

The Promising Role of miR-21 as a Cancer Biomarker and Its Importance in RNA-Based Therapeutics

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MicroRNAs are small noncoding transcripts that posttranscriptionally regulate gene expression via base-pairing complementarity. Their role in cancer can be related to tumor suppression or oncogenic function. Moreover, they have been linked to processes recognized as hallmarks of cancer, such as apoptosis, invasion, metastasis, and proliferation. Particularly, one of the first oncomiRs found upregulated in a variety of cancers, such as gliomas, breast cancer, and colorectal cancer, was microRNA-21 (miR-21). Some of its target genes associated with cancer are PTEN (phosphatase and tensin homolog), PDCD4 (programmed cell death protein 4), RECK (reversion-inducing cysteine-rich protein with Kazal motifs), and STAT3 (signal transducer activator of transcription 3). As a result, miR-21 has been proposed as a plausible diagnostic and prognostic biomarker, as well as a therapeutic target for several types of cancer. Currently, research and clinical trials to inhibit miR-21 through anti-miR-21 oligonucleotides and ADM-21 are being conducted. As all of the evidence suggests, miR-21 is involved in carcinogenic processes; therefore, inhibiting it could have effects on more than one type of cancer. However, whether miR-21 can be used as a tissue-specific biomarker should be analyzed with caution. Consequently, the purpose of this review is to outline the available information and recent advances regarding miR-21 as a potential biomarker in the clinical setting and as a therapeutic target in cancer to highlight its importance in the era of precision medicine.

MicroRNAs

In 1993, Ambros et al.¹ discovered in *Caenorhabditis elegans* that the *lin-4* gene (currently known as *lin-4* microRNA [miRNA]) could decrease the levels of *lin-14* protein through antisense complementary binding of the RNA transcripts. Later, other miRNA genes with the same mechanism of action were found in different species, including

humans. Thus, miRNAs were established as novel, small regulatory RNA molecules. These noncoding genes are single-stranded structures of 19–25 nt that can be found in intergenic or intragenic regions of the genome.²

As regulatory biomolecules, miRNAs need to be only partially complementary to induce the regulation of a target gene.³ Hence, a single miRNA can regulate a variety of genes, and a single gene can be targeted by many miRNAs.⁴ As a result, the identification of target genes is a complex process.⁵ Moreover, due to their broad regulatory activity, miRNAs are involved in a large number of processes, including development,^{6,7} proliferation,^{8–10} apoptosis,^{11,12} metabolism,^{13,14} differentiation,^{6,15} and metastasis,^{16,17} among others.¹⁸ Despite their broad involvement in a variety of processes, miRNA functions can be divided into two types: homeostatic regulation of gene expression and robustness in cellular responses.¹⁹ The first type involves the regulation of gene expression through the precise adjustment of the cell's requirements.^{20,21} As there are proteins that should be optimally expressed at low levels in certain cell types, miRNAs can fine-tune the expression of these. However, the second type of regulation is present on processes such as cell fate, its differentiation state, and stress responses,^{22–24} and its main objective is to dampen the protein production of the targeted messenger RNA (mRNA) almost completely.^{19,25} This regulation ensures accuracy and robustness by repressing the expression of mRNAs that linger from previous cell states or the products of leaky transcription.²⁵

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To synthesize a miRNA, a particular biogenesis pathway is followed. The biogenesis of miRNAs starts when RNA polymerase II (for intragenic or intergenic miRNAs)^{26–28} or RNA polymerase III (for some intergenic miRNAs)^{29–31} transcribes the miRNA gene and produces a primary transcript (pri-miRNA) with a 5' cap and polyadenylation at the 3' end.²⁸ Subsequently, Drosha (an RNase III), assisted by DGCR8 (DiGeorge syndrome critical region 8), cleaves the pri-miRNA sequence, releasing it as a precursor loop of approximately 70 nt called a pre-miRNA.^{32–34} Furthermore, exportin 5 (XPO5) in the nuclear membrane is capable of exporting the precursor sequences out of the nucleus.³⁵ Outside the nucleus, pre-miRNAs are cleaved by Dicer (an RNase III), aided by TRBP (transactivation response element RNA-binding protein), to become mature miRNAs of approximately 22 nt in length.^{36–38} Two mature miRNA sequences are produced, one of which is selected as a guide strand on the basis of a hydrogen bonding selection mechanism dependent on Argonaute (AGO) proteins.³⁹ Finally, this complex, along with AGO proteins, forms the RNA-induced silencing complex (RISC) for gene silencing by base pairing to the 3' untranslated region (UTR) of an mRNA.⁴⁰

