

The Role of Bacterial Outer Membrane Vesicles in Tumor Development: Facts, Potential Applications and Future Perspectives

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Abstract: Bacterial outer membrane vesicles (OMVs), nanosized lipid bilayer particles released by both Gram-negative and Gram-positive bacteria, are emerging as crucial mediators of host–microbe interactions in cancer biology. This review synthesizes current evidence on how OMVs modulate tumor initiation, progression, and therapeutic responses through multifaceted mechanisms aligned with the 14 hallmarks of cancer. Studies demonstrate that OMVs can either promote or inhibit neoplastic processes depending on their bacterial origin and cargo composition. Tumor-promoting OMVs enhance proliferative signaling, drive epithelial–mesenchymal transition, facilitate metastatic dissemination via barrier disruption and angiogenesis, and weaken antitumor immunity. Conversely, other OMVs exert antineoplastic effects by triggering intrinsic apoptosis, cell-cycle arrest, immunogenic cell death, and remodeling of the tumor immune microenvironment. Pre-clinical studies further highlight the translational potential of engineered OMVs as precision nano-vaccines, immunotherapeutic agents, and adjuvants that synergize with immune checkpoint blockade, chemotherapy, or photothermal therapy while minimizing systemic toxicity. Future directions could focus on mapping of OMV cargo–pathway–phenotype networks by multidisciplinary methods, programmable vesicle design using synthetic biology, and real-time microbiome-OMV monitoring in early-phase clinical trials to enable individualized onco-therapeutics. Collectively, OMVs represent a versatile platform to bidirectionally regulate oncogenesis and therapeutic responses. Exploiting their molecular plasticity through rational engineering and precision medicine frameworks would bring transformative potential for cancer prevention, diagnosis, and treatment.

Keywords: bacterial outer membrane vesicles, tumor microenvironment, carcinogenesis, metastasis, immunotherapy, nanomedicine

Introduction

Bacteria release membrane vesicles (MVs) of 20–400nm in diameter, with specific cargos and various functions.¹ MVs were initially found to be produced by blebbing from the outer membrane of Gram-negative bacteria and are therefore termed as outer membrane vesicles (OMVs). However, more recent studies have indicated that Gram-positive species and bacteria that do not Gram stain can also release OMVs.^{2,3} OMVs consist of a single membrane bilayer, and encapsulate periplasmic and cytosolic proteins, DNA and RNA, and virulence factors. Therefore, OMVs are considered as a specialized bacterial secretion pathway.^{4,5} Owing to their abundant cargos and membrane components, OMVs can affect diverse biological processes, including DNA transfer, cell-to-cell communication, the delivery of virulence factors and toxins to host cells, inflammatory and immunomodulatory effects, and so on.^{1,5,6}

In addition to canonical OMVs, the MVs repertoire is now recognized to be markedly heterogeneous. Outer-inner membrane vesicles (OIMVs) have two membrane bilayers derived from both outer and inner membranes.⁷ DNA is

specifically packed into these types of MVs.^{7,8} Biogenesis is thought to proceed via explosive cell lysis, a process that also generates explosive outer-membrane vesicles (EOMVs) that randomly contain cytoplasmic components.⁹ Cytoplasmic membrane vesicles (CMVs) are MVs released by Gram-positive bacteria, derived from “bubbling cell death” triggered by endolysin or other peptidoglycan-damaging enzymes or treatments.¹⁰ Tube-shaped membranous structures (TSMs) are tube-like protrusions of the cytoplasmic membrane of Gram-positive bacteria or of the outer membrane of Gram-negative bacteria, acting as intercellular bridges to trade cytoplasmic molecules between biofilm cells.¹¹ For more detailed classification and routes of formation of MVs, please refer to Toyofuku et al.¹² For brevity, all bacteria-derived membrane vesicles discussed below are indiscriminately referred to as OMVs (Figure 1).

As a subpopulation of extracellular vesicle (EVs), OMVs share the characteristics and functions of EVs. The term “extracellular vesicles” was first suggested in 2011 as mobile membrane-limited vesicles in extracellular environment, including exosomes, activation- or apoptosis-induced microparticles and apoptotic bodies.¹³ EVs can be released by all cellular organisms, representing that vesiculation is a fundamental process of living matter.¹⁴

In recent years, the microbiome has emerged as a crucial role in carcinogenesis and prognosis, and can synergize cancer diagnosis and treatment.¹⁵ Few microbes are direct carcinogens, but many of them could affect tumor development, including biogenesis, growth, invasion and metastasis^{16–19} Dysbiosis or imbalance in the microbiome has also been mechanistically linked to local and systemic disorders, influencing different organs, immune dysregulation and

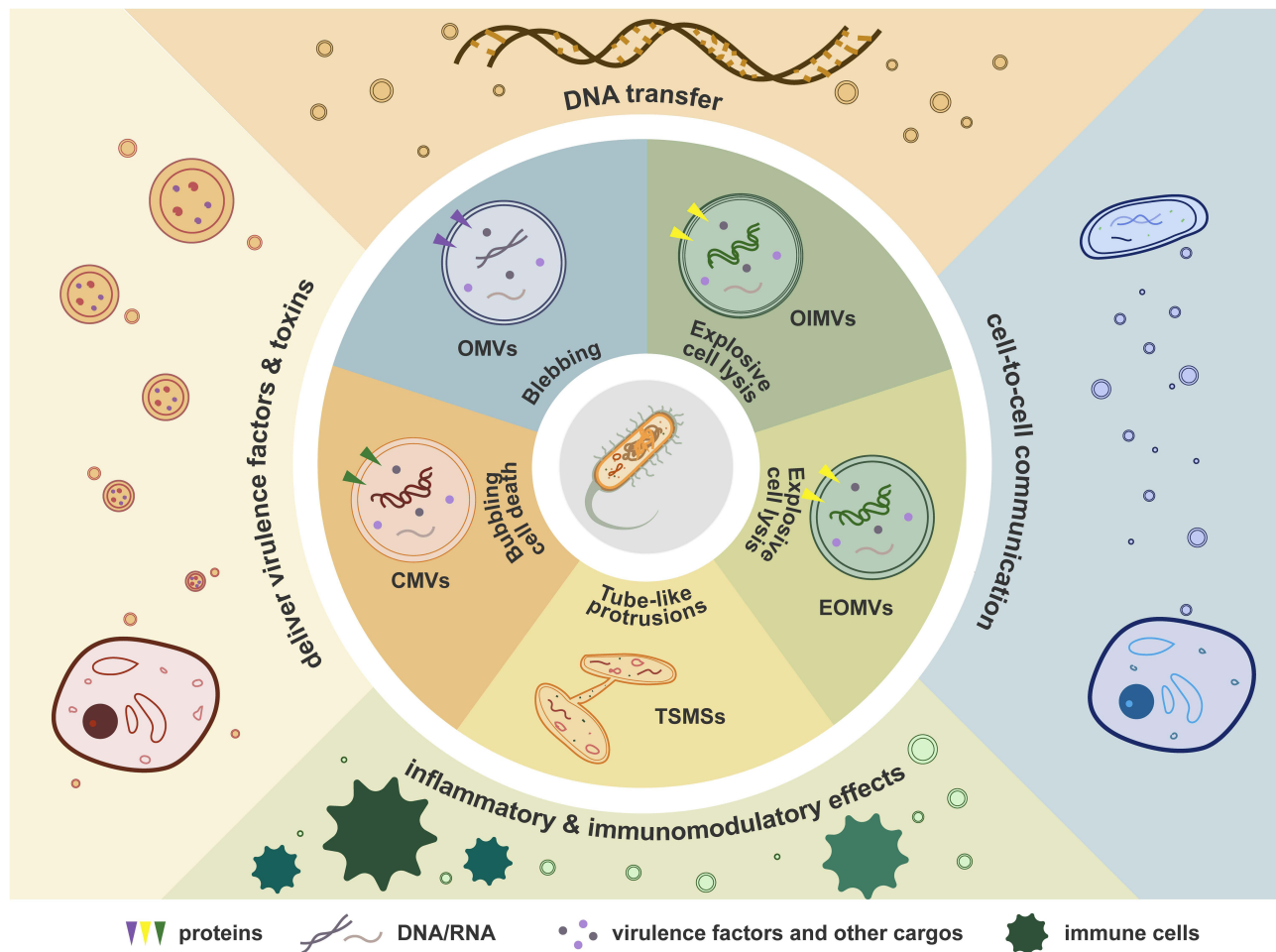


Figure 1 The biogenesis and functions of OMVs. Purple, green, and yellow arrowheads: trans-membrer proteins. Curve lines: DNA/RNA. Dots: virulence factors and other cargos. **Notes:** Triangles represent proteins; curved lines indicate DNA/RNA; dots denote virulence factors and other cargos.

