

REVIEW ARTICLE

Application of the microRNA-302/367 cluster in cancer therapy

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Abstract

As a novel class of noncoding RNAs, microRNAs (miRNAs) can effectively silence their target genes at the posttranscriptional level. Various biological processes, such as cell proliferation, differentiation, and motility, are regulated by miRNAs. In different diseases and different stages of disease, miRNAs have various expression patterns, which makes them candidate prognostic markers and therapeutic targets. Abnormal miRNA expression has been detected in numerous neoplastic diseases in humans, which indicates the potential role of miRNAs in tumorigenesis. Previous studies have indicated that miRNAs are involved in nearly the entire process of tumor development. MicroRNA-302a, miR-302b, miR-302c, miR-302d, and miR-367 are members of the miR-302/367 cluster that plays various biological roles in diverse neoplastic diseases by targeting different genes. These miRNAs have been implicated in several unique characteristics of cancer, including the evasion of growth suppressors, the sustained activation of proliferative signaling, the evasion of cell death and senescence, and the regulation of angiogenesis, invasion, and metastasis. This review provides a critical overview of miR-302/367 cluster dysregulation and the subsequent effects in cancer and highlights the vast potential of members of this cluster as therapeutic targets and novel biomarkers.

KEYWORDS

biological phenomena, cell dedifferentiation, MIRN302, neoplasm, therapy

Abbreviations: BAX, BCL2 associated X; BC, breast cancer; Bcl-2, B cell leukemia/lymphoma 2 apoptosis regulator; Bim, BCL2 like 11; CircRASSF2, Circular RNA RASSF2; CCN, cyclin; CCNA, cyclin A; CCND1, cyclin D1; CD49f, CD49 antigen-like family member F; CDK2, cyclin-dependent kinase 2; ceRNA, competing endogenous RNA; circRNAs, circular RNAs; COUPTFII, chicken ovalbumin upstream promoter transcription factor II; CSC, cancer stem cell; CXCR4, C-X-C chemokine receptor type 4; DCUN1D1, cullin neddylation 1 domain containing 1; DOCK4, dedicator of cytokinesis 4; E2F1, E2F transcription factor 1; E2F3, E2F transcription factor 3; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; EndMT, endothelial-mesenchymal transformation; EOMES, eomesodermin; ER, estrogen receptor; ErbB4, Receptor tyrosine-protein kinase erbB-4; Erk, extracellular regulated MAP kinase; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; GCMF, germ cell nuclear factor; GSK-3 β , glycogen synthase kinase-3 β ; HCC, hepatocellular carcinoma; HECC, human endometrial carcinoma cell; hESC, human embryonic stem cell; IGF-1R, insulin-like growth factor 1 receptor; iPSCs, induced pluripotent stem cells; JNK, c-Jun NH2-terminal kinase; KLF4, Kruppel Like Factor 4; KPNA2, NF- κ B-inducing kinase and karyopherin α 2; LATS2, large tumor suppressor kinase 2; lncRNA, long noncoding RNA; LSCC, laryngeal squamous cell carcinoma; Mcl-1, myeloid cell leukemia sequence 1; MIAT, myocardial infarction-associated transcript; miRNA, microRNA; mirPS, miRNA-induced pluripotent stem; MTDH, metadherin; ncRNA, noncoding RNA; NK, natural killer; Notch4, notch receptor 4; NR2F2, nuclear receptor subfamily 2 group F member 2; OC, ovarian cancer; OCT, organic cation/carnitine transporter; PCa, prostate cancer; P-gp, P-glycoprotein; PRP-1, proline-rich polypeptide 1; RACK1, receptor for activated C-kinase 1; S1pr1, sphingosine-1-phosphate receptor 1; SDC1, syndecan 1; SDF1, stromal cell-derived factor 1; SHH, sonic hedgehog; SOX2, SRY-box transcription factor 2; Tcf3, transcription factor 3; SSEA-4, stage-specific embryonic antigen-4; YAP, Yes-associated transcriptional regulator.

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1 | INTRODUCTION

MicroRNA, which was discovered in 1993, has become an emerging topic in biomedical research over the last few years. MicroRNAs are noncoding, single-stranded small RNAs of approximately 21 nucleotides. Previous studies found that miRNAs are important components of cellular development processes, including the regulation of cell stemness and the maintenance and progression of cardiovascular diseases, neurological diseases, immunological diseases, and cancer.¹ Specifically, miRNAs play a crucial role in tumor formation and are involved in nearly the entire process of tumor development. To regulate their target genes, miRNAs bind to complementary nucleotides within the 3'-UTR or ORF of coding mRNAs.² The genes are involved in a variety of biological processes, ranging from cell proliferation, apoptosis, growth, differentiation, and metabolism to vascularization and the immune response.

