



## Review Article

# Exosomal long noncoding RNAs and microRNAs in colorectal cancer

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### ABSTRACT

This review focuses on the multifaceted roles of exosomal noncoding RNAs (ncRNAs) in colorectal cancer (CRC), utilizing the provided document as the primary source of information. Exosomes, nanoscale vesicles ranging from 30 to 150 nm, act as crucial mediators of intercellular communication, encapsulating bioactive molecules such as microRNAs (miRNAs) and long ncRNAs (lncRNAs). The biogenesis of exosomes involves the endocytic pathway, including the formation of multivesicular bodies and subsequent release of intraluminal vesicles into the extracellular space. This process is regulated by the endosomal sorting complex required for transport (ESCRT) machinery and other ESCRT-independent mechanisms, as well as RNA-binding proteins (RBPs) that selectively package ncRNAs. MiRNAs, shorter single-stranded RNA molecules, regulate gene expression post-transcriptionally by binding to target mRNAs, leading to translational repression or mRNA degradation. LncRNAs, longer RNA molecules, are involved in chromatin remodeling and transcriptional regulation and act as competing endogenous RNAs that modulate miRNA availability. Exosomal ncRNAs play a crucial role in tumorigenesis, where certain miRNAs promote proliferation while others act as tumor suppressors. Furthermore, these ncRNAs are central to the epithelial–mesenchymal transition, a critical process that facilitates metastasis. They also play a role in chemoresistance by modulating drug metabolism and apoptotic pathways. Exosomal ncRNAs also show promise as diagnostic and prognostic biomarkers due to their presence in body fluids and their association with disease progression. Moreover, they hold potential as therapeutic agents through RNA-based therapeutics and exosome-based drug delivery. The challenges involve standardizing exosome research, elucidating the underlying mechanisms, and ensuring successful clinical translation.

**KEYWORDS:** Biomarkers, Colorectal cancer, Exosomal ncRNAs, Long noncoding RNAs, MicroRNAs

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## INTRODUCTION

Colorectal cancer (CRC) remains a major global health challenge, ranking as the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide. With an estimated 1.9 million new cases and over 900,000 deaths annually [1], CRC places a significant burden on healthcare systems. It is projected that by 2040, the global disease burden will rise to 3.2 million new cases and 1.6 million deaths [2]. Despite advancements in diagnostics, surgery, chemotherapy, and targeted therapies, the prognosis for patients with advanced CRC remains poor. Late-stage diagnosis, tumor heterogeneity, metastasis, and chemoresistance continue to hinder treatment efficacy and long-term survival. These challenges highlight the urgent need for innovative

approaches to CRC management, particularly in early detection and personalized therapy.

At the core of CRC progression lies the tumor microenvironment (TME), a dynamic and interactive ecosystem that profoundly influences tumor biology [3]. The TME consists of cancer cells, stromal cells (including fibroblasts, endothelial cells, and immune cells), soluble factors, and the extracellular matrix [4]. This microenvironment orchestrates key processes such as tumor growth, angiogenesis, immune evasion, and metastasis [4]. Interactions between tumor cells and the TME are highly complex, mediated by various

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extracellular signals – among which exosomes play a central role.

Exosomes, a subtype of extracellular vesicles (EVs), are nanosized vesicles ranging from 30 to 150 nm in diameter [5]. They are secreted by virtually all cell types [6] and facilitate intercellular communication by transporting bioactive molecules such as proteins, lipids, and nucleic acids, including DNA, mRNA, and noncoding RNAs (ncRNAs) [7]. The biogenesis of exosomes involves the inward budding of late endosomal membranes to form multivesicular bodies (MVBs), which fuse with the plasma membrane to release their vesicular contents into the extracellular space [5]. Once released, exosomes can travel through bodily fluids and deliver their cargo to recipient cells, thereby modulating cellular behavior and influencing the TME [4]. Exosomes are not merely passive carriers; their cargo reflects the physiological or pathological state of their originating cells, making them valuable sources of biomarkers for various diseases, including CRC [8].

Among the molecular cargo of exosomes, ncRNAs have garnered considerable attention for their regulatory roles in CRC pathogenesis [9]. ncRNAs, which include microRNAs (miRNAs) and long ncRNAs (lncRNAs), are pivotal regulators of gene expression [4,9]. miRNAs are small, single-stranded RNA molecules approximately 22 nucleotides in length that regulate gene expression post-transcriptionally by binding to complementary sequences in the 3'-untranslated regions (3'-UTRs) of target mRNAs [10]. This binding typically results in mRNA degradation or translational repression [11]. Depending on their targets, miRNAs can function as oncogenes or tumor suppressors.

Conversely, lncRNAs are longer RNA molecules with diverse functions, including epigenetic regulation, chromatin remodeling, and serving as competing endogenous RNAs (ceRNAs) that sequester miRNAs, thereby modulating their availability to target mRNAs [12]. In the context of CRC, exosomal miRNAs and ncRNAs have been implicated in various oncogenic processes, such as tumor initiation, proliferation, epithelial–mesenchymal transition (EMT), metastasis, and therapy resistance [8,12]. For instance, miRNAs such as miR-21 [13] and miR-92a [14] are frequently upregulated in CRC and promote tumor progression, while lncRNAs such as H19 [15,16] and plasmacytoma variant translocation 1 (PVT1) [17] contribute to chemoresistance and metastasis. Due to their stability in body fluids and tumor-specific expression, exosomal ncRNAs hold great potential as noninvasive biomarkers and therapeutic targets.

This review consolidates current research on exosomal ncRNAs in CRC, highlighting their critical roles in disease progression and potential clinical applications. Specifically, we discuss their involvement in key oncogenic mechanisms, evaluate their viability as diagnostic and prognostic biomarkers, and explore their potential in therapeutic strategies. By synthesizing the latest findings, this review aims to bridge the gap between fundamental research and clinical implementation, offering valuable insights into how exosomal ncRNAs could revolutionize CRC management and enhance patient outcomes.

## BIOGENESIS AND ROLE OF EXOSOMAL NONCODING RNAs

### Biogenesis of exosomes

The biogenesis of exosomes is a highly regulated, multistep process that begins within the endocytic pathway. It starts with the inward budding of the plasma membrane, forming early endosomes. These early endosomes mature into late endosomes or MVBs, characterized by the presence of intraluminal vesicles (ILVs) formed through the inward budding of the endosomal membrane. The MVBs either fuse with lysosomes for degradation or with the plasma membrane to release their ILVs as exosomes into the extracellular space [18].

The formation of exosomes is facilitated by the endosomal sorting complex required for transport machinery. Environmental cues, such as hypoxia and cellular stress, further influence the composition of exosomal cargo, tailoring their contents to the physiological or pathological state of the parent cell [19].

### Role of exosomal noncoding RNAs

Exosomal ncRNAs play critical roles in regulating gene expression and cellular processes, serving as messengers that mediate intercellular communication. These ncRNAs, once internalized by recipient cells through fusion with the plasma membrane or receptor-mediated endocytosis, exert their regulatory functions in diverse physiological contexts.

#### *miRNAs as posttranscriptional regulators*

MiRNAs are small, single-stranded RNA molecules, typically 18–24 nucleotides in length, that regulate gene expression at the posttranscriptional level [20]. Encapsulated within exosomes, miRNAs are delivered to recipient cells, where they bind to complementary sequences in the 3'-UTRs of target mRNAs [10]. This interaction leads to translational repression or mRNA degradation, finetuning protein expression.

MiRNAs encapsulated within exosomes serve as vital regulators of various physiological processes, exerting their influence across multiple domains of cellular function [21,22]. In the context of development, exosomal miRNAs play a pivotal role by modulating key transcription factors and signaling pathways [20], thereby directing cell differentiation and tissue patterning [21].

By fine-tuning these processes, miRNAs facilitate a balanced immune environment, enabling the body to respond effectively to pathogens while avoiding excessive inflammation.

In addition, exosomal miRNAs are central to metabolic regulation. By targeting enzymes and other regulatory molecules, they influence metabolic pathways that maintain energy balance and cellular health [23]. This ability to control metabolic flux highlights their importance in ensuring the proper functioning of cellular and systemic metabolic networks.

Furthermore, exosomal miRNAs contribute significantly to the regulation of cell proliferation and apoptosis. Specific

miRNAs either promote or inhibit these processes, ensuring that cellular homeostasis is preserved [23]. This delicate balance is crucial for maintaining proper tissue maintenance and preventing aberrant cell growth or premature cell death.