Abbreviations: OMVs, Outer membrane vesicles; OIMVs, Outer-inner membrane vesicles; EOMVs, Explosive outer-membrane vesicles; CMVs, Cytoplasmic membrane vesicles; TSMs, Tube-shaped membranous structures.

oncogenesis.²⁰ As the dominant secretory conduit of bacteria, OMVs are considered crucial in interaction between pathogens and host cells, both nearby and distant.^{21,22} Compared with eukaryotic extracellular vesicles, OMVs exhibit smaller size, higher intrinsic immunogenicity, and superior amenability to genetic engineering, while mass-production can be achieved cost-effectively through high-density fermentation.^{1,12,23} Besides, OMVs could target immune cells more and accumulate within inflammatory lesions, rendering them particularly suited for cargo delivery and immunomodulation.^{1,12} Due to their specific biostructure and activities, naturally secreted or bioengineered OMVs have been propelled to the forefront of next-generation vaccinology and targeted drug-delivery platforms.²⁴ However, the mechanisms through which OMVs affect various biological processes in cancer development have been largely neglected. This review aims to synthesize current evidence delineating the multifaceted roles of OMVs in the initiation, progression and metastatic outgrowth of diverse malignancies, and critically appraise their translational potential for cancer prevention, early detection and precision therapeutics.

Human cells make their way from normalcy to malignant tumors by acquiring various functional capabilities. The most widely endorsed classification is the hallmarks of cancer put forward by Hanahan in 2000, 2011, and 2022, including 14 distinct characteristics, which are crucial for their ability to form malignant tumors. The 14 hallmarks include core hallmark capabilities that define the neoplastic phenotype at the single-cell level, and enabling characteristics that supply the cellular and microenvironmental substrate for acquisition of these functional traits.^{25–27} OMVs involve the development of cancer bidirectionally by interfering with the acquisition of these capabilities and the effect of these functions through various mechanisms. In this review, we categorize the above mechanisms based on the 14 hallmarks, aiming at providing innovative perspectives for future research, pharmaceutical development, and therapeutic strategies.

The Impacts of OMVs on Tumor Development

The association between OMVs and human pathobiology has become an area of intensive investigation in recent years. Many OMVs are demonstrated significant in both localized and systemic diseases.²⁸ For example, *Porphyromonas gingivalis* OMVs has been found to be closely related to the development of cardiovascular disease, Alzheimer's disease, rheumatoid arthritis, diabetes mellitus, non-alcoholic hepatitis, and cancer,²⁹ among which cancer might be the one that gets the most attention. This part will therefore focus on the relationship between OMVs and cancer development.

Human oncogenesis is a multistep process driven by both well characterized and emerging influencing factors. The main capabilities necessary to tumor cells have been categorized by Hanahan into 14 hallmarks as is mentioned above. Based on Hanahan's theory, mechanisms through which OMVs promote or inhibit tumor development are summarized as regulating tumor cell proliferation, affecting metabolic reprogramming, mediating invasion and metastasis, and modulating immune microenvironment. All these data establish OMVs as a crucial regulator in connecting microbiome and tumor biology (Figure 2).

OMVs and Tumor Cell Proliferation

OMVs can regulate tumor cell proliferation by affecting their proliferative potential, apoptosis process, and metabolic progresses (Table 1).

OMVs Mediated Proliferative Ability

OMVs modulate neoplastic proliferation capacity at multiple molecular levels. *Helicobacter pylori* OMVs (*H.p* OMVs), a well-characterized cofactor in digestive system oncogenesis, has been reported to regulate the oncogenes or tumor suppressors genes in host cells in many studies. *H.p* OMVs induce the secretion of Oncostatin-M (OSM) and overexpression of its type II receptor OSMR β in human gastric adenocarcinoma cell line (AGS), while similar overexpression is detectable in many other cancer types, such as pancreatic cancer, breast cancer and colon cancer.^{30,31} Elevated OSM is found in precancerous lesions and early gastric cancer biopsies, and OSMR β is demonstrated to promote gastric cancer growth and metastasis upon treatment with OSM.^{32,33} The OSM/OSMR β signal axis involves in many physical and pathological processes, including enhancement of cancer cell proliferation, metastasis, regulation of inflammation. Collectively, the *H.p* OMVs- OSM/OSMR β axis functions as a context-dependent factor that converts bacterial signals

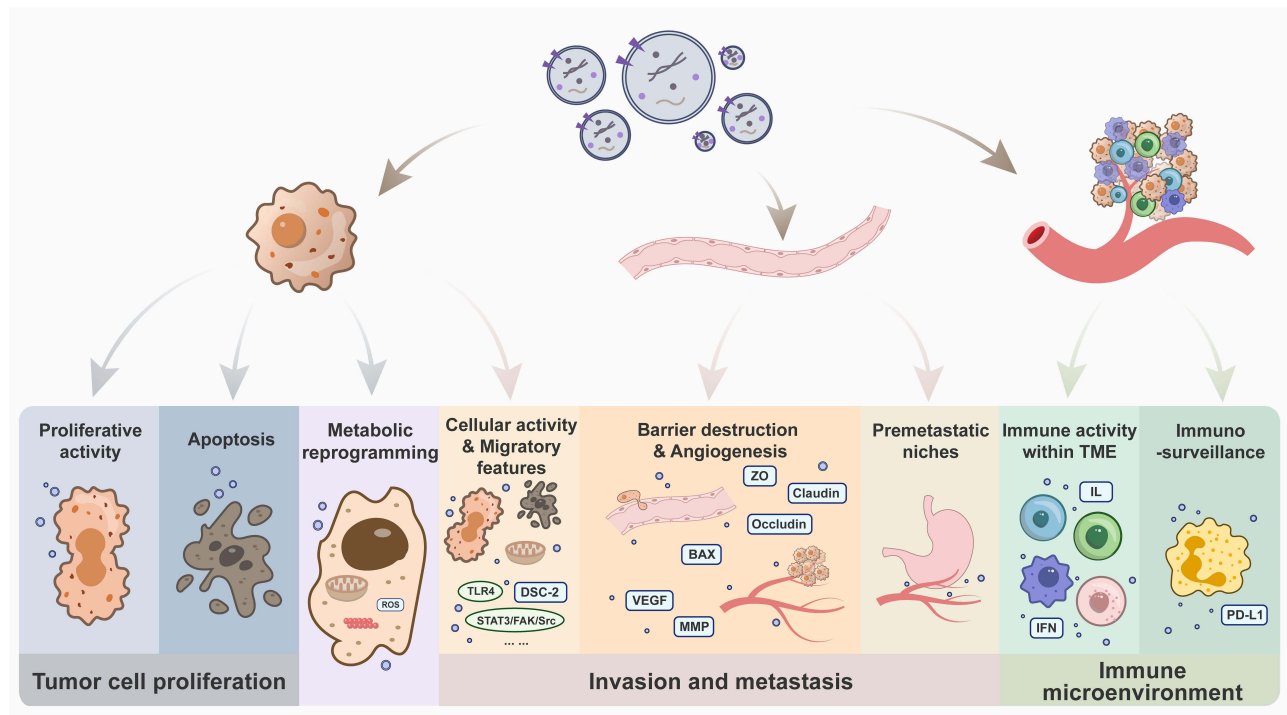


Figure 2 The impact of OMVs on tumor development.

into the full cascade of human tumor initiation and progression across anatomical sites.^{30–33} In breast cancer, OMVs derived from *Fusobacterium nucleatum* are also demonstrated to promote viability and proliferation through the activation of TLR4 pathway in vitro and in vivo.³⁴

Conversely, some studies indicate that certain OMVs inhibit tumor cell proliferation. *Salmonella Typhimurium* OMVs inhibit tumor growth and cell proliferation by downregulating Ki-67 expression.³⁵ *Escherichia coli* (*E. coli*) OMVs taken into cytoplasm and nucleus directly arrest cell cycle, blocking cells in the S phase.³⁶ *Bacillus licheniformis* OMVs increase reactive oxygen species (ROS) and decrease glutathione (GSH) to inhibit cell viability and proliferation.³⁷ In prostate cancer, *Akkermansia muciniphila* OMVs treated macrophages suppress the proliferation and invasion of tumor cells.³⁸ *Lactobacillus rhamnosus GG* OMVs are able to reduce the cell proliferation of HT29 (colorectal adenocarcinoma cell line) and SW480 (colorectal cancer cell line) by mechanism that still needs exploration.³⁹

OMVs Mediated Apoptosis

Apoptosis is a form of programmed cell death, essential for tissue homeostasis. It involves the activation, expression and regulation of a series of genes, such as the bcl-2 family and caspase family. By affecting the progress of apoptosis, OMVs can improve or reduce cell activity. OMVs from *Fusobacterium nucleatum* reduce ROS levels and oxidative stress response in colon cancer cells (CCC) to inhibit apoptosis.⁴⁰ Meanwhile, these vesicles promote the activity of superoxide dismutase (SOD) enzyme, which enhance the ability to fight free radicals and protect from oxidative damage, thus enhancing the activity of CCC.⁴⁰

Some OMVs induce cellular apoptosis, playing anti-tumor effects in vitro and in vivo. *Salmonella Typhimurium* OMVs elevate the expression of pro-apoptotic Bax gene, Beclin 1 (a marker for autophagy), and caspase-3 (a marker for cellular apoptosis), mediating non-inflammatory self-destruction of cancer cells.³⁵ OMVs released by *Lactobacillus rhamnosus GG* increase the apoptotic index (bax/bcl-2 expression ratio) in liver cancer, and lead to cancer cell death.⁴¹ Meanwhile, the supernatant of *Lactobacillus rhamnosus GG* is confirmed to upregulate pro-apoptotic genes including caspase-3, caspase-9 and Bax, and downregulate bcl-2, resulting in a decrease in expression levels of cyclin D1, cyclin E and ERBB2 genes, and then arrest cancer cells in the G0/G1 phase of the cell cycle, thus inhibiting the growth of HT-29 cancer cells and induce apoptosis.⁴² *Lentilactobacillus buchneri* OMVs are also demonstrated apoptotic activity