The has-miR-302/367 cluster, situated on chromosome band 4q25, is one of the most thoroughly studied groups of miRNAs. This cluster contains several members, including miR-302a/b/c/d and miR-367, which have various biological roles in diverse neoplastic diseases through different modes of gene regulation. This family is highly conserved and still evolving, and its potential targets and functions require further investigation.³ These targets regulate multiple biological behaviors in cells, including the cell cycle, EMT, epigenetic regulation, and vesicular transport.² This cluster also plays a significant role in hESC renewal and pluripotency maintenance as key regulatory factors and are involved in cellular stress response mechanisms.^{4,5} Cell stemness can be detected by markers such as Nanog and OCT4, which are core transcription factors that initiate their own pathways prior to regulation of the miR-302/367 family.³ Nanog, OCT4, and Tcf3, as classical transcription factors, can bind and occupy the promoter of miR-302 and regulate its expression.⁶ Researchers have found possible connections between the expression level of this cluster and tumor grade/state in many tissues. Significant changes in their expression levels were observed in tumor and adjacent tissue specimens. Most miRNAs in this cluster are tumor suppressors, but there are some exceptions.^{3,7}

Tumor initiation and progression are complex biological processes that occur in multiple stages and are influenced by several factors. Previous research showed that the miR302/367 cluster is involved in nearly the entire process of tumor development in various neoplasms. The miR-302/367 family participates in numerous hallmarks of cancer, regulating to some degree on the evasion of growth suppression, sustained activation of proliferative signaling, evasion of cell death and senescence, and induction of angiogenesis, invasion, and metastasis.^{3,7} This review highlights the status of miR-302/367 cluster dysregulation in cancer and reveals the potential of miR302/367 family members as therapeutic targets and novel biomarkers.

2 | REGULATORY NETWORK OF MIR-302/367 CLUSTER EXPRESSION

Many molecules regulate miRNA expression levels and functions, which occur both inside and outside the cell, through effects on

miRNA production and maturation. Cell viability and western blot assays showed that in glioblastoma, increased GSK-3 β activity promotes a positive feedback loop between DOCK4 and β -catenin, which in turn upregulates miR-302b production.⁸ Experimental results indicated that H3K9 activity is inhibited by the overexpression of PRP-1 in metastasis-derived chondrosarcoma, which leads to the downregulation of miR-302c, thereby achieving antiproliferative effects.⁹ MicroRNA-302/367, as well as OCT4, SOX2, KLF4, and c-MYC, can be activated by engineered zinc-finger transcription factors.¹⁰ OCT4, Nanog, SOX2, and EOMES clearly upregulate miR-302 gene expression, and Tcf3 inhibits miR-302 expression.^{10,11} In GC, real-time PCR analysis showed that the expression of the miR-302 cluster is upregulated by RACK1, and this result was confirmed in cell lines and clinical cases.¹² Electrophoretic mobility shift assays and chromatin ChIP assays confirmed that GCNF directly inhibits miR-302 by binding its promoter.¹³ In addition to transcription factors, miRNA sponges or decoys, termed ceRNAs, can regulate the levels of functional miRNAs. Competing endogenous RNAs include both protein-coding mRNAs and ncRNAs, such as long lncRNAs, pseudogenes, circRNAs, and viral RNAs, that release parental target mRNAs from miRNA control by competing with the miRNAs.¹⁴ Cross-talk between ceRNAs adds a new dimension to the regulation of miRNA expression and function. A large number of miR-302/367 family members can be encapsulated by exosomes and internalized by adjacent glioblastoma cells, thereby affecting cellular biological behavior.¹⁵ The regulation of miR-302/367 cluster expression in the nucleus, cytoplasm, and extracellular space and the intercellular exchange of miR-302/367 cluster members through exosomes contribute to the biological significance of the differential expression of this cluster (Figure 1).

3 | REGULATORY ROLE AND MECHANISMS OF MIR-302/367 CLUSTER REGARDING CELL STEMNESS

Stem cells can differentiate into other cell types and undergo self-renewal to produce more daughter cells. MicroRNA-302/367 can control the pluripotency and self-renewal of hESCs, iPSCs, and CSCs.¹⁶ The special ability of this cluster to change cell fate from somatic to pluripotent underscores its participation in regulating cell stemness through various pathways (Figure 2).

First, the miR-302b/367 cluster acts as a specific and critical regulator of hESC pluripotency and differentiation.^{3,17} Importantly, Oct3/4, Sox2, and Nanog, which are hESC-specific transcription factors, can transcriptionally regulate the miR-302/367 promoter.¹⁸ The downregulation of *Notch4*, a miR-302a target gene, leads to the downregulation of the Nodal signaling pathway.¹⁹ In addition, GCNF efficiently regulates the expression of CCND1 during hESC differentiation through the inhibition of miR-302a.¹³

Second, proper expression of this cluster is necessary for maintaining the stemness of iPSCs.²⁰ By suppressing both the CDK2 and CCND-CDK4/6 cell cycle pathways, this cluster can regulate iPSC tumorigenicity during the G₁-S phase transition.²¹