Through these diverse functions, exosomal miRNAs act as master regulators, facilitating intercellular communication and enabling precise control over developmental, immune, metabolic, and homeostatic processes [24,25].

The selective packaging of miRNAs into exosomes is mediated by RBPs and sequence-specific motifs, allowing precise intercellular communication [24]. Exosomal miRNAs facilitate rapid responses to environmental changes, supporting coordinated cellular activities across tissues.

Exosomal ncRNAs contribute significantly to physiological homeostasis and cellular function regulation through their involvement in biogenesis and signaling pathways. By enabling targeted delivery of regulatory molecules, exosomes serve as powerful mediators of intercellular communication [25]. Their ncRNA cargo – including lncRNAs and miRNAs – orchestrates complex regulatory networks and signaling pathways, underscoring their potential as biomarkers and therapeutic agents. Understanding the intricate processes governing exosomal biogenesis, signaling pathways, and cargo function will pave the way for leveraging these vesicles in diagnostic and therapeutic applications.

#### *Long noncoding RNAs as regulatory elements*

lncRNAs, transcripts longer than 200 nucleotides, play a critical role in regulating gene expression [26]. Within exosomes, lncRNAs serve as essential mediators of intercellular communication, influencing transcriptional, posttranscriptional, and epigenetic processes in recipient cells [26]. These versatile molecules exert their regulatory functions through a variety of sophisticated mechanisms.

One prominent mechanism by which lncRNAs regulate gene expression is through chromatin remodeling. By interacting with chromatin-modifying complexes, lncRNAs alter chromatin structure, facilitating or repressing the transcription of specific genes [27]. For instance, lncRNAs recruit histone modifiers to targeted genomic loci, enabling dynamic and context-specific regulation of gene expression [16]. This capability underscores their integral role in fine-tuning cellular responses to environmental cues.

In addition, lncRNAs influence transcriptional processes by directly interacting with transcription factors or RNA polymerase, modulating the transcriptional activity of target genes. This interaction can either enhance or suppress gene transcription, depending on the molecular context and the specific lncRNA involved [16,27]. Such transcriptional regulation highlights the versatility of lncRNAs in controlling gene networks.

Another critical function of lncRNAs is their role as ceRNAs [22]. In this capacity, lncRNAs act as molecular sponges for miRNAs, sequestering them and preventing their interaction with target messenger RNAs (mRNAs) [22]. By modulating miRNA availability, lncRNAs indirectly regulate

gene expression, contributing to the intricate balance of cellular regulatory networks.

Furthermore, lncRNAs influence RNA stability and localization by interacting with RNA-binding proteins (RBPs) [28]. Through these interactions, lncRNAs can stabilize or destabilize target RNAs, as well as guide them to specific cellular compartments. This ability to regulate RNA dynamics further emphasizes the multifaceted nature of lncRNA functions.

Through these diverse mechanisms, exosomal lncRNAs play a pivotal role in shaping cellular behavior and coordinating intercellular communication. Their capacity to modulate chromatin states, transcriptional activity, and RNA stability underscores their significance as key regulators of gene expression and cellular function.

Exosomal lncRNAs are involved in a wide array of physiological processes, including cell differentiation, immune modulation, and metabolic regulation. Their selective packaging into exosomes ensures the targeted delivery of these regulatory molecules, enabling precise control of recipient cell behavior.

## **FUNCTIONAL ROLES OF EXOSOMAL NONCODING RNAs IN COLORECTAL CANCER**

Exosomal ncRNAs have emerged as key players in the progression of CRC, influencing tumorigenesis, metastasis, and chemoresistance [9,25]. These vesicles, secreted by tumor and stromal cells, carry a wide range of bioactive molecules, including miRNAs, and lncRNAs [16], which regulate gene expression and cellular signaling in recipient cells [21]. This section highlights their functional roles in CRC progression.

### **Tumorigenesis and proliferation**

Exosomal ncRNAs play a pivotal role in the initiation and proliferation of CRC cells by modulating key signaling pathways and gene expression [Tables 1 and 2]. Certain miRNAs carried by exosomes act as oncogenes, driving uncontrolled cell division [20]. For instance, miR-21, significantly enriched in CRC exosomes, promotes the proliferation of colon adenocarcinoma cells by downregulating tumor suppressors such as programmed cell death protein 4 [20,37]. Similarly, miR-548am-5p expedites tumor growth by targeting RAR-related orphan receptor A [46], while miR-224-5p enhances cell proliferation, migration, and invasion through suppression of CKLF Like MARVEL transmembrane domain containing 4 [34].

Conversely, some exosomal miRNAs exert tumor-suppressive effects. For example, miR-16-5p, derived from bone marrow mesenchymal stem cells, inhibits CRC cell growth and induces apoptosis by targeting integrin alpha-2 [44,47]. In addition, miR-150-5p whose level was lowered in serum exosomes of CRC patients, acts as a brake on tumor development and growth and is correlated with tumor progression [30].

lncRNAs transported by exosomes also contribute to tumorigenesis. PVT1, a well-studied miRNA sponge oncogenic

**Table 1: Noncoding RNAs regulation of Wnt/ $\beta$ -catenin signaling in colorectal cancer proliferation**

Signaling pathway	ncRNA (lncRNA or miRNA)	Effect on CRC	References
Wnt/ $\beta$ -catenin	miR-16-5p	Inhibited CRC migration, invasion, and EMT by the miR-16-5p/HMGA2/ $\beta$ -catenin pathway. miR-16-5p overexpression or FOXK1 knockdown reduced CRC cell proliferation and angiogenesis of human umbilical vein endothelial cells cocultured with the supernatant of CRC cells	[29]
	miRNA-150-5p	Inhibits cell viability, proliferation, and colony formation and also inhibits CRC development by regulating $\beta$ -catenin	[30]
	lncRNA CCAT2	Stimulates Wnt cascade activity through TCF7L2, leading to MYC oncogenic activation	[31]
	lncRNA CASC11	Enhances Wnt/ $\beta$ -catenin signaling by targeting hnRNP-K in CRC cells	[32]
	lncRNA H19	Activates the $\beta$ -catenin pathway, promoting stemness in CRC cells. Promotes proliferation through targeting RB with miR-675	[15,16]
	lncRNA HNF1A-AS1	Controls the expression of $\beta$ -catenin, cyclinD1, and c-MYC, key components of the Wnt signaling pathway, thus influencing cell proliferation	[33]
	miR-224-5p	In exosomes from CRC cells, promotes malignant transformation of normal colon epithelial cells, and promotes proliferation, migration, and invasion of CRC cells	[34,35]
	miRNA-425-5p	Increases cell proliferation	[36]

CRC: Colorectal cancer, EMT: Epithelial–mesenchymal transition, miRNA: MicroRNAs, ncRNA: Noncoding RNAs, lncRNA: Long noncoding RNA, RB: RB transcriptional corepressor 1

**Table 2: Noncoding RNAs regulation of other signaling pathways in colorectal cancer proliferation**

Signaling pathway	ncRNA (lncRNA or miRNA)	Effect on CRC	References
PI3K/AKT/mTOR	miR-21	Promotes proliferation	[37]
PI3K/AKT/mTOR	miR-92a-3p	The ectopic expression of miR-92a enhanced CRC cell proliferation, migration, and invasion	[38]
PI3K/AKT/mTOR	lncRNA-422	Inhibits cell proliferation, migration, and invasion through regulating PI3K/AKT/mTOR pathway	[39]
PI3K/AKT/mTOR	lncRNA PlncRNA-1	Promotes proliferation through targeting the PI3K/AKT signaling cascade	[40]
PI3K/AKT/mTOR	lncRNA SNHG7	Promotes proliferation by regulating the PI3K/AKT/mTOR pathway	[41]
JAK/STAT	lncRNA HOTAIR	Up-regulates PD-L1 expression in B cells by increasing PKM2 stability, which increases STAT3 transcriptional activity, thereby contributing to cancer proliferation	[42]
TGF $\beta$	miR-423-5p	miR-423-5p acts as tumor promoter, leading to the proliferation, invasion, and angiogenesis of cancer. Targets CRC pathways, including the tumor suppressor genes SMAD2/3 and TGF $\beta$ R2	[43]
TGF $\beta$	lncRNA PVT1	Promotes abnormal proliferation and metastasis by upregulating EGFR and VEGFA	[17,44]
Other pathways	lncRNA BDNF-AS	lncRNA BDNF-AS induced inhibition of proliferation, migration, and invasion of CRC cells via inhibiting GSK-3 $\beta$ expression	[45]

CRC: Colorectal cancer, EMT: Epithelial–mesenchymal transition, miRNA: MicroRNAs, TGF $\beta$ : Transforming growth factor beta, ncRNA: Noncoding RNAs, lncRNA: Long noncoding RNA, EGFR: Epidermal growth factor receptor

lncRNA, sequesters the tumor-suppressive miR-3619-5p and regulates TRIM29 expression, promoting the growth and metastasis of CRC [17]. Similarly, H19, enriched in cancer-associated fibroblasts (CAF)-derived exosomes, enhances the stemness and chemoresistance of CRC cells and drives tumor progression [16].