Table 1 The Role of OMVs in the Regulation of Tumor Proliferation

Function	Bacteria	Cancer	Acting Pattern	Key Pathway/Ingredient	References	
Proliferative potential regulation	<i>Helicobacter pylori</i> <i>Fusobacterium nucleatum</i> <i>Salmonella Typhimurium</i>	Gastric adenocarcinoma	Induce the overexpression of OSM and OSMR β →promote proliferation	OSM/OSMR β signal axis	[30–33]	
		Breast cancer	Activate TLR4→promote viability and proliferation	TLR4	[34]	
		Colorectal carcinoma	Downregulate Ki-67 expression→inhibit cell viability and proliferation	Ki-67↓	[35]	
	<i>Escherichia coli</i> <i>Bacillus licheniformis</i>	Breast cancer				
		Hepatocellular carcinoma				
		Neuroblastoma	Arrest cell cycle in the S phase→inhibit cell proliferation	/	[36]	
	Apoptosis regulation	<i>Bacillus licheniformis</i>	Breast cancer	Increase ROS and decrease GSH→inhibit cell viability and proliferation	ROS↑; GSH↓	[37]
			Lung cancer			
		<i>Akkermansia muciniphila</i> <i>Lactobacillus rhamnosus GG</i>	Prostate cancer	Induce macrophages to suppress cell proliferation	/	[38]
			Colorectal cancer	Reduce cell proliferation	Unknown	[39]
<i>Fusobacterium nucleatum</i> <i>Salmonella Typhimurium</i> <i>Lactobacillus rhamnosus GG</i> <i>Lentilactobacillus buchneri</i> <i>Bifidobacterium</i> <i>Toxigenic Clostridioides difficile</i> <i>Escherichia coli</i> ; strain-A5922 <i>Escherichia coli</i> ; Strain ATCC 11775 <i>Lactocaseibacillus paracasei</i> PC-H1 <i>Lactobacillus plantarum</i>		Colon cancer	Reduce ROS levels and promote SOD enzyme activity→inhibit apoptosis	ROS↓; SOD↑	[40]	
		Colorectal carcinoma	Increase Bax expression, Beclin 1 and caspases-3→autophagy and apoptosis	Bax↑; Beclin 1↑; Caspases 3↑	[35]	
		Hepatocellular carcinoma				
		Liver cancer	Increase the apoptotic index→cell death	Bax/bcl-2↑	[41]	
		Colon cancer	(Supernatant) upregulate caspase-3, caspase-9 and Bax, and downregulate bcl-2→decrease the expression of cyclin D1, cyclin E and ERBB2 genes→arrest cancer cells in the G0/G1 phase→inhibit growth and induce apoptosis	Caspase-3↑; caspase-9↑; Bax↑; bcl-2↓; cyclin D1↓; cyclin E↓; ERBB2 gene↓	[42]	
		Colorectal adenocarcinoma	Increase Bax, caspase-3 and caspase-9 expression→arrest cancer cells in the G0/G1 phase and reduce cell viability→ induce apoptosis	Caspase-3↑; caspase-9↑; Bax↑	[43]	
	Gastric adenocarcinoma					
	Breast cancer	Increase Bax expression and decrease bcl-2→ induce apoptosis	Bax↑; bcl-2↓	[44]		
	Liver cancer	Increase caspase3 expression→ induce apoptosis	Caspase-3↑	[45]		
	Colon cancer	Increase ROS production→induce oxidative stress through Akt/mTOR pathways→mitophagy→cell death	ROS↑; Akt/mTOR pathway	[46]		
Metabolic reprogramming	<i>Escherichia coli</i> ; Strain ATCC 11775	Neuroblastoma	Increase phosphorylated P53, BAX protein and Cyt C in cytoplasm, and reduce antiapoptotic protein BCL-2 and Cyt C among mitochondrial proteins→activate mitochondrial apoptosis pathway and caspase-3→DNA damage and apoptosis	Mitochondrial apoptosis pathway caspase-3	[36]	
		Colorectal cancer	Activate the PDK1/AKT/Bcl-2 signaling pathway→inhibit growth and induce apoptosis	PDK1/AKT/Bcl-2 signaling pathway	[47]	
	<i>Lactocaseibacillus paracasei</i> PC-H1 <i>Lactobacillus plantarum</i>	Colorectal cancer	Attenuate PDK2 expression in p53-p21-dependent metabolic signaling→sensitize CRC/5FU cells to 5-FU→reverse metabolic shift and circumvent 5-FU resistance→inhibit proliferation and lead to apoptosis	PDK2↓	[48]	
		Colorectal cancer				
	<i>Fusobacterium nucleatum</i> <i>Staphylococcus aureus</i> <i>Toxigenic Clostridioides difficile</i>	Colorectal cancer	Regulate amino acid biosynthesis and metabolic pathways (eg central carbon metabolism, protein digestion, and uptake in cancer)→promote cell proliferation	/	[40]	
		/	Induce the necrosis of keratinocytes→disrupt skin barrier→chronic inflammatory	α -hemolysin	[49]	
		/	Induce the formation of LC3-GFP aggregates→an autophagy response in lung epithelial cells→immunostimulatory effect	LC3-GFP	[50]	
		Liver cancer	Induce mitochondrial dysfunction and increased intracellular ROS, and upregulate the expression of genes related to mitochondrial fission (FIS1 and DRP1), antioxidant status (GPX1), glycolysis (HK2, PDK1, LDHA and PKM2) and β -oxidation (CPT1A)	/	[45]	

Notes: →: The following is the next step of the procedure. ↑/↓: The pathway is activated/suppressed, or the expression of the ingredient is upregulated/downregulated. /: not applicable or not mentioned.

by increasing the expression of Bax, caspase-3 and caspase-9 genes, arresting AGS and HT-29 cells in the G0/G1 phase and reducing cell viability.⁴³ Likewise, the *Bifidobacterium* OMVs increase Bax expression and decrease bcl-2, inducing cellular apoptosis in triple-negative breast cancer,⁴⁴ and *Toxigenic Clostridioides difficile* OMVs upregulate caspase3 gene expression to increase apoptosis in liver cancer cells.⁴⁵ OMVs from *E. coli* strain-A5922 increase ROS production and induce oxidative stress via the activation of Akt/mTOR pathways, subsequently inducing mitophagy and culminating in cell death in CCC.⁴⁶ OMVs from another *E. coli* strain, ATCC 11775, upregulate the expression of phosphorylated P53, BAX protein and Cyt C in cytoplasm, and downregulate the expression of antiapoptotic protein BCL-2 and Cyt C among mitochondrial proteins. Thus, the mitochondrial apoptosis pathway and cleaved caspase-3 are activated, resulting in DNA damage and apoptosis in neuroblastoma.³⁶ *Lactocaseibacillus paracasei* PC-H1 OMVs activate the PDK1/AKT/Bcl-2 signaling pathway to inhibit the growth of colorectal cancer (CRC) cells and induce apoptosis.⁴⁷ OMVs derived from *Lactobacillus plantarum* sensitize CRC/5FU cells (5-fluorouracil-resistant CRC cells) to 5-FU by attenuating PDK2 expression in p53-p21-dependent metabolic signaling, to reverse the metabolic shift (such as the increased glycolysis) and circumvent 5-FU resistance, inhibiting cancer cell proliferation and leading to apoptosis.⁴⁸

OMVs Mediated Metabolic Reprogramming

Effects on metabolic progresses can lead to diverse outcomes of tumor cell proliferation. OMVs from *Fusobacterium nucleatum* regulate amino acid biosynthesis and metabolic pathways in CRC cells, such as central carbon metabolism, protein digestion, and uptake in cancer, thus promoting cell proliferation.⁴⁰ OMV-associated α -hemolysin of *Staphylococcus aureus* (*S. aureus*) induce necrosis of keratinocytes, disrupting skin barrier and resulting in chronic inflammatory.⁴⁹ *S. aureus* OMVs can also induce the formation of LC3-GFP aggregates and an autophagy response in lung epithelial cells, leading to the immunostimulatory effect in host cells.⁵⁰ *Toxigenic Clostridioides difficile* OMVs are demonstrated to induce mitochondrial dysfunction and increased intracellular ROS, and meanwhile upregulate the expression of genes related to mitochondrial fission (FIS1 and DRP1), antioxidant status (GPX1), glycolysis (HK2, PDK1, LDHA and PKM2) and β -oxidation (CPT1A) in liver cancer cells, with impacts on proliferation under exploration.⁴⁵

OMVs Mediated Invasion and Metastasis

Invasive and metastatic competence is considered one of the hallmarks of cancer, denoting the biologic capacity of malignant cells to detach from the primary neoplasm to a new organ and establish secondary tumors. The complex process of invasion and metastasis has been summarized in a series of steps, often termed as invasion-metastasis cascade. The complete course includes the local invasion, detachment of tumor cells, intravasation into nearby blood and lymphatic vessels, transit through the lymphatic and hematogenous systems, attachment to endothelial cells that line such vessels, extravasation from the lumina to the parenchyma of tissue or organs, the formation of small nodules, and finally the growth from micro-metastatic lesions into tumors, which is termed as colonization.^{26,51} Accumulating evidence indicates that OMVs modulate the cascade in diverse aspects (Table 2).