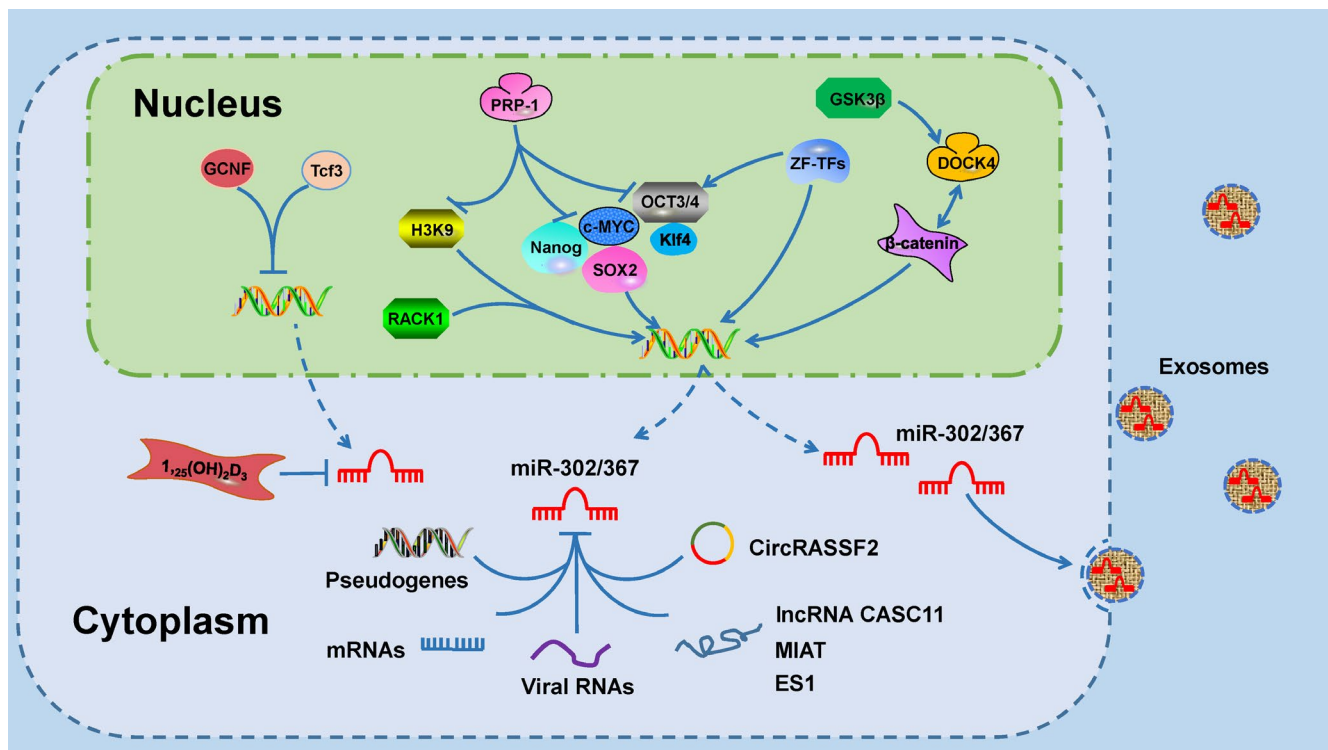


FIGURE 1 Regulatory network of microRNA (miR)-302/367 cluster expression. Many molecules regulate miRNA function inside and outside the cell by modulating miRNA production and maturation. The glycogen synthase kinase-3 β (GSK-3 β)/dedicator of cytokinesis 4 (DOCK4)/ β -catenin axis, zinc-finger transcription factors (ZF-TFs), Nanog, organic cation/carnitine transporter-3/4 (OCT3/4), and receptor for activated C-kinase 1 (RACK1) promote miR-302 expression, whereas the proline-rich polypeptide 1 (PRP-1)/H3K9 axis, transcription factor-3 (Tcf3), and germ cell nuclear factor (GCNF) inhibit miR-302/367 expression. Competing endogenous RNAs, including noncoding RNAs, pseudogenes, mRNAs, circular RNAs, and viral RNAs, can release parental target mRNAs from miRNA control by competition with the miRNA through their function as miRNA sponges or decoys. miR-302/367 can be encapsulated by exosomes and internalized by other cells, thereby affecting biological behavior. CASC11, Long noncoding RNA CASC11; CircRASSF2, circular RNA RASSF2; ES1, Long noncoding RNA ES1; Klf4, Kruppel like factor 4; lncRNA, long noncoding RNA; MIAT, myocardial infarction-associated transcript; SOX2, SRY-box transcription factor 2

Moreover, some researchers have found that differentiation ability can be inhibited by elevated p53 activity in miRNA-deficient iPSCs.²² Interestingly, miR-302 can repress the expression of *NR2F2*, a target gene verified by reporter luciferase assays and RT-PCR, and stimulate the expression of OCT4 to promote cell pluripotency.²³

In addition to its function in hESCs and iPSCs, this cluster also plays a vital role in CSCs, also called tumor-initiating cells.⁶ In the reproductive system, the miR-302 cluster can reportedly regulate male germline stem cell self-renewal through the posttranscriptional inhibition of gene expression. The miR-302 cluster can promote CD49f and OCT4 expression and downregulate P21 expression.²⁴ In addition, through an AKT1/OCT4-dependent pathway, miR-302 can regulate teratoma formation.²⁵ Similarly, tumor cell differentiation is modulated by the apparent downregulation of miR-302b in ESCC.²⁶ One study on HCC showed that the E2F7/AKT/ β -catenin/CCND1 pathway is regulated by miR-302a/d, which can inhibit the stemness of liver CSCs and the proliferation of tumor cells.²⁷ Furthermore, some evidence from a glioblastoma mouse model showed that the cell-to-cell transfer of miR-302/367 can result in the inhibition of CXCR4/SDF1, SHH, CCND, CCNA and E2F1, which are targets of miR-302/367.¹⁵

4 | FUNCTIONS AND MECHANISMS OF MIR-302 CLUSTER IN TUMOR DEVELOPMENT AND PROGRESSION

Tumor development is a complex biological process regulated by several signals and factors. Tumor cells exert different biological behaviors, including proliferation, apoptosis, metastasis, and drug resistance. Previous results show that the miR-302/367 cluster participates in nearly the entire process of tumor development in various neoplasms and exhibits different regulatory functions (Figure 3).^{3,7}

4.1 | Role of miR-302 cluster in sustaining proliferative signaling in cancer

The number of cells in normal tissue is essentially constant, and its regulation is guaranteed by a number of signaling pathways associated with cell proliferation. Increased cell proliferation is often observed in tumors, and tumor cells alter their biological behavior by promoting or inhibiting proliferative signals.²⁸ That is, tumor cells have the characteristic of sustained proliferation. The miR-302/367 family can affect the ability of tumor cells to proliferate