### Epithelial–mesenchymal transition and metastasis

The EMT is a critical process that enables CRC cells to gain migratory and invasive properties. Exosomal ncRNAs are central to orchestrating EMT by regulating cell adhesion, cytoskeletal dynamics, and signaling pathways [Tables 3 and 4].

Exosomal miR-21 promotes EMT in CRC by targeting genes involved in cell adhesion and migration, such as phosphatase and tensin homolog (PTEN) [37]. Similarly, miR-335-5p enhances CRC invasion and metastasis by suppressing RAS p21 protein activator 1, facilitating EMT [59], while exosomal miR-20a-5p from CAFs promotes CRC proliferation and migration by targeting PTEN and activating NF- $\kappa$ B/IL-6 signaling [60]. Another miRNA, miR-92a-3p [61], derived

from cancer-associated fibroblasts (CAFs), activates the Wnt/ $\beta$ -catenin pathway and inhibits mitochondrial apoptosis, driving metastatic progression [62]. In addition, CRC cell-derived exosomes can transfer multiple miRNAs, such as miR-25-3p, miR-130b-3p, and miR-425-5p, to macrophages, which in turn regulate PTEN expression through the activation of the PI3K/AKT signaling pathway, ultimately promoting EMT and the secretion of vascular endothelial growth factor, thereby accelerating cancer metastasis [50]. Furthermore, miR-17-5p can be transferred from CAFs to CRC cells, where it binds to the 3'-UTR of RUNX3 and interacts with MYC to activate the TGF- $\beta$  signaling pathway, promoting CRC progression [52]. In contrast, some miRNAs act as metastasis suppressors. For example, miR-193b is downregulated in the serum of CRC patients, and its low levels are correlated with tumor, node, metastasis stage and metastasis in CRC patients [55]. Other miRNAs such as miR-1224-5p can prevent the EMT, invasion, and migration of CRC cells [57].

Exosomal lncRNAs are also significant players in CRC metastasis. Several lncRNAs have been implicated

**Table 3: Noncoding RNAs regulation of Wnt/ $\beta$ -catenin signaling, PI3K/AKT/mTOR, and JAK/STAT pathways in colorectal cancer metastasis**

Signaling pathway	ncRNA (lncRNA or miRNA)	Effect on CRC	References
Wnt/ $\beta$ -catenin PI3K/AKT/mTOR	miR-16-5p	Inhibited CRC migration, invasion, and EMT by the miR-16-5p/HMGA2/ $\beta$ -catenin pathway. miR-16-5p overexpression or FOXK1 knockdown reduced CRC cell proliferation and angiogenesis of human umbilical vein endothelial cells cocultured with the supernatant of CRC cells	[29]
	LncRNA UCA1	miR-1224-5p suppresses the migration, invasion, and EMT of CRC cells <i>in vitro</i> and <i>in vivo</i> by directly targeting SP1	[48]
Wnt/ $\beta$ -catenin	lncRNA H19	Activates the $\beta$ -catenin pathway, promoting stemness in CRC cells. Promotes proliferation through targeting RB with miR-675	[15,16]
	lncRNA XIST	Promotes metastasis through modulating ZEB1 expression by competing with miR-200b-3p	[49]
PI3K/AKT/mTOR	miR-25-3p, miR-130b-3p, and miR-425-5p	Induces M2 polarization of macrophages, promoting EMT and metastasis	[50]
	miR-92a-3p	The ectopic expression of miR-92a enhanced CRC cell proliferation, migration and invasion	[38]
	lncRNA MALAT1	Promotes cell proliferation via activation of the PI3K/AKT/mTOR pathway and by sponging miR-26a/26b	[51]
JAK/STAT	lncRNA HOTAIR	HOTAIR contributes to CRC liver metastasis and lung metastasis	[42]

CRC: Colorectal cancer, EMT: Epithelial–mesenchymal transition, miRNA: MicroRNAs, ncRNA: Noncoding RNAs, lncRNA: Long noncoding RNA, RB: RB transcriptional corepressor 1

**Table 4: Noncoding RNAs regulation of transforming growth factor beta signaling and other pathway in colorectal cancer metastasis**

Signaling pathway	ncRNA (lncRNA or miRNA)	Effect on CRC	References
TGF $\beta$	miR-17-5p	Upregulation of miR-17-5p promoted CRC cell proliferation, metastasis, and invasion, while inhibiting apoptosis	[52]
	miR-146a-5p and miR-155-5p	Promotes lung metastasis via targeting ZBTB2 and SOCS1, activating cancer-associated fibroblasts	[53]
	miR-423-5p	Targets CRC pathways, including the tumor suppressor genes SMAD2/3 and TGF $\beta$ R2. miR-423-5p acts as tumor promoter, leading to proliferation, invasion, and angiogenesis of cancer	[43]
	lncRNA PVT1	Promotes promote abnormal proliferation and metastasis by upregulating EGFR and VEGFA	[17,44]
	lncRNA RPPH 1	Promotes CRC metastasis by interacting with TUBB3 and mediating macrophage M2 polarization	[54]
Other pathways	lncRNA BDNF-AS	lncRNA BDNF-AS induced inhibition of proliferation, migration, and invasion of CRC cells via inhibiting GSK-3 $\beta$ expression	[45]
	miR-193b	Low serum miR-193b was associated with TNM stage, grade and lymph node metastasis	[55]
	miRNA-375-3p	Promotes metastasis through regulation of $\beta$ -catenin, vimentin, ZEB1, and SNAIL	[56]
	miR-1224-5p	miR-1224-5p suppresses the migration, invasion, and EMT of CRC cells <i>in vitro</i> and <i>in vivo</i> by directly targeting SP1	[57]
	lncRNA GNAS-AS1	Promotes cell migration and invasion	[58]

CRC: Colorectal cancer, EMT: Epithelial–mesenchymal transition, miRNA: MicroRNAs, TGF $\beta$ : Transforming growth factor beta, ncRNA: Noncoding RNAs, lncRNA: Long noncoding RNA, TNM: Tumor, node, metastasis, EGFR: Epidermal growth factor receptor

in promoting EMT, a crucial step in the metastatic cascade. For instance, H19 promotes CRC metastasis by inducing EMT [16]. MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is another lncRNA that promotes CRC metastasis by sponging miR-26a and miR-26b, increasing fucosyltransferase 4 expression levels, and activating the PI3K/AKT/mTOR pathway [63]. Similarly, urothelial carcinoma-associated 1 (UCA1) is upregulated in serum exosomes from CRC patients, and its transfer to CRC cells enhances their proliferation and migration; it also regulates Myosin VI expression by sponging miR-1437 [48]. Conversely, some lncRNAs can suppress metastasis. For example, BDNF-AS acts as a tumor suppressor by inhibiting

EMT-induced migration [45]. Some lncRNAs also have complex interactions with proteins, such as RPPH 1, which interacts with  $\beta$ -III tubulin (TUBB3) [54].

