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Review

Promising nanotherapeutics of stem cell extracellular vesicles in liver regeneration

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ABSTRACT

Extracellular vesicles (EVs) have gainedsignificant attention due totheir crucialroles invarious biological systems. This review aims to explore the functions of EVs in both in physiological and pathological states of the liver, with a specific focus on the potential mechanisms and concrete evidence of EVs in liver regeneration processes. The review begins by emphasizing the importance of EVs in maintaining liver health and their involvement in different pathological conditions, starting from the liver's own EVs. Reviewing the role of EVs in liver diseases to reveal the impact of EVs in pathological processes (e.g., hepatitis, liver fibrosis, and cirrhosis) and elucidate their signaling functions at the molecular level. Subsequently, the work concentrates on the functions of EVs in liver regeneration, revealing their key role in repair and regeneration following liver injury by carrying growth factors, nucleic acids, and other bioactive molecules. This part not only theoretically clarifies the mechanisms of EVs in liver regeneration but also experimentally demonstrates their role in promoting liver cell proliferation, inhibiting apoptosis, regulating immune responses, and fostering angiogenesis, laying the groundwork for future clinical applications. Moreover, this work provides a comprehensive analysis of the challenges faced by existing EV-based therapies in liver regeneration and offers prospects for future research directions. It highlights that despite the tremendous potential of EVs in treating liver diseases, there are still technical challenges (e.g., EV isolation and purification, dosage control, and targeted delivery). To overcome these challenges, the review suggests improvements to current technologies and the development of new methods to realize the clinical application of EVs in treating liver diseases.

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1. Introduction

Extracellular vesicles (EVs) are a diverse group of membrane-bound that are entities released by cells into the extracellular milieu [1]. They are present ubiquitously across a wide range of biological systems, and their study has become a fascinating field of research with significant potential for diagnostic and therapeutic applications [2]. EVs, ranging in size from approximately 30 nm to 1 μm, encapsulate a microcosm of the cells, harboring proteins, lipids, and nucleic acids reflective of their cellular origin [3,4]. Their existence was once attributed to cellular debris [4,5]; however, it is now unequivocally established that EVs are purposefully secreted and play pivotal roles in intercellular communication [6]. The interest in EVs lies in their ability to transport molecular cargo between cells [7]. This intercellular exchange is not a mere random process but a highly regulated one that influences physiology of recipient cells [8]. The cargo within EVs is diverse and includes enzymes, signaling molecules, receptors, and genetic materials in the form of RNA or DNA (Fig. 1A). The composition of these vesicles is not static, but rather dynamic, and can change in response to the physiological condition of the parent cell and the surrounding environment.

The functional implications of EVs are extensive, impacting various cellular behaviors, including angiogenesis, immune modulation, cell proliferation, and tissue regeneration (Fig. 1B) [9–12]. They have been implicated in numerous physiological processes, such as the maintenance of homeostasis and the response to stress conditions [13,14]. In the pathological context, EVs have been shown to contribute to the progression of diseases, such as cancer, neurodegenerative disorders, ontogeny, and cardiovascular diseases (Fig. 1C) [15–18]. The dual role of EVs as facilitators of both normal physiological functions and pathological processes makes them an intriguing subject for intensive study and a potential target for therapeutic intervention.

Liver is the primary organ responsible for the metabolism of various substances, such as drug, glycogen, and alcohol [19]. Liver injury may be caused by multiple etiologies, including trauma, tumor, hypoxemia, toxicity [20,21]. Therefore, the liver regeneration is vital for patients [22,23]. This work not only comprehensively integrates the latest developments in EV-related liver research, but also provides valuable information for the development of EV-based liver regeneration treatment strategies, both theoretically and experimentally. By analyzing the multifaceted roles of EVs in liver physiology and disease, this review offers a new perspective and potential methods for the diagnosis and treatment of liver diseases. Simultaneously, by exploring and discussing the challenges of EV-based therapies in clinical applications, this review would trigger the possibility of transforming this cutting-edge technology into new means for treating liver diseases, heralding revolutionary changes in the biomedical field, particularly in the area of liver disease treatment.

2. Biogenesis and classification of EVs

The biogenesis of EVs is a complex and intricately regulated process that results in the formation of various types of EVs, each with distinct biophysical properties and functions [24]. The classification of EVs has evolved over time, and they are commonly categorized based on their size, biogenesis pathways, and surface markers [24–26]. The primary classes include exosomes, microvesicles (also known as ectosomes), and apoptotic bodies (Fig. 2) [27–30].

Exosomes are small EVs with a typical diameter range of 30~120 nm (Fig. 2) [13]. They originate within the endosomal network of a cell [31]. The genesis of exosomes begins with the inward budding of the endosomal membrane, leading to the formation of multivesicular bodies (MVBs) containing intraluminal vesicles [32]. Upon the fusion of MVBs with the plasma membrane, these intraluminal vesicles are released into the extracellular space as exosomes [33]. The process is highly selective, and the content of exosomes is a curated representation of the parent cell's cytosol, often enriched in specific proteins, lipids, and RNA molecules [34–36]. Microvesicles, are generally larger than exosomes, typically ranging from 100 nm to 1 μm in diameter, and are formed by the outward budding and fission of the plasma membrane (Fig. 2) [37]. The formation of microvesicles is a consequence of cytoskeletal protein reorganization and changes in lipid distribution within the plasma membrane [38]. This process is often stimulated by cellular activation or stress and results in the shedding of microvesicles directly into the extracellular space [39]. Apoptotic bodies are the largest class of EVs, typically ranging in size from 1 μm to 5 μm, and are released during the late stages of programmed cell death or apoptosis (Fig. 2) [40]. They contain cellular debris along with intact organelles and are characterized by the presence of phosphatidylserine on their surface [41]. Apoptotic bodies serve to package and remove cellular components during cell death, thereby preventing the leakage of potentially harmful substances into the tissue environment [42].