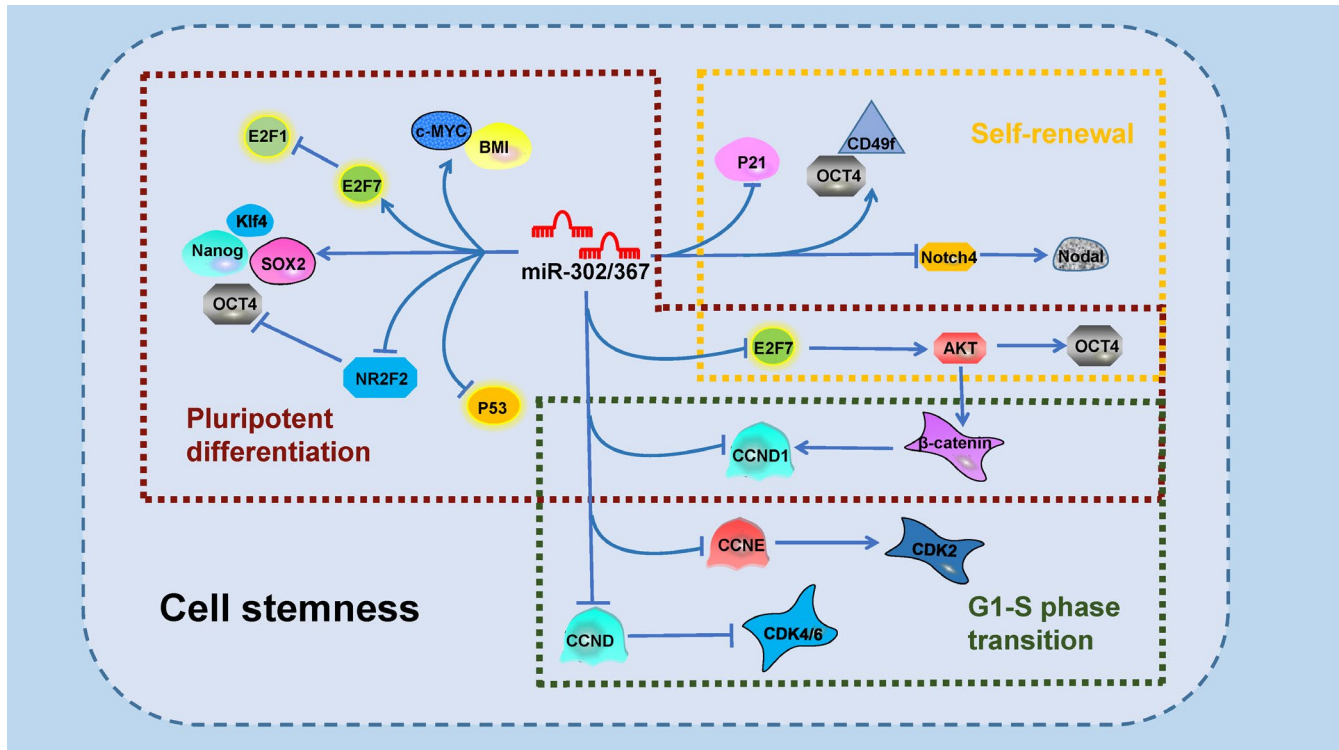


FIGURE 2 MicroRNA (miR)-302/367 cluster participates in the regulation of cell stemness. The miR-302/367 cluster controls the stemness of human embryonic stem cells, induced pluripotent stem cells, and cancer stem cells by regulating pluripotent differentiation, G₁-S phase transition, and self-renewal. The 3 major regulatory pathways are connected by nodal proteins such as AKT and organic cation/carnitine transporter 4 (OCT4). MYC, P21, cyclin D (CCND), and β-catenin are important in intracellular signaling networks in cancer. P21 and CCND are representative molecules involved in cytostasis and differentiation. Myc is a classical member of the proliferation and motility signaling networks. BMI, B cell-specific Moloney murine leukemia virus integration site 1; CCNE, cyclin E; CDK, cyclin-dependent kinase; E2F7, E2F transcription factor 7; Klf4, Kruppel like factor 4; NR2F2, nuclear receptor subfamily 2 group F member 2; SOX2, SRY-box transcription factor 2

continuously by regulating the cell cycle and other processes (Figure 3).

Members of this cluster exhibit tumor suppressor activity. In general, the growth ability of epithelial ovarian cancer, ESCC, HCC, BC, and glioma cells can be inhibited by miR-302b.²⁹⁻³² According to previous studies, miR-302b plays a tumor suppressor role in ESCC by repressing cell proliferation through the posttranscriptional downregulation of ErbB4.²⁶ Similarly, miR-302c functions as a tumor suppressor in BC by regulating ER to regulate cell proliferation and differentiation.³² The miR-302/367 family inhibits melanoma and colorectal cancer cells by suppressing proliferation, and *Ascl2* knockdown can arrest tumor growth by elevating miR-302b, which significantly inhibits colon cancer progenitor cell function.^{33,34} Evidence from *in vivo* and *in vitro* experiments indicates that *DOCK4* can restrain glioblastoma progenitor cell proliferation by upregulating nuclear β-catenin and subsequently increasing the expression of the miR-302/367 family.⁸

Although miR-302 was shown to inhibit clonal cell proliferation, tumor formation, and growth in some studies, other studies reached the opposite conclusion. The miR-302d/LATS2 axis can promote cell proliferation by targeting the Hippo pathway in cardiomyocytes.³⁵ In addition, upregulation of the miR-302/367 cluster in PCa has protumorigenic effects *in vivo* and *in vitro* through enhanced cell proliferation,

sphere formation, and migration through the miR-302/367/LATS2/YAP pathway.³⁶ However, in another PCa study, miR-302a functioned as a tumor suppressor by influencing the AKT-GSK-3β-CCND1 and AKT-p27^{Kip1}/p21^{Cip1} pathways.³⁷ The second PCa study did not verify the conclusions in animal models, and the clinical sample size was not large enough. Therefore, this conclusion needs further verification.

