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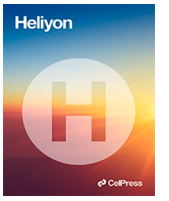

Research article

Extracellular vesicles for the treatment of ulcerative colitis: A systematic review and meta-analysis of animal studies

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ABSTRACT

Background: Extracellular vesicles (EVs) are being considered as a potential therapeutic option for ulcerative colitis (UC), and numerous preclinical studies have been conducted on the use of EVs for UC.
Methods: A systematic review was conducted to compare the therapeutic effects of mammalian EVs and placebo on UC in animal models, along with a meta-analysis comparing naïve (unmodified) EVs and placebo. The search was performed in four databases (PubMed, Web of Science, Scopus, and EMBASE) up to September 13th, 2023. The primary outcomes included disease activity index (DAI), colonic mucosal damage index (CMDI), and adverse effects (PROSPERO ID: CRD42023458039).
Results: A total of 69 studies were included based on pre-determined criteria, involving 1271 animals. Of these studies, 51 measured DAI scores, with 98 % reporting that EVs could reduce DAI scores. Additionally, 5 studies reported CMDI and all showed that EVs could significantly reduce CMDI. However, only 3 studies assessed adverse effects and none reported any significant adverse effects. The meta-analysis of these studies (40 studies involving 1065 animals) revealed that naïve EVs could significantly decrease the DAI score (SMD = −3.00; 95 % CI: −3.52 to −2.48) and CMDI (SMD = −2.10; 95 % CI: −2.85 to −1.35).
Conclusion: The results indicate that mammalian EVs have demonstrated therapeutic benefits in animal models of UC; however, the safety profile of EVs remains inadequate which highlights the need for further research on safety outcomes.

1. Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD), similar to Crohn's disease and indeterminate colitis [1]. It is an idiopathic, chronic inflammatory disorder of the colonic mucosa, beginning in the rectum and typically extending continuously through part or all of the colon. However, some patients with proctitis or left-sided colitis may have inflammation extending to the

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caecum [2]. Symptoms may include bloody diarrhea, frequent bowel movements, abdominal pain, fatigue, and fecal incontinence [3].

Population aging and earlier diagnoses have led to an increasing incidence and prevalence of UC, especially in newly industrialized countries [4]. The pathogenesis of UC is complex and not fully understood, but it is believed to involve genetics, immune response, epithelial barrier defects, and dysbiosis in initiating and sustaining inflammation [5].

Treatment for UC depends on disease severity and the affected parts of the colon. Mild to moderate UC limited to the rectum and lower colon is typically treated with oral or topical 5-aminosalicylic acid (5-ASA), while oral corticosteroids may be prescribed for patients who do not respond well to 5-ASA treatment. Patients with moderate to severe UC may receive intravenous corticosteroids (short courses), biologic monoclonal antibody medications or small molecule drugs. Surgery may be necessary for patients with severe UC that does not improve with maximal medical treatment or for complications related to colorectal cancer prevention or treatment [6].

Despite significant progress in research on UC, remission rates do not exceed 20–30 % in induction clinical trials and only reach 30–60 % among patients in real-life settings [5], indicating a need for more effective new therapies development.

Extracellular vesicles (EVs) are particles enclosed by a phospholipid bilayer that can be secreted by various cells. They contain a variety of bioactive molecules, including proteins, lipids, and nucleic acids, and have been found to play important roles in intercellular communication as well as the regulation of both physiological and pathological processes [7]. Therefore, EVs can be targeted for treating diseases, used as a direct therapeutic application based on their endogenous bioactivity, or applied in drug delivery [8]. In addition, there is a growing focus on researching the role of EVs in the treatment, diagnosis, and prognostic markers of tumors. EVs have been found to mitigate the nephrotoxicity caused by the cisplatin [9], and showed potential as oncolytic agents [10]. Liquid biopsy techniques, including EVs, are gaining increased attention for tumor diagnosis [11]. Immune checkpoints play a crucial role in regulating immune responses. In the tumor microenvironment, malignant cells can exploit the immunosuppressive effects of inhibitory immune checkpoints to promote tumor progression [12]. Despite the occurrence of immune-related adverse events [13], blocking inhibitory immune checkpoints has proven to be an effective strategy for cancer treatment; among these, PD-L1 is extensively studied as an EV immune checkpoint [12]. Additionally, along with albumin [14] and Royal Marsden Hospital score [15], EVs also hold promise for prognostic markers [16,17].

Further research has increasingly recognized the important roles of EVs in the immune system [18], their contribution to vascular and epithelial barrier function in inflamed intestines and wound healing [19], their ability to modulate the intestinal microenvironment [20], and their anti-inflammatory properties [21]. EV therapy shows promising prospects in treatingUC, supported by existing preclinical evidence. This review aims to evaluate the effect of EVs in UC animal models to facilitate clinical translation of EV therapy.

2. Methodology

This review adheres to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [22] and has been registered in the International Prospective Register of Systematic Reviews (PROSPERO; protocol ID: CRD42023458039). Compared to the protocol, we have included additional content on utilizing the guidelines specified by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) for assessing evidence.

2.1. Search strategy

We conducted a comprehensive search of electronic bibliographic databases including PubMed, Web of Science, Scopus, and EMBASE. The MeSH headings and keywords used in the search were “exosom*”, “secretom*”, “extracellular vesicle*”, “cell-derived microparticle*”, “microvesicle*”, “proctocolitis”, “ulcerative colitis”, “colitis”, “inflammatory bowel disease”, “IBD”, “UC”, and “ulcerat*" (Additional file 1). There were no restrictions on publication date, but the search was completed on September 13th, 2023.

All identified articles were imported into EndNote 20.6 and duplicates were removed. Two reviewers (Y.L. and Z.Y.) independently screened all studies based on pre-defined inclusion and exclusion criteria. Titles and abstracts were initially reviewed, followed by a full-text assessment of selected studies. Any discrepancies during the selection process were resolved by a third reviewer (M.C.).

2.2. Eligibility criteria

The studies included in this review met all of the following criteria, presented in a “PICOS” format.

2.2.1. Inclusion criteria

1. Population: *In vivo* mammalian animal model of UC.
2. Intervention: Mammalian EV without any restrictions on modification and route of administration.
3. Comparator: The comparator would be no treatment, placebo, or standard treatment. If there were multiple control groups, a non-active control group (i.e., placebo) would be chosen.
4. Outcome: The primary outcome was disease activity index (DAI), colonic mucosal damage index (CMDI), and adverse effects. The secondary outcome was colon length, histological score, and body weight. Studies that did not report any of the above outcomes were excluded.
5. Study design: comparative pre-clinical studies. Controlled studies with a separate control group. No language restrictions.

2.2.2. Exclusion criteria

1. Population: UC model with co-morbidities.
2. Intervention: No characterization of EVs (including morphology, size distribution, or EV markers) or if EVs are used only in combination with other drugs.
3. Study design: Cross-over studies.

2.3. Data extraction

Four reviewers, divided into two groups (Y.L. and Z.Y., D.Z. and F.Z.), independently extracted data from each article. In cases where discrepancies could not be resolved after discussion, two additional reviewers (D.Z. and M.C.) were consulted. Data was extracted from the study text, tables, figures, supplementary materials, and cited references for methods using an online application WebPlotDigitizer [23] when necessary. If an outcome was measured at multiple time points, only the data from the last time point was included.

The extracted data included study characterization (first author, publication year, and country of the corresponding author/s), study population (species, gender, age, weight), animal model (sample size and modeling method), intervention details (dose, comparator, frequency, route of administration and follow-up period), information on EV (source, modification, collection medium supplementation, vehicles, isolation and characterization method, characterization result, labeling and tracking, and immune-biocompatibility), outcomes (DAI, CMDI, and adverse effects as the primary outcome; colon length, histological score, and body weight as the secondary outcome), and the reported mechanisms from the study.

In addition to the above information if mesenchymal stem cells (MSCs) were the source of EVs their characterization would also be extracted.

2.4. Quality and risk of bias assessment

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias (RoB) tool [24] was utilized to assess the risk of bias in this review. The SYRCLE's RoB tool consists of six categories, encompassing a total of ten items. These categories include: selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (random housing and blinding of intervention), detection bias (random outcome assessment and blinding of outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting), and other sources of bias.

We evaluated the characterization outcome of MSCs (one of the source of EVs) and EVs in included studies based on the International Society for Cellular Therapy (ISCT) [25] minimal criteria and the Minimal information for studies of extracellular vesicles 2018 (MISEV2018) [26], respectively.

The ISCT minimal criteria included the following: 1) adherence to plastic, 2) specific surface antigen expression (positive for CD105, CD73, and CD90; negative for CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR), and 3) *in vitro* differentiation (osteoblasts, adipocytes, and chondroblasts).

MISEV2018 recommends using the term "extracellular vesicle" (EV) as the general name for particles that are naturally released from cells and are enclosed by a lipid bilayer, and cannot replicate. To characterize EVs, MISEV2018 provides recommendations as follows: 1) quantitation, 2) characterization of at least three positive protein markers of EVs (including at least one transmembrane/lipid-bound protein and cytosolic protein) and one negative protein marker, and 3) characterization of single vesicles by two different but complementary techniques. To determine the purity and reliability of the quantity measure, MISEV2018 also suggested reporting the ratios of proteins: particles, lipids: particles, or lipids: proteins, along with global quantification estimates.

The quality of the outcomes was assessed in accordance with the guidelines specified by the GRADE [27]. We utilized the GRADE profiler (GRADEpro GDT) to import data from Review Manager Software 5.4.1 for creating 'Summary of findings' tables.

The assessment of quality and RoB was completed by two independent reviewers (Y.L. and Z.Y.) and any discrepancies were resolved through discussion with a third reviewer (M.C.).

2.5. Meta-analysis/statistical analysis

The meta-analysis was conducted using Review Manager Software 5.4.1 to analyze five outcomes: DAI, CMDI, colon length, histological score, and body weight. The inclusion criteria for the meta-analysis required a minimum of three studies reporting the same outcome between naïve (unmodified) EVs and placebo controls using the same scale. Descriptive summaries were provided for outcomes that could not be assessed by meta-analysis, such as adverse effects.

We used inverse variance for meta-analysis. A random-effects model was utilized for the meta-analysis with the standardized mean difference (SMD). Study confidence interval and total confidence interval were all 95 %. Heterogeneity was evaluated using the I^2 index, and if $I^2 > 75$ %, further heterogeneity analysis was conducted by excluding studies one by one. If a confirmed source of heterogeneity was identified, the remaining studies would undergo meta-analysis, followed by qualitative and heterogeneity analysis of this study. Subgroup analyses were performed based on compliance with MISEV2018 guidelines and EV source (derived from MSCs or others). Additionally, a sensitivity analysis was carried out by systematically excluding studies from the results of the meta-analysis one at a time. Publication bias was assessed using funnel plots.

3. Results

3.1. Literature search

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guideline [22].

The search process is illustrated in Fig. 1. After conducting a systematic search of four databases, a total of 3391 records were identified. Following our eligibility criteria, we excluded 1787 duplicates, 1485 records through title and abstract screening, and 49 studies through full-text screening as shown in Fig. 1. Consequently, our qualitative synthesis included 69 studies comprising of 70 articles (2 records [28,29] representing one study) and involving a total of 1271 animals. There were no studies in the search results that met the inclusion criteria but were excluded.

3.2. Study characteristics

The 69 studies were published between 2010 and 2023. Fig. 2a shows a steady increase in the number of published studies each year, especially after 2019, indicating a significant surge in interest in utilizing EVs in UC. The studies were conducted in nine countries, with China contributing to 75 % (n = 53) of the total research output.

3.3. Characteristics of UC animal models

3.3.1. Animal species

Animal models were distributed as follows: mice (n = 60) and rats (n = 9) in UC models (Fig. 2c).

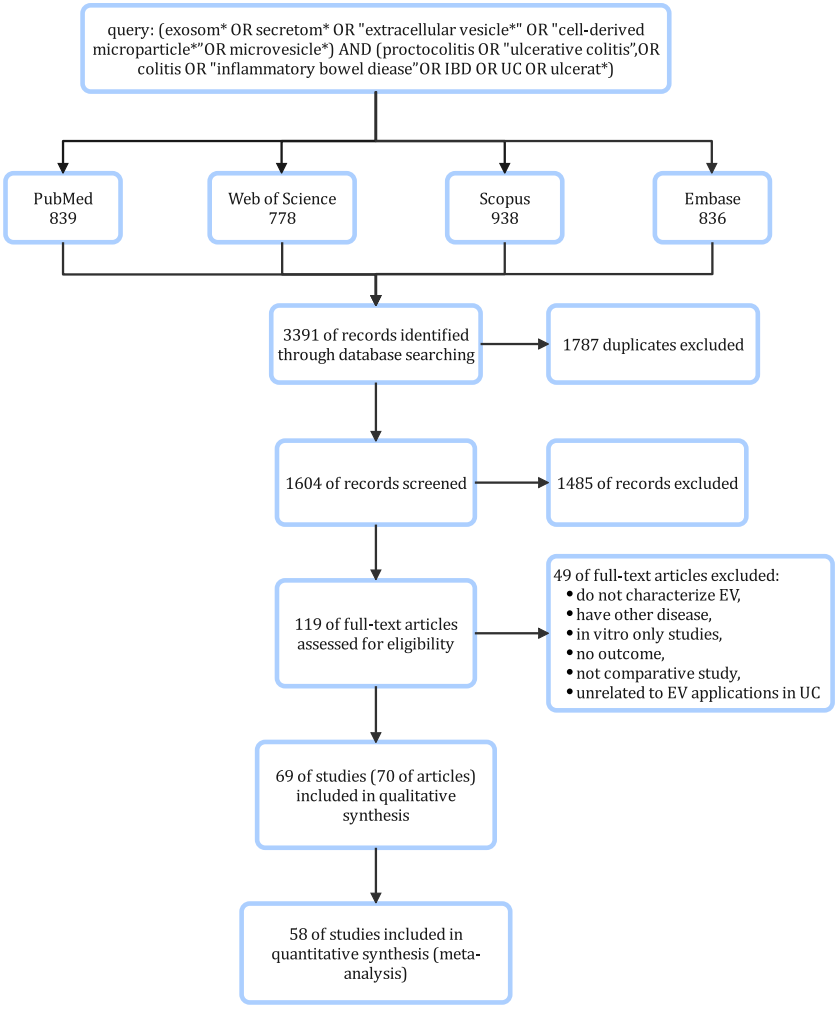

Fig. 1. PRISMA flow chart summarizing study screening and selection procedure.