Effects on Tumor Cells

The effects of OMVs on tumor cells mainly include altered cellular activity and acquisition of migratory features. Among the OMVs mentioned above, some have been demonstrated to attenuate invasive and migrative capacity by inhibiting tumor cell proliferation and thus affecting cellular activity. *Salmonella Typhimurium* OMVs downregulate Ki-67 expression and increase Bax expression, Beclin 1 and caspases-3, inhibiting tumor cell proliferation. The inhibition also impact the invasive and migratory viability of tumor cells, playing significant anti-tumor effects.³⁵ Similarly, *Lentilactobacillus buchneri* OMVs decrease migration viability by upregulating apoptotic activity.⁴³ In addition, macrophages treated by *Akkermansia muciniphila* OMVs suppress the proliferation and invasion of prostate cancer cells, of which concrete procedures will be summarized in the following parts.³⁸

OMVs exert bidirectional control over the acquisition of metastatic competence, either licensing or constraining the molecular and cellular programs that drive tumor cell dissemination. *Fusobacterium nucleatum* OMVs enhance the

Table 2 The Role of OMVs in the Mediation of Tumor Invasion and Metastasis

Function	Bacteria	Cancer	Acting Pattern	Key Pathway/Ingredient	References
Alter cellular activity	<i>Salmonella Typhimurium</i>	Colorectal carcinoma Breast cancer Hepatocellular carcinoma	Downregulate Ki-67 expression and increase Bax expression, Beclin 1 and caspases-3→reduce tumor cell activity	Ki-67↓; Bax↑; Beclin 1↑; Caspases 3↑	[35]
	<i>Lentilactobacillus buchneri</i>	Colorectal adenocarcinoma Gastric adenocarcinoma	Increase Bax, caspase-3 and caspase-9 expression→reduce tumor cell activity	Caspase-3↑; caspase-9↑; Bax↑	[43]
Acquisition of migratory features	<i>Akkermansia muciniphila</i>	Prostate cancer	Induce macrophages to suppress cell proliferation→reduce tumor cell activity	/	[38]
	<i>Fusobacterium nucleatum</i>	Breast cancer Colorectal cancer Oral cancer	Activate TLR4→promote migration and invasion Increase mitochondrial fusion→promote migration and invasion Activate intracellular autophagy pathways→induce EMT phenotype→downregulate E-cadherin and upregulate Vimentin and N-cadherin→promote migration and invasion	TLR4 / E-cadherin↓; Vimentin↑; N-cadherin↑	[34] [40, 52] [53]
Barrier destruction	<i>Helicobacter pylori</i>	Gastric adenocarcinoma	Induce the overexpression of OSM and OSMRβ→promote migration and invasion	OSM/OSMRβ signal axis	[30–33]
	<i>Porphyromonas gingivalis</i>	Oral squamous cell carcinoma	Contain sRNA23392→decrease DSC-2 expression→reduce adhesion ability→promote migration and invasion	DSC-2↓	[54]
	<i>Fusobacterium nucleatum</i>	Colorectal cancer	Downregulate miR-574-5p expression→activate miR-574-5p/CARD3-dependent autophagy→downregulate ZO-1 and claudin-1→epithelial barrier dysfunction in IECs	miR-574-5p axis; ZO-1, Claudin-1↓	[55]
	Enteropathogenic <i>Escherichia coli</i> (EPEC)	Colorectal cancer	Downregulate ZO-1, ZO-2, occludin, claudin-14 expression and alter the subcellular localization of ZO-1, occludin and F-actin cytoskeleton→intestinal epithelial barrier dysfunction	ZO-1, ZO-2, occludin, claudin-14↓	[56, 57]
	<i>Escherichia coli</i> Nissle 1917 (EcN)	Colorectal cancer	Upregulate ZO-1, claudin-14 and downregulate claudin-2→positively modulate the intestinal epithelial barrier; retain ZO-1 and occludin at tight junctions in the cell boundaries and ameliorate F-actin disorganization→counteract EPEC-altered transepithelial resistance and paracellular permeability	ZO-1, claudin-14↑, claudin-2↓	[56, 57]
	<i>Limosilactobacillus reuteri</i>	Colon cancer	Counteract the detrimental effect of enterotoxigenic <i>E. coli</i> (ETEC)→protect the integrity of epithelial barrier	/	[58]
Angiogenesis	<i>Limosilactobacillus johnsoni</i>	Colon cancer	Increase ZO-1 and occludin mRNA expression and reduce IL-1β, caspase-3, and bax gene expression in intestinal epithelial cells→improve intestinal barrier function	ZO-1, occludin↑; IL-1β, caspase-3, bax↓	[59]
	<i>Limosilactobacillus mucosae</i>	Colon cancer	Increase the expression of ZO-1 and occludin in intestinal epithelial cells→enhance tight junction function and decrease the gut permeability→improve intestinal barrier integrity	ZO-1, occludin↑	[55, 60]
	<i>Akkermansia muciniphila</i>	Colon cancer	Increase the expression of ZO-1 and occludin in intestinal epithelial cells→enhance tight junction function and decrease the gut permeability→improve intestinal barrier integrity	ZO-1, occludin↑	[55, 60]
	<i>Salmonella Typhimurium</i>	Colorectal carcinoma Breast cancer Hepatocellular carcinoma	Inhibit the expression of VEGF gene→anti-angiogenesis	VEGF↓	[35]
	<i>Escherichia coli</i> Strain ATCC 11775	Neuroblastoma	Decrease the expression of MMP-2, MMP-9, VEGF, CD24→inhibit migration and invasion	MMP-2, MMP-9, VEGF, CD24↓	[36]
Establishing premetastatic niches in distant organs	<i>Helicobacter pylori</i>	Extragastric neoplasia	OMV-CagA→delivered to distant organs and tissues→localize in proximity to ZO-1 in junctional area, and induce strong histone H1 affinity to ATP in differentiated and polarized epithelial monolayers→effects on gene transcription→different results of cancer development	/	[61–63]

Notes: →: The following is the next step of the procedure. ↑/↓: The pathway is activated/suppressed, or the expression of the ingredient is upregulated/downregulated. /: not applicable or not mentioned.

migration and invasion of breast cancer cells via TLR4,³⁴ and have similar effects on colorectal cancer cells by increasing mitochondrial fusion.^{40,52} It can also promote cell migration and invasion of oral cancer by activating intracellular autophagy pathways and inducing the EMT (epithelial–mesenchymal transition) phenotype in cancer cells, downregulating E-cadherin and upregulating Vimentin and N-cadherin.⁵³ *Helicobacter pylori* OMVs induce OSM secretion and OSMR β overexpression, activate the STAT3/FAK/Src pathway, and finally prompt the migration and invasion potential of AGS.^{30–33} *Porphyromonas gingivalis* OMVs contain abundant sRNA23392, which is found to target and degrade Desmocollin-2 (DSC-2), a type of desmosomes, acting as a cadherin-type transmembrane adhesion molecule. With the decreased expression of DSC-2, the adhesion ability of oral squamous cell carcinoma cells is reduced, leading to the enhancement of migration and invasion ability.⁵⁴

Effects on Vascular Systems

Hematogenous and lymphatic dissemination share a mechanistic prerequisite: the destruction of endothelial junctions that permits tumor cells to traverse the vessel wall in a process collectively termed trans-endothelial migration (TEM).⁶⁴ Meanwhile, individualities exist in both metastases. For example, cytokines such as vascular endothelial growth factor C/D (VEGFC/D) and insulin-like growth factor 1 (IGF-1) secreted by cancer cells can induce lymphatic vessel formation and drive lymphatic metastasis,^{65–67} while neutrophils, monocytes-derived VEGF and metastasis-associated inflammatory macrophages can contribute to extravasation in hematogenous metastasis.^{60,64,68} As most studies explore the two systems with a same model rather than focus on their distinctions, we frame the impact of OMVs on vascular systems according to their underlying biologic processes—barrier destruction and angiogenesis—rather than by the anatomic distinction between blood and lymphatic vessels.

The integrity of endothelial barrier is guaranteed by tight junction proteins, which mainly consist of the claudin family, occludin family, and Zona Occludens (ZO) family. *Fusobacterium nucleatum* OMVs downregulate the expression of miR-574-5p to activate mir-574-5p/CARD3-dependent autophagy, reducing ZO-1 and claudin-1 and thus leading to epithelial barrier dysfunction in intestinal epithelial cells (IECs).⁵⁵ The enteropathogenic *E. coli* (EPEC) OMVs downregulate ZO-1, ZO-2, occludin, claudin-14 expression and alter the subcellular localization of ZO-1, occludin and F-actin cytoskeleton. However, *E. coli* Nissle 1917 (EcN) are found to positively modulate the intestinal epithelial barrier by upregulating ZO-1, claudin-14 and downregulating claudin-2, which can also counteract EPEC-altered transepithelial resistance and paracellular permeability, by retaining ZO-1 and occludin at tight junctions in the cell boundaries and ameliorating F-actin disorganization by factors free-released or associated with OMVs.^{56,57} Likewise, *Limosilactobacillus reuteri* OMVs protect the integrity of epithelial barrier from the detrimental effect of enterotoxigenic *E. coli* (ETEC).⁵⁸ Apart from the above, *Limosilactobacillus johnsoni* and *Limosilactobacillus mucosae* OMVs improve intestinal barrier function by increasing ZO-1 and occludin mRNA expression and reducing IL-1 β , caspase-3, and bax gene expression in intestinal epithelial cells.⁵⁹ *Akkermansia muciniphila* OMVs enter intestinal epithelial cells to stimulate the expression of ZO-1 and occludin, enhancing tight junction function and decreasing the gut permeability, thus improving the intestinal barrier integrity^{55,60} (Figure 3).