There are many studies on the mechanism of action of these family members in regard to the regulation of cell proliferation. miR-302a can restrain the proliferation of colon cancer, cervical cancer, melanoma, and colorectal cancer cells.^{33,37-39} Cyclins and CDKs are key regulatory factors that participate in DNA synthesis, chromosome separation, and cell division and are intimately involved in cell cycle regulation. Alterations in the AKT-GSK-3β-CCND1 and AKT-p27^{Kip1}/p21^{Cip1} pathways lead to decreased AKT expression through miR-302/367 family activity in ESCC and cervical cancer.³⁷⁻³⁹ MicroRNA-302b can suppress HCC cell proliferation by targeting the EGFR/AKT2/CCND1 pathway.^{29,30} As a novel direct target gene, *SDC1* can be downregulated by miR-302a, leading to the suppression of OC cell proliferation.³⁹ Recent research found that the miR-302/CDK1 axis can inhibit lung cancer cell proliferation, and this axis can be downregulated by the lncRNA *CASC11*.⁴⁰ Another lncRNA, *MIAT*, can act as an upstream regulator of miR-302b, promoting the proliferation of BC cells.⁴¹ Similarly, in LSCC,

progression and a worse patient prognosis.⁴⁶ Another study found that RACK1 downregulation can decrease miR-302c expression, resulting in increased interleukin-8 secretion and thereby promoting metastasis.¹² As a critical regulator of metastasis, CXCR4 is downregulated at the expression level by miR-302a, leading to decreased invasion and metastasis abilities of BC cells.⁴⁵ The role of the miR-302/367 cluster in BC is complex, and various studies have reached different conclusions. Estrogen receptor was shown by quantitative PCR and western blot analysis to be downregulated by miR-302c, and luciferase reporter assays confirmed that CXCR4 and ER can be directly targeted by miR-302c. In addition, the ER pathway can mediate invasion and migration and play an antitumor role.³² The downregulation of miR-302b by its upstream regulator, MIAT, can also promote BC cell migration.⁴¹ High lncRNA ES1 transcript levels in high-grade and P53-mutated BC tissues can lead to miR-302 upregulation and promote cell proliferation and migration.⁴⁷ In specific BC cell lines, some scholars have found that vitamin C can reduce the reprogramming efficiency of the miR-302/367 cluster by downregulating *TET1* gene expression, which reverses the inhibition of cell invasion and proliferation by this cluster.⁴⁸ Insulin-like growth factor-1R, can be directly targeted by miR-302a, which plays a tumor suppressor role by inhibiting the invasion and migration of osteosarcoma cells.⁴⁹ One recent study indicated that miR-302-3p can suppress cervical cancer metastasis through actions on its direct target, defective in DCUN1D1.⁵⁰ In contrast, the circRASSF2/miR-302b-3p/IGF-1R axis is protumorigenic in LSCC.⁴² Moreover, in ESCC, miR-302b overexpression can attenuate lymph node metastasis by suppressing ErbB4.²⁶

4.3 | Role of miR-302 cluster in resisting cancer cell death

Tumor cell apoptosis can occur at any stage of tumorigenesis as a mechanism by the host to prevent tumor progression, but abnormal cells can protect themselves from programmed cell death by apoptosis.²⁶ Recent discoveries have shown that the miR-302/367 cluster participates in signaling pathways that control apoptosis (Figure 3).

Among the increased miRNAs in mucoepidermoid carcinoma, miR-302a shows the most pronounced change.⁵¹ In OC, miR-302a downregulates SDC1 to increase tumor cell apoptosis.³⁹ All-trans retinoic acid can upregulate miR-302b by way of a retinoic acid receptor- α -mediated pathway, whereas high miR-302b expression promotes tumorigenic behaviors by directly targeting and downregulating E2F3, an important transcriptional regulator of glioblastoma proliferation and apoptosis.⁵² Correspondingly, Akt/pAkt, Bcl-2, Bim, and caspase-3 levels can be regulated by miR-302b to enhance osteosarcoma cell apoptosis.⁴³ One study of malignant pleural mesothelioma indicated that miR-302b can inhibit tumor cell tumor-sphere growth by targeting Mcl-1 to inhibit proliferation and induce apoptosis.⁵³ Additionally, in HECCs, miR-302b/c/d was confirmed to promote apoptosis by downregulating zinc-finger E-box binding homeobox 1 and Bcl-2 and upregulating BAX.⁵⁴

4.4 | Role of miR-302 cluster in inducing tumor angiogenesis

Adequate angiogenesis and unstable vascular permeability provide excellent conditions for tumor survival and metastasis.³³ Some studies have found that the miR-302/367 family is closely related to tumor-associated angiogenesis.⁵⁵ (Figure 3).

