al. demonstrated that macrophages infected with intracellular bacteria (such as Mycobacterium tuberculosis) can release ApoBDs to transfer antigens to DCs, subsequently activating CD8 T cells and promoting specific immunity. This study provides a new reference point for vaccine design in antibacterial immunity [183]. Notably, in studies of systemic lupus erythematosus (SLE), DCs have shown a stronger ability to phagocytose apoptotic blebs compared to ApoBDs, mediating more effective antigen presentation and autoimmune responses. This suggests that the type of ApoEVs should be a critical factor in vaccine design for different diseases [187]. Additionally, ApoBDs from DCs carrying HIV-1 can serve as vaccines to activate T cells for the intervention and treatment of HIV-1 infection [186]. Thus, ApoEVs can act as natural antigen carriers, delivering various antigens and possessing specific

Table 2 Applications based on natural ApoEVs

Origin of ApoEVs			Recipient cell	Applications		Functions	Ref
Types	Name	ApoEVs types		Types	Diseases		
Immune cell	Alveolar macrophage	ApoBDs	/	Diagnosis	Lung carcinoma	Cancer diagnosis	[171]
Cancer cell	Prostate cancer	ApoBDs	/	Diagnosis	Prostate adenocarcinoma	Cancer diagnosis	[168]
Cancer cell	Prostate cancer	ApoBDs		Diagnosis	Prostate cancer	Cancer diagnosis and prognostics	[169]
Cancer cell	Pancreatic tumor	ApoBDs	/	Diagnosis	PDAC	Assess drug sensitivity and toxicity	[170]
Blood	/	ApoBDs	/	Diagnosis	Pemphigus Vulgaris	Assessing disease progression	[172]
Nerve cell	Cerebellar granule cells	ApoBDs	/	Diagnosis	CJD	CJD diagnosis	[175]
/	/	ApoBDs	/	Diagnosis	PCD	Predicting the potential for continued villous atrophy following GFD	[174]
/	/	ApoBDs	/	Diagnosis	CD	CD diagnosis	[173]
/	/	ApoBDs	/	Diagnosis	GVHD	GVHD diagnosis	[248]
/	/	ApoBDs	/	Diagnosis	Neurological disorder	Prognosis and monitoring of cerebrovascular and neurodegenerative disease activities	[176]
Immune cell	DC	ApoEVs	T cells	Immunomodulation	AML	DC-vaccines	[179]
Immune cell	Macrophage	ApoEVs	CD8 T cell	Immunomodulation	MTI	DC-vaccines	[183]
Immune cell	Macrophage	ApoEVs	DC	Immunomodulation	MTI	DC-vaccines	[184]
Immune cell	Macrophage	ApoEVs	DC	Immunomodulation	CL	DC-vaccines	[181]
Immune cell	Macrophage	ApoEVs	DC	Immunomodulation	MTI	DC-vaccines	[185]
Immune cell	DC	ApoBDs	T cells	Immunomodulation	HIV 1 infection	DC-vaccines	[186]
Leukemic cell	Leukemic B cells	ApoBDs	DC	Immunomodulation	B-CLL	DC-vaccines	[180]
Leukemic cell	HL60	ApoEVs	DC	Immunomodulation	AML	DC-vaccines	[182]
Leukemic cell	32Dcl3	ApoEVs	DC	Immunomodulation	SLE	DC-vaccines	[187]
Stem cell	AMSCs	ApoBDs	Macrophages	Immunomodulation	Skin wounds	Promoting M2 polarization of macrophages	[140]
Stem cell	AMSCs	ApoBDs	Macrophages	Immunomodulation	Diabetic wounds	Promoting M2 polarization of macrophages	[141]
Stem cell	mBMMSCs	ApoEVs	Macrophages	Immunomodulation	Periodontitis	Inhibiting pro-inflammatory phenotypes	[249]
Stem cell	mBMMSCs	ApoEVs	T cells	Immunomodulation	Lupus, arthritis	Ameliorating of inflammation	[192]

Table 2 (continued)

Origin of ApoEVs			Recipient cell	Applications		Functions	Ref
Types	Name	ApoEVs types		Types	Diseases		
Stem cell	hBMMSCs	ApoEVs	Macrophages	Immunomodulation	Type 2 diabetes	Promoting M2 polarization of macrophages	[117]
Immune cell	Macrophage	ApoBDs	Macrophages	Immunomodulation	OA	Promoting M2 polarization of macrophages	[243]
Immune cell	Macrophage	ApoEVs	Macrophages	Immunomodulation	RA	Promoting M2 polarization of macrophages	[193]
Immune cell	Murine thymocytes	ApoEVs	Macrophages	Immunomodulation	Colitis	Promoting anti-inflammatory phenotype	[190]
ECs	HUVECs	ApoExos	ECs	Immunomodulation	Endothelial injury	Promoting NF-κB activation	[189]
Stem cell	mBMMSCs	ApoExos	MMC	Cancer therapy	MM	Inducing MMC apoptosis	[208]
Cancer cell	EG7	ApoBDs	T cells	Cancer therapy	T lymphoma	Inhibiting CTL responses and antitumor immunity	[207]
Cancer cell	PROb	ApoBDs	DCs	Cancer therapy	Colon cancer	Activating CTL responses	[204]
Cancer cell	LUAD cell	ApoEVs	A549	Cancer therapy	LUAD	Promoting LUAD metastasis and stemness	[151]
Cancer cell	HL60	ApoEVs	DCs	Cancer therapy	Leukemia	Inducing tumor-directed immunity	[250]
Cancer cell	C6	ApoEVs	DCs	Cancer therapy	GMB	Activating CTL responses	[206]
Cancer cell	GBM cells	ApoEVs	GBM cells	Cancer therapy	GMB	Promoting therapy resistance and aggressive migratory phenotype	[152]
Cancer cell	Melanoma cells	ApoEVs	DCs	Cancer therapy	Melanoma	Activating CTL responses	[251]
Cancer cell	Lymphoma cells	ApoEVs	Macrophages	Cancer therapy	Lymphoma	Inhibiting CTL responses	[212]
Stem cell	mBMMSCs	ApoEVs	MSCs	Regeneration and healing	Skin and hair disorders	Promoting wound healing and hair growth	[218]
Stem cell	hESCs	ApoEVs	MSCs	Regeneration and healing	Skin wound	Promoting skin wound healing	[150]
Stem cell	MSCs	ApoEVs	ECs	Regeneration and healing	Skin wound	Promoting skin wound healing	[142]
Stem cell	BMMSCs	ApoEVs	stem cells	Regeneration and healing	Skin wound	Promoting skin wound healing	[49]
Tissue	Adipose tissue	ApoEVs	Fibroblasts, ECs	Regeneration and healing	Skin wound	Promoting skin wound healing	[252]
ECs	HUVECss	ApoEVs	ECs	Regeneration and healing	Skin wound	Enhancing Angiogenesis	[253]
Stem cell	hDPSCs	ApoEVs	ECs	Regeneration and healing	Ischemic-hypoxic injury	Enhancing Angiogenesis	[224]

