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Role of cell-free network communication in alcohol-associated disorders and liver metastasis

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Abstract

The aberrant use of alcohol is a major factor in cancer progression and metastasis. Contributing mechanisms include the systemic effects of alcohol and the exchange of bioactive molecules between cancerous and non-cancerous cells along the brain-gut-liver axis. Such interplay leads to changes in molecular, cellular, and biological functions resulting in cancer progression. Recent investigations have examined the role of extracellular vesicles (EVs) in cancer mechanisms in addition to their contribution as diagnostic biomarkers. Also, EVs are emerging as novel cell-free mediators in pathophysiological scenarios including alcohol-mediated gut microbiome dysbiosis and the release of nanosized EVs into the circulatory system. Interestingly, EVs in cancer patients are enriched with oncogenes, miRNA, lipids, and glycoproteins whose delivery into the hepatic microenvironment may be enhanced by the detrimental effects of alcohol. Proof-of-concept studies indicate that alcohol-associated liver disease is impacted by the effects of exosomes, including altered immune responses, reprogramming of stromal cells, and remodeling of the extracellular matrix. Moreover, the culmination of alcohol-related changes in the liver likely contributes to enhanced hepatic metastases and poor outcomes for cancer patients. This review summarizes the numerous aspects of exosome communications between organs with emphasis on the relationship of EVs in alcohol-associated diseases and cancer metastasis. The potential impact of

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EV cargo and release along a multi-organ axis is highly relevant to the promotion of tumorigenic mechanisms and metastatic disease. It is hypothesized that EVs target recipient tissues to initiate the formation of prometastatic niches and cancer progression. The study of alcohol-associated mechanisms in metastatic cancers is expected to reveal a better understanding of factors involved in the growth of secondary malignancies as well as novel approaches for therapeutic interventions.

Key Words: Exosomes; Extracellular vesicles; Alcohol-associated liver disease; Colorectal cancer; Liver metastasis; Interorgan communication

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Core Tip: Alcohol consumption is an independent risk factor for cancer development as well as the promotion of metastatic disease, a major cause of morbidity and mortality in cancer patients. The identification of mechanisms and potential therapeutic targets for metastases remains to be determined for many cancers. Interorgan communication involving extracellular vesicles (EVs) is considered a vital process in the promotion of tumorigenic pathways and the spread of disease. Understanding the role of EVs in organ-organ communication networks will likely contribute to the development of future opportunities to combat cancer metastasis.

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INTRODUCTION

The consumption of alcohol in chronic and/or aberrant drinking patterns correlates with a substantial burden of disease worldwide. A recent study conducted by the National Survey on Drug Use and Health stated that in the United States alone, 73.1% of adults regularly use alcohol and nearly 15 million people have an alcohol use disorder[1]. Based on World Health Organization reports, alcohol use has a negative impact on health and quality of life, creating more than 5% of global disease burden and premature deaths[2,3]. The processing of alcohol in the body significantly affects multiple organs including the liver, gut, lungs, heart and brain[4-7]. A prominent alcohol-related disorder is alcohol-associated liver disease (AALD) that is initially facilitated by ethanol metabolism in the liver[8]. However, AALD is a complex disease with factors from other organs also contributing to its development and progression. Notable contributing factors include cells of the innate immune system and bacteria of the alcohol-altered gut microbiota[9,10]. Overall, the interplay between alcohol-affected organs clearly plays a role in the outcomes of AALD as well as additional adverse consequences such as alcohol-related cancer development and metastatic disease.

Alcohol is an identified carcinogenic factor in several cancers including head and neck, esophageal, liver, breast, pancreatic, and colorectal[11,12]. Recent reports indicate that alcohol consumption is the third and fourth largest contributor of all primary cancers in women and men, respectively[3]. Further, studies have shown that alcohol associates with an increased risk of secondary cancers of the upper aerodigestive tract (*i.e.* oral cavity, pharynx and esophagus) as well as metastases of colorectal cancers[13,14]. Multiple mechanisms are attributed to alcohol-induced cancer risk including toxic products and reactive oxygen species generated by ethanol metabolism. Additionally, cellular factors produced in response to injury such as protein, lipids and microRNAs can be packaged and released in extracellular vesicles (EVs)[15]. The EVs can migrate to modulate neighboring cells and/or distant tissues, acting in many cases as tumorigenic signaling molecules. Multiple cell types including endothelial cells, epithelial cells, neuronal cells, immune cells, and cancer cells can secrete nanosized EVs as part of their normal physiology, as well as during the

pathophysiology of disease[16]. Recent studies have suggested that during pathophysiological conditions exosomes have multiple roles in disease progression. Interestingly, tumor-derived exosomes have been implicated as regulatory factors in cancer progression by promoting cancer cell proliferation, migration, and the establishment of a premetastatic niche for drug-resistant cells[17,18]. Overall, EVs have the capability to contribute to the progression of AALD as well as alcohol-related advanced or secondary cancers. A better understanding of the integrated cell-cell communication between cancer cells and normal cells is critical for the development of new therapeutic options. Research into the complex interactions of diverse organs by EVs is a focus of new and clinically relevant areas of study. Here, we review studies on exosome biology and EV communication networks associated with alcohol-related disorders and metastatic cancers.

EXOSOME CHARACTERISTICS

Exosome biogenesis

Exosomes were first identified in 1981 as cell-derived, membrane-bound enzymatic vesicles[19]. Subsequently, it was demonstrated that exosomes are nano-sized (30 to 150 nm) lumen vesicles that originate from the endosomal system[20]. Further, it was elucidated that EV biogenesis is a sequential process in which multivesicular bodies (MVBs) form following membrane invagination of intraluminal vesicles[18,20]. A small fraction of MVBs fuse with the plasma membrane and are released into the extracellular milieu[21]. The regulation of MVB fusion and release can involve cholesterol content as seen in B-lymphocytes where membrane fusion and exosome release were only observed for the high cholesterol pool of MVBs[22]. Additionally, several reports have shown that exosome release depends on the cell polarity and the contribution of specific components of apical or basolateral membranes[23-25]. Overall, existing evidence indicates that different MVB populations exist inside cells and that select pools are involved in extracellular release[25] as well as the scavenging of plasma membrane proteins[26] to maintain cellular homeostasis during the EV maturation process[27,28].

