

miR-324-5p in oncology: Molecular mechanisms and clinical outlook (Review)

KUN ZHANG^{1,2*}, ZHEN XU^{2,3*}, YAN BIN LUO², CHAO QI LIU² and YONG QIN ZHOU²

¹The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang, Hubei 443002, P.R. China; ²Hubei Key Laboratory of Tumor Microenvironment and Immunotherapy, College of Basic Medical Science, China Three Gorges University, Yichang, Hubei 443002, P.R. China; ³Department of Obstetrics and Gynecology, Affiliated Renhe Hospital of China Three Gorges University, Yichang, Hubei 443002, P.R. China

Received May 5, 2026; Accepted September 10, 2026

DOI: 10.3892/or.2026.9209

Abstract. MicroRNAs (miRNAs or miRs) represent a class of regulatory non-coding RNAs that control post-transcriptional gene expression and influence multiple cancer-associated biological processes. Among these molecules, miR-324-5p exhibits cancer type-dependent functions, displaying tumor-suppressive activity in certain types of malignancy while promoting oncogenic phenotypes in others. The molecular basis responsible for these opposing effects remains to be fully clarified. The present review summarizes evidence and integrates pan-cancer analyses from The Cancer Genome Atlas to characterize the molecular mechanisms of miR-324-5p across different types of malignancies, with emphasis on experimental findings from animal models investigating tumor growth and metastasis. The clinical relevance of miR-324-5p, including its diagnostic, prognostic and therapeutic implications, is also discussed, together with challenges limiting clinical translation, particularly tumor-specific delivery strategies and *in vivo* stability. Further elucidation of the molecular contexts governing miR-324-5p activity may facilitate its application in precision oncology.

Contents

1. Introduction
2. Cancer associated with miR-324-5p
3. miR-324-5p as a potential cancer biomarker

4. Potential of miR-324-5p as a therapeutic target
5. Challenges and prospects

1. Introduction

Cancer development results from interactions among genetic abnormalities, epigenetic changes and alterations in cellular regulatory networks, which collectively shape tumor initiation and progression (1). Aberrations in genomic non-coding regions can influence transcriptional control and participate in malignant transformation and tumor evolution (2). Among non-coding RNAs (ncRNAs), microRNAs (miRNAs or miRs) constitute key post-transcriptional regulators that have attracted considerable research interest due to their involvement in physiological regulation and pathological processes (3,4). Typically consisting of 17-25 nucleotides, miRNAs regulate gene expression through sequence-dependent interactions with target mRNAs, typically involving binding to the 3'-untranslated regions (UTRs) and altering downstream protein production. These interactions result in suppression of protein translation or degradation of target transcripts, affecting diverse cellular functions (4). In cancers, miRNAs may display tumor-suppressive or -promoting activities by modulating specific molecular targets and signaling networks, thereby influencing tumorigenesis, tumor growth, metastasis and drug resistance (5-7). The emerging diagnostic, prognostic and therapeutic potential of miRNAs has generated increasing interest in their clinical translation in oncology (7,8).

The MIR324 gene is located on chromosome 17p13.1 (9), and miR-324-5p is processed through the canonical miRNA biogenesis pathway. The primary miRNA transcript undergoes sequential cleavage by Drosha and Dicer to generate mature miR-324-5p (10). miR-324-5p exhibits variable expression across malignancies, with both upregulation and downregulation (11-13). Specifically, miR-324-5p expression is upregulated in several types of cancer, including gastric cancer (GC) (14,15), lung adenocarcinoma (LUAD) (16,17) and papillary thyroid carcinoma (PTC) (18,19). Conversely, downregulation of miR-324-5p has been observed in colorectal cancer (CRC) (20), liver cancer (21,22), glioblastoma (GBM) (23,24) and melanoma (12). Furthermore, miR-324-5p

Correspondence to: Professor Yong Qin Zhou, Hubei Key Laboratory of Tumor Microenvironment and Immunotherapy, College of Basic Medical Science, China Three Gorges University, 8 Da Xue Road, Yichang, Hubei 443002, P.R. China
E-mail: 13545855812@163.com

*Contributed equally

Key words: microRNA-324-5p, cancer, therapeutic target, biomarker, molecular mechanism

modulates tumorigenesis and cancer progression by influencing diverse molecular pathways, including JAK2/STAT3 signaling, rho-associated kinase 1 (ROCK1) and ETS proto-oncogene 1, transcription factor (ETS1)/Sp1 transcription factor (SP1)-mediated regulatory mechanisms (25-27). The tissue-specific and cancer-type-dependent expression patterns of miR-324-5p underscore its potential clinical value. Accumulating evidence has revealed the biological functions of miR-324-5p across cancer contexts (14,16,20,22,24). The present review summarizes the functional roles of miR-324-5p in cancer types, highlighting its regulatory mechanisms, biomarker potential and therapeutic implications in oncology.

2. Cancer associated with miR-324-5p

The Cancer Genome Atlas (TCGA) is a large-scale cancer research initiative that provides multidimensional molecular profiling datasets from diverse tumor cohorts, including genomic, transcriptomic, epigenomic and copy-number information (28). To characterize miR-324-5p expression patterns across distinct tumor entities, differential expression analysis was conducted using TCGA cancer cohorts (Supplementary Material 1; Table SI). Mature miRNA isoform expression data for hsa-miR-324-5p (miRBase accession no. MIMAT0000761) (29,30) were retrieved from the TCGA/National Cancer Institute Genomic Data Commons (GDC) repository using the TCGAbiolinks R package (version 2.34.1; Bioconductor; accessed on July 21, 2026) (31). hsa-miR-324-5p expression was normalized to counts per million mature miRNA reads (CPM) and transformed as $\log_2(\text{CPM} + 1)$. Tumor-normal expression differences were assessed using a two-sided unpaired Wilcoxon rank-sum test.

Significant increases in miR-324-5p expression were observed in TCGA tumor cohorts, including head and neck squamous cell carcinoma, LUAD, uterine corpus endometrial carcinoma, lung squamous cell carcinoma (LUSC) and bladder urothelial carcinoma (BLCA; Fig. 1; Table SI). Conversely, decreased miR-324-5p expression was observed in colorectal adenocarcinoma (COAD), rectum adenocarcinoma (READ), pancreatic adenocarcinoma (PAAD) and kidney chromophobe. By contrast, no significant tumor-normal difference was detected in skin cutaneous melanoma and kidney renal papillary cell carcinoma.

Biological functions of miR-324-5p across malignancies

Oncogenic effects of miR-324-5p upregulation in cancer

Lung cancer (LC). LC is a significant public health challenge, with ~2.6 million new cases and 1.9 million deaths worldwide in 2024, accounting for 12.8% of all cancer cases and 19.1% of cancer deaths, respectively (32). Clinical studies have demonstrated altered circulating miR-324-5p levels in patients with non-small cell lung cancer (NSCLC), including increased plasma levels in one cohort (33,34). F-box protein 11 (FBXO11), the mammalian ortholog of the *Caenorhabditis elegans* E-box protein DRE-1, is a member of the E3 ubiquitin ligase complex and is involved in multiple biological processes, including apoptosis, DNA replication and transcriptional regulation (35,36). Mechanistically, long ncRNA (lncRNA) GATA6-AS1 acts as a competing endogenous RNA (ceRNA) by sequestering miR-324-5p, thereby restoring FBXO11 expression and

suppressing LC cell proliferation and invasiveness (17). Conversely, elevated miR-324-5p expression negatively regulates the tumor-suppressive activity of FBXO11, leading to decreased cisplatin sensitivity and enhanced chemoresistance in LC cells (16). miR-324-5p may contribute to benzo[a]pyrene-7,8-diol-9,10-epoxide-induced malignant transformation of human bronchial epithelial cells via modulation of the circ_0067716-mediated miR-324-5p-DNA damage-regulated autophagy modulator 1 (DRAM1) axis (37). Additionally, analysis of TCGA sequencing data showed higher miR-324-5p expression in LUAD and LUSC than in normal tissue samples, while cell-based experiments showed that miR-324-5p upregulation promotes proliferation and invasion in A549 human lung adenocarcinoma cells (38). However, another cell-based study found that miR-324-5p upregulation decreases prostate cancer-associated transcript 1 (PCAT-1)-induced proliferation of A549 cells and promotes apoptosis, potentially through the PCAT-1/miR-324-5p/RAP1A (RAP1A member of RAS oncogene family) axis (39).

GC. GC ranks fifth worldwide in both incidence and mortality, with ~980,000 new cases and 642,000 deaths estimated globally in 2024 (32). Evidence indicates elevated miR-324-5p expression in both GC tissues and cell lines (15). Mechanistically, inhibition of miR-324-5p suppresses GC cell proliferation and invasive behavior while promoting apoptosis, potentially through relief of miR-324-5p-mediated suppression of PTEN, a downstream target negatively regulated by miR-324-5p (15). Moreover, circ_0049447 modulates GC progression through sequestration of miR-324-5p, potentially involving a protein tyrosine phosphatase receptor Δ (PTPRD)-related mechanism (40). miR-324-5p has been identified as a component of a plasma small extracellular vesicle (sEV)-miRNA signature associated with distant lymph node metastasis (41). However, another study found that miR-324-5p expression is lower in five paired clinical GC than in adjacent normal tissues (14). Mechanistically, inhibition of miR-324-5p reverses the antitumor effects induced by circ_0091994 knockdown (14).

Breast cancer (BC). In 2024, ~2.4 million new breast cancer cases and 694,000 BC-related deaths were estimated worldwide, accounting for 11.8% of all new cancer cases and 7.1% of all cancer deaths, respectively (32). miR-324-5p contributes to BC progression (42), with increased expression associated with unfavorable clinical outcomes (43). TCGA transcriptomic profiling combined with multi-omics analyses predicted miR-324-5p as a potential regulator of Friend leukemia virus integration 1 (FLI1) in breast invasive carcinoma (BRCA) (44). miR-324-5p expression shows an inverse association with FLI1 levels, while increased miR-324-5p abundance is associated with poorer overall survival (OS) (44). Furthermore, FLI1 was implicated in the regulation of the BRCA immune microenvironment in a cell-type-specific manner (44). In addition, the Multicase-Control Spain (MCC-Spain) project reported elevated circulating miR-324-5p levels in BC cases compared with controls (45). Mechanistically, miR-324-5p promotes BC progression by suppressing glutathione peroxidase 3, thereby enhancing cellular proliferation, migration and invasiveness (46). Additionally, miR-324-5p regulates BC cell motility and invasiveness

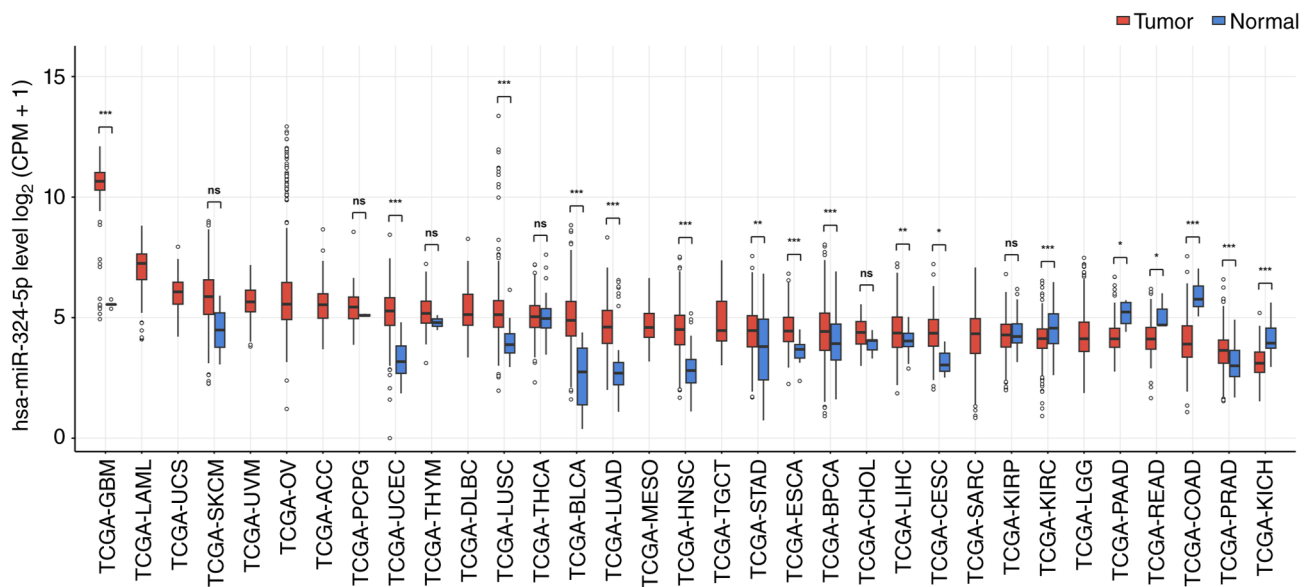


Figure 1. hsa-miR-324-5p expression between tumor and normal samples across TCGA cancer cohorts. hsa-miR-324-5p expression levels were normalized to CPM and transformed as $\log_2(\text{CPM}+1)$. Samples were compared using a two-sided Wilcoxon rank-sum test when both groups contained ≥ 2 samples. Statistical significance was based on unadjusted P-values. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. CPM, counts per million; ns, not significant; TCGA, The Cancer Genome Atlas; LAML, acute myeloid leukemia; UCS, uterine carcinosarcoma; SKCM, skin cutaneous melanoma; UVM, uveal melanoma; ACC, adrenocortical carcinoma; OV, ovarian serous cystadenocarcinoma; PCPG, pheochromocytoma and paraganglioma; UCEC, uterine corpus endometrial carcinoma; DLBC, lymphoid neoplasm diffuse large B cell lymphoma; THYM, thymoma; THCA, thyroid carcinoma; LUSC, lung squamous cell carcinoma; BLCA, bladder urothelial carcinoma; MESO, mesothelioma; TGCT, testicular germ cell tumors; LUAD, lung adenocarcinoma; HNSC, head and neck squamous cell carcinoma; ESCA, esophageal carcinoma; LIHC, liver hepatocellular carcinoma; CHOL, cholangiocarcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; STAD, stomach adenocarcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; SARC, sarcoma; READ, rectum adenocarcinoma; PAAD, pancreatic adenocarcinoma; KIRC, kidney renal clear cell carcinoma; COAD, colon adenocarcinoma; PRAD, prostate adenocarcinoma; KICH, kidney chromophobe; GBM, glioblastoma multiforme.

through mechanisms involving NF- κ B signaling, a pathway involved in epithelial-mesenchymal transition (EMT) and metastatic dissemination (47). miR-324-5p downregulation results in increased CUE domain-containing 2 (CUEDC2) expression, which inhibits I κ B kinase (IKK) phosphorylation and decreases NF- κ B activity, ultimately limiting BC cell invasion and migration (47). These findings demonstrate the intricate regulatory mechanisms by which miR-324-5p influences BC progression.