Table 2 (continued)

Origin of ApoEVs			Recipient cell	Applications		Functions	Ref
Types	Name	ApoEVs types		Types	Diseases		
Stem cell	hDPSCs	ApoEVs	hBMMSCs	Regeneration and healing	Osteoporosis	Promoting bone formation	[254]
Stem cell	hBMMSCs	ApoEVs	MSCs	Regeneration and healing	Osteoporosis	Promoting osteogenesis	[219]
Stem cell	hBMMSCs	ApoEVs	MSCs	Regeneration and healing	Osteoporosis	Promoting osteogenesis	[164]
Stem cell	hBMMSCs	ApoEVs	MSCs	Regeneration and healing	Osteoporosis	Promoting osteogenesis	[255]
Stem cell	mBMMSCs	ApoEVs	MSCs	Regeneration and healing	Bone loss	Enhancing bone mass	[153]
Stem cell	rBMMSCs	ApoEVs	ECs	Regeneration and healing	Defect fabrication	Promoting osteogenesis	[256]
Bone cell	Osteoclast	ApoBDs	MC3T3-E1	Regeneration and healing	/	Promoting osteogenesis	[247]
Immune cell	RAW264.7	ApoEVs	MSCs	Regeneration and healing	/	Promoting osteogenesis	[143]
Stem cell	mBMMSCs	ApoEVs	C2C12	Regeneration and healing	TA injury	Promoting muscle regeneration	[220]
Stem cell	mBMMSCs	ApoEVs	mBMMSCs	Regeneration and healing	Irradiation-induced injury	Ameliorating irradiation-induced DNA damage	[222]
Stem cell	hUMSCs	ApoEVs	HEI-OC1	Regeneration and healing	NIHL	Attenuating NIHL	[257]
Stem cell	SHED	ApoEVs	ECs	Regeneration and healing	Ischemic Retinopathy	Regulate the angiogenic activation	[245]
CTCs	Tendon Cells	ApoBDs	Tendon Cells, BMMSCs	Regeneration and healing	Tendon injury	Promoting cell proliferation and migration	[223]
Immune cell	Macrophages	ApoBDs	Epithelial cells	Regeneration and healing	Pneumonia	Promoting proliferation of epithelial cells	[144]
Blood cell	hRBC	ApoEVs	hBMMSCs	Regeneration and healing	Calvarial defects	Enhancing bone regeneration	[154]
ECs	HUVECs	ApoBDs	ECs	Regeneration and healing	Atherosclerosis	Limiting atherosclerosis	[147]

GFD Gluten free diet, *PDAC* Pancreatic ductal adenocarcinoma, *CJD* Creutzfeldt-Jakob disease, *PCD* Pediatric celiac disease, *CD* Celiac disease, *GCHD* Graft versus host disease, *AML* Acute myeloid leukemia, *CL* Cutaneous leishmaniasis, *MTI* Mycobacterium tuberculosis infection, *32Dcl3* Murine 32D clone 3 (32Dcl3) cells (H-2 ^{k}), *AMSCs* Adipose mesenchymal stem cells, *CTL* Cytotoxic T lymphocytes, *LUAD* Lung adenocarcinoma, *GMB* Glioblastoma, *hDPSCs* Human deciduous pulp stem cells, *hUMSCs* Human umbilical mesenchymal stem cells, *NIHL* Noise-induced hearing loss, *CTCs* Connective tissue cells

cell-targeting capabilities. However, further improvements in vaccine storage stability and production purity are needed.

In addition to serving as novel vaccines, ApoEVs are also involved in regulating the balance of inflammation. ApoEVs express multiple "Eat-me" signals on their surface, which are recognized by phagocytes to mediate the endocytosis of ApoEVs [60]. The entry and digestion of ApoEVs within phagocytes is a clearance pathway for ApoEVs in the body, producing anti-inflammatory responses and suppressing tissue inflammation [188]. Currently, inflammation-related diseases involving ApoEVs include cardiovascular diseases [189], digestive system diseases [190], metabolic diseases [117, 141], and autoimmune diseases [191, 192]. For example, adipose-derived mesenchymal stem cell ApoBDs can promote macrophage M2 polarization and accelerate skin injury repair [140]. ApoEVs from macrophages and osteoclasts can inhibit joint inflammation and repair cartilage damage and bone erosion in rheumatoid arthritis (RA), showing promising prospects in RA treatment [193]. Moreover, BMMSC ApoEVs can reprogram macrophages in the

liver of type 2 diabetes, promoting their transformation to an anti-inflammatory phenotype [117]. Mechanistically, the anti-inflammatory effects of ApoEVs can be achieved by delivering cargoes to recipient cells or through ligand-receptor binding [140, 192].

Additionally, ApoEVs have been found to carry endogenous danger signals that trigger detrimental immune and thrombotic processes. These vesicles, originating from apoptotic monocytes/macrophages, are loaded with oxidized membrane phospholipids, which activate endothelial cells and induce the low-density lipoprotein receptor-1(LOX-1)-dependent expression of intercellular adhesion molecule-1 (ICAM-1), subsequently attracting leukocytes to the vasculature [194]. Importantly, these ApoEVs expose PS on their membrane and also contain tissue factors (TFs), indicating that they could represent novel mediators of atherothrombosis [195, 196]. Interestingly, a recent study showed that macrophage phagocytosis of different types of apoptotic cells can produce different phenotypic transcriptome changes, with receptors on macrophage surfaces playing a crucial role in macrophage functional differentiation. Altering the number of apoptotic cells given to macrophages does not lead to the acquisition of a tissue remodeling phenotype. This suggests that the identity of perceived apoptotic cells, rather than their quantity, is key in phagocyte remodeling [197].