Endosome pathways identified in the regulation of exosome biogenesis include endosomal sorting complex required for transport (ESCRT)-dependent and independent pathways (Figure 1). Studies have eloquently described ESCRT pathways showing the direct control of ESCRT-mediated membrane machinery[29] and ESCRT-independent regulation of EV budding and release by factors such as sphingolipid ceramide[30,31]. It was also demonstrated that vesicle formation and trafficking involve functional proteins such as Rab GTPases, heat shock proteins (HSP70 or HSP90), tetraspanins (CD9, CD63, and CD81), and integrins[18,32]. Further, the role of sphingomyelin, phosphatidylcholine, diacylglycerol, and ceramide as exosome membrane lipids was described[33]. Altogether, these studies suggest that distinct exosome biogenesis pathways, in addition to specific sorting and cargo mechanisms, dictate diverse biological functions and effects of EVs on recipient cells.

Exosome sorting and cargo delivery

A significant feature of exosomes is the morphological and size profile of the vesicles. Based on size, EVs are classified into large exosome vesicles (90-120 nm), small exosome vesicles (60-80 nm), or non-membranous nanoparticles called exomeres (35 nm)[34]. While both large and small size exosomes can respond to signaling pathways such as IL-2/STAT5, density gradient centrifugation studies revealed differences in lipid compositions between various sized EVs[34]. Moreover, subpopulations of low-density and high-density exosomes can have differential effects on gene expression profiles.

In addition to EV size, the characterization of exosome cargo is important to the understanding of EV effects in healthy and pathophysiological scenarios. Exosomes contain distinct ratios of molecular constituents such as nucleic acids, proteins, lipids, and metabolites that vary depending upon their cellular conditions, cells of origin, epigenetic changes, and metabolomic stages[35]. Moreover, studies have described various RNA species that are components of exosome cargo including microRNAs (miRNAs), rRNAs, tRNAs, or long noncoding RNAs (lncRNAs)[36]. The role of miRNAs as EV cargo is an emerging area of study, especially in oncology. In cancer cells, exosomes are highly enriched in miRNAs compared to parent cells indicating that miRNAs are sorting into the exosome cargo[37-39]. Several studies have identified exosomal miRNAs as serum biomarkers for the prediction of cancer progression and

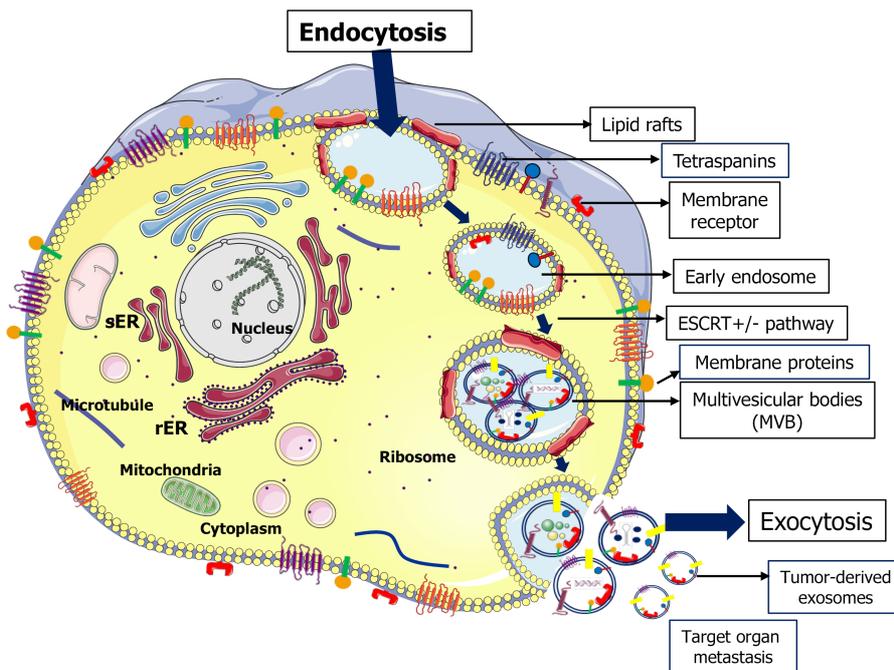


Figure 1 Extracellular vesicle biogenesis. Pathways involved in extracellular vesicle (EV) generation from the endocytosis of cargo components to release of targeting exosomes. EV biogenesis is achieved by endosomal sorting complex required for transport (ESCRT)-dependent or ESCRT-independent pathways. Several cytoplasmic and nuclear molecules can be sorted in the EVs such as ubiquitin-related proteins, heat shock proteins, miRNAs, and cytoskeleton proteins. ESCRT: Endosomal sorting complex required for transport; sER: Smooth endoplasmic reticulum; rER: Rough endoplasmic reticulum.

metastasis[40-42]. Significantly, the differential expression of exosomal miRNA was noted to have a role in the regulation of tumor progression and metastasis in various cancer models[43-45]. However, the mechanisms involved in the loading and sorting of molecules into exosome vesicles remain to be elucidated. Towards those efforts, Villarroya-Beltri *et al*[46], have identified a sequence motif that controls the miRNA loading into exosomes. In addition, Kirsten rat sarcoma (KRAS) oncogene-dependent miRNA sorting into exosomes was found to play a key role in colorectal cancer cell (CRC) since CRC cells expressing mutant KRAS have distinct miRNA profiles compared to wild-type cells[47]. In another study, it was shown that the hyperactivation of mutated KRAS inhibited the localization of the regulatory protein Argonaute 2 into exosomes[48]. The sorting of exosome mRNAs and enrichment of 3' UTR fragments also demonstrates the importance of exosomal RNA effects in recipient cells[49,50]. Also, tumor-derived exosomes can carry double stranded DNA and genomic DNA fragments that reflect the mutational status of oncogene and tumor suppressor genes[51,52]. And finally, ubiquitination has been noted to have a role in the packaging of target proteins into exosomes[53-55].