The biogenesis of EVs is not merely a structural process but also one of profound biological significance [43]. The mechanisms governing EV formation are tightly linked with their ultimate function. For instance, the molecular composition of EVs is determined during their formation, which in turn dictates their interaction with target cells and their ability to transfer functional biomolecules [44]. Therefore, understanding the biogenesis of EVs provides essential insights into their roles in health and disease.

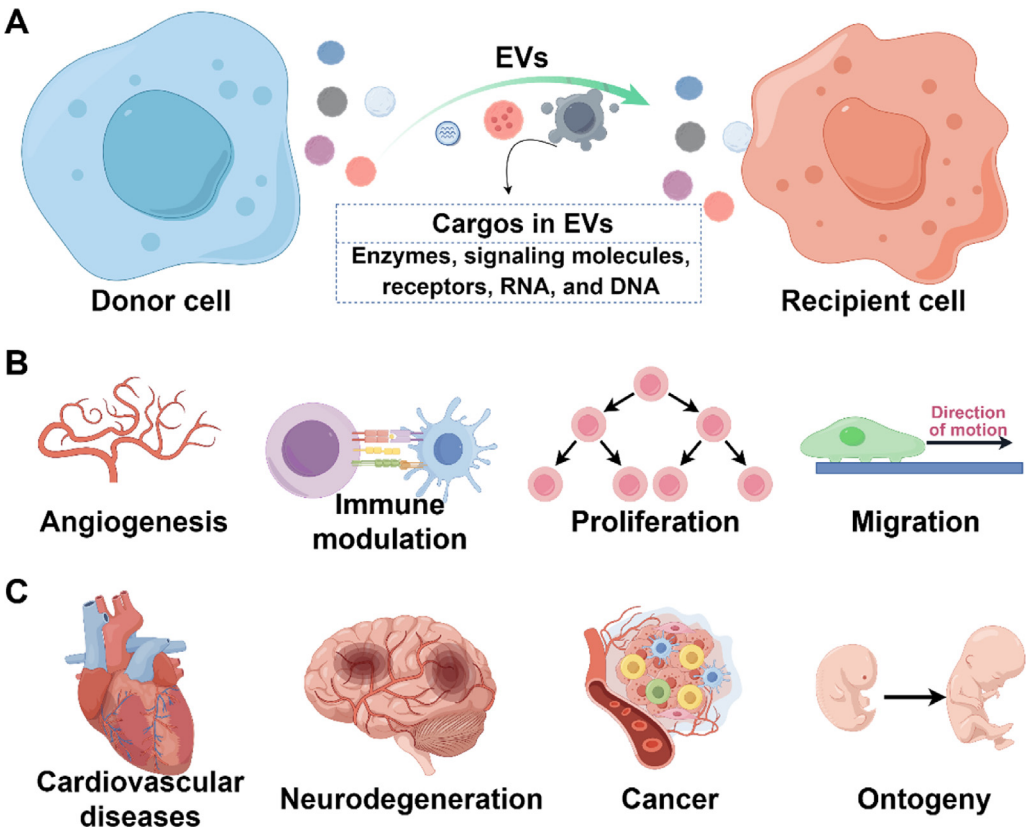

Fig. 1. The intrigue surrounding EVs stems from their ability to act as vehicles for the transfer of molecular cargo between cells. (A) The cargo contained within EVs varies, encompassing a wide array of components, such as enzymes, signaling molecules, receptors, and genetic material in the form of RNA and DNA. (B) The functional implications of EVs are vast, affecting various aspects of cellular behaviors, including angiogenesis, immune modulation, cell proliferation, and tissue regeneration. (C) In the pathological context, EVs have been shown to contribute to the progression of diseases, such as cancer, neurodegenerative disorders, ontogeny, and cardiovascular diseases.

3. General functions of EVs

EVs are highly skilled regulators of intercellular communication, possessing the remarkable ability to influence the behavior of recipient cells. The functions of EVs can be broadly categorized into several areas, including modulation of the immune response [45], transfer of genetic information, alteration of the tumor microenvironment, and facilitation of cellular waste management [46].

In the field of immunomodulation, EVs play a dual role, capable of both enhancing and suppressing immune responses [45]. For instance, EVs can present antigens and activate immune cells, such as dendritic cells, thereby promoting the adaptive immune response [47,48]. Conversely, they may carry immunosuppressive molecules that dampen inflammatory reactions and induce tolerance, thus contributing to the maintenance of immune homeostasis (Fig. 3) [49,50].

The transfer of genetic information via EVs is another pivotal function that has profound implications for gene regulation and the alteration of recipient cell phenotypes [51]. EVs can shuttle RNA species, including messenger RNA (mRNA) and microRNA (miRNA), between donor cells and recipient cells [52]. This transfer can result in the modulation of gene expression in recipient cells, thereby influencing cellular functions and behaviors [53]. For example, the delivery of miRNA by EVs can lead to the post-transcriptional regulation of target genes, with significant effects on cell proliferation, differentiation, and apoptosis (Fig. 3).