Autoimmune diseases arise when structural changes or biochemical modifications in self-components lead to epitope spreading, revealing cryptic epitopes and forming "altered self" molecules. This process breaks immune tolerance and triggers autoimmunity [198]. In autoimmune diseases, excessive apoptosis and impaired clearance of apoptotic cells disrupt immune tolerance, leading to autoimmunity [199]. During apoptosis, positively charged autoantigens bind to the negatively charged plasma membrane PS and are released via ApoEVs during membrane budding, establishing a molecular foundation for antigen presentation [198]. In conditions such as SLE and RA, defective clearance of ApoBDs results in cellular debris accumulation, exposing self-antigens and activating autoreactive B and T lymphocytes, perpetuating autoimmune pathology [200]. The accumulation of ApoBDs, enriched with post-translationally modified or oxidized proteins, nucleic acids, and lipids, exacerbates their immunogenicity in autoimmune diseases [201]. This alteration enhances recognition by pattern recognition receptors, particularly Toll-like receptors (TLRs), amplifying the autoimmune response. The balance between apoptotic body clearance and immune tolerance is delicate, and disruption in this process triggers a cascade of autoimmune events. Understanding the molecular mechanisms governing apoptotic body formation, modification, and clearance is essential for grasping autoimmune pathology. In summary, the upstream and downstream relationship between ApoEVs and phagocytes, especially immune cells, as well as the functional molecules carried by ApoEVs themselves, hold great potential for immune regulation and molecular delivery in various diseases.

Cancer therapy

Apoptotic tumors can communicate with adjacent cells through ApoEVs [202]. Although the structural characteristics, contents, and functional properties of ApoEVs in cancer remain unclear, growing evidence suggests that ApoEVs play multifaceted roles in mediating tumor immunity, transformation, and metastasis [46, 203]. During apoptosis, tumor cells can present tumor antigens to APCs via ApoEVs [204]. These antigens are then captured by APCs, which activate CD4 helper and cytotoxic lymphocytes to drive immune responses, promoting tumor regression [166, 205]. For example, subcutaneous injection of irradiated C6 cell-derived MVs increases the infiltration of helper T cells, cytotoxic T cells, and regulatory T cells into tumors, reducing the size of glioblastomas [206]. Contradictorily, there are differing conclusions about whether apoptotic cell vesicles stimulate or suppress immunity in tumors. Tumor-derived ApoEVs can downregulate the immune-stimulatory function of antigen-specific DCs, and evidence suggests that this immunosuppressive effect is mediated by transforming growth factor-β1 (TGF-β1) [207]. Therefore, the role of ApoEVs in tumor immunity may need to be carefully differentiated according to different tumor environments and types.

Apart from immune response intervention, Kou et al. found that ApoEVs from MSCs can induce multiple myeloma (MM) cell apoptosis and inhibit MM cell growth by binding FasL to Fas on the tumor cell membrane [208]. ApoEVs derived from lung adenocarcinoma cells can deliver ALDH 1A1 to normal lung adenocarcinoma cells, activating the NF-κB signaling pathway by increasing aldehyde dehydrogenase enzyme activity in recipient tumor cells, thus promoting metastasis, self-renewal, and chemoresistance [151]. These findings underscore the diversity of functions and mechanisms of ApoEVs in tumors.

The process of tumor metastasis involves the migration of tumor cells from the primary site to draining lymph nodes, eventually spreading sequentially from the nearest to the furthest lymph nodes [209]. In the subcapsular sinus of the lymph node, macrophages are the first to encounter antigens and play a role in presenting captured antigens to APCs, including B cells [210]. CD169 + macrophages are crucial for tumor tolerance and immunogenic responses. By immunizing with apoptotic tumor cells, CD169 + macrophages cross-present tumor antigens to CD8 + T cells,

mediating cytotoxic anti-cancer immune responses [211]. However, a study showed that CD169 − / − mice had significantly enhanced in vivo cytotoxic T lymphocyte responses to antigen-pulsed ApoVs, indicating a suppressive role for CD169 + macrophages in ApoV-associated antigen presentation [212]. Therefore, we must re-examine the direct impact of CD169-captured EVs ("seeds") and CD169 ("soil" receptors) on tumor metastasis. Despite the conflicting conclusions, the unique immune properties and carrier characteristics of ApoEVs still offer valuable tools for future cancer therapy. This necessitates a deeper understanding of the complexity of interactions between ApoEVs and the immune system, as well as recipient cells, to further elucidate the relationship between ApoEVs and tumor progression.

Regeneration and healing

ApoEVs derived from stem cells, endothelial cells, and immune cells have been shown to directly and indirectly promote injury repair and regeneration [213–217]. For instance, treatment with exogenous MSC-derived ApoEVs can activate the proliferation and differentiation of skin and hair follicle mesenchymal stem cells via the Wnt/β-catenin pathway, accelerating wound healing [218]. Liu et al. found that MSC-derived ApoEVs can promote osteogenesis and inhibit osteoclast formation [219]. Additionally, MSC-ApoEVs have been shown to promote muscle regeneration and increase the proportion of multinucleated cells in cardiotoxin-induced tibialis anterior (TA) injury [220]. The mechanisms through which ApoEVs promote tissue regeneration and repair are understood to revolve around their ability to deliver DNA, RNA, and proteins that directly enhance the proliferation and functional remodeling of recipient cells [143, 221, 222].

It is noteworthy that ApoEVs' reparative effects can also be achieved through indirect actions on recipient cells. For example, endothelial cell-derived ApoEVs can enhance vascular cells' production of CXCL12, which promotes the incorporation of Sca-1 + progenitor cells and confers features of plaque stability [147]. Beyond bone and skin injury repair, ApoEVs have been reported to have therapeutic effects in various other fields, such as radiation injury [222], pneumonia [144], atherosclerosis [147], tendon injury [223], and ischemic injury [224]. In summary, ApoEVs primarily originate from stem cells, endothelial cells, and immune cells, which are already extensively studied for their roles in regeneration and repair. This suggests that injury and repair are closely linked, and the components of damaged cells seem to have a specific targeting relationship with the cells involved in repair. Future research into injury-derived ApoEVs promoting repair is promising. Leveraging this potential targeting relationship may provide a foundation for developing safe and effective therapeutic systems.

Applications based on engineered ApoEVs

Although ApoEVs from various cell types show significant intervention effects in immune regulation, tumor therapy, and tissue regeneration, natural cell-derived ApoEVs often do not meet therapeutic requirements due to the complexity of their components and limitations in production and preservation. Engineering modifications can enable the loading of specific therapeutic molecules, precise targeting, multifunctional synergy, and controlled release [225, 226]. These modifications can significantly enhance the therapeutic effects of ApoEVs while avoiding potential risks, thus offering broader prospects for their future clinical applications [227]. In this section, we systematically summarize the applications of engineered ApoEVs in biotherapy, aiming to provide broader perspectives for future research (Fig. 6) (Table 3).