Another important aspect of exosome cargo and sorting mechanisms is the lipid content of exosome membranes such as cholesterol, sphingomyelin, and glycosphingolipids that have specific roles in protein sorting into exosomes[33,56]. Data indicates that subdomains of the plasma membrane (lipid rafts) enriched with distinct proteins on exosome membranes mediate exosome signaling as well as molecule sorting into exosomes[57,58]. Further, mechanistic studies demonstrated the release of factors such as flotillin-1 and stomatin into the external medium *via* EVs associated with lipid microdomains[59]. Another study showed a positive regulation of sphingosine 1-phosphate (S1P) by sphingosine kinases that enabled S1P receptors to be continuously active on EVs[31]. The continuous activation of S1P has been shown to regulate CD63, CD81, and flotillin-mediated sorting into exosomes through inhibitory G protein-coupled S1P receptors located on MVBs[31]. This suggests that G protein receptor-mediated S1P signaling on MVEs is mainly involved in the ESCRT-independent exosome cargo. Collectively, these studies suggest that distinct molecular constituents such as proteins, lipids, and nucleic acids play an essential role in exosome maturation culminating in effective sorting and extracellular release of EV cargo. The molecular, cellular, and biological functions that result from the released EVs is a critical area of research, especially in the evolving era to understand the mechanisms of alcohol-associated diseases including cancer.

THE EFFECTS OF ALCOHOL ON EXOSOME COMMUNICATION

Alcohol and liver-associated EVs

Clinical manifestations of AALD include steatosis, steatohepatitis, fibrosis, and cirrhosis[8,60]. The liver is sensitized to triggers such as oxidative stress and endotoxins in early phases of AALD resulting in cellular damage and development of advanced disease. Further, consequences of ethanol metabolism lead to alterations in the function of hepatic cells as well as the recruitment of circulating cells and molecules that contribute to organ dysfunction. Previous reviews have comprehensively described the emerging role of EVs during the pathogenesis of alcohol-mediated diseases[61-63]. In brief, alcohol-mediated stresses result in elevated EV generation and release from hepatocytes as well as non-parenchymal cells. The released EVs can modulate gene expression and function of target cells contributing to the perpetuation of liver damage. Examples of the effect of EV cargo (*i.e.* miRNA, proteins, and lipids) include changes in macrophage phenotype and the activation status of hepatic stellate cells. Altogether, EVs generated in the liver are key players in alcohol-mediated liver inflammatory and profibrogenic mechanisms. In addition to EV-mediated intra-organ signaling, communication to extra-hepatic tissues can occur, as well as bidirectional exosome communication between organs such as liver, brain, gut, and lung.

Gut-Liver axis

Alcohol-induced impairments to the intestinal epithelial barrier result in increased gut permeability and release of bacterial products into the circulation[9,64]. The released products can perpetuate gut-barrier dysfunction, as well as contribute to hepatic injury, as the liver is the primary organ to receive and detoxify gut-derived factors. The translocation of intestinal products to the liver is involved in several diseases including obesity, metabolic syndrome, and non-alcoholic and alcoholic liver diseases. In the setting of alcohol, the gut-liver axis sustains bilateral communications between the intestine and the liver leading to gut-dysbiosis and progression of liver injury[65, 66]. Notably, the transfer of gut-derived toxins to the liver due to alcohol consumption is considered a pivotal event in the development and severity of AALD. Clinical data indicates that drinking patterns correlate with processes of the gut-liver axis as changes in intestinal permeability increase with the degree of alcohol consumption [64]. Next-generation sequencing data further confirmed the association between chronic alcohol consumption and altered gut microbiome functions in mice and humans[67,68]. Overall, alcohol consumption is linked to multiple changes in the gut including intestinal epithelial barrier dysfunction, alterations in gut epithelial and mucosal cells, and changes to the intestinal microbiota. As a result, bacterial products (*i.e.* endotoxin and other pathogen-associated molecular patterns) translocate to the liver and contribute to the production of proinflammatory pathways. Despite the current understanding of alcohol's effects on the gut microbiome, the role of EVs in the transfer of gut-derived products is not defined. However, emerging data indicates the EVs significantly contribute to alcohol-related liver inflammation.

The effects of alcohol on the intestinal microbiome and the translocation of injurious factors to the liver is an area of extensive research. It is well characterized that alcohol consumption results in the dysbiosis of bacterial and fungal intestinal species and the release of products including lipopolysaccharide (LPS) from the leaky gut[69,70]. In search of contributing mechanisms, studies have described alcohol-induced reductions in the expression of tight junction proteins as well as direct injury to gut epithelial cells [71,72]. The overexpression miRNA has been implicated in tight junction alterations as the knockdown of miRNA-21 prevented ethanol-induced disruption of tight junctions through the restoration of associated transmembrane proteins such as occludin and zonula occludens-1 (ZO-1)[71,73]. Additionally, the blockade of miRNA-122a was found to be protective against tight junction alterations in Caco-2 cells[74]. It is suggested that EVs generated during alcohol-induced changes to the intestinal barrier contain cargo such as miRNAs, LPS, and bacterial products that target the liver and contribute to AALD. Indeed, a recent study by Lamas-Paz *et al*[75] demonstrated that EVs derived from alcohol-affected intestinal epithelial cells contributed to hepatocellular injury. Further, it is likely that ethanol-mediated changes in intestinal barrier and microbiome composition result in the release of bacterial EVs. For example, in addition to its role as a soluble factor, LPS can also be packaged into EVs for transport from the injured gut. This is supported by a recent report indicating the presence and activity of bacterial EVs in patients with intestinal barrier dysfunction[76]. The role of bacterial EVs in alcohol consuming patients remains to be characterized along with the

therapeutic potential of targeting such EVs.

The mechanistic role of bacterial products in the progression of alcohol-associated diseases has led to the study of the gut microbiota as a therapeutic target in patients with alcohol use disorders[77]. Currently, probiotics (living bacterial cultures), prebiotics (promoters of beneficial or commensal bacteria), and antibiotics, all serve as potential therapies for alcohol-associated diseases[78]. For instance, *Lactobacillus rhamnosus* is protective against alcohol-induced liver injury in mice[79]. Further, the administration of prescribed probiotics is promising as a protective barrier against alcohol-induced gut permeability and AALD[80]. The use of prebiotics may also be beneficial as certain diets (*i.e.* oats, flaxseed) protect against alcohol-induced oxidative stress and hepatic inflammation[81,82]. Similarly, antibiotic treatment can attenuate alcohol-induced endotoxemia by preventing the overgrowth of harmful bacteria in the gut[83]. Overall, insight into the mechanistic utility of targeting exosomes generated by the alcohol-altered gut warrants investigation for the development of effective therapeutics against disease progression related to the gut-liver axis.

