Cytokine and Growth Factor Reviews 73 (2023) 173–184

Contents lists available at ScienceDirect

Cytokine and Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Extracellular vesicles as a novel mediator of interkingdom communication

Jumin Huang [a,b,1], Xuanrun Wang [a,b,1], Ziming Wang [a,b,1], Liyan Deng [d,e], Yuwei Wang [f], Yuping Tang [f,*], Lianxiang Luo [d,e,**], Elaine Lai-Han Leung [a,b,c,***]

[a] Cancer Centre, Faculty of Health Sciences, Universty of Macau, Macao Special Administrative Region of China
[b] MOE Frontiers Science Centre for Precision Oncology, University of Macau, Macao Special Administrative Region of China
[c] State Key Laboratory of Quality Research in Chinese Medicine, University of Macau, Macao Special Administrative Region of China
[d] The Marine Biomedical Research Institute, Guangdong Medical University, China
[e] The Marine Biomedical Research Institute of Guangdong Zhanjiang, Zhanjiang, Guangdong, China
[f] Key Laboratory of Shaanxi Administration of Traditional Chinese Medicine for TCM Compatibility, Shaanxi University of Chinese Medicine, Xi'an 712046, Shaanxi, China

ARTICLE INFO

Keywords:
Extracellular vesicles
Gut microbiota
Herbal medicine
Multi-omics

ABSTRACT

Extracellular vesicles (EVs) are nanosized lipid bilayer-delimited particles secreted from almost all types of cells including bacteria, mammals and plants, and are presumed to be mediators of intercellular communication. Bacterial extracellular vesicles (BEVs) are nanoparticles with diverse diameters, ranging from 20 to 400 nm. BEVs are composed of soluble microbial metabolites, including nucleic acid, proteins, lipoglycans, and short-chain fatty acids (SCFAs). In addition, EVs may contain quorum sensing peptides that are endowed with the ability to protect bacteria against bacteriophages, form and maintain bacterial communities, and modulate the host immune system. BEVs are potentially promising therapeutic modalities for use in vaccine development, cancer immunotherapy regimens, and drug delivery cargos. Plant-derived EVs (PEVs), such as EVs derived from herbal medicines, can be absorbed by the gut microbiota and influence the composition and homeostasis of gut microbiota. This review highlights the roles of BEVs and PEVs in bacterial and plant physiology and discusses crosstalk among gut bacteria, host metabolism and herbal medicine. In summary, EVs represent crucial communication messengers in the gut microbiota, with potential therapeutic value in the delivery of herbal medicines.

1. Introduction

Extracellular vesicles (EVs) are nanosized lipid bilayer-delimited particles secreted from almost all types of cells including bacteria, mammals and plants, which are presumed to be mediators of intercellular communication [1–4]. EVs possess a functional versatility that extends beyond their conventional role as waste carriers. The current focus of interest in the field of EVs centers on their ability to mediate intercellular crosstalk of diverse cargo molecules, ranging from nucleic acids, and lipids to proteins [4–8]. In this regard, EVs have the potential to serve as amplifiers of signaling mechanisms during both normal cell homeostasis and pathological development [9].

In the 1960 s, bacterial extracellular vesicles (BEVs) were first observed in *Escherichia coli* (*E. coli*). Subsequently, extensive research focused on BEVs has aimed to elucidate the mechanisms underlying their biogenesis, composition, and functional properties. BEVs are

Abbreviations: BCG, *Bacillus Calmette Guérin*; BEVs, Bacterial extracellular vesicles; CMVs, Cytoplasmic membrane vesicles; cryo-TEM, Cryo-transmission electron microscopy; *E. coli,*, *Escherichia coli*; EOMVs, Explosive outer-membrane vesicles; Evs, Extracellular vesicles; G^-, Gram-negative; G^+, Gram-positive; I3A, Indole-3-carboxaldehyde; LPS, Lipopolysaccharide; MW, Molecular weight; NFIL3, Nuclear factor interleukin 3-regulated protein; OIMVs, Outer-inner membrane vesicles; OMVs, Outer membrane vesicles; PGN, Peptidoglycan; SCFAs, Short-chain fatty acids; SEC, Size exclusion chromatography; TCMs, Traditional Chinese medicines; TEM, Transmission electron microscopy; TLRs, Toll-like receptor.

* Corresponding author.
** Corresponding author at: The Marine Biomedical Research Institute, Guangdong Medical University, China.
*** Corresponding author at: Cancer Center, Faculty of Health Sciences, University of Macau, Macao Special Administrative Region of China.
E-mail addresses: yupingtang@sntcm.edu.cn (Y. Tang), luolianxiang321@gdmu.edu.cn (L. Luo), lhleung@um.edu.mo (E.L.-H. Leung).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cytogfr.2023.08.005
Received 28 July 2023; Received in revised form 17 August 2023; Accepted 18 August 2023
Available online 21 August 2023

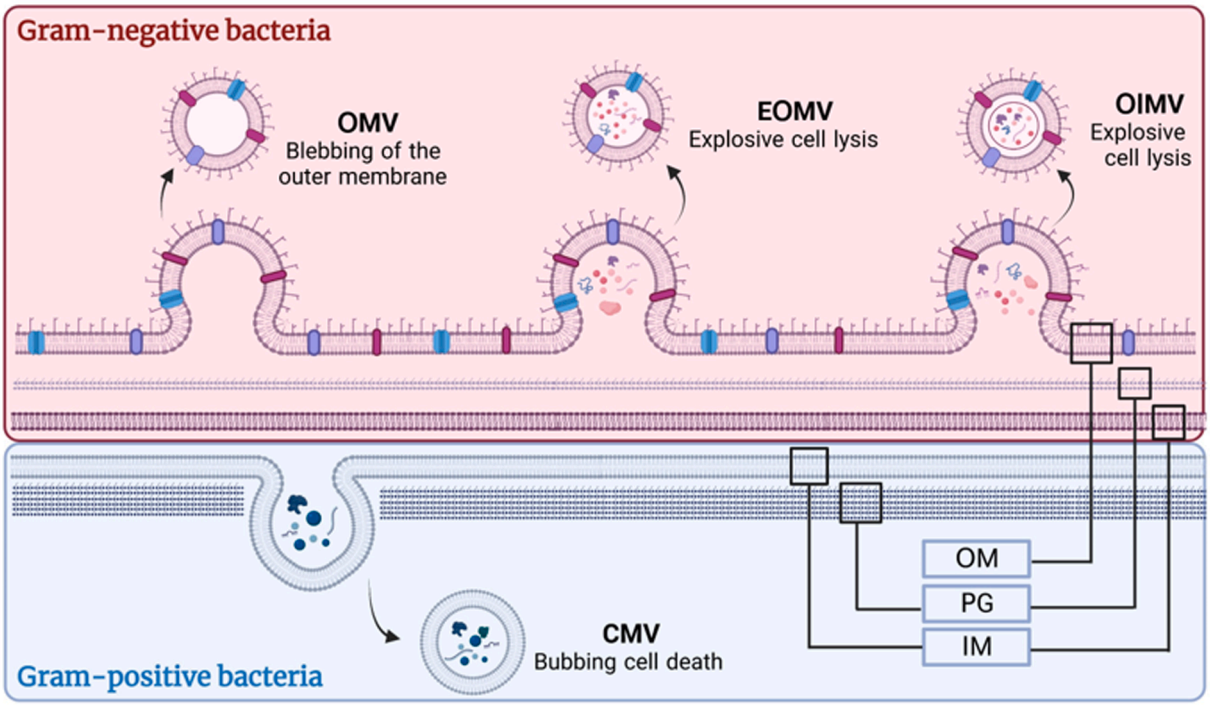


Fig. 1. The formation pathways of different types of bacterial extracellular vesicles. G^- bacteria have two main routes for vesicle formation: blebbing of the outer membrane and explosive cell lysis. When biosynthetic peptidoglycan is unbalanced, or hydrophobic molecules insert into the outer membrane to cause cell membrane disorder, the outer membrane blisters and outer membrane vesicles (OMVs) are generated. The cell wall peptidoglycan layer is degraded by autolysin, the inner membrane protrudes outward, and the cytoplasmic contents such as DNA enter the vesicles. Finally, the vesicles are extruded from the cell surface together with the surrounding outer membrane to form outer inner membrane vesicles (OIMVs). When bacterial chromosomal DNA is damaged, it will induce oxidative stress response and trigger cell death and lysis, resulting in the re circulation of cell membrane fragments, aggregation and random encapsulation of cytoplasmic material, forming explosive outer membrane vesicles (EOMVs). The biogenesis mechanism of extracellular vesicles released by G^+ bacteria is bubbling cell death. Endolysins degrade the PG layer and trigger bubbling cell death and production of CMV in G+ bacteria. LPS: lipopolysaccharide, OM: other membrane, PG: peptidoglycan, IM: Inner membrane.

nanoparticles with diverse diameters, ranging from 20 to 400 nm. BEVs are composed of soluble microbial metabolites, including nucleic acids, proteins, lipoglycans, short-chain fatty acids (SCFAs), and quorum sensing peptides, which are endowed with the ability to protect bacteria against bacteriophages, form and maintain bacterial communities, and modulate the host immune system [10–13]. Recently, accumulating evidence has supported the notion that microbial-derived EVs can serve as an important orchestrator in the mutual communications between gut microbiota and the host [14]. BEVs produced by gut microbiota can translocate to systemic circulation to control host metabolism, epithelial barrier integrity and immune responses [15]. In this regard, BEVs are potentially promising therapeutic modalities for use in vaccines, cancer immunotherapy regimens, and drug delivery cargos.

Plant-derived EVs (PEVs), such as EVs derived from herbal medicines, can be absorbed by the gut microbiota and influence the composition and homeostasis of gut microbiota. Owing to the pivotal role of the gut microbiota in maintaining the host physiological state, its dysbiosis can lead to a wide range of disorders, such as cancer and neurological and metabolic disorders. Ginger-derived EVs can be selectively taken up by Lactobacillaceae in a lipid-dependent manner in vivo, and the increased Lactobacillus abundance in turn enhances gut barrier integrity and alleviates colitis by producing indole-3-carboxaldehyde (I3A) to induce the secretion of IL-22 [16]. Thus, it is tempting to surmise that EVs may be an important mediator among the host, gut microbiota, and plants, such as diets or traditional Chinese medicines (TCMs).

In this review, we mainly highlight and discuss the roles of BEVs and PEVs and aim to provide new insight regarding the crosstalk among herbal medicine, gut bacteria and hosts. We surmise that EVs are crucial messengers between the gut microbiota and host, and that EVs represent a new approach to revealing the mechanism of herbal medicines.

2. Bacteria-derived EVs: Composition, structure, and functions

EVs can be further classified based on their biogenesis, size, and biophysical properties, such as exosomes (40–200 nm), microvesicles (200–2000 nm) and apoptotic bodies (500–2000 nm) [17–19]. In recent years, vesicles derived from microbial communities have become an emerging research direction, with bacterial groups being the most extensively studied [20].

2.1. A brief introduction of BEVs

Bacteria can be divided into gram-positive (G^+) bacteria and gram-negative (G^-) bacteria based on their morphology, structure, and staining characteristics. BEVs are nanovesicles generated by bacteria, ranging from 20 to 300 nanometers, which are natural phenomena in the normal growth process of bacteria [21]. The discovery of BEVs can be traced back to the 1960 s [22]. In recent decades, research on bacteria has mainly focused on G^- bacteria, such as *Helicobacter pylori* [23], *Salmonella* sp. [24], *Borrelia burgdorferi* [25], *Campylobacter jejuni* [26], and *Acinetobacter baumannii* [27]. Due to the thick cell wall of G^+ bacteria, it was believed that they are unable to secrete BEVs. In 2009, researchers isolated BEVs from *Staphylococcus aureus*, providing the first evidence that G^+ bacteria can also secrete BEVs [28].