Immunomodulation

To meet the demands for precision, efficiency, and stability in the practical application of inflammation regulation, engineered ApoEVs have been developed. These ApoEVs primarily utilize the PS-mediated "eat-me" signal to target immune cells for modulation [228]. They can be categorized into mimetic and engineered systems based on their fabrication techniques, typically reprogramming macrophages and microglia to enhance reparative cell populations and diminish inflammatory responses. For instance, Jin et al. created eNAB^{HAL} by coating mesoporous silica nanoparticles loaded with hexyl 5-aminolevulinate hydrochloride (HAL) with neutrophil-derived apoptotic body membranes (NABM), mimicking apoptotic neutrophils to reprogram macrophages and resolve inflammation after myocardial infarction (MI) (Fig. 7A-B). eNAB^{HAL}, carrying adhesion molecules from neutrophil ApoBDs, targets damaged cardiac endothelial cells (Fig. 7C-D). In vitro co-culture systems demonstrated that eNAB^{HAL} is engulfed by cardiomyocytes and macrophages, with a notable accumulation in macrophages, indicating specific targeting (Fig. 7E). Within macrophages, eNAB^{HAL} promotes M2 polarization via phagocytosis and HAL's antioxidant properties (Fig. 7F). Consistently, in vivo studies revealed that eNAB^{HAL} exhibits significant cardiac accumulation (Fig. 7G), increases the number of CD206 + macrophages (Fig. 7H), facilitates myocardial repair post-MI, and reduces infarct size and fibrosis (Fig. 7I) [229].

Beyond engineered ApoEVs, mimicking ApoEVs using biomaterials, such as PS-functionalized liposomes to target macrophages, is also a viable approach. These mimetic systems are specifically recognized and phagocytosed by macrophages within atherosclerotic plaques, aiding in inflammation resolution and plaque stabilization [230]. Notably, the exposure of PS on ApoEVs often results in rapid clearance by monocytes/macrophages, limiting their circulation time, especially during intravenous administration.

Table 3 Applications based on engineered ApoEVs

Therapeutic systems			Target cells	Applications		Functions	Ref
Type	Materials	Cargoes		Types	Diseases		
Mimicking	Lipid-PEG	QD	Macrophages	Immunomodulation	/	Imaging or drug delivery	[258]
Mimicking	Zwitterionic polymers	/	Macrophages	Immunomodulation	/	Inhibiting inflammation	[259]
Mimicking	Poly (HEMA-co-MPS)	/	Macrophages	Immunomodulation	/	Inhibiting inflammation	[260]
Mimicking	Lipid-PEG	PIO	Macrophages	Immunomodulation	Atherosclerosis	Upregulating anti-inflammatory macrophages	[230]
Mimicking	Lipid	JQ1	Macrophages	Immunomodulation	OA	Reducing synovial inflammation	[240]
Mimicking	PLGA	/	Macrophages	Immunomodulation	Footpad inflammation	Inhibiting inflammation	[228]
Mimicking	PLGA						
Mimicking	Poly (MPS)	/	Macrophages	Immunomodulation	/	Inhibiting inflammation	[261]
Mimicking	Poly (BMA-st-HEMA)	/	Microglia	Immunomodulation	Neurodegenerative disease	Inhibiting inflammation	[262]
Mimicking	Poly (BMA-co-HEMA-co-MPS)	/	Microglia	Immunomodulation	Neurological disorders	Inhibiting inflammation	[263]
Mimicking	MSNs	microRNA-21, curcumin	Macrophages	Immunomodulation	Skin would	Promoting M2 polarization	[29]
Mimicking	PLGA	SOD/CAT, CSF	Macrophages, monocytes	Immunomodulation	Lung injury	Promoting inflammation resolution	[264]
Engineering	ApoBDs	ASO	Microglia	Immunomodulation	PD	Inhibiting inflammation	[265]
Engineering	ApoBDs	CAT	Microglia	Immunomodulation	Ischemic stroke	Inhibiting inflammation	[266]
Engineering	ApoBDs	HAL	Macrophages	Immunomodulation	MI	Promoting inflammation resolution	[229]
Engineering	ApoBDs	FP1Au NPs	Macrophages	Immunomodulation	Septic arthritis	Detecting intracellular pathogen	[267]
Mimicking	PLGA	TMP195	DCs	Cancer therapy	HCC	Enhancing immunotherapy and chemotherapy for tumors	[268]
Mimicking	Lipid-PEG	/	Macrophages	Cancer therapy	Breast tumor	Improving anticancer activity	[31]
Engineering	ApoBDs	IR820	Macrophages	Cancer therapy	Breast tumor	Regulating tumor microenvironment	[231]
Engineering	ApoBDs	CPT, PR104A	Tumor cells	Cancer therapy	Breast tumor	Facilitating deep penetration of drugs	[269]
Engineering	ApoBDs	cGAMP	DCs	Cancer therapy	Breast tumor	Boosting the adaptive immunity	[270]
Engineering	ApoBDs	Saporin, siRNA	Tumor cells	Cancer therapy	Breast tumor	Reducing clearance and enhancing targeted accumulation	[271]

Table 3 (continued)

Therapeutic systems			Target cells	Applications		Functions	Ref
Type	Materials	Cargoes		Types	Diseases		
Engineering	ApoBDs	CpG ODN	Macrophages	Cancer therapy	Breast tumor	Targeting and polarizing macrophages	[234]
Engineering	ApoEVs	HDDT	DCs	Cancer therapy	Breast tumor	Boosting systemic immune responses	[232]
Engineering	ApoEVs	BTZ	Tumor cells	Cancer therapy	MM	Improving therapeutic efficacy in tumor killing	[233]
Engineering	ApoBDs	DOX, IGG	Macrophages	Cancer therapy	Glioma	Enhancing photothermal-chemotherapeutic effect	[272]
Engineering	ApoBDs	AuNR-CpG	Monocytes	Cancer therapy	Lymphoma	Enhancing intratumoral accumulation and immunostimulation	[235]
Mimicking	Lipid	/	Macrophages	Regeneration and healing	Skin would	Promoting M2-like macrophage polarization	[273]
Engineering	ApoBDs	DFO	ECs	Regeneration and healing	Diabetic wounds	Promoting new blood vessel formation	[236]
Engineering	ApoBDs	/	ECs, macrophages	Regeneration and healing	Intrauterine adhesions	Reducing fibrosis and promoting endometrial regeneration	[274]
Engineering	ApoEVs	/	Platelet	Regeneration and healing	Traumatic hemorrhage	Enhancing coagulation	[275]
Engineering	ApoEVs	/	ECs, macrophages	Regeneration and healing	Implant osseointegration	Promoting angiogenesis and osteogenesis	[276]
Engineering	ApoEVs	/	Macrophages	Regeneration and healing	Osteonecrosis	Promoting M2-like macrophage polarization	[277]
Engineering	ApoEVs		α-M	Regeneration and healing	Ischemic stroke	Regulating immunological response, angiogenesis, and cell proliferation	[237]