Liver-Brain axis

It is well described that manifestations of AALD lead to a spectrum of symptoms in the brain such as cerebral edema and hepatic encephalopathy. Of notable involvement, ammonia and other harmful substances produced by the alcohol-injured liver can reach the brain causing injury and neuroinflammation. However, mechanisms related to exosome communication networks of the liver-brain axis remain to be characterized. Reports to date indicate that the coadministration of alcohol and LPS result in altered profiles of cytokines such as TNF- α , MCP-1, IL-1 β in the gut, liver, and brain[84]. Other studies demonstrate that the lack of the tumor necrosis factor receptor 1 results in the accumulation of TNF- α in mouse serum, gut, and liver; and that alcohol intake potentiates long-lasting levels of proinflammatory cytokines in the brain[85]. A recent study demonstrated that TNF- α inhibition reduced systemic inflammation and improved symptoms[86]. Additionally, chronic alcohol consumption not only influences brain inflammation but also interferes with stress-mediated psychiatric behavior through the disruption of the hypothalamic-pituitary-adrenal (HPA) axis [87]. The alcohol-mediated neutralization of the HPA axis could be a potential mechanism by which systemic inflammation continues in individuals who have an addiction to alcohol.

Besides chronic alcohol addiction, the loss of gut barrier integrity is a causative factor of endotoxin transport during sepsis and brain inflammation. Alcohol-induced gut dysbiosis is thought to not only play a role in alcohol dependency but also in the regulation of effects including neuro and endocrine signaling and immune system alterations[64,68]. However, a connective factor such as EVs in the gut-liver-brain axis has yet to be identified. Interestingly, the blood-brain barrier (BBB) serves as a defensive barrier against the extravasation of tumor cells and pathogens[88]. However, cancer cells can destruct the BBB structure to mediate migration during brain metastasis[89]. Overall, it is suggested that EVs facilitate cell network communications through the delivery of their cargo (*i.e.* proteins, mRNA, and miRNAs) to trigger the breakdown of the BBB through EV-induced changes in tight-junction proteins including ZO-1, N-cadherin, and actin.

Liver-Lung axis

Excessive alcohol use is a major factor in the enhanced risk of acute respiratory distress syndrome (ARDS)[90]. Chronic alcohol exposure in the liver-lung axis is linked to hepatopulmonary syndrome, bacterial infection, and increased mortality from ARDS [90,91]. Recently, Siore *et al*[92] reported that pulmonary edema and acute lung damage occur through the activation of inflammatory responses and oxidative stress involving liver-lung axis communications. It was shown that alcohol administration results in elevated levels of the TNF- α responsive chemokines, macrophage inflammatory protein, and keratinocyte chemoattractant. Further, the enhanced chemokine expression is associated with the recruitment of pulmonary neutrophils. Additional studies indicated that the liver-lung axis is bidirectional for the communication and effects involved in alcohol-enhanced hepatopulmonary injury. For instance, ventilator-induced lung injury in a mouse model resulted in significant inflammatory responses produced in cultured hepatic sinusoidal endothelial by perfusate from injured lungs [93]. In relationship to the role of the liver-lung axis in alcohol-related cancers, several studies have investigated the role of metastatic determinants[94,95]. In particular, tumor-derived exosomes may have a significant role in cancer cell metastasis that is mediated by cell adhesion molecules such as integrins, tenascin, and periostin[96-98]. In summary, the role of EVs in the interplay between pulmonary disease, AALD and

alcohol-associated cancers is a needed area of research for the identification of potential therapeutic targets.

EXOSOMES AND CANCER: ROLE OF ALCOHOL-MEDIATED EFFECTS

The interorgan communication mediated by EVs is clearly a factor of pathophysiology in various disease states. The role of alcohol-induced EV communication in the development and progression of cancer is not defined and is an area of clinical importance due to the prevalence of alcohol consumption and associated risk of cancers. Thus, investigations into the role of EVs in the initiation and severity of cancers aims to gain insight into the relationship of comorbid conditions related to the effects of alcohol consumption. Moreover, realization of the importance of EVs in cancer progression and metastasis has increased exponentially, as have their potential application in therapy and diagnosis[99]. The contribution of EVs in pathological processes is far reaching since tumor-secreted exosomes can mediate angiogenesis, modulate the immune system, and facilitate the generation of pre-metastatic niches[96, 100]. Indeed, EVs have been identified as key mediators of communication networks within and between organ systems, highlighting the clinical importance of exosome function[18,101,102]. Existing web-based online bioinformatic tools including high-throughput techniques (*i.e.* ExoCarta, EVpedia, Vesiclepedia catalog, and Ingenuity Pathway Analysis, IPA) are beneficial to the scientific community in EV research[103, 104]. These resources assist in the characterization of EV molecular and pathophysiology mechanisms through the identification of key functional elements. Based on IPA data, EV cargo delivery depends on the content of bioactive molecules such as mRNA, enzymes, proteins, DNA and lipids that can dictate the role of EVs in disease progression and diagnostic functions (Figure 2).

The clinical assessment of EVs in body fluids provides another measure towards the understanding of exosomes as diagnostic biomarkers and therapeutic targets. Biomolecule-loaded EVs from blood are stable for more than 90 days under normal storage conditions making EV analyses more useful compared to other less-stable measures of cell-free DNAs and circulating tumor cells that are used as liquid biopsies [105,106]. Examples of exosome-related identification in serum samples include prostate cancer-derived exosomes[107]; and exosome cargo containing an androgen receptor variant that is a biomarker of metastatic prostate cancer[108]. Several studies have also reported the sensitivity of EV miRNA composition as biomarkers in disease identification that can be isolated from various body fluids including blood, saliva, and urine[109,110]. A noted example is the oncogenic signature of miR-21 as a biomarker for various cancers including colorectal[111], breast[112], brain[113], and liver[114]. Concerning diseases of the liver, it has been shown that the concentration of EVs in the circulation is enhanced in the setting of AALD, nonalcoholic fatty liver disease, viral hepatitis, and hepatocellular carcinoma indicating the clinical significance of EV-mediated communication and subsequent effects[66]. Overall, clinical measures as well as bioinformatic programs are valuable in deciphering EV-mediated mechanisms and are useful tools for the characterization of alcohol-associated EVs in development and progression cancers.