Although the production of EVs is a natural phenomenon, G^- and G^+ bacteria synthesize vesicles in different ways (Fig. 1). G^- bacteria employ two primary routes for vesicle formation: blebbing of the outer membrane and explosive cell lysis [10]. The disruption of biosynthetic peptidoglycan or the incorporation of hydrophobic molecules into the

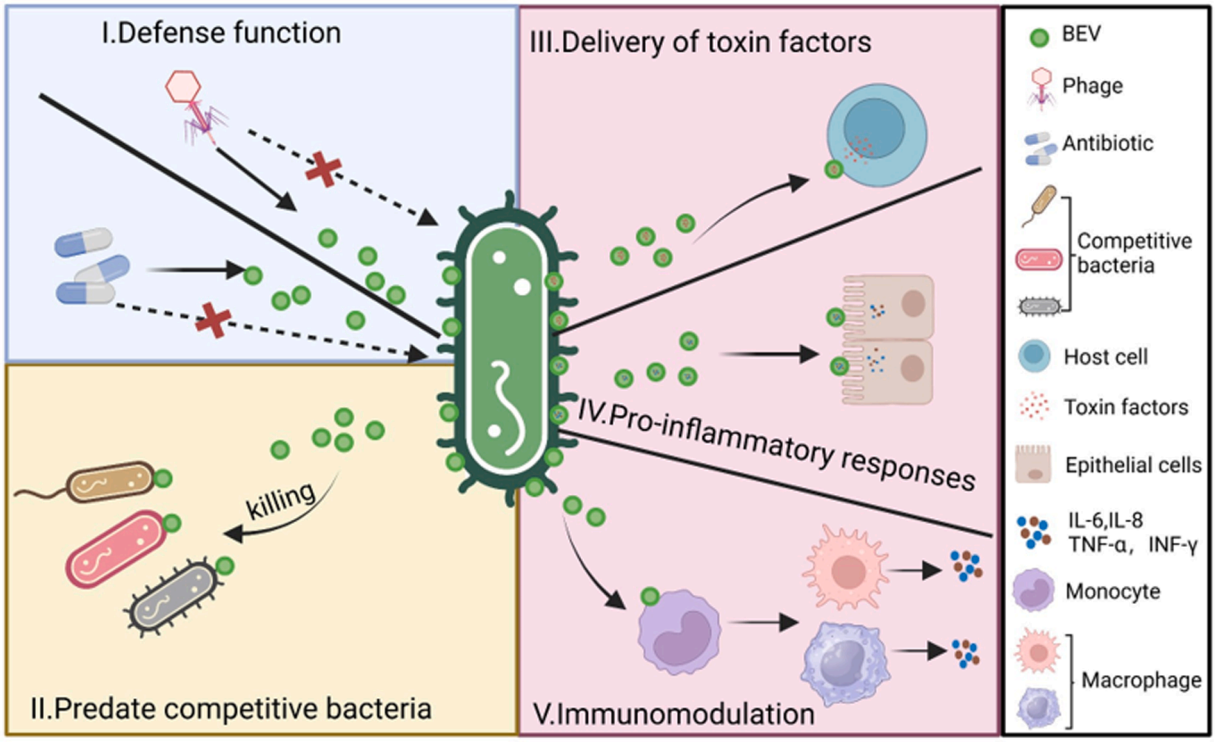


Fig. 2. The biological functions of BEVs. BEVS are nanoparticles that can carry protein, toxin, peptidoglycan, LPS and genetic material (DNA, RNA). The nucleic acid components in BEVs include plasmids, chromosomes, and even phage DNA, which are generally hundreds of BP to hundreds of KB in length. BEVS translocated and interacted with the recipient cells after they separated from the parent cells, and then transferred the goods to the recipient cells, thus triggering the downstream signal transduction pathway. BEVS containing different inclusions have different functions, including binding bacteriophages, inactivating antibiotics, protecting host bacteria, delivering virulence factors to human cells, affecting human health, delivering toxic substances, predating competitive bacteria, mediating nutrient delivery, regulating immunity and inflammation, etc.

outer membrane of bacterial cells can result in an imbalanced outer membrane structure. This imbalanced state leads to the formation of outer membrane blisters and the subsequent release of outer membrane vesicles (OMVs) [29]. Explosive cell lysis is initiated by bacteriophage-derived endolysin which can degrade the peptidoglycan cell wall. Upon degradation of the peptidoglycan, the cell rounds up and explodes, leading to the formation of fragmented membrane remnants. These remnants then undergo self-assembly into two distinct types of vesicles: outer-inner membrane vesicles (OIMVs) and explosive outer-membrane vesicles (EOMVs) [21]. In structural terms, OMVs are spherical particles 20–250 nm in size, with an inner leaflet of phospholipids and an outer leaflet of lipopolysaccharide (LPS). EOMVs have only one outer membrane, whereas OIMVs have two membrane bilayers [30]. Both EOMVs and OIMVs exhibit a random assortment of cytoplasmic components, such as outer membrane proteins, plasma membrane proteins, virulence factors, DNA, and RNA. However, it should be noted that a distinguishing characteristic of OMVs is their enriched content of outer membrane proteins and lipids, which makes them distinct from EOMVs and OIMVs [31]. The main mechanism by which G^+ bacteria generate vesicles involves the induction of bubbling cell death by endolysin, resulting in the release of cytoplasmic membrane vesicles (CMVs). These CMVs encompass not only the cell membrane but also cytoplasmic components [32]. The variability of the types and sizes of these BEVs further emphasizes the intricate and multifaceted nature of the EV generation process.

To date, although a handful of models have been proposed to elucidate extracellular vesicles by G^- and G^+ bacteria, the underlying mechanisms of BEV biogenesis in bacteria still require extensive and comprehensive investigation. The production of BEVs is regulated by a variety of factors, including genetic regulation, bacterial nutritional conditions, exposure to environmental stressors, and growth periods [33–35]. Growth conditions, including pH, oxygen content, nutritional levels, and antibiotics, can also affect the cargo packaging of BEVs [36,37]. The capacity of bacteria to selectively sort BEV contents in response to their local environment illustrates the intricate nature of extracellular vesicle biogenesis and underscores the need for additional research into the specific mechanisms governing cargo selection.

2.2. Isolation and purification of BEVs

The guidelines for the separation and characterization of EVs cover the entire process from sample collection to separation and characterization [38]. In contrast, there is currently a lack of standards for BEVs. The extraction of BEVs typically involves the following steps: cultivation, removal of bacteria, isolation of BEVs from culture medium, and purification. It is crucial to optimize the harvesting time, as the blebbing process of BEVs is influenced both quantitatively and qualitatively by the bacterial growth phase [39,40]. Once bacterial cultures have been established, centrifugation at low or differential speeds and sterile filtration are used to remove viable bacteria, cellular detritus, macromolecules of substantial size, and membrane amalgamations [41]. To improve the separation efficiency and purity, ultrafiltration centrifugation, density gradient centrifugation or size exclusion chromatography (SEC) are necessary to separate the non-BEVs [42,43]. In summary, there are currently no standardized methods available for the isolation and purification of BEVs. The selection of a specific method depends on several different conditions, such as the purity requirements and sample size.

Due to the small size of BEVs, the resolution of conventional optical microscopes is inadequate for observation. Electron microscopy, particularly transmission electron microscopy (TEM), is commonly employed for the characterization of various biological samples [44].

Table 1
The function of BEVs in disease.

Site of action	Bacteria	Function	Refs
Gut	*H. pylori*	BEVs may contribute to proinflammatory effects on epithelial and immune cells by promoting IL-6, IL-8, TNF- α, and IFN-γ production	[100]
	V. cholerae	BEVs carriers for many virulence	[101]
	pathogenic *E. coli*	factors such as cholera toxin, heat-labile enterotoxin, hemolysin, and Shiga toxin, resulting in endothelial cytotoxicity, apoptosis, and inflammatory cytokine release	[102]
	Neisseria gonorrhoeae	BEVs induced mitochondrial	[103]
	E. coli	apoptosis and NLRP3	
	P. aeruginosa	inflammatory vesicle activation, resulting in the release of LI-1β	
	probiotic *E. coli Nissle* 1917	BEVs enhanced the intestinal mucosal barrier by upregulating the tight junction proteins ZO-1, ZO-2 and claudin-14	[104]
	probiotic *A. muciniphila*	BEVs improved tight junctions and gut permeability by activating AMPK	[47]
	pathogenic *E. coli*	BEVs stimulated inflammatory production via the TLRs pathway in intestinal epithelial cells, and even induce endothelial and epithelial mitochondria-associated apoptosis via toxin delivery	[105]
Cancer	LPS-depleted *E. coli*	BEVs targeted cancer cells in vivo and reduce tumor burden by constant CXCL10 and IFN-γ production and antitumor immune responses	[106]
	H. pylori	BEVs containing CagA and VacA are suggested to hold capabilities of alteration of cell growth, damage to DNA, and aneuploidy, implying its tight association with gastric cancer	[107]
	P. gingivalis	BEVs can release sRNA23392 to promote invasion and migration of OSCC cells	[108]
Brain	*E. coli*	BEVs induced permeabilization of the mitochondrial membranes and trigger the mitochondrial apoptotic pathway in human brain microvascular endothelial cells	[109]
	Aggregatibacter actinomycetemcomitans	BEVs could travel across the BBB and successfully upregulate TNF-α expression.	[110]
Bone	*A. muciniphila*	BEVs promoted osteoblasts and inhibited osteoclasts	[81]
	Lactobacillus animalis	BEVs could selectively ferry various functional proteins and deliver them to the femoral head, whereby BEVs prevent trabecular bone damage and bone loss by improving the activity and function of endothelial and bone cells	[111]
Lung	*S. aureus*	BEVs could induce Th1 and Th17 neutrophilic pulmonary inflammation in a TLR2–dependent manner	[112]

Additionally, cryo-transmission electron microscopy (cryo-TEM) is used to characterize BEVs, where samples are first rapidly frozen and then imaged by cryo-TEM without the addition of any heavy metals or fixatives, providing a better picture of the original structure of BEVs than TEM [45].

2.3. Communication between BEVs and host physiology

BEVs have been discovered to play an essential role in a range of physiological and pathological processes. The function of BEVs varies from binding bacteriophages, inactivating antibiotics, and transmitting virulence factors to human cells, depending on their diverse contents (Fig. 2). Substantial evidence supports the notion that BEVs can translocate into the host's circulation and metabolic tissues, such as liver, adipose tissues, and skeletal muscle both in obese mice and humans through the leaked gut barrier, leading to obesity-associated tissue inflammation and insulin resistance [46–48]. The OMVs secreted by *E. coli* inhibit colon cancer tumor growth by inducing the production of the antitumor cytokines CXCL10 and IFN-γ (Table 1). Lipids play a crucial role in the composition of BEVs and bacterial-host interactions. Most BEVs enter nonphagocytic host cells through the utilization of lipid rafts [49–53]. Various lipids have been found to be selectively enriched in BEVs, such as LPS, cardiolipin, sphingolipids, phosphatidylethanolamine and phosphatidylglycerol [54]. In particular, phosphatidylglycerol and stearic acid are associated with the fluidity and rigidity of BEV membranes [55]. Phospholipid enrichment has been observed in OMVs produced via sulfate depletion [56]. One of the phospholipid transport proteins was even found to be involved in the formation of OMVs [57].

In addition to the lipids that are significantly enriched in BEVs, other cargoes of BEVs, including microRNAs, bacterial membrane components, and cellular signaling molecules, are associated with lipid metabolism. For instance, peptidoglycan (PGN) is a unique and important structural element in the cell wall of G^- bacteria. It is embedded in the relatively thick cell wall alongside other polymers. PGN directly activates Toll-like receptor 2 (TLR2) on adipocytes to inhibit beige adipocytes [58]. LPS has been reported to inhibit intestinal ANGPTL4 transcription through activation of the TLR4/STAT3 pathway, leading to increased expression of the nuclear factor interleukin 3-regulated protein (NFIL3) and resulting in increased ileal lipid uptake [58]. Furthermore, the TLRs enriched in BEVs also mediate lipid metabolism disorders in chronic inflammation of obese intestinal mucosa. TLR1 mediates the regulation of the intestinal metabolite triacylglycerol, participates in intestinal lipid metabolism, and effectively improves the utilization of short-chain fatty acids [58]. *M. bovis Bacillus Calmette Guérin* (BCG) induced NF-κB activation and increased PPARγ expression in a TLR2-dependent manner, leading to liposome formation [58]. In addition, the microRNAs carried by BEVs affect lipid metabolism. Increased levels of miR-30c and miR-130a in T84 cells were demonstrated to be infected with the clinical AIEC LF82 strain [58]. Both miR-30c and miR-130a have been reported to be involved in lipid metabolism. miRNA-30c has been reported to reduce hyperlipidemia and atherosclerosis by reducing lipid synthesis and apolipoprotein B-containing lipoprotein production [58]. miR-130a/b has been found to regulate metabolism-related inflammatory processes by modulating the translational levels of PPARγ and other key genes participating in lipid metabolism [58]. Taken together, the above studies suggest a potential mechanism by which BEVs are involved in lipid homeostasis. However, the exact mechanism by which BEVs affect lipid metabolism must be further explored.

3. Plant-derived extracellular vesicles (PEVs): Properties and therapeutic applications

Edible plants are profoundly linked to humanity, both as a source of nutrition and as a form of medicine. Furthermore, edible plants can be cultivated in abundance to help fight diseases, and one of the most attractive recently newly discovered treatment mechanisms is the mediation of interkingdom communication by PEVs. Consequently, this mechanism fuels growing interest in exploring the potential of PEVs in addressing various medical conditions.