PTC. PTC is the predominant histological subtype of thyroid cancer. In 2024, ~959,000 new thyroid cancer cases and 48,000 deaths were estimated worldwide (32). Increased miR-324-5p expression in PTC tissue is associated with unfavorable clinicopathological characteristics, including lymph node metastasis (LNM), greater tumor size and advanced clinical stages (19). Molecular studies indicate that miR-324-5p promotes PTC cell proliferation, migration and invasion (18,19). Mechanistically, miR-324-5p directly targets PTPRD and suppresses its protein expression, leading to CCAAT enhancer-binding protein δ (CEBPD) upregulation (18). Elevated CEBPD expression enhances the migratory and invasive capacities of human umbilical vein endothelial cells within the tumor microenvironment and promotes macrophage polarization toward an M2-like phenotype (18). Additionally, enhanced CEBPD levels reinforce M2 macrophage polarization by increasing IL-4 and IL-13 production (18), thereby contributing to PTC progression. Collectively, these observations highlight the contribution of miR-324-5p to PTC development and progression.

Pancreatic cancer (PC). PC is characterized by an unfavorable prognosis. In 2024, ~531,000 new cases and 491,000 deaths from PC were estimated worldwide (32), reflecting its high mortality burden. miRNAs participate in PC development by regulating molecular targets involved in tumor proliferation and apoptosis, including KLF3 (48,49). Evidence has implicated miR-324-5p in PC progression (48,49). Increased miR-324-5p expression is detected in PC cell lines and patient-derived tumor samples (49). Inhibition of miR-324-5p suppresses PC cell proliferation and induces apoptosis, whereas restoration of miR-324-5p expression enhances proliferative activity and attenuates apoptotic responses (49). Mechanistically, Krüppel-like factor 3 (KLF3) is a direct downstream target of miR-324-5p. By suppressing KLF3 expression, miR-324-5p promotes PC cell proliferation and decreases apoptosis (49). Collectively, these data demonstrate that miR-324-5p contributes to PC progression through regulation of the KLF3-mediated molecular pathway. In addition, a recent study implicates miR-324-5p in trace element metabolism (50,51). In pancreatic ductal adenocarcinoma (PDAC) specimens, miR-324-5p abundance is negatively associated with selenium, manganese and zinc levels, suggesting a potential role for miR-324-5p in trace element homeostasis (50).

Bladder cancer. Bladder cancer is a common malignant neoplasm of the urinary system. In 2024, ~635,000 new bladder cancer cases and 228,000 deaths were estimated worldwide (32). Elevated urinary miR-324-5p levels have been detected in patients with bladder cancer (52), while increased miR-324-5p abundance has also been observed in surgically resected bladder cancer tissues compared with matched

adjacent tumor tissues (53). Mechanistic investigations have implicated miR-324-5p in bladder cancer progression through regulatory associations involving regulator of calcineurin 1 (RCAN1), FosB proto-oncogene, AP-1 transcription factor subunit (FOSB) and eyes absent homolog 4 (EYA4) (52,53). In particular, suppression of RCAN1 and EYA4 expression was associated with enhanced bladder cancer cell proliferation, colony formation and invasive behavior (53). Taken together, these findings indicate that miR-324-5p may contribute to bladder cancer progression by promoting oncogenic processes.

Oncogenic effects of miR-324-5p downregulation in cancer

CRC. In 2024, >2 million new CRC cases and ~918,000 deaths were estimated worldwide, making CRC the third most commonly diagnosed cancer and the second leading cause of cancer death globally (32). TCGA analysis identified decreased miR-324-5p levels in COAD and READ samples compared with corresponding normal tissue. One study reported decreased miR-324-5p levels in CRC cells (20). Overexpression of miR-324-5p inhibited CRC cell proliferation, migration and invasion (20). Mechanistically, embryonic lethal abnormal vision Drosophila-like protein 1 (ELAVL1) was a direct target of miR-324-5p. Through regulation of invasion-associated effectors, including MMP9, urokinase-type plasminogen activator (uPA) and uPA receptor (uPAR), ELAVL1 influences CRC tumor growth and metastatic potential (20). Moreover, hsa_circ_0079662 serves as a ceRNA that sequesters miR-324-5p, preventing miRNA-mediated repression of homeobox A9 (HOXA9) and enhancing tumor cell proliferation and oxaliplatin resistance (54). Additionally, miR-324-5p directly regulates superoxide dismutase 2 (SOD2), resulting in decreased stemness characteristics of CRC cells and improved responsiveness to chemotherapy with folinic acid, oxaliplatin and fluorouracil (FOLFOX) (55). Supplementation with dietary n-3 polyunsaturated fatty acids experimentally elevates miR-324-5p levels, counteracting carcinogen-induced suppression and protecting against colorectal carcinogenesis (56). These effects may involve Dicer-mediated miRNA biogenesis. Reduced Dicer expression is associated with advanced CRC and poorer overall survival (57), whereas experimental Dicer deficiency disrupts miRNA maturation, promotes cytoskeletal remodeling and intestinal inflammation, and increases tumor formation (58). Decreased miR-324-5p levels are reported in intestinal epithelial cells from Dicer-deficient mice and in Dicer-deficient CRC cell lines (58). Mechanistically, miR-324-5p is a downstream component of the Dicer-mediated miRNA network, regulating high-mobility group box-containing protein 3 (HMGXB3) and WAS protein family member 2 (WASF-2). This regulatory pathway contributes to preservation of intestinal epithelial integrity, decreased inflammatory responses and inhibited tumor initiation (58). Furthermore, one study in COAD revealed that augmenting miR-324-5p levels or suppressing the lncRNA VPS9D1-AS1 limits tumor cell proliferation (59). VPS9D1-AS1 serves as a ceRNA by sequestering miR-324-5p, thereby decreasing miR-324-5p-mediated repression of integrin subunit $\alpha 2$ (ITGA2) and driving COAD progression (59). Kang *et al* (60) observed decreased miR-324-5p expression in F-box and WD repeat domain-containing 7 (FBXW7)-mutant rectal neuroendocrine tumors (NETs), where miR-324-5p was

among seven miRNAs identified through NanoString-based profiling of tumor samples. The altered expression pattern of miR-324-5p may reflect FBXW7-associated molecular changes and the biological heterogeneity of rectal NETs (60).

Hepatocellular carcinoma (HCC). In 2024, ~843,000 new liver cancer cases and 732,000 deaths were estimated worldwide (32). HCC represents the predominant histological subtype of primary liver cancer (61). Recent studies have highlighted miR-324-5p-associated regulatory networks involved in HCC progression, immune regulation and prognosis (62,63). Notably, miR-324-5p expression is reduced in hepatitis C virus (HCV)-infected HCC cells but is restored following IFN- α treatment, indicating its involvement in the molecular interplay between HCV infection and the response to IFN- α therapy (64). Similarly, HCC tissue and cell-based models exhibit diminished miR-324-5p expression (27). Conversely, increasing miR-324-5p expression inhibits the invasive capacity and metastatic properties of HCC cells (27). Mechanistically, miR-324-5p regulates ETS1 and SP1, resulting in the downregulation of MMP2 and MMP9, and decreased HCC cell invasion and migration (27). In addition to direct target regulation, miR-324-5p activity in HCC is modulated by multiple lncRNAs (27). For example, LINC00491 serves as a ceRNA that binds miR-324-5p, thereby increasing ROCK1 expression and promoting liver cancer progression (22). Similarly, lnc RP11-85G21.1, is abundantly expressed in HCC cells and cell-derived exosomes, where it promotes malignant phenotypes by modulating the functional availability of miR-324-5p (65). LINC01134 interacts with miR-324-5p and attenuates its suppressive effect on insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) (13). Through stabilization of Yin Yang-1 (YY1) transcripts, IGF2BP1 increases YY1 expression, which promotes LINC01134 transcription and creates a positive feedback mechanism contributing to HCC progression (13). Moreover, evidence suggests an association between C-type lectin domain family 4s (CLEC4s) member expression in HCC tissue and infiltration of immune cells (66). miR-324-5p, a potential regulator of CLEC4s, may modulate the HCC immune microenvironment by targeting CLEC4s expression (66). In HCC, exosomal circGSE1 interacts with miR-324-5p and modulates the transforming growth factor β receptor 1 (TGFBRI)/Smad3 pathway, resulting in enhanced regulatory T cell (Treg) expansion and immune evasion (21). In addition, (1E,4E)-1,5-bis(2-bromophenyl)penta-1,4-dien-3-one (GL63), a synthetic derivative of curcumin, displays antitumor activity, partially by modulating circZNF83, a ceRNA within the miR-324-5p regulatory network, thereby attenuating malignant phenotypes and promoting apoptosis in HCC cells (25). Collectively, these data elucidate the intricate molecular pathways involving miR-324-5p in HCC pathogenesis and therapy.

Cervical cancer (CC). In 2024, ~604,000 new CC cases and 280,000 deaths were estimated worldwide (32). Accumulating evidence implicates miR-324-5p in the regulation of CC progression and treatment response through diverse molecular pathways (11,67). Decreased expression of miR-324-5p is reported in CC tissue, indicating its potential involvement in tumor development (11). Mechanistically, LINC00511 attenuates miR-324-5p activity through competitive binding, resulting in DRAM1 dysregulation and altered proliferative

and migratory behavior of CC cells (11). Moreover, re-expression of miR-324-5p increases the radiosensitivity of CC cells by enhancing apoptosis and limiting migratory capacity (67). This radiosensitizing effect is mediated, at least partially, by directly targeting ELAVL1, followed by modulation of apoptosis-associated proteins, including Bcl-2 and Bax (67). Therefore, miR-324-5p may be a critical regulator of CC pathogenesis and treatment-associated responses.

Ovarian cancer (OC). In 2024, ~331,000 new ovarian cancer cases and 204,000 deaths were estimated worldwide (32). Dysregulated miRNA profiles contribute to OC progression and therapeutic response (68). Survival analyses of serous OC cohorts reveal that patients with lower miR-324-5p levels exhibit poorer OS (69,70) and, in one cohort, with shorter progression-free intervals (69). Notably, although miR-324-5p downregulation is associated with olaparib resistance, inhibition of the ataxia telangiectasia and Rad3-related/checkpoint kinase 1 (ATR/CHK1) pathway suppresses miR-324-5p expression and enhances olaparib sensitivity in BRCA2-mutant olaparib-resistant ovarian cancer cells (PEO1-OR) (69). Computational network analysis has identified G2/M checkpoint-associated genes, including tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein 0, as potential downstream effectors of miR-324-5p, suggesting miR-324-5p may influence cell cycle regulation during the development of olaparib resistance (69). Furthermore, circZNF609 promotes OC progression by sequestering miR-324-5p and relieving miR-324-5p-mediated repression of voltage-dependent anion channel 1 expression (71). Conversely, enforced miR-324-5p expression impairs OC cell proliferative and invasive capacity and decreases xenograft tumor growth, highlighting its tumor-suppressive function in OC (71).

Multiple myeloma (MM). MM is a hematological malignancy driven by the uncontrolled expansion of clonal plasma cells in the bone marrow microenvironment, which is associated with clinical complications, including renal dysfunction, hypercalcemia, anemia and osteolytic bone lesions (72). Increased miR-324-5p expression may attenuate MM progression by suppressing cell proliferation, viability and cancer stem-like characteristics (73,74). miR-324-5p enhances the therapeutic response to bortezomib (BTZ) by regulating multidrug resistance-associated proteins and apoptosis-associated members of the Bcl-2 family (73). At the molecular level, circATIC participates in a ceRNA regulatory network by sequestering miR-324-5p, thereby relieving miR-324-5p-mediated suppression of hepatocyte growth factor (HGF) expression. This regulatory pathway enhances proliferation, migration, invasion and glycolytic reprogramming while attenuating apoptosis in MM cells (74). Collectively, these results identify miR-324-5p as a key target downstream of circATIC and regulator of MM biology.

GBM. GBM is a highly aggressive primary brain malignancy (75). Despite multimodal treatment, the median overall survival is ~15 months, and the 5-year survival rate remains ~5.8% (76). In GBM tissue, decreased miR-324-5p abundance is observed with upregulation of circ_0079593 and X-box binding protein 1 (XBP1) (77). Mechanistically, miR-324-5p directly binds XBP1 and suppresses its expression, thereby limiting GBM cell proliferation (77). By contrast,

circ_0079593 promotes GBM progression by competitive binding with miR-324-5p, thereby increasing XBP1 expression (77). Furthermore, lncRNA NEAT1 contributes to GBM progression through a ceRNA mechanism, in which it binds miR-324-5p and prevents miR-324-5p-mediated suppression of potassium channel tetramerization domain-containing 20 (KCTD20). Restoration of KCTD20 expression promotes GBM cell proliferation and attenuates apoptosis (78). circ-SERPINE2 contributes to GBM progression by competitively binding miR-324-5p and miR-361-3p, thereby relieving miRNA-mediated suppression of Bcl-2 and promoting malignant cell survival (79). Conversely, miR-324-5p exerts inhibitory effects on GBM by targeting enhancer of zeste homolog 2 (EZH2), thereby limiting tumor growth and increasing temozolomide (TMZ) responsiveness (23). Therefore, miR-324-5p appears to function as a negative regulator of GBM progression.

Hematological malignancy. Hematological malignancy comprise neoplastic disorders involving molecular alterations and exhibiting substantial variability in treatment response. A circulating miRNA study in diffuse large B cell lymphoma (DLBCL) have linked miR-324-5p to responsiveness to rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (80). Tumor miRNA profiling demonstrated an association between miR-324-5p expression and survival outcomes in patients with DLBCL (81). Circulating and tissue-derived miR-324-5p display divergent expression patterns in DLBCL, with elevated serum levels observed in refractory patients but decreased expression detected in tumors from non-responsive cases (80). Re-expression of miR-324-5p impairs DLBCL cell survival through thioredoxin reductase 1 regulation and ferroptosis induction, providing functional evidence for its context-dependent antitumor activity (80). Bioinformatics suggests a potential role for miR-324-5p in acute myeloid leukemia (AML) (82). A miRNA profiling study identified miR-324-5p among the miRNAs upregulated in primary AML samples compared with normal bone marrow (83). A bioinformatic analysis predicted a hsa_circ_0058058/miR-324-5p regulatory axis involving adaptor-associated protein complex 1 γ 1 subunit (APIG1) and SP1, suggesting this network may contribute to AML progression (82). Collectively, the aforementioned studies indicate that miR-324-5p has context-dependent roles in hematological malignancy with its biological effects varying across disease entities and regulatory networks. The expression patterns, regulatory mechanisms and biological functions of miR-324-5p across different cancer types are summarized in Table I.

miR-324-5p mediates tumor-associated pathways. The major tumor-associated signaling pathways involving miR-324-5p are summarized in Fig. 2.