The miR-302/367 family could be a promising target for regulating oncogenesis by inhibiting angiogenesis in melanoma and colorectal cancer.³³ Mechanistically, miR-302c can inhibit angiogenesis by regulating EndMT and its direct functional target, MTDH in HCC.⁵⁶ Moreover, miR-302 represses the expression of the steroid/thyroid hormone receptor NR2F2, also known as COUPTFII, to inhibit angiogenesis.²³ In-depth research found that the miR-302/367 cluster can limit tumor growth by restricting neovascularization and improving vascular stability through the Erk1/2-Klf2-S1pr1/VE-cadherin pathway.⁵⁵

4.5 | Role of miR-302 cluster in protumorigenic inflammation

Immune-related molecules are important players in neoplastic disease. During tumor formation and progression, some miRNAs are linked to the immune response. Certain miRNAs are involved in the immune network controlled by estrogen.⁵⁷ MicroRNA-302c can regulate interferon expression induced by influenza A virus and downregulate innate immune responses induced by enterovirus 71 by targeting KPNA2, which is intracellularly associated with JNK1/JNK2 and p38. This finding indicates that miR-302c has immunomodulatory function in cancer.⁵⁸ According to some researchers, miR-302c downregulation can enhance the sensitivity of cancer cells to NK cell-mediated cytotoxicity; 1,25-(OH)₂D₃ was confirmed to participate in this process, in which the NKG2D ligands MICA/B and ULBP2 are upregulated⁵⁹ (Figure 3).

4.6 | Role of miR-302 cluster in evading growth suppressors in cancer

Cancer cells can induce and maintain positive growth-stimulating signals and evade growth-suppressive signals, in which the miR-302 cluster is involved. Epithelial-mesenchymal transition and cell reprogramming are effective means by which tumors evade growth inhibition. Several molecular mechanisms, including miRNA regulation, might directly and/or indirectly lead to phenotypic variations in tumor cell plasticity.^{19,33,56,60} Metastatic melanoma cells can be epigenetically reprogrammed in an embryonic microenvironment through changes in the expression of miR-302a and miR-27b, suggesting that the metastatic phenotype and plasticity of tumor cells can be regulated by these 2 miRNAs.¹⁹ The upregulation of the miR-302 family in A-375 melanoma and HT-29 colorectal cancer cells reverses EMT through a reprogramming process.³³ Similarly,

the EC-specific miR-302c inhibits HCC growth and angiogenesis by regulating EndMT through MTDH.⁵⁶ The upregulation of miR-302 by lncRNA ES1 promotes EMT in BC cells.⁴⁷ Jhdm1a/1b, a vitamin C-dependent H3K36 demethylase, can cooperate with Oct4 to activate the miR-302/367 cluster, leading to cell reprogramming.⁶¹ In addition, a study found that miR-302 can increase the efficiency of reprogramming through the repression of NR2F2,²³ while some scholars found that vitamin C can reduce the reprogramming efficiency of the miR-302/367 cluster by downregulating *TET1* gene expression in specific BC cell lines.⁴⁸ The miR-302/367 cluster can promote somatic cell reprogramming and induce dedifferentiation into PSCs.^{2,62,63} In the initial stage of reprogramming to generate iPSCs, the miR-302/367 cluster efficiently induces EMT and promotes cell morphological changes.² The transformation of human adipose-derived stem cells into iPSCs was more efficient in the presence of miR-302.⁶⁰ For example, ESC markers such as Nanog, OCT3/4, SOX2, SSEA-3, and SSEA-4 are detected in miR-302-transfected skin cancer cells, indicating that miR-302 has the ability to gradually and continuously reprogram tumor cells into an ESC-like pluripotent state.⁴ In addition, the miR-302/367 cluster can effectively reprogram human fibroblasts into functional neurons.⁶⁴

4.7 | Other effects of miR-302/367 cluster

In addition to the above regulatory effects, the miR-302/367 family also affects cancer cell behavior by regulating cell resistance to chemotherapeutic drugs/radiotherapy and cellular energy metabolism (Figure 4).

First, miRNAs play important roles in modulating cell sensitivity to chemotherapeutic drugs. Studies have found that miR-302a/b can enhance the drug susceptibility of testicular and OC cells.⁶⁵ MicroRNA-302b can promote HCC cell resistance to human adipose-derived stem cells through 2 possible targets, Mcl-1 and dihydropyrimidine dehydrogenase.⁶⁶ Low miR-302s expression is found in BC cells, especially in those resistant to chemotherapy.^{67,68} The MAP/ERK kinase kinase 1 can be cooperatively inhibited by miR-302s to suppress P-gp, leading to an increased susceptibility of BC cells to adriamycin.⁶⁷ Expression of BCRP can also be cooperatively inhibited by miR-302s to increase the sensitivity of BC cells to mitoxantrone.⁶⁹ Some reports stated that miR-302 overexpression enhances the sensitivity of drug-resistant hepatoma cells. Testicular germ cell tumors showed increased miR-302a expression when treated with cisplatin, leading to increased drug sensitivity.⁶⁵ The antiosteosarcoma effect of epirubicin can be enhanced by miR-302b.⁴³ Second, according to clinical results, different miRNAs show different responses to preoperative radiochemotherapy in locally advanced GC patients,⁷⁰ indicating the potential application of microarray technology in candidates' selection based on radio/chemotherapy sensitivity. Finally, NR2F2, which can regulate development, differentiation and metabolism, can be repressed by the miR-302 clusters, which provides a mechanism for the deregulation of cellular energetics in cancer cells.²³ An inverse correlation between

miR-302b and EphA2 expression was identified in GC, but the underlying mechanism is not clear.