Role of exosomes in cancer progression

Mechanisms of tumor development and progression are dynamic, multi-step processes that occur in response to the accumulation of genetic alterations in damaged cells. An integral component of tumor development is thought to be the communication between cancerous and non-cancerous cells that is mediated by nanosized vesicles [115]. Research to date indicates that cancer cell microvesicles actively transfer oncogenic molecules from primary cancer cells to intercellular populations. Indeed, tumor-derived exosomes can regulate cancer progression by stimulating oncogene overexpression, stromal cell remodeling, immune system modulation, and angiogenesis[115]. The transfer of tumorigenic material *via* EVs is implicated in the modulation of morphological changes and the enhancement of anchorage-independent growth capacity of cancer cells. Similarly, tumor-derived exosomes can act as survival factors that bind to and activate anti-apoptotic pathways[116].

The knowledge that exosomes are potential stimulators in cancer progression indicates that EVs can promote angiogenesis and changes in the microenvironment [117]. In this regard, tumor-derived exosomes can influence mesenchymal stem cell differentiation facilitating cancer cell proliferation and disease progression[118]. Moreover, the exosome-mediated transfer of lncRNAs as tumor-promoting material

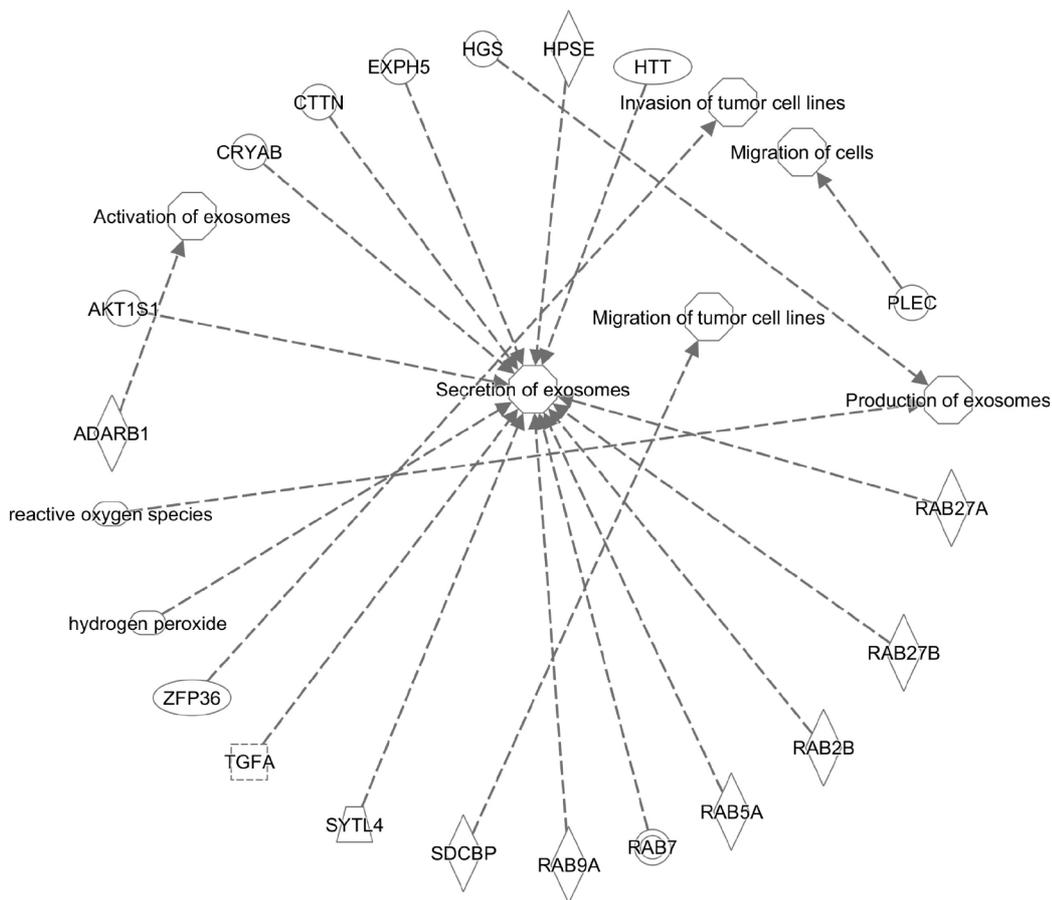


Figure 2 Exosome network analysis. Based on Ingenuity Pathway Analysis, distinct molecules from different tissues and cells can be involved in exosome secretion during cancer progression as well as in diagnostic functions. CRYAB: Chaperone alphaB-crystallin; CTTN: Cortactin; EXPH5: Exophilin 5; HGS: Hepatocyte growth factor; HPSE: Heparinase; HTT: Huntingtin protein; PLEC: Plectin; RAB27A: Ras-related protein Rab-27A; RAB27B: Ras-related protein Rab-27B; RAB2B: Ras-related protein Rab-2B; RAB5A: Ras-related protein Rab-5A; RAB7: Ras-related protein Rab-7; RAB9A: Ras-related protein Rab-9A; SDCBP: Syndecan binding protein; SYTL4: Synaptotagmin like 4; TGFA: Transforming growth factor alpha; ZFP36: Zinc finger protein 36; ADARB1: Adenosine deaminase RNA specific B1; AKT1S1: AKT1 Substrate 1.

has been shown during the transformation of non-malignant cells[119]. The role of EVs enriched with miRNAs has also been shown in cell-cell communications and conversion of cells to populations with enhanced motility[120]. Specific examples include the role of miR-17-92 and miRNA-92a as potent promoters of angiogenesis and oncogenic activity[121]. Likewise, miR-135b-5p[122], miR-30a-5p[40], miR-150-5p [123], miR-183-5p[124], miR-155[125], miR-497[126], miR-181b-5p[127], miR-375[128, 129] and the miR-200 family[110,130,131] have been shown to be effective markers of cancer progression. The clinical evaluation of EV miRNA cargo provides insightful information into processes involved in the various stages of cancer from detection to metastasis as summarized in Table 1.