### Chemoresistance

The development of resistance to chemotherapy is a major challenge in CRC treatment. Exosomal ncRNAs contribute to chemoresistance by modulating drug metabolism, DNA damage repair, and apoptotic pathways, enabling tumor cells to evade therapeutic stress [Table 5].

miRNAs are significantly involved in mediating chemoresistance. For example, exosomal miR-92a-3p, which is transferred from CAFs to CRC cells, enhances cell

**Table 5: Noncoding RNAs regulation of transforming growth factor beta signaling and other pathway in colorectal cancer chemoresistance**

Signaling pathway	ncRNA (lncRNA or miRNA)	Effect on CRC	References
Wnt/ $\beta$ -catenin	miR-19b	Exo-miR-19b transfer from CRC to recipient cells, has been linked with enhanced stemness and radioresistance, via FBX downregulation and Wnt/ $\beta$ -catenin pathway activation	[64]
	lncRNA H19, CRNDE, UCA1 and HOTAIR	lncRNA H19 was enhanced in hypoxia- or oxaliplatin-treated CRC cells; moreover, H19 contributed to drug resistance in CRC cells	[15,16,65]
PI3K/AKT/mTOR	miR-21-5p, miR-1246, miR-1229-5p, miR-135b, miR-425 and miR-96-5p	Promotes chemoresistance: miR-21-5p, miR-1246, miR-1229-5p, miR-135b, miR-425 and miR-96-5p are also upregulated in exosomes from culture media of resistant cells	[66]
	miR-92a-3p	High expression of exosomal miR-92a-3p in serum was highly linked with metastasis and chemotherapy resistance in CRC patients	[38]
	miRNA-223	Enhances drug resistance	[64]
	lncRNA NEAT1	Activates Wnt/ $\beta$ -catenin signaling and promotes CRC progression by interacting with DDX5, negatively regulates miR-335, and promotes tumor metastasis <sup>36</sup> . NEAT1 silencing promoted the 5-Fu sensitivity and apoptosis and repressed the invasion and the expression of resistance-correlated proteins including P-gp and GST- $\pi$ in CRC cells	[67]
NA	miR-210	miR-210-3p downregulation enables resistant cells to counteract the toxic effect of the drug	[68]
	lncRNA CCAL	Promotes OX resistance by regulating HuR	[69]
	lncRNA HOTTIP	Promotes CRC, elevated levels in mitomycin-resistant cancer cells, inhibition reduces resistance	[70]

NA: Not applicable, CRC: Colorectal cancer, EMT: Epithelial–mesenchymal transition, miRNA: MicroRNAs, 5-Fu: 5-fluorouracil, ncRNA: Noncoding RNAs, lncRNA: Long noncoding RNA

stemness, EMT, and chemoresistance [62]. This miRNA has been linked with resistance to 5-fluorouracil (5-FU) and OX (oxaliplatin) [62,64]. Similarly, exosomal miR-223, derived from macrophages, has been correlated with enhanced drug resistance in ovarian cancer through the PTEN/AKT/PI3K pathway, suggesting a potential parallel mechanism in CRC [64]. Moreover, a panel of six exosomal miRNAs has been linked with 5-FU/OX resistance via the PI3K-Akt, FoxO, and autophagy pathways [66]. Specifically, increased expression of miR-92a-3p in CRC tissues has been shown to activate the Wnt/ $\beta$ -catenin pathway and inhibit mitochondrial apoptosis, contributing to 5-FU/OX resistance [62]. Another study found that exosomal miR-19b transfer from CRC cells to recipient cells is associated with enhanced stemness and radioresistance via FBX downregulation and Wnt/ $\beta$ -catenin pathway activation [64]. In addition, exosomal miR-210 promotes EMT and metastasis in 5-FU-treated cells [68]. Conversely, some miRNAs can also promote chemosensitivity. For example, miR-423-5p is a target of lncRNA PGM5-AS1, and increasing its levels via PGM5-AS1 can reverse the oxaliplatin resistance of CRC cells [43].

lncRNAs also play a crucial role in CRC chemoresistance. Exosomal CCAL has been correlated with oxaliplatin resistance, activating  $\beta$ -catenin and suppressing apoptosis by directly interacting with the mRNA stabilizing protein HuR [69]. Additionally, exosomal H19 mediates oxaliplatin resistance by activating the  $\beta$ -catenin pathway. Several other lncRNAs, such as CRNDE, UCA1, and HOTAIR, have been associated with oxaliplatin or irinotecan resistance through the modulation of cell proliferation, apoptosis, and cell energetic metabolism under hypoxic conditions [15]. Moreover, lncRNA PGM5-AS1 can reverse oxaliplatin resistance by acting as a sponge for miR-423-5p. Furthermore, lncRNA GNAS-AS1

is upregulated in 5-FU-resistant cells and potentially promotes increased exosome release [58]. lncRNA NEAT1 also facilitates 5-FU chemoresistance in CRC by inducing autophagy and supporting the maintenance of stemness [67]. Similarly, lncRNA HOTTIP, transported via exosomes, promotes resistance to mitomycin by activating survival pathways [70].

In summary, exosomal ncRNAs weave a complex narrative in CRC progression, acting as mediators of tumorigenesis, metastasis, and chemoresistance [Tables 1-5]. The complex interplay between these ncRNAs and various signaling pathways highlights their potential as therapeutic targets. Further research into their specific mechanisms of action could lead to the development of novel strategies for overcoming drug resistance and improving treatment outcomes in CRC patients.

## CLINICAL APPLICATIONS OF EXOSOMAL NONCODING RNAs IN COLORECTAL CANCER

Exosomal ncRNAs have emerged as transformative players in the clinical management of CRC. Their unique characteristics – including stability in bodily fluids, accessibility through noninvasive sampling, and involvement in key cancer-related processes – position them as powerful tools for diagnostics, prognostics, and therapeutics. By offering a window into the molecular underpinnings of CRC, exosomal ncRNAs are paving the way for precision medicine.

### Diagnostic biomarkers

The potential of exosomal ncRNAs as diagnostic biomarkers lies in their ability to provide early and accurate detection of CRC through noninvasive methods. These molecules, encapsulated within exosomes, circulate in bodily

fluids such as blood, urine, and stool, making them easily accessible for diagnostic purposes [13,71].

One of the most extensively studied biomarkers is miR-21, which is frequently upregulated in CRC patients [13]. Exosomal miR-21 levels in serum and plasma are significantly higher in patients with colorectal adenomas and early-stage CRC [37], highlighting its value as an early detection marker. Similarly, miR-92a has shown the ability to distinguish CRC patients from healthy individuals with high sensitivity and specificity [62]. Panels of miRNAs, such as a six-miRNA signature (miR-19a, miR-20a, miR-150, miR-143, miR-145, and let-7a), further enhance diagnostic accuracy by capturing the multifaceted nature of CRC progression [72].

Exosomal lncRNAs are also gaining attention as diagnostic tools. For example, CCAT2 [73] and HOTTIP [70], encapsulated in circulating exosomes, are significantly upregulated in CRC patients, providing additional molecular insights into tumor biology. Furthermore, miRNAs derived from peritoneal lavage fluid, such as miR-199b-5p, miR-150-5p, and miR-218-5p, represent an untapped reservoir of diagnostic markers, particularly for advanced CRC [71].

Recent research, exemplified by Miyazaki *et al.*, highlights the potential of exosomal miRNAs for pre-operative identification of lymph node metastasis (LNM) in patients with pathological high-risk T1 CRC [74]. Due to the low positive predictive value of current pathological risk factors for LNM, existing guidelines often lead to overtreatment in this population. Miyazaki *et al.*'s [74] investigation of cell-free and exosomal miRNAs in pre-operative serum revealed that a panel of four miRNAs (miR-181b, miR-193b, miR-195, and miR-411) effectively detects LNM, with exosomal miRNAs demonstrating superior diagnostic performance compared to their cell-free counterparts.

The combination of exosomal ncRNAs with traditional biomarkers like carcinoembryonic antigen has the potential to significantly improve diagnostic power, offering a more comprehensive approach to CRC detection.

### Prognostic biomarkers

In addition to their diagnostic utility, exosomal ncRNAs serve as robust prognostic markers, offering insights into disease progression, recurrence, and treatment outcomes. These molecules provide a molecular snapshot of tumor behavior, helping clinicians tailor treatment strategies.

For instance, low levels of exosomal miR-4772-3p have been linked to shorter time to recurrence and reduced overall survival in stage II and III CRC patients [75]. Similarly, elevated levels of miR-19a and miR-21 in serum exosomes correlate with poor prognosis, aggressive tumor behavior, and a higher likelihood of metastasis [76,77].

The prognostic value of exosomal lncRNAs is also being explored. Dysregulated lncRNAs, such as those derived from CAFs, influence tumor-stromal interactions and are associated with worse outcomes. In addition, analyzing exosomal ncRNAs from tumor-draining veins provides more accurate

prognostic information than peripheral samples, as these vesicles are directly influenced by the TME.