TGF- β pathway. TGF- β signaling is extensively involved in tumor biology and influences multiple cell programs, including differentiation, cell motility, EMT and metastatic spread (84). In prostate cancer, lncRNA PCAT7 promotes TGFBR1 expression by competitively interacting with miR-324-5p, thereby increasing TGF- β pathway activity. This PCAT7/miR-324-5p/TGFBR1 regulatory pathway contributes to prostate cancer bone metastasis (85).

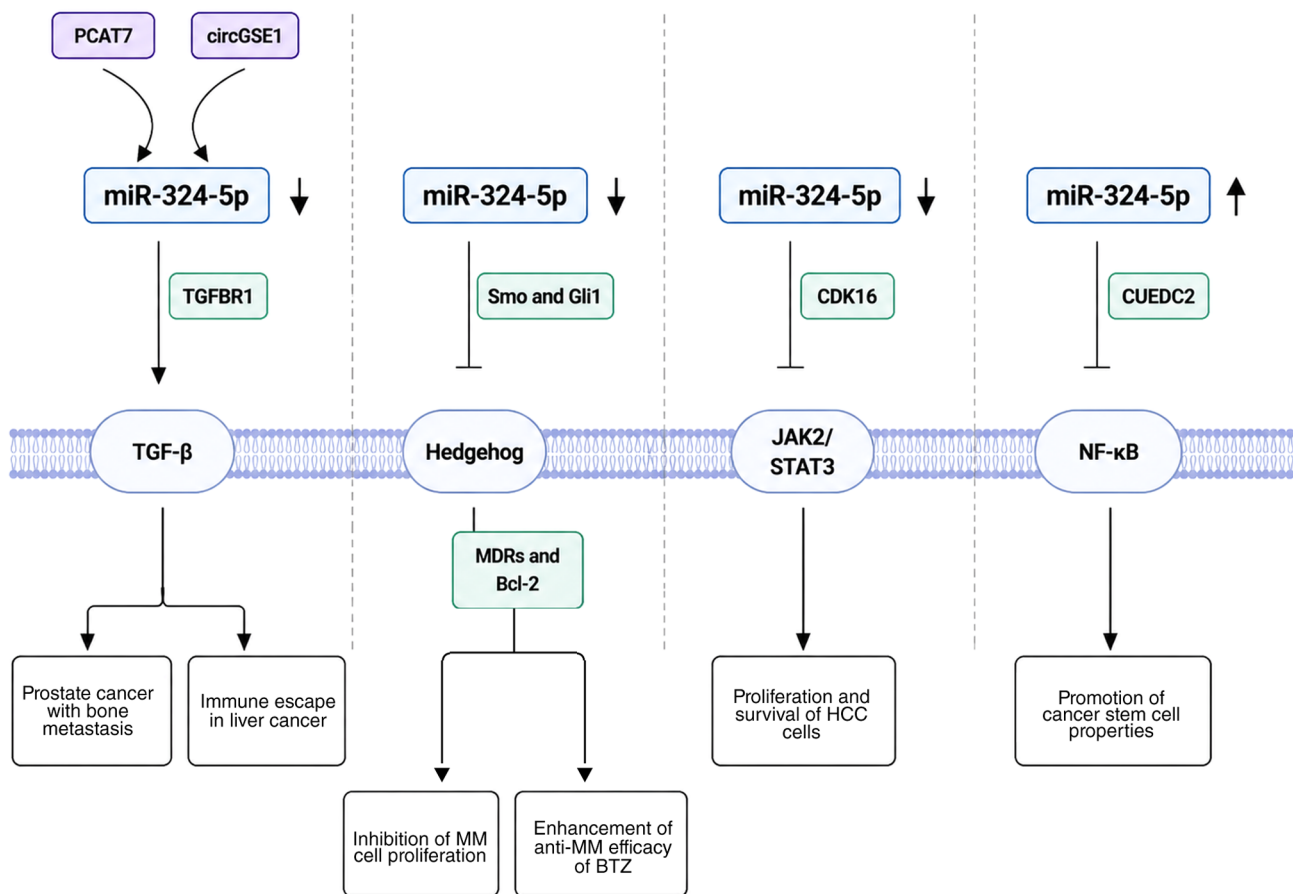


Figure 2. miR-324-5p-mediated tumor pathway. miR-324-5p-associated regulatory networks modulate TGFBR1 expression and TGF- β signaling. In MM, miR-324-5p suppresses the Hedgehog pathway through Smo and Gli, decreases MDR protein and Bcl-2 expression and enhances bortezomib sensitivity. In HCC, increased miR-324-5p activity suppresses CDK16 expression and attenuates JAK2/STAT3 signaling. miR-324-5p downregulates CUEDC2, thereby affecting NF- κ B-associated cancer stem-like phenotypes. CUEDC2, CUE domain-containing 2; TGFBR1, transforming growth factor β receptor 1; Smo, smoothened; Gli1, Gli family zinc finger 1; MM, multiple myeloma; CDK16, cyclin-dependent kinase 16; HCC, hepatocellular carcinoma; circ, circular; miR, microRNA.

Conversely, exosome-derived circGSE1 in HCC decreases miR-324-5p availability, resulting in TGFBR1 upregulation, Smad3-dependent signaling activation and increased Treg infiltration, thereby promoting tumor immune escape (21). The influence of miR-324-5p on TGF- β signaling is shaped by tumor-specific ceRNA networks, resulting in distinct functional outcomes across cancer types.

Hedgehog (Hh) pathway. Hh signaling is key to developmental patterning and the maintenance of tissue homeostasis in adulthood, whereas aberrant pathway activity is associated with tumor progression (86). In MM, the miR-324-5p locus resides within a chromosomal region typically affected by 17p deletion. Patients with del(17p) frequently exhibit decreased miR-324-5p expression, resulting in enhanced Hh pathway activity (73). Experimental findings show miR-324-5p negatively regulates the Hh pathway by targeting Gli family zinc finger 1 and Smoothened, with consequent inhibition of MM cell proliferation, survival and stem-like behavior (73). Moreover, restoration of miR-324-5p expression disrupts the supportive interactions between MM and bone marrow stromal cells (73). Increased miR-324-5p levels also regulate multidrug resistance-associated proteins and Bcl-2 family members via Hh pathway inhibition, thereby improving the

therapeutic response to BTZ in MM (73). Overall, miR-324-5p influences MM progression and treatment sensitivity through Hh pathway regulation, suggesting this miRNA may represent a candidate for therapeutic intervention in MM.

JAK2/STAT3 pathway. JAK2/STAT3 signaling coordinates cellular activities and has been implicated in both tumorigenesis and malignant progression (87). Zhao *et al* (25) reported that GL63, a curcumin derivative, suppresses circZNF83 expression, thereby restoring miR-324-5p availability and relieving circZNF83-mediated sequestration of miR-324-5p. Increased miR-324-5p activity suppresses cyclin-dependent kinase 16 (CDK16), leading to decreased JAK2/STAT3 signaling activity and suppression of malignant phenotypes in HCC (25). Thus, GL63 may inhibit JAK2/STAT3 signaling in HCC via the circZNF83/miR-324-5p/CDK16 regulatory pathway (25).

NF- κ B pathway. NF- κ B signaling regulates tumor cell biology, including proliferation, inflammatory activity, survival and immune escape (88). Persistent disturbance of this pathway may promote malignant transformation, invasion and metastatic dissemination. Elevated miR-324-5p levels are detected in NSCLC cells and other malignancies carrying

Table I. Expression, regulatory mechanisms and biological functions of miR-324-5p in cancer.

A, LC				
Regulation	Biological effects	Regulatory pathway/targets	Role	(Refs.)
Up	Contributes to LC initiation	circ_0067716/miR-324-5p/ DRAM1	Tumor-promoting	(37)
Up	Enhances proliferation and invasiveness of LC cells via FBXO11 regulation	GATA6-AS1/miR-324-5p/ FBXO11	Tumor-promoting	(17)
Up	Promotes LC progression by suppressing FBXO11-mediated tumor inhibition and decreasing cisplatin sensitivity	miR-324-5p/FBXO11	Tumor-promoting	(16)
Up	Promotes LC cell proliferation and invasion	GPX3, RCAN1 and MGAT3	Tumor-promoting	(38)
Not assessed	Suppresses LC cell proliferation and promotes apoptosis by targeting RAP1A	PCAT-1/miR-324-5p/RAP1A	Tumor-suppressive	(39)
B, GC				
Up	Enhances metastatic behavior by regulating GC cell migration and invasion	circ_0049447/miR-324-5p/ PTPRD	Tumor-promoting	(40)
Down	Suppresses GC cell viability and promotes apoptosis via HMGA1 regulation	hsa_circ_0091994/miR-324- 5p/HMGA1	Tumor-suppressive	(14)
Up	Promotes GC cell proliferation, migration and invasion while inhibiting apoptosis via PTEN regulation	miR-324-5p/PTEN	Tumor-promoting	(15)
C, Breast cancer				
Up	Promotes tumor progression by enhancing proliferation, migration, invasion and unfavorable clinical outcomes	miR-324-5p/GPX3	Tumor-promoting	(46)
Up	Elevated circulating miR-324-5p contributes to diagnostic prediction of breast cancer	Circulating miR-324-5p; seven-miRNA signature	Biomarker	(45)
Up	Regulates cell motility and invasiveness via modulation of NF-κB signaling and EMT-associated processes	miR-324-5p/CUEDC2/ NF-κB	Tumor-promoting	(47)
D, PTC				
Up	Promotes proliferation, migration and invasion of thyroid cancer cells and remodels the tumor microenvironment via endothelial activation and M2 macrophage polarization	miR-324-5p/PTPRD; miR- 324-5p/PTPRD/CEBPD	Tumor-promoting	(18)
E, PC				
Up	Promotes PC cell proliferation and suppresses apoptosis, thereby facilitating tumor progression	miR-324-5p/KLF3	Tumor-promoting	(49)
F, Bladder cancer				
Up	Promotes proliferation, colony formation and invasion of bladder cancer cells	miR-324-5p/RCAN1 and EYA4	Tumor-promoting	(53)

Table I. Continued.

G, CRC				
Regulation	Biological effects	Regulatory pathway/targets	Role	(Refs.)
Down	Restores miR-324-5p-mediated inhibition of tumor growth and invasion	miR-324-5p/ELAVL1	Tumor-suppressive	(20)
Down	Suppresses CRC cell proliferation and invasion by regulating invasion-associated proteins	miR-324-5p/ELAVL1/ MMP9/uPA/uPAR	Tumor-suppressive	(18)
Down	Promotes tumor cell proliferation and oxaliplatin resistance by sequestering miR-324-5p, thereby decreasing miR-324-5p-mediated suppression of HOXA9	hsa_circ_007966/miR-324-5p/HOXA9	Tumor-promoting (ceRNA-mediated)	(54)
Down	Restores miR-324-5p expression and suppresses colorectal carcinogenesis by counteracting carcinogen-induced miRNA downregulation	Dietary n-3 PUFA/miR-324-5p	Tumor-suppressive	(55)
Down	Decreases cancer stem cell-like properties and enhances FOLFOX sensitivity via SOD2 regulation	miR-324-5p/SOD2	Tumor-suppressive	(55)
Down	Loss of Dicer-mediated miR-324-5p regulation promotes intestinal inflammation, barrier disruption and tumor initiation	Dicer/miR-324-5p/ HMGXB3/WASF-2	Tumor-suppressive	(58)
Down	Inhibits COAD cell proliferation by restoring miR-324-5p activity	VPS9D1-AS1/miR-324-5p/ITGA2	Tumor-suppressive	(59)
H, HCC				
Down	Suppresses invasion and migration by inhibiting extracellular matrix remodeling	miR-324-5p/ETS1, SP1/ MMP2 and MMP9	Tumor-suppressive	(27)
Down	Enhances proliferation and migration via modulation of miR-324-5p availability	lnc85/miR-324-5p	Tumor-suppressive	(65)
Down	Facilitates tumor progression by activation of an IGF2BP1/YY1 positive feedback loop	LINC01134/miR-324-5p/ IGF2BP1/YY1	Tumor-promoting (ceRNA-mediated)	(13)
Down	May regulate the immune microenvironment via modulation of CLEC4s expression and immune cell infiltration	miR-324-5p/CLEC4s	Context-dependent	(66)
Down	Promotes immune evasion by enhancing Treg expansion via TGF- β signaling activation	Exosomal circGSE1/miR-324-5p/TGFBR1/Smad3	Tumor-promoting (immune regulation)	(21)
Down	GL63 inhibits tumor growth by suppressing the JAK2/STAT3 pathway	GL63/circZNF83/miR-324-5p/CDK16/JAK2/STAT3	Tumor-suppressive	(25)
I, Gallbladder cancer				
Down	Inhibits migration, invasion and EMT of gallbladder cancer cells	miR-324-5p/TGFB2	Tumor-suppressive	(90)
J, CC				
Down	Promotes proliferation and migration via regulation of DRAM1 expression	RNA LINC00511/ceRNA/ miR-324-5p/DRAM1	Tumor-suppressive	(11)

Table I. Continued.

J, CC				
Regulation	Biological effects	Regulatory pathway/targets	Role	(Refs.)
Down	Enhances radiosensitivity by promoting apoptosis and suppressing migration	miR-324-5p/ELAVL1/Bax/Bcl-2	Tumor-suppressive	(67)
K, Endometrial cancer				
Down	Inhibits the proliferation, migration and invasion of endometrial cancer cells	miR-324-5p/HMGA1	Tumor-suppressive	(91)
L, OC				
Down	Low miR-324-5p expression is associated with poor OS and shorter PFI and influences olaparib resistance	ATR/CHK1	Context-dependent	(68)
Down	Modulates G2/M checkpoint regulation and olaparib response via YWHAQ-associated mechanisms	miR-324-5p/YWHAQ	Tumor-suppressive	(68)
Down	Suppresses proliferation, migration, invasion and tumor growth by inhibiting VDAC1 expression	circZNF609/miR-324-5p/VDAC1	Tumor-suppressive	(71)
M, MM				
Down	Suppresses proliferation, viability and cancer stem-like properties and enhances bortezomib sensitivity	miR-324-5p/Bcl-2 family and multidrug resistance-associated proteins	Tumor-suppressive	(73)
Down	Promotes proliferation, migration, invasion and glycolytic reprogramming via HGF activation following miR-324-5p sequestration	circATIC/miR-324-5p/HGF	Tumor-promoting (ceRNA-mediated)	(74)
N, GBM				
Down	Suppresses proliferation by directly targeting XBP1	miR-324-5p/XBP1	Tumor-suppressive	(77)
Down	Promotes proliferation by sequestering miR-324-5p, thereby decreasing miR-324-5p-mediated suppression of XBP1	circ_0079593/miR-324-5p/XBP1	Tumor-promoting (ceRNA-mediated)	(77)
Down	Enhances proliferation and decreases apoptosis through KCTD20 activation	NEAT1/miR-324-5p/KCTD20	Tumor-promoting (ceRNA-mediated)	(78)
Down	Promotes malignant cell survival via Bcl-2 upregulation	circSERPINE2/miR-324-5p/miR-361-3p/Bcl-2	Tumor-promoting (ceRNA-mediated)	(79)
Down	Inhibits tumor growth and increases temozolomide sensitivity via EZH2 suppression	miR-324-5p/EZH2	Tumor-suppressive	(23)

Table I. Continued.