5 | MICRORNA-302 CLUSTER IS AN EMERGING POTENTIAL BIOMARKER OR TARGET FOR CANCER THERAPY

The functions of miRNAs in various tumors have been widely recognized, and basic research on this topic has rapidly progressed. A miR-34 mimic was made into a miRNA analogue drug called MRX34, which recently entered phase I clinical trials with the aim of treating cancer patients with liver involvement.⁷¹ This trial will provide reference data for the further clinical application of miR-302/367-based therapeutics. The miR-302 cluster is involved in most biological processes and could provide many promising biomarkers or therapeutic targets for neoplastic disease. All of these different mechanisms provide directions for studying the biological significance of the miR-302 family in specific tumors and strategies for clinical use (Figure 4).

The technology for extracting miRNA from serum is very well developed.⁷² The simultaneous downregulation of miRNA-302a/d and overexpression of E2F7 showed a potential correlation with poor median overall survival and progression-free survival in HCC patients.⁷³ As highly sensitive and specific miRNAs, miR-367-3p and miR-371 are good diagnostic factors for malignant germ cell tumors, including seminomas and embryonal carcinomas.⁷⁴

Compared with traditional tumor markers, the serum levels of the miR-302/367 family members, which include stem cell-associated miRNAs, have significant advantages in sensitivity and specificity in diagnosing some patients with metastatic cancer.⁷⁵ Exosomes are a novel research tool for studying the mechanisms of miRNAs in tumors, and exosomal miRNAs can predict the unique characteristics of some neoplasms.⁷⁶ Therefore, exosomal miRNAs could potentially predict the disease stage, forecast the prognosis, and guide the treatment of advanced carcinomas. Given the tumor suppressor properties of the miR-302/367 cluster, some researchers overexpressed miR-302/367 in mice; these miRNAs were secreted in exosomes, which were then absorbed by tumor cells. This strategy effectively altered glioblastoma progression in the brains of mice.¹⁵

Many tumors show rapid progression and poor prognosis, and current methods have limited efficacy for aggressive carcinomas. As the miR-302/367 family is involved in nearly the entire process of tumor development in various neoplasms and exerts different regulatory functions, these family members can be used to detect or treat cancer in numerous ways. MicroRNA-302/367 levels could indicate tumor progression, and mimics or corresponding regulatory factors can be used to therapeutically regulate the various biological characteristics of tumor cells at different stages. Reprogramming tumor cells with miRNAs, for example, could provide a new way to treat cancer, and this approach may have additional advantages in more aggressive tumors.³³ Chemically modified antisense oligonucleotides could efficiently and specifically inhibit miRNA function. Accordingly, miRNA-targeted antisense oligonucleotides are novel therapies for