### Recent clinical trials on exosomal noncoding RNAs in colorectal cancer

Currently, there are several clinical trials (NCT04523389, NCT06654622, NCT06342440, NCT03962088, and NCT05375604) [78-82] actively exploring exosomal molecules, particularly exosomal miRNAs, as biomarkers for CRC. The objectives of these studies encompass multiple important clinical aspects, including the development of novel diagnostic markers (NCT04523389 and NCT06342440) [78,80], predicting LNM [74], detecting minimal residual disease after surgery (NCT06654622) [79], and monitoring treatment response (NCT03962088 and NCT05375604) [81,82]. A study identified circulating exosomal miR-185-5p as a potential biomarker for detecting advanced adenoma and CRC, highlighting its clinical relevance in early detection [83]. Another clinical investigation demonstrated that exosomal miR-652-3p could serve as a predictive biomarker for regorafenib resistance in CRC patients, offering insights into treatment response mechanisms (NCT03010722) [84]. These findings support the growing clinical interest in leveraging exosomal ncRNAs for precision oncology in CRC.

### Therapeutic applications

Exosome-based ncRNAs, proteins, and lipids each have distinct advantages and disadvantages as biomarkers or therapeutic agents. Exosomal ncRNAs, such as miRNAs and lncRNAs, play crucial roles in gene regulation and can modulate multiple cancer-related pathways, making them highly versatile therapeutic targets [7,9]. Their stability within exosomes protects them from rapid degradation, allowing for effective systemic delivery. However, challenges such as off-target effects, immunogenicity, and delivery specificity remain obstacles for clinical translation [9].

In contrast, exosomal proteins serve as direct functional mediators of intercellular communication and can act as disease-specific biomarkers or therapeutic agents. They are often more stable than free ncRNAs but may have limited regulatory potential compared to ncRNAs [85]. Exosomal lipids contribute to vesicle stability and can influence signaling pathways; however, their role as therapeutic agents is less explored due to their complexity in functional modulation [85].

Exosomal ncRNAs are not only biomarkers but also hold immense promise as therapeutic agents and delivery vehicles. Their natural ability to transfer molecular information between cells makes them ideal candidates for innovative treatment strategies.

### RNA-based therapeutics

Targeting oncogenic ncRNAs is a promising therapeutic avenue. For instance, inhibiting miR-21 using anti-miR oligonucleotides suppresses tumor growth and enhances chemosensitivity in preclinical models [86]. Similarly, silencing lncRNAs like H19 or HOTTIP, which drive CRC progression, sensitizes tumors to chemotherapeutic agents such as 5-FU and oxaliplatin [65].

### Exosome-based drug delivery

Exosomes themselves are being engineered as delivery vehicles for therapeutic molecules. These vesicles can be loaded with chemotherapeutic agents, siRNAs, or antisense oligonucleotides and targeted specifically to tumor cells. For example, human umbilical cord mesenchymal stem cell (hUC-MSC)-derived exosomes loaded with anti-miR-146b-5p demonstrated potent antitumor effects in preclinical CRC models [87]. Similarly, exosome-based delivery of miR-375-3p has shown efficacy in modulating EMT in CRC [56].

### Immunotherapy

Exosomal ncRNAs also play a role in enhancing immune responses. By targeting immune-regulatory ncRNAs, such as miR-208b, researchers aim to restore antitumor immunity [88]. In addition, exosome-based vaccines that carry tumor-associated antigens can prime the immune system to recognize and attack CRC cells [89], representing a novel approach to cancer immunotherapy.

## CHALLENGES AND FUTURE DIRECTIONS

Exosomal ncRNAs are at the forefront of innovative research in CRC, offering transformative potential for diagnostics, therapeutics, and prognostics. However, the field faces numerous challenges that must be addressed to realize its full clinical potential. These challenges span the standardization of research methodologies, the elucidation of underlying mechanisms, and the successful translation of findings into clinical applications.

### Standardization in exosome research

A major obstacle in exosome research is the lack of standardized protocols for isolating, characterizing, and quantifying exosomes. The heterogeneity of EVs, including exosomes, microvesicles, and apoptotic bodies, complicates efforts to achieve consistent results across studies. Common isolation techniques, such as ultracentrifugation, density gradient separation, and immunoaffinity capture, yield varying levels of purity and efficiency [3,87]. For instance, ultracentrifugation can lead to contamination with other vesicles and proteins, while immunoaffinity capture may introduce bias based on the antibodies used.

The development of advanced techniques, including microfluidic devices, nanoparticle tracking analysis [90], and transmission electron microscopy [90,91], has improved the characterization of exosomes. However, discrepancies in exosome composition, influenced by their cellular origin and environmental conditions, add another layer of complexity. Moreover, the instability of exosomal RNA during sample handling and storage further underscores the need for standardized protocols that cover the entire workflow, from sample collection to data analysis.

The MISEV2018 guidelines [92], developed by the International Society for EVs, represent a step forward in addressing these issues. However, efforts to create reference materials and universal standards for exosome isolation and analysis must continue to ensure reproducibility and reliability in exosome research.

### Mechanistic research

Despite significant progress in understanding the roles of exosomal ncRNAs, many mechanistic aspects remain elusive. The biogenesis of exosomes, involving the inward budding of late endosomes to form MVBs [5], is a highly regulated process. However, the specific mechanisms that govern the selective loading of ncRNAs, such as miRNAs and lncRNAs, into exosomes remain incompletely understood.

RBPs like hnRNPA2B1 [93] and YBX1 [94,95] have been implicated in this process, suggesting that specific RNA motifs may determine cargo selection. For instance, KRAS-MEK signaling has been shown to regulate Ago2 sorting into exosomes, highlighting the complexity of cargo-loading pathways [96]. Similarly, the interactions between exosomes and recipient cells – including mechanisms of internalization and specificity – require further investigation.

The downstream effects of exosomal ncRNAs on cellular processes are another critical area of research. While exosomal miRNAs such as [37,76,86,97,98] and miR-146a [53,87] are known to influence tumor progression and immune modulation, the precise molecular interactions they mediate remain poorly defined. Understanding these interactions at a systems level, including the role of circRNAs and passenger-strand miRNAs, will provide a more comprehensive picture of exosome biology and its impact on CRC.

### Translation into clinical practice

Bridging the gap between laboratory discoveries and clinical implementation presents significant challenges. While exosomes offer a noninvasive platform for diagnostics and therapeutics, their clinical translation faces hurdles related to scalability, targeting efficiency, and regulatory approval.

In diagnostics, the identification of robust biomarkers remains a priority. Exosomal miRNAs such as miR-21 [37] and miR-92a [62] have shown promise in detecting early-stage CRC, but large-scale clinical validation is necessary [21]. In addition, the integration of exosomal biomarkers with existing diagnostic tools, such as liquid biopsies, requires the development of cost-effective assays with high sensitivity and specificity.

Therapeutically, engineered exosomes represent a promising approach for delivering RNA-based drugs and other therapeutic agents [87,88,91,99]. For example, exosomes have been used to deliver siRNA targeting FAK in cetuximab-resistant CRC cells, demonstrating their potential to overcome drug resistance [91]. However, challenges such as efficient loading of therapeutic cargo, precise targeting to tumor cells, and large-scale production must be addressed [21]. Advances in biomanufacturing, including the use of hUC-MSC-derived exosomes, are paving the way for scalable and clinically viable solutions [87].

Ethical and regulatory considerations also play a critical role in the clinical translation of exosome-based therapies. Ensuring the safety and efficacy of these interventions, particularly in the context of genetic manipulation, requires robust preclinical and clinical testing. Collaborative efforts among researchers, clinicians, and regulatory bodies are

essential to establish guidelines that facilitate the safe and effective use of exosomal therapies.

## THE FUTURE OF EXOSOMAL NONCODING RNAS IN COLORECTAL CANCER MANAGEMENT

Exosomal ncRNAs have emerged as crucial regulators in CRC, marking a paradigm shift in disease management. These bioactive molecules – including miRNAs and lncRNAs – are selectively packaged into exosomes and play vital roles in intercellular communication, modulating tumor initiation, progression, metastasis, and therapy resistance. Their stability in body fluids and tumor specificity make them promising biomarkers for noninvasive liquid biopsies, aiding in early detection and patient stratification. In addition, engineered exosomes hold potential as therapeutic vehicles, delivering targeted treatments with precision.

Despite their promise, challenges remain in standardizing isolation techniques, understanding selective RNA packaging, and ensuring safety in clinical applications. Addressing these hurdles through cutting-edge technologies, including artificial intelligence and multiomics approaches, will accelerate discoveries and unlock the full potential of these molecular messengers. With continued research and innovation, exosomal ncRNAs are poised to transform CRC care, making diagnosis more precise, prognosis more reliable, and treatment more effective, ultimately paving the way for personalized medicine in oncology.