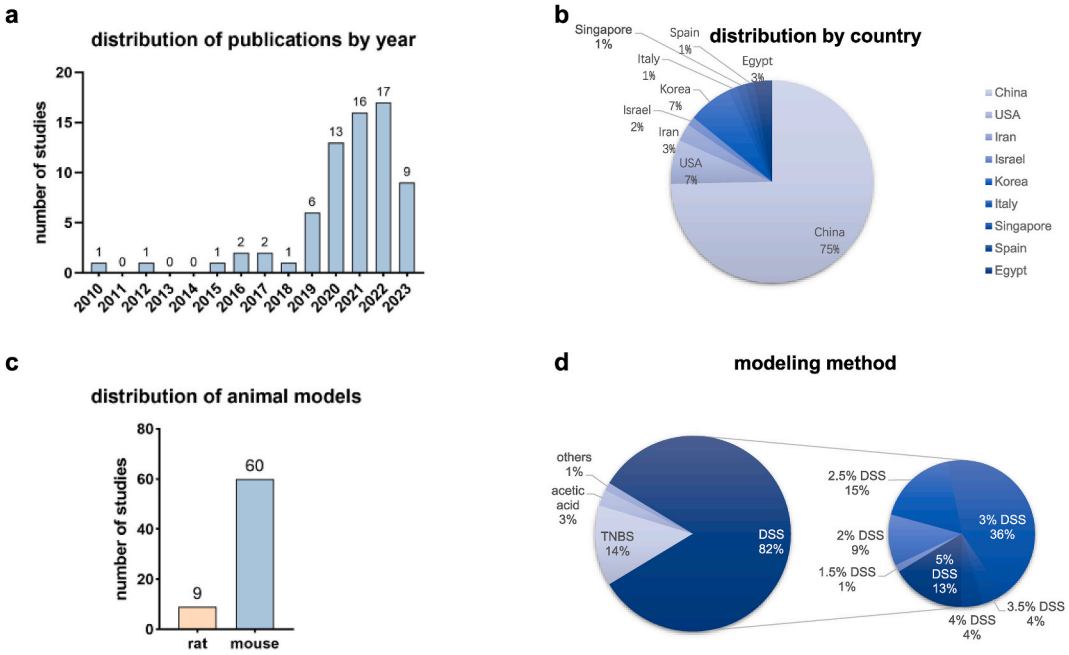

Fig. 2. Overview of publish year (a), region(b), animal models (c), and modeling method (d).

3.3.2. *Modeling method*

In Fig. 2d, it is demonstrated that dextran sulfate sodium (DSS) was the most frequently utilized drug for modeling (n = 62, 82 %). Trinitrobenzene sulfonic acid (TNBS) was employed in 14 % of studies (n = 10), while acetic acid was used in 3 % (n = 2). Only one study (1 %) established a UC model by injecting $CD4^{+}CD45R^{Bhigh}$ T cells into $Rag1^{-/-}$ mice. The concentration of 3 % (n = 27) was the most commonly utilized in DSS modeling. Additionally, two studies [30,31] used both DSS and TNBS, two studies [32,33] used two different concentrations of DSS, and one study [34] used two different concentrations of DSS and TNBS to create UC models.

3.4. *Intervention characteristics*

3.4.1. *Cellular origin of EVs*

As illustrated in Fig. 3, EVs were obtained from various sources including cells (n = 60, 87 %), biofluid (n = 7, 10 %), faeces (n = 1,

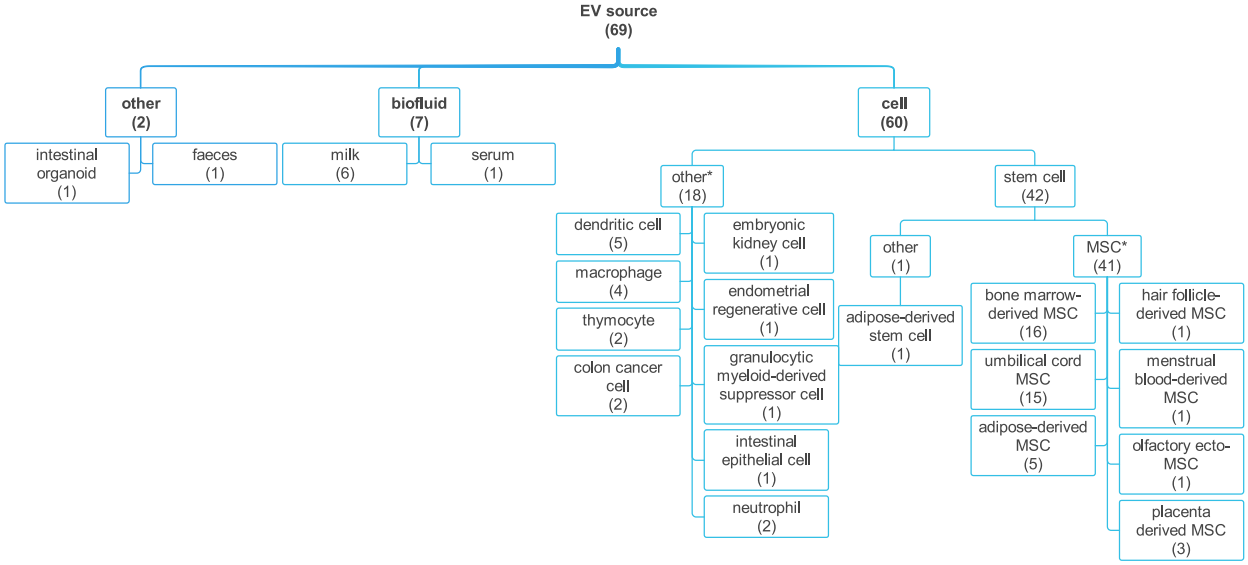

Fig. 3. Sources of EVs used for the treatment of UC. *: One study utilized EVs derived from two distinct cell sources.

1 %), or intestinal organoids (n = 1, 1 %). Among the EVs derived from cells, the majority were sourced from stem cells (n = 42, 70 %), with MSC being the most commonly utilized type (n = 41,68 %). One study [35] employed both umbilical cord MSC and placenta-derived MSC. Another study [36] used both granulocytic myeloid-derived suppressor cells and neutrophils. The milk-derived EVs originated from human and cow milk and one study [37] utilized EVs from both sources.

With the exception of one study that did not specify a particular species of EV source, most studies used xenogeneic EVs (n = 37, 54 %) followed by allogeneic EVs (n = 30, 44 %). Only one study (1 %) [38] used autologous EVs.

3.4.2. Modification of EVs

A total of 34 studies utilized EV modification. Preconditioning (n = 14) and genetic modification (n = 14) were both common methods used to modify EVs. Table 1 shows that EV modification involves various stimuli and genes, mostly applied to the source of EVs.

Three studies employed both preconditioning and genetic modification techniques. Cao [39] transfected HCT116 cells with miR-149-3p mimics and treated them with Enterotoxigenic *Bacteroides fragilis* before harvesting the EVs. Zhu [40] cultured cells in normoxic or hypoxic incubators, then transfected them with a HIF-1α inhibitor or a control lentiviral vector. Gu [41] conducted two experiments: one stimulated MSCs with or without lipopolysaccharide (LPS) (10 μg/mg), and another transfected MSCs with 100 nM scrambled sequences and inhibitors of miR-181a, respectively.

Three other studies used different methods to modify EVs: Chen [42] collected EVs from the serum of pediatric patients who were positive for *Helicobacter pylori* (Hp) or healthy volunteers. Deng [43] utilized the layer-by-layer self-assembly technique to encapsulate human placenta MSC-EVs using biodegradable N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride and oxidized konjac glucomannan, both polysaccharide derivatives. Rao [44] packaged triptolide (TP) into EVs by mixing and incubating them in a shaking incubator.

Out of the 34 studies that utilized modified EVs, only 12 of them (35 %) assessed the potential impact of processing on EVs. Only one study (3 %) [44] evaluated the encapsulation efficiency when loading drugs into EVs. Four studies [40,45–47] reported no change in morphology, size distribution, and surface markers of EVs. Three studies [48–50] found that higher quantity of EVs after modification. One study [51] reported that after IL-10 treatment, dendritic cell-derived EVs expressed decreased levels of MHC II, CD80, and CD40, moderate levels of CD86, and a high level of IL-10. Additionally, two studies [43,52] showed that modification can enhance the

Table 1
The modifications of EVs.

Ref.	Modification	Modification category
[54]	*S. japonicum soluble* eggs antigen	Preconditioning
[51]	IL-10	Preconditioning
[55]	IL-13, IL-10, or IL-1β	Preconditioning
[36]	N^{ω}-hydroxy-nor-Arginine	Preconditioning
[48]	Irradiated with 30 Gy	Preconditioning
[56]	Thapsigargin	Preconditioning
[49]	IL-6, TNF-α, and IL-1β	Preconditioning
[53]	INF-γ	Preconditioning
[57]	Morphine	Preconditioning
[58]	A defined medium	Preconditioning
[59]	*Dendrobium officinale* polysaccharide and LPS	Preconditioning
[50]	$CoCl_2$ (hypoxia mimetic agent)	Preconditioning
[47]	TNF-α and IFN-γ	Preconditioning
[33]	TNF-α	Preconditioning
[60]	miR-146a	GM
[61]	miR-195a-3p	GM
[62]	PD-L1	GM
[63]	miR-146a	GM
[52]	EphB2	GM
[64]	TGF-β1 or *LacZ*	GM
[65]	miR-590-3p	GM
[66]	miR-378a-3p	GM
[31]	TSG-6	GM
[45]	TSG-6	GM
[46]	IL-10	GM
[67]	PD-L1	GM
[68]	miR-129-5p	GM
[69]	miR-223	GM
[39]	Enterotoxigenic *Bacteroides fragilis* and miR-149-3p	P&G
[41]	LPS or miR-181a	P&G
[40]	Hypoxic cultured and transfected with HIF-1α inhibitor	P&G
[42]	Hp-positive pediatric patients or healthy volunteers-derived serum	Other
[43]	A layer-by-layer self-assembly technique was used to encapsulate human placenta MSC-EVs	Other
[44]	TP was packed in EVs	Other

Abbreviations: LPS: lipopolysaccharide; TSG-6: tumor necrosis factor-stimulated gene-6; Hp: *Helicobacter pylori*; GM: genetic modification; P&G: preconditioning and genetic modification; TP: triptolide.

targeting ability of EVs in the colons. One study [53] reported that IFN-γ treatment slightly increases MSC-secreted EV numbers and significantly increases expression of CD9 and CD81 in EVs. Another study [41] reported that LPS stimulation increased expression TSG101, CD81, CD63, and miR-181a in EVs.

3.4.3. EV preparation

3.4.3.1. EV collection conditions. A total of 61 studies collected EVs from conditioned medium. Since serum contains EVs, 24 studies [30,31,33,40,41,46,48,49,52,57,62–64,70–80] chose a serum-free medium and 26 studies used a medium with EV-depleted serum to culture cells before extracting EVs. However, only nine of those studies that used medium with EV-depleted serum described how they depleted the serum of EVs without reporting the changes in particle count [44,51,53,56,66–68,81,82]. Three studies reported using commercial EV-depleted serum [35,45,83], and fourteen studies did not describe the details of their EV-depletion protocol [34,43,47, 50,61,65,69,84–90]. In the remaining studies, one made the condition of source cells in PBS for 48 h [58], another study used a complete medium [91], and nine studies did not report what they used before extracting EVs.

Except for 13 studies that did not report the duration of cell culture conditioning, more than half (n = 31) in the remaining studies treated cells for 48 h before harvest. Seven studies treated cells for 24 h and five studies treated cells for 72 h. Besides, two studies treated cells for 48–72 h [41,66], and 3 studies treated cells for 6 h [48], 20 h [36], and 24–48 h [85], respectively.

3.4.3.2. EV separation techniques. Fig. 4a shows the various separation techniques used for extracting EVs. Out of 32 studies, EVs were separated using a single method with ultracentrifugation being the most frequently used (n = 19), followed by commercial kits (n = 9). Some studies also utilized ultrafiltration (UF), size exclusion chromatography, and differential centrifugation. Due to the advantages and disadvantages of each method, researchers have started combining separation techniques for EV isolation [92]. Thirty-seven studies employed two techniques simultaneously, with ultracentrifugation and UF being the most common combination (n = 28). Additionally, eight studies combined commercial kits with ultrafiltration, while one study combined commercial kits with ultracentrifugation. Further details can be found in [Additional file 2].

3.4.4. Characterization of EV preparations

Absolute purification of EVs from other entities is deemed impossible, as stated by MISEV2018 [26]. Therefore, it is essential to characterize EVs using multiple and complementary techniques.

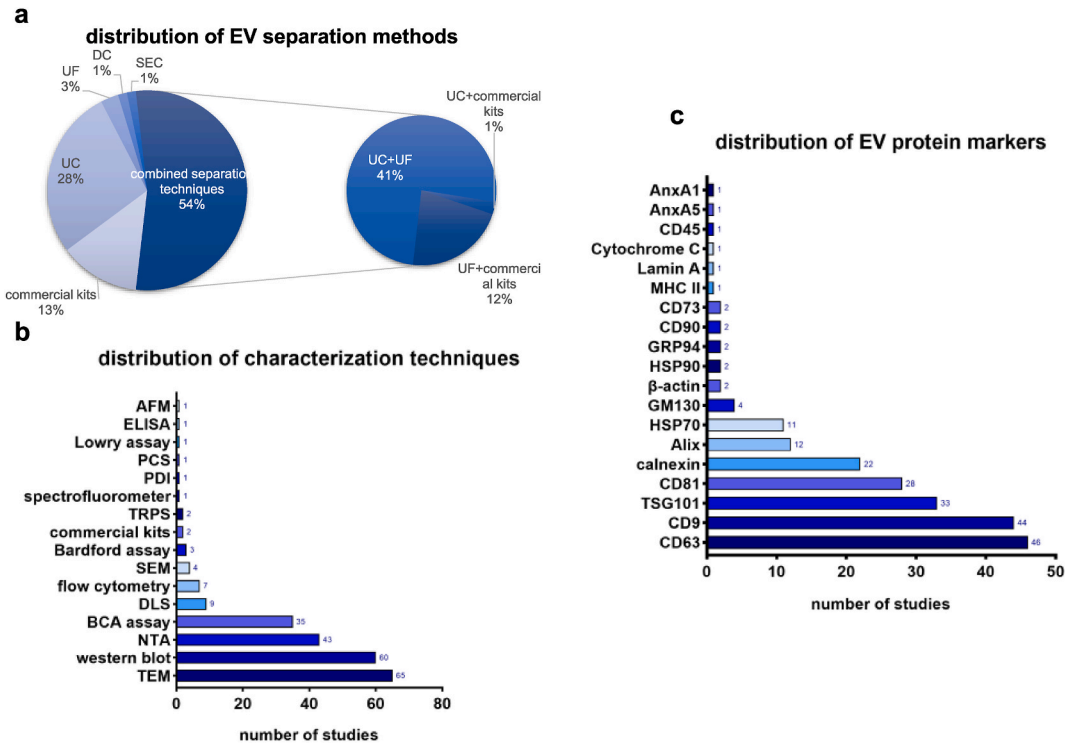

Fig. 4. Distribution of separation methods(a), characterization techniques (b), and protein markers of EVs (c). UC: ultracentrifugation; UF: ultrafiltration; SEC: size exclusion chromatography; DC: differential centrifugation. AFM: atomic force microscopy; PCS: photon correlation spectroscopy; PDI: polydispersity index; TRPS: Tunable Resistive Pulse Sensing; SEM: scanning electron microscopy; DLS: dynamic light scattering; NTA: nanoparticle tracking analysis; TEM: transmission electron microscopy.