In the context of cancer, EVs have been demonstrated to alter the tumor microenvironment by promoting angiogenesis, remodeling the extracellular matrix, and even conferring drug resistance [54–56]. Tumor-derived EVs can manipulate stromal cells to support tumor growth and metastasis [57]. Additionally, EVs can facilitate the removal of cellular waste, including misfolded proteins and damaged organelles, which is crucial for maintaining cellular homeostasis and preventing pathological conditions (Fig. 3) [58].

The burgeoning interest in EVs is justified by their multifaceted roles and their potential for innovative therapeutic strategies [59]. By harnessing the natural abilities of EVs, it may be possible to design interventions that can selectively deliver therapeutic agents, modulate immune responses, and correct genetic defects [59–61]. As we continue to unravel the complexities of EVs, their promise in the field of regenerative medicine, particularly in relation to liver regeneration, remains a compelling avenue for future research and clinical applications [62–64].

4. Role of EVs in liver physiology and disease

4.1. EVs in liver homeostasis

The intricate processes that maintain liver homeostasis are a testament to the organ's remarkable capacity for regeneration and repair [65]. Central to the maintenance of liver health are the EVs, which are lipid bilayer-enclosed structures released by cells into the extracellular milieu [66]. These vesicles are not merely cellular detritus; rather, they are a sophisticated system of intercellular communication that carries proteins, lipids, RNA, and DNA to distant cells, thereby influencing various physiological processes [67]. Here, we will discuss how to promote EV secretion by these

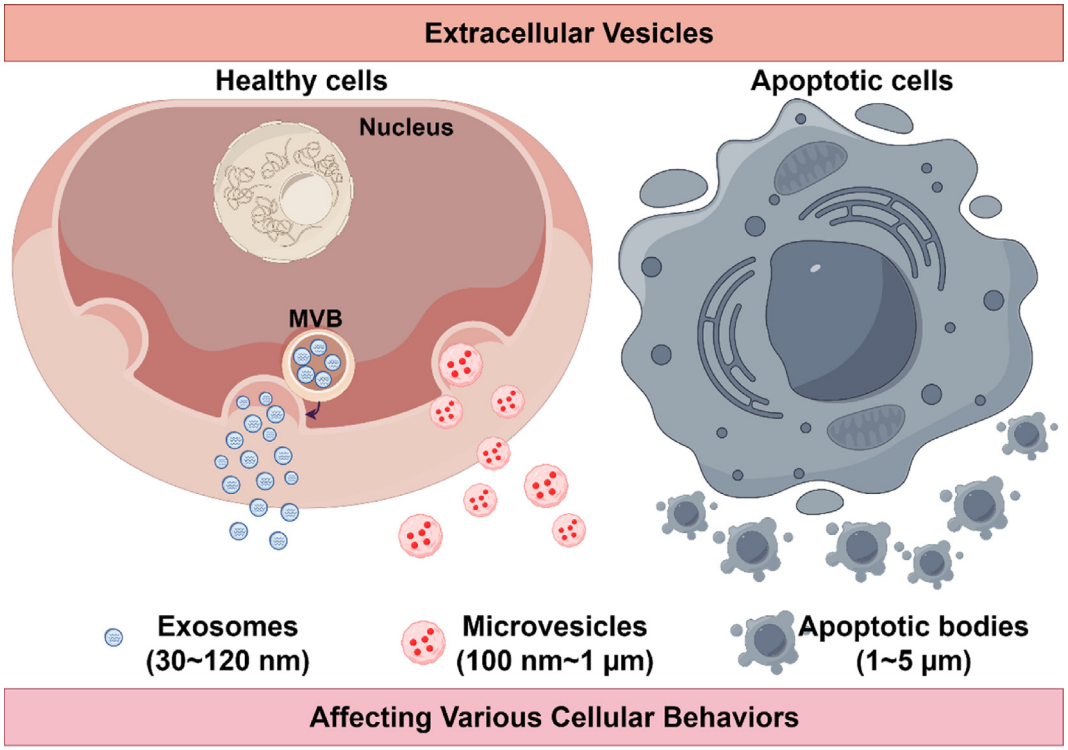

Fig. 2. The primary classes include exosomes, microvesicles (also known as ectosomes), and apoptotic bodies. Exosomes are small EVs with a typical diameter range of 30~120 nm. The genesis of exosomes begins with the inward budding of the endosomal membrane, leading to the formation of multivesicular bodies (MVBs) containing intraluminal vesicles. Microvesicles, are generally larger than exosomes, typically ranging from 100 nm to 1 μm in diameter, and are formed by the outward budding and fission of the plasma membrane. Apoptotic bodies are the largest class of EVs, typically ranging in size from 1 μm to 5 μm, and are released during the late stages of programmed cell death or apoptosis.

liver cells and how EVs can affect other cells. Additionally, we will outline some liver conditions for which EVs can serve as biomarkers.

In the hepatic environment, EVs are secreted by hepatocytes, Kupffer cells, stellate cells, and endothelial cells (Fig. 4A) [68]. This diverse range of cellular origins endows EVs with a heterogeneous nature, reflecting the multifaceted roles they play in liver function. Hepatocyte-derived EVs, for instance, have been shown to be instrumental in the transfer of metabolic enzymes and liver-specific RNA, thus participating in the regulation of systemic metabolism. Furthermore, EVs derived from liver macrophages, namely Kupffer cells, are involved in the modulation of immune responses [68]. They do so by presenting antigens and facilitating the communication between innate and adaptive immune systems, which is crucial in maintaining immunological tolerance and preventing unwarranted inflammation that can lead to tissue damage.