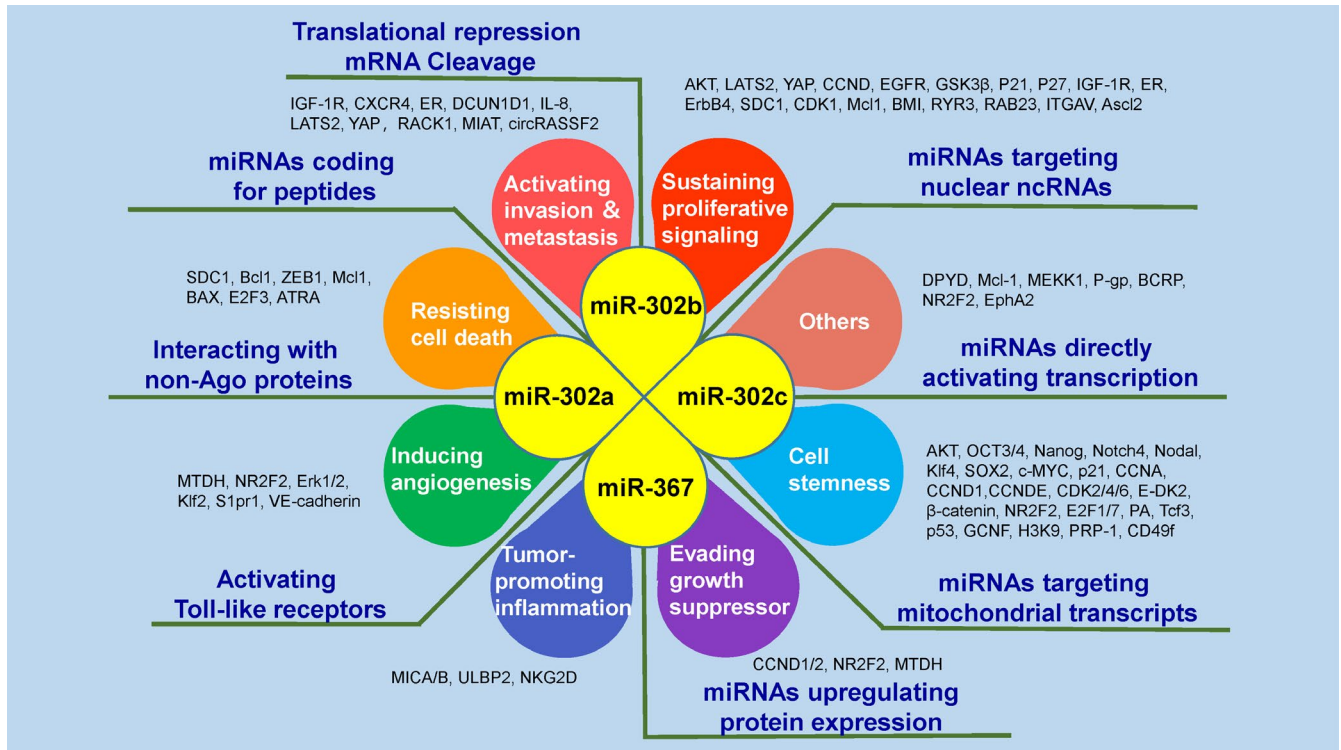


FIGURE 4 MicroRNA (miR-302/367 cluster regulates tumorigenesis and development through multiple mechanisms. The miR-302/367 cluster has been implicated in several of the hallmarks of cancer through interactions with various molecules and pathways, and the members of this cluster function by classical and noncanonical mechanisms of miRNA action. Through the networks that regulate the above characteristics, this cluster can affect the biological behaviors of tumor cells and induce different outcomes of cancer cells. As numerous hallmarks of cancer are regulated by the miR-302/367 cluster, there are a variety of candidates for cancer treatment that involve different molecular targets and strategies. ATRA, All-trans retinoic acid; BMI, B cell-specific Moloney murine leukemia virus integration site 1; CCND, cyclin D; CDK, cyclin-dependent kinase; CXCR4, C-X-C chemokine receptor type 4; DCUN1D1, cullin neddylation 1 domain containing 1; E2F3, E2 transcription factor 3; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ErbB4, Receptor tyrosine-protein kinase erbB-4; GCNF, germ cell nuclear factor; GSK-3 β , glycogen synthase kinase-3 β ; IGF-1R, insulin-like growth factor 1 receptor; IL-8, interleukin-8; ITGAV, Integrin alpha-V; Klf2, Kruppel like factor 2; LATS2, large tumor suppressor kinase 2; Mcl-1, myeloid cell leukemia sequence 1; MEKK, MAP/ERK kinase kinase 1; MIAT, myocardial infarction-associated transcript; MICA/B, major histocompatibility complex class I chain-related proteins A and B; MTDH, metadherin; NR2F2, nuclear receptor subfamily 2 group F member 2; PRP-1, proline-rich polypeptide 1; RAB23, Ras-related protein 23; RACK1, receptor for activated C-kinase 1; RYR3, Ryanodine receptor 3; SDC1, syndecan 1; VE-cadherin, vascular E-cadherin; YAP, Yes-associated transcriptional regulator; ULBP2, UL16-binding protein 2; ZEB1, zinc finger E-boxbinding 1

several neoplastic diseases.⁷⁷ The downregulation of miRNAs in some metastatic tumors indicates that miRNA mimics could be effective therapeutics for inhibiting cancer and preventing metastasis.⁴⁵ Exogenous miR-302a has shown potential therapeutic value in treating OC.³⁹ Recent studies found that somatic pluripotency was more efficiently induced through the miR-302/367 family without the need for exogenous transcription factors than through the standard approach involving OCT4/SOX2/KLF4/MYC. Basic research in an orthotopic xenograft mouse model of glioblastoma showed that cell-based therapy with miR-302/367-expressing cells inhibits tumor growth.¹⁵ Currently, researchers can generate high-quality iPSCs from most fibroblast sources by transfection with reprogramming-modified mRNAs and mature ESC-specific miR-367/302 mimics.⁷⁸ Cancer stem cells also represent a new approach to understanding the origin of cancer. Cancer cells transfected with miR-302s, also called mirPS cells, offer new options for blocking cancer progression.⁴ Furthermore, this cluster offers new options for antiangiogenic

therapy in cancer.⁵⁶ As miRNA array technology can be used to screen miRNAs related to radiochemotherapy sensitivity in many cancers, it can provide biomarkers for miRNA-targeted therapy and personalized radiochemotherapy.⁷⁰ A synthetic RNA-based CRISPR-Cas9 system that is responsive to the miR-Cas9 switch is under development.⁷⁹

6 | CONCLUSION AND PERSPECTIVE

The miR-302 cluster shows multimodal tumor suppression in many cancers and is critically important in regulating cell stemness, multiple hallmarks of cancer cells, the cell response to chemotherapy drugs, and tumor immunity in classic and alternative ways, suggesting that this miRNA cluster has great value in the detection and treatment of neoplastic disease.

After the corresponding specificity is obtained, the circulating miR-302/367 family will have considerable value in disease diagnosis

and prognosis and in monitoring disease progression. In recent experiments, the malignant biological characteristics of some cancer cells have been successfully changed by regulating the miR302/367 cluster in vitro, which has laid a foundation for the clinical application of miRNAs. Viral vector transduction and miRNA synthesis (mimic or inhibitor) techniques can be used to overexpress or inhibit intracellular miRNA expression. Cell-based therapy offers an alternative approach to miR-302/367 cluster-related treatment. MicroRNA mimics are promising for clinical use, but further study is needed to determine the mechanism of uptake by tumor cells in a patient's body and how they function within tumor cells. As the miR-302/367 family can increase the sensitivity of some tumor cells to radiotherapy or chemotherapy, the corresponding miRNA treatments could be applied before these therapies.

The miR-302/367 family has been shown to efficiently reprogram human somatic cells to iPSCs; however, the safety and controllability of differentiation potential pose challenges in this kind of mirPS cell therapy for cancer. The new miR-Cas9 switch technique has potential in the clinical application of this cluster. Further studies are necessary to verify the potential, feasibility, and safety of such treatments. Because the miR-302/367 family has diverse expression patterns and specific tumor characteristics in different cancers at different stages, and the resulting biological behavior is dictated by interactions among complex target genes, it is of vital importance to identify the target genes and node proteins of this cluster. Therefore, a stable, safe, feasible, and convenient protocol is required for the application of the miR-302/367 cluster as a promising biomarker and therapeutic.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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