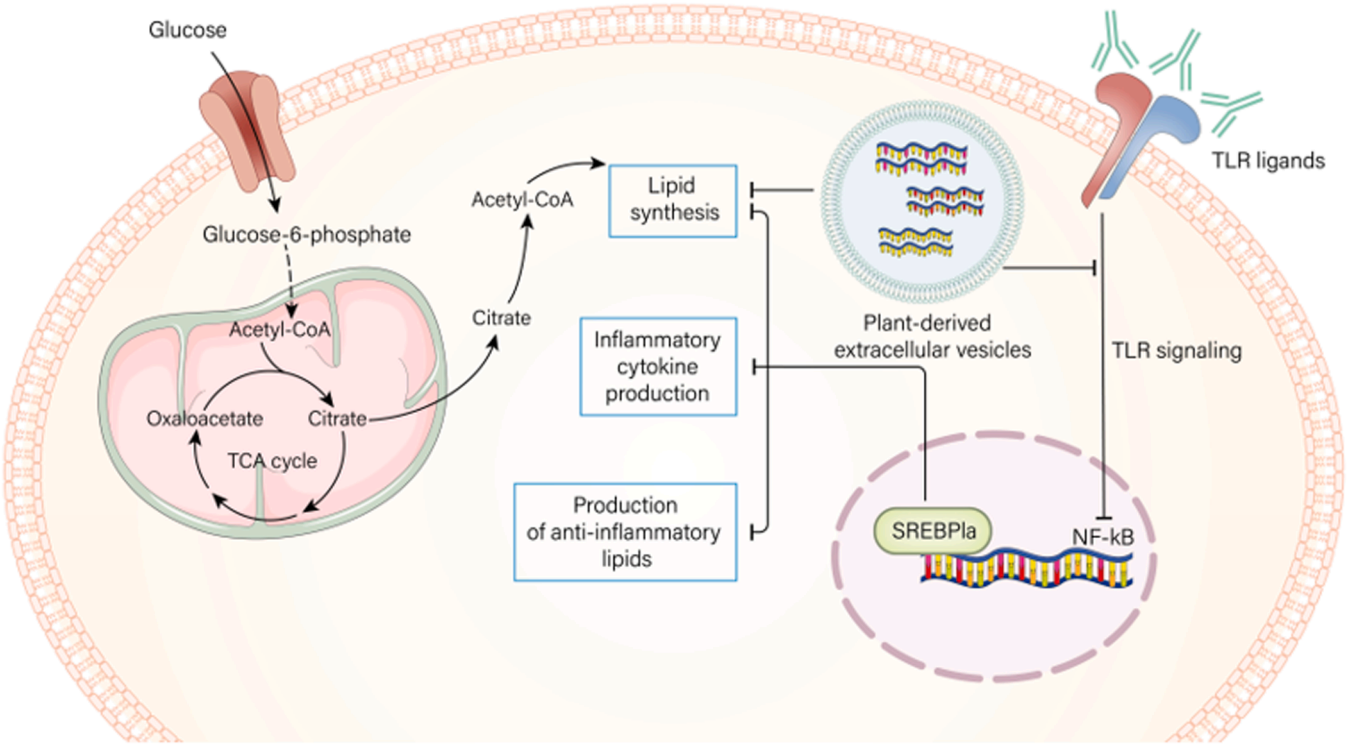


Fig. 3. Interactions between plant-derived EVs and lipid metabolism. Plant-derived extracellular vesicles regulate NF-kB transcription and thus affect lipid synthesis, inflammatory cytokine production and anti-inflammatory lipid production in vivo, mainly through TLR signaling and other pathways.

3.1. Properties of PEVs

PEVs have emerged as a potential drug delivery system due to their natural origin, biocompatibility, and stability in the human body after oral administration. These EVs can be divided into three categories based on their size: exosomes, microvesicles, and apoptotic bodies. PEVs have been shown to exhibit low toxicity, possess the ability to cross biological barriers to specific sites, and do not cause inflammation or necrosis, distinguishing them from traditional liposomes, which makes them a promising drug delivery system [59].

3.2. Therapeutic potential of PEVs

PEVs are a potential biotherapeutic agent, both in their natural form and as an engineered drug carrier. PEVs has been demonstrated to possess biological activity and can be employed to prevent and treat a variety of ailments, including metabolic syndrome, cancer, and inflammatory diseases. Shogaols carried by ginger-derived EVs can protect against the development of liver-associated diseases by inducing Nrf2 in a TLR4/TRIF-dependent manner [60]. Salvia dominica hairy root-derived EVs demonstrated a powerful and particular proapoptotic effect in pancreatic and breast cancer cells, with no adverse effects on noncancerous cells. Trichome root-derived EVs exhibited

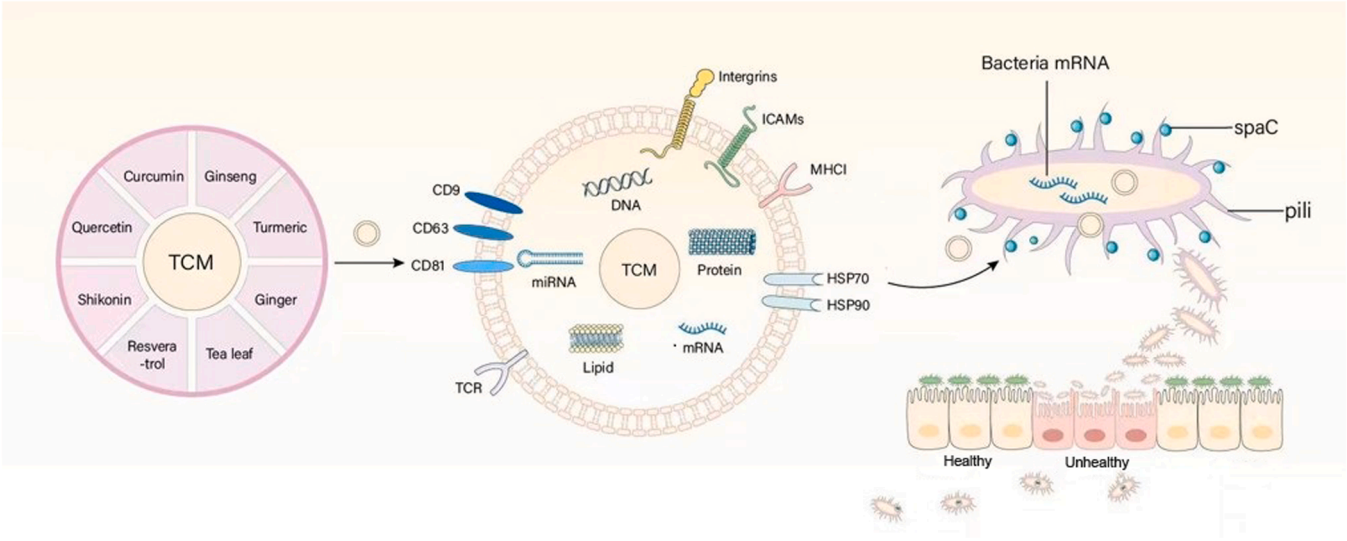


Fig. 4. Interactions between TCM-derived EVs and intestinal flora. The extracellular vesicles secreted by TCM carry relevant bioactive substances that are taken up by the intestinal flora and alter the DNA or RNA of the bacteria, which in turn acts as a treatment for intestinal diseases.

antiproliferative and proapoptotic effects in Mia PaCa-2 cells that were comparable to, or even superior to, those of gemcitabine, a chemotherapeutic drug used to treat a range of solid tumors [61]. Nanoparticles derived from ginseng have the potential to slow the progression of melanoma by changing the polarization of macrophages, thereby providing a novel strategy for cancer immunotherapy [62]. Vesicles derived from garlic chives have been identified as an NLRP3 inflammatory inhibitor and have been demonstrated to inhibit NLRP3 inflammasome activation and chronic inflammation in obese mice due to diet [63]. ExosomesNsp12Nsp13 released by lung epithelial cells exposed to SARS-CoV-2 Nsp12 and Nsp13 can be taken up by lung macrophages to trigger inflammatory cascade signaling and induce apoptosis in lung epithelial cells. Ginger-derived EVs with miRNA aly-miR396a-5p and rlcv-miR-rL1–28–3p can effectively abolish exosomes Nsp12Nsp13-mediated lung inflammation via inhibition of Nsp12 and spike genes [64]. Curcumin-derived EVs have been demonstrated to attenuate the catabolic effects of IL-1β-induced osteoarthritis by promoting cell viability and migration, reducing apoptosis and ERK1/2 protein phosphorylation, PI3K/Akt and p38 MAPK thereby exerting regulatory control over proinflammatory signaling pathways [65]. Collectively, PEVs can serve as biomarkers for diagnosing and monitoring a variety of diseases, such as cancer and cardiovascular diseases.

3.3. Crosstalk between PEVs and host physiology

PEVs interact with host physiology in a complex manner, exerting influence on various aspects, such as controlling immune responses, intercellular communication, and gene expression. Furthermore, the host's physiological state, such as inflammatory responses and lipid metabolism disorders, can affect the quantity and composition of EVs that are produced and released. Lipid metabolic pathways, including the production, transport, and decomposition of cholesterol and triglycerides, govern the transport and exchange of these EVs (Fig. 3). The lipid bilayer arrangement of ginger-derived EVs primarily comprises phospholipids and glycerol lipids. Specifically, phosphatidic acid (PA) has been identified as a key component in the uptake of ginger-derived EVs by *Lactobacillus rhamnosus* and *Porphyromonas gingivalis*. PA facilitates vesicular endocytosis and can interact with proteins expressed on the bacterial surface, highlighting its pivotal role in the uptake process [66]. EVs have the potential to alter a wide range of signaling pathways within tissues in different physiopathological states of the organism (Fig. 4). Crewe C et al. found that in the context of metabolic regulation, small EVs mediate forms of intercellular communication within adipose tissue that contribute to multifaceted crosstalk between adipocytes and stromal vascular cells by increasing or inhibiting certain lipid metabolism synthesis pathways [67]. The pathogenesis-related 1 protein of Arabidopsis is sorted in the endoplasmic reticulum in a C-terminal-dependent manner and is then transported by phosphatidylinositol-3-phosphate-positive extracellular vesicles [68]. Lipid metabolism and the individual lipid molecules involved are vital for plants to detect pathogenic bacteria and avoid the host's immune system [69]. Lipid metabolism abnormalities can disrupt the production and release of EVs, which can modify intercellular information transfer and regulation. Therefore, it is imperative to understand the connection between EVs, host physiology, and lipid metabolism for the purpose of biological and medical research.

4. EVs orchestrate communication among TCMs, the microbiome, and host and the corresponding novel bioinformatic analysis platform

EVs serve as crucial mediators within the intercellular network, facilitating various physiological processes and pathological alterations through their communication functions. Various types of molecular cargo present in EVs are believed to play specific roles in biological or biomedical procedures. In the contemporary era of multiomics science, the utilization of systems biology methodologies offers novel insights and avenues for investigating EVs. Therefore, the utilization of integrative bioinformatics in multiomics analysis can provide robust support for examining the collective or multiperspective depiction of EVs and can contribute to comprehending the intercellular trajectories of EVs.

4.1. The role and function of BEVs in bacterial interactions

Bacterial cells within the microbial community participate in intricate interactions with each other. BEVs play a role in supporting the survival of producer bacteria within their ecological niche through various mechanisms, including cooperation, competition, or antagonism toward other bacterial species [70]. In the context of this conceptual framework, it is posited that BEVs may serve as a mechanism for microbial defense, providing protective capabilities against a range of harmful agents including phages, antibiotics, reactive oxygen species, and antimicrobial peptides [71]. BEVs possess the capacity to confer metabolic advantages. The acquisition of nutrients and the survival of bacteria are heavily dependent on the presence of transportation mechanisms for siderophores, amino acids, and fatty acids. BEVs can also include hydrolase-type enzymes, which aid in the breakdown of proteins and complex polysaccharides that are present in the environment in which bacteria are situated, which has the potential to make it easier for the microbial community to acquire nutrients [72]. The outer membrane vesicles lacked certain outer membrane proteins, while a significantly high quantity of proteins was found exclusively within the vesicles. A significant portion of the proteins found in the outer membrane vesicles exhibited an acidic nature and were identified as hydrolases, specifically proteases and glycosidases. Furthermore, certain hydrolases were demonstrated to be enzymatically active in vitro [73]. Moreover, these mechanisms may act as a competitive strategy against other bacterial species. In this respect, it is noteworthy that BEVs possess degradative enzymes, such as murein hydrolases, peptidoglycan hydrolases, or endopeptidases, which are employed to eradicate rival bacteria [74]. Horizontal gene transfer is a plausible mechanism for the acquisition of antibiotic resistance. This process involves the transfer of antibiotic resistance genes through BEVs [75]. The utilization of BEVs as a mechanism of communication may provide a unique advantage by facilitating the horizontal transfer of genes that confer resistance to other bacteria. In short, BEVs exhibit diverse cargo, providing evidence of their involvement in several crucial bacterial processes such as antibiotic resistance, biofilm formation, survival, virulence factor production, quorum sensing, and interbacterial communication [76].

Bacteria in the intestinal microenvironment can engage in communication with their immediate surroundings by releasing a diverse range of chemical compounds, which include toxins, quorum sensing molecules, and nucleic acids. BEVs have a significant function in modulating the in vivo equilibrium of intestinal bacteria by acting as carriers of signaling molecules [77]. *Clostridium butyricum* derived EVs can alleviate bacterial dysbiosis in colitis mice, resulting in a substantial reduction in the abundance of two prominent bacterial pathogens, *Escherichia coli* and *Shigella flexneri* [78]. Alternatively, BEVs might be disrupted to minimize the colonization of pathogenic bacteria, or they could be exploited to boost probiotic colonization, which is crucial for the therapeutic impact of probiotics [79].