DOX Doxorubicin, *TKI* Tyrosine kinase inhibitor, *PEG* Polyethylene glycol, *QD* Quantum dot, *PIO* Pioglitazone, *PLGA* Poly (lactic-co-glycolic acid), *MPS* Methacryloyloxyethyl phosphorylserine, *MSNs* Mesoporous silica nanoparticles, *SOD/CAT* Superoxide dismutase/catalase, *CSF* Colony-stimulating factor, *ASO* Antisense oligonucleotide, *PD* Parkinson's disease, *HCC* Hepatocellular carcinoma, *cGAMP* 2′,3′-cyclic guanosine monophosphate-adenosine monophosphate, *HDDT* HA – dendrimer (Den) – DOX – tyrosine kinase inhibitor (TKI), *DFO* Deferoxamine

Cancer therapy

The application of ApoEVs in cancer therapy has gained increasing attention, with various mimetic and engineered systems based on ApoEVs demonstrating significant antitumor effects across different cancer types [231, 232]. Shi et al. discovered that during the apoptosis of MSCs, particles loaded with bortezomib (BTZ) encapsulated in polycarbonate (PC) could be incorporated into ApoEVs, facilitating therapy for MM (Fig. 8A). Notably, most PC-vesicles were produced during the mid-apoptosis stage, approximately 3–4.5 h after staurosporine (STS) induction (Fig. 8B). Morphological analysis of these vesicles revealed that PC-apoVs maintained a complete membrane

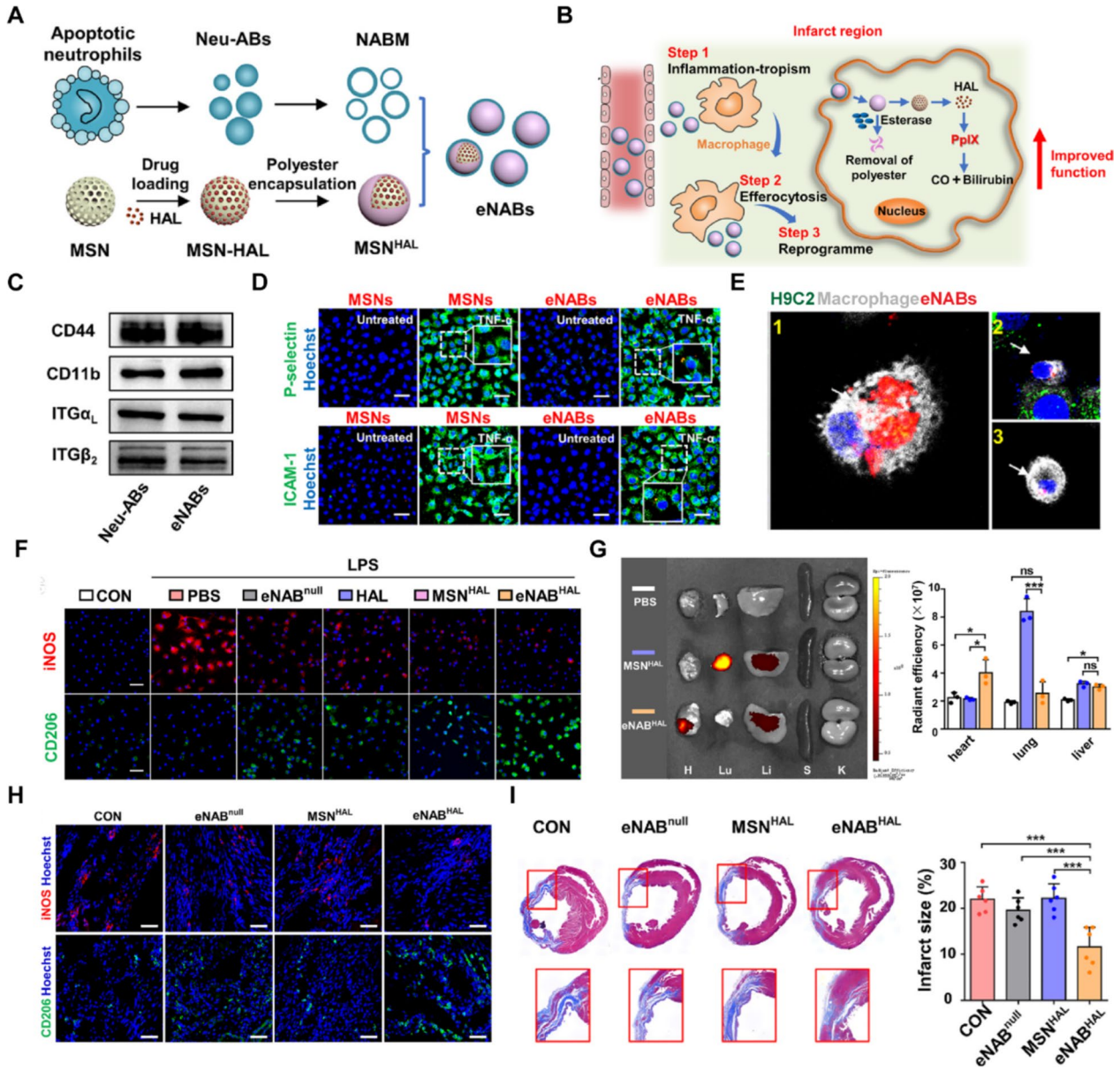

Fig. 7 Engineered neutrophil apoptotic bodies for MI treatment. **A** Scheme of the eNABs construction. **B** Diagram depicting eNABs therapy for modulating inflammation in MI. **C** eNABs retain adhesion molecules inherited from Neu-ABs. **D** Fluorescence images representing eNABs adhering to inflamed endothelium in vitro. Scale bar = 50 μm. **E** Fluorescence images showing eNABs (red) engulfed by macrophages (white) and cardiomyocytes (green) in a coculture setting. **F** Fluorescence images representing macrophage phenotypes and the proportion of iNOS/CD206-positive cells. Scale bar = 50 μm. **G** Fluorescence imaging ex vivo of the major organs 3 h after injection. **H** Fluorescence images showing representative iNOS + and CD206 + macrophages in heart tissue sections. Scale bar = 50 μm. **I** Masson's trichrome staining and infarct size quantification. Reprinted with permission from Ref [229]

structure, indistinguishable from natural apoVs, suggesting that PC loading does not compromise the structural integrity of ApoEVs (Fig. 8C). The loading efficiency of PC nanoparticles in the apoptotic encapsulation system (AES) of apoptotic MSCs was markedly enhanced compared to ultrasonic encapsulation, indicating that AES is a superior technique for generating PC-apoVs (Fig. 8D). In vitro tumor cytotoxicity assays indicated that PC-apoVs accumulated more significantly in cultured MM cells (5TGM1), exhibiting potent antitumor effects (Fig. 8E-F). In a 5TGM1-induced MM mouse model, intravenous administration of BTZ/PC-apoVs significantly suppressed MM cell growth and pathological infiltration, as assessed by live imaging (Fig. 8G-H). Hematoxylin and eosin (H&E) staining of