O, DLBCL				
Regulation	Biological effects	Regulatory pathway/targets	Role	(Refs.)
Down (tumor tissue); up (serum)	Inhibits lymphoma cell survival and promotes ferroptosis via TXNRD1 suppression	miR-324-5p/TXNRD1	Context-dependent	(80)

LC, Lung cancer; DRAM1, DNA damage-regulated autophagy modulator 1; FBXO11, F-box protein 11; GC, gastric cancer; PTPRD, protein tyrosine phosphatase receptor Δ ; GPX3, glutathione peroxidase 3; IKK, I κ B kinase; CUEDC2, CUE domain-containing 2; PTC, papillary thyroid carcinoma; CEBPD, CCAAT enhancer-binding protein Δ ; PC, pancreatic cancer; KLF3, Krüppel-like factor 3; CRC, colorectal cancer; ELAVL1, embryonic lethal abnormal vision Drosophila-like protein 1; HOXA9, homeobox A9; SOD2, superoxide dismutase 2; HMGB3, HMG-box containing 3; WASF-2, WAS protein family member 2; ITGA2, integrin subunit α 2; COAD, colorectal adenocarcinoma; HCC, hepatocellular carcinoma; ROCK1, rho-associated kinase 1; ETS1, E26 transformation-specific 1; SP1, specificity protein 1; IGF2BP1, insulin-like growth factor 2 mRNA binding protein 1; YY1, Yin Yang-1; CLEC4s, C-type lectin 4s; TGFBR1, transforming growth factor β receptor 1; GL63, (1E,4E)-1,5-bis(2-bromophenyl) penta-1,4-dien-3-one; CDK16, cyclin-dependent kinase 16; EMT, epithelial-mesenchymal transition; CC, cervical cancer; ceRNA, competing endogenous RNA; HMGA1, high mobility group A1; OC, Ovarian cancer; VDAC1, voltage-dependent anion channel 1; MM, Multiple myeloma; Gli1, Gli family zinc finger 1; Smo, Smoothened; Hh, Hedgehog; BTZ, bortezomib; MRP, multidrug resistance-associated protein; HGF, hepatocyte growth factor; GBM, glioblastoma; XBP1, X-box binding protein 1; KCTD20, potassium channel tetramerization domain-containing 20; EZH2, zeste homolog 2; TMZ, temozolomide; DLBCL, diffuse large B cell lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; TXNRD1, thioredoxin reductase 1; MGAT3, mannosyl (β -1,4-) glycoprotein β -1,4-N-acetylglucosaminyltransferase; PCAT-1, prostate cancer associated transcript 1.

gain-of-function TP53 mutations (89). At the molecular level, mutant p53 enhances miR-324-5p transcription via the c-Myc signaling pathway, resulting in CUEDC2 down-regulation and NF- κ B activation. This regulatory pathway promotes key cancer stem-like properties, including increased proliferation, survival and chemotherapy resistance (89). Conversely, miR-324-5p silencing restores CUEDC2 expression, suppresses NF- κ B signaling activity and attenuates cancer-like phenotypes (89). Available evidence implicates the TP53/c-Myc/miR-324-5p/CUEDC2 pathway in coupling miR-324-5p activity with NF- κ B signaling and cancer stem-like properties (89).

miR-324-5p affects tumor growth and metastasis in animal models. Several *in vivo* studies have evaluated the tumor-regulatory functions of miR-324-5p in cancer models (Table II) (14,22,49,58,59,79,90,91). These studies indicate that miR-324-5p-related interventions can influence tumor growth, metastasis and treatment-associated phenotypes through mechanisms involving direct target gene regulation, EMT-associated changes, ceRNA networks and intestinal barrier-associated pathways. These *in vivo* findings complement cell-based evidence and provide additional support for the context-dependent role of miR-324-5p in tumor regulation.

miR-324-5p regulates tumor growth, invasion and metastasis through target-gene-associated pathways. Integrin and focal adhesion kinase (FAK) signaling pathways are key regulators of tumor invasion and metastatic progression (92,93). ITGA9 is directly regulated by miR-324-5p through interaction with the ITGA9 3'UTR (94). In rhabdomyosarcoma (RMS), miR-324-5p-mediated suppression of ITGA9 limits proliferative and invasive behavior *in vitro* and decreases tumor growth

in mouse xenograft models (94). Decreased ITGA9 levels are accompanied by reduced FAK phosphorylation, indicating that the miR-324-5p/ITGA9 pathway may regulate RMS progression via FAK-related signaling (94). In a COAD xenograft model, enforced miR-324-5p expression and VPS9D1-AS1 silencing markedly inhibits tumor growth. VPS9D1-AS1 regulates the miR-324-5p/ITGA2 pathway by limiting miR-324-5p activity, thereby favoring ITGA2 expression and COAD progression (59).

Beyond integrin-associated pathways, miR-324-5p is associated with EMT regulation and metastatic dissemination. In gallbladder carcinoma (GBC), elevated miR-324-5p expression decreases cell motility, invasion and EMT-related phenotypes, leading to fewer hepatic metastatic lesions *in vivo* (90). Conversely, miR-324-5p is downregulated in liver cancer tissue and cells models relative to corresponding non-tumor controls and its restoration suppresses tumor-associated phenotypes (22,27). Mechanistically, miR-324-5p limits tumor expansion and metastatic potential by modulating ROCK1 (22).

miR-324-5p regulates tumor growth by modulating transcriptional factors. In endometrial cancer xenografts, miR-324-5p inhibits subcutaneous tumor formation by suppressing high mobility group AT-hook 1 (HMGA1) expression (91). In a PC xenograft model, miR-324-5p promotes xenograft tumor growth by targeting KLF3. Conversely, inhibition of miR-324-5p markedly attenuates tumor growth (49).

miR-324-5p regulates tumor progression via ceRNA networks. circRNAs and lncRNAs modulate miR-324-5p activity in ceRNA networks, thereby affecting tumor-associated phenotypes. In a GBM xenograft model, loss of circSERPINE2 markedly inhibits tumor growth (79). Mechanistically,

Table II. Animal models investigating miR-324-5p-mediated tumor progression and regulatory mechanisms.

Cancer	Animal model	Treatment	Intervention	Key <i>in vivo</i> phenotypic outcomes	miR-324-5p-associated pathways	(Refs.)
Gastric cancer	BALB/c nude xenograft model	Not applicable	hsa_circ_0091994 knockdown	Decreased tumor growth; miR-324-5p restoration	hsa_circ_0091994/miR-324-5p/HMGA1	(14)
Liver cancer	Nude mouse xenograft and metastasis models	Xenograft tumors and lung metastasis	LINC00491 overexpression	Increased tumor growth and metastasis and proliferation markers	LINC00491/miR-324-5p/ROCK1	(22)
PDAC	BALB/c nude subcutaneous xenograft model	miR-324-5p antagomir (5 nmol, every 5 days for 35 days)	miR-324-5p inhibition	Decreased tumor volume/weight and PCNA-positive cells	miR-324-5p/KLF3	(49)
Colo-rectal cancer	Intestinal epithelial Dicer-deficient mouse model (DSS/AOM-DSS CRC models)	miR-324-5p agomiR (10 nmol); 3% DSS-induced colitis and AOM/DSS-induced CRC models	Dicer deletion to decrease miR-324-5p; miR-324-5p agomiR rescue treatment in DSS model	Dicer deficiency promotes intestinal injury and tumorigenesis; miR-324-5p rescue restores intestinal protection	Dicer/miR-324-5p/HMGXB3/WASF-2	(58)
COAD	Nude mouse xenograft	Transfection before implantation	VPS9D1-AS1 knockdown	Decreased tumor growth	VPS9D1-AS1/miR-324-5p/ITGA2 ceRNA	(59)
Glioblastoma	Nude mouse xenograft model	~1x10 ⁶ cells injected subcutaneously	circSERPINE2 knockdown	Decreased tumor growth and proliferation; increased apoptosis	circSERPINE2/miR-361-3p/miR-324-5p/BCL2	(79)
GBC	BALB/c nude xenograft model	1x10 ⁶ SGC-996 cells	miR-324-5p overexpression or TGFB2 knockdown	Decreased incidence of liver metastasis and fewer liver metastatic foci	TGFB2 downstream of miR-324-5p	(90)
GBC	BALB/c nude xenograft model	Stable SGC-996 xenograft model	miR-324-5p overexpression	Decreased liver metastasis and metastatic foci	miR-324-5p/TGFB2/EMT-associated metastasis	(90)
Endometrial carcinoma	BALB/c nude xenograft model; circ_0067835 knockdown	Cell-based shRNA xenograft model	circ_0067835 knockdown	Decreased tumor growth, proliferation and EMT; increased apoptosis	circ_0067835/miR-324-5p/HMGA1	(91)
RMS	SCID xenograft model	1x10 ⁶ RD cells injected into the gastrocnemius muscle; doxycycline (1 mg/ml) initiated 1 week later	miR-324-5p overexpression	Decreased tumor growth and ITGA9/Ki-67/CD31	miR-324-5p/ITGA9/FAK-associated signaling	(94)

RMS, rhabdomyosarcoma; SCID, severe combined immunodeficiency; ITGA9, integrin $\alpha 9$; FAK, focal adhesion kinase; COAD, colorectal adenocarcinoma; VPS9D1-AS1, VPS9D1 antisense RNA 1; ceRNA, competing endogenous RNA; GBC, gallbladder carcinoma; TGFB2, transforming growth factor $\beta 2$; EMT, epithelial-mesenchymal transition; ROCK1, rho-associated kinase 1; PCNA, proliferating cell nuclear antigen; KLF3, Krüppel-like factor 3; HMGA1, high mobility group A1; DSS, dextran sodium sulfate; AOM, azoxymethane; HMGXB3, HMG-box containing protein 3; WASF-2, Wiskott-Aldrich syndrome protein family member 2; agomiR, miRNA agonist.

circSERPINE2 sequesters miR-324-5p and miR-361-3p, decreasing suppression of Bcl-2 and favoring GBM progression (79). In GC xenografts, higher miR-324-5p abundance is associated with smaller and lighter tumors (14). Silencing hsa_circ_0091994 increases miR-324-5p availability, resulting in decreased HMGA1 expression and suppression of GC progression (14).

miR-324-5p regulates tumorigenesis through cytoskeletal remodeling and intestinal barrier maintenance. Altered Dicer expression is implicated in CRC (57), and experimental Dicer deficiency reduces miR-324-5p expression, supporting a role for Dicer-mediated miRNA processing in the regulation of miR-324-5p (58). In a CRC model, Dicer deficiency markedly decreases miR-324-5p expression in mice, resulting in abnormal cytoskeletal alterations in intestinal epithelial cells, compromised barrier function, enhanced inflammatory response and increased tumor formation (58). Conversely, intraperitoneal delivery of miR-324-5p agonist alleviates dextran sulfate sodium-induced intestinal inflammation and tissue injury, restores epithelial barrier integrity and decreases HMGXB3 and WASF-2 expression, thereby suppressing CRC progression (58).

3. miR-324-5p as a potential cancer biomarker

Application of miR-324-5p in cancer diagnosis. miRNAs, which are detectable in both intracellular and extracellular environments, have attracted attention for their potential utility in cancer detection, prognostic evaluation and assessment of therapeutic response (95). Combined RNA sequencing and reverse transcription-quantitative (RT-q)PCR have detected enrichment of miR-324-5p in plasma-derived exosomes from patients with NSCLC, indicating potential biomarker relevance (33). Similarly, elevated miR-324-5p levels are observed in urine specimens from individuals with bladder cancer and show an inverse association with the expression of the lncRNA miR-497-HG (52). These clinical observations are complemented by a separate functional study showing that miR-324-5p promotes bladder cancer cell proliferation, colony formation, migration and invasion (53). In PTC, higher miR-324-5p levels are linked to LNM, greater tumor burden and more advanced disease stage, supporting its potential utility for patient stratification and risk assessment (19). Gómez-Acebo *et al* (45) reported higher circulating miR-324-5p levels in patients with breast cancer than in healthy controls and incorporated this miRNA into a seven-miRNA diagnostic signature (OR=1.60, 95% CI: 1.08-2.37), supporting its potential value as a diagnostic marker. Collectively, evidence points to a potential diagnostic application for miR-324-5p, although validation is needed to establish its clinical biomarker utility.

Association between miR-324-5p levels and patient prognosis. miR-324-5p expression patterns are associated with clinical outcomes across several types of malignancies. In glioma, miR-324-5p has been identified as an independent prognostic indicator (23). In GC, RT-qPCR has revealed elevated miR-324-5p levels in tumor tissue and cell lines, which are associated with lymphatic metastasis, advanced disease stage and poor survival (15). Another study profiled

miRNAs in plasma-derived small extracellular vesicles from patients with GC using sEV-miRNA sequencing followed by RT-qPCR validation and found that miR-324-5p abundance is associated with organ-specific metastatic patterns, suggesting potential prognostic relevance (41). By contrast, lower miR-324-5p levels in GBC are linked to adverse clinical outcomes (90). This association may be partly explained by miR-324-5p-mediated regulation of TGFB2, which affects GBC cell migration, invasion and EMT (90). In triple-negative breast cancer, next-generation sequencing of 51 specimens showed that elevated miR-324-5p levels were associated with shorter OS (43). Overall, these observations indicate that miR-324-5p may have prognostic value across multiple tumor types.