4.2. The role and function of BEVs in bacteria-host interactions

Similar to interbacterial communication, the communication facilitated by BEVs enables interaction between bacteria and their respective hosts. BEVs exhibit diverse mechanisms of interaction with human host cells, including binding to host receptors, internalization of EV contents into the host cell, and the integration of EVs into the cytoplasm of the host cells [80]. It has been noted that bacterial effectors are transported into specific cells, thereby facilitating the regulation of host cell pathways and physiological processes. *Akkermensia muciniphila*-induced EVs

exhibit a remarkable ability to infiltrate and accumulate within bone tissues, thereby enhancing osteogenic activity and inhibiting of osteoclast formation in ovariectomy-induced osteoporotic mice [81]. BEVs contain adhesin-type molecules that enable their attachment to host cells, as well as microbial molecules that are recognized by immune receptors involved in the induction of inflammatory and defense responses [82]. The relationship between host cells and the intestinal mucosal microbiota is characterized by its dynamic nature, mutual benefits, and high complexity [83]. Bidirectional communication between the microbiota and the host within the gut ecosystem does not necessarily rely on direct cellular interactions. BEVs that are released from host cells and the microbiota are both significant contributors to interkingdom communication. To effectively communicate with the host, bacteria need EVs that can penetrate the mucin layer and reach cells lining the gut mucosal surface. In obese mouse models, it has been demonstrated that *Akkermansia muciniphila* and its EVs may help reduce some of the detrimental effects of obesity. The effects of EVs derived from *Akkermansia muciniphila* were found to be more significant than those of the bacterium itself in reducing inflammation, easing intestinal permeability, and reversing the effects of obesity [84]. The administration of pasteurized *Akkermansia muciniphila* and its EVs has been observed to promote the growth of beneficial microbiota while inhibiting the proliferation of pathogenic bacteria. The aforementioned procedure plays a role in maintaining a balance within the intestinal environment, consequently resulting in a decrease in obesity rates and the advancement of general well-being [85]. In addition, BEVs combined with tumor tissue-derived EVs exhibited a more robust immune response in melanoma and colon mouse models [86]. Increasing research points to the importance of EVs generated by gut microbiota or host intestinal cells in mediating interkingdom crosstalk [87].

4.3. Interactions between BEVs and TCMs

The gut microbiome is a crucial factor in host nutrient metabolism, preservation of the structural integrity of the intestinal mucosal barrier, immune regulation, and protection against various pathogens [88]. TCMs have been observed to have a significant impact on the gut microbiota, leading to alterations in its composition. After oral administration of TCM ingredients, they are often not directly absorbed by the host, but instead enter the intestines, where they are transformed by the intestinal microbiota. In NSCLC patients treated with anti-PD-1 inhibitors, *Bacteroides vulgatus* and *Parabacteroides distasonis* were found to be highly represented in responders compared to nonresponders. When the gut microbiota was transplanted from nonresponders to germ-free mice, combination treatment with ginseng polysaccharides and anti-PD-1 inhibitors reshaped the gut microbiota from nonresponders to responders by increasing the abundance of *Bacteroides vulgatus* and *Parabacteroides distosoins* [89]. BaWeiBaiDuSan (BWBDS), a TCM prescription, was demonstrated to increase the abundance of *Lactobacillus johnsonii* which can promote the secretion of IL-10 of M2 macrophage to alleviate sepsis-induced liver injury in a cecal ligation and puncture (CLP) mouse model [90]. Collectively, these findings indicate that TCMs can act as postbiotics by harnessing gut microbiota to maintain host balance and health.

BEVs have the capacity to serve as critical mediators in the communication between microorganisms and their host across different biological kingdoms. These EVs are believed to play a significant role in maintaining the balance and stability of the gut microbiota, which in turn contributes to the overall health of the intestines. Furthermore, they are thought to be implicated in the development and progression of metabolic diseases [91]. BEVs have the potential to interact with diverse bioactive constituents found in herbal medicines. These interactions have the capacity to influence the biotransformation and metabolism of various chemical components, including but not limited to flavonoids, saponins, alkaloids, and anthraquinones. BEVs exert their influence on the host in various physiological and pathophysiological contexts, thereby modulating the pharmacological effects of herbal medicines. Hence, it is imperative to examine the correlation between BEVs and TCMs, as this will facilitate researchers in exploring the pharmacological impacts of TCMs on the human body, as well as the causal association between intestinal microorganisms and various diseases.

4.4. The crosstalk between PEVs and gut microbiota

PEVs maintain intestinal homeostasis and intestinal barrier function by modulating the gut microbiota. Lei C et al. found that lemon-like exosome nanoparticles improve colitis caused by *Clostridium difficile* infection by regulating probiotics [92]. Specifically, treatment with lemon-like exosome nanoparticles increased the levels of AhR ligands, such as indole-3-lactic acid and indole-3-formaldehyde. This increase subsequently leads to the induction of IL-22. Additionally, the elevated levels of lactic acid resulting from this treatment have been found to reduce the shedding of *Clostridium difficile* in feces. This reduction is achieved by inhibiting the growth of *Clostridium difficile* and impeding indole biosynthesis [92]. Furthermore, plant-derived nanoparticles can be absorbed by the gut microbiota. These nanoparticles carry specific RNAs that can alter the composition of the microbiota and impact host physiology (Fig. 4). Ginger-derived exosome nanoparticles show a preference for uptake by Lactobacillaceae in a lipid-dependent manner. These nanoparticles have been found to contain microRNAs that specifically target various genes in *Lactobacillus rhamnosus*. Ginger-derived exosome nanoparticles mdo-miR7267–3p mediated an increase in indole-3-carboxaldehyde production targeting *Lactobacillus rhamnosus* monooxygenase ycnE, induced IL-22 production and improved intestinal barrier function in mice with colitis [16]. Maca-derived EVs could cross the brain-blood barrier and increase serotonin production to recover depression-like behaviors, as well as increase of *Enterococcus*, *Lactobacillus*, and *Escherichia_Shigella* [93]. Altogether, the interactions between PEVs and the gut microbiota represent a fascinating area of research with potential implications for human health. Further studies are needed to better understand the specific mechanisms underlying these interactions and to explore the therapeutic potential of PEVs for promoting a healthy gut microbiota and preventing disease.

4.5. EV-based integrated multiomics platforms for TCM microbiome study

The utilization of EVs and the integration of multi-omics platforms to study TCMs and the microbiome signifies a novel and pioneering methodology. Various multiomics platforms, such as 16 S rRNA amplicon sequencing, shotgun metagenomics, epigenetics, transcriptomics, proteomics, and metabolomics, are employed to perform comprehensive analyses with the goal of identifying the functional components of EVs. Proteomic and lipidomic analysis provides a possibility to reveal the compositions of EVs. Garcia-Martin et al. identified six common markers (ENO1, GPI, HSPA5, YWHAB, CSF1R, and CNTN1) of exosomes/small EVs from five cell lines representing metabolically important tissues by quantitative proteomic analysis. They also revealed the unique small EV proteome in each cell type and validated the prediction via lipodystrophy mice with decreased white adipose and increased brown adipose tissue, which provides an effective prediction method to understand the origin of exosomes/small EVs in vivo [94]. Lipidomic profiles showed the presence of fatty acids, such as myristic, palmitic, octadecenoic, stearic, and eicosenoic acids in G^+ bacteria [95–97]. To determine the bacterial origin of plasma derived BEVs, Jones et al. employed a rapid and cost-effective isolation method of EBV-associated DNA in plasma. Subsequently, they utilized 16 S rRNA sequencing to identify the bacterial species present in these EVs [98]. This method may hold promise as a less invasive and potentially more accurate method for evaluating gut dysbiosis in the context of various diseases. In addition, the recent proliferation of diverse databases and bioinformatics tools dedicated to EVs has the potential to accelerate the pace of EV investigation, enabling researchers to focus on applied research and the discovery of biomarkers

Table 2
Extracellular-related databases.

Resource	Latest version	Statistics	Features	Refs
ExoCarta	12/09/2016	include 286 exosomal studies, 41,860 exosomal proteins, 7784 RNAs and 1116 lipid molecules	a web-based compendium of exosomal proteins, RNAs and lipids	[113]
EVpedia	27/09/2021	include 6879 publications, 172 080 vesicular components from 263 high-throughput datasets	a web portal contains identification of orthologous vesicular components and bioinformatic tools	[114]
Vesiclepedia	15/08/2018	include 1254 EV studies, 38166 RNA entries, 349988 protein entries and 639 lipid/metabolite entries	a web-based database; incorporate the entire data into FunRich	[115]
EV-TRACK	31/05/2023	include 2910 EV-related articles and multiple sample types or isolation methods of 7514 experiments (until 2023–05–31)	a crowdsourcing knowledgebase that centralizes EV biology and methodology	[116]
ExoRBase	19/11/2021	include 19643 mRNAs, 15645 lncRNAs and 79084 circRNAs obtained from human blood, urine, cerebrospinal fluid and bile samples	an online resource available for exploring different long RNA biotypes of EVs in human normal and cancerous biofluids	[117]
miREV	26/06/2021	include 428 miRNA sequencing data sets	an online database for the analysis of EV enriched samples; find reference transcripts in studies with comparable experimental setups	[118]
EVAtlas	13/08/2021	include the expression of seven types of ncRNA (miRNA, snoRNA, piRNA, snRNA, rRNA, tRNA and Y RNA) from 2030 smRNA-seq datasets of EVs	a state-of-the-art EV ncRNA repository deposited comprehensive expression profiles	[119]

associated with EVs. Several widely used and significant research tools that are presently accessible here encompass ExoCarta, EVpedia, Vesiclepedia, EV-TRACK, ExoRBase, miREV, and EVAtlas. These resources will be briefly outlined in Table 2.

5. Conclusion and perspectives

EVs can serve as a shuttle system to orchestrate the crosstalk among the host, TCMs, and gut microbes. In 2021, the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined the term "postbiotics" as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" [99]. Thus, BEVs derived from probiotics may be new postbiotics to support health. To magnify the effect of BEVs, we can use synthetic biology technology to modify and obtain more purified recombinant BEVs. However, current findings mainly focus on preclinical studies, and clinical proof is still needed prior to advancing microbial EV technology and its potential utilization in diagnostic and therapeutic interventions in precision medicine. First, a standard method to isolate and characterize BEVs is lacking, which makes it difficult to evaluate the accuracy and reproductivity. Second, since BEVs have the capability to reach host tissues, whether they may elicit negative effects on the host is unclear. Hence, the safety of BEVs must be assessed and confirmed before carrying out clinical trials. In summary, the mechanistic study of interkingdom crosstalk of EVs among TCMs, microbes, and host cells is still in its infancy and great efforts are required to fully illustrate this phenomenon. Further understanding of the crosstalk of TCM-host-microbiota via EVs will help to reveal the pharmacodynamic mechanism of TCMs, improve the quality control of TCMs and ensure safe, stable and effective clinical treatment. Identification of BEVs in blood can provide a new avenue for diagnosis and treatment.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

This work was funded by regular grants (File no. 0063/2022/A2) supported by the Macau Science and Technology Development Fund. This work was also supported by the 2020 Young Qihuang Scholar funded by the National Administration of Traditional Chinese Medicine, the National Natural Science Foundation of China (82003779 and 82001995), and the FDCT Funding Scheme for Postdoctoral Researchers of Higher Education Institutions (0017/2021/APD). This work is also financially supported by the Start-up Research Grant of University of Macau (SRG2022–00020-FHS), the 2023 State Key Laboratory of Quality Research in Chinese Medicine (UM) Internal Research Grant (IRG) (SKL-QRCM-IRG2023–001), and the FSCPO project (type 1) from Faculty of Health Science, University of Macau.