Alternatively, the translation repression mechanism is still the subject of debate. Mechanisms inhibiting translation include cap-dependent translation inhibition,^{41–43} cap-independent translation mechanisms,^{41,44,45} premature termination (the drop-off theory),^{42,46,47} and the recruitment of proteolytic enzymes that can degrade a polypeptide as it is being produced.⁴² mRNA decay is another mechanism of miRNA inhibition in which mRNAs bound to miRNAs accumulate in P-bodies inside the cytoplasm where they are degraded by the 5' to 3' exonuclease activity of Xrn1 (5'-3' exoribonuclease 1).^{48–50} miRNA biogenesis and repression mechanisms are complex processes that are tightly regulated and have not yet been fully elucidated. Their dynamics and effects are of special interest to have a better understanding of many complex diseases, such as cancer.

miRNAs and Cancer

As reported previously, miRNAs are involved in a variety of processes inside the cell, some of which are considered hallmarks of cancer (e.g., metastasis,^{16,17} cell proliferation,^{9,51} apoptosis,^{11,12}). Furthermore, it has been reported that more than 50% of miRNAs are located at fragile sites or regions where deletion or amplification tends to occur in human cancers.⁵² Accordingly, miRNA expression in cancer cells is dysregulated (more commonly upregulated) in comparison to normal cells.^{53–55} It has been found that miRNA expression profiles can classify poorly differentiated tumors more successfully than mRNA expression profiles, which highlights their importance in cancer.^{56–58} These findings were obtained after Lu et al.⁵⁸ analyzed 17 poorly differentiated tumors with non-diagnostic histologic appearance. Overall, poorly differentiated tumors had lower levels of miRNAs compared with more differentiated tumors. Even at low levels, miRNA profiles could establish a better diagnosis by classifying tumors into 11 categories (including colon, ovary, lung, breast, lymphoblastic lymphoma), unlike the mRNA-based classification.