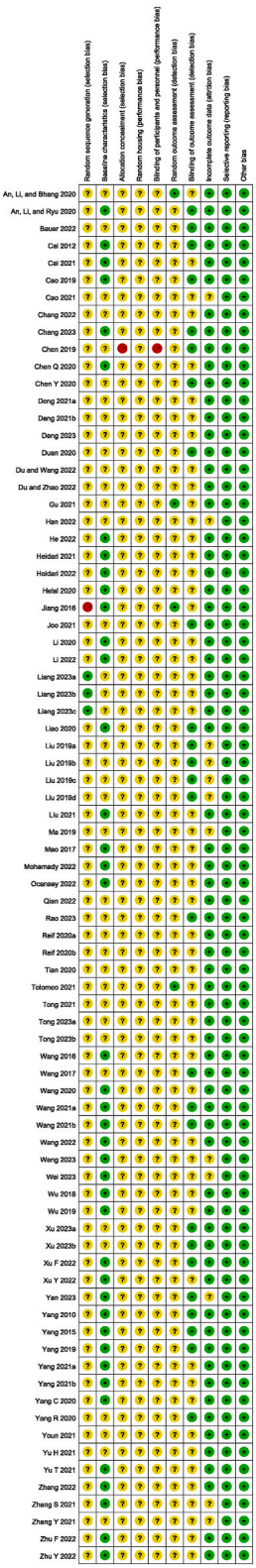

Fig. 5. Risk of bias assessment based on SYRCLE's ROB tool represented by RevMen 5.4.

Fig. 4b illustrates the various techniques utilized for characterizing EVs, including quantification, morphology, size distribution, and protein markers. Except for three studies [77,79,80], the remaining studies employed more than one technique for EV characterization. The most commonly used techniques were transmission electron microscopy (TEM), western blotting, nanoparticle tracking analysis (NTA), and BCA assay for evaluating EV morphology, protein markers, size distribution, and protein concentration (as a proxy for quantification of EVs), respectively. TEM was also utilized for size distribution analysis while NTA and Tunable Resistive Pulse Sensing were used for determining EV concentration. Almost all studies detected the size distribution (n = 66; 96 %), protein markers (n = 66; 96 %), and morphology of EVs (n = 65; 94 %). However, despite MISEV2018's recommendation that global quantification of EVs should be provided in studies characterizing them, only 67 % of studies (n = 46) described their quantification methods for EVs with only 37 % (n = 17) reporting their results.

Western blotting was the most frequently employed technique (n = 59) for characterizing EV protein markers. Six studies utilized flow cytometry, and one study employed both techniques. As depicted in Fig. 4c, tetraspanin transmembrane proteins CD63 (n = 46), CD9 (n = 44), CD81 (n = 28), as well as cytosolic proteins TSG101 (n = 33), Alix (n = 12), HSP70 (n = 11) were among the most frequently detected protein markers, along with negative marker calnexin (n = 22). Approximately thirty-three percent of studies identified at least four different protein markers but four did not characterize negative protein markers.

3.4.5. EV administration and dosage regimen

3.4.5.1. Dose. Out of the 69 studies, 4 (6 %) did not specify the dose of EV. Forty-seven studies (68 %) utilized the amount of protein as a measure of EV quantity, ranging from 10 μg to 100 mg. Conversely, 7 studies (10 %) counted the number of particles to assess EV quantity, with a range of 10^8 to 2×10^{10} particles [29,49,60,69,83,93,94]. Only one study (1 %) used the amount of EV source cells (10 million human adipose-derived MSCs) to estimate the quantity of EV [77], while ten studies (14 %) based their EV dose on the weight of the animal [32,37,41,46,62,68,71,95–97]. Additionally, among 65studies, only7 (10 %) evaluated the dose-response [32,71,75,85,95,96,98]. More detail is shown in [Additional file 3].

3.4.5.2. Administration route. Intravenous injection (n = 36, 53 %), particularly via the tail vein (n = 28, 41 %), was the most commonly utilized route for EV administration. Intraperitoneal (n = 24, 35 %) and oral (n = 7, 10 %) routes were also frequently employed in numerous studies. Furthermore, one study [43] compared the oral and intravenous administration of layer-by-layer coated EVs for UC therapy and found that oral administration effectively alleviated UC using half the number of EVs. Another study [81] injected EVs *in situ* at the injured colon mesangial margin.

3.4.6. Labeling and tracking of EVs

More than half of the studies (n = 36, 52 %) reported on the labeling and tracking of EVs. With the exception of 8 studies, most used a single type of fluorescent dye to label EVs. The most commonly used dyes were PKH26 (n = 12, 27 %), DiR (n = 12, 27 %), and Dil (n = 7, 16 %). One study [69] utilized Exo-CD63-GFP lentivirus to fluorescently label CD63 in macrophages and extracted CD63-GFP-labeled EVs after establishing a stable cell line for observing EV tracking *in vitro*.

The majority of the studies (n = 21, 58 %) reported tracking EVs in only one of the following settings: *in vivo* (n = 2, 6 %), *ex vivo* (post-mortem) (n = 12, 33 %), or *in vitro* (n = 7, 19 %). Eight studies (22 %) tracked EVs both *ex vivo* and *in vitro* [33,43,44,50,59,63,95,97], while five (14 %) tracked EVs both *in vivo* and *ex vivo* [73,85,86,88,90]. Only two studies (6 %) labeled and tracked EVs in all three settings: *in vivo*, *ex vivo*, and *in vitro* [32,76].

Despite UC mainly affecting the colon, twelve studies examined labeled EVs in other organs both *in vivo* and *ex vivo*. For more details see [Additional file 4].

3.5. Quality assessment

3.5.1. Risk of bias assessment

Fig. 5 illustrates the results of the risk of bias assessment using SYRCLE's ROB tool [24].

Out of the sixty-seven studies (97 %), all declared randomization, with only one study [30] specifically mentioning the use of a random number table for grouping. Another study [98] divided mice based on their body weight, which was considered to have a high risk of bias.

In terms of baseline comparability, 39 studies (57 %) reported comparable baseline characteristics, while thirty studies (43 %) did not report body weight at the beginning of modeling, which is crucial in assessing UC severity.

Most studies did not report allocation concealment, random housing, and blinding. Only one study [48] explicitly stated that experimenters were not blinded during grouping.

Merely four studies (6 %) indicated that multiple fields of view would be randomly selected under the microscope for outcome assessment [41,47,49,98], and only twenty-one studies (30 %) reported experimenter blinding during outcome assessment.

Sixty studies (87 %) had a low risk of attrition bias, while nine studies (13 %) [34,35,39,46,57,58,68,83,97] had an unclear risk due to lack of reporting sample size for each group.

All studies were assessed to have a low risk of reporting bias after comparing their methods and results; however, no prior protocol was available.

The specific details of bias risk assessment could be found in the [Additional file 5].

3.5.2. Adherence to ISCT criteria for MSC characterization

More than half of the studies (n = 41, 59 %) utilized MSC-derived EVs. Only 9 studies (22 %) examined all the required items as per ISCT guidelines, but with incomplete surface antigen and differentiation outcomes [33,35,47,63,70,71,76,78,85]. Another 10 studies (24 %) only examined two items. Seven studies [40,49,52,53,62,67,75] assessed criteria 1 and 2, while three [31,45,89] evaluated criteria 2 and 3. Three studies (7 %) only looked at one item; one study [58] examined criteria 1 and two others [34,87] examined criteria 2. Lastly, 19 studies (46 %) did not report the characterization of MSCs. Additionally, 4 studies mentioned that they obtained MSCs commercially or from other institutes.

3.5.3. Adherence to MISEV2018 for EV nomenclature, characterization, and purity

For nomenclature, only 35 % of the studies (n = 24) adhered to the recommendation of MISEV2018. Specifically, 61 % (n = 42) continued to use the term "exosome", while 1 % (n = 1) used "microvesicle", and 3 % of studies (n = 2) utilized both "extracellular vesicle" and "exosome". Additionally, it was found that 40 studies (58 %) published after 2019 did not comply with the MISEV2018

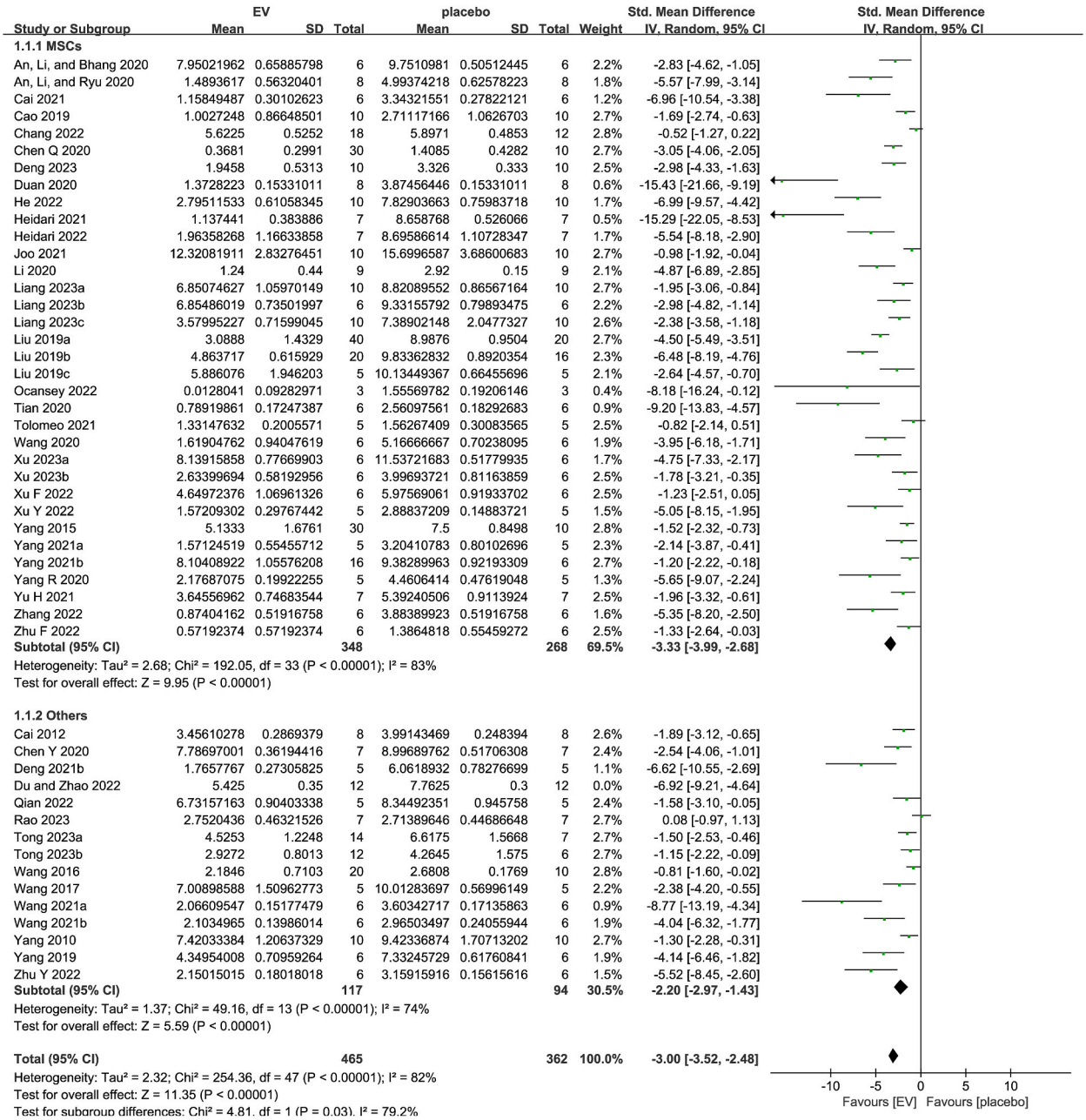

Fig. 6. Forest plot of the efficacy of EVs in decreasing the DAI scores.

guidelines for EV nomenclature.

In terms of characterization, only a mere10 studies (14 %) [28–30,32,76,81,86,87,95,97,98] fully met the established criteria.

As for purification and quantitation methods employed in the research analyzed, only one study (1 %) [95] measured particle: protein as a purifying analysis of EVs by using NTA.

3.5.4. Quality of the evidence

The quality of evidence was evaluated as very low to low by using GRADE. More detail was shown in [Additional file 6].

3.6. Synthesis of results

3.6.1. DAI

3.6.1.1. All included studies. A total of 51 studies measured DAI scores. As shown in [Additional file 7], almost all of them reported that EVs could significantly decrease DAI (n = 50, 98 %), except for one study [69].

Initially, we included 41 studies for meta-analysis. However, as the heterogeneity exceeded 75, a heterogeneity analysis was conducted. The results showed that excluding one study [28] lowered the I^2 of the non-MSC-derived subgroup from 80 to 74.

Subsequently, we included 40 studies for meta-analysis, with 616 animals in the MSCs subgroup and 211 animals in the non-MSC-derived subgroup (Fig. 6). The results indicated that intervention with EVs could significantly reduce the DAI scores (in the total: SMD = −3.00, 95 % CI: −3.52 to −2.48, $p < 0.00001$; in the MSCs subgroup: SMD = −3.33, 95 % CI: −3.99 to −2.68, $p < 0.00001$; in the non-MSCs subgroup: SMD = −2.20, 95 % CI: −2.97 to −1.43, $p < 0.00001$). The certainty of the evidence was low.

Due to high heterogeneity, a descriptive summary was conducted for the outcome of DAI scores. As shown in Table 2, naïve EVs and modified EVs could decrease the DAI scores, and compared with the naïve EVs, the modified EVs usually had a better effect.

Additionally, different from other studies using total protein to characterize the quantities of EVs, the excluded study [28,29] used particles numbers, may be the source of heterogeneity.

3.6.1.2. Studies that characterized EVs based on MISEV2018. We also conducted a meta-analysis on the DAI scores of studies that characterized EV as required by MISEV2018. The analysis included 7 studies. The results showed that EVs were effective in reducing DAI scores, which is consistent with our earlier finding.

However, high heterogeneity was observed ($I^2 = 82$ in the total meta-analysis, $I^2 = 78$ in the MSC-derived EVs subgroup, and $I^2 = 90$ in the non-MSCs-derived EVs subgroup), so we performed a heterogeneity analysis. The heterogeneity analysis demonstrated that the heterogeneity index decreased ($I^2 = 56$ in the total meta-analysis, $I^2 = 52$ in the MSC-derived EVs subgroup, and $I^2 = 0$ in the non-MSCs-derived EVs subgroup) when we excluded one study from each of the two subgroups.

Therefore, we included only 5 studies in the final analysis, with 88 animals treated with MSC-derived EVs and 39 animals treated with non-MSC-derived EVs (Fig. 7). The results showed that regardless of source, EV treatment significantly reduced DAI scores (in the total: SMD = −2.27, 95 % CI: −3.05 to −1.49, $p < 0.00001$; in the MSCs subgroup: SMD = −2.79, 95 % CI: −3.78 to −1.79, $p < 0.00001$; in the non-MSCs subgroup: SMD = −1.33, 95 % CI: −2.07 to −0.59, $p = 0.0005$).

The increased heterogeneity observed may be attributed to differences in administration method and dosage among studies. Unlike other studies that administered EVs through i.v. or i.p., one study administered them directly at injury colon mesangial margin after laparotomy [81]. Another study intervened with animals using a dosage of 3.0×10^9 particles per gram body weight of EVs as previously mentioned [28].