References

[1] D.G. Robinson, Y. Ding, L. Jiang, Unconventional protein secretion in plants: a critical assessment, Protoplasma 253 (1) (2016) 31–43.
[2] B.L. Deatherage, B.T. Cookson, Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life, Infect. Immun. 80 (6) (2012) 1948–1957.
[3] J.S. Schorey, Y. Cheng, P.P. Singh, V.L. Smith, Exosomes and other extracellular vesicles in host-pathogen interactions, EMBO Rep. 16 (1) (2015) 24–43.
[4] S. Rani, A. Lai, S. Nair, S. Sharma, A. Handberg, F. Carrion, A. Moller, C. Salomon, Extracellular vesicles as mediators of cell-cell communication in ovarian cancer and beyond - a lipids focus, Cytokine Growth Factor Rev. (2023).
[5] M.V. Chiantore, G. Mangino, M. Iuliano, L. Capriotti, P. Di Bonito, G. Fiorucci, G. Romeo, Human papillomavirus and carcinogenesis: novel mechanisms of cell communication involving extracellular vesicles, Cytokine Growth Factor Rev. 51 (2020) 92–98.
[6] M. Hegde, A. Kumar, S. Girisa, M.S. Alqahtani, M. Abbas, A. Goel, K.M. Hui, G. Sethi, A.B. Kunnumakkara, Exosomal noncoding RNA-mediated spatiotemporal regulation of lipid metabolism: Implications in immune evasion and chronic inflammation, Cytokine Growth Factor Rev. (2023).
[7] L. Dini, S. Tacconi, E. Carata, A.M. Tacconi, C. Vergallo, E. Panzarini, Microvesicles and exosomes in metabolic diseases and inflammation, Cytokine Growth Factor Rev. 51 (2020) 27–39.
[8] S. Han, P. Underwood, S.J. Hughes, From tumor microenvironment communicants to biomarker discovery: selectively packaged extracellular vesicular cargoes in pancreatic cancer, Cytokine Growth Factor Rev. 51 (2020) 61–68.
[9] G. van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat. Rev. Mol. Cell Biol. 19 (4) (2018) 213–228.
[10] M. Kaparakis-Liaskos, R.L. Ferrero, Immune modulation by bacterial outer membrane vesicles, Nat. Rev. Immunol. 15 (6) (2015) 375–387.
[11] V. Gujrati, S. Kim, S.H. Kim, J.J. Min, H.E. Choy, S.C. Kim, S. Jon, Bioengineered bacterial outer membrane vesicles as cell-specific drug-delivery vehicles for cancer therapy, ACS Nano 8 (2) (2014) 1525–1537.
[12] A. Chronopoulos, R. Kalluri, Emerging role of bacterial extracellular vesicles in cancer, Oncogene 39 (46) (2020) 6951–6960.
[13] D.T. Hughes, V. Sperandio, Inter-kingdom signalling: communication between bacteria and their hosts, Nat. Rev. Microbiol 6 (2) (2008) 111–120.
[14] J. Tulkens, G. Vergauwen, J. Van Deun, E. Geeurickx, B. Dhondt, L. Lippens, M. A. De Scheerder, I. Miinalainen, P. Rappu, B.G. De Geest, K. Vandecasteele, D. Laukens, L. Vandekerckhove, H. Denys, J. Vandesompele, O. De Wever,

A. Hendrix, Increased levels of systemic LPS-positive bacterial extracellular vesicles in patients with intestinal barrier dysfunction, Gut 69 (1) (2020) 191–193.
[15] L. Gul, D. Modos, S. Fonseca, M. Madgwick, J.P. Thomas, P. Sudhakar, C. Booth, R. Stentz, S.R. Carding, T. Korcsmaros, Extracellular vesicles produced by the human commensal gut bacterium Bacteroides thetaiotaomicron affect host immune pathways in a cell-type specific manner that are altered in inflammatory bowel disease, J. Extra Vesicles 11 (1) (2022), e12189.
[16] Y. Teng, Y. Ren, M. Sayed, X. Hu, C. Lei, A. Kumar, E. Hutchins, J. Mu, Z. Deng, C. Luo, K. Sundaram, M.K. Sriwastva, L. Zhang, M. Hsieh, R. Reiman, B. Haribabu, J. Yan, V.R. Jala, D.M. Miller, K. Van Keuren-Jensen, M.L. Merchant, C. J. McClain, J.W. Park, N.K. Egilmez, H.G. Zhang, Plant-derived exosomal MicroRNAs shape the gut microbiota, Cell Host Microbe 24 (5) (2018) 637–652, e8.
[17] M. Colombo, G. Raposo, C. Thery, Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles, Annu Rev. Cell Dev. Biol. 30 (2014) 255–289.
[18] J.C. Akers, D. Gonda, R. Kim, B.S. Carter, C.C. Chen, Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies, J. Neurooncol. 113 (1) (2013) 1–11.
[19] H.P. Nguyen, R.J. Simpson, L.A. Salamonsen, D.W. Greening, Extracellular vesicles in the intrauterine environment: challenges and potential functions, Biol. Reprod. 95 (5) (2016) 109.
[20] J.F. Cryan, K.J. O'Riordan, C.S.M. Cowan, K.V. Sandhu, T.F.S. Bastiaanssen, M. Boehme, M.G. Codagnone, S. Cussotto, C. Fulling, A.V. Golubeva, K. E. Guzzetta, M. Jaggar, C.M. Long-Smith, J.M. Lyte, J.A. Martin, A. Molinero-Perez, G. Moloney, E. Morelli, E. Morillas, R. O'Connor, J.S. Cruz-Pereira, V. L. Peterson, K. Rea, N.L. Ritz, E. Sherwin, S. Spichak, E.M. Teichman, M. van de Wouw, A.P. Ventura-Silva, S.E. Wallace-Fitzsimons, N. Hyland, G. Clarke, T. G. Dinan, The microbiota-gut-brain axis, Physiol. Rev. 99 (4) (2019) 1877–2013.
[21] M. Toyofuku, N. Nomura, L. Eberl, Types and origins of bacterial membrane vesicles, Nat. Rev. Microbiol. 17 (1) (2019) 13–24.
[22] S.N. Chatterjee, J. Das, Electron microscopic observations on the excretion of cell-wall material by vibrio cholerae, J. Gen. Microbiol. 49 (1) (1967) 1–11.
[23] L. Turner, J. Praszkier, M.L. Hutton, D. Steer, G. Ramm, M. Kaparakis-Liaskos, R. L. Ferrero, Increased outer membrane vesicle formation in a helicobacter pylori tolB mutant, Helicobacter 20 (4) (2015) 269–283.
[24] W. Elhenawy, M. Bording-Jorgensen, E. Valguarnera, M.F. Haurat, E. Wine, M. F. Feldman, LPS remodeling triggers formation of outer membrane vesicles in salmonella, mBio 7 (4) (2016).
[25] R.J. Shoberg, D.D. Thomas, Specific adherence of Borrelia burgdorferi extracellular vesicles to human endothelial cells in culture, Infect. Immun. 61 (9) (1993) 3892–3900.
[26] A. Elmi, E. Watson, P. Sandu, O. Gundogdu, D.C. Mills, N.F. Inglis, E. Manson, L. Imrie, M. Bajaj-Elliott, B.W. Wren, D.G. Smith, N. Dorrell, Campylobacter jejuni outer membrane vesicles play an important role in bacterial interactions with human intestinal epithelial cells, Infect. Immun. 80 (12) (2012) 4089–4098.
[27] C. Rumbo, E. Fernandez-Moreira, M. Merino, M. Poza, J.A. Mendez, N.C. Soares, A. Mosquera, F. Chaves, G. Bou, Horizontal transfer of the OXA-24 carbapenemase gene via outer membrane vesicles: a new mechanism of dissemination of carbapenem resistance genes in Acinetobacter baumannii, Antimicrob. Agents Chemother. 55 (7) (2011) 3084–3090.
[28] E.Y. Lee, D.Y. Choi, D.K. Kim, J.W. Kim, J.O. Park, S. Kim, S.H. Kim, D. M. Desiderio, Y.K. Kim, K.P. Kim, Y.S. Gho, Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles, Proteomics 9 (24) (2009) 5425–5436.
[29] S. Roier, F.G. Zingl, F. Cakar, S. Schild, Bacterial outer membrane vesicle biogenesis: a new mechanism and its implications, Micro Cell 3 (6) (2016) 257–259.
[30] S. Wang, J. Gao, Z. Wang, Outer membrane vesicles for vaccination and targeted drug delivery, Wiley Inter. Rev. Nanomed. Nanobiotechnol. 11 (2) (2019), e1523.
[31] J. Li, F. Azam, S. Zhang, Outer membrane vesicles containing signalling molecules and active hydrolytic enzymes released by a coral pathogen Vibrio shilonii AK1, Environ. Microbiol. 18 (11) (2016) 3850–3866.
[32] M. Toyofuku, G. Carcamo-Oyarce, T. Yamamoto, F. Eisenstein, C.C. Hsiao, M. Kurosawa, K. Gademann, M. Pilhofer, N. Nomura, L. Eberl, Prophage-triggered membrane vesicle formation through peptidoglycan damage in Bacillus subtilis, Nat. Commun. 8 (1) (2017) 481.
[33] K.E. Bonnington, M.J. Kuehn, Outer membrane vesicle production facilitates LPS remodeling and outer membrane maintenance in salmonella during environmental transitions, mBio 7 (5) (2016).
[34] S.W. Kim, J.S. Seo, S.B. Park, A.R. Lee, J.S. Lee, J.W. Jung, J.H. Chun, J.M. S. Lazarte, J. Kim, J.H. Kim, J.W. Song, C. Franco, W. Zhang, M.W. Ha, S.M. Paek, M. Jung, T.S. Jung, Significant increase in the secretion of extracellular vesicles and antibiotics resistance from methicillin-resistant Staphylococcus aureus induced by ampicillin stress, Sci. Rep. 10 (1) (2020) 21066.
[35] J. Bos, L.H. Cisneros, D. Mazel, Real-time tracking of bacterial membrane vesicles reveals enhanced membrane traffic upon antibiotic exposure, Sci. Adv. 7 (4) (2021).
[36] N. Orench-Rivera, M.J. Kuehn, Environmentally controlled bacterial vesicle-mediated export, Cell Microbiol 18 (11) (2016) 1525–1536.
[37] M. Toyofuku, S. Zhou, I. Sawada, N. Takaya, H. Uchiyama, N. Nomura, Membrane vesicle formation is associated with pyocin production under denitrifying conditions in Pseudomonas aeruginosa PAO1, Environ. Microbiol 16 (9) (2014) 2927–2938.
[38] C. Thery, K.W. Witwer, E. Aikawa, M.J. Alcaraz, J.D. Anderson, R. Andriantsitohaina, A. Antoniou, T. Arab, F. Archer, G.K. Atkin-Smith, D. C. Ayre, J.M. Bach, D. Bachurski, H. Baharvand, L. Balaj, S. Baldacchino, N. N. Bauer, A.A. Baxter, M. Bebawy, C. Beckham, A. Bedina Zavec, A. Benmoussa, A.C. Berardi, P. Bergese, E. Bielska, C. Blenkiron, S. Bobis-Wozowicz, E. Boilard, W. Boireau, A. Bongiovanni, F.E. Borras, S. Bosch, C.M. Boulanger, X. Breakefield, A.M. Breglio, M.A. Brennan, D.R. Brigstock, A. Brisson, M.L. Broekman, J. F. Bromberg, P. Bryl-Gorecka, S. Buch, A.H. Buck, D. Burger, S. Busatto, D. Buschmann, B. Bussolati, E.I. Buzas, J.B. Byrd, G. Camussi, D.R. Carter, S. Caruso, L.W. Chamley, Y.T. Chang, C. Chen, S. Chen, L. Cheng, A.R. Chin, A. Clayton, S.P. Clerici, A. Cocks, E. Cocucci, R.J. Coffey, A. Cordeiro-da-Silva, Y. Couch, F.A. Coumans, B. Coyle, R. Crescitelli, M.F. Criado, C. D'Souza-Schorey, S. Das, A. Datta Chaudhuri, P. de Candia, E.F. De Santana, O. De Wever, H.A. Del Portillo, T. Demaret, S. Deville, A. Devitt, B. Dhondt, D. Di Vizio, L.C. Dieterich, V. Dolo, A.P. Dominguez Rubio, M. Dominici, M.R. Dourado, T.A. Driedonks, F. V. Duarte, H.M. Duncan, R.M. Eichenberger, K. Ekstrom, S. El Andaloussi, C. Elie-Caille, U. Erdbrugger, J.M. Falcon-Perez, F. Fatima, J.E. Fish, M. Flores-Bellver, A. Forsonits, A. Frelet-Barrand, F. Fricke, G. Fuhrmann, S. Gabrielsson, A. Gamez-Valero, C. Gardiner, K. Gartner, R. Gaudin, Y.S. Gho, B. Giebel, C. Gilbert, M. Gimona, I. Giusti, D.C. Goberdhan, A. Gorgens, S.M. Gorski, D.W. Greening, J. C. Gross, A. Gualerzi, G.N. Gupta, D. Gustafson, A. Handberg, R.A. Haraszti, P. Harrison, H. Hegyesi, A. Hendrix, A.F. Hill, F.H. Hochberg, K.F. Hoffmann, B. Holder, H. Holthofer, B. Hosseinkhani, G. Hu, Y. Huang, V. Huber, S. Hunt, A. G. Ibrahim, T. Ikezu, J.M. Inal, M. Isin, A. Ivanova, H.K. Jackson, S. Jacobsen, S. M. Jay, M. Jayachandran, G. Jenster, L. Jiang, S.M. Johnson, J.C. Jones, A. Jong, T. Jovanovic-Talisman, S. Jung, R. Kalluri, S.I. Kano, S. Kaur, Y. Kawamura, E. T. Keller, D. Khamari, E. Khomyakova, A. Khvorova, P. Kierulf, K.P. Kim, T. Kislinger, M. Klingeborn, D.J. Klinke 2nd, M. Kornek, M.M. Kosanovic, A. F. Kovacs, E.M. Kramer-Albers, S. Krasemann, M. Krause, I.V. Kurochkin, G. D. Kusuma, S. Kuypers, S. Laitinen, S.M. Langevin, L.R. Languino, J. Lannigan, C. Lasser, L.C. Laurent, G. Lavieu, E. Lazaro-Ibanez, S. Le Lay, M.S. Lee, Y.X. F. Lee, D.S. Lemos, M. Lenassi, A. Leszczynska, I.T. Li, K. Liao, S.F. Libregts, E. Ligeti, R. Lim, S.K. Lim, A. Line, K. Linnemannstons, A. Llorente, C.A. Lombard, M.J. Lorenowicz, A.M. Lorincz, J. Lotvall, J. Lovett, M.C. Lowry, X. Loyer, Q. Lu, B. Lukomska, T.R. Lunavat, S.L. Maas, H. Malhi, A. Marcilla, J. Mariani, J. Mariscal, E.S. Martens-Uzunova, L. Martin-Jaular, M.C. Martinez, V.R. Martins, M. Mathieu, S. Mathivanan, M. Maugeri, L.K. McGinnis, M.J. McVey, D. G. Meckes Jr., K.L. Meehan, I. Mertens, V.R. Minciacchi, A. Moller, M. Moller Jorgensen, A. Morales-Kastresana, J. Morhayim, F. Mullier, M. Muraca, L. Musante, V. Mussack, D.C. Muth, K.H. Myburgh, T. Najrana, M. Nawaz, I. Nazarenko, P. Nejsum, C. Neri, T. Neri, R. Nieuwland, L. Nimrichter, J.P. Nolan, E.N. Nolte-'t Hoen, N. Noren Hooten, L. O'Driscoll, T. O'Grady, A. O'Loghlen, T. Ochiya, M. Olivier, A. Ortiz, L.A. Ortiz, X. Osteikoetxea, O. Ostergaard, M. Ostrowski, J. Park, D.M. Pegtel, H. Peinado, F. Perut, M.W. Pfaffl, D. G. Phinney, B.C. Pieters, R.C. Pink, D.S. Pisetsky, E. Pogge von Strandmann, I. Polakovicova, I.K. Poon, B.H. Powell, I. Prada, L. Pulliam, P. Quesenberry, A. Radeghieri, R.L. Raffai, S. Raimondo, J. Rak, M.I. Ramirez, G. Raposo, M. S. Rayyan, N. Regev-Rudzki, F.L. Ricklefs, P.D. Robbins, D.D. Roberts, S. C. Rodrigues, E. Rohde, S. Rome, K.M. Rouschop, A. Rughetti, A.E. Russell, P. Saa, S. Sahoo, E. Salas-Huenuleo, C. Sanchez, J.A. Saugstad, M.J. Saul, R. M. Schiffelers, R. Schneider, T.H. Schoyen, A. Scott, E. Shahaj, S. Sharma, O. Shatnyeva, F. Shekari, G.V. Shelke, A.K. Shetty, K. Shiba, P.R. Siljander, A. M. Silva, A. Skowronek, O.L. Snyder 2nd, R.P. Soares, B.W. Sodar, C. Soekmadji, J. Sotillo, P.D. Stahl, W. Stoorvogel, S.L. Stott, E.F. Strasser, S. Swift, H. Tahara, M. Tewari, K. Timms, S. Tiwari, R. Tixeira, M. Tkach, W.S. Toh, R. Tomasini, A. C. Torrecilhas, J.P. Tosar, V. Toxavidis, L. Urbanelli, P. Vader, B.W. van Balkom, S.G. van der Grein, J. Van Deun, M.J. van Herwijnen, K. Van Keuren-Jensen, G. van Niel, M.E. van Royen, A.J. van Wijnen, M.H. Vasconcelos, I.J. Vechetti Jr., T.D. Veit, L.J. Vella, E. Velot, F.J. Verweij, B. Vestad, J.L. Vinas, T. Visnovitz, K. V. Vukman, J. Wahlgren, D.C. Watson, M.H. Wauben, A. Weaver, J.P. Webber, V. Weber, A.M. Wehman, D.J. Weiss, J.A. Welsh, S. Wendt, A.M. Wheelock, Z. Wiener, L. Witte, J. Wolfram, A. Xagorari, P. Xander, J. Xu, X. Yan, M. Yanez-Mo, H. Yin, Y. Yuana, V. Zappulli, J. Zarubova, V. Zekas, J.Y. Zhang, Z. Zhao, L. Zheng, A.R. Zheutlin, A.M. Zickler, P. Zimmermann, A.M. Zivkovic, D. Zocco, E.K. Zuba-Surma, 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. Extra Vesicles 7 (1) (2018) 1535750.
[39] S. Santos, L.J. Arauz, J. Baruque-Ramos, I. Lebrun, S.M. Carneiro, S.A. Barreto, R. P. Schenkman, Outer membrane vesicles (OMV) production of neisseria meningitidis serogroup B in batch process, Vaccine 30 (42) (2012) 6064–6069.
[40] W.D. McCaig, A. Koller, D.G. Thanassi, Production of outer membrane vesicles and outer membrane tubes by francisella novicida, J. Bacteriol. 195 (6) (2013) 1120–1132.
[41] A. Gorringe, D. Halliwell, M. Matheson, K. Reddin, M. Finney, M. Hudson, The development of a meningococcal disease vaccine based on neisseria lactamica outer membrane vesicles, Vaccine 23 (17–18) (2005) 2210–2213.
[42] A. Kulp, M.J. Kuehn, Biological functions and biogenesis of secreted bacterial outer membrane vesicles, Annu Rev. Microbiol. 64 (2010) 163–184.
[43] J. Klimentova, J. Stulik, Methods of isolation and purification of outer membrane vesicles from gram-negative bacteria, Microbiol. Res 170 (2015) 1–9.
[44] J. Tulkens, O. De Wever, A. Hendrix, Analyzing bacterial extracellular vesicles in human body fluids by orthogonal biophysical separation and biochemical characterization, Nat. Protoc. 15 (1) (2020) 40–67.