Some OMVs downregulate angiogenesis to inhibit migration and invasion. *Salmonella Typhimurium* derived OMVs play the role of anti-angiogenesis by inhibiting the expression of VEGF gene.³⁵ OMVs of *E. coli* (strain ATCC 11775) decrease the expression of MMP-2, MMP-9, VEGF, CD24 to inhibit migration and invasion of neuroblastoma cells.³⁶

OMVs Mediated Formation of Premetastatic Niches in Distant Organs

Some OMVs counteract with distant organs and thus affect cancer development. CagA is a bacterial oncoprotein,⁶¹ and its delivery to distant organs and tissues mediated by *Helicobacter pylori* OMVs may be involved in the development of extragastric neoplasia.⁶² OMV-CagA is found to localize in proximity to ZO-1 in junctional area, and induce strong histone H1 affinity to ATP in differentiated and polarized epithelial monolayers, which may have possible effects on gene transcription, leading to different results of cancer development.⁶³

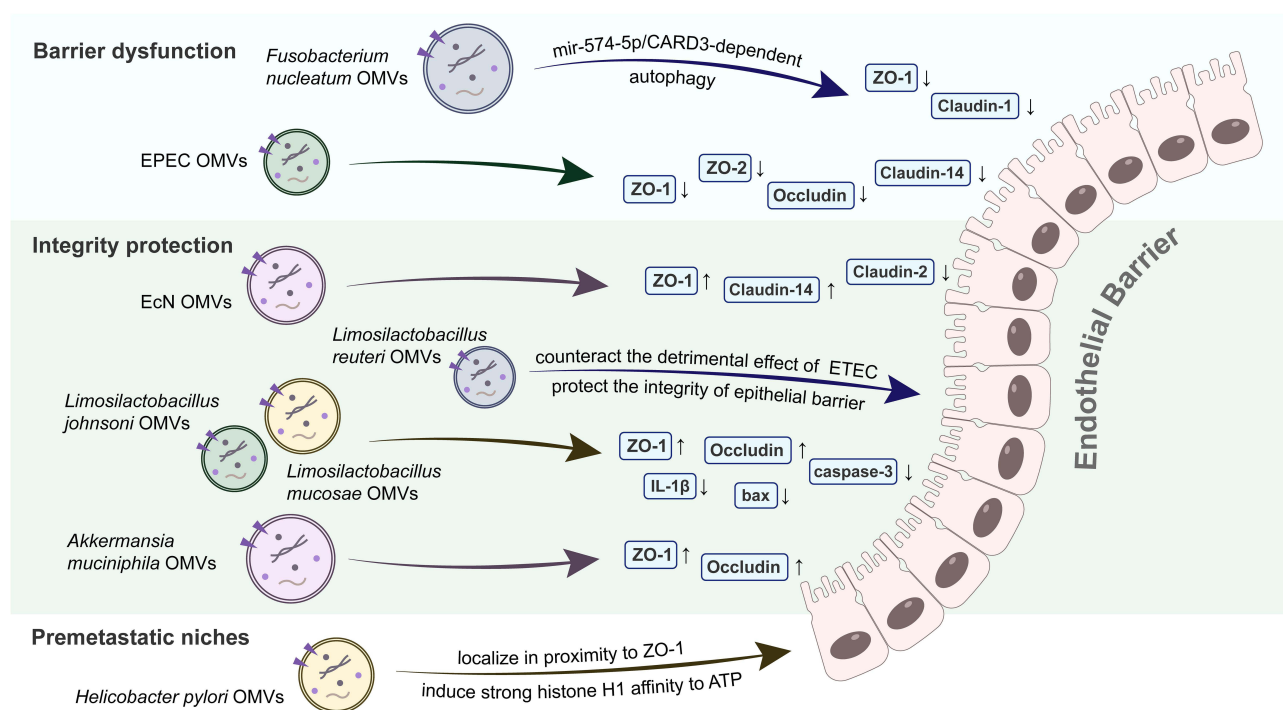


Figure 3 The influence of OMVs on tight junction of endothelial barrier. Upward arrows: upregulate the expression of the molecules. Downward arrows: upregulate the expression of the molecules.

Modulation of the Immune Microenvironment

The effects of OMVs on immune microenvironment are listed in [Table 3](#).

OMVs Mediated Regulating Immune Activity within the Tumor Microenvironment (TME)

Tumor microenvironment (TME) is a highly integrated and dynamically evolving ecosystem composed of neoplastic cells, heterogeneous immune infiltrates and extracellular-matrix constituents. This multicellular niche governs every stage of tumorigenesis, from initial transformation to metastatic outgrowth.⁷⁹ OMVs either amplify or attenuate the immune activity in TME, engaged in the modulation of immune microenvironment, thereby exerting control over cancer progression.

Some OMVs enhance the immune activity by mobilizing immune cells. *E. coli* OMVs are demonstrated to induce infiltration and activation of cancer antigen-specific stem-like CD8+ T cells with high expression of TCF1 and PD-1 into the TME.^{69,70} *E. coli* MG1655 Δ pal Δ lpxM OMVs show anti-tumor immunity by mediating the expansion of V γ 9V Δ 2 T cells, which then develop robust cytolytic activity against breast cancer and leukaemia cells.⁷¹ *Salmonella Typhimurium* derived OMVs are successfully exploited to passively target tumor mass by EPR (enhanced permeability and retention) effect and presents its content of PAMPs (pathogen-associated molecular patterns), and thus awaken patients' immune system to defeat tumor. The anti-tumor effect is mediated by upregulating CD49b expression and then increasing NK cells infiltration.³⁵ *Akkermansia muciniphila* OMVs play antitumor immunity against prostate cancer by elevating the proportion of granzyme B-positive (GZMB+) and interferon γ -positive (IFN- γ +) lymphocytes in CD8+ T cells and causing macrophage recruitment, with increased tumor-killing M1 macrophages and decreased immunosuppressive M2 macrophages.³⁸ Also, *Akkermansia muciniphila* OMVs are found to promote CTL-related immune response against CRC with Amuc_2172 as active principle.⁷² In the meanwhile, some OMVs trigger anti-tumor responses by specifically targeting and accumulating in tumor tissue, thus inducing antitumor cytokines CXCL10 and interferon- γ .⁷³

By avoiding immune destruction, some OMVs and their components weaken the immune activity within TME. OMVs from *Helicobacter suis* translocate across the epithelial layers and deliver γ -glutamyl transpeptidase (GGT) to mucosal lymphocytes, inhibiting their proliferation.⁷⁴ OMVs from *Helicobacter pylori* induce the expression of

Table 3 The Role of OMVs in the Modulation of Immune Microenvironment

Function	Bacteria	Cancer	Acting Pattern	Key Pathway/Ingredient	References	
Regulating immune activity within TME	<i>Escherichia coli</i>	/	Induce infiltration and activation of cancer antigen-specific stem-like CD+8 T cells with high expression of TCF1 and PD-1 into the TME→mobilize immune cells to enhance the immune activity	/	[69, 70]	
	<i>Escherichia coli</i> ; MGI655 Δ pal; Δ lpxM	Breast cancer Leukaemia	Mediate the expansion of V γ 9V Δ 2 T cells→robust cytolytic activity→enhance the anti-tumor immunity	/	[71]	
	<i>Salmonella Typhimurium</i>	Colorectal carcinoma Breast cancer Hepatocellular carcinoma	Upregulate CD49b expression→increase NK cell infiltration→passively target tumor mass by EPR effect and presents its content of PAMPs→awaken the immune system to defeat tumor	CD49b \uparrow ; EPR effect, PAMPs	[35]	
	<i>Akkermansia muciniphila</i>	Prostate cancer	Elevate the proportion of GZMB+ and IFN- γ + lymphocytes in CD8+ T cells and cause macrophage recruitment, with increased tumor-killing M1 macrophages and decreased immunosuppressive M2 macrophages→mobilize immune cells to enhance the immune activity	/	[38]	
	<i>Escherichia coli</i> W3110 Δ msbB; <i>Lactobacillus acidophilus</i>	Colorectal cancer Colorectal cancer Breast cancer Melanoma	Amuc_2172→promote CTL-related immune response Target and accumulate in tumor tissue→induce antitumor cytokines CXCL10 and interferon- γ →enhance immune activity	Amuc_2172 CXCL10 \uparrow ; IFN- γ \uparrow	[72] [73]	
	<i>Helicobacter suis</i>	Gastric cancer	Translocate across the epithelial layers→deliver GGT to mucosal lymphocytes→inhibit their proliferation→weaken immune activity	/	[74]	
	<i>Helicobacter pylori</i>	Gastric cancer	Induce the expression of monocytes COX-2→increase PGE2 and IL-10→inhibit human T cell responses→weaken immune activity	COX-2 \uparrow ; PGE2 \uparrow ; IL-10 \uparrow	[69]	
			Transport sncRNA into host cells→inhibit IL-8 expression→weaken the innate immune response of infected individuals to eliminate <i>H. pylori</i>	IL-8 \downarrow	[70]	
	Affecting immunosurveillance	<i>Staphylococcus aureus</i>	/	Contain many virulence factors→activate NETs→promote the immune evasion of <i>S. aureus</i>	/	[75–77]
		<i>Porphyromonas gingivalis</i>	Oral carcinoma	Induce PD-L1 expression in a RIP2-dependent fashion→the escape of tumor cells from immunosurveillance	PD-L1 \uparrow	[78]