Another component identified in cancer progression is the release of cancer-associated fibroblasts (CAFs) from exosomes. CAF-derived EVs can play a key role in tumor progression by enabling the transfer of oncogenic molecules such as amino acids, lipids, and TCA-cycle intermediates to confer glycolysis modulation and carboxylation in cancer cells[132]. Tumor-derived exosomes have also been shown to be involved in the stimulation of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 (ICAM-1) enhancing the process of neovascularization in endothelial cells in the microenvironment[133]. Moreover, recent studies suggest that EVs are important in mediating cellular communication between cancer cells and other cells of the microenvironment such as immune cells, neutrophils, natural killer (NK) cells, dendritic cells, T cells, and macrophages. For example, cancer-derived exosomes can alter macrophage polarization[134], induce the recruitment of neutrophils to the tumor site[135], decrease the cytotoxic activity of NK cells[136], or inhibit T-cell proliferation mechanisms[137]. Altogether, it is evident that exosomes can mediate cancer progression through a variety of pathways and cellular communications leading to cancer cell proliferation and spread to distant sites.

Table 1 Summary of exosome miRNA signatures as cancer biomarkers

Exosomal miRNA	Expression profile	Mode of action	Type of cancer	Ref.
miR-320d	Upregulated	Predicts metastasis	CRC	Tang <i>et al</i> [41]
miR-106b-3p	Upregulated	Promotes metastasis	CRC	Liu <i>et al</i> [163]
miR-6803-5p	Upregulated	Prognosis marker	CRC	Yan <i>et al</i> [42]
miR-874	Upregulated	Prognosis marker	GC	Zhang <i>et al</i> [164]
miR-30a-5p	Downregulated	Diagnostic tool	CRC	Sun <i>et al</i> [40]
miR-21	Upregulated	Diagnostic tool	CRC	Bastaminejad <i>et al</i> [111]
miR-135b-5p	Upregulated	Metastatic marker	CRC	Li <i>et al</i> [122]
miR-150-5p	Downregulated	Prognosis, marker	CRC	Zou <i>et al</i> [123]
miR-183-5p	Upregulated	Angiogenesis	CRC	Shang <i>et al</i> [124]
miR-155	Upregulated	Diagnostic tool	CRC	Lv <i>et al</i> [125]
miR-16-5p	Upregulated	Regulation of ITGA2	CRC	Xu <i>et al</i> [165]
miR-497	Downregulated	Prognosis marker	CRC	Zou <i>et al</i> [126]
miR-4461	Downregulated	Regulation of COPB2	CRC	Chen <i>et al</i> [43]
miR-146a	Upregulated	Invasion and metastasis	BC	Yang <i>et al</i> [45]
miR-125a-3p, miR-320c	Upregulated	Stage I marker	CC	Wang <i>et al</i> [166]
miR-4772-3p	Upregulated	Stage II & III marker	CC	Liu <i>et al</i> [167]
miR-21, miR-10b	Upregulated	Metastatic marker	HCC	Tian <i>et al</i> [44]
miR-1290, miR-375	Upregulated	Prognostic marker	CRPC	Huang <i>et al</i> [129]
miR-373, miR-200a, miR-200b, miR-200c	Upregulated	Tumor progression	EOC	Meng <i>et al</i> [131]
mir-181b-5p	Downregulated	Diagnostic tool	GC	Yun <i>et al</i> [127]

CRC: Colorectal cancer; GC: Gastric cancer; ITGA2: Integrin alpha 2; COPB2: COPI coat complex subunit beta 2; BC: Breast cancer; Stage I, II, III: North American Association of Central Cancer Registries Stages I, II, III and IV; CC: Colon cancer; HCC: Hepatocellular carcinoma; CRPC: Castration-resistant prostate cancer; EOC: Epithelial ovarian cancer.

Exosomes role in cancer metastasis

Metastasis is one of the most common causative factors in cancer-related death. Cancer metastasis is a multi-step process for the development of secondary cancers. In 1889, Stephen Paget described the “seed and soil” theory, in which metastasis depends on the interaction between primary cancer cells as the seed and secondary host microenvironments designated as the soil[138]. Involved mechanisms were found to include changes to the extracellular matrix architecture and associated reprogramming of normal cells. Clinically significant interactions between cancer cells and the cells of secondary organ sites have been shown to involve hepatocytes, bone marrow progenitor cells, CAFs, macrophages, and neutrophils. However, regulatory mechanisms of secondary organ-specific metastasis are poorly understood. Towards that understanding, studies have indicated that tumor-derived exosomes assist in the priming of premetastatic niches by delivering prometastatic factors. In particular, the integrin expression profile of tumor derived EVs can act as functional “ZIP codes” during metastatic organotropism to direct metastatic cancer cells to target tissue/organs[96]. Proteomic and clinical data support the role of exosome-sorted integrins as vital players in the development of cancer metastasis. For instance, $\alpha\beta4$ and $\alpha\beta1$ integrins are associated with lung metastasis and $\alpha\beta5$ is involved with liver metastasis[139]. Also, tumor-derived exosomes are involved in the activation of the Src kinase pathway and the upregulation of pro-inflammatory S100 genes during the establishment of premetastatic niches[140]. Thus, cell-cell communication mediated by EVs appears to be a critical element during premetastatic niche formation in cancer development (Figure 3). Review of the literature indicates the variety of cancer types and stromal cell-derived exosome molecules can initiate signals during the reprogramming of the tumor microenvironment (Table 2). Thus, exosome-mediated

Table 2 Extracellular vesicle components in cancer progression and metastasis

EV cargo	Type of molecule	Action on recipient cells/tissue	Type of cancer	Ref.
CEA	Protein	Inflammation	Colorectal	Yokoyama <i>et al</i> [162]
KRAS	Protein	Invasiveness in recipient cells	Colorectal	Beckler <i>et al</i> [152]
ITG	Protein	Metastatic organotropism	Breast	Hoshino <i>et al</i> [96]
TNC	ECM protein	Stem cell niche formation	Breast	Oskarsson <i>et al</i> [97]
MIF	Protein	Liver premetastatic niche formation	Pancreatic	Costa-Silva <i>et al</i> [168]
ZFAS1	lncRNA	Cancer growth/metastasis	Gastric	Pan <i>et al</i> [119]
Amino acids, lipids, TCA-cycle intermediates	Metabolites	Cancer growth	Prostate	Zhao <i>et al</i> [132]

CEA: Carcinoembryonic antigen; KRAS: Kirsten rat sarcoma viral oncogene; ITG: Integrins; TNC: Tenascin C; MIF: Macrophage migration inhibitory factor; ZFAS1: ZNF1 antisense RNA1; lncRNA: Long non-coding RNA; ECM: Extracellular matrix.