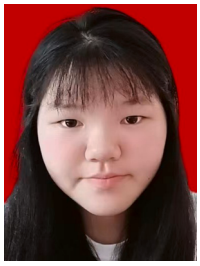
[45] R. Szatanek, M. Baj-Krzyworzeka, J. Zimoch, M. Lekka, M. Siedlar, J. Baran, The methods of choice for extracellular vesicles (EVs) characterization, Int J. Mol. Sci. 18 (6) (2017).
[46] Z. Luo, Y. Ji, H. Gao, F.C. Gomes Dos Reis, G. Bandyopadhyay, Z. Jin, C. Ly, Y. J. Chang, D. Zhang, D. Kumar, W. Ying, CRIg(+) macrophages prevent gut microbial DNA-containing extracellular vesicle-induced tissue inflammation and insulin resistance, Gastroenterology 160 (3) (2021) 863–874.
[47] C. Chelakkot, Y. Choi, D.K. Kim, H.T. Park, J. Ghim, Y. Kwon, J. Jeon, M.S. Kim, Y.K. Jee, Y.S. Gho, H.S. Park, Y.K. Kim, S.H. Ryu, Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions, Exp. Mol. Med 50 (2) (2018), e450.
[48] Y. Liu, K.A.Y. Defourny, E.J. Smid, T. Abee, Gram-positive bacterial extracellular vesicles and their impact on health and disease, Front Microbiol 9 (2018) 1502.
[49] A. Elmi, F. Nasher, H. Jagatia, O. Gundogdu, M. Bajaj-Elliott, B. Wren, N. Dorrell, Campylobacter jejuni outer membrane vesicle-associated proteolytic activity promotes bacterial invasion by mediating cleavage of intestinal epithelial cell E-cadherin and occludin, Cell Microbiol 18 (4) (2016) 561–572.
[50] N. Furuta, K. Tsuda, H. Omori, T. Yoshimori, F. Yoshimura, A. Amano, Porphyromonas gingivalis outer membrane vesicles enter human epithelial cells via an endocytic pathway and are sorted to lysosomal compartments, Infect. Immun. 77 (10) (2009) 4187–4196.
[51] S.J. Bauman, M.J. Kuehn, Pseudomonas aeruginosa vesicles associate with and are internalized by human lung epithelial cells, BMC Microbiol 9 (2009) 26.
[52] S.W. Sharpe, M.J. Kuehn, K.M. Mason, Elicitation of epithelial cell-derived immune effectors by outer membrane vesicles of nontypeable Haemophilus influenzae, Infect. Immun. 79 (11) (2011) 4361–4369.
[53] N.C. Kesty, K.M. Mason, M. Reedy, S.E. Miller, M.J. Kuehn, Enterotoxigenic Escherichia coli vesicles target toxin delivery into mammalian cells, EMBO J. 23 (23) (2004) 4538–4549.
[54] I. Barak, K. Muchova, The role of lipid domains in bacterial cell processes, Int J. Mol. Sci. 14 (2) (2013) 4050–4065.
[55] Y. Tashiro, A. Inagaki, M. Shimizu, S. Ichikawa, N. Takaya, T. Nakajima-Kambe, H. Uchiyama, N. Nomura, Characterization of phospholipids in membrane vesicles derived from Pseudomonas aeruginosa, Biosci. Biotechnol. Biochem 75 (3) (2011) 605–607.
[56] M.J.H. Gerritzen, D.E. Martens, J.P. Uittenbogaard, R.H. Wijffels, M. Stork, Sulfate depletion triggers overproduction of phospholipids and the release of outer membrane vesicles by Neisseria meningitidis, Sci. Rep. 9 (1) (2019) 4716.
[57] S. Roier, F.G. Zingl, F. Cakar, S. Durakovic, P. Kohl, T.O. Eichmann, L. Klug, B. Gadermaier, K. Weinzerl, R. Prassl, A. Lass, G. Daum, J. Reidl, M.F. Feldman, S. Schild, A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria, Nat. Commun. 7 (2016) 10515.
[58] T. Feng, W. Zhang, Z. Li, Potential mechanisms of gut-derived extracellular vesicle participation in glucose and lipid homeostasis, Genes (Basel) 13 (11) (2022).
[59] S. Rome, Biological properties of plant-derived extracellular vesicles, Food Funct. 10 (2) (2019) 529–538.
[60] X. Zhuang, Z.B. Deng, J. Mu, L. Zhang, J. Yan, D. Miller, W. Feng, C.J. McClain, H. G. Zhang, Ginger-derived nanoparticles protect against alcohol-induced liver damage, J. Extra Vesicles 4 (2015) 28713.
[61] E. Boccia, M. Alfieri, R. Belvedere, V. Santoro, M. Colella, P. Del Gaudio, M. Moros, F. Dal Piaz, A. Petrella, A. Leone, A. Ambrosone, Plant hairy roots for the production of extracellular vesicles with antitumor bioactivity, Commun. Biol. 5 (1) (2022) 848.
[62] M. Cao, H. Yan, X. Han, L. Weng, Q. Wei, X. Sun, W. Lu, Q. Wei, J. Ye, X. Cai, C. Hu, X. Yin, P. Cao, Ginseng-derived nanoparticles alter macrophage polarization to inhibit melanoma growth, J. Immunother. Cancer 7 (1) (2019) 326.
[63] B. Liu, X. Li, H. Yu, X. Shi, Y. Zhou, S. Alvarez, M.J. Naldrett, S.D. Kachman, S. H. Ro, X. Sun, S. Chung, L. Jing, J. Yu, Therapeutic potential of garlic chive-derived vesicle-like nanoparticles in NLRP3 inflammasome-mediated inflammatory diseases, Theranostics 11 (19) (2021) 9311–9330.
[64] Y. Teng, F. Xu, X. Zhang, J. Mu, M. Sayed, X. Hu, C. Lei, M. Sriwastva, A. Kumar, K. Sundaram, L. Zhang, J.W. Park, S.Y. Chen, S. Zhang, J. Yan, M.L. Merchant, X. Zhang, C.J. McClain, J.K. Wolfe, R.S. Adcock, D. Chung, K.E. Palmer, H. G. Zhang, Plant-derived exosomal microRNAs inhibit lung inflammation induced by exosomes SARS-CoV-2 Nsp12, Mol. Ther. 29 (8) (2021) 2424–2440.
[65] S. Li, S. Stockl, C. Lukas, M. Herrmann, C. Brochhausen, M.A. Konig, B. Johnstone, S. Grassel, Curcumin-primed human BMSC-derived extracellular vesicles reverse IL-1beta-induced catabolic responses of OA chondrocytes by upregulating miR-126-3p, Stem Cell Res Ther. 12 (1) (2021) 252.
[66] Z. Qiao, K. Zhang, J. Liu, D. Cheng, B. Yu, N. Zhao, F.J. Xu, Biomimetic electrodynamic nanoparticles comprising ginger-derived extracellular vesicles for synergistic anti-infective therapy, Nat. Commun. 13 (1) (2022) 7164.
[67] C. Crewe, N. Joffin, J.M. Rutkowski, M. Kim, F. Zhang, D.A. Towler, R. Gordillo, P.E. Scherer, An endothelial-to-adipocyte extracellular vesicle axis governed by metabolic state, Cell 175 (3) (2018) 695–708, e13.
[68] T. Pecenkova, R. Pleskot, V. Zarsky, Subcellular localization of arabidopsis pathogenesis-related 1 (PR1) protein, Int. J. Mol. Sci. 18 (4) (2017).
[69] A.R. Cavaco, A.R. Matos, A. Figueiredo, Speaking the language of lipids: the cross-talk between plants and pathogens in defence and disease, Cell Mol. Life Sci. 78 (9) (2021) 4399–4415.
[70] R.M. Stubbendieck, P.D. Straight, Multifaceted interfaces of bacterial competition, J. Bacteriol. 198 (16) (2016) 2145–2155.
[71] W.P.J. Smith, B.R. Wucher, C.D. Nadell, K.R. Foster, Bacterial defences: mechanisms, evolution and antimicrobial resistance, Nat. Rev. Microbiol. (2023).
[72] N. Diaz-Garrido, J. Badia, L. Baldoma, Microbiota-derived extracellular vesicles in interkingdom communication in the gut, J. Extra Vesicles 10 (13) (2021).
[73] W. Elhenawy, M.O. Debelyy, M.F. Feldman, Preferential packing of acidic glycosidases and proteases into bacteroides outer membrane vesicles, Mbio 5 (2) (2014).
[74] S. Bose, S. Aggarwal, D.V. Singh, N. Acharya, Extracellular vesicles: an emerging platform in gram-positive bacteria, Micro Cell 7 (12) (2020) 312–322.
[75] S. Domingues, K.M. Nielsen, Membrane vesicles and horizontal gene transfer in prokaryotes, Curr. Opin. Microbiol. 38 (2017) 16–21.
[76] L. Kern, S.K. Abdeen, A.A. Kolodziejczyk, E. Elinav, Commensal inter-bacterial interactions shaping the microbiota, Curr. Opin. Microbiol. 63 (2021) 158–171.
[77] X. Liang, N.N. Dai, K.L. Sheng, H.Q. Lu, J.M. Wang, L.P. Chen, Y.Z. Wang, Gut bacterial extracellular vesicles: important players in regulating intestinal microenvironment, Gut Microbes 14 (1) (2022).
[78] L. Ma, W. Lyu, Y. Song, K. Chen, L. Lv, H. Yang, W. Wang, Y. Xiao, Anti-inflammatory effect of clostridium butyricum-derived extracellular vesicles in ulcerative colitis: impact on host micrornas expressions and gut microbiome profiles, Mol. Nutr. Food Res 67 (13) (2023), e2200884.
[79] J.A. Molina-Tijeras, J. Galvez, M.E. Rodriguez-Cabezas, The immunomodulatory properties of extracellular vesicles derived from probiotics: a novel approach for the management of gastrointestinal diseases, Nutrients 11 (5) (2019).
[80] M. Yanez-Mo, P.R.M. Siljander, Z. Andreu, A.B. Zavec, F.E. Borras, E.I. Buzas, K. Buzas, E. Casal, F. Cappello, J. Carvalho, E. Colas, A. Cordeiro-da Silva, S. Fais, J.M. Falcon-Perez, I.M. Ghobrial, B. Giebel, M. Gimona, M. Graner, I. Gursel, M. Gursel, N.H.H. Heegaard, A. Hendrix, P. Kierulf, K. Kokubun, M. Kosanovic, V. Kralj-Iglic, E.M. Kramer-Albers, S. Laitinen, C. Lasser, T. Lener, E. Ligeti, A. Line, G. Lipps, A. Llorente, J. Lotvall, M. Mancek-Keber, A. Marcilla, M. Mittelbrunn, I. Nazarenko, E.N.M. Nolte-t' Hoen, T.A. Nyman, L. O'Driscoll, M. Olivan, C. Oliveira, E. Pallinger, H.A. del Portillo, J. Reventos, M. Rigau, E. Rohde, M. Sammar, F. Sanchez-Madrid, N. Santarem, K. Schallmoser, M. S. Ostenfeld, W. Stoorvogel, R. Stukelj, S.G. Van der Grein, M.H. Vasconcelos, M. H.M. Wauben, O. De Wever, Biological properties of extracellular vesicles and their physiological functions, J. Extra Vesicles 4 (2015).
[81] J.H. Liu, C.Y. Chen, Z.Z. Liu, Z.W. Luo, S.S. Rao, L. Jin, T.F. Wan, T. Yue, Y.J. Tan, H. Yin, F. Yang, F.Y. Huang, J. Guo, Y.Y. Wang, K. Xia, J. Cao, Z.X. Wang, C. G. Hong, M.J. Luo, X.K. Hu, Y.W. Liu, W. Du, J. Luo, Y. Hu, Y. Zhang, J. Huang, H. M. Li, B. Wu, H.M. Liu, T.H. Chen, Y.X. Qian, Y.Y. Li, S.K. Feng, Y. Chen, L.Y. Qi, R. Xu, S.Y. Tang, H. Xie, Extracellular vesicles from child gut microbiota enter into bone to preserve bone mass and strength, Adv. Sci. (Weinh.) 8 (9) (2021) 2004831.
[82] J. Pizarro-Cerda, P. Cossart, Bacterial adhesion and entry into host cells, Cell 124 (4) (2006) 715–727.
[83] J. Badia, L. Baldomà, Membrane vesicles from the gut microbiota and their interactions with the host, Bact. Membr. vesicles: Biog., Funct. Appl. (2020) 189–217.
[84] F. Ashrafian, A. Shahriary, A. Behrouzi, H.R. Moradi, S.K.A. Raftar, A. Lari, S. Hadifar, R. Yaghoubfar, S.A. Badi, S. Khatami, F. Vaziri, S.D. Siadat, Akkermansia muciniphila-derived extracellular vesicles as a mucosal delivery vector for amelioration of obesity in mice, Front Microbiol 10 (2019).
[85] F. Ashrafian, S.K.A. Raftar, A. Lari, A. Shahryari, S. Abdollahiyan, H.R. Moradi, M. Masoumi, M. Davari, S. Khatami, M.D. Omrani, F. Vaziri, A. Masotti, S. D. Siadat, Extracellular vesicles and pasteurized cells derived from Akkermansia muciniphila protect against high-fat induced obesity in mice, Micro Cell Fact. 20 (1) (2021).
[86] K.S. Park, K. Svennerholm, R. Crescitelli, C. Lasser, I. Gribonika, J. Lotvall, Synthetic bacterial vesicles combined with tumour extracellular vesicles as cancer immunotherapy, J. Extra Vesicles 10 (9) (2021), e12120.
[87] R.A.N. Palomino, C. Vanpouille, P.E. Costantini, L. Margolis, Microbiota-host communications: bacterial extracellular vesicles as a common language, Plos Pathog. 17 (5) (2021).
[88] E. Rinninella, P. Raoul, M. Cintoni, F. Franceschi, G.A.D. Miggiano, A. Gasbarrini, M.C. Mele, What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases, Microorganisms 7 (1) (2019) 14.
[89] J. Huang, D. Liu, Y. Wang, L. Liu, J. Li, J. Yuan, Z. Jiang, Z. Jiang, W.W. Hsiao, H. Liu, I. Khan, Y. Xie, J. Wu, Y. Xie, Y. Zhang, Y. Fu, J. Liao, W. Wang, H. Lai, A. Shi, J. Cai, L. Luo, R. Li, X. Yao, X. Fan, Q. Wu, Z. Liu, P. Yan, J. Lu, M. Yang, L. Wang, Y. Cao, H. Wei, E.L.-H. Leung, Ginseng polysaccharides alter the gut microbiota and kynurenine/tryptophan ratio, potentiating the antitumour effect of antiprogrammed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy, Gut 71 (2022) 734–745.
[90] X. Fan, C. Mai, L. Zuo, J. Huang, C. Xie, Z. Jiang, R. Li, X. Yao, X. Fan, Q. Wu, P. Yan, L. Liu, J. Chen, Y. Xie, E.L.-H. Leung, Herbal formula BaWeiBaiDuSan alleviates polymicrobial sepsis-induced liver injury via increasing the gut microbiota Lactobacillus johnsonii and regulating macrophage anti-inflammatory activity in mice, Acta Pharm. Sin. B (2022).
[91] A. Villard, J.M. Boursier, R. Andriantsitohaina, Microbiota-derived extracellular vesicles and metabolic syndrome, Acta Physiol. 231 (4) (2021).
[92] C. Lei, J. Mu, Y. Teng, L. He, F. Xu, X. Zhang, K. Sundaram, A. Kumar, M. K. Sriwastva, M.B. Lawrenz, L. Zhang, J. Yan, W. Feng, C.J. McClain, X. Zhang, H. G. Zhang, Lemon exosome-like nanoparticles-manipulated probiotics protect mice from C. d iff Infection, iScience 23 (10) (2020), 101571.
[93] R. Hong, L. Luo, L. Wang, Z.-L. Hu, Q.-R. Yin, M. Li, B. Gu, B. Wang, T. Zhuang, X.-Y. Zhang, Y. Zhou, W. Wang, L.-Y. Huang, B. Gu, S.-H. Qi, Lepidium meyenii Walp (Maca)-derived extracellular vesicles ameliorate depression by promoting 5-HT synthesis via the modulation of gut–brain axis, iMeta n/a(n/a) e116.