3.6.2. CMDI

Out of the total number of studies, only 5 reported CMDI [51,54,55,74,75], and all of them (n = 5, 100 %) demonstrated a positive impact on improving CMDI.

The meta-analysis included data from 60 animals treated with MSC-derived EVs and 42 animals treated with EVs derived from other sources. The results indicated that treatment with EVs had a beneficial effect on CMDI. This is shown in Fig. 8 (in the total: SMD = −2.10, 95 % CI: −2.85 to −1.35, $p < 0.00001$; in the MSCs subgroup: SMD = −1.77, 95 % CI -2.69 to −0.85, $p = 0.0002$; in the non-MSCs subgroup: SMD = −2.57, 95 % CI -3.87 to −1.27, $p = 0.0001$). The certainty of the evidence was very low.

Furthermore, we have summarized the results that were not included in the meta-analysis in Table 3. Both naïve and modified EVs demonstrated a favorable therapeutic effect.

Table 2
Summary of the DAI results.

EV types	Results	n	Percent
Naïve	Decreased DAI scores	23	45 %
Modified	Decreased DAI scores	6	12 %
	No effect	1	2 %
Naïve and modified	Both decreased DAI scores but modified EVs had a better effect	14	27 %
	Only naïve EVs decreased DAI scores	4	8 %
	Only modified EVs decreased DAI scores	3	6 %

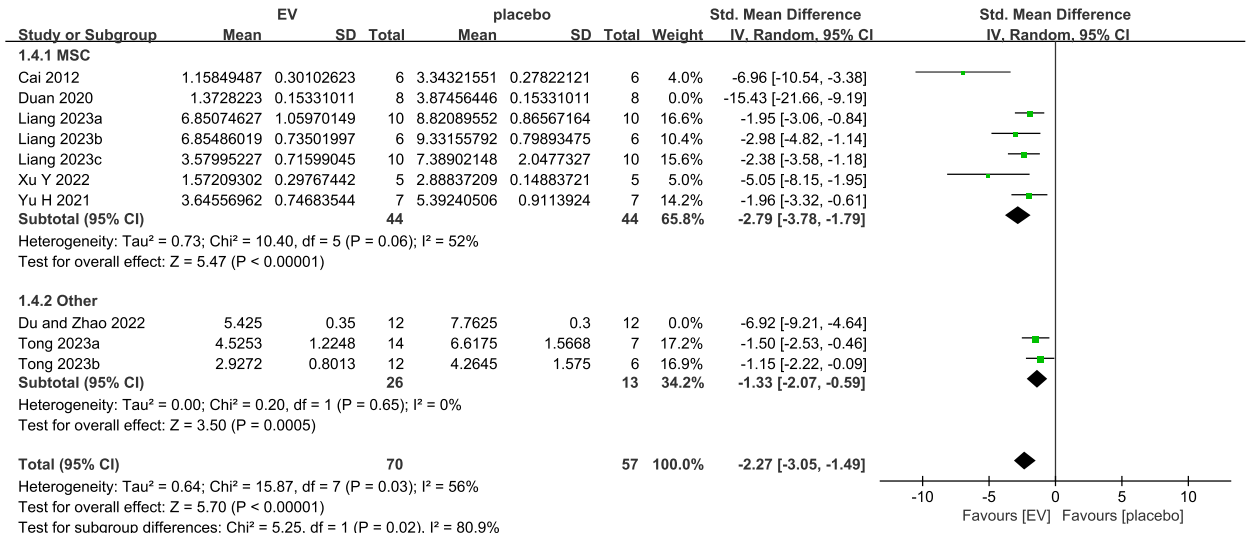

Fig. 7. Forest plot of DAI scores of studies that characterized EV based on MISEV2018.

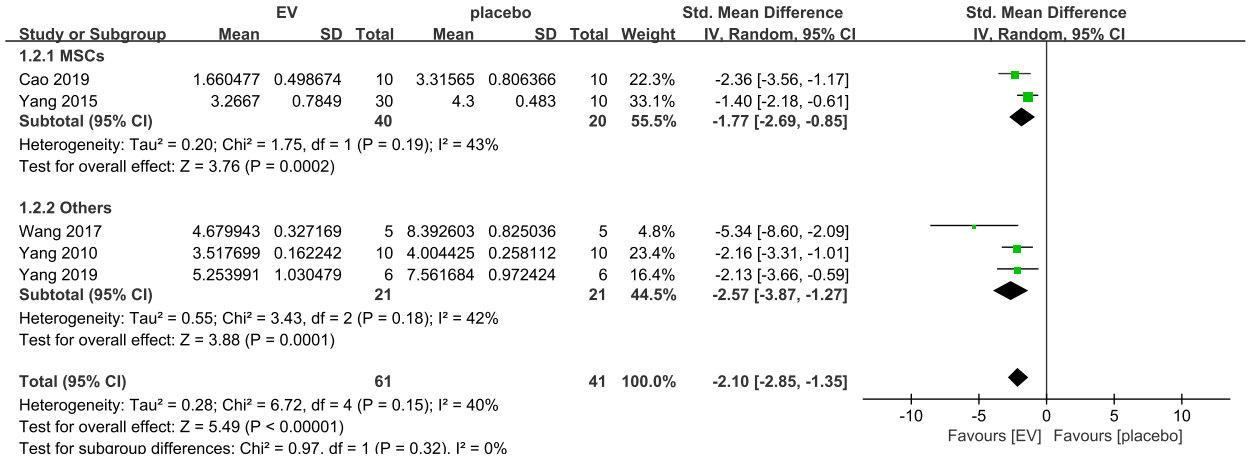

Fig. 8. Forest plot of the efficacy of EVs in decreasing the CMDI.

3.6.3. Adverse effects

There were only three studies (4 %) conducted to evaluate the adverse effects of EVs, and all of them found no toxicity in animals. Reif [37] administered milk-derived EVs to mice for seven days and observed no signs of illness, weight loss, or intolerance. Xu [67] assessed the safety of PD-L1 overexpression MSC-derived extracellular vesicles (MSC-sEVs-PD-L1) by conducting H&E staining, flow cytometry, blood biochemical examination, and blood routine examination. The study found that while MSC-sEVs-PD-L1 slightly affected the macrophage population in the liver, it did not significantly impact other immune contexts of major tissues and was therefore biocompatible in mice. Rao [44] used EVs to deliver TP to mice and evaluated its toxicity by performing pathological staining, biochemistry examination, and by comparing the survival rate of mice treated with TP or doubled doses of EVs carrying TP. The results suggested that EVs carrying TP could effectively reduce the toxicity of TP. The certainty of the evidence was very low.

Table 3
Summary of the CMDI results.

EV types	Results	n	Percent
Naïve	Decreased CMDI	2	40 %
Naïve and modified	Both decreased CMDI and no difference	1	20 %
	Both decreased CMDI but modified EVs had a better effect	1	20 %
	Only modified EVs decreased CMDI	1	20 %

3.6.4. Colon length

3.6.4.1. All included studies. Out of the 69 studies reviewed, 53 quantitatively reported the length of the colon after EV interventions compared to the control group, while 10 studies reported qualitatively. With the exception of 2 studies [49,69], all remaining studies (n = 61, 97 %) reported that EVs could increase colon length.

A total of 44 studies were included in a meta-analysis, which indicated that EVs could significantly increase colon length. However, high heterogeneity was observed in the MSC-derived EV subgroups (I^2 = 76). To address this heterogeneity, further analysis was

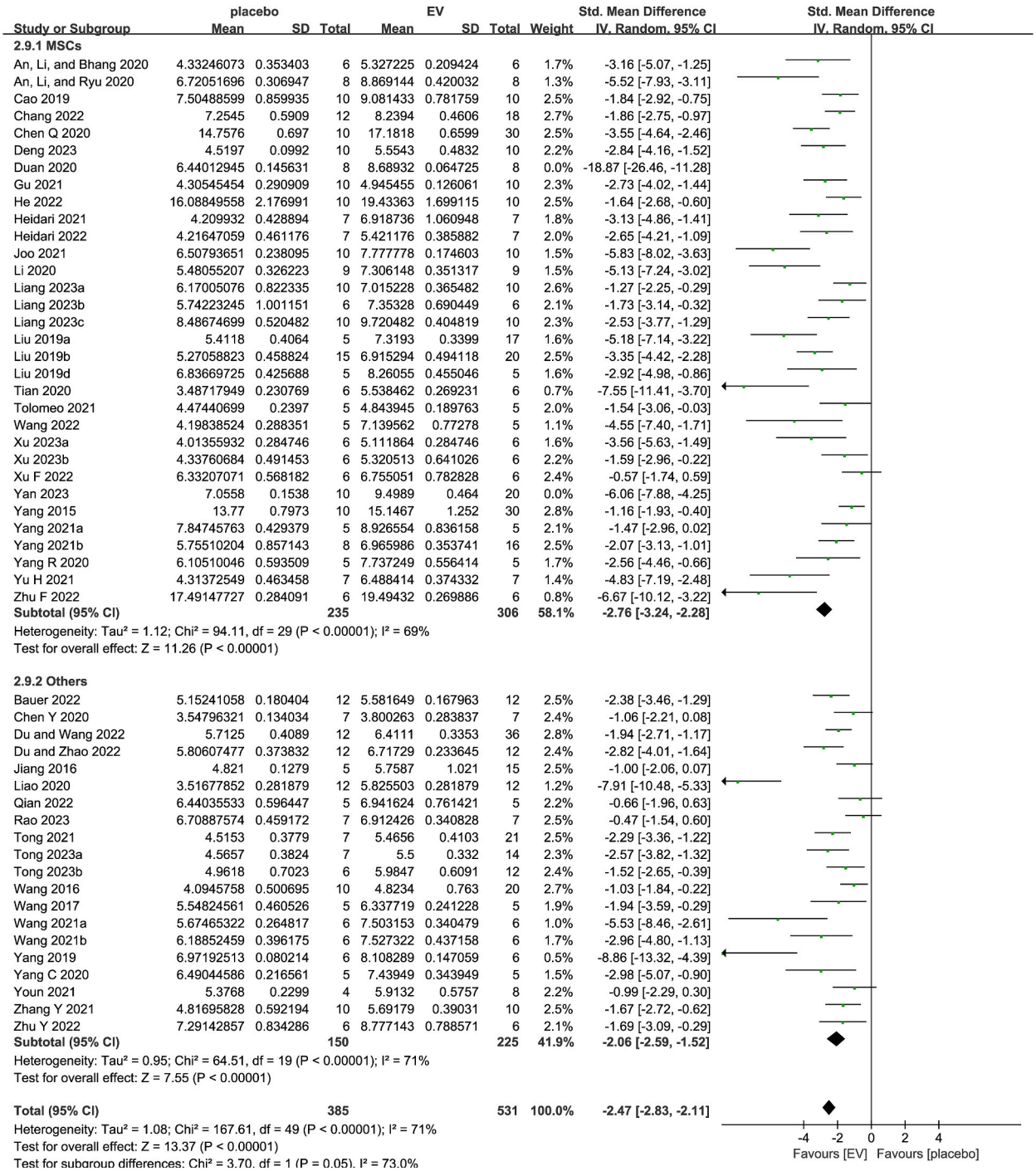

Fig. 9. Forest plot of the efficacy of EVs in increasing the colon length.

conducted and I^2 was reduced to 73 by excluding one of the two studies.

Finally, we included 42 studies in the meta-analysis with a total of 541 animals in the MSC subgroup and 375 animals in the non-MSC subgroup. As shown in Fig. 9, EVs had a significant effect on increasing the colon length regardless of their source (in the total: SMD = −2.47, 95 % CI: −2.83 to −2.11, $p < 0.00001$; in the MSCs subgroup: SMD = −2.76, 95 % CI -3.24 to −2.28, $p < 0.00001$; in the non-MSCs subgroup: SMD = −2.06, 95 % CI -2.59 to −1.52, $p < 0.00001$). The certainty of the evidence was low.

Due to some excluded studies from meta-analysis, a brief summary of results is provided as shown in Table 4. The results indicate that EVs could reduce shortening of the colon.

Regarding heterogeneity analysis results, it is noted that one excluded study may have caused increased heterogeneity due to its administration method [81]. The other study may have resulted in increased heterogeneity because it involved concurrent use of two different MSC-derived EVs [35].

3.6.4.2. Studies that characterized EVs based on MISEV2018. We also conducted a meta-analysis of studies that strictly characterized EVs according to MISEV2018 guidelines. A total of 7 studies were included in the meta-analysis. The results revealed high heterogeneity in the subgroup of MSC-derived EVs ($I^2 = 85$). Further analysis showed that excluding one study [81] would decrease the heterogeneity to 65 in the MSC-derived EVs subgroup (Fig. 10), possibly due to differences in route of administration as previously described.

Therefore, we included 6 studies, involving 66 animals treated with MSC-derived EVs and 111 animals treated with non-MSCs-derived EVs (in the total: SMD = −2.07, 95 % CI: −2.64 to −1.50, $p < 0.00001$; in the MSCs subgroup: SMD = −2.28, 95 % CI -3.46 to −1.11, $p = 0.0001$; in the non-MSCs subgroup: SMD = −2.01, 95 % CI -2.68 to −1.34, $p < 0.00001$).

3.6.5. Histological score

3.6.5.1. All included studies. Out of the 69 analyzed studies, only 2 did not report the outcome of histological staining, and 25 studies reported HE staining outcomes without a histological score. Except for one [69], the remaining studies (n = 66, 99 %) all reported that EVs could alleviate pathological changes and reduce the histological scores.

A meta-analysis was conducted on 36 studies, which included 474 animals treated with MSC-derived EVs and 298 animals treated with non-MSCs-derived EVs. As shown in Fig. 11, there was high heterogeneity in the data ($I^2 = 80$ in total and the MSC-derived EVs subgroup). The heterogeneity analysis indicated that no single study had a significant influence on the outcome.

The results of the meta-analysis showed that the non-MSCs-derived EVs could decrease the histological scores significantly (in the total: SMD = −2.61, 95 % CI: −3.11 to −2.11, $p < 0.00001$; in the MSCs subgroup: SMD = −3.11, 95 % CI -3.79 to −2.42, $p < 0.00001$; in the non-MSCs subgroup: SMD = −1.93, 95 % CI -2.56 to −1.30, $p < 0.00001$). The certainty of the evidence was low.

Due to high heterogeneity, we conducted a descriptive summary of results (Table 5).

3.6.5.2. Studies that characterized EVs based on MISEV2018. Another meta-analysis, strictly following the MISEV2018 guidelines, was conducted. A total of 5 studies were included in this analysis. The findings indicated that regardless of the source, EVs were effective in reducing histological scores. However, there was high heterogeneity observed in the subgroup of MSC-derived EVs ($I^2 = 81$). It was found that excluding one study [81] resulted in a decrease of heterogeneity to 0. As previously mentioned, the administration methods used in these studies may be a potential source of heterogeneity.

Finally, a total of 4 studies involving 66 animals treated with MSC-derived EVs and 63 animals treated with non-MSCs-derived EVs were included. As depicted in Fig. 12, it was evident that EVs had a significant effect on decreasing histological scores (in the total: SMD = −2.06, 95 % CI: −2.51 to −1.61, $p < 0.00001$; in the MSCs subgroup: SMD = −1.92, 95 % CI -2.53 to −1.30, $p < 0.00001$; in the non-MSCs subgroup: SMD = −2.24, 95 % CI -2.91 to −1.56, $p < 0.00001$).