## CONCLUSION

Exosomal ncRNAs have emerged as significant players in the pathogenesis of CRC, presenting novel opportunities for clinical applications. These ncRNAs, including miRNAs and lncRNAs, are key exosomes, facilitating intercellular communication and playing critical roles in tumor progression. This review underscores the multifaceted contributions of exosomal ncRNAs in CRC and highlights their potential to revolutionize its diagnosis, prognosis, and therapy, while also acknowledging the imperative need for further research to overcome existing challenges.

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## Data availability statement

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209-49.
- Morgan E, Arnold M, Gini A, Lorenzoni V, Cabañas CJ, Laversanne M, et al. Global burden of colorectal cancer in 2020 and 2040: Incidence and mortality estimates from GLOBOCAN. *Gut* 2023;72:338-44.
- Zhang Y, Huo M, Li W, Zhang H, Liu Q, Jiang J, et al. Exosomes in tumor-stroma crosstalk: Shaping the immune microenvironment in colorectal cancer. *FASEB J* 2024;38:e23548.
- Yue M, Hu S, Sun H, Tuo B, Jia B, Chen C, et al. Extracellular vesicles remodel tumor environment for cancer immunotherapy. *Mol Cancer* 2023;22:203.
- Auger C, Christou N, Brunel A, Perraud A, Verdier M. Autophagy and extracellular vesicles in colorectal cancer: Interactions and common actors? *Cancers (Basel)* 2021;13:1039.
- Mannavola F, Salerno T, Passarelli A, Tucci M, Internò V, Silvestris F. Revisiting the role of exosomes in colorectal cancer: Where are we now? *Front Oncol* 2019;9:521.
- Yimin E, Lu C, Zhu K, Li W, Sun J, Ji P, et al. Function and mechanism of exosomes derived from different cells as communication mediators in colorectal cancer metastasis. *iScience* 2024;27:109350.
- Umwali Y, Yue CB, Gabriel AN, Zhang Y, Zhang X. Roles of exosomes in diagnosis and treatment of colorectal cancer. *World J Clin Cases* 2021;9:4467-79.
- Farzam OR, Eslami S, Jafarizadeh A, Alamdari SG, Dabbaghpour R, Nobari SA, et al. The significance of exosomal non-coding RNAs (ncRNAs) in the metastasis of colorectal cancer and development of therapy resistance. *Gene* 2025;937:149141.
- Fang Z, Rajewsky N. The impact of miRNA target sites in coding sequences and in 3'UTRs. *PLoS One* 2011;6:e18067.
- Sin C, Chiarugi D, Valleriani A. Single-molecule modeling of mRNA degradation by miRNA: Lessons from data. *BMC Syst Biol* 2015; 9(Suppl 3): S2.
- Yang M, Sun M, Zhang H. The interaction between epigenetic changes, EMT, and exosomes in predicting metastasis of colorectal cancers (CRC). *Front Oncol* 2022;12:879848.
- Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, et al. Circulating exosomal microRNAs as biomarkers of colon cancer. *PLoS One* 2014;9:e92921.
- Fu F, Jiang W, Zhou L, Chen Z. Circulating exosomal miR-17-5p and miR-92a-3p predict pathologic stage and grade of colorectal cancer. *Transl Oncol* 2018;11:221-32.
- Sun F, Liang W, Qian J. The identification of CRNDE, H19, UCA1 and HOTAIR as the key lncRNAs involved in oxaliplatin or irinotecan resistance in the chemotherapy of colorectal cancer based on integrative bioinformatics analysis. *Mol Med Rep* 2019;20:3583-96.
- Ren J, Ding L, Zhang D, Shi G, Xu Q, Shen S, et al. Carcinoma-associated fibroblasts promote the stemness and chemoresistance of colorectal cancer by transferring exosomal lncRNA H19. *Theranostics* 2018;8:3932-48.
- Sun Z, Li X, Shi Y, Yao Y. LncRNA PVT1 facilitates the growth and metastasis of colorectal cancer by sponging with miR-3619-5p to regulate TRIM29 expression. *Cancer Rep (Hoboken)* 2024;7:e2085.
- Xu R, Greening DW, Zhu HJ, Takahashi N, Simpson RJ. Extracellular vesicle isolation and characterization: Toward clinical application. *J Clin Invest* 2016;126:1152-62.
- Abramowicz A, Widlak P, Pietrowska M. Different types of cellular stress affect the proteome composition of small extracellular vesicles: A mini review. *Proteomes* 2019;7:23.
- Dong W, Wu D, Xu S, Sun Q, Ci X. Construction of a miRNA-mRNA network related to exosomes in colon cancer. *Dis Markers* 2022;2022:2192001.
- Ma J, Wang P, Huang L, Qiao J, Li J. Bioinformatic analysis reveals an exosomal miRNA-mRNA network in colorectal cancer. *BMC Med Genomics* 2021;14:60.