[94] R. Garcia-Martin, B.B. Brandao, T. Thomou, E. Altindis, C.R. Kahn, Tissue differences in the exosomal/small extracellular vesicle proteome and their potential as indicators of altered tissue metabolism, Cell Rep. 38 (3) (2022), 110277.
[95] S.L. Sado-Kamdem, L. Vannini, M.E. Guerzoni, Effect of alpha-linolenic, capric and lauric acid on the fatty acid biosynthesis in Staphylococcus aureus, Int. J. Food Microbiol. 129 (3) (2009) 288–294.
[96] M.A. Lambert, C.W. Moss, Cellular fatty acid composition of Streptococcus mutans and related streptococci, J. Dent. Res. 55 (1976) A96–A102.
[97] M. Bielaszewska, C. Ruter, A. Bauwens, L. Greune, K.A. Jarosch, D. Steil, W. Zhang, X. He, R. Lloubes, A. Fruth, K.S. Kim, M.A. Schmidt, U. Dobrindt, A. Mellmann, H. Karch, Host cell interactions of outer membrane vesicle-associated virulence factors of enterohemorrhagic Escherichia coli O157: Intracellular delivery, trafficking and mechanisms of cell injury, PLoS Pathog. 13 (2) (2017), e1006159.
[98] E. Jones, R. Stentz, A. Telatin, G.M. Savva, C. Booth, D. Baker, S. Rudder, S. C. Knight, A. Noble, S.R. Carding, The origin of plasma-derived bacterial extracellular vesicles in healthy individuals and patients with inflammatory bowel disease: a pilot study, Genes (Basel) 12 (10) (2021).
[99] S. Salminen, M.C. Collado, A. Endo, C. Hill, S. Lebeer, E.M.M. Quigley, M. E. Sanders, R. Shamir, J.R. Swann, H. Szajewska, G. Vinderola, The international scientific association of probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics, Nat. Rev. Gastroenterol. Hepatol. 18 (9) (2021) 649–667.
[100] M.F. Gonzalez, P. Diaz, A. Sandoval-Borquez, D. Herrera, A.F.G. Quest, Helicobacter pylori outer membrane vesicles and extracellular vesicles from helicobacter pylori-infected cells in gastric disease development, Int. J. Mol. Sci. 22 (9) (2021).
[101] F.G. Zingl, H.B. Thapa, M. Scharf, P. Kohl, A.M. Muller, S. Schild, Outer membrane vesicles of vibrio cholerae protect and deliver active cholera toxin to host cells via porin-dependent uptake, mBio 12 (3) (2021), e0053421.
[102] C. Rueter, M. Bielaszewska, Secretion and delivery of intestinal pathogenic escherichia coli virulence factors via outer membrane vesicles, Front Cell Infect. Microbiol. 10 (2020) 91.
[103] P. Deo, S.H. Chow, M.L. Han, M. Speir, C. Huang, R.B. Schittenhelm, S. Dhital, J. Emery, J. Li, B.T. Kile, J.E. Vince, K.E. Lawlor, T. Naderer, Mitochondrial dysfunction caused by outer membrane vesicles from Gram-negative bacteria activates intrinsic apoptosis and inflammation, Nat. Microbiol. 5 (11) (2020) 1418–1427.
[104] C.S. Alvarez, J. Badia, M. Bosch, R. Gimenez, L. Baldoma, Outer membrane vesicles and soluble factors released by probiotic escherichia coli Nissle 1917 and commensal ECOR63 enhance barrier function by regulating expression of tight junction proteins in intestinal epithelial cells, Front Microbiol 7 (2016) 1981.
[105] M. Bielaszewska, M. Marejkova, A. Bauwens, L. Kunsmann-Prokscha, A. Mellmann, H. Karch, Enterohemorrhagic Escherichia coli O157 outer membrane vesicles induce interleukin 8 production in human intestinal epithelial cells by signaling via Toll-like receptors TLR4 and TLR5 and activation of the nuclear factor NF-kappaB, Int J. Med Microbiol 308 (7) (2018) 882–889.
[106] O.Y. Kim, H.T. Park, N.T.H. Dinh, S.J. Choi, J. Lee, J.H. Kim, S.W. Lee, Y.S. Gho, Bacterial outer membrane vesicles suppress tumor by interferon-gamma-mediated antitumor response, Nat. Commun. 8 (1) (2017) 626.
[107] S.B. Amatya, S. Salmi, V. Kainulainen, P. Karihtala, J. Reunanen, Bacterial extracellular vesicles in gastrointestinal tract cancer: an unexplored territory, Cancers (Basel) 13 (21) (2021).
[108] D. Liu, S. Liu, J. Liu, L. Miao, S. Zhang, Y. Pan, sRNA23392 packaged by Porphyromonas gingivalis outer membrane vesicles promotes oral squamous cell carcinomas migration and invasion by targeting desmocollin-2, Mol. Oral. Microbiol. 36 (3) (2021) 182–191.
[109] M. Bielaszewska, C. Ruter, L. Kunsmann, L. Greune, A. Bauwens, W. Zhang, T. Kuczius, K.S. Kim, A. Mellmann, M.A. Schmidt, H. Karch, Enterohemorrhagic Escherichia coli hemolysin employs outer membrane vesicles to target mitochondria and cause endothelial and epithelial apoptosis, PLoS Pathog. 9 (12) (2013), e1003797.
[110] E.C. Han, S.Y. Choi, Y. Lee, J.W. Park, S.H. Hong, H.J. Lee, Extracellular RNAs in periodontopathogenic outer membrane vesicles promote TNF-alpha production in human macrophages and cross the blood-brain barrier in mice, FASEB J. 33 (12) (2019) 13412–13422.
[111] C.Y. Chen, S.S. Rao, T. Yue, Y.J. Tan, H. Yin, L.J. Chen, M.J. Luo, Z. Wang, Y. Y. Wang, C.G. Hong, Y.X. Qian, Z.H. He, J.H. Liu, F. Yang, F.Y. Huang, S.Y. Tang, H. Xie, Glucocorticoid-induced loss of beneficial gut bacterial extracellular vesicles is associated with the pathogenesis of osteonecrosis, Sci. Adv. 8 (15) (2022) eabg8335.
[112] M.R. Kim, S.W. Hong, E.B. Choi, W.H. Lee, Y.S. Kim, S.G. Jeon, M.H. Jang, Y. S. Gho, Y.K. Kim, Staphylococcus aureus-derived extracellular vesicles induce neutrophilic pulmonary inflammation via both Th1 and Th17 cell responses, Allergy 67 (10) (2012) 1271–1281.
[113] S. Keerthikumar, D. Chisanga, D. Ariyaratne, H. Saffar, S. Anand, K.N. Zhao, M. Samuel, M. Pathan, M. Jois, N. Chilamkurti, L. Gangoda, S. Mathivanan, ExoCarta: a web-based compendium of exosomal cargo, J. Mol. Biol. 428 (4) (2016) 688–692.
[114] D.K. Kim, J. Lee, S.R. Kim, D.S. Choi, Y.J. Yoon, J.H. Kim, G. Go, D. Nhung, K. Hong, S.C. Jang, S.H. Kim, K.S. Park, O.Y. Kim, H.T. Park, J.H. Seo, E. Aikawa, M. Baj-Krzyworzeka, B.W.M. van Balkom, M. Belting, L. Blanc, V. Bond, A. Bongiovanni, F.E. Borras, L. Buee, E.I. Buzas, L. Cheng, A. Clayton, E. Cocucci, C.S. Dela Cruz, D.M. Desiderio, D. Di Vizio, K. Ekstrom, J.M. Falcon-Perez, C. Gardiner, B. Giebel, D.W. Greening, J.C. Gross, D. Gupta, A. Hendrix, A.F. Hill, M.M. Hill, E.N. Hoen, D.W. Hwang, J. Inal, M.V. Jagannadham, M. Jayachandran, Y.K. Jee, M. Jorgensen, K.P. Kim, Y.K. Kim, T. Kislinger, C. Lasser, D.S. Lee, H. Lee, J. van Leeuwen, T. Lener, M.L. Liu, J. Lotvall, A. Marcilla, S. Mathivanan, A. Moller, J. Morhayim, F. Mullier, I. Nazarenko, R. Nieuwland, D.N. Nunes, K. Pang, J. Park, T. Patel, G. Pocsfalvi, H. del Portillo, U. Putz, M.I. Ramirez, M. L. Rodrigues, T.Y. Roh, F. Royo, S. Sahoo, R. Schiffelers, S. Sharma, P. Siljander, R.J. Simpson, C. Soekmadji, P. Stahl, A. Stensballe, E. Stepien, H. Tahara, A. Trummer, H. Valadi, L.J. Vella, S.N. Wai, K. Witwer, M. Yanez-Mo, H. Youn, R. Zeidler, Y.S. Gho, EVpedia: a community web portal for extracellular vesicles research, Bioinformatics 31 (6) (2015) 933–939.
[115] M. Pathan, P. Fonseka, S.V. Chitti, T. Kang, R. Sanwlani, J. Van Deun, A. Hendrix, S. Mathivanan, Vesiclepedia 2019:a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles, Nucleic Acids Res. 47 (D1) (2019) D516–D519.
[116] J. Van Deun, P. Mestdagh, P. Agostinis, O. Akay, S. Anand, J. Anckaert, Z. A. Martinez, T. Baetens, E. Beghein, L. Bertier, G. Berx, J. Boere, S. Boukouris, M. Bremer, D. Buschmann, J.B. Byrd, C. Casert, L. Cheng, A. Cmoch, D. Daveloose, E. De Smedt, S. Demirsoy, V. Depoorter, B. Dhondt, T.A. P. Driedonks, A. Dudek, A. Elsharawy, I. Floris, A.D. Foers, K. Gartner, A.D. Garg, E. Geeurickx, J. Gettemans, F. Ghazavi, B. Giebel, T.G. Kormelink, G. Hancock, H. Helsmoortel, A.F. Hill, V. Hyenne, H. Kalra, D. Kim, J. Kowal, S. Kraemer, P. Leidinger, C. Leonelli, Y.X. Liang, L. Lippens, S. Liu, A. Lo Cicero, S. Martin, S. Mathivanan, P. Mathiyalagan, T. Matusek, G. Milani, M. Monguio-Tortajada, L. M. Mus, D.C. Muth, A. Nemeth, E.N.M. Nolte-'t Hoen, L. O'Driscoll, R. Palmulli, M.W. Pfaffl, B. Primdal-Bengtson, E. Romano, Q. Rousseau, S. Sahoo, N. Sampaio, M. Samuel, B. Scicluna, B. Soen, A. Steels, J.V. Swinnen, M. Takatalo, S. Thaminy, C. Thery, J. Tulkens, I. Van Audenhove, S. van der Grein, A. Van Goethem, M. J. van Herwijnen, G. Van Niel, N. Van Roy, A.R. Van Vliet, N. Vandamme, S. Vanhauwaert, G. Vergauwen, F. Verweij, A. Wallaert, M. Wauben, K. W. Witwer, M.I. Zonneveld, O. De Wever, J. Vandesompele, A. Hendrix, EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research, Nat. Methods 14 (3) (2017) 228–232.
[117] H.Y. Lai, Y.C. Li, H.N. Zhang, J. Hu, J.T. Liao, Y. Su, Q. Li, B. Chen, C.P. Li, Z. Wang, Y. Li, J.L. Wang, Z.Q. Meng, Z.H. Huang, S.L. Huang, exoRBase 2.0: an atlas of mRNA, lncRNA and circRNA in extracellular vesicles from human biofluids, Nucleic Acids Res. 50 (D1) (2022) D118–D128.
[118] A. Hildebrandt, B. Kirchner, E.N.M. Nolte-'t Hoen, M.W. Pfaffl, miREV: an online database and tool to uncover potential reference RNAs and biomarkers in small-RNA sequencing data sets from extracellular vesicles enriched samples, J. Mol. Biol. 433 (15) (2021).
[119] C.J. Liu, G.Y. Xie, Y.R. Miao, M.X. Xia, Y. Wang, Q. Lei, Q. Zhang, A.Y. Guo, EVAtlas: a comprehensive database for ncRNA expression in human extracellular vesicles, Nucleic Acids Res. 50 (D1) (2022) D111–D117.