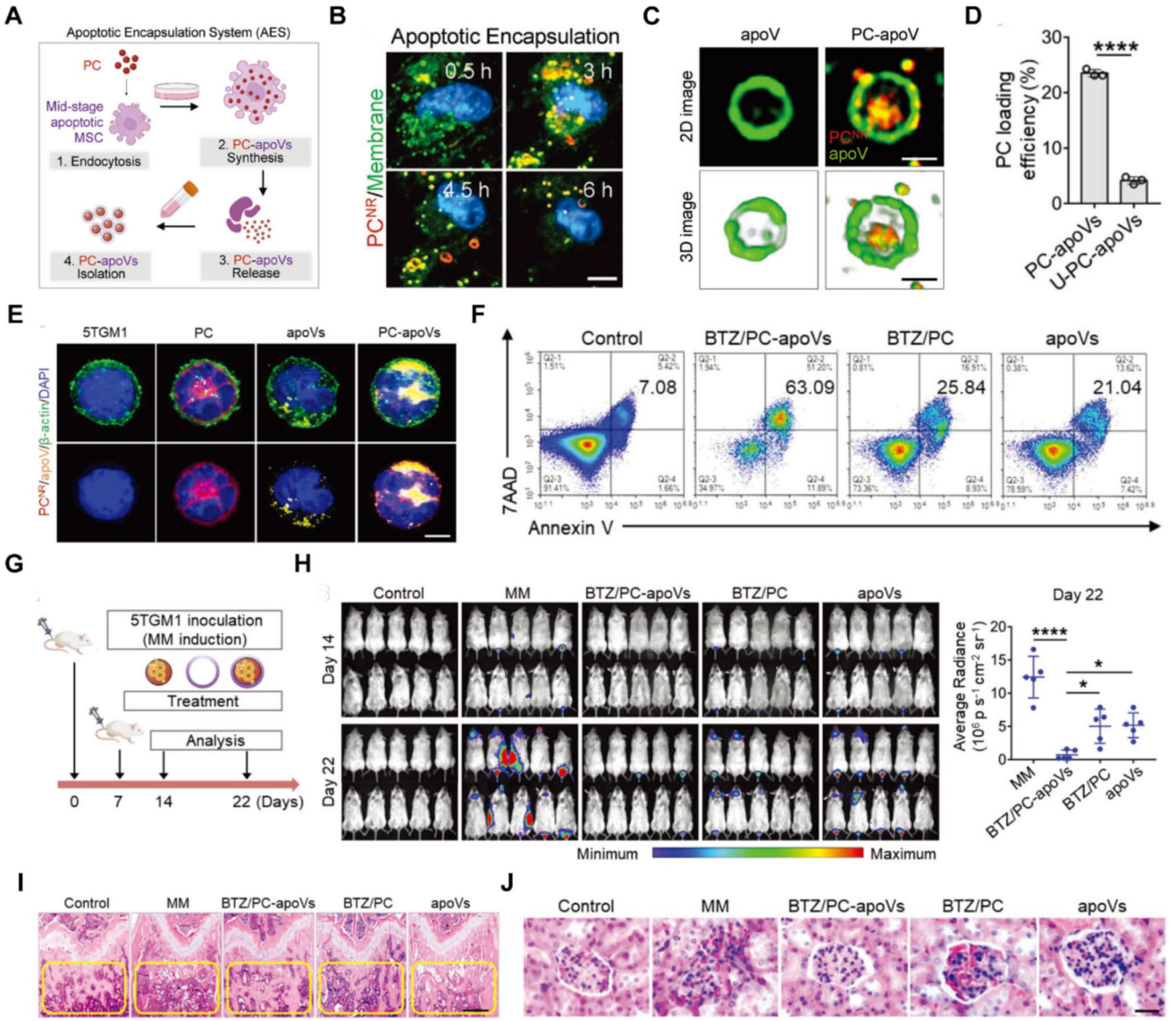

Fig. 8 Nano-bortezomib encapsulation in apoptotic stem cell-derived vesicles for enhanced multiple myeloma therapy. **A** A detailed schematic diagram depicting the step-by-step process of PC-apoV generation. **B** The process of generating and secreting PC-apoVs in apoptotic MSCs. Scale bar = 10 μm. **C** Comprehensive 2D and 3D visualizations of apoVs and PC-apoVs. Scale bar = 1 μm. **D** The loading efficiency of PC nanoparticles in PC-apoVs and U-PC-apoVs. **E** Representative immunofluorescence images showing MM cells (5TGM1) co-incubated with PC, apoVs, or PC-apoVs for a duration of 12 h. Scale bar = 10 μm. **F** Flow cytometry data demonstrated the proportion of Annexin V-positive apoptotic 5TGM1 cells post-treatment with BTZ/PC-apoVs, BTZ/PC, and natural apoVs. **G** Illustration of experimental design. **H** In vivo fluorescence imaging of mice and quantification. **I** H&E staining of the distal femurs. Scale bar = 0.5 mm. **J** H&E staining of kidney tissues. Scale bar = 20 μm. Reprinted with permission from Ref [233]

the distal femurs in MM mice showed reduced trabecular bone area (Fig. 8I). Additionally, BTZ/PC-apoV therapy also ameliorated acute kidney injury caused by increased IgG2b deposits in renal tubules, providing robust evidence supporting the therapeutic potential of engineered ApoEVs in cancer treatment (Fig. 8J) [233].