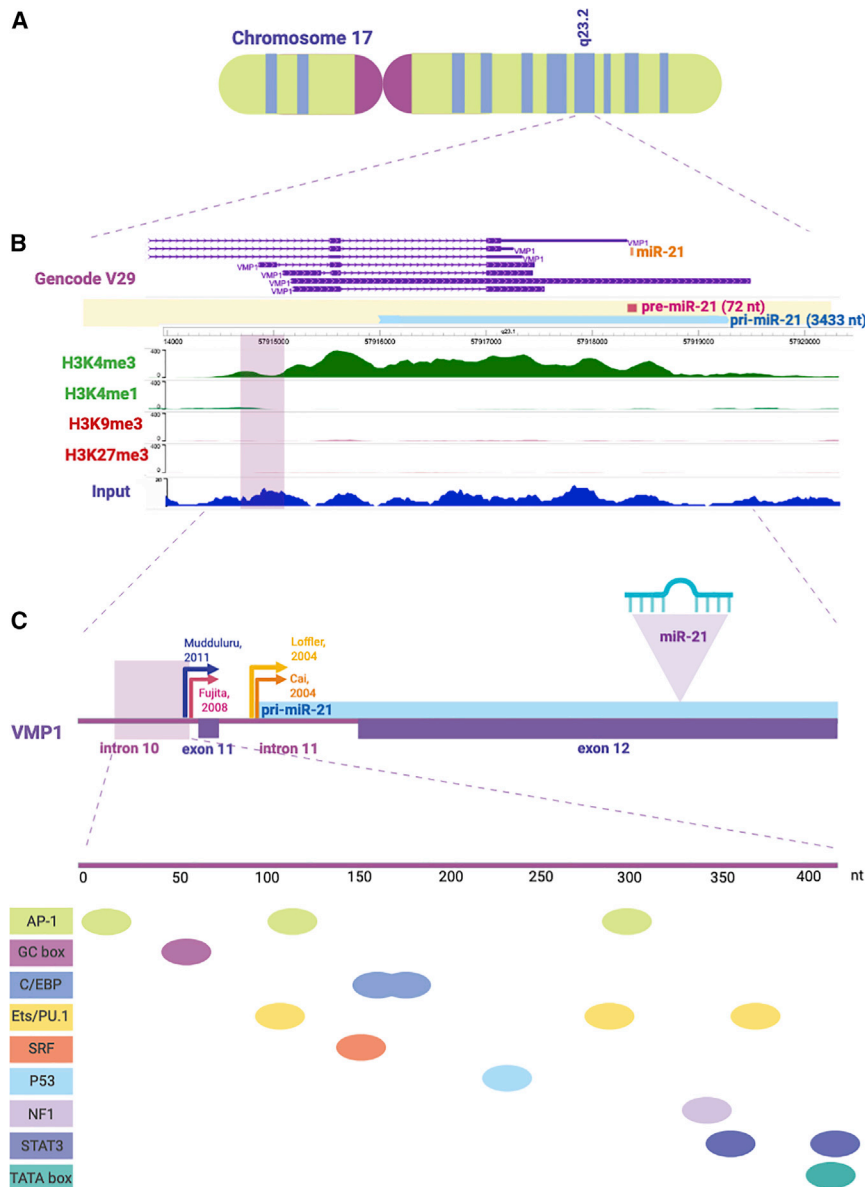
In particular, miRNAs involved in cancer are divided into two categories: oncogenes (oncomiRs) and tumor suppressors.⁵⁹ OncomiRs act by promoting tumor development by inhibiting tumor suppressor genes; some examples of these small RNAs are homologous miRNA (miR)-221/222,⁶⁰ miR-27a,^{61,62} and miR-21.^{63–65} In contrast, tumor suppressor miRNAs inhibit oncogenes, thereby suppressing tumor development. Some miRNAs that serve as tumor suppressors are miR-145,⁶⁶ the let-7 family,⁶⁷ and miR-205.^{68,69} In either role, miRNAs have been shown to be crucial for processes considered to be hallmarks of cancer.

miR-21

miR-21 was one of the first mammalian miRNAs identified.^{28,70} Regarding its structure, miR-21 is found on chromosome 17 (17q.23.1) in the 11th intron of the *TMEM49* (transmembrane protein 49) gene, precursor of VMP1 (vacuole membrane protein 1).^{28,71} It has its own highly conserved promoter.⁷² In 2008, Fujita et al.⁷³ described a putative promoter region (miPPR-21) containing TATA, GC, and CCAAT boxes, as well as binding sites for activator protein 1 (AP-1), E-26 transformation specific/PU.1 (Ets/PU.1), CCAAT/enhancer-binding protein α (C/EBP α), nuclear factor I (NFI), serum response factor (SRF), p53, and signal transducer activator of transcription 3 (STAT3)⁷⁴ (Figure 1). When transcribed, the miR-21 gene produces a 3,433-nt long pri-miRNA (pri-miR-21). Subsequently, it is cleaved into a 72-nt-long pre-miRNA loop, which is the source of the miR-21-5p and miR-21-3p mature transcript (21 and 20 nt long, respectively).⁷⁵

Alternatively, the epigenetic regulation of miR-21 has not been thoroughly studied, but in research conducted by Ferraro et al.,⁷⁶ it was found that histone posttranslational modifications such as H3K27me3 and H3K9me2 were completely absent, whereas the marks related to active transcription, H3K9-14ac, H3K4me3, and H3K27ac, were higher on the promoter of miR-21 in cell lines with an epithelial-mesenchymal transition (EMT) phenotype.⁷⁶ However, further studies are needed to understand the relationship between the miRNA promoter sequence, histone marks, and the expression of miRNAs, particularly miR-21.⁷⁶