4. Potential of miR-324-5p as a therapeutic target

Accumulating evidence indicates miRNAs participate in carcinogenesis by controlling target transcripts (95,96). Therapeutic alteration of miRNA function has been investigated as a strategy to reshape oncogenic signaling networks (97). Given its context-dependent functions across malignancies, miR-324-5p may be therapeutically modulated through lncRNA/circRNA regulatory networks or by exogenous miRNA mimics or inhibitors (12,20,22,59,74,77,78,91). In addition, miR-324-5p is implicated in cancer-specific responses to chemotherapy and radiotherapy, as well as in the regulation of tumor-associated immune processes (16,21,23,54,55,66,67,69,73) (Fig. 3). Further development of such strategies requires tumor-specific determination of whether miR-324-5p activity should be restored or inhibited.

Strategies for regulating miR-324-5p

lncRNA strategies. lncRNAs interact with miRNAs through regulatory networks involved in tumor initiation and progression (98). Several lncRNAs have been reported to alter the availability or activity of miR-324-5p, with effects on malignant phenotypes (12,59,78). In GBM, lncRNA NEAT1 sequesters miR-324-5p, thereby weakening its inhibitory effect on KCTD20 and facilitating tumor cell proliferation (78). A similar regulatory mechanism operates in COAD, where lncRNA VPS9D1-AS1 overexpression suppresses miR-324-5p activity and elevates ITGA2 expression. The VPS9D1-AS1/miR-324-5p/ITGA2 pathway promotes COAD cell proliferation and decreases apoptosis (59). In melanoma, HLA complex group 18 (HCG18) expression is elevated in both tumor specimens and cultured cells, and modulates the miR-324-5p/CDK16 pathway (12). By decreasing miR-324-5p-mediated suppression of CDK16, HCG18 enhances malignant cell proliferation, motility and invasiveness while decreasing apoptotic activity (12). Although these lncRNAs act in different tumor settings, they converge on miR-324-5p and regulate malignant phenotypes through distinct downstream effectors, including KCTD20, ITGA2 and CDK16 (12,59,78). Whether these networks are independent or share common upstream regulatory features remains unclear. Comparison of their expression patterns, intracellular localization and interaction with miR-324-5p may clarify this issue.

circRNA-based strategies. circRNAs influence gene expression through several mechanisms, including functional interaction with miRNAs (99). One mechanism

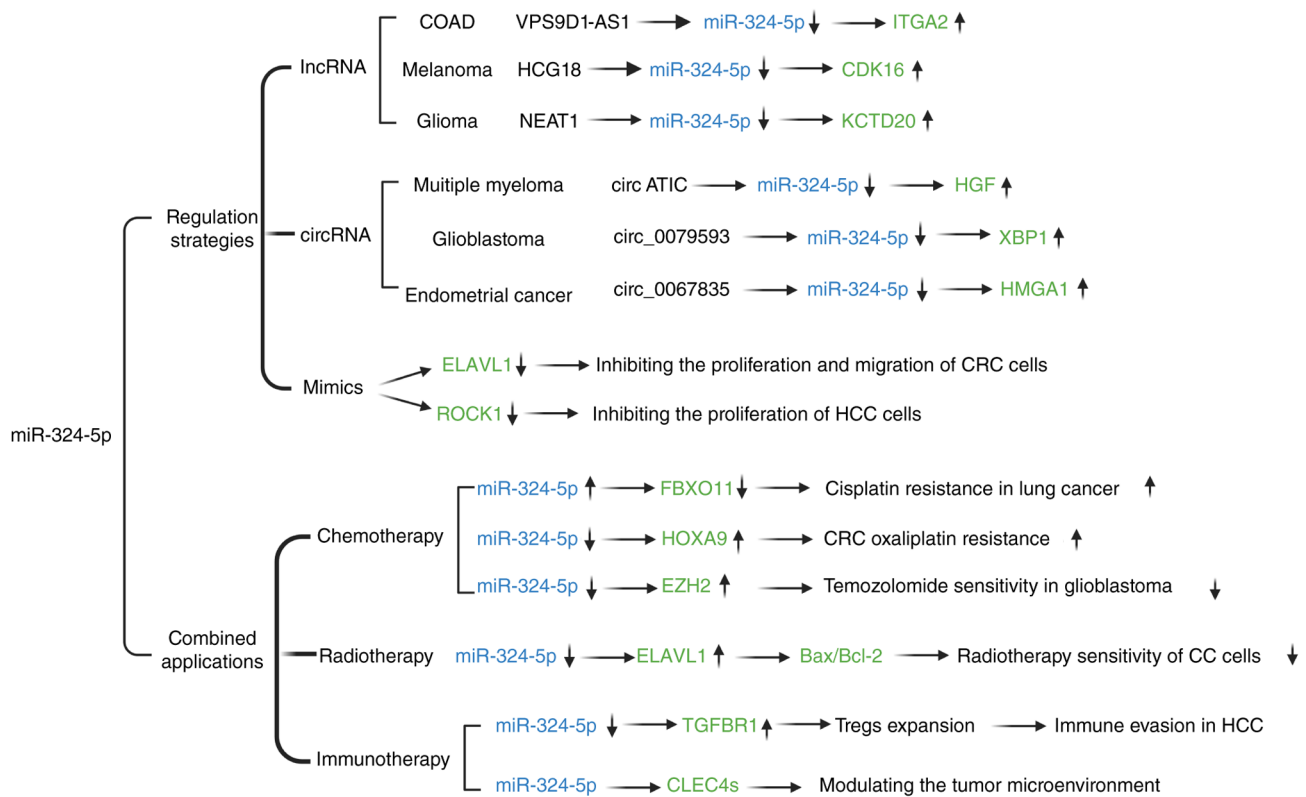


Figure 3. miR-324-5p-mediated tumor therapeutic strategies. miR-324-5p is therapeutically modulated through lncRNA/circRNA regulatory networks or by exogenous miRNA mimics and inhibitors. These strategies may alter tumor growth, invasion and apoptosis and may modify responses to chemotherapy, radiotherapy and tumor-associated immune regulation through cancer-specific downstream targets and signaling pathways. ITGA2, integrin subunit $\alpha 2$; CDK16, cyclin-dependent kinase 16; KCTD20, potassium channel tetramerization domain containing 20; HGF, hepatocyte growth factor; XBP1, X-box binding protein 1; HMGA1, high mobility group A1; ELAVL1, embryonic lethal abnormal vision Drosophila-like protein 1; CRC, colorectal cancer; ROCK1, rho-associated kinase 1; HCC, hepatocellular carcinoma; FBXO11, F-box protein 11; HOXA9, homeobox A9; EZH2, zeste homolog 2; TGFBR1, transforming growth factor β receptor 1; CC, cervical cancer; CLEC4S, C-type lectin 4s; circ, circular RNA; miR, microRNA; lnc, long non-coding; COAD, colon adenocarcinoma.

involves sequestration of miRNAs, thereby decreasing miRNA-mediated suppression of downstream targets and influencing tumor development (100). Interactions between circRNAs and miR-324-5p are implicated in the regulation of multiple tumor-associated phenotypes (74,77,91). In MM, circATIC binds miR-324-5p, thereby increasing HGF expression and promoting cell proliferation, invasion, migration and glycolysis while suppressing apoptosis (74). In GBM, circ_0079593 is upregulated and associated with decreased miR-324-5p expression (77). This circRNA is implicated in GBM progression via the miR-324-5p/XBP1 regulatory pathway (77). Similarly, circ_0067835 is upregulated in endometrial carcinoma and negatively associated with miR-324-5p levels (91). By serving as a ceRNA, circ_0067835 increases HMGA1 levels and promotes endometrial carcinoma progression (91). Although these circRNAs are involved in different tumor contexts, they converge on miR-324-5p and influence malignant phenotypes through distinct downstream effectors (74,77,91). To the best of our knowledge, whether these regulatory events occur independently or share common upstream determinants has not been fully explored. Future studies comparing miR-324-5p interactions and upstream regulatory mechanisms may clarify this. Further investigation may reveal whether individual circRNAs or broader miR-324-5p regulatory networks represent feasible therapeutic targets.

Therapeutic application of miR-324-5p overexpression or inhibition. Therapeutic modulation of miR-324-5p using mimics or antagonists has demonstrated potential in preclinical cancer models (20,22,58). In CRC cell systems, miR-324-5p mimics directly target ELAVL1, resulting in decreased levels of invasion-associated proteins, including MMP-9, uPA and uPAR, and suppressing CRC cell proliferation, migration and invasive behavior (20). Conversely, inhibition of miR-324-5p increases ELAVL1 expression together with invasion-associated factors, thereby promoting CRC cell growth and invasiveness (20). This supports the possibility that restoring miR-324-5p activity may represent a therapeutic strategy for CRC. Similarly, in liver cancer, treatment with miR-324-5p mimics suppresses the proliferative, migratory and invasive capacity of HUH-7 and SK-Hep1 cells while inducing apoptosis, whereas miR-324-5p inhibition produces opposing cell responses (22). Mechanistically, LINC00491 promotes HCC progression by serving as a ceRNA that sequesters miR-324-5p, thereby decreasing miR-324-5p-mediated suppression of ROCK1 (22). Restoration of miR-324-5p through mimics counteracts the oncogenic effects associated with LINC00491, supporting the therapeutic relevance of miR-324-5p modulation (22). Taken together, the aforementioned preclinical studies suggest that miR-324-5p-based interventions may have therapeutic value in certain cancer contexts. However, clinical translation of miRNA mimics remains limited by challenges, including

delivery efficiency, molecular stability, immune-associated responses and off-target effects, which require further optimization before clinical application (101).

Regulation of miR-324-5p in combination with chemotherapy, radiotherapy and immunotherapy. miR-324-5p is implicated in treatment response across cancer, particularly in relation to chemotherapy, radiotherapy and tumor-associated immune regulation (16,21,23,54,55,66,67,69,73).

Chemotherapy. Resistance to chemotherapy is a key barrier limiting the effectiveness of anticancer treatment. Increasing evidence indicates that miRNAs contribute to chemotherapy sensitivity and resistance through post-transcriptional regulation of target genes (95,102). miR-324-5p influences chemotherapy responses across multiple cancer types. In LC, increased miR-324-5p expression decreases FBXO11 levels, thereby decreasing cisplatin sensitivity and promoting chemoresistance (16). In OC, miR-324-5p is implicated in the response to olaparib: In olaparib-resistant PEO1-OR cells, changes in miR-324-5p expression are observed following combined treatment with olaparib and ATR/CHK1 pathway inhibitors, which were associated with resensitization to olaparib (69). In CRC, miR-324-5p suppresses cell proliferation through negative regulation of HOXA9 (54). Conversely, hsa_circ_0079662 modulates the miR-324-5p/HOXA9 pathway by sequestering miR-324-5p, thereby relieving suppression of HOXA9 and enhancing colorectal tumor growth and oxaliplatin resistance (54). miR-324-5p decreases chemoresistance and cancer stem-like properties in CRC by directly targeting SOD2, thereby increasing sensitivity to FOLFOX (55). In GBM, elevated miR-324-5p expression suppresses EZH2, inhibits cell proliferation and enhances TMZ sensitivity (23). Overall, evidence links miR-324-5p to chemotherapy responsiveness through cancer-specific regulatory mechanisms and suggests potential therapeutic relevance in drug-resistant disease (16,23,54,55,69).

Radiotherapy. Increased miR-324-5p expression sensitizes CC cells to irradiation-induced apoptosis and inhibits cell migration (67). Mechanistically, miR-324-5p directly targets ELAVL1, leading to alterations in the apoptotic protein profile, including Bax and Bcl-2, thereby increasing cell sensitivity to irradiation (67).

Immune regulation. Previous studies have established that miRNAs participate in tumor immune surveillance and immune escape through regulation of immune-associated pathways (99,100,103). Studies have linked miR-324-5p-associated regulatory networks with features of the tumor immune microenvironment (44,104). In HCC, exosomal circGSE1 modulates the miR-324-5p/TGFBR1/Smad3 pathway, thereby promoting Treg expansion and immune evasion (21). CLEC receptors contribute to immune cell migration and tumor-associated processes (105). miR-324-5p has been suggested to regulate CLEC4s family members, thereby influencing the tumor microenvironment and immune responses in HCC (66). Collectively, evidence links miR-324-5p-associated regulatory networks to tumor immune modulation, including immune cell infiltration, Treg expansion and immune escape,

although the direct therapeutic relevance remains to be established (21,44,104).

Taken together, the aforementioned studies highlight the complex regulatory role of miR-324-5p across cancers, involving diverse expression patterns, signaling pathways, *in vivo* phenotypes, biomarker applications and therapeutic implications. Several miR-324-5p-associated target genes or downstream effectors recur across different malignancies, but their functional consequences are not always consistent across tumor contexts. These recurrent targets, together with their reported functional outcomes and evidence categories, are summarized in Table III.

5. Challenges and prospects

Despite its potential diagnostic and therapeutic value in cancer, the clinical translation of miR-324-5p remains challenging because of its complex regulatory behavior and context-specific biological functions. miR-324-5p displays divergent effects among different types of malignancies. For example, miR-324-5p exhibits tumor-suppressive functions and decreased expression in CC (11) and HCC (27), whereas elevated expression and tumor-promoting effects have been reported in LC and breast cancer (33,43). miR-324-5p may exert different biological effects within the same cancer type. In PDAC tissue and cells, miR-324-5p expression is elevated and exerts an oncogenic role through KLF3 regulation (49). By contrast, a bioinformatics analysis of PAAD suggested decreased miR-324-5p expression, with higher expression associated with a more favorable prognosis, and predicted a regulatory axis involving the lnc AL049555.1, miR-324-5p and ring finger protein 169 (RNF169) (106). This evidence indicates that the functional output of miR-324-5p may be influenced by the tumor-specific molecular context and its associated regulatory networks, rather than solely by its expression. Therefore, elucidating the molecular determinants that govern miR-324-5p function is key for accurately assessing its diagnostic and therapeutic relevance.

At the molecular level, specific downstream targets, ceRNA regulatory networks and genetic variants represent three molecular dimensions that warrant consideration when interpreting the biological outcomes of miR-324-5p regulation. For example, KLF3 in pancreatic cancer (49), ROCK1 in liver cancer (22), CUEDC2 in breast cancer (47) and ITGA9 in RMS (94) represent distinct downstream effectors involved in different malignant processes, emphasizing the importance of tumor-specific molecular environments in determining miR-324-5p function. ceRNA interactions mediated by lncRNAs and circRNAs regulate miR-324-5p availability and activity of miR-324-5p (74,78). Moreover, genetic variations affecting miRNA-binding regions contribute to functional variability (107). Abid *et al* (107) identified PRKCG 3'UTR variants that alter binding sites for cancer-associated miRNAs, including miR-324-5p, suggesting genetic backgrounds influence miRNA-mediated regulation. Incorporating molecular information into future biomarker and therapeutic studies may facilitate more precise patient stratification and evaluation of miR-324-5p clinical potential.

The variable biological effects of miR-324-5p across tumor types complicate the interpretation of its clinical value.