3.6.6. Body weight

Of the 69 studies reviewed, 58 reported a change in body weight. With the exception of four studies [37,68,69,72], nearly all of the studies (n = 54, 93 %) indicated that EVs could improve body weight loss.

A total of 17 studies were eligible for meta-analysis, which involved 180 animals treated with MSC-derived EVs and 68 animals treated with other-derived EVs. As depicted in Fig. 13, the use of EVs led to a significant reduction in body weight loss (in the total:

Table 4
Summary of the colon length results.

EV types	Results	n	Percent
Naïve	Increased colon length	33	52 %
Modified	Increased colon length	7	11 %
	No effect	1	2 %
Naïve and modified	Both increased colon length	1	2 %
	Both increased colon length but modified EVs had a better effect	8	13 %
	Only naïve EVs increased colon length	6	10 %
	Only modified EVs increased colon length	6	10 %
	No effect	1	2 %

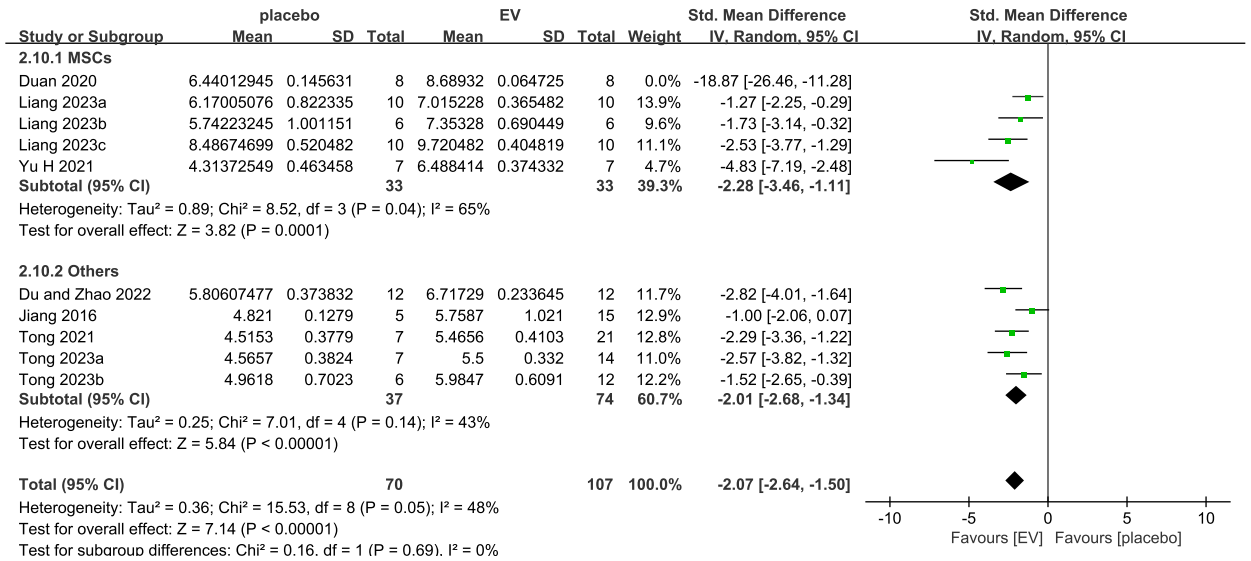

Fig. 10. Forest plot of colon length of studies that characterized EV based on MISEV2018.

SMD = −3.04, 95 % CI: −3.83 to −2.25, $p < 0.00001$; in the MSCs subgroup: SMD = −3.68, 95 % CI -4.76 to −2.59, $p < 0.00001$; in the non-MSCs subgroup: SMD = −2.06, 95 % CI -3.10 to −1.03, $p < 0.0001$). The certainty of the evidence was very low.

Due to variations in how weight change was reported across studies, some were not included in the meta-analysis; therefore, we provided a brief summary of the results of weight changes as shown in Table 6.

3.6.7. Publication bias

The funnel plot was utilized to assess publication bias for the primary outcomes. As depicted in Fig. 14, there is no indication of publication bias.

3.6.8. Sensitivity analysis

We conducted a sensitivity analysis for each meta-analysis and did not observe any significant changes in the outcomes.

4. Discussion

4.1. General interpretation of the results

We conducted a systematic review of the available evidence on the therapeutic efficacy and safety of EVs in animal studies. A total of 69 studies were included, but only 54 studies were used for meta-analysis to assess the effects of naïve EV treatment according to our eligibility criteria.

UC is a chronic and refractory disease that requires therapy to induce and maintain remission. The initial treatment should focus on normalizing bowel frequency, controlling bleeding and urgency, as well as promoting mucosal healing and histologic remission, which have been associated with improved clinical outcomes [99,100]. In the study, we utilized DAI scores and weight changes to represent clinical symptoms, while CMDI, colon length, and histological scores were used to represent pathological results. Consistent with a previous systematic review [101] assessing the efficacy of EVs for colitis, our findings indicated that naïve EVs was remarkably effective in alleviating symptoms and improving pathology.

Most studies have indicated that EVs play a role in regulating immunity and anti-inflammatory responses. Additionally, some research suggests that EVs contribute to repairing the intestinal mucosal barrier, regulating intestinal microbiota, and alleviating oxidative stress. One study [88] proposes that EVs can alleviate ulcerative colitis by inhibiting neddylation, while another study [91] suggests their potential use in treating ulcerative colitis through ubiquitination. Two studies [85,93] have highlighted the ability of extracellular vesicles to inhibit pyroptosis and exert therapeutic effects. Furthermore, one study [84] has suggested that EVs may improve UC by regulating lymphangiogenesis. Finally, two other studies [68,72] have mentioned that EVs alleviate UC by the inhibition of ferroptosis.

We had originally planned to perform subgroup analyses based on the source of EVs (MSCs vs. others), given that MSC transplantation has become the most widely used stem cell therapy for IBD due to several advantages [102]. Our results showed that both subgroups were effective in treating UC; however, there was high heterogeneity. Although we conducted some analysis on possible source of heterogeneity, it was not comprehensive enough. Additionally, differences in methodological design and lack of raw data reporting may also contribute to heterogeneity.

EVs have been extensively researched as drug transporters due to their ability to enter a cell through various cellular pathways,

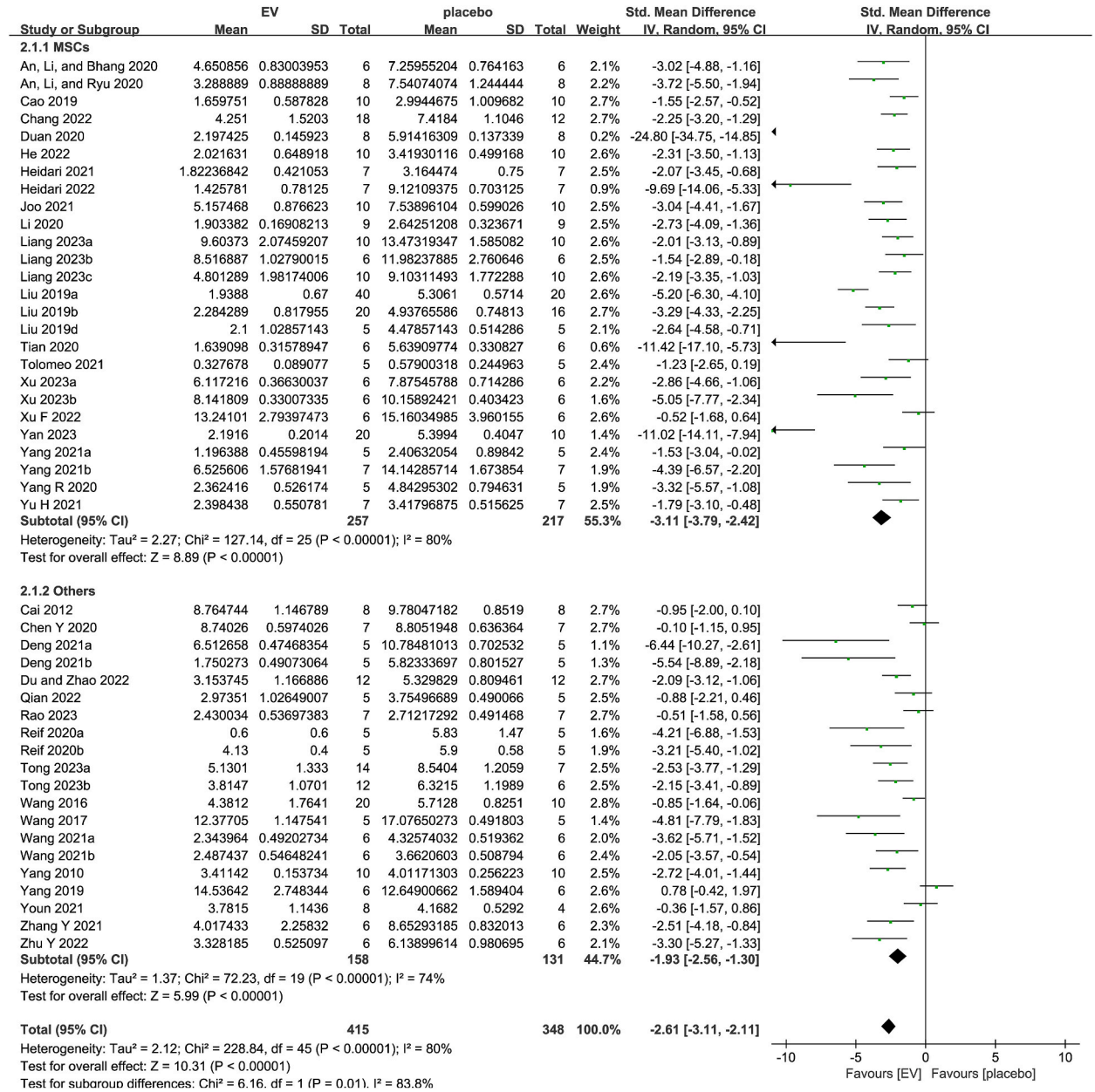

Fig. 11. Forest plot of the efficacy of EVs in decreasing the histological scores.

Table 5
Summary of the histological score results.

EV types	Results	n	Percent
Naïve	Reduced the histological damage	34	51 %
Modified	Reduced the histological damage	7	10 %
	No effect	1	1 %
Naïve and modified	Both reduced the histological damage	1	1 %
	Both reduced the histological damage but modified EVs had a better effect	9	13 %
	Only naïve EVs reduced the histological damage	6	9 %
	Only modified EVs reduced the histological damage	9	13 %

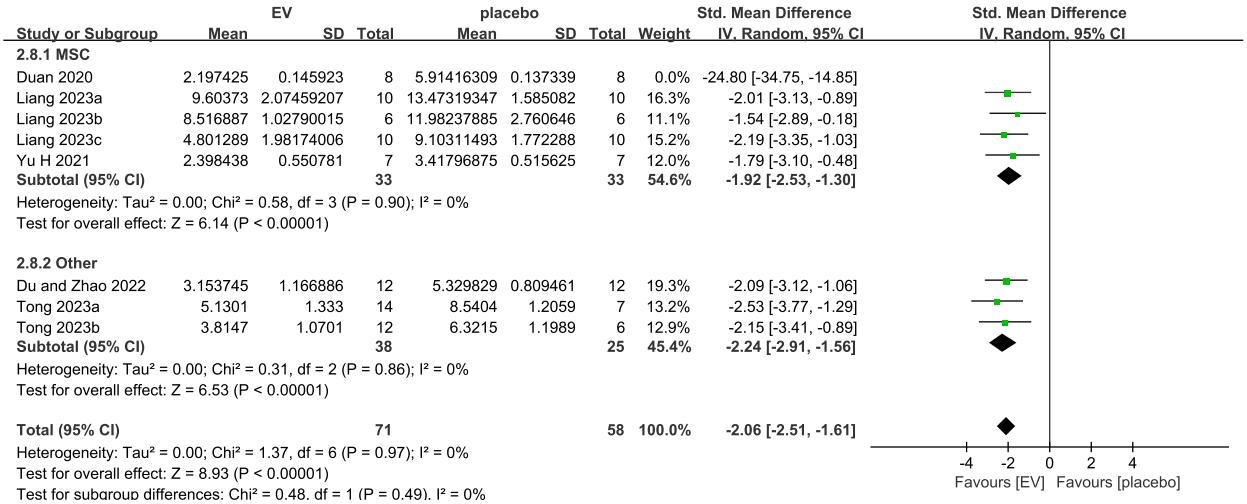

Fig. 12. Forest plot of histological scores of studies that characterized EV based on MISEV2018.

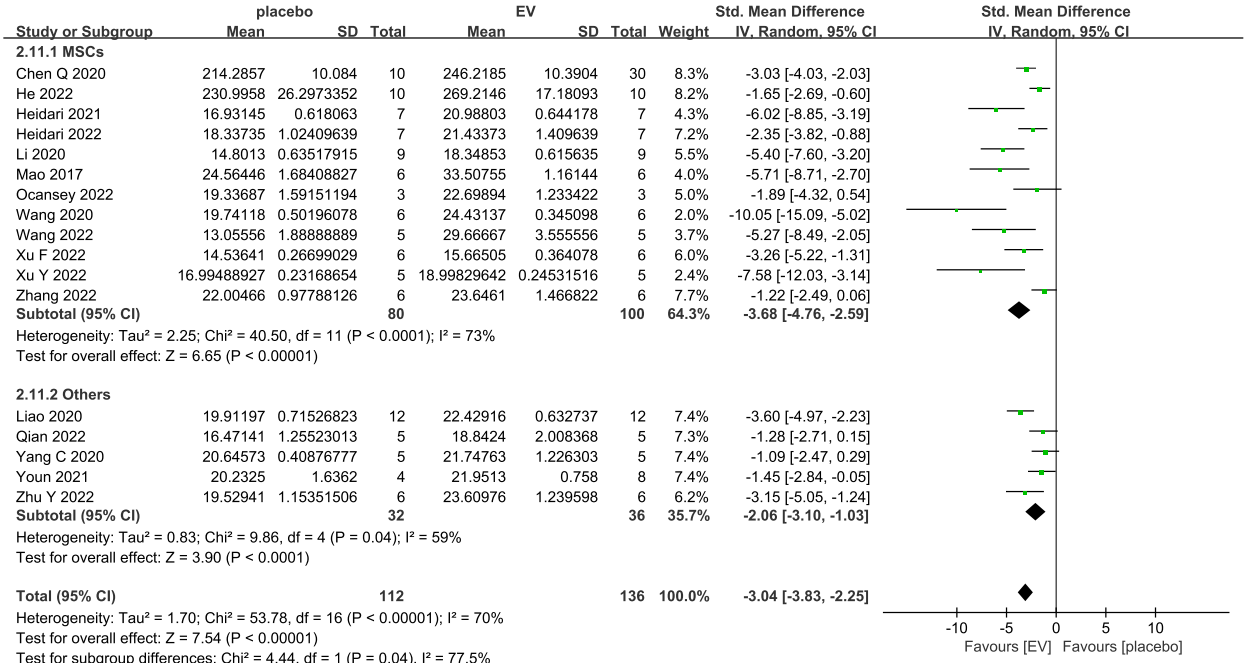

Fig. 13. Forest plot of the efficacy of EVs in decreasing the body weight loss.