22. Russo F, Fiscono G, Conte F, Rizzo M, Paci P, Pellegrini M. Interplay between long noncoding RNAs and MicroRNAs in cancer. *Methods Mol Biol* 2018;1819:75-92.
23. Reif S, Elbaum Shiff Y, Golan-Gerstl R. Milk-derived exosomes (MDEs) have a different biological effect on normal fetal colon epithelial cells compared to colon tumor cells in a miRNA-dependent manner. *J Transl Med* 2019;17:325.
24. Gao T, Shu J, Cui J. A systematic approach to RNA-associated motif discovery. *BMC Genomics* 2018;19:146.
25. Noh GT, Kwon J, Kim J, Park M, Choi DW, Cho KA, et al. Verification of the role of exosomal microRNA in colorectal tumorigenesis using human colorectal cancer cell lines. *PLoS One* 2020;15:e0242057.
26. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in development and disease: Background, mechanisms, and therapeutic approaches. *Physiol Rev* 2016;96:1297-325.
27. Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. *Cancer Cell* 2016;29:452-63.
28. Shaath H, Vishnubalaji R, Elango R, Kardousha A, Islam Z, Qureshi R, et al. Long non-coding RNA and RNA-binding protein interactions in cancer: Experimental and machine learning approaches. *Semin Cancer Biol* 2022;86:325-45.
29. Cai K, Yang Y, Guo ZJ, Cai RL, Hashida H, Li HX. Amentoflavone inhibits colorectal cancer epithelial-mesenchymal transition via the miR-16-5p/HMGA2/ $\beta$ -catenin pathway. *Ann Transl Med* 2022;10:1009.
30. Zhao YJ, Song X, Niu L, Tang Y, Song X, Xie L. Circulating exosomal miR-150-5p and miR-99b-5p as diagnostic biomarkers for colorectal cancer. *Front Oncol* 2019;9:1129.
31. Ling H, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, et al. CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. *Genome Res* 2013;23:1446-61.
32. Zhang Z, Zhou C, Chang Y, Zhang Z, Hu Y, Zhang F, et al. Long non-coding RNA CASC11 interacts with hnRNP-K and activates the WNT/ $\beta$ -catenin pathway to promote growth and metastasis in colorectal cancer. *Cancer Lett* 2016;376:62-73.
33. Zhang X, Xiong Y, Tang F, Bian Y, Chen Y, Zhang F. Long noncoding RNA HNF1A-AS1 indicates a poor prognosis of colorectal cancer and promotes carcinogenesis via activation of the Wnt/ $\beta$ -catenin signaling pathway. *Biomed Pharmacother* 2017;96:877-83.
34. Wu F, Yang J, Shang G, Zhang Z, Niu S, Liu Y, et al. Exosomal miR-224-5p from colorectal cancer cells promotes malignant transformation of human normal colon epithelial cells by promoting cell proliferation through downregulation of CMTM4. *Oxid Med Cell Longev* 2022;2022:5983629.
35. Yang LM, Zheng Q, Liu XJ, Li XX, Veronica L, Chen Q, et al. Exosome-transmitted miR-224-5p promotes colorectal cancer cell proliferation via targeting ULK2 in p53-dependent manner. *Biomed Environ Sci* 2024;37:71-84.
36. Angius A, Pira G, Scanu AM, Uva P, Sotgiu G, Sadari L, et al. MicroRNA-425-5p expression affects BRAF/RAS/MAPK pathways in colorectal cancers. *Int J Med Sci* 2019;16:1480-91.
37. Sun LH, Tian D, Yang ZC, Li JL. Exosomal miR-21 promotes proliferation, invasion and therapy resistance of colon adenocarcinoma cells through its target PDCD4. *Sci Rep* 2020;10:8271.
38. Zhang G, Zhou H, Xiao H, Liu Z, Tian H, Zhou T. MicroRNA-92a functions as an oncogene in colorectal cancer by targeting PTEN. *Dig Dis Sci* 2014;59:98-107.
39. Shao Q, Xu J, Deng R, Wei W, Zhou B, Yue C, et al. Long non-coding RNA-422 acts as a tumor suppressor in colorectal cancer. *Biochem Biophys Res Commun* 2018;495:539-45.
40. Song W, Mei JZ, Zhang M. Long noncoding RNA PlncRNA-1 promotes colorectal cancer cell progression by regulating the PI3K/Akt signaling pathway. *Oncol Res* 2018;26:261-8.
41. Guo X, Liang X, Wang Y, Cheng A, Qin C, Zhang H, et al. Construction and comprehensive prognostic analysis of a lncRNA-miRNA-mRNA regulatory network and tumor immune cell infiltration in colorectal cancer. *Front Genet* 2021;12:652601.
42. Xie Z, Xia J, Jiao M, Zhao P, Wang Z, Lin S, et al. Exosomal lncRNA HOTAIR induces PDL1(+) B cells to impede anti-tumor immunity in colorectal cancer. *Biochem Biophys Res Commun* 2023;644:112-21.
43. Hui B, Lu C, Wang J, Xu Y, Yang Y, Ji H, et al. Engineered exosomes for co-delivery of PGM5-AS1 and oxaliplatin to reverse drug resistance in colon cancer. *J Cell Physiol* 2022;237:911-33.
44. Lai SW, Chen MY, Bamodu OA, Hsieh MS, Huang TY, Yeh CT, et al. Exosomal lncRNA PVT1/VEGFA axis promotes colon cancer metastasis and stemness by downregulation of tumor suppressor miR-152-3p. *Oxid Med Cell Longev* 2021;2021:9959807.
45. Zhi H, Lian J. LncRNA BDNF-AS suppresses colorectal cancer cell proliferation and migration by epigenetically repressing GSK-3 $\beta$  expression. *Cell Biochem Funct* 2019;37:340-7.
46. Li F, Zhang M, Yin X, Zhang W, Li H, Gao C. Exosomes-derived miR-548am-5p promotes colorectal cancer progression. *Cell Mol Biol (Noisy-le-grand)* 2023;69:104-10.
47. Xu Y, Shen L, Li F, Yang J, Wan X, Ouyang M. microRNA-16-5p-containing exosomes derived from bone marrow-derived mesenchymal stem cells inhibit proliferation, migration, and invasion, while promoting apoptosis of colorectal cancer cells by downregulating ITGA2. *J Cell Physiol* 2019;234:21380-94.
48. Luan Y, Li X, Luan Y, Zhao R, Li Y, Liu L, et al. Circulating lncRNA UCA1 promotes malignancy of colorectal cancer via the miR-143/MYO6 axis. *Mol Ther Nucleic Acids* 2020;19:790-803.
49. Chen DL, Chen LZ, Lu YX, Zhang DS, Zeng ZL, Pan ZZ, et al. Long noncoding RNA XIST expedites metastasis and modulates epithelial-mesenchymal transition in colorectal cancer. *Cell Death Dis* 2017;8:e3011.
50. Wang D, Wang X, Si M, Yang J, Sun S, Wu H, et al. Exosome-encapsulated miRNAs contribute to CXCL12/CXCR4-induced liver metastasis of colorectal cancer by enhancing M2 polarization of macrophages. *Cancer Lett* 2020;474:36-52.
51. Xu J, Xiao Y, Liu B, Pan S, Liu Q, Shan Y, et al. Exosomal MALAT1 sponges miR-26a/26b to promote the invasion and metastasis of colorectal cancer via FUT4 enhanced fucosylation and PI3K/Akt pathway. *J Exp Clin Cancer Res* 2020;39:54.
52. Zhang Y, Wang S, Lai Q, Fang Y, Wu C, Liu Y, et al. Cancer-associated fibroblasts-derived exosomal miR-17-5p promotes colorectal cancer aggressive phenotype by initiating a RUNX3/MYC/TGF- $\beta$ 1 positive feedback loop. *Cancer Lett* 2020;491:22-35.
53. Wang D, Wang X, Song Y, Si M, Sun Y, Liu X, et al. Exosomal miR-146a-5p and miR-155-5p promote CXCL12/CXCR7-induced metastasis of colorectal cancer by crosstalk with cancer-associated fibroblasts. *Cell Death Dis* 2022;13:380.
54. Liang ZX, Liu HS, Wang FW, Xiong L, Zhou C, Hu T, et al. LncRNA RPPH 1 promotes colorectal cancer metastasis by interacting with TUBB3 and by promoting exosomes-mediated macrophage M2 polarization. *Cell Death Dis* 2019;10:829.
55. Xu J, Zhao J, Zhang R. Prognostic significance of serum miR-193b in colorectal cancer. *Int J Clin Exp Pathol* 2017;10:9509-14.
56. Rezaei R, Baghaei K, Amani D, Piccin A, Hashemi SM, Asadzadeh Aghdaei H, et al. Exosome-mediated delivery of functionally active miRNA-375-3p mimic regulate epithelial mesenchymal transition (EMT) of colon cancer cells. *Life Sci* 2021;269:119035.
57. Li J, Peng W, Yang P, Chen R, Gu Q, Qian W, et al. MicroRNA-1224-5p inhibits metastasis and epithelial-mesenchymal transition in colorectal cancer by targeting SP1-mediated NF- $\kappa$ B signaling pathways. *Front Oncol* 2020;10:294.