As reported previously, miR-21 is subject to transcriptional regulation, but the mechanisms involved in its posttranscriptional regulation have also been described. For example, in smooth muscle cells, bone morphogenic proteins (BMPs) and transforming growth factor β (TGF- β) stimulate the maturation of miR-21 via the recruitment of SMAD proteins (SMAD1 and SMAD5 are specific for BMPs, and SMAD-3 is specific for TGF- β) that stabilize the DROSHA microprocessor complex.⁷⁷ In another study, miR-21 expression was found to be downregulated in MCF-7 cells when exposed to estradiol. These results indicate that the estrogen receptor is a negative regulator through the inhibition of DROSHA activity.⁷⁸ Consequently, these studies show that the regulation of miR-21 is a complex process that involves transcriptional and posttranscriptional steps along the biogenesis pathway.



miR-21 Pathways

Cell Proliferation

miR-21 has been linked to cell proliferation through some of its targets, such as programmed cell death protein 4 (*PDCD4*), sprouty RTK signaling antagonist 2 (*SPRY2*), phosphatase and tensin homolog (*PTEN*), and reversion-inducing cysteine-rich protein with Kazal motifs (*RECK*). In pancreatic cancer cells, miR-21 can promote epidermal growth factor (EGF)-induced proliferation by targeting *SPRY2*, which inhibits growth factor-induced cell proliferation.⁷⁹ Additionally, mechanistic studies have revealed that the signaling pathways that miR-21 targets to modulate cell proliferation are mitogen-activated protein kinase/extracellular receptor kinase (MAPK/ERK) and phosphatidylinositol 3-kinase/protein kinase

Figure 1. The Genomic and Epigenomic Landscape of miR-21

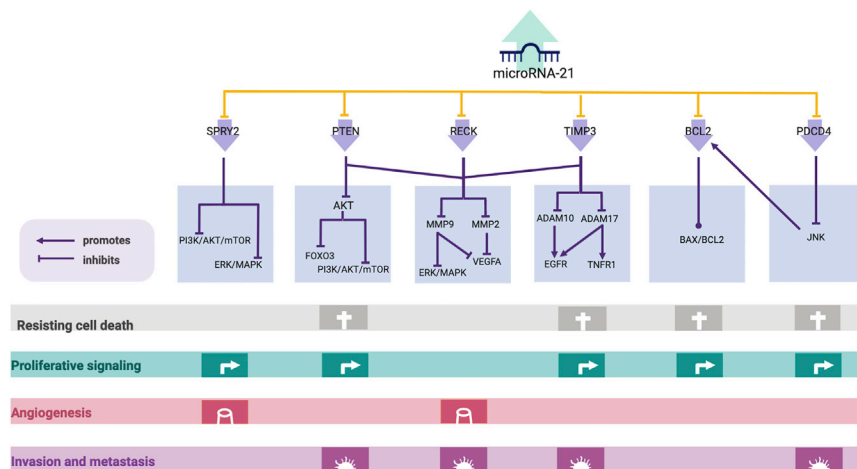
(A) Genomic location of miR-21: miR-21 is located on chromosome 17 (17q.23.1) in the 10th intron of the *TMEM49* gene precursor of the VMP1 protein. The site is recognized as a fragile site of the neuroblastoma histotype, where HPV16 integration events occur.⁵² (B) Epigenetic landscape of miR-21. H3K4me3 and H3K4m1 marks are associated with active transcription (in green), and chromatin immunoprecipitation sequencing (ChIP-seq) histograms (in blue) in the IMR90 cell line were retrieved from the Wash U Epigenome Browser (GRCh37).¹⁴⁴ Chromatin marks such as H3K27me3 and H3K9me3 are completely absent from the miRNA promoter, whereas H3K4me3 and H3K4me1 marks, which are related to active transcription, are present on the promoter of miR-21. (C) Putative promoter region of miR-21 according to Fujita et al.⁷³ (purple region). Transcription start sites for miR-21 described by Mudduluru et al.⁷⁴ (blue arrow) and Fujita et al.⁷³ (pink arrow) are found in the 10th intron of the VMP1 gene. Transcription start sites described by Cai et al.²⁸ (orange arrow) and Löffler et al.¹⁴⁵ (yellow arrow) are found on the 11th intron of the same gene. Transcription factor binding regions described by Fujita et al.⁷³ at the putative promoter region are shown. The putative miRNA promoter region contains several binding sites for AP-1, Ets/PU.1, C/EBP, NFI, SRF, p53, and STAT3.