Notes: →: The following is the next step of the procedure. \uparrow/\downarrow : The pathway is activated/suppressed, or the expression of the ingredient is upregulated/downregulated. /: not applicable or not mentioned.

monocytes COX-2 to increase PGE2 and IL-10, inhibiting T cell responses.⁸⁰ Meanwhile, *Helicobacter pylori* OMVs are found to transport sncRNA into host cells to inhibit IL-8 expression, weakening the innate immune response of infected individuals to eliminate *Helicobacter pylori*, which may worsen chronic gastritis and gastric cancer.⁸¹

Affecting Immunosurveillance

The theory of immune surveillance proposes that cells and tissues are under the monitor of immune system, which is responsible for recognizing and eliminating the vast majority of incipient cancer cells and thus nascent tumors.²⁶ Certain OMVs drive neoplastic progression by subverting immunosurveillance. OMVs derived from *S. aureus* are enriched in virulence factors which trigger a specialized mode of neutrophil death named as NETs (Neutrophil Extracellular Traps), to foster bacterial immune evasion of *S. aureus* from neutrophils, and establish a chronically inflamed microenvironment, thereby creating a pro-carcinogenic niche that facilitates tumor progression.^{75–77} *Porphyromonas gingivalis* OMVs induce programmed death ligand 1 (PD-L1) expression on human oral carcinoma cells in a receptor-interacting serine/threonine-protein kinase 2 (RIP2)-dependent fashion, which can then induce the dysfunction and apoptosis of T cells through the interaction between PD-L1 and programmed death 1 (PD1) on the surface of T cells, thus elevating the escape of tumor cells from immunosurveillance.^{78,82}

Clinical Application and Potential

Owing to their distinctive structural characteristics, OMVs exhibit substantial potential for clinical translation. Natural or bioengineered OMVs serve as effective carriers to transport bacteria-, virus- and human-derived proteins, functioning as mucosal biologics, vaccine and drug delivery platform. In this section, the application of OMVs will be reviewed under three main categories: vaccines, immunotherapeutic agents and adjunctive therapy, and some other therapeutic options will also be briefly mentioned (Table 4 and Figure 4).

Meanwhile, it is necessary to ensure the quality and purity of the extracted OMVs before application to avoid risk bacterial infection and intoxication. While eukaryotic extracellular vesicles isolation is standardized in Minimal Information for Studies of Extracellular Vesicles (MISEV) updated in 2023 by the International Society for Extracellular Vesicles (ISEV),¹⁰⁴ OMVs purification lacks consensus and the extraction methods should be carefully chosen according to specific bacterial species and culture conditions. Classical workflows include ultracentrifugation, ultrafiltration and characterization with nanoparticle tracking analysis, and newer techniques for isolation and characterization include acoustic trapping and resistive pulse sensing, respectively.¹⁰⁵ Selecting appropriate centrifugation and filtration strategies will help researchers with purification, and in order to confirm the absence of viable bacteria, parallel validation by microbial planting is necessary.²³

OMVs-Based Vaccines

Many studies have explored the clinical potential of OMVs, one of which OMVs-based vaccines constitute a major application. The first generation of FDA-approved OMVs-based vaccine is meningococcal group B vaccine in children, which is under the trade name Bexsero[®], preventing infectious disease.^{83,84} Numerous additional OMVs are still being studied about the mechanisms by which they can function as a part of vaccines. *Helicobacter pylori* OMVs (ExHp-CD) are found to induce TH2-biased immune responses to resist *H. pylori* infection and induce huge production of IL-10 and IL-4. Thus, ExHp-CD can be considered as antigen-presenting vesicles and acellular vaccine. ExHp-CD and its associated cargos can also serve as effective immunogenic candidates.^{85,86} OMVs of some types of *S. aureus* can protect mice against *S. aureus* infection.^{75,87,88} Engineered *S. aureus* OMVs with non-toxic Hla_{H35L} and the LukE monomer packaged are demonstrated to protect immunized mice against lethal sepsis.⁸⁹ Some scientists engineer the Gram-negative bacterium *Bacteroides thetaiotaomicron* (*Bt*) to incorporate bacteria-, virus- and human-derived proteins into its OMVs to deliver the proteins to the respiratory and gastrointestinal tract. *Bt* OMVs with *Typhimurium*-derived vaccine antigens and influenza A virus (IAV)-derived vaccine antigens in or on the outer membrane can elicit antigen-specific immune and antibody responses in mucosal tissues and systemically, thus protecting against infection. Furthermore, *Bt* OMVs can stably express the human therapeutic protein, keratinocyte growth factor-2 (KGF-2), which reduce disease severity and promote intestinal epithelial repair and recovery when delivered orally in colitis models.⁹⁰ Trained

Table 4 OMVs with Clinical Application Potential

Function	Bacteria	Name	Clinical Applications	Acting Pattern/Key Cargo	References
OMV-based vaccines	<i>Neisseria meningitidis</i> serogroup B	Bexsero®	Protect children from meningococcal group B	/	[83, 84]
	<i>Helicobacter pylori</i>	ExHp-CD	Present antigen and resist <i>H. pylori</i> infection	Induce TH2-biased immune responses and production of IL-10 and IL-4	[85, 86]
	<i>Staphylococcus aureus</i>	/	Protect mice against <i>S. aureus</i> infection	/	[75, 87, 88]
		Engineered <i>S. aureus</i> OMVs	Protect immunized mice against lethal sepsis	Non-toxic Hla _{H35L} and the LukE monomer	[89]
	<i>Bacteroides thetaiotaomicron</i>	Engineered Bt OMVs	Deliver bacteria-, virus- and human-derived proteins to the respiratory and gastrointestinal tract to protect against infection	Elicit antigen-specific immune and antibody responses in mucosal tissues and systemically	[90]
Immunotherapeutic agents			Reduce disease severity and promote intestinal epithelial repair and recovery in colitis	Express KGF-2	
	/	OMV-SIRP α @CaP/GM-CSF	Target tumor-associated macrophages to enhance TlrV-generated antitumor activity	/[91]	[92]
	Gram-negative bacteria	Gram-negative bacterial OMVs deprived of LPS	Eradicate established tumors and induce long-term immune responses	Specifically target and accumulate in tumor tissue and induce the production of CXCL10 and IFN- γ	[73]
	/	SyBV	Activate mouse bone marrow-derived dendritic cells; co-immunized as adjuvants to elicit tumor regression	Th-1 type T cell immunity and balanced antibody production	[91]
	/	PEG/Se@OMV-CD47nb	Promote phagocytosis of tumor cells by TAMs, and result in TME remodeling and stimulation of T cell-mediated immunity	Simultaneously bind to TAMs and tumor cells to induce TAM sensitization and CD47 blockade	[93]
	/	OMV-LYTAC	Reduce tumor growth	PD-1/PD-L1 pathway inhibition and immune activation	[94]
	/	OMV-NICE	Enhance the recruitment of macrophages and T cells to the tumor region	Target CD47/SIRP α and PD-1/PD-L1 pathways simultaneously	[95]
	<i>Escherichia coli</i>	OMV/SaFeFA	Activate ferroptosis and stimulate the immune responses	Be anchored with ferrous ions vis electrostatic interactions and loaded with the STING agonist-4, followed by tumor-targeting DSPE-PEG-FA decoration	[96]
<i>Escherichia coli</i> Nissle 1917	OMV ^{Mel} @CaP	Reduce systemic toxicity and elevate the efficiency of tumor photothermal/immunotherapy	Melanin-loaded OMVs coated with CaP; co-stimulation of OMV ^{Mel} as immunoadjuvants and DAMPs	[97]	

Adjunctive therapy	<i>Salmonella Typhimurium</i>	/	As an adjuvant to the standard chemotherapy to avoid chemotherapy-related complications or side effects and reduce the rate of drug resistance	/	[35]
	<i>Bacillus licheniformis</i>	/	Be combined with doxorubicin	Enhance mitochondrial dysfunction, DNA damage, and apoptosis	[37]
	<i>Escherichia coli</i>	/	Synergistic effects with anti-PD-I combination therapy, inhibiting tumor growth	/	[69, 70]
	/	SyBV	Synergistically enhanced by anti-PD-I inhibitor	/	[91]
	<i>Akkermansia muciniphila</i>	/	Overcome primary resistance to PD-I blockade in tumor immunotherapy	Selectively promote the proliferation of beneficial bacteria through membrane fusion to restore the disturbed balance of the gut microbiota	[98]
	<i>Staphylococcus aureus</i>	/	Enhance tamoxifen efficacy when treating breast cancer	Increase p-erk and p-akt levels	[99]
	<i>Escherichia coli</i>	I-P-OMVs	Induce the transdermal photo-trail-programmed treatment in skin melanoma	Modified with $\alpha\upsilon\beta 3$ integrin targeting ligand and indocyanine green	[100]
	/	DOX-OMV	Biologic nanocarriers for chemotherapeutic agents; recruit TME macrophages to synergize with DOX to inhibit lung cancer progression	Doxorubicin-loaded	[101]
Other therapeutic options	/	IR780@PLGA@HM	Tumor targeting, immune regulating, and Sonodynamic abilities to treat breast cancer bone metastasis	/	[102]
	/	Enzyme-powered bacterial omv nanorobots	Enhance sirna delivery and immune stimulation	Cell-penetrating peptide on the surface for tumor targeting and penetration; effectively safeguard sirna	[103]

Notes: /: not applicable or not mentioned.