Dr. Jumin Huang, Ph.D. She received her doctoral degree in Macau University of Science and Technology. Currently, she is a postdoctoral fellow in University of Macau. Her main research direction is traditional Chinese medicine facilitating tumor immunity by modulating gut microbiota. Her research results were published in *Gut* (ESI highly cited paper), *Seminars in Cancer Biology*, *Antioxidants & Redox Signaling*, *Pharmacological Research*, etc. She has published more than 20 SCI papers and 2 Chinese patents. She is also the young editorial board of iMeta.

Xuanrun Wang, she received her Master's degree from the Macau University of Science and Technology in 2022. She is currently a doctor's student at University of Macau. The member of the Macau Clinical Oncology Society and the Chinese Medicine Immunology Professional Committee of the World Federation of Chinese Medicine Societies. Her main research direction is the research and development of innovative Chinese medicine anti-tumor Drug development and clinical research. During master's degree, she published 9 related SCI articles, with cumulative impact factor (IF) of 75.3.

Wang Ziming, He received his Master's degree in Medicine from the University of Chinese Academy of Sciences (UCAS) and is now a current PhD student at the University of Macau. His main research interests are multi-omics technology and precision oncology.



Liyan Deng, she is a student at Guangdong Medical University. Currently, she focuses on the mechanism of ferroptosis and has published 4 papers in Inflamm Bowel Dis, Antioxidants, and Inflamm Res.

Dr.Yuwei Wang, Ph.D. He is a lecturer from Shaanxi University of Chinese Medicine(Shaanxi, China). He received his Ph. D. degree in Chinese Medicine from Macau University of Science and Technology (Macau, China) and then worked as a postdoctoral fellow at the State Key Laboratory of Quality Research in Chinese Medicine of Macau University of Science and Technology. His study has been focused on anticancer drug design and has published more than 40 peer-reviewed journal articles.

Yu-Ping Tang (born 1970 in Bazhong, Sichuan Province), Professor and Dean of College of Pharmacy, Shaanxi University of Chinese Medicine. He obtained his Bachelor degree (Major: medicobotany) from China Pharmaceutical University in 1993. Afterwards, he joined Jiangsu Tianqing Pharmaceutical Co. Ltd. as assistant engineer. Two years later, he came back to China Pharmaceutical University, and obtained his PhD (Major: medicinal chemistry; supervisor: Professor Fengchang Lou) in 2000. As a postdoctoral researcher in Professor Biao Yu's group he worked for two years at Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. He visited Arizona State University as postdoctoral and faculty research fellow in Professor George R. Pettit's group for four years, he devoted to the isolation and structure study on the antitumor constituents from some natural resources there. In 2006 he came back to China and joined Nanjing University of Chinese Medicine, and was given an accelerated promotion to a full professor one year later. In 2017 he joined Nanjing University of Chinese Medicine, and became the dean of College of Pharmacy. His research interests mainly focused on the complex functional constituents and the compatibility interaction of traditional Chinese medicine with over 300 publications including review articles.

Dr. Lianxiang Luo, a master tutor, holds the position of associate researcher at the Institute of Marine Medicine of Guangdong Medical University. An individual who holds a Doctorate from Macau University of Science and Technology, is a postdoctoral fellow, and has founded the official account of "Luo Luo Research Group". The primary focus of his research is in the area of cell death mechanism and targeted drug research. Over the past five years, he has authored or been the corresponding author of more than 50 papers published in prestigious journals such as Theranostics, Pharmacological Research, and Aging and Disease, with an H index of 17. His work has been cited more than 1000 times, with a single paper receiving more than 100 citations. Additionally, he has applied for over 20 international and domestic patents, with nine of them being authorized. Working as a deputy editor of Heliyon and an editorial board member of BMC Cancer.

Prof. Elaine Leung is a professor at the University of Macau (UM) Faculty of Health Sciences (FHS), the 2020 Young Qihuang Scholar of the "Tens of millions" talent project of the People Republic of China, the Deputy Editor in Chief of Pharmacological Research (IF 10.334, Q1 interval). Prof. Leung has long worked in main research direction is about the investigation of Traditional Chinese medicines (TCMs) for the treatment of lung cancer, particularly on the holistic modulation function of TCMs. She is also interested in applying multi-omics technologies for precision oncology. Her research results were published in *Nature(outlook)*, *Gut*, *JAMA Oncology*, etc. She has already published over 150 SCI-E papers and owns 45 international invention patents.