B (PI3K/AKT).⁷⁹ As in pancreatic cancer, in non-small-cell lung cancer (NSCLC), miR-21 is associated with cell proliferation through *PDCD4*⁸⁰ and through *PTEN* and *RECK* in Gejiu squamous cell lung carcinoma (a type of NSCLC).⁸¹ Overall, miR-21 plays an important role in cell proliferation in diverse types of cancer, including NSCLC and pancreatic cancer, through pathways involved in the carcinogenic process (Figure 2).

Migration and Invasion

miR-21 has been identified as a miRNA involved in metastasis and invasion (metasmiR).^{82,83}

Particularly in breast, prostate, hepatocellular carcinoma, and colon cancer cell lines, it has been found to target genes linked to decreased metastatic potential, such as *PDCD4*,⁸⁴ metalloproteinase inhibitor 3 (*TIMP3*),^{85,86} tropomyosin 1 (*TPM1*),⁸⁷ serpin peptidase inhibitor/clade B (*SERPINB5*) coding for Maspin,⁸⁸ and *PTEN*.⁸⁹ *In vitro*, the overexpression of miR-21 is correlated with the downregulation of *TIMP3* and an increase in the invasiveness of melanoma cell lines WM1552c and WM793b but not with migration potential.⁸⁵ Regarding human hepatocellular carcinoma, it was found that miR-21 controls migration and invasion by targeting *PTEN*.⁶³ *PTEN* can suppress the expression of MMP-9 and MMP-2 through FAK dephosphorylation. As seen here, miR-21 plays an important role in processes that lead to metastasis, such as migration and invasion, not only



and inactivation of TSC2 or by the phosphorylation of PRAS40,¹⁵² which affects cell growth, proliferation, survival, and motility.¹⁵³ Alternatively, RECK can negatively control two matrix metalloproteinases, MMP-2 and MMP-9, which inhibit angiogenesis and invasion.^{154,155} In addition, TIMP3 negatively regulates ADAM10 and ADAM17, two metalloproteinases that are upregulated in cancer, and receptor substrates such as Notch receptors, transforming growth factor β (TGF- β), HER2, HER4, and VEGFR2, among others.¹⁵⁶ In contrast, BCL2 expression can be induced by miR-21 binding to its 3' UTR, which decreases apoptosis and increases proliferation¹¹⁵ by dysregulating the BAX/BCL2 ratio.¹⁵⁷ Finally, PDCD4, a miR-21 target, can interfere with JNK activation and lead to the upregulation of BCL2-like proteins and affect apoptosis, proliferation, and migration.¹⁵⁸

through the direct repression of its targets but also through the effectors of its targets.

Apoptosis

Another process that miR-21 has been associated with is apoptosis. In breast cancer, apoptosis is modulated through the miRNA targeting of B cell lymphoma 2 (*BCL2*); in addition, the targeting of *PDCD4* by miR-21 has also been confirmed. An *in vivo* study also confirmed this association, which translates to the miR-21 targeting of various genes related to apoptosis in more than one cancer and demonstrated that miR-21 is a regulator of this process.⁹⁰ In the previous study, it was found that miR-21 inhibits negative regulators of the Ras/MAPK kinase (MEK)/ERK pathways, and it was linked to the inhibition of apoptosis in a xenograft mouse model of NSCLC.⁹⁰ Moreover, the expression of some miRNA targets associated with apoptosis was measured and found to be decreased, such as the case of *PDCD4*, apoptotic protease-activating factor 1 (*APAF1*), caspase 3 (*CASP3*), Fas ligand (*FASLG*), and Ras homolog family member B (*RHOB*).⁹⁰ In summary, miR-21 can influence apoptotic processes through some of its targets and lead to their inhibition, thereby contributing to carcinogenic processes.

miR-21: A Biomarker for Diagnosis, Prognosis, and Prediction

As described above, miR-21 is one of the oncomiRs identified that has been linked to carcinogenic processes. Additionally, it has been found to be upregulated in several cancers. These findings highlight the importance of miR-21 as a plausible molecular biomarker^{91–93} (Figure 3).