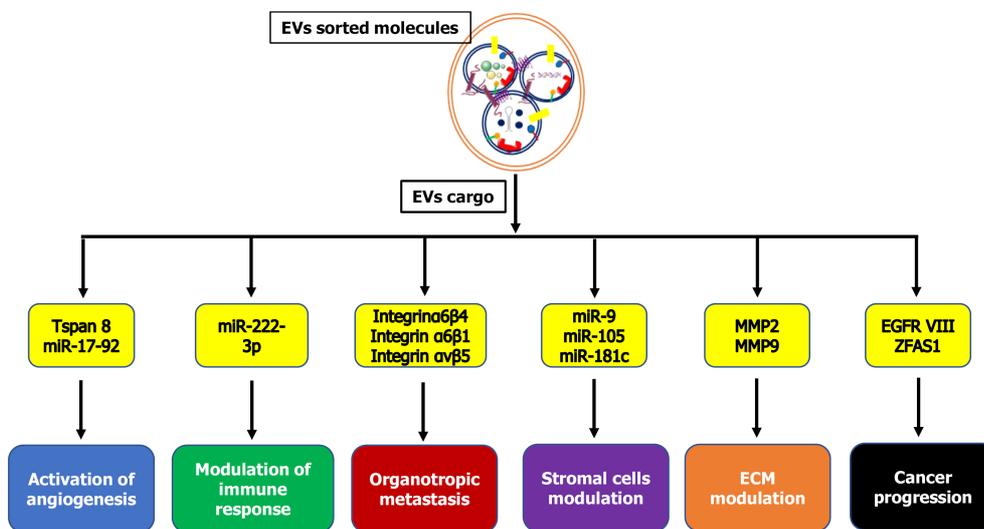


Figure 3 Exosome-mediated functions in the pre-metastatic niche. The role of tumor-derived exosomes along the establishment and progression of metastatic disease. Extracellular vesicle cargo can be involved in the initiation and regulation of cancer by promoting immune responses, angiogenesis, extracellular matrix modulation, stromal cell changes and metastatic organotropism. EVs: Extracellular vesicles; Tspan8: Tetraspanin-8; MMP2: Matrix metalloproteinase 2; MMP9: Matrix metalloproteinase 9; EGFR VIII: Epidermal growth factor receptor variant III; ZFAS1: ZNF1 antisense RNA1; ECM: Extracellular matrix.

intracellular signaling as well as organ-organ communication can influence cancer progression and changes to host and tumor microenvironments to facilitate metastatic disease.

CLINICAL IMPLICATIONS OF EXOSOME COMMUNICATION NETWORKS

Role of EVs in alcohol and colorectal cancer disease

Recent studies indicate an alarming increased rate of morbidity and mortality from alcohol use disorders in the United States[141]. Of particular clinical significance is the disease burden related to alcohol use in colorectal cancer and associated liver metastasis. CRC is a leading cause of cancer mortality with the majority of deaths due to the development of colorectal liver metastasis (CRLM) as the liver is the foremost site of distant metastatic spread in CRC patients[142,143]. Epidemiological studies suggest that chronic alcohol consumption is one of the major causative factors of colorectal cancer mortality in both men and women[144]. Alcohol use correlates with CRLM at colorectal cancer diagnosis as well as hepatic metastases that occur over time. Further, alcohol-associated CRLM requires intensive follow-up and treatment due to poor liver function and unresectable lesions. Despite advancements in surgical interventions and chemotherapeutics, CRLM morbidity and mortality are leading healthcare concerns

emphasizing the significant need to determine contributing mechanisms. The involvement of EV signaling during CRC progression in the setting of alcohol is not known. Thus, understanding EV communication networks and the role of EVs as biomarkers can significantly contribute to the development of strategies to address the serious public health issues associated with alcohol use and cancers.

The development of CRC is a multi-step process involving the malignant transformation of normal cells of the colon. The contribution of ethanol metabolism and related metabolites in colon carcinogenesis has been investigated for some time. A variety of pathways attributed to the effects of alcohol have been identified in the promotion CRC including genetic abnormalities, epigenetic dysregulation, cell signaling, and changes in the tumor microenvironment[13]. However, the role of alcohol during CRC spread to other organs is less understood. In particular, the contribution of alcohol during liver metastasis is emerging as a critical area of study given the substantial mortality associated with CRLM and need to identify targetable mechanisms. Current literature indicates that alcohol creates a hepatic microenvironment susceptible to CRC seeding and growth. Attributable mechanisms include the sensitization of resident macrophages (Kupffer cells, KCs) to endotoxin-induced signaling, the production of inflammatory factors, and the activation of fibroblastic cells that promote disease rather than wound-healing[145]. Moreover, it is likely that targetable mechanisms of CRLM involve communication networks between alcohol-affected macrophages and cancer cell-associated factors. The contribution of EVs in alcohol-associated CRLM is not defined but is clearly considered an important process to characterize.