Table 6
Summary of the body weight results.

EV types	Results	n	Percent
Naïve	Reduced body weight loss	27	47 %
	No effect	2	3 %
Modified	Reduced body weight loss	6	10 %
	No effect	1	2 %
Naïve and modified	Both reduced body weight loss	3	5 %
	Both reduced body weight loss but modified EVs had a better effect	4	7 %
	Only naïve EVs reduced body weight loss	5	9 %
	Only modified EVs reduced body weight loss	9	16 %
	No effect	1	2 %

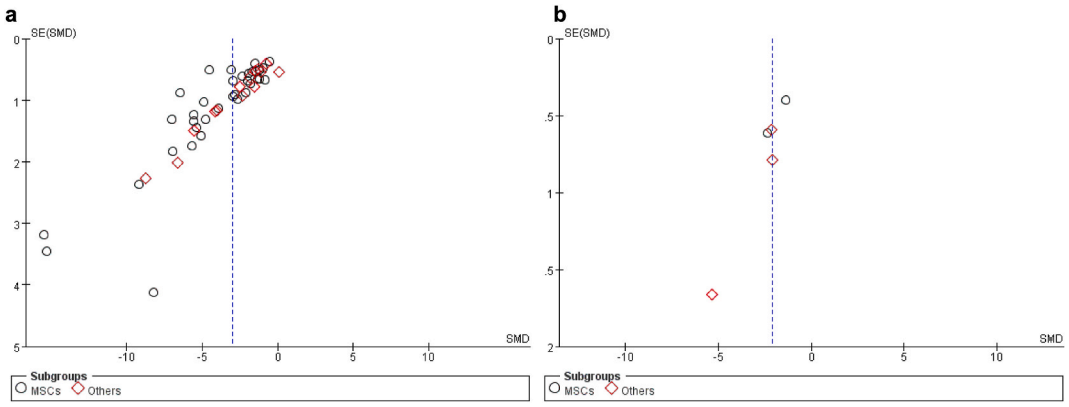

Fig. 14. (a) Funnel plot with the DAI scores. (b) Funnel plot with the CMDI.

excellent transcellular permeability, adaptability to various techniques applicable for loading, the possibility of transporting across the blood-brain barrier, and their biocompatibility [103]. The processing of EVs is utilized for mechanism research, enhancing therapeutic efficacy, or serving as carriers for drug delivery. Modified EVs showed greater effectiveness in our studies compared to naïve EVs. However, more than half of the studies using modified EVs did not assess the influence on EVs, which may be one of the potential mechanisms affecting therapeutic efficacy.

Animal experiments aim to find solutions to clinical problems; however, there are still hurdles in developing EV therapy. Factors such as the source, mechanisms, isolation procedure and storage conditions of EVs can all impact the translation of EV therapy [104]. Some of these challenges are also evident in the studies that we have included.

Various sources of EVs are considered to have an impact on UC treatment; however, identification of the source is still insufficient. Our review found that MSCs were the most commonly used source of EVs, but none of the studies strictly followed ISCT guidelines for identifying MSCs. Furthermore among 61 studies that harvested EV from medium, 24 did not describe their depletion method of exogenous EVs or even report specific culture media conditions. It is evident that researchers still pay insufficient attention to sourcing and handling methods for obtaining high-quality EVs.

As recommended in MISEV2018 [26], over half of the studies included in our analysis utilized a combination of methods for EV separation, which is advantageous for their isolation and concentration. However, only ten studies strictly adhered to the criteria outlined in the MISEV2018 when characterizing EVs. Interestingly, when we included only these ten studies in another meta-analysis, and the results indicated that heterogeneity was generally lower after conducting heterogeneity analysis. This suggests that incomplete characterization of EVs can impact the components present and thus influence the results, possibly contributing to the high heterogeneity observed in the original meta-analysis.

It is important to note that there is currently no standardized dosage for EVs, and just over half of the studies have labeled and tracked the EVs used. Most studies have only utilized intravenous administration, with oral delivery being preferred in chronic conditions like UC for improved patient compliance and comfort [105]. Only one study [43] compared therapeutic effects between intravenous and oral administration, reporting that orally administered processed EVs had better therapeutic effects. Therefore, further research is necessary to determine optimal dosage and administration route for EV therapy.

It has been discovered that EVs contain multiple biomolecules, making them significant for intercellular exchange. Despite many studies claiming their safety, there are still potential issues with their use. For example, most therapeutic EVs are produced by immortalized cell lines, which may carry carcinogens [106]. Out of the 69 studies evaluated, only 3 assessed the adverse effects. While all three showed no observed adverse effects caused by EVs, it is evident that there is still insufficient research on adverse effects in animal experiments. The lack of research on adverse effects is a challenge for the clinical translation of EV treatment.

As highlighted in a survey [107] over a decade ago, reporting omissions were still common in our included studies. Many details such as initial weight, sample size, randomized methods, implementation of blinding or not, and study design were not reported. As a result, most studies only received an unclear risk when conducting a bias risk assessment. The quality of the included studies will greatly affect the reliability of the conclusions [107,108], and potential biases caused by reporting omissions also pose a challenge for clinical translation or EV therapy.

4.2. Limitations of the evidence

After evaluating the quality of evidence in accordance with the GRADE guidelines, EVs may reduce DAI and histological score and increase colon length. The evidence is very uncertain about the effect of EVs on CMDI, adverse effect, and body weight. Downgrading is due to unclear bias risk, high heterogeneity, and small sample size.

The inadequate design and reporting of animal research has long been a problem, compounded by high data heterogeneity, publication bias, and other issues, leading to a generally low quality of evidence in animal experiments [109]. The ARRIVE guidelines [110] may offer some improvement in addressing this issue. In the included studies, insufficient methodological descriptions are

common, and the guidelines outline the essential minimum information that should be included in the manuscript. By strictly adhering to these guidelines, the quality of evidence may be enhanced.

4.3. Limitations of the review

This review has some potential limitations. Firstly, the data in the article are most extracted from online software rather than raw data, which may affect the statistical results. Secondly, the quality of evidence is low and the risk of bias is mostly unclear, leading to uncertainty in our conclusions. Finally, there are significant differences in modeling, intervention, and outcome measurement among different animal experiments. Although we have imposed some limitations on animal species, EV sources, and EV processing, there are still significant differences between them and the actual situation of human diseases.

4.4. Future prospects

While the results of this study demonstrate significant therapeutic effects of extracellular vesicles in animal experiments, there are still underlying limitations. It is possible that future research efforts adhering strictly to various guidelines will expedite the clarification of the efficacy and mechanism of action of EVs. Efficient, high-purity, and low-cost extraction and separation of extracellular vesicles are also future goals in the field. Although naïve EVs necessitate further investigation, modified EVs may offer enhanced effectiveness and safety as pharmaceutical agents. Therefore, there may be an increase in research on the processing of EVs in the future. In addition, due to the prolonged course of ulcerative colitis, long-term medication may be necessary. Therefore, while most animal experiments use intravenous administration, oral administration may be more suitable for future clinical practice. Research on the transformation of administration methods is also crucial.

5. Conclusion

In summary, EVs has been found to be effective in animal models of UC, regardless of their sources. However, there are still some deficiencies in the experimental design, which makes it challenging to translate the findings into clinical applications. Animal experiments have demonstrated the efficacy and potential of EVs, which is undoubtedly good news for future clinical medication. However, the quality of the evidence and the risk of bias are not optimistic. It may be helpful if future research strictly adheres to the requirements of randomized controlled trials and guidelines. It is also important to not only encourage therapeutic studies but also pay attention to the potential adverse effects caused by EVs. We believe that EV therapy holds promising prospects for difficult-to-treat diseases like UC.

Abbreviations

EVs	extracellular vesicles
UC	ulcerative colitis
DAI	disease activity index
CMDI	colonic mucosal damage index
MISEV2018	Minimal information for studies of extracellular vesicles 2018
IBD	inflammatory bowel disease
5-ASA	5-aminosalicylic acid
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PROSPERO	International Prospective Register of Systematic Reviews
GRADE	Grading of Recommendations Assessment, Development and Evaluation
MSCs	mesenchymal stem cells
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
RoB	risk of bias
ISCT	International Society for Cell & Gene Therapy
SMD	standardized mean difference
UF	ultrafiltration
SEC	size exclusion chromatography
TEM	transmission electron microscopy
NTA	nanoparticle tracking analysis
TRPS	Tunable Resistive Pulse Sensing
ISEV	International Society for Extracellular Vesicles
MSC-sEVs-PD-L1	PD-L1 overexpression MSC-derived extracellular vesicles

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability statement

The data associated with the study has not been deposited into a publicly available repository. All data generated or analyzed during this study are sourced from cited literature and included in this published article and supplementary material.

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CRediT authorship contribution statement

Yu-Jing Li: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Ze-Yu Yu:** Writing – review & editing, Investigation, Data curation. **Di Zhang:** Investigation, Formal analysis. **Fu-Rong Zhang:** Investigation. **Dong-Mei Zhang:** Writing – review & editing. **Meng Chen:** Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36890.

References

[1] S. Flynn, S. Eisenstein, Inflammatory bowel disease presentation and diagnosis, Surg. Clin. 99 (6) (2019) 1051–1062, https://doi.org/10.1016/j.suc.2019.08.001.
[2] I. Ordás, L. Eckmann, M. Talamini, D.C. Baumgart, W.J. Sandborn, Ulcerative colitis, Lancet (London, England) 380 (9853) (2012) 1606–1619, https://doi.org/10.1016/s0140-6736(12)60150-0.
[3] J.P. Segal, J.F. LeBlanc, A.L. Hart, Ulcerative colitis: an update, Clin. Med. 21 (2) (2021) 135–139, https://doi.org/10.7861/clinmed.2021-0080.
[4] L. Du, C. Ha, Epidemiology and pathogenesis of ulcerative colitis, Gastroenterol. Clin. N. Am. 49 (4) (2020) 643–654, https://doi.org/10.1016/j.gtc.2020.07.005.
[5] C. Le Berre, S. Honap, L. Peyrin-Biroulet, Ulcerative colitis, Lancet (London, England) 402 (10401) (2023) 571–584, https://doi.org/10.1016/s0140-6736(23)00966-2.
[6] National Institute for Health and Care Excellence: Guidelines, Ulcerative Colitis: Management, London: National Institute for Health and Care Excellence (NICE) Copyright © NICE, 2019.; 2019.
[7] S. Moghassemi, A. Dadashzadeh, M.J. Sousa, H. Vlieghe, J. Yang, C.M. León-Félix, et al., Extracellular vesicles in nanomedicine and regenerative medicine: a review over the last decade, Bioact. Mater. 36 (2024) 126–156, https://doi.org/10.1016/j.bioactmat.2024.02.021.
[8] Y. Takahashi, Y. Takakura, Extracellular vesicle-based therapeutics: extracellular vesicles as therapeutic targets and agents, Pharmacol. Therapeut. 242 (2023) 108352, https://doi.org/10.1016/j.pharmthera.2023.108352.
[9] R. Franzin, A. Stasi, G. De Palma, A. Picerno, C. Curci, S. Sebastiano, et al., Human adult renal progenitor cells prevent cisplatin-nephrotoxicity by inducing CYP1B1 overexpression and miR-27b-3p down-regulation through extracellular vesicles, Cells 12 (12) (2023), https://doi.org/10.3390/cells12121655.
[10] N. Shuwari, C. Inoue, I. Ishigami, K. Jingushi, M. Kamiya, S. Kawakami, et al., Small extracellular vesicles carrying reovirus, tumor antigens, interferon-β, and damage-associated molecular patterns for efficient tumor treatment, J. Contr. Release : official journal of the Controlled Release Society 374 (2024) 89–102, https://doi.org/10.1016/j.jconrel.2024.07.079.
[11] A. Rizzo, A.D. Ricci, S. Tavolari, G. Brandi, Circulating tumor DNA in biliary tract cancer: current evidence and future perspectives, Cancer Genomics Proteomics 17 (5) (2020) 441–452, https://doi.org/10.21873/cgp.20203.
[12] W. Zhang, M. Ou, P. Yang, M. Ning, The role of extracellular vesicle immune checkpoints in cancer, Clin. Exp. Immunol. 216 (3) (2024) 230–239, https://doi.org/10.1093/cei/uxae026.
[13] F.G. Dall'Olio, A. Rizzo, V. Mollica, M. Massucci, I. Maggio, F. Massari, Immortal time bias in the association between toxicity and response for immune checkpoint inhibitors: a meta-analysis, Immunotherapy 13 (3) (2021) 257–270, https://doi.org/10.2217/imt-2020-0179.
[14] D.C. Guven, T.K. Sahin, E. Erul, A. Rizzo, A.D. Ricci, S. Aksoy, et al., The association between albumin levels and survival in patients treated with immune checkpoint inhibitors: a systematic review and meta-analysis, Front. Mol. Biosci. 9 (2022) 1039121, https://doi.org/10.3389/fmolb.2022.1039121.
[15] T.K. Sahin, A. Rizzo, S. Aksoy, D.C. Guven, Prognostic significance of the royal marsden hospital (RMH) score in patients with cancer: a systematic review and meta-analysis, Cancers 16 (10) (2024), https://doi.org/10.3390/cancers16101835.