Table III. miR-324-5p cross-cancer targets.

Target gene	Cancer	Association with miR-324-5p	Functional effect	Study-specific evidence	(Refs.)
CDK16	Melanoma	Directly suppressed	Promotes proliferation, migration and invasion and suppresses apoptosis	Dual-luciferase validation; CDK16 regulation; rescue experiments	(12)
	Hepatocellular carcinoma	Directly suppressed	Promotes proliferation, migration and invasion; inhibits apoptosis	Dual-luciferase validation; CDK16 regulation; rescue experiments; pathway-level xenograft support	(25)
HMGA1	Gastric cancer	Directly suppressed	Promotes GC cell viability and suppresses apoptosis	Dual-luciferase validation; HMGA1 rescue; pathway-level xenograft support	(14)
	Endometrial carcinoma	Directly suppressed	Promotes proliferation, migration and invasion	Dual-luciferase validation; pathway rescue; pathway-level xenograft support	(91)
ELAVL1	Colorectal cancer	Directly suppressed	Promotes proliferation, migration and invasion	Dual-luciferase validation; ELAVL1 expression regulation; downstreamuPA/uPAR/MMP-9 changes	(20)
	Cervical cancer	Directly suppressed	Promotes radioresistance and malignant behavior under IR	Dual-luciferase validation; ELAVL1 expression regulation; ELAVL1 rescue under IR	(67)
TGFBR1	Hepatocellular carcinoma	Directly suppressed	Promotes Treg expansion and immune escape	Dual-luciferase validation; TGFBR1 regulation; miR-324-5p/TGFBR1/Smad3 rescue; pathway-level <i>in vivo</i> support	(21)
TGFBR1	Prostate cancer	Directly suppressed	Promotes migration, invasion and bone metastasis	Dual-luciferase validation; TGFBR1 regulation; TGFBR1 rescue; pathway-level bone-metastasis support	(85)
ROCK1	Liver cancer	Directly suppressed	Promotes proliferation, migration and invasion	Dual-luciferase validation; mRNA/protein regulation; rescue with miR-324-5p mimic or ROCK1 knockdown	(22)
ROCK1	Laryngeal squamous cell cancer	Directly suppressed	Tumor-promoting; supports cell viability and cell cycle progression	Dual-luciferase validation; ROCK1 expression regulated by miR-324-5p; ROCK1 rescue	(26)
CUEDC2	Breast cancer	Suppressed	Inhibits NF- κ B-dependent migration and invasion	CUEDC2 regulation; miR-324-5p mimic rescue; NF- κ B pathway evidence	(47)
	NSCLC	Suppressed	Suppresses NF- κ B-dependent cancer stemness	CUEDC2 regulation; complementation rescue; miR-324-5p inhibition rescue; CCLE/TCGA support	(89)

Table III. Continued.

Target gene	Cancer	Association with miR-324-5p	Functional effect	Study-specific evidence	(Refs.)
CUEDC2	Colon cancer/ TAMs	Directly suppressed	Restraints inflammatory macrophage dysfunction and CAC development	3'UTR luciferase validation; CUEDC2 rescue; Cuedc2-KO CAC model	(108)

miR, microRNA; CDK16, cyclin-dependent kinase 16; HMGA1, high mobility group A1; GC, gastric cancer; ELAVL1, embryonic lethal abnormal vision Drosophila-like protein 1; uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; MMP-9, matrix metalloproteinase 9; IR, ionizing radiation; TGFBR1, transforming growth factor β receptor 1; Treg, regulatory T cell; SMAD3, SMAD family member 3; ROCK1, rho-associated kinase 1; mRNA, messenger RNA; CUEDC2, CUE domain-containing 2; NF- κ B, nuclear factor κ B; NSCLC, non-small cell lung cancer; CCLE, Cancer Cell Line Encyclopedia; TCGA, The Cancer Genome Atlas; TAMs, tumor-associated macrophages; 3'UTR, 3' untranslated region; KO, knockout; CAC, colitis-associated cancer.

Inconsistent findings have also been reported within the same cancer type, particularly in LC (17,38,39) and GC (14,15,40). In LC, studies have suggested a tumor-promoting role for miR-324-5p (38,39). Upregulation of miR-324-5p promotes A549 cell proliferation and invasion (38), while miR-324-5p mimic attenuates the inhibitory effects of GATA6-AS1 on proliferation and invasion in NCI-H1975 and A549 cells (17). However, another cell-based study showed that miR-324-5p upregulation decreases PCAT-1-induced proliferation in A549 cells and promotes apoptosis (39). Although all aforementioned studies involved A549 cells, the reported effects of miR-324-5p on proliferation were not consistent, indicating that cell line identity alone is insufficient to explain this discrepancy.

Table IV compares the tumor type/subtype, sample and clinical background, miR-324-5p-associated findings, experimental models and major methods, as well as regulatory targets or ceRNA axes. The studies differed in study design and evidence source (Table IV). Lin *et al* (38) combined population-level expression analysis with cell functional experiments, Wang *et al* (17) used paired clinical tissue together with ceRNA and rescue experiments, whereas Huang *et al* (39) conducted a cell-based mechanistic study without an independent patient cohort. These differences may affect the comparability between studies. The regulatory context also differed between studies. Wang *et al* (17) focused on the GATA6-AS1/miR-324-5p/FBXO11 regulatory association, whereas Huang *et al* (39) investigated the PCAT-1/miR-324-5p/RAP1A regulatory association. Each study validated its respective regulatory axis, but FBXO11- and RAP1A-associated pathways were not directly compared under matched experimental conditions in A549 cells. Therefore, the available evidence does not establish that differences in regulatory context account for the opposite effects on proliferation.

Similarly, inconsistent findings have also been reported in GC: Certain studies show elevated miR-324-5p expression in GC tissue (15,40), whereas another study reported decreased expression in GC tissues (14). Functionally, miR-324-5p exerts tumor-promoting effects, with higher expression associated with an unfavorable prognosis (15), whereas another study suggested a tumor-suppressive role (14).

With respect to patient cohorts and disease stage, Zheng *et al* (15) included 122 patients with TNM stage I-IV disease, Tang *et al* (40) used 30 paired samples for the miR-324-5p expression comparison, whereas Xie *et al* (14) analyzed five paired clinical tissue samples and did not report detailed staging information (14). Mechanistically, Zheng *et al* (15) showed that inhibition of miR-324-5p suppressed GC cell proliferation, migration and invasion, and identified PTEN as a direct target negatively regulated by miR-324-5p. Tang *et al* (40) reported that circ_0049447 may modulate GC progression via sequestration of miR-324-5p, potentially involving a PTPRD-related mechanism. By contrast, Xie *et al* (14) found that inhibition of miR-324-5p reversed the antitumor effects induced by circ_0091994 knockdown. Differences in cohort size, stage and mechanistic context may affect the comparability of these findings. Because the aforementioned studies did not perform direct comparisons using matched clinical samples or experimental models or standardized detection and analytical conditions, the available evidence remains insufficient to explain the opposite results reported across studies.

Establishing standardized workflows for sample collection, miRNA detection, data normalization and interpretation at the clinical level, together with validation in independent prospective cohorts, is essential for determining the clinical utility of miR-324-5p as a biomarker (109). At the mechanistic level, establishing standardized functional models, matched experimental conditions and direct pathway comparisons is essential for clarifying the context-dependent function of miR-324-5p and assessing mechanistic reproducibility. Integrating miR-324-5p with other molecular markers and multi-omics datasets may provide a more comprehensive understanding of its regulatory context. Computational approaches, including machine learning-based analyses, may provide additional support for interpreting multidimensional datasets (110).

The development of miR-324-5p-based therapeutic strategies is constrained by challenges associated with miRNA stability and delivery. Rapid degradation, systemic clearance, limited tumor targeting and potential immune activation compromise therapeutic efficacy and contribute to unintended biological effects (111). Delivery platforms, including exosomes (112), poly (lactic-co-glycolic acid)-based

Table IV. miR-324-5p in LC and GC.

Tumor type	Sample cohort	Clinical context/treatment	miR-324-5p effects	Experimental model	Methods	Target/ceRNA	(Refs.)
GC	Paired GC/adjacent tissue (n=5); GEO/TCGA datasets	Stage not reported	Downregulated in paired GC tissue; associated with circ_0091994/miR-324-5p/HMGA1-associated functional effects	AGS, MKN45, HGC-27; xenograft	GEO/TCGA analysis; RT-qPCR; CCK-8, apoptosis, scratch and luciferase assay; xenograft	circ_0091994/miR-324-5p/HMGA1	(14)
GC	122 primary GC with adjacent normal tissue	TNM I-IV	Upregulated in GC; promotes proliferation, migration and invasion and inhibits apoptosis; higher expression associated with poorer prognosis	AGS, MKN45	RT-qPCR; gain/loss-of-function experiments; proliferation/apoptosis, Transwell and luciferase assay	miR-324-5p/PTEN	(15)
LC	Paired LC/adjacent tissue (n=100)	Clinical cohort includes mixed histology	miR-324-5p mimic attenuates GATA6-AS1-mediated inhibition of proliferation and invasion	NCI-H1975, A549	RT-qPCR; CCK-8; Transwell assay and dual-luciferase assay; rescue experiments	GATA6-AS1/miR-324-5p/FBXO11	(17)
LUAD; LUSC	TCGA: LUAD, 458 tumor/46 normal; LUSC, 342 tumor/45 normal	Not applicable	Upregulated in LUAD/LUSC; overexpression increases A549 proliferation and invasion	A549 cells	TCGA small RNA profiling; RT-qPCR; miR mimic; proliferation/invasion assay	GPX3, RCAN1, MGAT3; predicted/association-supported candidates	(38)
LC	Cell-based study	Not applicable	miR-324-5p attenuates PCAT-1-associated proliferation and promotes apoptosis	A549, SPC-A-1, NCI-H1734 and H460 cells; 16HBE cells; subsequent mechanistic experiments performed in A549 cells	RT-qPCR; RNA pull-down, luciferase and viability/apoptosis assay	PCAT-1/miR-324-5p/RAP1A	(39)
GC	Paired GC/adjacent tissue (n=80); miR-324-5p subset: 30 pairs	pTNM I-III	Upregulated in the 30-pair GC tissue comparison	AGS, HGC-27 and MGC-803 cells; xenograft model	RT-qPCR; CCK-8, Transwell and luciferase assay; xenograft	circ_0049447/miR-324-5p; PTPRD-associated mechanism	(40)

miR, microRNA; LC, lung cancer; GC, gastric cancer; ceRNA, competing endogenous RNA; GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; circ, circular RNA; HMGA1, high mobility group A1; RT-qPCR, reverse transcription quantitative polymerase chain reaction; CCK-8, Cell Counting Kit-8; TNM, tumor-node-metastasis; GATA6-AS1, GATA6 antisense RNA 1; FBXO11, F-box protein 11; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; RNA, ribonucleic acid; GPX3, glutathione peroxidase 3; RCAN1, regulator of calcineurin 1; MGAT3, mannosyl (β -1,4-) glycoprotein β -1,4-N-acetylglucosaminyltransferase; PCAT-1, prostate cancer associated transcript 1; RAP1A, RAP1A member of RAS oncogene family; pTNM, pathological tumor-node-metastasis; PTPRD, protein tyrosine phosphatase receptor type D.

nanoparticles (113) and targeted liposome systems (114), have been explored to protect miRNA stability and facilitate intracellular delivery. Stimuli-responsive approaches, such as pH- or enzyme-sensitive materials (115), and tumor-directed strategies, including CD44-targeted delivery systems (116), may improve delivery efficiency and specificity. However, most of these approaches remain in the preclinical stage.

From a translational perspective, combining miR-324-5p-based approaches, including mimics or inhibitors, with chemotherapy, radiotherapy or immunotherapy may enable simultaneous modulation of complementary cancer-associated pathways and improve treatment sensitivity (117,118). However, the feasibility of such combinations may depend on tumor type, molecular subtype, dominant downstream targets of miR-324-5p and the direction of its dysregulation. Future studies should identify patient subsets that may benefit from miR-324-5p restoration or inhibition. Studies should prioritize validation using standardized preclinical models and comprehensive evaluation of therapeutic efficacy and safety (118). Although clinical experience from other miRNA-based therapeutics provides valuable guidance (119), to the best of our knowledge, direct clinical evidence supporting miR-324-5p-based therapy remains unavailable. Carefully designed multicenter and prospective studies are necessary before miR-324-5p-based strategies can be integrated into precision oncology approaches.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