Beyond direct drug delivery to tumor cells, ApoEVs can also manipulate tumor immunity through their unique interactions with phagocytic cells, thereby exerting antitumor effects. Li and colleagues engineered cancer cell ApoBDs loaded with CpG oligodeoxyribonucleotides (CpG ODN) as cancer vaccines to enhance immunotherapy by alleviating immunosuppression via cascade amplification. CpG ODN induces polarization of macrophages towards the M1 phenotype, leading to significant production of TNF-α, which activates cell division control protein 42 (Cdc42) [234]. Intriguingly, ApoEVs are extensively phagocytosed by monocytes in the bloodstream, a feature that can be exploited to enhance

their delivery to deep tumor tissues. Following this concept, Tan et al. developed ApoBDs-encapsulated nanomedicine using CpG immunoadjuvant-modified gold-silver nanorods (AuNR-CpG) as a model. Once injected into the vasculature, AuNR-CpG is ingested by monocytes that then leverage their natural tumor-homing capabilities to actively infiltrate the tumor core. This cell-mediated delivery system not only efficiently ablates primary tumors but also triggers a robust immune response, preventing tumor metastasis and recurrence [235]. Thus, modifying ApoEVs to enhance their tumor specificity and deep tissue infiltration capabilities represents a promising research direction for future cancer therapies.

Regeneration and healing

The regenerative and reparative applications of ApoEVs are driven not only by their rich content of bioactive molecules but also by their specific interactions with immune cells, endothelial cells, and stem cells [213]. Artificial modification or mimetic techniques can enhance the therapeutic potential of ApoEVs, enabling multifaceted synergistic therapies [236]. Recently, researchers have created MAP-functionalized α-M-loaded ApoVs (α-M/ApoV-MAP) by functionalizing α-mangostin (α-M)-induced MSC-derived ApoVs with a metalloproteinase-activatable cell-penetrating peptide (MAP) (Fig. 9A). Both α-M/ApoV and OX-ApoV (ApoVs without α-M) exhibited Annexin V positivity, indicating the presence of PS on their surfaces (Fig. 9B). Coomassie brilliant blue staining revealed the substantial presence of MSC-derived proteins within ApoVs, indicating their potential to transport active molecules from MSCs to target cells (Fig. 9C). In vitro drug release studies demonstrated that α-M release from α-M/ApoV and α-M/ApoV-MAP was significantly slower than from free α-M, with MAP modification not altering the release profile of α-M in α-M/ApoV (Fig. 9D). In an oxygen–glucose deprivation/reoxygenation (OGD/R) model, OX-ApoV, α-M/ApoV, and free α-M all showed significant reactive oxygen species (ROS) scavenging, suggesting neuroprotective effects under ischemic conditions (Fig. 9E). Additionally, the effects of ApoVs on the proliferation, migration, and tube formation of brain capillary endothelial cells (BCECs) were evaluated. The results showed that OX-ApoV and α-M/ApoV promoted robust tubular structure formation in BCECs on Matrigel, highlighting the pro-angiogenic properties of ApoVs (Fig. 9F-G). In the presence of matrix metalloproteinases (MMP-2/9), MAP can be cleaved to expose an arginine-rich cell-penetrating peptide (RRRRRRRRR), facilitating the entry of ApoVs into cells at injury sites and their accumulation in lesion areas. Brain fluorescence imaging indicated that α-M/ApoV-MAP accumulation in the ischemic hemisphere was significantly higher than that of α-M/ApoV from 12 h post-administration, with this enhanced accumulation persisting up to 48 h (Fig. 9H). This improved targeting ability significantly reduced brain infarct size (Fig. 9I) [237].

Challenges and prospects

ApoEVs can be described as the final emissaries of a cell, delivering critical signals to recipient cells during their clearance [159, 238]. In contrast to exosomes and MVs, ApoEVs exhibit distinct characteristics in terms of production, metabolism, size, cargoes, and molecular markers [166]. As our understanding of their microstructure and properties deepens, ApoEVs are increasingly recognized for their potential in various biomedical applications.

From a functional perspective, ApoEVs offer inherent advantages over other types of EVs. Their high yield addresses the supply constraints in ApoEV-based applications. Specific molecules on ApoEV surfaces, such as PS, facilitate targeted interactions with phagocytes, enhancing the precision of EV-based therapies in inflammation and tissue regeneration [67]. Furthermore, ApoEVs are enriched with various cargoes, especially nucleic acids, which are pivotal in gene regulation. ApoEVs also play a role in pathogen dissemination. For example, ApoBDs derived from T cells infected with HIV-1 can facilitate the transmission of HIV-1 to HK2 cells, HRPTEC, and primary renal tubular cells [45]. Inhibiting the formation of ApoEVs can mitigate pathogen spread, providing new intervention targets for infectious diseases. Additionally, ApoEVs are implicated in coagulation. For instance, melanoma-derived ApoEVs induce coagulation more efficiently than exosomes, and tumor-derived ApoEVs exhibit greater procoagulant activity than their parent cells, primarily due to the presence of TFs and PS [239]. Future research may uncover more mechanisms, including those involved in embryonic development.

The engineering of ApoEV-based systems primarily focuses on several key aspects: I) the targeted recognition of ApoEVs by immune cells, significantly influencing antigen presentation and the remodeling of immune cell functions; II) the communication between ApoEVs and stem cells or endothelial cells in the local microenvironment, which plays a crucial role in tissue regeneration and injury repair; III) the targeted interaction of ApoEVs with the circulating monocyte/macrophage system, enabling ApoEVs to act as intermediate carriers for the delivery of live cells. Current methodologies often involve the direct modification of natural ApoEVs or the incorporation of therapeutic agents and the utilization of biocompatible materials to replace natural ApoEVs as carriers, modifying them with specific markers such as PS [240]. Although these systems vary in their