EVs released by liver cells can interact with and affect various other cell types within and outside the liver, influencing processes such as inflammation, fibrosis, regeneration, and metastasis. For examples, EVs can modulate the activity of immune cells, affecting both innate and adaptive immunity. EVs can transfer functional molecules that alter the metabolic state or gene expression of nearby hepatocytes. Fibroblasts and other stromal cells can be influenced by EVs, potentially impacting tissue remodeling and fibrosis. Circulating EVs can reach distant organs and affect their function, which is relevant in metastatic processes. EVs have emerged as promising biomarkers for several liver conditions due to their ability to reflect the pathophysiological state of the liver. Some key liver conditions where EVs can serve as biomarkers include. In liver fibrosis and cirrhosis, EVs derived from activated stellate cells may contain markers of fibrogenesis. Levels of certain miRNAs in circulating EVs correlate with the stage of fibrosis. For Hepatitis, viral hepatitis can alter the cargo of EVs released by hepatocytes and immune cells. EV-associated proteins or nucleic acids specific to hepatitis viruses can be detected. In liver cancer (hepatocellular carcinoma, HCC), EVs from HCC cells often carry oncogenic proteins and nucleic acids. Specific miRNA signatures in serum EVs are associated with HCC progression and recurrence. In alcoholic liver disease (ALD), EVs might reflect ethanol-induced liver injury and inflammation. Changes in EV composition can indicate the severity of ALD. Nonalcoholic fatty liver disease (NAFLD), EVs could be used to monitor metabolic changes and progression to nonalcoholic steatohepatitis (NASH). Lipid content in EVs might correlate with hepatic steatosis.

Furthermore, the role of EVs extends into the realm of liver regeneration. Hepatocyte proliferation, a key feature of liver regeneration, is modulated by EVs through the delivery of growth factors, such as hepatocyte growth factor (HGF) and epidermal growth factor (EGF) [69]. These growth factors, encapsulated within EVs, are protected from enzymatic degradation in the extracellular space and can be efficiently delivered to target cells, thereby enhancing signal specificity and potency (Fig. 4A).

In addition to growth factors, EVs are known to transport miRNAs that can post-transcriptionally regulate gene expression in recipient cells (Fig. 4A). For example, miRNA-122, an abundant liver-specific miRNA, is packed into EVs and can modify gene expression patterns in adjacent cells, influencing lipid metabolism and cholesterol homeostasis [70]. This mechanism highlights a novel layer of regulation wherein EVs serve as vehicles for the horizontal transfer of genetic information, orchestrating complex physiological responses at the cellular and systemic levels.

EVs are indispensable components of the hepatic landscape, intricately involved in maintaining liver homeostasis. They facilitate a myriad of functions ranging from metabolic regulation to

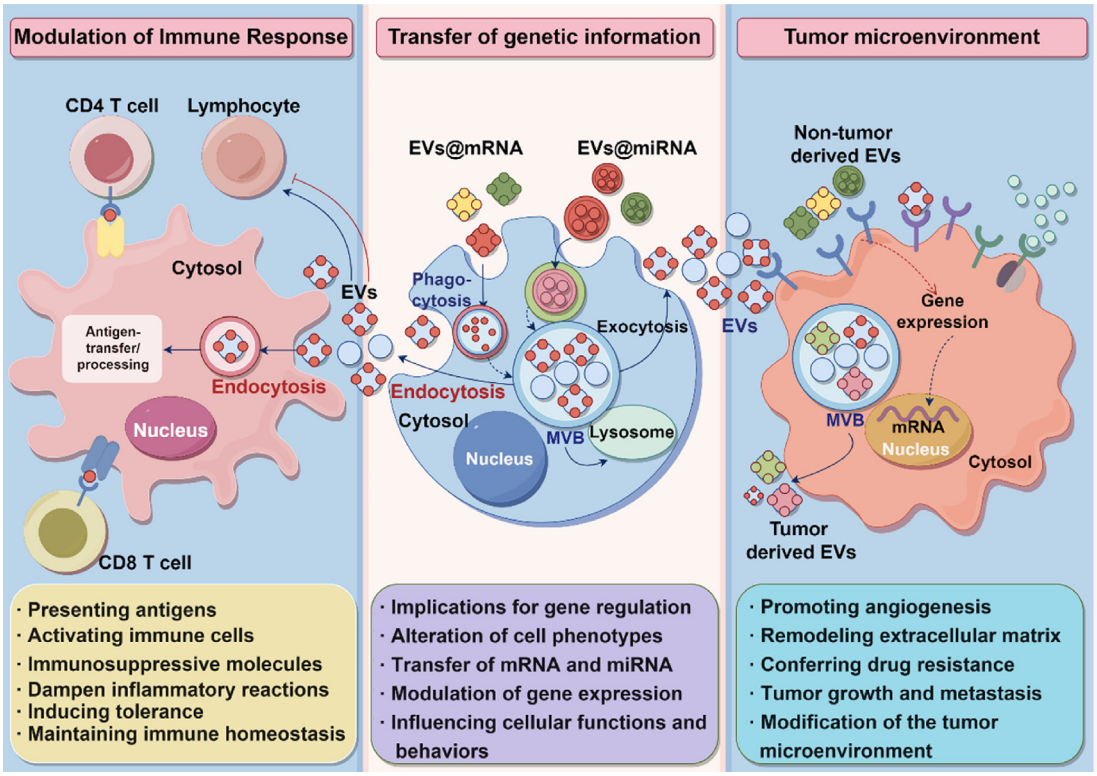

Fig. 3. The general functions of EVs can be categorized into several broad areas, including modulation of the immune response, transfer of genetic information, alteration of the tumor microenvironment, and facilitation of cellular waste management. EVs play a dual role, capable of both enhancing and suppressing immune responses, and also can regulate the maintenance of immune homeostasis. EVs can shuttle RNA species, including messenger RNA (mRNA) and microRNA (miRNA), between cells, which can lead to the post-transcriptional regulation of target genes, with significant effects on cell proliferation, differentiation, and apoptosis. EVs have been shown to modify the tumor microenvironment by promoting angiogenesis, remodeling the extracellular matrix, and even conferring drug resistance. Tumor-derived EVs can manipulate stromal cells to support tumor growth and metastasis.