The use of miRNAs as biomarkers is currently on the rise due to their stability and presence in bodily fluids, which makes them easy to

Figure 2. miR-21 Targets and Pathways Associated with the Hallmarks of Cancer

The upregulation of miR-21 can lead to the downregulation of target genes involved in the carcinogenic process, such as *SPRY2*,¹⁴⁶ *PTEN*,⁹⁷ *RECK*,¹⁴⁷ *TIMP3*,⁶⁴ *BCL2*,¹¹⁵ and *PDCD4*.⁸⁴ In addition, *SPRY2* can negatively regulate the PI3K/AKT/mTOR and ERK/MAPK pathways by controlling the trafficking of EGFR and HER2 through the endosome and by inhibiting Raf1, respectively.¹⁴⁸ Therefore, in its absence, uncontrolled proliferation is observed, but angiogenesis is suppressed.¹⁴⁹ *PTEN*, another miR-21 target, can also control the PI3K/AKT/mTOR pathway by inhibiting AKT activation. In this case, AKT can inhibit FOXO3, which regulates cell survival, growth, and differentiation by inducing the expression of proapoptotic BCL2 family proteins and Fas and TRAIL ligands or by enhancing cyclin-dependent kinase inhibitors (CDKIs).^{150,151} Additionally, AKT can activate mTORC1 by the phosphorylation

assay.^{94–96} As previously mentioned, miR-21 is upregulated in many cancers, such as lung, ovarian, breast, stomach, prostate, colon, thyroid, and pancreatic cancers, as well as gliomas (Table 1). Furthermore, miR-21 targets important tumor suppressor genes as well as genes involved in carcinogenesis, such as *PTEN*,⁹⁷ *PDCD4*,⁷⁶ and *RECK*.⁹⁸ As a result, various studies have proposed miR-21 as a potential molecular biomarker for diagnosis, prediction, and prognosis, as well as a new therapeutic target in various types of cancer.^{94,95,99}

miR-21 as a Diagnostic Biomarker

miR-21 has demonstrated its potential as a diagnostic biomarker for different types of cancer, such as breast, colorectal, and pancreatic cancers. In particular, Iorio et al.⁵⁶ found that in breast cancer samples (76 primary tissue samples), miR-21 was progressively upregulated. It was also found to be a plausible sex-independent biomarker, as a study in male breast cancer demonstrated its overexpression.¹⁰⁰ Despite several studies reporting the upregulation of this miRNA in breast cancer, its sensitivity was inconsistent. In 2016, Gao et al.¹⁰¹ reviewed 11 studies from 10 articles to evaluate this miRNA as a biomarker. They found a pooled sensitivity and specificity of 0.72 and 0.8, respectively. The area under the curve (AUC) of the summary receiving operating characteristic (SROC) was 0.8517. These results were higher than those of other markers, such as carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE) (with a sensitivity of 0.48 and 0.39, respectively). Due to its relatively high specificity and sensitivity in comparison with other markers, miR-21 is recommended for further large-scale studies to validate its clinical application in diagnosis.

As in breast cancer, miR-21 dysregulation can also be used as a diagnostic biomarker in pancreatic cancer. In an article published in 2017,