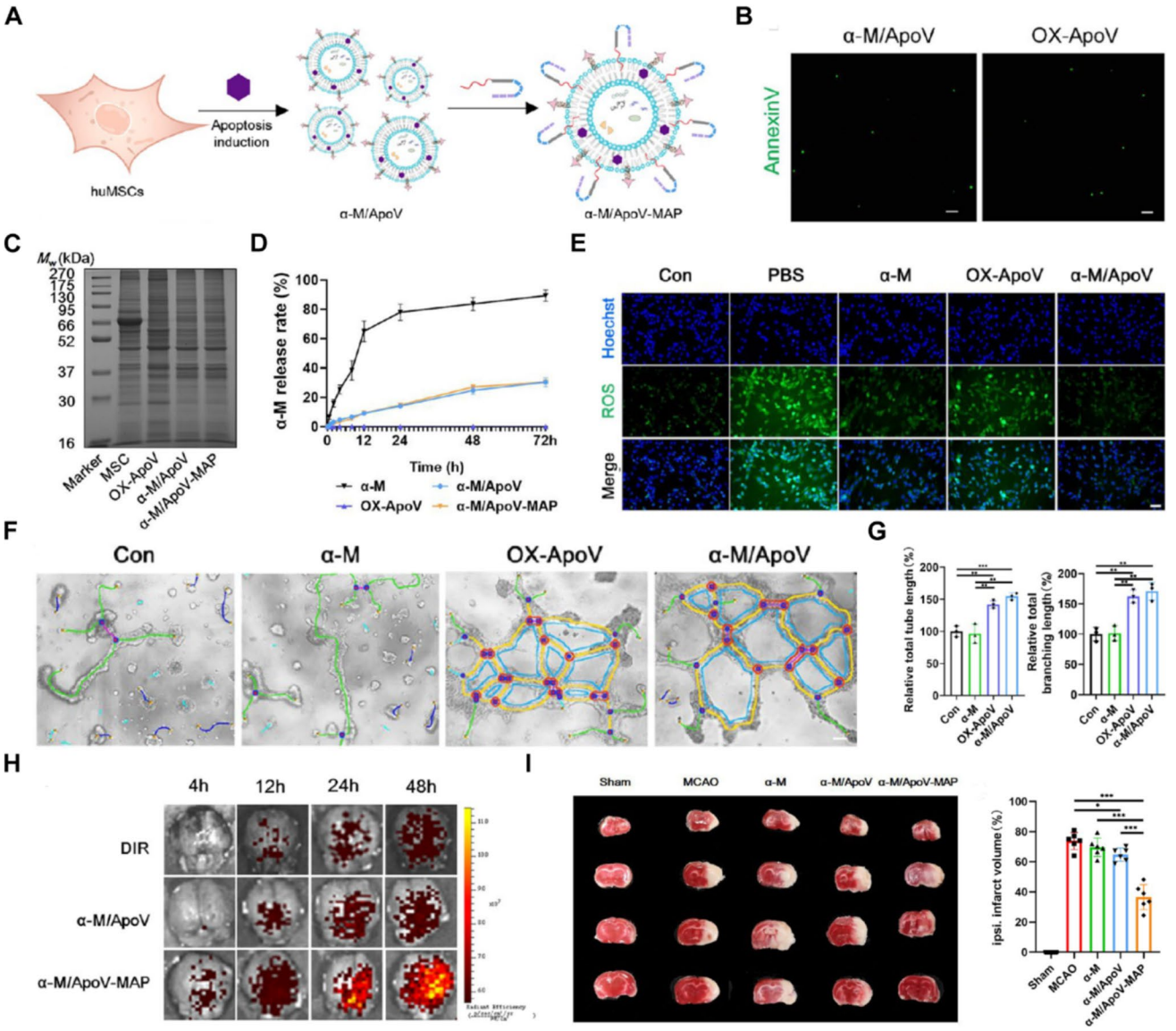

Fig. 9 Precision-engineered apoptotic vesicle delivery mechanism for ischemic stroke therapy. **A** Preparation of α-M/ApoV-MAP. **B** Representative micrographs illustrating Annexin V (green) staining in ApoVs. Scale bar = 20 μm. **C** Analysis of protein composition in MSCs and ApoVs using Coomassie staining. **D** α-M release profiles of ApoVs. **E** ROS level of OGD/R-treated PC-12 cells. Scale bar = 50 μm. **F** Tube formation assay. Scale bar = 50 μm. **G** Quantification of tube formation. **H** Fluorescence intensity of ApoVs in lesion region (**I**) TTC staining of brain slices and quantification of infarct area. Reprinted with permission from Ref [237]

functional mechanisms and pathways, the underlying theoretical principles are largely unified.

However, the clinical translation of ApoEVs encounters several significant challenges, such as the coagulation issues mentioned earlier. Additionally, the inherent heterogeneity of ApoEVs is a major concern. ApoEVs derived from identical cell sources can exhibit considerable variation in size, cargoes, and biological properties. This heterogeneity can adversely affect their ability to traverse physiological barriers and their targeting efficiency, leading to variable and sometimes opposing functional outcomes. Moreover, the properties and functions of ApoEVs are influenced by the induction, isolation, and preservation methods employed. Most experimental protocols induce apoptosis in parental cells using agents such as STS, serum deprivation, or UV irradiation [241]. While some studies have compared the impact of different apoptotic induction methods on ApoEVs functionality, comprehensive investigations are necessary to fully understand these effects. Gradient centrifugation remains the primary technique for isolating ApoEVs, yet different centrifugation speeds significantly affect the enrichment of their cargoes and impurities.

Future research needs to focus on the precise mechanisms of ApoEV formation and cargoes loading. Understanding how ApoEVs select their cargoes will provide more possibilities for their application and clinical translation. Additionally, standardized guidelines for the nomenclature, isolation, concentration, storage, and standard procedures of ApoEVs need to be established to enhance the reliability and reproducibility of EVs research. Furthermore, studies on the short-term and long-term side effects of exogenous ApoEV therapy, as well as on the metabolic pathways and metabolic safety, need to be strengthened. Overall, while the research on ApoEVs is challenging, their immense potential warrants continuous exploration.

Conclusion

In conclusion, ApoEVs are not simply cellular debris from dying cells. ApoEVs play a significant regulatory role in intercellular communication, making them promising candidates for various biomedical applications such as disease diagnosis, immunomodulation, cancer therapy, regenerative therapy, and drug delivery. The diverse cargoes carried by ApoEVs and their specific interactions with recipient cells underpin these functions. Despite substantial progress in the field of ApoEVs, several challenges remain for their clinical application. Further research is necessary to identify the therapeutic components in ApoEVs and understand the mechanisms by which ApoEVs exert their effects on target cells. Additionally, strategies must be developed to harness the phagocytic function of immune cells while mitigating the rapid in vivo clearance of ApoEVs. According to recent studies, ApoEVs are expected to become superior drug carriers, opening new avenues for biomedical applications and disease treatment.

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Authors' contributions D.H. was responsible for the initial drafting of the manuscript, the organization of the tables, and the creation of the figures. Z.L. undertook comprehensive revisions of the manuscript and refined the figures. F.W. contributed to further revisions and modifications of the manuscript content. K.C. and D.S. provided conceptual guidance for the overall structure and writing approach, and they supervised the preparation of the manuscript. All authors contributed to the editing and final composition of the manuscript.

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Data availability No data was used for the research described in the article.

Declarations

Competing Interest Author Ke Cheng is an Associate Editor for Med-X. The paper was handled by another Editor and has undergone a rigorous peer review process. Author Ke Cheng was not involved in the journal's peer review of, or decisions related to, this manuscript.

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