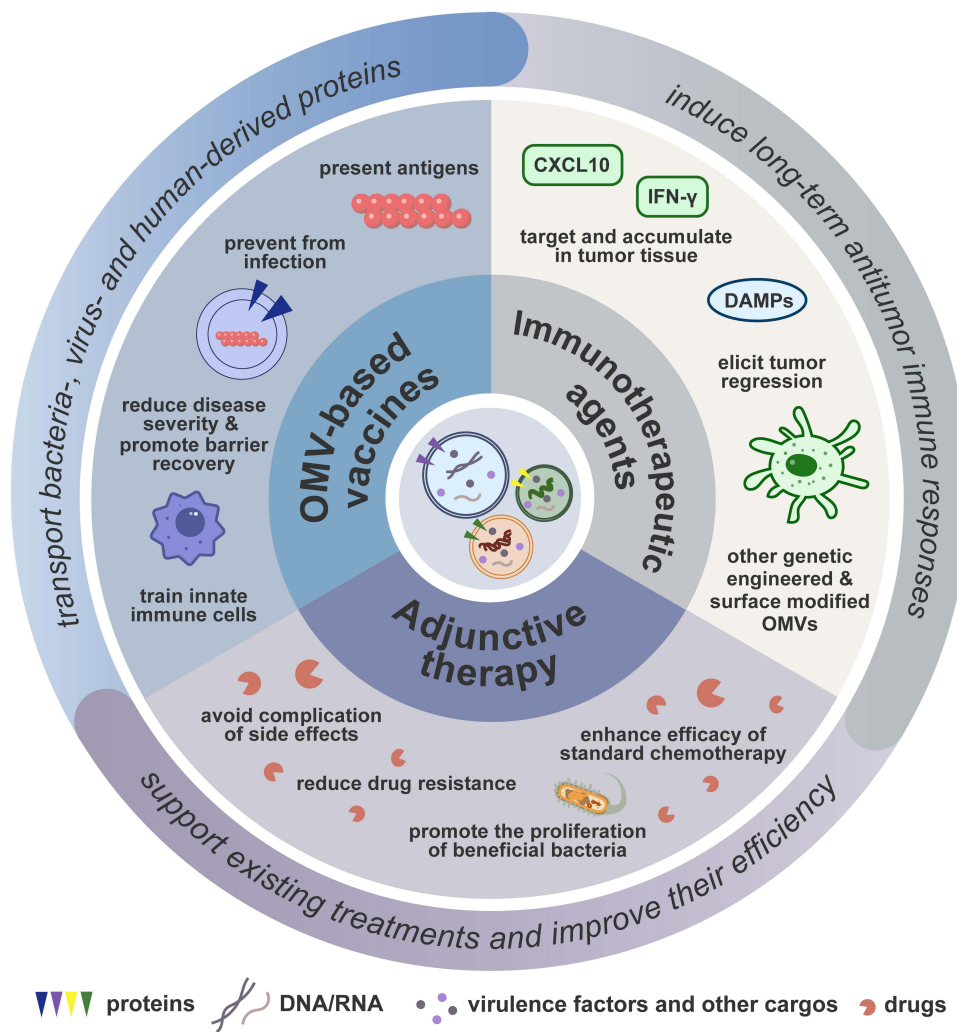


Figure 4 The clinical application and potential of OMVs. Purple, green, and yellow arrowheads: trans-member proteins. Curve lines: DNA/RNA. Dots: virulence factors and other cargos.

immunity-related vaccines (TirV) is an emerging therapeutic tumor vaccine form, which can potentially foster antitumor activity by training innate immune cells and their derived hematopoietic progenitor cells without specific antigens. OMV nanohybrids (OMV-SIRP α @CaP/GM-CSF), an OMV-based TirV, have been developed to target tumor-associated macrophages to enhance TirV-generated antitumor activity, which shows possibilities of broad-spectrum vaccines and development of the next-generations of tumor vaccines.⁹²

Immunotherapeutic Agents

OMVs can also serve as potent immunotherapeutic agents that elicit durable antitumor immunity for the treatment of various cancers. Kim et al have found gram-negative bacterial OMVs deprived of bacterial endotoxin lipopolysaccharide (LPS) can fully eradicate established tumors without notable adverse effects and induce long-term antitumor immune responses. When administered systemically, these OMVs specifically target and accumulate in tumor tissue and induce the production of antitumor cytokines CXCL10 and IFN- γ , leading to this IFN- γ -dependent antitumor effect.⁷³ Park's team have produced a kind of artificial OMV named as Synthetic Bacterial Vesicles (SyBV), which avoid activating the innate immune system and thus do not induce a severe toxic response. SyBV is found effective in activation of mouse bone marrow-derived dendritic cells. And co-immunization with SyBV and melanoma extracellular vesicles can elicit tumor regression in melanoma-bearing mice through Th-1 type T cell immunity and balanced antibody production.⁹¹

Feng et al have developed an OMV-based, controllable two-way adaptor, PEG/Se@OMV-CD47nb through genetic engineering and surface modification. OMV-CD47nb can simultaneously bind to both TAMs (tumor-associated macrophages) and tumor cells to induce TAM sensitization and CD47 blockade that synergistically promote robust phagocytosis of tumor cells by TAMs, and this multiple mechanism-based activation of macrophage phagocytosis will result in TME remodeling and stimulation of T cell-mediated immunity meanwhile. In addition, the surface PEG/Se layer modification endows the formulation with radiation-triggered controlled release of OMV-CD47nb, mitigating the side effects and enabling the precise release of OMV-CD47nb at the tumor site, thereby increasing the safety window of intravenous injection of the formulation.⁹³ Su et al have designed a delivery platform developed by fusing a genetically engineered transferrin receptor-mediated lysosome-targeting chimera (TfR-LYTAC) to the surface of OMVs, which can be efficiently internalized via TfR-mediate endocytosis and targets PD-L1 for lysosomal degradation in cultured cells. The engineered OMV-LYTAC combines PD-1/PD-L1 pathway inhibition by LYTAC with immune activation by OMVs, significantly reducing tumor growth *in vivo*.⁹⁴ Sun conjugated two types of nanobodies targeting CD47/SIRP α and PD-1/PD-L1 pathways onto the surfaces of the OMVs simultaneously and constructed an OMV-based nanosized immune cell engager (OMV-NICE) with dual-targeting abilities. OMV-NICE enhance the recruitment of macrophages and T cells to the tumor region and show superior antitumor activity compared to those of unconjugated nanobodies or OMVs alone.⁹⁵ OMV/SaFeFA, derived from *E. coli*, is anchored with ferrous ions vis electrostatic interactions and loaded with the STING agonist-4, followed by tumor-targeting DSPE-PEG-FA decoration, aiming to activate ferroptosis and stimulate the immune responses. OMV/SaFeFA shows tumor-targeting capabilities, eliciting robust antitumor responses while minimizing side effects.⁹⁶ Chen et al have developed melanin-loaded OMVs from EcN by genetic engineering and then coated them with calcium phosphate (CaP) to reduce systemic toxicity in tumor therapy, and named the nanoparticles as OMV^{MeI}@CaP. Under the co-stimulation of OMV^{MeI} as immunoadjuvants and the damage-associated molecular patterns (DAMPs) released by the photothermal effect, the efficiency of tumor photothermal/immunotherapy can be largely boosted through triggering antitumor immune responses.⁹⁷