KZ wrote the manuscript and constructed figures. ZX performed the literature review and wrote the manuscript. YBL performed the literature review. CQL revised the manuscript. YQZ conceived the study. KZ and YQZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Zhu Y, Wang C, Becker SA, Hurst K, Nogueira LM, Findlay VJ and Camp ER: miR-145 antagonizes SNAIL-Mediated stemness and radiation resistance in colorectal cancer. *Mol Ther* 26: 744-754, 2018.
2. Wang X, Geng H, Yao Z, Jiang Y, Chen C, Lu Z, Tian S, Zhang M, Liu R, Feng C, *et al*: Mapping genetic regulation of transcription to identify functional variants and genes associated with pancreatic cancer risk. *Adv Sci (Weinh)* 13: e17184, 2026.
3. Chang M, Papazyan T, Pons-Tostivint E and Fradin D: Unlocking the power of non-coding RNAs: Toward real-time cancer monitoring in precision oncology. *Mol Cancer* 25: 34, 2026.
4. Leng X, Zhang M, Xu Y, Wang J, Ding N, Yu Y, Sun S, Dai W, Xue X, Li N, *et al*: Non-coding RNAs as therapeutic targets in cancer and its clinical application. *J Pharm Anal* 14: 100947, 2024.
5. Calin GA and Croce CM: MicroRNA signatures in human cancers. *Nat Rev Cancer* 6: 857-866, 2006.
6. Ventura A and Jacks T: MicroRNAs and cancer: Short RNAs go a long way. *Cell* 136: 586-591, 2009.
7. Fadaka AO, Akinsoji T, Klein A, Madiehe AM, Meyer M, Keyster M, Sikhivihilu LM and Sibuyi NRS: Stage-specific treatment of colorectal cancer: A microRNA-nanocomposite approach. *J Pharm Anal* 13: 1235-1251, 2023.
8. Lim SY, Boyd SC, Diefenbach RJ and Rizos H: Circulating MicroRNAs: Functional biomarkers for melanoma prognosis and treatment. *Mol Cancer* 24: 99, 2025.
9. Kadkhoda S, Hussien BM, Eslami S and Ghafouri-Fard S: A review on the role of miRNA-324 in various diseases. *Front Genet* 13: 950162, 2022.
10. Wei X, Ke H, Wen A, Gao B, Shi J and Feng Y: Structural basis of microRNA processing by Dicer-like 1. *Nat Plants* 7: 1389-1396, 2021.
11. Zhang X, Wang Y, Zhao A, Kong F, Jiang L and Wang J: Long non-coding RNA LINC00511 accelerates proliferation and invasion in cervical cancer through targeting miR-324-5p/DRAM1 axis. *Onco Targets Ther* 13: 10245-10256, 2020.
12. Zhang C, Lv H, Zhang F and Ji A: LncRNA HCG18 facilitates melanoma progression by modulating miR-324-5p/CDK16 axis. *Am J Transl Res* 14: 1246-1257, 2022.
13. Rong Z, Wang Z, Wang X, Qin C and Geng W: Molecular interplay between linc01134 and YY1 dictates hepatocellular carcinoma progression. *J Exp Clin Cancer Res* 39: 61, 2020.
14. Xie Y, Liu Z and Zhu H: Knockdown of hsa_circ_0091994 constrains gastric cancer progression by suppressing the miR-324-5p/HMGAl axis. *Aging (Albany NY)* 13: 20598-20608, 2021.
15. Zheng Z, Li J, An J, Feng Y and Wang L: High miR-324-5p expression predicts unfavorable prognosis of gastric cancer and facilitates tumor progression in tumor cells. *Diagn Pathol* 16: 5, 2021.
16. Ba Z, Zhou Y, Yang Z, Xu J and Zhang X: miR-324-5p upregulation potentiates resistance to cisplatin by targeting FBXO11 signalling in non-small cell lung cancer cells. *J Biochem* 166: 517-527, 2019.
17. Wang Z, Pan L, Yang L, Lv P, Mai S and Wang Y: Long non-coding RNA GATA6-AS1 sponges miR-324-5p to inhibit lung cancer cell proliferation and invasion. *Onco Targets Ther* 13: 9741-9751, 2020.
18. Yang Y, Xia S, Ni X, Zhang L, Wang W, Chen L and Zhan W: MiR-324-5p/PTPRD/CEBPD axis promotes papillary thyroid carcinoma progression via microenvironment alteration. *Cancer Biol Ther* 21: 522-532, 2020.
19. Yang Y, Xia S, Ni X, Zhang L, Wang W, Kong Y, Wang Y, Ye L and Zhan W: MiR-324-5p assists ultrasonography in predicting lymph node metastasis of unifocal papillary thyroid microcarcinoma without extracapsular spread. *Oncotarget* 8: 83802-83816, 2017.
20. Gu C, Zhang M, Sun W and Dong C: Upregulation of miR-324-5p inhibits proliferation and invasion of colorectal cancer cells by targeting ELAVL1. *Oncol Res* 27: 515-524, 2019.
21. Huang M, Huang X and Huang N: Exosomal circGSE1 promotes immune escape of hepatocellular carcinoma by inducing the expansion of regulatory T cells. *Cancer Sci* 113: 1968-1983, 2022.
22. Wang W, Yang T, Li D, Huang Y, Bai G and Li Q: LINC00491 promotes cell growth and metastasis through miR-324-5p/ROCK1 in liver cancer. *J Transl Med* 19: 504, 2021.
23. Zhi T, Yu T, Pan M, Nie E, Wu W, Wang X, Liu N, You Y, Wang Y and Zhang J: EZH2 alteration driven by microRNA-524-5p and microRNA-324-5p promotes cell proliferation and temozolomide resistance in glioma. *Oncotarget* 8: 96239-96248, 2017.

24. Xu HS, Zong HL, Shang M, Ming X, Zhao JP, Ma C and Cao L: MiR-324-5p inhibits proliferation of glioma by target regulation of GLI1. *Eur Rev Med Pharmacol Sci* 18: 828-832, 2014.
25. Zhao JA, Nie W, Dong L, Liu W and Wei W: A curcumin analog GL63 inhibits the malignant behaviors of hepatocellular carcinoma by inactivating the JAK2/STAT3 signaling pathway via the circular RNA zinc finger protein 83/microRNA-324-5p/cyclin-dependent kinase 16 axis. *J Gastroenterol Hepatol* 36: 2967-2977, 2021.
26. Liu F, Xiao Y, Ma L and Wang J: Regulating of cell cycle progression by the lncRNA CDKN2B-AS1/miR-324-5p/ROCK1 axis in laryngeal squamous cell cancer. *Int J Biol Markers* 35: 47-56, 2020.
27. Cao L, Xie B, Yang X, Liang H, Jiang X, Zhang D, Xue P, Chen D and Shao Z: MiR-324-5p suppresses hepatocellular carcinoma cell invasion by counteracting ECM degradation through Post-transcriptionally downregulating ETS1 and SP1. *PLoS One* 10: e0133074, 2015.
28. Danaher P, Warren S, Lu R, Samayoa J, Sullivan A, Pekker I, Wallden B, Marincola FM and Cesano A: Pan-cancer adaptive immune resistance as defined by the Tumor Inflammation Signature (TIS): Results from the cancer genome atlas (TCGA). *J Immunother Cancer* 6: 63, 2018.
29. Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM and Ruvkun G: Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc Natl Acad Sci USA* 101: 360-365, 2004.
30. Kozomara A, Birgaoanu M and Griffiths-Jones S: miRBase: From microRNA sequences to function. *Nucleic Acids Res* 47: D155-D162, 2019.
31. Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, Sabedot TS, Malta TM, Pagnotta SM, Castiglioni I, *et al*: TCGAbiolinks: An R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res* 44: e71, 2016.
32. Sung H, Filho AM, Laversanne M, Ferlay J, Siegel RL, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide for 34 cancers in 186 countries. *CA Cancer J Clin* 76: e70090, 2026.
33. Ni Y, Zhang W, Mu G, Gu Y, Wang H, Wei K, Xia Y, Xie X, Ge Q, Tan T and Wang J: Extracellular RNA profiles in non-small cell lung cancer plasma. *J Thorac Dis* 15: 2742-2753, 2023.
34. Zaporozhchenko IA, Morozkin ES, Ponomaryova AA, Rykova EY, Cherdynseva NV, Zheravin AA, Pashkovskaya OA, Pokushalov EA, Vlassov VV and Laktionov PP: Profiling of 179 miRNA expression in blood plasma of lung cancer patients and Cancer-Free individuals. *Sci Rep* 8: 6348, 2018.
35. Skaar JR, Pagan JK and Pagano M: Mechanisms and function of substrate recruitment by F-box proteins. *Nat Rev Mol Cell Biol* 14: 369-381, 2013.
36. Fielenbach N, Guardavaccaro D, Neubert K, Chan T, Li D, Feng Q, Hutter H, Pagano M and Antebi A: DRE-1: An evolutionarily conserved F box protein that regulates *C. elegans* developmental age. *Dev Cell* 12: 443-455, 2007.
37. Zhao S, Xiao M, Li L, Zhang H, Shan M, Cui S, Zhang L, Zhang G, Wu S, Jin C, *et al*: A unique circ_0067716/EIF4A3 double-negative feedback loop impacts malignant transformation of human bronchial epithelial cells induced by benzo(a) pyrene. *Sci Total Environ* 923: 171349, 2024.
38. Lin MH, Chen YZ, Lee MY, Weng KP, Chang HT, Yu SY, Dong BJ, Kuo FR, Hung LT, Liu LF, *et al*: Comprehensive identification of microRNA arm selection preference in lung cancer: miR-324-5p and -3p serve oncogenic functions in lung cancer. *Oncol Lett* 15: 9818-9826, 2018.
39. Huang N, Dai W, Li Y, Sun J, Ma C and Li W: LncRNA PCAT-1 upregulates RAPIA through modulating miR-324-5p and promotes survival in lung cancer. *Arch Med Sci* 16: 1196-1206, 2020.
40. Tang KW, Guo ZX, Wu ZH, Zhou C, Sun J, Wang X, Song YX and Wang ZN: Circ_0049447 acts as a tumor suppressor in gastric cancer through reducing proliferation, migration, invasion, and epithelial-mesenchymal transition. *Chin Med J (Engl)* 134: 1345-1355, 2021.
41. Zhang C, Yang J, Chen Y, Jiang F, Liao H, Liu X, Wang Y, Kong G, Zhang X, Li J, *et al*: miRNAs derived from plasma small extracellular vesicles predict organo-tropic metastasis of gastric cancer. *Gastric Cancer* 25: 360-374, 2022.
42. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, Ji X, Liu W, Huang B, Luo W, *et al*: Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes Dis* 5: 77-106, 2018.
43. Turashvili G, Lightbody ED, Tyryshkin K, SenGupta SK, Elliott BE, Madarnas Y, Ghaffari A, Day A and Nicol CJB: Novel prognostic and predictive microRNA targets for triple-negative breast cancer. *FASEB J*: fj201800120R, 2018.
44. Pei J, Peng Y, Ma K, Lan C, Zhang T, Li Y, Chen X and Gao H: Integrated analysis reveals FLI1 regulates the tumor immune microenvironment via its cell-type-specific expression and transcriptional regulation of distinct target genes of immune cells in breast cancer. *BMC Genomics* 25: 250, 2024.
45. Gómez-Acebo I, Llorca J, Alonso-Molero J, Díaz-Martínez M, Pérez-Gómez B, Amiano P, Belmonte T, Molina AJ, Burgui R, Castaño-Vinyals G, *et al*: Circulating miRNAs signature on breast cancer: the MCC-Spain project. *Eur J Med Res* 28: 480, 2023.
46. Lou W, Ding B, Wang S and Fu P: Overexpression of GPX3, a potential biomarker for diagnosis and prognosis of breast cancer, inhibits progression of breast cancer cells in vitro. *Cancer Cell Int* 20: 378, 2020.
47. Song L, Liu D, Zhao Y, He J, Kang H, Dai Z, Wang X, Zhang S and Zan Y: Sinomenine inhibits breast cancer cell invasion and migration by suppressing NF- κ B activation mediated by IL-4/miR-324-5p/CUEDC2 axis. *Biochem Biophys Res Commun* 464: 705-710, 2015.
48. Fuller RN, Morcos A, Bustillos JG, Molina DC and Wall NR: Small non-coding RNAs and pancreatic ductal adenocarcinoma: Linking diagnosis, pathogenesis, drug resistance, and therapeutic potential. *Biochim Biophys Acta Rev Cancer* 1879: 189153, 2024.
49. Wan Y, Luo H, Yang M, Tian X, Peng B, Zhan T, Chen X, Ding Y, He J, Cheng X, *et al*: miR-324-5p contributes to cell proliferation and apoptosis in pancreatic cancer by targeting KLF3. *Mol Ther Oncolytics* 18: 432-442, 2020.
50. Pisano A, Sabalic A, Forte G, Fenu G, Bocca B, Etzi F, Tutedde D, Trignano C, Fiorito G, Massányi P and Madeddu R: Relationship between heavy metals and miRNAs in pancreatic ductal adenocarcinoma. *J Trace Elem Med Biol* 92: 127754, 2025.
51. Fenu G, Griñán-Lisón C, Pisano A, Hernández-Camarero P, López-Ruiz E, Griñán-Lisón C, Giovannetti E, Marchal JA and Perán M: Unveiling the microRNA landscape in pancreatic ductal adenocarcinoma patients and cancer cell models. *BMC Cancer* 24: 1308, 2024.
52. Eissa S, Safwat M, Matboli M, Zaghoul A, El-Sawalhi M and Shaheen A: Measurement of urinary level of a specific competing endogenous RNA network (FOS and RCAN mRNA/miR-324-5p, miR-4738-3p, /lncRNA miR-497-HG) Enables diagnosis of bladder cancer. *Urol Oncol* 37: 292.e19-292.e27, 2019.
53. Tsai KW, Kuo WT and Jeng SY: microRNA-324 plays an oncogenic role in bladder cancer cell growth and motility. *Transl Cancer Res* 9: 707-716, 2020.
54. Lai M, Liu G, Li R, Bai H, Zhao J, Xiao P and Mei J: Hsa_circ_0079662 induces the resistance mechanism of the chemotherapy drug oxaliplatin through the TNF- α pathway in human colon cancer. *J Cell Mol Med* 24: 5021-5027, 2020.
55. Bamodu OA, Yang CK, Cheng WH, Tzeng DTW, Kuo KT, Huang CC, Deng L, Hsiao M, Lee WH and Yeh CT: 4-Acetyl-Antroquinonol B Suppresses SOD2-enhanced cancer stem Cell-Like phenotypes and chemoresistance of colorectal cancer cells by Inducing hsa-miR-324 re-Expression. *Cancers (Basel)* 10: 269, 2018.
56. Davidson LA, Wang N, Shah MS, Lupton JR, Ivanov I and Chapkin RS: n-3 Polyunsaturated fatty acids modulate carcinogen-directed non-coding microRNA signatures in rat colon. *Carcinogenesis* 30: 2077-2084, 2009.
57. Faggad A, Kasajima A, Weichert W, Stenzinger A, Elwali NE, Dietel M and Denkert C: Down-regulation of the microRNA processing enzyme Dicer is a prognostic factor in human colorectal cancer. *Histopathology* 61: 552-561, 2012.
58. Sun LN, Xing C, Zhi Z, Liu Y, Chen LY, Shen T, Zhou Q, Liu YH, Gan WJ, Wang JR, *et al*: Dicer suppresses cytoskeleton remodeling and tumorigenesis of colorectal epithelium by miR-324-5p mediated suppression of HMGXB3 and WASF-2. *Oncotarget* 8: 55776-55789, 2017.
59. Huang G, Yang Y, Lv M, Huang T, Zhan X, Kang W and Hou J: VPS9D1-AS1, a novel long-non-coding RNA, acts as a tumor promoter by regulating the miR-324-5p/ITGA2 axis in colon adenocarcinoma. *Am J Transl Res* 14: 955-966, 2022.
60. Kang HS, Park HY, Lim H, Son IT, Kim MJ, Kim NY, Kim MJ, Nam ES, Cho SJ and Kwon MJ: Different miRNAs related to FBXW7 mutations or high mitotic indices contribute to rectal neuroendocrine tumors: A pilot study. *Int J Mol Sci* 24: 6329, 2023.
61. Forner A, Reig M and Bruix J: Hepatocellular carcinoma. *Lancet* 391: 1301-1314, 2018.