immune surveillance, and their role in liver regeneration underscores their potential as therapeutic agents [33,70]. The dynamics of EV secretion and the selective cargo loading are areas of ongoing research that promise to unveil new insights into the mechanisms by which EVs contribute to liver health.

4.2. EVs in liver diseases

When the delicate balance of liver homeostasis is disrupted, disease ensues. EVs are deeply implicated in the pathogenesis of a spectrum of liver diseases, including hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Fig. 4B) [23,71]. They act as harbingers of disease, altering the cellular communications and modulating the extracellular environment to favor pathological processes [67].

In the context of hepatitis, both viral and non-viral, EVs can have dual roles. On one hand, they can potentiate the immune response by carrying viral antigens to dendritic cells, thereby enhancing antigen presentation and facilitating the clearance of the virus [5]. On the other hand, EVs can also transmit pro-inflammatory signals that exacerbate liver injury. For example, EVs containing pro-inflammatory cytokines, such as TNF-α and IL-6, can aggravate the inflammatory milieu, leading to hepatocyte death and further propagation of the disease [10,12].

Liver fibrosis, the excessive accumulation of extracellular matrix proteins, is a common consequence of chronic liver diseases. EVs contribute to the fibrogenic process by transporting transforming growth factor-beta (TGF-β), a potent inducer of fibrogenesis, to hepatic stellate cells (HSCs) [72]. Upon activation by such factors, HSCs transform into myofibroblasts and begin producing collagen, thereby perpetuating the fibrotic response. Moreover, EVs can carry miRNAs that modulate the expression of genes involved in fibrosis, such as miRNA-19b, which targets the downregulation of tissue inhibitor of metalloproteinases 3 (TIMP3) [73], an inhibitor of matrix metalloproteinases that degrade the extracellular matrix.

In the progression to cirrhosis, EVs are reflective of the ongoing hepatocellular damage and impaired regenerative capacity. They contain markers of cell death (e.g., cytochrome *c*) and can induce further apoptosis in neighboring cells, thereby amplifying the cycle of cell death and fibrosis [54,74]. The escalating burden of fibrotic tissue eventually leads to cirrhosis, where the architecture of the liver is fundamentally altered, impairing its function [74].

The role of EVs in HCC is particularly noteworthy. These vesicles can facilitate tumor progression through multiple mechanisms [75]. EVs from HCC cells can modify the behavior of non-tumorigenic cells, inducing angiogenesis and creating a supportive tumor microenvironment. They can also carry oncogenic proteins and nucleic acids, transforming recipient cells and promoting metastasis [75]. Additionally, EVs can confer drug resistance by exporting chemotherapeutic agents out of tumor cells, thus undermining the efficacy of cancer treatments.

In conclusion, EVs play multifaceted roles in the pathology of liver diseases. They are not passive participants but active drivers of disease progression, influencing inflammation, fibrosis, and tumorigenesis [25]. The molecular cargo of EVs reflects the state of their cells of origin and can provide insights into disease mechanisms. As such, understanding the role of EVs in liver diseases is