Adjunctive Therapy

Beyond their intrinsic activity, certain OMVs function as adjuvants that augment established therapies and enhance therapeutic efficacy. Acting as monotherapy or an adjuvant to the standard chemotherapy, *Salmonella Typhimurium* OMVs both have antitumor effects and can help avoid chemotherapy-related complications or side effects and reduce the rate of drug resistance.³⁵ *Bacillus licheniformis* OMVs can be combined with doxorubicin to enhance mitochondrial dysfunction, DNA damage, and apoptosis when treating breast and lung cancer.³⁷ *E. coli* OMVs have synergistic effects with anti-PD-1 combination therapy, inhibiting tumor growth substantially.^{69,70} The immunotherapeutic effect of the SyBV mentioned above can be synergistically enhanced by anti-PD-1 inhibitor, and SyBV shows significantly greater adjuvant activity than other classical adjuvants.⁹¹ *Akkermansia muciniphila* OMVs restore disturbed balance of the gut microbiota by selectively promoting the proliferation of beneficial bacteria through membrane fusion, and thus overcome primary resistance to PD-1 blockade in tumor immunotherapy.⁹⁸ *S. aureus* OMVs increase p-ERK and p-AKT levels, while tamoxifen inhibits breast cancer cell growth by modulating PI3K/AKT, ERK, and IGF receptors, thereby enhancing tamoxifen efficacy when treating breast cancer.⁹⁹ OMVs derived from transgenic *E. coli*, modified with $\alpha_v\beta_3$ integrin targeting ligand and indocyanine green (named as I-P-OMVs), are developed to induce the transdermal photo-TRAIL-programmed treatment in skin melanoma.¹⁰⁰

Additionally, researchers have proposed loading OMVs with chemotherapeutics to integrate the advantages of nanotherapy, immunotherapy, and chemotherapy into a single platform. Especially, surface modification with cancer-targeting ligands can minimize off-tumor toxicity and multidrug resistance.³⁵ Doxorubicin-loaded OMVs (DOX-OMV) are prepared and demonstrated to function as both biologic nanocarriers for chemotherapeutic agents and to recruit TME macrophages to synergize with DOX, inhibiting lung cancer progression.¹⁰¹ Nanoparticles, IR780@PLGA@HM, are constructed by fusing breast cancer cell membranes and OMVs to form a hybrid membrane (HM) and then encapsulating IR780-loaded PLGA, with tumor targeting, immune regulating, and Sonodynamic abilities to treat breast cancer bone metastasis.¹⁰²

Other Therapeutic Options

Besides therapies mentioned above, multiple therapeutic methods with OMVs participated are emerging. For example, Tang et al develop the enzyme-powered bacterial OMV nanorobots by surface bioengineering the robotic body with cell-penetrating peptide for tumor targeting and penetration, which is further enhanced by active propulsion of nanorobots. These nanorobots can also effectively safeguard the loaded gene silencing tool, siRNA, from enzymatic degradation. Systematic *in vitro* and *in vivo* studies in rodent models have demonstrated that these OMV nanorobots can substantially enhance siRNA delivery and immune stimulation.¹⁰³

Notably, OMVs are endowed with pathogen-associated molecular pattern (PAMP)-rich membranes and biogenesis-dependent cargoes encompassing proteins and nucleic acids.²³ Consequently, every contemplated clinical application must rigorously address formulation safety, including scrutiny of endotoxin burden, immunogenic potential, and inter-batch variability. For example, in order to manage the immunogenicity of OMVs, calcium phosphate (CaP) shells can be employed to cover the surface of OMVs to alleviate the side effects of intravenous administration.¹⁰⁶ Moreover, in the PEG/Se@OMV-CD47nb mentioned above, the surface PEG/Se layer endows the nanoparticles with radiation-triggered controlled release of OMV-CD47nb, also mitigating the side effects observed from intravenous injection of naked OMVs.⁹³

Future Perspectives

Over the past few years, studies have established that OMVs can bidirectionally modulate tumor proliferation, metastasis and the immune microenvironment. Nevertheless, mechanistic dissection largely remains descriptive and confined to cataloguing discrete signaling pathways. By integrating published OMV cargo profiles with tumor-phenotype datasets and applying deep-learning algorithms, future work could construct a tripartite knowledge graph of “OMV cargo - host pathway - tumor outcome”. Mining this graph for recurrent patterns will provide perspectives for clarifying how cargo heterogeneity among OMVs of distinct bacterial origins dictates their pro- or anti-tumor selectivity, and how the same OMV elicits divergent phenotypes when exposed to different cancer type or microenvironment. Such a framework will yield molecular signatures for distinguishing “risk-associated” from “therapeutic” bacterial sources. Meanwhile, through interdisciplinary integration, the knowledge graph can be coupled with an artificial intelligence-driven predictive model. By means of transfer learning, this model could be extrapolated to novel bacterial strains or uncharacterized cargo, enabling *in-silico* screening and risk/efficacy prediction of OMV functionalities, and remarkably compressing the period from discovery to preclinical validation.

In addition to natural OMVs, engineered OMVs are a research focus in recent years. Diverse technologies have emerged to modify OMVs, including displaying tumor antigens on the vesicle surface for vaccination purposes by genetically editing the parental bacterium, presenting immunomodulatory ligands for onco-immune reprogramming, encapsulating therapeutic payloads for targeted delivery, or by coated and hybrid membrane technologies, constructing hybrid nanoplatfroms that exert antitumor effects.^{107,108} Looking forward, researchers can exploit CRISPR-Cas systems and synthetic biology to install controllable switches at OMV biogenesis checkpoints, thereby enabling precision cargo loading and programmed release. Integration with existing immunotherapeutic formulations—for example, the PEG/Se@OMV-CD47nb construct mentioned above—could establish sequential regimens in which immune activation precedes drug release, thereby enhancing therapeutic efficacy and elevating OMVs from passive vectors to intelligent therapeutic systems.

Moreover, the clinical application of OMVs could be extended to encompass early-stage prevention, diagnostic assistance, post-metastatic maintenance, and so on. In imminent Phase I trials, the patients’ metagenomic profiling of microbiota and parallel characterization of circulating OMVs could be performed in real time to establish a “bacterial source–OMV–efficacy” monitoring framework. This will explore the potential of precision-medicine paradigms such as “same disease, different treatment” and “different diseases, same treatment,” thereby maximizing the clinical value of each OMV formulation. For patients with established metastases, it should be investigated whether OMVs engineered to carry specific cargoes can re-program dormant micro-metastatic niches, thereby prolonging progression-free survival.

However, OMV-based therapeutics still face many challenges in its route to clinical deployment, including the absence of a standardized isolation protocol and quality control specification, side effects of intravenous administration, and the potential intoxication. Besides, most pre-clinical studies relied on model antigens or murine tumor antigens, and thus the adaptability of the delivery systems to human tumor antigens needs further study. The OMV-based strategies delineated above largely remain confined to pre-clinical investigation, with efficacy documented solely in animal models. It is anticipated that an expanding repertoire of OMV-based formulations will successfully advance through clinical translation.

Meanwhile, it remains to be systematically determined whether—and to what extent—OMVs release is modulated by extrinsic cues such as microbiome composition, dietary constituents, or antibiotic exposure, and how these variables subsequently shape cancer susceptibility or therapeutic outcomes. Elucidating and ultimately taking advantage of these modifiable determinants could markedly enhance the efficiency of OMV-based interventions in clinical appliances.

Conclusions

In conclusion, OMVs can bidirectionally modulate tumor development by influencing the cancer cells through their diverse molecular cargos in various pathways. Promising results from studies show that natural or engineered OMVs could serve as effective vaccines, immune-therapeutics and adjuvants and can also synergize with chemotherapy or checkpoint blockade to overcome resistance. To advance these findings, future efforts could combine them with different intelligent models through multidisciplinary tools, the isolation and purification process should be further standardized, and more factors of OMVs release should be studied, guiding the clinical transformation of OMVs from basic research to precision cancer prevention and treatment.

Abbreviations

EVs, Extracellular vesicles; MVs, Membrane vesicles; OMVs, Outer membrane vesicles; OIMVs, Outer-inner membrane vesicles; EOMVs, Explosive outer-membrane vesicles; CMVs, Cytoplasmic membrane vesicles; TSMSs, Tube-shaped membranous structures; *h.p* OMVs, *Helicobacter pylori* OMVs; OSM, Oncostatin-M; OSMR β , Oncostatin-M type II receptor; AGS, Human gastric adenocarcinoma cell line; ROS, Reactive oxygen species; GSH, Glutathione; CCC, Colon cancer cells; SOD, Superoxide dismutase; CRC, Colorectal cancer cell; CRC/5FUR cells, 5-fluorouracil-resistant CRC cells; EMT, Epithelial–mesenchymal transition; DSC-2, Desmocollin-2; TEM, Trans-endothelial migration; VEGFC/D, Vascular endothelial growth factor C/D; IGF-1, Insulin-like growth factor 1; ZO, Zona Occludens; IECs, intestinal epithelial cells; EPEC, enteropathogenic *E. coli*; EcN, *E. coli* Nissle 1917; ETEC, enterotoxigenic *E. coli*; TME, Tumor microenvironment; EPR effect, enhanced permeability and retention effect; PAMPs, Pathogen-associated molecular patterns; GZMB+, Granzyme B-positive; IFN- γ +, Interferon γ -positive; GGT, γ -glutamyl transpeptidase; PD-L1, Programmed death ligand 1; RIP2, Receptor-interacting serine/threonine-protein kinase 2; IAV, Influenza A virus; KGF-2, Keratinocyte growth factor-2; TlrV, Trained immunity-related vaccines; SyBV, Synthetic Bacterial Vesicles; TAMs, Tumor-associated macrophages; TfR-LYTAC, transferrin receptor-mediated lysosome-targeting chimera; OMV-NICE, OMV-based nanosized immune cell engager; CaP, Calcium phosphate; DAMPs, damage-associated molecular patterns; HM, Hybrid membrane.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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