EVs represent a new form of communication in colorectal cancer progression and liver metastasis. The fact that EVs can deliver cargo (*i.e.* RNAs, lipids, proteins) between cells and organs indicates the potential of playing a key role in metastatic disease[146,147]. CRC proliferation and migration can induce the release of EVs and other tumor-derived factors that can promote prometastatic niche formation, vascular changes, inflammation, and immunosuppression in host microenvironments. Several studies have recently described the contributions of EV cargo as prime mechanisms of CRC metastasis. For example, proteomic data revealed a distinct profile of metastatic factors, signal transduction molecules, and lipid raft-associated components in EVs obtained from metastatic CRC cells[148]. The contribution of mRNA components from CRC-derived EVs in cancer progression has also been shown for miRNAs (*i.e.* miR-21, miR-192 and miR-221) as well as natural antisense RNAs such as Leucine Rich Repeat Containing 24, MDM2 Proto-Oncogene, and Cyclin Dependent Kinase Inhibitor 1A [149]. Moreover, the role of genetic mutations in CRC patients are of interest. In particular, KRAS mutations are frequently associated with CRC metastasis and the regulation of exosome composition and release in CRC cells[150,151]. In addition, many oncogenic proteins (*e.g.* KRAS, Src family kinases, integrins) are highly enriched in mutant KRAS-derived exosomes indicating a role in CRC progression and metastasis[152]. Together, these observations provide novel insight into the role of EVs and the therapeutic potential of targeting the CRC-generated EVs during metastatic disease.

There is a growing body evidence suggesting that tumor-derived exosomes are crucial factors that influence differentiation in the microenvironment through particular signaling pathways[153]. For example, CRC cell-derived EVs have been shown to promote angiogenesis and tumor growth in the host microenvironment through the hyper-activation of Wnt/ β -catenin signaling. As a result, hypoxic metastatic niches provide CRC cells protection from chemotherapy and attack from immune cells[154]. Another signaling pathway implicated in colon tumorigenesis is the activation of proinflammatory cellular kinases. A recent study by Talwar *et al*[155] demonstrated that phosphorylated p38 γ is activated in CRC tumorigenesis. Further, it is suggested that the activation of p38 γ may be associated with immunoglobulin adhesion molecules such as carcinoembryonic antigen (CEA) and biliary glycoprotein (BGP). In support, the expression of CEA and BGP have been linked to hepatic metastasis in various preclinical models and in CRC patients with ongoing efforts to define the mechanistic role of CEA during CRLM[156-158]. Key studies have shown a direct relationship between CEA and the metastatic potential of CRC cells, and that CEA stimulation results in the production of tumorigenic factors by Kupffer cells[159-161]. Current works are evaluating the role of alcohol on KC function to determine if ethanol-sensitized macrophages are more responsive to CEA leading to advanced metastatic disease. To date, studies have shown that the alcohol-injured liver provides a permissive environment for CRLM and that CEA-mediated inflammatory mechanisms may play a key role[157,162]. However, the role of tumor-derived and alcohol-associated EVs in the process of metastatic mechanisms involving MAPK signaling or

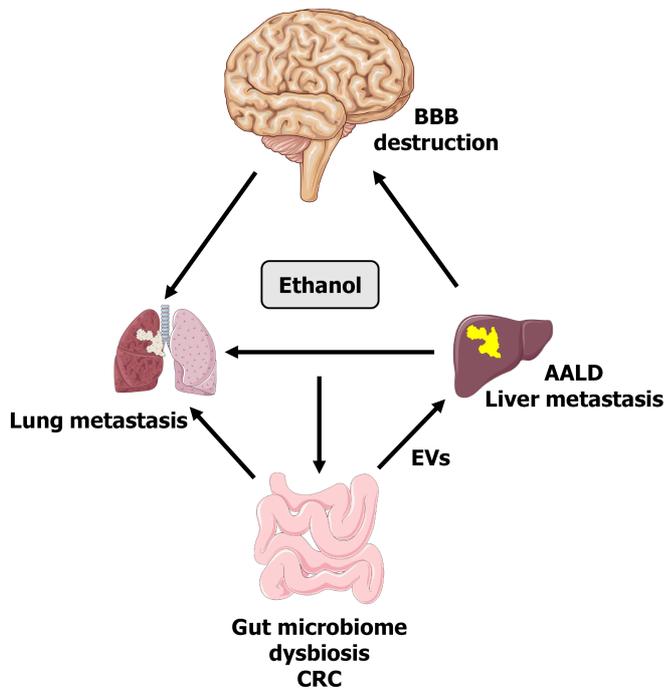


Figure 4 Model of extracellular vesicle interactions along the gut/liver/lung/brain axis during alcohol disorders and cancer progression. Alcohol exposure leads to enhanced gut microbiome dysbiosis, the development of alcohol-associated liver disease, lung inflammation, and neurological manifestations. The potential role of tumor- and alcohol-derived extracellular vesicles (EVs) in advanced malignancies is a potential consequence of EV organ-organ communication during alcohol disorders. BBB: Blood brain barrier; CRC: Colorectal cancer; AALD: Alcohol-associated liver disease.

carcinoembryonic antigen-related cell adhesion molecules is unknown. Further, the effectiveness of blocking EV-mediated communication in the alcohol-injured liver during CRLM also remains to be defined. Overall, the characterization of exosome cargo and communication networks in the transformation of CRC cells and reprogramming of the tumor microenvironment is an important area of translational research, especially in the context of complex comorbidities associated with aberrant alcohol intake.

CONCLUSION

In recent years, investigations into the role of EVs in cancer progression and AALD have increased in a remarkable manner. The elucidation of EV communication networks to date have indicated the powerful role of EVs as metastatic cancer markers and inducers of varied biological effects. Extensive work is ongoing to characterize the biogenesis and effects of distinct EV populations generated from different cell types and diseases. The unique features of EV size and cargo contents can produce hallmark effects on recipient cells. Therefore, the heterogeneity of exosome populations will dictate studies on the role and outcomes of exosome networks during disease states. For example, understanding the diversity of EVs released during gut microbiome dysbiosis, migration, and organ-organ communication aims to reveal the association of AALD and hepatic CRC metastasis. The complexity of interorgan communication and the involvement of mediators such as EVs, cytokines, and chemokines is the ongoing focus of translational research. Related to alcohol-associated diseases, it is proposed that EV-mediated communication affects multi-organ damage as well as cancer metastasis along the liver/gut/lung/brain axis (Figure 4). Future studies will likely focus on the characterization of exosomal components involved in alcohol's effects and cancer cell metastasis to secondary organs. Moreover, further investigation is needed to explore the role of exosome-mediated cell-free networks in the detection of alcohol-related tumors and microenvironment interactions for the development of targeted therapeutics.

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