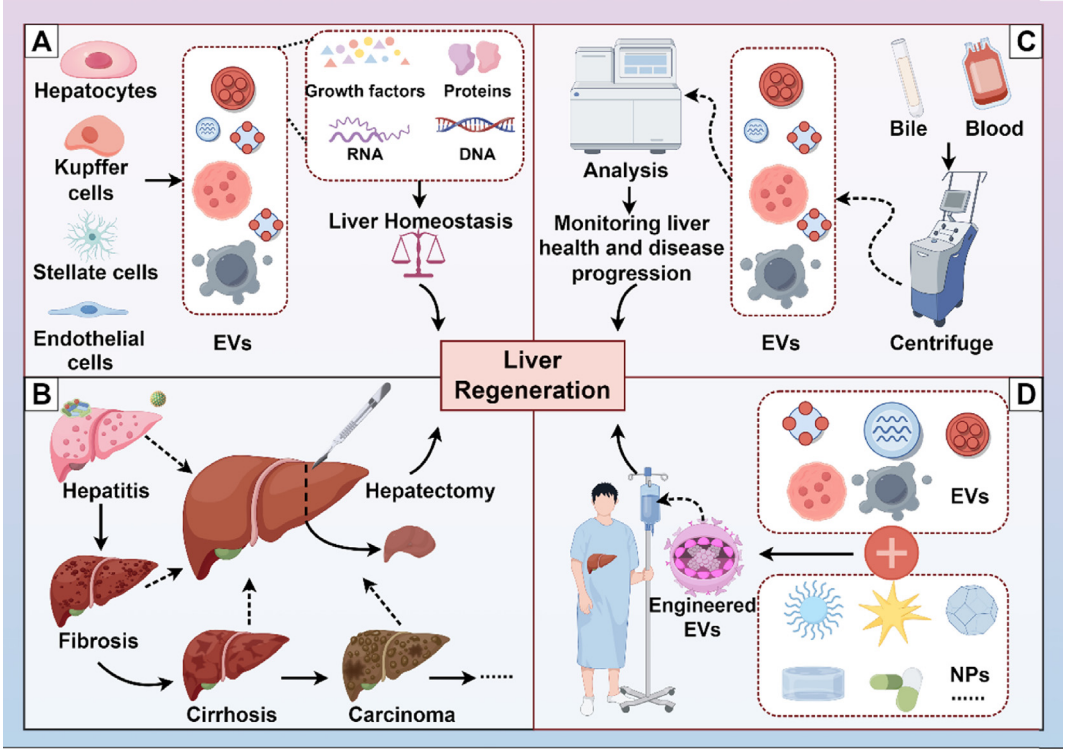

Fig. 4. The role of EVs in liver physiology and diseases. (A) In the hepatic environment, EVs are secreted by hepatocytes, Kupffer cells, stellate cells, and endothelial cells. a sophisticated system of intercellular communication that carries proteins, growth factors, lipids, RNA, and DNA to distant cells, thereby influencing various physiological processes of liver homeostasis. (B) EVs are deeply implicated in the pathogenesis of a spectrum of liver diseases, including hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. (C) EVs stand as a promising source of biomarkers for liver diseases. They can be isolated from body fluids, including blood and bile, providing a minimally invasive means to monitor liver health and disease progression. (D) Potential therapeutic uses of EVs and EV-based nanoparticles in promoting liver regeneration.

critical for the development of novel diagnostic and therapeutic strategies.

4.3. EVs as biomarkers for liver conditions

The diagnostic potential of EVs in liver conditions lies in their cargo, which mirrors the physiological and pathological state of the liver. As such, EVs stand as a promising source of biomarkers for liver diseases. They can be isolated from body fluids, including blood and bile, providing a minimally invasive means to monitor liver health and disease progression (Fig. 4C) [32].

In hepatocellular carcinoma, for instance, EVs carry tumor-specific proteins and nucleic acids that can serve as biomarkers for early detection. Alpha-fetoprotein (AFP), a well-established marker for HCC, has been detected in EVs isolated from the plasma of patients with HCC [76]. Additionally, EVs containing glypican-3, another HCC marker, have been proposed as a diagnostic tool for the detection of early-stage HCC, where traditional biomarkers may be less effective [77].

Beyond cancer, EVs can also reflect the fibrogenic activity within the liver. EVs enriched in fibrosis-associated proteins, such as collagen and fibronectin, indicate active fibrogenesis and can be used to assess the severity of fibrosis. Similarly, miRNAs involved in the regulation of fibrosis, such as miRNA-33a and miRNA-122, have been identified in circulating EVs and correlate with the extent of liver fibrosis [70].

Furthermore, EVs are being investigated as biomarkers for drug-induced liver injury (DILI), a significant concern in clinical pharmacology [78,79]. EVs can provide early signs of hepatotoxicity by carrying liver-specific enzymes like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) before their levels rise significantly in the serum [78,80]. This early detection is crucial for preventing severe liver damage and for the safe management of pharmacotherapy.

In summary, the utility of EVs as biomarkers for liver conditions is a burgeoning area of research. Their ability to encapsulate and protect a wide array of bioactive molecules makes them an invaluable tool in the non-invasive diagnosis and monitoring of liver diseases [26]. Furthermore, as our understanding of EV biology deepens, the specificity and sensitivity of EV-based diagnostics are expected to improve, offering new avenues for the management of liver health.

5. EVs in liver regeneration: mechanisms and evidence

5.1. Mechanisms of liver regeneration mediated by EVs

The intricate process of liver regeneration is a quintessential example of the body's remarkable ability to repair and restore itself following injury. These vesicles are laden with a plethora of bioactive molecules, including proteins, lipids, RNA, and DNA, which play pivotal roles in intercellular communication [14]. As we delve into the mechanisms of liver regeneration mediated by EVs, it is imperative to acknowledge the multifaceted nature of this process.

One of the primary mechanisms by which EVs contribute to liver regeneration is through the transfer of genetic information [72]. miRNAs and other non-coding RNAs encapsulated within EVs can modulate gene expression in recipient cells. This modulation is critical in controlling the cell cycle and apoptosis, thereby fostering an environment conducive to liver regeneration. For instance, the miRNA-122 packaged within EVs has been shown to downregulate

the expression of cyclin G1, a regulator of the cell cycle, thereby facilitating hepatocyte proliferation [60,66].

Furthermore, the protein cargo of EVs also plays a significant role in liver regeneration. Growth factors and cytokines, such as HGF and TGF-β, can be transported via EVs [79]. These factors are instrumental in activating signaling pathways that lead to hepatocyte proliferation and liver tissue remodeling. The ability of EVs to act as vehicles for such proteins allows for a localized and controlled delivery of regenerative signals directly to the site of liver injury.

The lipid composition of EVs is another critical component that influences liver regeneration. The lipids can modify the local tissue environment, modulating inflammatory responses and promoting tissue repair. Phosphatidylserine, for example, present on the surface of EVs, has been implicated in the immunomodulatory effects observed during liver regeneration [81,82]. This phospholipid can elicit anti-inflammatory responses by interacting with receptors on immune cells, thus attenuating excessive inflammation that could otherwise impair the regenerative process.