[16] C. Genova, S. Marconi, G. Chiorino, F. Guana, P. Ostano, S. Santamaria, et al., Extracellular vesicles miR-574-5p and miR-181a-5p as prognostic markers in NSCLC patients treated with nivolumab, Clin. Exp. Med. 24 (1) (2024) 182, https://doi.org/10.1007/s10238-024-01427-8.
[17] B. Boonkaew, N. Satthawiwat, N. Pinjaroen, N. Chuaypen, P. Tangkijvanich, Circulating extracellular vesicle-derived microRNAs as novel diagnostic and prognostic biomarkers for non-viral-related hepatocellular carcinoma, Int. J. Mol. Sci. 24 (22) (2023), https://doi.org/10.3390/ijms242216043.
[18] E.I. Buzas, The roles of extracellular vesicles in the immune system, Nat. Rev. Immunol. 23 (4) (2023) 236–250, https://doi.org/10.1038/s41577-022-00763-8.
[19] Q. Shen, Z. Huang, J. Yao, Y. Jin, Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease, J. Adv. Res. 37 (2022) 221–233, https://doi.org/10.1016/j.jare.2021.07.002.
[20] X. Liang, N. Dai, K. Sheng, H. Lu, J. Wang, L. Chen, et al., Gut bacterial extracellular vesicles: important players in regulating intestinal microenvironment, Gut Microb. 14 (1) (2022) 2134689, https://doi.org/10.1080/19490976.2022.2134689.
[21] D. Levy, T.J. Solomon, S.M. Jay, Extracellular vesicles as therapeutics for inflammation and infection, Curr. Opin. Biotechnol. 85 (2024) 103067, https://doi.org/10.1016/j.copbio.2024.103067.
[22] M.J. Page, J.E. McKenzie, P.M. Bossuyt, I. Boutron, T.C. Hoffmann, C.D. Mulrow, et al., The PRISMA 2020 statement: an updated guideline for reporting systematic reviews, BMJ (Clinical research ed) 372 (2021) n71, https://doi.org/10.1136/bmj.n71.
[23] D. Drevon, S.R. Fursa, A.L. Malcolm, Intercoder reliability and validity of WebPlotDigitizer in extracting graphed data, Behav. Modif. 41 (2) (2017) 323–339, https://doi.org/10.1177/0145445516673998.
[24] C.R. Hooijmans, M.M. Rovers, R.B. de Vries, M. Leenaars, M. Ritskes-Hoitinga, M.W. Langendam, SYRCLE's risk of bias tool for animal studies, BMC Med. Res. Methodol. 14 (2014) 43, https://doi.org/10.1186/1471-2288-14-43.
[25] S. Viswanathan, Y. Shi, J. Galipeau, M. Krampera, K. Leblanc, I. Martin, et al., Mesenchymal stem versus stromal cells: international society for cell & gene therapy (ISCT®) mesenchymal stromal cell committee position statement on nomenclature, Cytotherapy 21 (10) (2019) 1019–1024, https://doi.org/10.1016/j.jcyt.2019.08.002.
[26] C. Théry, K.W. Witwer, E. Aikawa, M.J. Alcaraz, J.D. Anderson, R. Andriantsitohaina, et al., Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, J. Extracell. Vesicles 7 (1) (2018) 1535750, https://doi.org/10.1080/20013078.2018.1535750.
[27] G. Guyatt, A.D. Oxman, E.A. Akl, R. Kunz, G. Vist, J. Brozek, et al., GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables, J. Clin. Epidemiol. 64 (4) (2011) 383–394, https://doi.org/10.1016/j.jclinepi.2010.04.026.
[28] C. Du, S. Quan, Y. Zhao, X. Nan, R. Chen, X. Tang, et al., Bovine milk-derived extracellular vesicles prevent gut inflammation by regulating lipid and amino acid metabolism, Food Funct. 14 (4) (2023) 2212–2222, https://doi.org/10.1039/d2fo03975c.
[29] C. Du, Y. Zhao, K. Wang, X. Nan, R. Chen, B. Xiong, Effects of milk-derived extracellular vesicles on the colonic transcriptome and proteome in murine model, Nutrients 14 (15) (2022), https://doi.org/10.3390/nu14153057.
[30] X. Liang, C. Li, J. Song, A. Liu, C. Wang, W. Wang, et al., HucMSC-exo promote mucosal healing in experimental colitis by accelerating intestinal stem cells and epithelium regeneration via Wnt signaling pathway, Int. J. Nanomed. 18 (2023) 2799–2818, https://doi.org/10.2147/ijn.S402179.
[31] S. Yang, X. Liang, J. Song, C. Li, A. Liu, Y. Luo, et al., A novel therapeutic approach for inflammatory bowel disease by exosomes derived from human umbilical cord mesenchymal stem cells to repair intestinal barrier via TSG-6, Stem Cell Res. Ther. 12 (1) (2021) 315, https://doi.org/10.1186/s13287-021-02404-8.
[32] L. Tong, S. Zhang, Q. Liu, C. Huang, H. Hao, M.S. Tan, et al., Milk-derived extracellular vesicles protect intestinal barrier integrity in the gut-liver axis, Sci. Adv. 9 (15) (2023) eade5041, https://doi.org/10.1126/sciadv.ade5041.
[33] H. Xu, J. Fu, L. Chen, S. Zhou, Y. Fang, Q. Zhang, et al., TNF-A enhances the therapeutic effects of MenSC-derived small extracellular vesicles on inflammatory bowel disease through macrophage polarization by miR-24-3p, Stem Cell. Int. 2023 (2023) 2988907, https://doi.org/10.1155/2023/2988907.
[34] H. Liu, Z. Liang, F. Wang, C. Zhou, X. Zheng, T. Hu, et al., Exosomes from mesenchymal stromal cells reduce murine colonic inflammation via a macrophage-dependent mechanism, JCI insight 4 (24) (2019), https://doi.org/10.1172/jci.insight.131273.
[35] Y. Yan, K. Li, J. Jiang, L. Jiang, X. Ma, F. Ai, et al., Perinatal tissue-derived exosomes ameliorate colitis in mice by regulating the Foxp3 + Treg cells and gut microbiota, Stem Cell Res. Ther. 14 (1) (2023) 43, https://doi.org/10.1186/s13287-023-03263-1.
[36] Y. Wang, J. Tian, X. Tang, K. Rui, X. Tian, J. Ma, et al., Exosomes released by granulocytic myeloid-derived suppressor cells attenuate DSS-induced colitis in mice, Oncotarget 7 (13) (2016) 15356–15368, https://doi.org/10.18632/oncotarget.7324.
[37] S. Reif, Y. Elbaum-Shiff, N. Koroukhov, I. Shilo, M. Musseri, R. Golan-Gerstl, Cow and human milk-derived exosomes ameliorate colitis in DSS murine model, Nutrients 12 (9) (2020), https://doi.org/10.3390/nu12092589.
[38] C. Yang, M. Zhang, J. Sung, L. Wang, Y. Jung, D. Merlin, Autologous exosome transfer: a new personalised treatment concept to prevent colitis in a murine model, Journal of Crohn's & colitis 14 (6) (2020) 841–855, https://doi.org/10.1093/ecco-jcc/jjz184.
[39] Y. Cao, Z. Wang, Y. Yan, L. Ji, J. He, B. Xuan, et al., Enterotoxigenic bacteroidesfragilis promotes intestinal inflammation and malignancy by inhibiting exosome-packaged miR-149-3p, Gastroenterology 161 (5) (2021), https://doi.org/10.1053/j.gastro.2021.08.003, 1552-66.e12.
[40] F. Zhu, C. Wei, H. Wu, B. Shuai, T. Yu, F. Gao, et al., Hypoxic mesenchymal stem cell-derived exosomes alleviate ulcerative colitis injury by limiting intestinal epithelial cells reactive oxygen species accumulation and DNA damage through HIF-1α, Int. Immunopharm. 113 (Pt A) (2022) 109426 https://doi.org/10.1016/j.intimp.2022.109426.
[41] L. Gu, F. Ren, X. Fang, L. Yuan, G. Liu, S. Wang, Exosomal MicroRNA-181a derived from mesenchymal stem cells improves gut microbiota composition, barrier function, and inflammatory status in an experimental colitis model, Front. Med. 8 (2021) 660614, https://doi.org/10.3389/fmed.2021.660614.
[42] Y. Chen, J. Huang, H. Li, P. Li, C. Xu, Serum exosomes derived from Hp-positive gastritis patients inhibit MCP-1 and MIP-1α expression via NLRP12-Notch signaling pathway in intestinal epithelial cells and improve DSS-induced colitis in mice, Int. Immunopharm. 88 (2020) 107012, https://doi.org/10.1016/j.intimp.2020.107012.
[43] C. Deng, Y. Hu, M. Conceição, M.J.A. Wood, H. Zhong, Y. Wang, et al., Oral delivery of layer-by-layer coated exosomes for colitis therapy, J. Contr. Release : official journal of the Controlled Release Society 354 (2023) 635–650, https://doi.org/10.1016/j.jconrel.2023.01.017.
[44] Q. Rao, G. Ma, M. Li, H. Wu, Y. Zhang, C. Zhang, et al., Targeted delivery of triptolide by dendritic cell-derived exosomes for colitis and rheumatoid arthritis therapy in murine models, Br. J. Pharmacol. 180 (3) (2023) 330–346, https://doi.org/10.1111/bph.15958.
[45] J.H. An, Q. Li, M.O. Ryu, A.R. Nam, D.H. Bhang, Y.C. Jung, et al., TSG-6 in extracellular vesicles from canine mesenchymal stem/stromal is a major factor in relieving DSS-induced colitis, PLoS One 15 (2) (2020) e0220756, https://doi.org/10.1371/journal.pone.0220756.
[46] S. Zhang, Y. Dong, Y. Wang, W. Sun, M. Wei, L. Yuan, et al., Selective encapsulation of therapeutic mRNA in engineered extracellular vesicles by DNA aptamer, Nano Lett. 21 (20) (2021) 8563–8570, https://doi.org/10.1021/acs.nanolett.1c01817.
[47] J.H. An, Q. Li, D.H. Bhang, W.J. Song, H.Y. Youn, TNF-α and INF-γ primed canine stem cell-derived extracellular vesicles alleviate experimental murine colitis, Sci. Rep. 10 (1) (2020) 2115, https://doi.org/10.1038/s41598-020-58909-4.
[48] H. Chen, S. Kasagi, C. Chia, D. Zhang, E. Tu, R. Wu, et al., Extracellular vesicles from apoptotic cells promote TGFβ production in macrophages and suppress experimental colitis, Sci. Rep. 9 (1) (2019) 5875, https://doi.org/10.1038/s41598-019-42063-7.
[49] A.M. Tolomeo, I. Castagliuolo, M. Piccoli, M. Grassi, F. Magarotto, G. De Lazzari, et al., Extracellular vesicles secreted by mesenchymal stromal cells exert opposite effects to their cells of origin in murine sodium dextran sulfate-induced colitis, Front. Immunol. 12 (2021) 627605, https://doi.org/10.3389/fimmu.2021.627605.
[50] W. Qian, L. Huang, Y. Xu, W. Lu, W. Wen, Z. Guo, et al., Hypoxic ASCs-derived exosomes attenuate colitis by regulating macrophage polarization via miR-216a-5p/HMGB1 Axis, Inflamm. Bowel Dis. 29 (4) (2023) 602–619, https://doi.org/10.1093/ibd/izac225.
[51] X. Yang, S. Meng, H. Jiang, T. Chen, W. Wu, Exosomes derived from interleukin-10-treated dendritic cells can inhibit trinitrobenzene sulfonic acid-induced rat colitis, Scand. J. Gastroenterol. 45 (10) (2010) 1168–1177, https://doi.org/10.3109/00365521.2010.490596.
[52] T. Yu, S. Chu, X. Liu, J. Li, Q. Chen, M. Xu, et al., Extracellular vesicles derived from EphB2-overexpressing bone marrow mesenchymal stem cells ameliorate DSS-induced colitis by modulating immune balance, Stem Cell Res. Ther. 12 (1) (2021) 181, https://doi.org/10.1186/s13287-021-02232-w.

[53] R. Yang, H. Huang, S. Cui, Y. Zhou, T. Zhang, Y. Zhou, IFN-γ promoted exosomes from mesenchymal stem cells to attenuate colitis via miR-125a and miR-125b, Cell Death Dis. 11 (7) (2020) 603, https://doi.org/10.1038/s41419-020-02788-0.

[54] L. Wang, Z. Yu, S. Wan, F. Wu, W. Chen, B. Zhang, et al., Exosomes derived from dendritic cells treated with schistosoma japonicum soluble egg antigen attenuate DSS-induced colitis, Front. Pharmacol. 8 (2017) 651, https://doi.org/10.3389/fphar.2017.00651.

[55] R. Yang, Y. Liao, L. Wang, P. He, Y. Hu, D. Yuan, et al., Exosomes derived from M2b macrophages attenuate DSS-induced colitis, Front. Immunol. 10 (2019) 2346, https://doi.org/10.3389/fimmu.2019.02346.

[56] H. Joo, M.K. Oh, J.Y. Kang, H.S. Park, D.H. Chae, J. Kim, et al., Extracellular vesicles from thapsigargin-treated mesenchymal stem cells ameliorated experimental colitis via enhanced immunomodulatory properties, Biomedicines 9 (2) (2021), https://doi.org/10.3390/biomedicines9020209.

[57] Y. Zhang, Y. Yan, J. Meng, M. Girotra, S. Ramakrishnan, S. Roy, Immune modulation mediated by extracellular vesicles of intestinal organoids is disrupted by opioids, Mucosal Immunol. 14 (4) (2021) 887–898, https://doi.org/10.1038/s41385-021-00392-9.

[58] Z.J. Ma, Y.H. Wang, Z.G. Li, Y. Wang, B.Y. Li, H.Y. Kang, et al., Immunosuppressive effect of exosomes from mesenchymal stromal cells in defined medium on experimental colitis, International journal of stem cells 12 (3) (2019) 440–448, https://doi.org/10.15283/ijsc18139.

[59] H. Liu, J. Liang, Y. Zhong, G. Xiao, T. Efferth, M.I. Georgiev, et al., Dendrobium officinale polysaccharide alleviates intestinal inflammation by promoting small extracellular vesicle packaging of miR-433-3p, J. Agric. Food Chem. 69 (45) (2021) 13510–13523, https://doi.org/10.1021/acs.jafc.1c05134.

[60] K.M. Bauer, M.C. Nelson, W.W. Tang, T.R. Chiaro, D.G. Brown, A. Ghazaryan, et al., CD11c+ myeloid cell exosomes reduce intestinal inflammation during colitis, JCI insight 7 (19) (2022), https://doi.org/10.1172/jci.insight.159469.

[61] F. Liao, X. Lu, W. Dong, Exosomes derived from T regulatory cells relieve inflammatory bowel disease by transferring miR-195a-3p, IUBMB Life (2020), https://doi.org/10.1002/iub.2385.

[62] H. He, Q. Chen, H. Fan, X.Y. Leng, F. Zhu, F. Gao, et al., Extracellular vesicles produced by bone marrow mesenchymal stem cells overexpressing programmed death-ligand 1 ameliorate dextran sodium sulfate-induced ulcerative colitis in rats by regulating Th17/Treg cell balance through PTEN/PI3K/AKT/mTOR axis, J. Gastroenterol. Hepatol. 37 (12) (2022) 2243–2254, https://doi.org/10.1111/jgh.15987.

[63] H. Wu, H. Fan, Z. Shou, M. Xu, Q. Chen, C. Ai, et al., Extracellular vesicles containing miR-146a attenuate experimental colitis by targeting TRAF6 and IRAK1, Int. Immunopharm. 68 (2019) 204–212, https://doi.org/10.1016/j.intimp.2018.12.043.

[64] Z.J. Cai, W. Zhang, F. Yang, L. Yu, Z. Yu, J.H. Pan, et al., Immunosuppressive exosomes from TGF-beta 1 gene-modified dendritic cells attenuate Th17-mediated inflammatory autoimmune disease by inducing regulatory T cells, Cell Res. 22 (3) (2012) 607–610, https://doi.org/10.1038/cr.2011.196.

[65] F. Deng, J. Yan, J. Lu, M. Luo, P. Xia, S. Liu, et al., M2 macrophage-derived exosomal miR-590-3p attenuates DSS-induced mucosal damage and promotes epithelial repair via the LATS1/YAP/β-catenin signalling Axis, Journal of Crohn's & colitis. 15 (4) (2021) 665–677, https://doi.org/10.1093/ecco-jcc/jjaa214.

[66] P. Li, H.Y. Zhang, J.Z. Gao, W.Q. Du, D. Tang, W. Wang, et al., Mesenchymal stem cells-derived extracellular vesicles containing miR-378a-3p inhibit the occurrence of inflammatory bowel disease by targeting GATA2, J. Cell Mol. Med. 26 (11) (2022) 3133–3146, https://doi.org/10.1111/jcmm.17176.