58. Azwar S, Ng CT, Zahari Sham SY, Seow HF, Chai M, Ghazali MF, et al. Possible involvement of long non-coding RNAs GNAS-AS1 and MIR205HG in the modulation of 5-fluorouracil chemosensitivity in colon cancer cells through increased extracellular release of exosomes. *Noncoding RNA* 2024;10:25.
59. Sun X, Lin F, Sun W, Zhu W, Fang D, Luo L, et al. Exosome-transmitted miRNA-335-5p promotes colorectal cancer invasion and metastasis by facilitating EMT via targeting RASA1. *Mol Ther Nucleic Acids* 2021;24:164-74.
60. Ghofrani-Shahpar M, Pakravan K, Razmara E, Amooie F, Mahmoudian M, Heshmati M, et al. Cancer-associated fibroblasts drive colorectal cancer cell progression through exosomal miR-20a-5p-mediated targeting of PTEN and stimulating interleukin-6 production. *BMC Cancer* 2024;24:400.
61. Escalante PI, Quiñones LA, Contreras HR. Exploring the impact of MiR-92a-3p on FOLFOX chemoresistance biomarker genes in colon cancer cell lines. *Front Pharmacol* 2024;15:1376638.
62. Hu JL, Wang W, Lan XL, Zeng ZC, Liang YS, Yan YR, et al. CAFs secreted exosomes promote metastasis and chemotherapy resistance by enhancing cell stemness and epithelial-mesenchymal transition in colorectal cancer. *Mol Cancer* 2019;18:91.
63. Zhang SH, Zhang SG, Zhou P, Wei X, Mao XD, Lin SG, et al. LncRNA MALAT1 affects high glucose-induced endothelial cell proliferation, apoptosis, migration and angiogenesis by regulating the PI3K/Akt signaling pathway. *Eur Rev Med Pharmacol Sci* 2019;23:8551-9.
64. Lampropoulou DI, Pliakou E, Aravantinos G, Filippou D, Gazouli M. The role of exosomal non-coding RNAs in colorectal cancer drug resistance. *Int J Mol Sci* 2022;23:1473.
65. Wang M, Han D, Yuan Z, Hu H, Zhao Z, Yang R, et al. Long non-coding RNA H19 confers 5-Fu resistance in colorectal cancer by promoting SIRT1-mediated autophagy. *Cell Death Dis* 2018;9:1149.
66. Jin G, Liu Y, Zhang J, Bian Z, Yao S, Fei B, et al. A panel of serum exosomal microRNAs as predictive markers for chemoresistance in advanced colorectal cancer. *Cancer Chemother Pharmacol* 2019;84:315-25.
67. Azidoost S, Ghaedrahmati F, Anbiyae O, Ahmad Ali R, Cheraghzadeh M, Farzaneh M. Emerging roles for lncRNA-NEAT1 in colorectal cancer. *Cancer Cell Int* 2022;22:209.
68. Pranzini E, Leo A, Rapizzi E, Ramazzotti M, Magherini F, Giovannelli L, et al. miR-210-3p mediates metabolic adaptation and sustains DNA damage repair of resistant colon cancer cells to treatment with 5-fluorouracil. *Mol Carcinog* 2019;58:2181-92.
69. Deng X, Ruan H, Zhang X, Xu X, Zhu Y, Peng H, et al. Long noncoding RNA CCAL transferred from fibroblasts by exosomes promotes chemoresistance of colorectal cancer cells. *Int J Cancer* 2020;146:1700-16.
70. Ghafouri-Fard S, Dashti S, Taheri M. The HOTTIP (HOXA transcript at the distal tip) lncRNA: Review of oncogenic roles in human. *Biomed Pharmacother* 2020;127:110158.
71. Roman-Canal B, Tarragona J, Moiola CP, Gatiús S, Bonnin S, Ruiz-Miró M, et al. EV-associated miRNAs from peritoneal lavage as potential diagnostic biomarkers in colorectal cancer. *J Transl Med* 2019;17:208.
72. Maminezhad H, Ghanadian S, Pakravan K, Razmara E, Rouhollah F, Mossahebi-Mohammadi M, et al. A panel of six-circulating miRNA signature in serum and its potential diagnostic value in colorectal cancer. *Life Sci* 2020;258:118226.
73. Wang L, Duan W, Yan S, Xie Y, Wang C. Circulating long non-coding RNA colon cancer-associated transcript 2 protected by exosome as a potential biomarker for colorectal cancer. *Biomed Pharmacother* 2019;113:108758.
74. Miyazaki K, Wada Y, Okuno K, Murano T, Morine Y, Ikemoto T, et al. An exosome-based liquid biopsy signature for pre-operative identification of lymph node metastasis in patients with pathological high-risk T1 colorectal cancer. *Mol Cancer* 2023;22:2.
75. Liu C, Eng C, Shen J, Lu Y, Takata Y, Mehdizadeh A, et al. Serum exosomal miR-4772-3p is a predictor of tumor recurrence in stage II and III colon cancer. *Oncotarget* 2016;7:76250-60.
76. Schee K, Boye K, Abrahamsen TW, Fodstad Ø, Flatmark K. Clinical relevance of microRNA miR-21, miR-31, miR-92a, miR-101, miR-106a and miR-145 in colorectal cancer. *BMC Cancer* 2012;12:505.
77. Tsukamoto M, Iinuma H, Yagi T, Matsuda K, Hashiguchi Y. Circulating exosomal MicroRNA-21 as a biomarker in each tumor stage of colorectal cancer. *Oncology* 2017;92:360-70.
78. Study Details | Contents of Circulating Extracellular Vesicles: Biomarkers in Colorectal Cancer Patients | ClinicalTrials.Gov. Available from: <https://clinicaltrials.gov/study/NCT04523389>. [Last accessed on 2025 Apr 06].
79. Study Details | Exosome-Based Detection of Molecular Residual Disease in Stage II-III Colorectal Cancer | ClinicalTrials.Gov. Available from: <https://clinicaltrials.gov/study/NCT06654622>. [Last accessed on 2025 Apr 06].
80. Study Details | Early Detection of Advanced Adenomas and Colorectal Cancer (AACRC)|ClinicalTrials.Gov. Available from: <https://clinicaltrials.gov/study/NCT06342440>. [Last accessed on 2025 Apr 06].
81. Study Details | Timisnar – Biomarkers Substudy (Timisnar-mirma) (TiMiSNAR-miRNA)|ClinicalTrials.Gov. Available from: <https://clinicaltrials.gov/study/NCT03962088>. [Last accessed on 2025 Apr 06].
82. Study Details | A Study of exoASO-STAT6 (CDK-004) in Patients With Advanced Hepatocellular Carcinoma (HCC) and Patients With Liver Metastases From Either Primary Gastric Cancer or Colorectal Cancer (CRC)|ClinicalTrials.Gov. Available from: <https://clinicaltrials.gov/study/NCT05375604>. [Last accessed on 2025 Apr 06].
83. Shi YJ, Fang YX, Tian TG, Chen WP, Sun Q, Guo FQ, et al. Discovery of extracellular vesicle-delivered miR-185-5p in the plasma of patients as an indicator for advanced adenoma and colorectal cancer. *J Transl Med* 2023;21:421.
84. Hedayat S, Cascione L, Cunningham D, Schirripa M, Lampis A, Hahne JC, et al. Circulating microRNA analysis in a prospective co-clinical trial identifies MIR652-3p as a response biomarker and driver of regorafenib resistance mechanisms in colorectal cancer. *Clin Cancer Res* 2024;30:2140-59.
85. Mosquera-Heredia MI, Morales LC, Vidal OM, Barceló E, Silvera-Redondo C, Vélez JI, et al. Exosomes: Potential disease biomarkers and new therapeutic targets. *Biomedicines* 2021;9:1061.
86. Ding T, Cui P, Zhou Y, Chen C, Zhao J, Wang H, et al. Antisense oligonucleotides against miR-21 inhibit the growth and metastasis of colorectal carcinoma via the DUSP8 pathway. *Mol Ther Nucleic Acids* 2018;13:244-55.
87. Yu S, Liao R, Bai L, Guo M, Zhang Y, Zhang Y, et al. Anticancer effect of hUC-MSC-derived exosome-mediated delivery of PMO-miR-146b-5p in colorectal cancer. *Drug Deliv Transl Res* 2024;14:1352-69.
88. Ning T, Li J, He Y, Zhang H, Wang X, Deng T, et al. Exosomal miR-208b related with oxaliplatin resistance promotes Treg expansion in colorectal cancer. *Mol Ther* 2021;29:2723-36.
89. Shakerian N, Darzi-Eslam E, Afsharoori F, Bana N, Noorabad Ghahroodi F, Tarin M, et al. Therapeutic and diagnostic applications of exosomes in colorectal cancer. *Med Oncol* 2024;41:203.
90. Hui B, Zhou C, Xu Y, Wang R, Dong Y, Zhou Y, et al. Exosomes secreted by *Fusobacterium nucleatum*-infected colon cancer cells transmit resistance to oxaliplatin and 5-FU by delivering hsa\_circ\_0004085. *J Nanobiotechnology* 2024;22:62.
91. Geng Y, Xia W, Zheng X, Chen L, Zhou Y, Feng J, et al. Targeted delivery of FAK siRNA by engineered exosomes to reverse cetuximab resistance via activating paraptosis in colon cancer. *Apoptosis* 2024;29:1959-77.

92. Zhang Y, Lan M, Chen Y. Minimal Information for Studies of Extracellular Vesicles (MISEV): Ten-year evolution (2014-2023). *Pharmaceutics* 2024;16:1394.
93. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez J, Martín-Cofreces N, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat Commun* 2013;4:2980.
94. Shurtleff MJ, Temoche-Diaz MM, Karfilis KV, Ri S, Schekman R. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. *Elife* 2016;5:e19276.
95. Suresh PS, Tsutsumi R, Venkatesh T. YBX1 at the crossroads of non-coding transcriptome, exosomal, and cytoplasmic granular signaling. *Eur J Cell Biol* 2018;97:163-7.
96. McKenzie AJ, Hoshino D, Hong NH, Cha DJ, Franklin JL, Coffey RJ, et al. KRAS-MEK signaling controls Ago2 sorting into exosomes. *Cell Rep* 2016;15:978-87.
97. Shi J. Considering exosomal miR-21 as a biomarker for cancer. *J Clin Med* 2016;5:42.
98. Zhang J, Xiao Z, Lai D, Sun J, He C, Chu Z, et al. miR-21, miR-17 and miR-19a induced by phosphatase of regenerating liver-3 promote the proliferation and metastasis of colon cancer. *Br J Cancer* 2012;107:352-9.
99. Lin D, Zhang H, Liu R, Deng T, Ning T, Bai M, et al. iRGD-modified exosomes effectively deliver CPT1A siRNA to colon cancer cells, reversing oxaliplatin resistance by regulating fatty acid oxidation. *Mol Oncol* 2021;15:3430-46.