In summary, the mechanisms by which EVs mediate liver regeneration are complex and multifaceted. Through the delivery of genetic material, proteins, and lipids, EVs orchestrate a symphony of biological processes that culminate in the successful regeneration of liver tissue. These processes involve the modulation of gene expression, activation of proliferative signaling pathways, remodeling of tissue architecture, and regulation of the immune response, all of which are essential for the restoration of liver function.

5.2. Preclinical studies on EVs in liver regeneration

In pursuit of elucidating the role of EVs in liver regeneration, numerous preclinical studies have been conducted [63,83]. These studies have revealed that EVs are not merely bystanders but active participants that can significantly influence the outcome of liver regeneration [83]. The following discourse provides an exposition of the various preclinical studies that have laid the groundwork for understanding the regenerative capabilities of EVs.

Rodent models of partial hepatectomy have been a fundamental tool in studying liver regeneration. In these models, the administration of EVs derived from stem cells, such as mesenchymal stem cells (MSCs), has demonstrated an enhancement in the regenerative capacity of the liver [84]. The EVs derived from MSCs were found to carry pro-regenerative molecules, such as Wnt/β-catenin signaling pathway components, which were implicated in the activation of hepatocyte proliferation [85–87]. Additionally, these studies have shown that EVs can reduce oxidative stress and mitigate inflammation, thereby creating an environment that favors regeneration [87].

Another focal point of preclinical studies is the analysis of EVs in the context of liver injury induced by hepatotoxins [88]. When EVs were introduced in models of toxin-induced liver injury, a reduction in hepatocyte apoptosis was observed. This cytoprotective effect was attributed to the transfer of anti-apoptotic miRNAs and proteins via EVs [89]. Moreover, these studies provided insights into the role of EVs in modulating the extracellular matrix and vascular remodeling, which are critical for the restoration of liver architecture [90–92].

Preclinical studies have also shed light on the potential of EVs to modulate the immune response during liver regeneration. It has been shown that EVs can alter the phenotype of immune cells, promoting the polarization of macrophages towards a pro-regenerative M2 phenotype [93]. This immunomodulatory capacity of EVs could prove beneficial in reducing the hepatic inflammation that often accompanies liver injury and impedes regeneration [94,95].

In conclusion, preclinical studies have been invaluable in uncovering the potential of EVs in liver regeneration. Through the use of various models of liver injury, these studies have provided a robust body of evidence demonstrating the cytoprotective, pro-proliferative, and immunomodulatory effects of EVs (Fig. 4D) [95,96]. This preclinical evidence sets the stage for the exploration of EVs as therapeutic agents in the context of liver regeneration [95,96].

5.3. Potential therapeutic uses of EVs in promoting liver regeneration

With the groundwork laid by extensive preclinical studies, the potential therapeutic uses of EVs in promoting liver regeneration have come to the forefront [63,96–98]. The therapeutic landscape is filled with numerous possibilities, each more promising than the last, as we consider the role of EVs in liver regeneration. In this section, we will explore the burgeoning field of EV-based therapies and their potential applications in the context of liver regeneration [98–100].

One of the primary therapeutic uses of EVs is their potential to serve as a cell-free alternative to stem cell therapies. Given the challenges associated with stem cell transplantation, such as immune rejection and tumorigenicity, EVs derived from stem cells offer a safer and possibly more efficacious option [100,101]. The use of EVs could circumvent these issues, providing a means to deliver the regenerative benefits of stem cells without the associated risks [101,102].

Another promising therapeutic application of EVs is their use as drug delivery vehicles [103]. The natural propensity of EVs to home to specific tissues, including the liver, can be harnessed to deliver therapeutic agents directly to the site of injury [104]. This targeted delivery could enhance the efficacy of drugs while minimizing systemic side effects [105,106]. Moreover, the ability to engineer EVs to express specific targeting ligands further increases their potential as precision medicine tools for liver regeneration [106,107].

The role of EVs in gene therapy is also an area of immense therapeutic potential [102]. By loading EVs with therapeutic nucleic acids, such as siRNA or miRNA mimics, one can modulate the gene expression profile of hepatocytes to promote regeneration [108]. This approach could be particularly beneficial in addressing the underlying genetic causes of liver diseases that impede regeneration [109].

In conclusion, the potential therapeutic uses of EVs in promoting liver regeneration are extensive and diverse. EVs have the potential to revolutionize the treatment of liver diseases by providing cell-free, targeted, and gene therapy-based approaches [110]. While the journey from laboratory research to clinical application is filled with challenges, the evidence gathered thus far provides a solid foundation for the continued exploration of EVs as novel therapeutic agents in the field of liver regeneration.

6. Challenges and future perspectives

6.1. Current challenges in EV research and application

The realm of EVs has been steadily expanding, with a growing interest in their multifaceted roles within biological systems, particularly in the context of liver regeneration. However, despite the remarkable potential of EV-based therapies, there are significant challenges that currently hamper their translation from bench to bedside. One of the primary obstacles is the heterogeneity of EV

populations [111]. EVs are not a uniform group of entities but rather a varied collection of vesicles with different biophysical and molecular characteristics [110]. This diversity presents a considerable challenge in the standardization of EV preparations for therapeutic applications [112]. The complexity is compounded by the lack of universally accepted biomarkers for EV subpopulations, making it difficult to precisely isolate and characterize the vesicles that possess the most therapeutic potential [113].