[67] F. Xu, Z. Fei, H. Dai, J. Xu, Q. Fan, S. Shen, et al., Mesenchymal stem cell-derived extracellular vesicles with high PD-L1 expression for autoimmune diseases treatment, Advanced materials (Deerfield Beach, Fla) 34 (1) (2022) e2106265, https://doi.org/10.1002/adma.202106265.

[68] Z. Wei, S. Hang, D.K. Wiredu Ocansey, Z. Zhang, B. Wang, X. Zhang, et al., Human umbilical cord mesenchymal stem cells derived exosome shuttling mir-129-5p attenuates inflammatory bowel disease by inhibiting ferroptosis, J. Nanobiotechnol. 21 (1) (2023) 188, https://doi.org/10.1186/s12951-023-01951-x.

[69] X. Chang, Y.H. Song, T. Xia, Z.X. He, S.B. Zhao, Z.J. Wang, et al., Macrophage-derived exosomes promote intestinal mucosal barrier dysfunction in inflammatory bowel disease by regulating TMIGD1 via mircroRNA-223, Int. Immunopharm. 121 (2023) 110447, https://doi.org/10.1016/j.intimp.2023.110447.

[70] N. Heidari, H. Abbasi-Kenarsari, S. Namaki, K. Baghaei, M.R. Zali, S. Ghaffari Khaligh, et al., Adipose-derived mesenchymal stem cell-secreted exosome alleviates dextran sulfate sodium-induced acute colitis by Treg cell induction and inflammatory cytokine reduction, J. Cell. Physiol. 236 (8) (2021) 5906–5920, https://doi.org/10.1002/jcp.30275.

[71] Q. Chen, X. Duan, M. Xu, H. Fan, Y. Dong, H. Wu, et al., BMSC-EVs regulate Th17 cell differentiation in UC via H3K27me3, Mol. Immunol. 118 (2020) 191–200, https://doi.org/10.1016/j.molimm.2019.12.019.

[72] Y. Zhu, H. Qin, C. Sun, B. Shao, G. Li, Y. Qin, et al., Endometrial regenerative cell-derived exosomes attenuate experimental colitis through downregulation of intestine ferroptosis, Stem Cell. Int. 2022 (2022) 3014123, https://doi.org/10.1155/2022/3014123.

[73] F. Mao, Y. Wu, X. Tang, J. Kang, B. Zhang, Y. Yan, et al., Exosomes derived from human umbilical cord mesenchymal stem cells relieve inflammatory bowel disease in mice, BioMed Res. Int. 2017 (2017) 5356760, https://doi.org/10.1155/2017/5356760.

[74] L. Cao, H. Xu, G. Wang, M. Liu, D. Tian, Z. Yuan, Extracellular vesicles derived from bone marrow mesenchymal stem cells attenuate dextran sodium sulfate-induced ulcerative colitis by promoting M2 macrophage polarization, Int. Immunopharm. 72 (2019) 264–274, https://doi.org/10.1016/j.intimp.2019.04.020.

[75] J. Yang, X.X. Liu, H. Fan, Q. Tang, Z.X. Shou, D.M. Zuo, et al., Extracellular vesicles derived from bone marrow mesenchymal stem cells protect against experimental colitis via attenuating colon inflammation, oxidative stress and apoptosis, PLoS One 10 (10) (2015) e0140551, https://doi.org/10.1371/journal.pone.0140551.

[76] H. Yu, X. Yang, X. Xiao, M. Xu, Y. Yang, C. Xue, et al., Human adipose mesenchymal stem cell-derived exosomes protect mice from DSS-induced inflammatory bowel disease by promoting intestinal-stem-cell and epithelial regeneration, Aging and disease 12 (6) (2021) 1423–1437, https://doi.org/10.14336/ad.2021.0601.

[77] Y. Li, J. Altemus, A.L. Lightner, Mesenchymal stem cells and acellular products attenuate murine induced colitis, Stem Cell Res. Ther. 11 (1) (2020) 515, https://doi.org/10.1186/s13287-020-02025-7.

[78] N. Heidari, H. Abbasi-Kenarsari, S. Namaki, K. Baghaei, M.R. Zali, Z. Mirsanei, et al., Regulation of the Th17/Treg balance by human umbilical cord mesenchymal stem cell-derived exosomes protects against acute experimental colitis, Exp. Cell Res. 419 (1) (2022) 113296, https://doi.org/10.1016/j.yexcr.2022.113296.

[79] O.K. Helal, A.E.L. Mansy, O.M. Mohamed, Radwan H. El Ahmed, The possible therapeutic effect of rebamipide and stem cell derivative microvesicles on experimentally provoked colitis in rats: histological and immuno hisochemical study, Egyptian Journal of Histology 43 (1) (2020) 340–352, https://doi.org/10.21608/ejh.2019.10575.1100.

[80] R.E. El-Desoky Mohamady, S.K. Elwia, S.M. Abo El Wafa, M.A. Mohamed, Effect of mesenchymal stem cells derived exosomes and green tea polyphenols on acetic acid induced ulcerative colitis in adult male albino rats, Ultrastruct. Pathol. 46 (2) (2022) 147–163, https://doi.org/10.1080/01913123.2022.2039825.

[81] L. Duan, H. Huang, X. Zhao, M. Zhou, S. Chen, C. Wang, et al., Extracellular vesicles derived from human placental mesenchymal stem cells alleviate experimental colitis in mice by inhibiting inflammation and oxidative stress, Int. J. Mol. Med. 46 (4) (2020) 1551–1561, https://doi.org/10.3892/ijmm.2020.4679.

[82] Y.X. Wang, C. Lin, L.J. Cui, T.Z. Deng, Q.M. Li, F.Y. Chen, et al., Mechanism of M2 macrophage-derived extracellular vesicles carrying lncRNA MEG3 in inflammatory responses in ulcerative colitis, Bioengineered 12 (2) (2021) 12722–12739, https://doi.org/10.1080/21655979.2021.2010368.

[83] Y. Wang, Y. Zhang, B. Lu, J. Xi, D.K.W. Ocansey, F. Mao, et al., hucMSC-ex alleviates IBD-associated intestinal fibrosis by inhibiting ERK phosphorylation in intestinal fibroblasts, Stem Cell. Int. 2023 (2023) 2828981, https://doi.org/10.1155/2023/2828981.

[84] L. Zhang, J. Yuan, D. Kofi Wiredu Ocansey, B. Lu, A. Wan, X. Chen, et al., Exosomes derived from human umbilical cord mesenchymal stem cells regulate lymphangiogenesis via the miR-302d-3p/VEGFR3/AKT axis to ameliorate inflammatory bowel disease, Int. Immunopharm. 110 (2022) 109066, https://doi.org/10.1016/j.intimp.2022.109066.

[85] Y. Chang, Y. Zhang, Y. Jiang, L. Zhao, C. Lv, Q. Huang, et al., From hair to colon: hair follicle-derived MSCs alleviate pyroptosis in DSS-induced ulcerative colitis by releasing exosomes in a paracrine manner, Oxid. Med. Cell. Longev. 2022 (2022) 9097530, https://doi.org/10.1155/2022/9097530.

[86] X. Cai, Z.Y. Zhang, J.T. Yuan, D.K.W. Ocansey, Q. Tu, X. Zhang, et al., hucMSC-derived exosomes attenuate colitis by regulating macrophage pyroptosis via the miR-378a-5p/NLRP3 axis, Stem Cell Res. Ther. 12 (1) (2021) 416, https://doi.org/10.1186/s13287-021-02492-6.

[87] Y. Xu, X. Tang, A. Fang, J. Yan, D. Kofi Wiredu Ocansey, X. Zhang, et al., HucMSC-Ex carrying miR-203a-3p.2 ameliorates colitis through the suppression of caspase11/4-induced macrophage pyroptosis, Int. Immunopharm. 110 (2022) 108925, https://doi.org/10.1016/j.intimp.2022.108925.

[88] G. Wang, J. Yuan, X. Cai, Z. Xu, J. Wang, D.K.W. Ocansey, et al., HucMSC-exosomes carrying miR-326 inhibit neddylation to relieve inflammatory bowel disease in mice, Clin. Transl. Med. 10 (2) (2020) e113, https://doi.org/10.1002/ctm2.113.
[89] J. Tian, Q. Zhu, Y. Zhang, Q. Bian, Y. Hong, Z. Shen, et al., Olfactory ecto-mesenchymal stem cell-derived exosomes ameliorate experimental colitis via modulating Th1/Th17 and treg cell responses, Front. Immunol. 11 (2020) 598322, https://doi.org/10.3389/fimmu.2020.598322.
[90] D.K.W. Ocansey, Z. Zhang, X. Xu, L. Liu, S. Amoah, X. Chen, et al., Mesenchymal stem cell-derived exosome mitigates colitis via the modulation of the gut metagenomics-metabolomics-farnesoid X receptor axis, Biomater. Sci. 10 (17) (2022) 4822–4836, https://doi.org/10.1039/d2bm00559j.
[91] Y. Wu, W. Qiu, X. Xu, J. Kang, J. Wang, Y. Wen, et al., Exosomes derived from human umbilical cord mesenchymal stem cells alleviate inflammatory bowel disease in mice through ubiquitination, Am. J. Tourism Res. 10 (7) (2018) 2026–2036.
[92] R. Tenchov, J.M. Sasso, X. Wang, W.S. Liaw, C.A. Chen, Q.A. Zhou, Exosomes—Nature's lipid nanoparticles, a rising star in drug delivery and diagnostics, ACS Nano 16 (11) (2022) 17802–17846, https://doi.org/10.1021/acsnano.2c08774.
[93] D. Wang, H. Xue, J. Tan, P. Liu, C. Qiao, C. Pang, et al., Bone marrow mesenchymal stem cells-derived exosomes containing miR-539-5p inhibit pyroptosis through NLRP3/caspase-1 signalling to alleviate inflammatory bowel disease, Inflamm. Res. : official journal of the European Histamine Research Society [et al] 71 (7–8) (2022) 833–846, https://doi.org/10.1007/s00011-022-01577-z.
[94] Y.J. Youn, S. Shrestha, Y.B. Lee, J.K. Kim, J.H. Lee, K. Hur, et al., Neutrophil-derived trail is a proinflammatory subtype of neutrophil-derived extracellular vesicles, Theranostics 11 (6) (2021) 2770–2787, https://doi.org/10.7150/thno.51756.
[95] L. Tong, H. Hao, Z. Zhang, Y. Lv, X. Liang, Q. Liu, et al., Milk-derived extracellular vesicles alleviate ulcerative colitis by regulating the gut immunity and reshaping the gut microbiota, Theranostics 11 (17) (2021) 8570–8586, https://doi.org/10.7150/thno.62046.
[96] C. Du, K. Wang, Y. Zhao, X. Nan, R. Chen, S. Quan, et al., Supplementation with milk-derived extracellular vesicles shapes the gut microbiota and regulates the transcriptomic landscape in experimental colitis, Nutrients 14 (9) (2022), https://doi.org/10.3390/nu14091808.
[97] G. Han, H. Cho, H. Kim, Y. Jang, H. Jang, D.E. Kim, et al., Bovine colostrum derived-exosomes prevent dextran sulfate sodium-induced intestinal colitis via suppression of inflammation and oxidative stress, Biomater. Sci. 10 (8) (2022) 2076–2087, https://doi.org/10.1039/d1bm01797g.
[98] L. Jiang, Y. Shen, D. Guo, D. Yang, J. Liu, X. Fei, et al., EpCAM-dependent extracellular vesicles from intestinal epithelial cells maintain intestinal tract immune balance, Nat. Commun. 7 (2016) 13045, https://doi.org/10.1038/ncomms13045.
[99] D.T. Rubin, A.N. Ananthakrishnan, C.A. Siegel, B.G. Sauer, M.D. Long, ACG clinical guideline: ulcerative colitis in adults, Am. J. Gastroenterol. 114 (3) (2019) 384–413, https://doi.org/10.14309/ajg.0000000000000152.
[100] C. Le Berre, L. Peyrin-Biroulet, Selecting end points for disease-modification trials in inflammatory bowel disease: the SPIRIT consensus from the IOIBD, Gastroenterology 160 (5) (2021), https://doi.org/10.1053/j.gastro.2020.10.065, 1452-60.e21.
[101] J.J. Hou, W.W. Li, X.L. Wang, A.H. Ma, Y.H. Qin, Efficacy of extracellular vesicles as a cell-free therapy in colitis: a systematic review and meta-analysis of animal studies, Front. Pharmacol. 14 (2023) 1260134, https://doi.org/10.3389/fphar.2023.1260134.
[102] C.M. Tian, Y. Zhang, M.F. Yang, H.M. Xu, M.Z. Zhu, J. Yao, et al., Stem cell therapy in inflammatory bowel disease: a review of achievements and challenges, J. Inflamm. Res. 16 (2023) 2089–2119, https://doi.org/10.2147/jir.S400447.
[103] M. Nowak, J. Górczyńska, K. Kołodzińska, J. Rubin, A. Choromańska, Extracellular vesicles as drug transporters, Int. J. Mol. Sci. 24 (12) (2023), https://doi.org/10.3390/ijms241210267.
[104] T. Burnouf, V. Agrahari, V. Agrahari, Extracellular vesicles as nanomedicine: hopes and hurdles in clinical translation, Int. J. Nanomed. 14 (2019) 8847–8859, https://doi.org/10.2147/ijn.S225453.
[105] S. Sadeghi, W.K. Lee, S.N. Kong, A. Shetty, C.L. Drum, Oral administration of protein nanoparticles: an emerging route to disease treatment, Pharmacol. Res. 158 (2020) 104685, https://doi.org/10.1016/j.phrs.2020.104685.
[106] O.M. Elsharkasy, J.Z. Nordin, D.W. Hagey, O.G. de Jong, R.M. Schiffelers, S.E. Andaloussi, et al., Extracellular vesicles as drug delivery systems: why and how? Adv. Drug Deliv. Rev. 159 (2020) 332–343, https://doi.org/10.1016/j.addr.2020.04.004.
[107] C. Kilkenny, N. Parsons, E. Kadyszewski, M.F. Festing, I.C. Cuthill, D. Fry, et al., Survey of the quality of experimental design, statistical analysis and reporting of research using animals, PLoS One 4 (11) (2009) e7824, https://doi.org/10.1371/journal.pone.0007824.
[108] J.P. Higgins, D.G. Altman, P.C. Gøtzsche, P. Jüni, D. Moher, A.D. Oxman, et al., The Cochrane Collaboration's tool for assessing risk of bias in randomised trials, BMJ (Clinical research ed) 343 (2011) d5928, https://doi.org/10.1136/bmj.d5928.
[109] Z. Bahadoran, P. Mirmiran, K. Kashfi, A. Ghasemi, Importance of systematic reviews and meta-analyses of animal studies: challenges for animal-to-human translation, J Am Assoc Lab Anim Sci 59 (5) (2020) 469–477, https://doi.org/10.30802/aalas-jaalas-19-000139.
[110] N. Percie du Sert, V. Hurst, A. Ahluwalia, S. Alam, M.T. Avey, M. Baker, et al., The ARRIVE guidelines 2.0: updated guidelines for reporting animal research, PLoS Biol. 18 (7) (2020) e3000410, https://doi.org/10.1371/journal.pbio.3000410.