62. Wang C, Zhang X, Yu J, Bu J, Gu X, Wang Y, Zhu X and Lin J: Spotlights on extracellular vesicles in hepatocellular carcinoma diagnosis and treatment: An update review. *Front Bioeng Biotechnol* 11: 1215518, 2023.
63. You J, Xia H, Huang Z, He R, Zhao X, Chen J, Liu S, Xu Y and Cui Y: Research progress of circulating non-coding RNA in diagnosis and treatment of hepatocellular carcinoma. *Front Oncol* 13: 1204715, 2023.
64. Zhang X, Daucher M, Armistead D, Russell R and Kottlilil S: MicroRNA expression profiling in HCV-infected human hepatoma cells identifies potential anti-viral targets induced by interferon- α . *PLoS One* 8: e55733, 2013.
65. Huang X, Sun L, Wen S, Deng D, Wan F, He X, Tian L, Liang L, Wei C, Gao K, *et al*: RNA sequencing of plasma exosomes revealed novel functional long noncoding RNAs in hepatocellular carcinoma. *Cancer Sci* 111: 3338-3349, 2020.
66. Zhang Y, Wei H, Fan L, Fang M, He X, Lu B and Pang Z: CLECs as potential therapeutic targets in hepatocellular carcinoma microenvironment. *Front Cell Dev Biol* 9: 681372, 2021.
67. Fan MJ, He PJ, Lin XY, Yang CR, Li CZ and Xing LG: MicroRNA-324-5p affects the radiotherapy response of cervical cancer via targeting ELAV-like RNA binding protein 1. *Kaohsiung J Med Sci* 36: 965-972, 2020.
68. Chen SN, Chang R, Lin LT, Chern CU, Tsai HW, Wen ZH, Li YH, Li CJ and Tsui KH: MicroRNA in ovarian cancer: Biology, pathogenesis, and therapeutic opportunities. *Int J Environ Res Public Health* 16: 1510, 2019.
69. Biegała L, Kołat D, Gajek A, Szymczak-Pajor I, Marczak A, Śliwińska A and Rogalska A: Uncovering miRNA-mRNA regulatory networks related to olaparib resistance and resensitization of BRCA2(MUT) ovarian cancer PEO1-OR cells with the ATR/CHK1 pathway inhibitors. *Cells* 13: 867, 2024.
70. Kuznetsov VA, Tang Z and Ivshina AV: Identification of common oncogenic and early developmental pathways in the ovarian carcinomas controlling by distinct prognostically significant microRNA subsets. *BMC Genomics* 18: 692, 2017.
71. Ren L, Huo X and Zhao Y: CircZNF609/miR-324-5p/voltage-dependent anion channel 1 axis promotes malignant progression of ovarian cancer cells. *iScience* 27: 110861, 2024.
72. Cowan AJ, Green DJ, Kwok M, Lee S, Coffey DG, Holmberg LA, Tuazon S, Gopal AK and Libby EN: Diagnosis and management of multiple myeloma: A review. *JAMA* 327: 464-477, 2022.
73. Tang B, Xu A, Xu J, Huang H, Chen L, Su Y, Zhang L, Li J, Fan F, Deng J, *et al*: MicroRNA-324-5p regulates stemness, pathogenesis and sensitivity to bortezomib in multiple myeloma cells by targeting hedgehog signaling. *Int J Cancer* 142: 109-120, 2018.
74. Wu B, Wang F, Wang Y, Deng X and Wu W: CircATIC contributes to multiple myeloma progression via miR-324-5p-Dependent regulation of HGF. *Biochem Genet* 60: 2515-2532, 2022.
75. Lapointe S, Perry A and Butowski NA: Primary brain tumours in adults. *Lancet* 392: 432-446, 2018.
76. Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS and Khasraw M: Management of glioblastoma: State of the art and future directions. *CA Cancer J Clin* 70: 299-312, 2020.
77. Wang P, Wang T, Dong L, Xu Z, Guo S and Chang C: Circular RNA circ_0079593 facilitates glioma development via modulating miR-324-5p/XBP1 axis. *Metab Brain Dis* 37: 2389-2403, 2022.
78. Zhang J, Li Y, Liu Y, Xu G, Hei Y, Lu X and Liu W: Long non-coding RNA NEAT1 regulates glioma cell proliferation and apoptosis by competitively binding to microRNA-324-5p and upregulating KCTD20 expression. *Oncol Rep* 46: 125, 2021.
79. Li D, Li L, Chen X, Yang W and Cao Y: Circular RNA SERPINE2 promotes development of glioblastoma by regulating the miR-361-3p/miR-324-5p/BCL2 signaling pathway. *Mol Ther Oncolytics* 22: 483-494, 2021.
80. Regazzo G, Vari G, Marchesi F, Díaz Méndez AB, Di Giuliani M, Sacconi A, Palombi F, Lulli V, Goeman F, Novello M, *et al*: A predictive serum miRNA signature impacts diffuse large B-cell lymphoma cell viability via inhibition of EGLN1 and TXNRD1 regulators of ferroptosis. *Br J Haematol* 207: 802-812, 2025.
81. Lim EL, Trinh DL, Scott DW, Chu A, Krzywinski M, Zhao Y, Robertson AG, Mungall AJ, Schein J, Boyle M, *et al*: Comprehensive miRNA sequence analysis reveals survival differences in diffuse large B-cell lymphoma patients. *Genome Biol* 16: 18, 2015.
82. Misir S, Ozer Yaman S, Petrovic N, Šami A, Akidan O, Hepokur C and Aliyazicioglu Y: Identification of a Novel hsa_circ_0058058/miR-324-5p axis and Prognostic/Predictive molecules for acute myeloid leukemia outcome by bioinformatics-based analysis. *Biology (Basel)* 13: 487, 2024.
83. Dixon-McIver A, East P, Mein CA, Cazier JB, Molloy G, Chaplin T, Andrew Lister T, Young BD and Debernardi S: Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. *PLoS One* 3: e2141, 2008.
84. Tauriello DVF, Sancho E and Batlle E: Overcoming TGF β -mediated immune evasion in cancer. *Nat Rev Cancer* 22: 25-44, 2022.
85. Lang C, Dai Y, Wu Z, Yang Q, He S, Zhang X, Guo W, Lai Y, Du H, Wang H, *et al*: SMAD3/SP1 complex-mediated constitutive active loop between lncRNA PCAT7 and TGF- β signaling promotes prostate cancer bone metastasis. *Mol Oncol* 14: 808-828, 2020.
86. Jing J, Wu Z, Wang J, Luo G, Lin H, Fan Y and Zhou C: Hedgehog signaling in tissue homeostasis, cancers, and targeted therapies. *Signal Transduct Target Ther* 8: 315, 2023.
87. Yu H, Lee H, Herrmann A, Buettner R and Jove R: Revisiting STAT3 signalling in cancer: New and unexpected biological functions. *Nat Rev Cancer* 14: 736-746, 2014.
88. Taniguchi K and Karin M: NF- κ B, inflammation, immunity and cancer: Coming of age. *Nat Rev Immunol* 18: 309-324, 2018.
89. Ghatak D, Datta A, Roychowdhury T, Chattopadhyay S and Roychowdhury S: MicroRNA-324-5p-CUEDC2 axis mediates gain-of-function mutant p53-driven cancer stemness. *Mol Cancer Res* 19: 1635-1650, 2021.
90. Zhang X, Zhang L, Chen M and Liu D: miR-324-5p inhibits gallbladder carcinoma cell metastatic behaviours by downregulation of transforming growth factor beta 2 expression. *Artif Cells Nanomed Biotechnol* 48: 315-324, 2020.
91. Liu Y, Chang Y and Cai Y: Circ_0067835 sponges miR-324-5p to induce HMGA1 expression in endometrial carcinoma cells. *J Cell Mol Med* 24: 13927-13937, 2020.
92. Huang S, Hu J, Hu M, Hou Y, Zhang B, Liu J, Liu X, Chen Z and Wang L: Cooperation between SIX1 and DHX9 transcriptionally regulates integrin-focal adhesion signaling mediated metastasis and sunitinib resistance in KIRC. *Oncogene* 43: 2951-2969, 2024.
93. Sun Q, Ye Z, Qin Y, Fan G, Ji S, Zhuo Q, Xu W, Liu W, Hu Q, Liu M, *et al*: Oncogenic function of TRIM2 in pancreatic cancer by activating ROS-related NRF2/ITGB7/FAK axis. *Oncogene* 39: 6572-6588, 2020.
94. Molist C, Navarro N, Giralt I, Zarzosa P, Gallo-Oller G, Pons G, Magdaleno A, Moreno L, Guillén G, Hladun R, *et al*: miRNA-7 and miRNA-324-5p regulate alpha9-Integrin expression and exert anti-oncogenic effects in rhabdomyosarcoma. *Cancer Lett* 477: 49-59, 2020.
95. He B, Zhao Z, Cai Q, Zhang Y, Zhang P, Shi S, Xie H, Peng X, Yin W, Tao Y and Wang X: miRNA-based biomarkers, therapies, and resistance in Cancer. *Int J Biol Sci* 16: 2628-2647, 2020.
96. Yan Y, Liu S, Wen J, He Y, Duan C, Nabavi N, Ashrafzadeh M, Sethi G, Liu L and Ma R: Advances in RNA-based cancer therapeutics: Pre-clinical and clinical implications. *Mol Cancer* 24: 251, 2025.
97. Zhang C, Qin C, Dewanjee S, Bhattacharya H, Chakraborty P, Jha NK, Gangopadhyay M, Jha SK and Liu Q: Tumor-derived small extracellular vesicles in cancer invasion and metastasis: Molecular mechanisms, and clinical significance. *Mol Cancer* 23: 18, 2024.
98. Li C, Lagunas-Rangel FA, Du L, Sun C and Schiöth HB: LncRNA PVT1 in human cancers: Genomic complexity, isoforms, functional elements, mechanism of action, subcellular localization and possible role as a therapeutic target. *Mol Cancer* 25: 2026, 2026.
99. Ma Y, Wang T, Zhang X, Wang P and Long F: The role of circular RNAs in regulating resistance to cancer immunotherapy: Mechanisms and implications. *Cell Death Dis* 15: 312, 2024.
100. Zhang W, Xu C, Yang Z, Zhou J, Peng W, Zhang X, Li H, Qu S and Tao K: Circular RNAs in tumor immunity and immunotherapy. *Mol Cancer* 23: 171, 2024.
101. Zhang W, Jiang A, Jia BK, Jin Y, Chen Y, Li Z, Liao Y, Zhang H, Lin Z, Fang X and Wang L: Progress in RNA-Targeted therapeutics for human diseases. *MedComm (2020)* 7: e70607, 2026.
102. To KKW, Huang Z, Zhang H, Ashby CR Jr and Fu L: Utilizing non-coding RNA-mediated regulation of ATP binding cassette (ABC) transporters to overcome multidrug resistance to cancer chemotherapy. *Drug Resist Updat* 73: 101058, 2024.
103. Eichmüller SB, Osen W, Mandelboim O and Seliger B: Immune modulatory microRNAs involved in tumor attack and tumor immune escape. *J Natl Cancer Inst*: 109, 2017 doi: 10.1093/jnci/djx034.

104. Guan L, Hao Q, Shi F, Gao B, Wang M, Zhou X, Han T and Ren W: Regulation of the tumor immune microenvironment by cancer-derived circular RNAs. *Cell Death Dis* 14: 132, 2023.
105. Hoober JK, van Vliet SJ, Dudziak D and Eggink LL: Editorial: Sentinel CLECs at Immunological Decision Nodes. *Front Immunol* 11: 2066, 2020.
106. Chen Y, Wang SX, Mu R, Luo X, Liu ZS, Liang B, Zhuo HL, Hao XP, Wang Q, Fang DF, *et al*: Dysregulation of the miR-324-5p-CUEDC2 axis leads to macrophage dysfunction and is associated with colon cancer. *Cell Rep* 7: 1982-1993, 2014.
107. Wang J, Chen H, Deng Q, Chen Y, Wang Z, Yan Z, Wang Y, Tang H, Liang H and Jiang Y: High expression of RNF169 is associated with poor prognosis in pancreatic adenocarcinoma by regulating tumour immune infiltration. *Front Genet* 13: 1022626, 2022.
108. Abid F, Khan K, Ashraf NM, Badshah Y, Shabbir M, Trembley JH, Afsar T, Almajwal A and Razak S: Genetic variants at PRKCG splice and UTR sites promote cancer susceptibility by disrupting epigenetic and miRNA Regulatory Network. *J Cancer* 15: 6644-6657, 2024.
109. Fam MK, Ismail NI, Zahari AI, Ghazali NA and Hassan N: Biosensor technologies in cancer: Tools for early detection and prognostic monitoring. *Biomark Res* 14: 8, 2025.
110. Pei J, Perumal NC, Meng P, Long Q and Palanisamy CP: Extracellular vesicle-based biosensors for Alzheimer's disease: A new frontier in precision diagnostics. *Ageing Res Rev* 113: 102904, 2026.
111. Forterre A, Komuro H, Aminova S and Harada M: A comprehensive review of cancer MicroRNA therapeutic delivery strategies. *Cancers* 12: 1852, 2020.
112. Kim YK, Choi Y, Nam GH and Kim IS: Functionalized exosome harboring bioactive molecules for cancer therapy. *Cancer Letters* 489: 155-162, 2020.
113. Ramasubramanian L, Bahatyrevich N, Hao D, Pivetti C, Loll EG, Paxton ZJ, Gidda SS, Kumar P, Farmer DL, Panitch A, *et al*: Development of novel targeted, Membrane-Coated polymer hybrid nanoparticles for endothelial repair following balloon angioplasty in rat carotid artery model. *Circulation* 148: A12931, 2023.
114. Cheng Z, Fobian SF, Gurrieri E, Amin M, D'Agostino VG, Falahati M, Zalba S, Debets R, Garrido MJ, Saeed M, *et al*: Lipid-based Nanosystems: The next generation of cancer immune therapy. *J Hematol Oncol* 17: 53, 2024.
115. Ding D and Jiang X: Advances in immunogenic cell death for cancer immunotherapy. *Small Methods* 7: e2300354, 2023.
116. Liu X, Adorno-Cruz V, Chang YF, Jia Y, Kawaguchi M, Dashzeveg NK, Taftaf R, Ramos EK, Schuster EJ, El-Shennawy L, *et al*: EGFR inhibition blocks cancer stem cell clustering and lung metastasis of triple negative breast cancer. *Theranostics* 11: 6632-6643, 2021.
117. Alexandre D, Baptista PV and Cruz C: Harnessing microRNAs in lung cancer: The future of diagnosis and precision therapy. *Biochim Biophys Acta Rev Cancer* 1881: 189535, 2026.
118. Qiu C, Wu Y, Shi Q, Guo Q, Zhang J, Meng Y, Wang C, Xia F, Wang J and Xu C: Advanced strategies for nucleic acids and small-molecular drugs in combined anticancer therapy. *Int J Biol Sci* 19: 789-810, 2023.
119. Rupaimoole R and Slack FJ: MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov* 16: 203-222, 2017.



Copyright © 2026 Zhang et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.