Another significant challenge lies in the scalability of EV production [114]. For EV-based therapies to be viable on a clinical scale, it is imperative to develop reliable and efficient methods for the production of EVs in large quantities without compromising their integrity or functionality. Current techniques of EV isolation and purification, such as ultracentrifugation, filtration, and size-exclusion chromatography, are often labor-intensive, time-consuming, and may lead to the loss of EVs or their bioactive contents [114–116]. Moreover, these methods typically yield EVs with varying purity levels, which can affect the reproducibility and efficacy of therapeutic outcomes.

The quantification and dosing of EVs pose additional challenges. There is a critical need for accurate and consistent methodologies to quantify EVs and establish optimal dosing regimens [116]. Determining the appropriate dose of EVs for therapeutic purposes is not straightforward, as it involves considerations of not only the number of vesicles but also their molecular cargo and the dynamic state of the recipient's liver environment [117]. Furthermore, the stability and storage of EVs are areas that require attention. Ensuring that EVs maintain their structural and functional integrity during storage and transportation is essential for their efficacy as therapeutics.

Lastly, the targeted delivery of EVs to the liver or specific liver cell types remains an intricate task. The innate homing capabilities of EVs to specific tissues are beneficial, yet they may not be sufficient for targeted therapy [118]. Engineering EVs to enhance their specificity and uptake by hepatocytes or other relevant liver cells could enhance the efficacy of EV-based therapies [24,119]. However, this necessitates advancements in the understanding of EV biology, as well as the development of novel engineering techniques.

6.2. Future directions in EV-based liver regeneration therapies

In contemplating the future of EV-based therapies for liver regeneration, it is essential to focus on the development of innovative strategies to overcome the aforementioned challenges [120]. One promising direction is the utilization of cutting-edge technologies such as nanotechnology, bioengineering, and synthetic biology to refine the isolation, purification, and modification of EVs. For instance, the application of microfluidic devices could revolutionize the EV isolation process, offering a high-throughput and automated platform that minimizes vesicle loss and maintains the integrity of EVs [121]. The engineering of EVs to express specific surface proteins or to encapsulate desired therapeutic cargo is another avenue that holds great promise. By manipulating the EV membrane or loading EVs with specific RNA, DNA, or proteins, it becomes possible to tailor EVs for targeted delivery and enhanced therapeutic efficacy [122,123]. This level of customization would not only improve the specificity of EV-based therapies but also reduce potential off-target effects. Another significant area for future research is the exploration of the therapeutic potential of synthetic or designer EVs [124]. These are artificially created vesicles that mimic the natural properties of EVs and can be engineered to carry a specific set of bioactive molecules [125]. The advantage of synthetic EVs is that they can be produced consistently and in large quantities, circumventing the barriers associated with the isolation and scalability of natural EVs [126]. Furthermore, the development of robust animal models and preclinical studies is crucial to gain a deeper understanding of EV behavior *in vivo*, including their distribution, clearance, and interaction with the immune system. Such studies will provide valuable insights into the optimization of dosing regimens and the safety profile of EV-based therapies.

6.3. Regulatory and ethical considerations in the use of EVs

As the field of EV-based therapies progresses towards clinical application, it is imperative to address the regulatory and ethical considerations that accompany the use of EVs. Regulatory frameworks must be established to ensure the safety, quality, and efficacy of EV-based products [127]. Such frameworks will require the standardization of EV characterization methods, the establishment of quality control benchmarks, and the implementation of rigorous clinical trial protocols. Additionally, ethical considerations arise with the use of EVs, particularly when they are derived from human cells. Issues such as donor consent, privacy, and the potential for genetic modification of EVs must be carefully considered [128,129]. It is essential to develop ethical guidelines that balance the potential benefits of EV-based therapies with the respect for donor autonomy and privacy [128]. While EV-based therapies hold immense potential for liver regeneration, their clinical realization is contingent upon overcoming significant scientific and technical challenges. Through concerted efforts in research and development, along with the establishment of appropriate regulatory and ethical frameworks, the future of EV-based therapies in liver regeneration looks bright, with the promise of revolutionizing the treatment of liver diseases and enhancing patient outcomes.

7. Conclusion

The work provides a comprehensive and systematic integration of the latest advancements in EV-related studies in liver. It not only elucidates theoretical mechanisms through which EVs contribute to liver regeneration but also empirically validates the promise of EVs in promoting liver cell proliferation, inhibiting apoptosis, moderating immune responses, and supporting angiogenesis. Despite the tremendous potential EVs hold in treating liver conditions, significant challenges such as isolation purity, dosage control, and targeted delivery remain. Addressing these issues, the review suggests improvements to current techniques and the development of new methods to enable the clinical application of EVs for liver disease treatment. Overall, the review offers invaluable resources both theoretically and experimentally for the advancement of liver regeneration therapeutic strategies employing EVs. By dissecting the multifaceted role of EVs in liver physiology and disease, the work presents new perspectives and potential methods for the diagnosis and treatment of liver diseases. Moreover, exploring and addressing the challenges of EV-based therapies' clinical applications would open up possibilities for translating this cutting-edge technology into a new approach for treating liver diseases, signaling revolutionary changes in the field of biomedicine, especially in the domain of liver disease treatment.

Ethics approval and consent to participate

This work does not include animal experiments and human experiments.

Consent for publication

All authors have seen and approved the manuscript during the sub-mission process. All authors have accepted responsibility for the entire content of this manuscript and approved its final version and submission.

Availability of data and material

This is a review paper without data and material.

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Authors' contributions

N. Guo and Y. Wang contributed to review of the literature and manuscript writing. Z. Y. Wen and X. F. Fan designed the concept of the review and offered scientific editing. All authors were responsible for the designed critical revision of the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

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