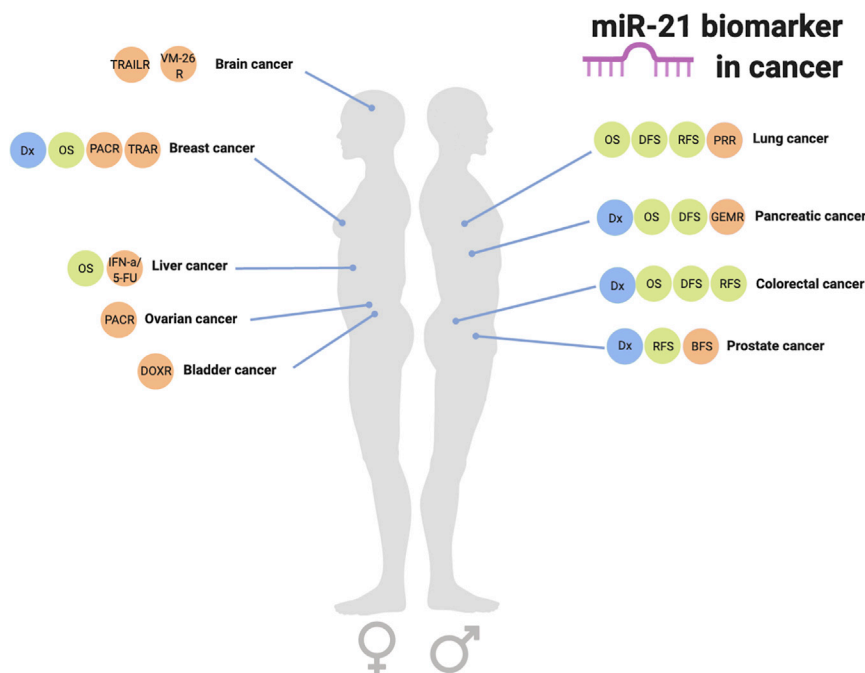


Figure 3. The Plausible Applications of miR-21 as a Diagnostic, Prognostic, and Predictive Biomarker in Several Types of Cancer

Diagnostic biomarker applications (Dx) are shown in dark green and can be used in breast, pancreatic, colorectal, and prostate cancers. Plausible prognostic biomarkers are shown in light green, and their type is indicated by OS (overall survival), DFS (disease-free survival), BFS (biochemical-free survival), and RFS (recurrence-free survival). Prognosis parameters are found in breast, liver, lung, pancreatic, colorectal, and prostate cancers. Plausible predictive biomarkers are found in brain, breast, liver, ovarian, bladder, lung, pancreatic, and prostate cancers. The types of predictive biomarkers are also indicated in purple: TRAILR (tumor necrosis factor-related apoptosis-inducing ligand resistance), VM-26 R (teniposide resistance), PACR (paclitaxel resistance), TRAR (trastuzumab resistance), IFN- α /5-FU (interferon- α /5-fluorouracil resistance), DOXR (doxorubicin resistance), PRR (platinum resistance), GEMR (gemcitabine resistance).

it was found that miR-21 is upregulated in the sera of pancreatic cancer patients, with a sensitivity of 0.77 and specificity of 0.8.¹⁰² In this case, the AUC of the SROC for the Asian population was 0.78. The same study recommended the use of circulating miR-21 alone or in combination with carbohydrate antigen 19-9 (CA-19-9) for a better diagnosis of pancreatic cancer, but the authors agreed that further studies are needed to unveil the clinical significance of miR-21.¹⁰² The overexpression of miR-21 is also found in serum samples from patients with colorectal cancer.¹⁰³ In this study, the authors proposed a three-miRNA panel for the non-invasive diagnosis of colorectal cancer by the use of miR-21, miR-19a-3p, and miR-425-5p. The authors of this research refer to the high sensitivity and specificity in colorectal cancer serum samples (0.875 and 0.744, respectively) with an area under the ROC curve of 0.88, as found by Wang and Zhang.¹⁰⁴ Furthermore, in another study, circulating miR-21 was analyzed as an early detection biomarker for colorectal cancer and was reported to have a sensitivity and specificity of 0.77 and 0.84, respectively,¹⁰⁵ with an AUC of the ROC of 0.81.

Due to its relatively easy detection, miR-21 is proposed as a highly convenient diagnostic biomarker alongside prostate-specific antigen (PSA) and miR-141, with a high value of AUC when graphing sensitivity and specificity of 0.88.¹⁰⁶ In prostate cancer, miR-21 expression, alongside other biomarkers, has been associated with the pathological stage, lymph node metastasis, and extracapsular extension.¹⁰⁷ Analyses have revealed that miR-21 expression could be a predictor of biochemical-free survival (BFS) in prostate cancer patients.¹⁰⁸ In this way, we hypothesize that the role of miR-21 as a diagnostic biomarker can be accompanied by its use as a prognosis biomarker.

The upregulation of miR-21 in several types of cancer, such as breast, pancreatic, prostate, and colorectal cancers, makes it possible to use miR-21 as a non-invasive diagnostic biomarker; however, its use might have to be broad and not for a particular type of cancer, as it is upregulated in more than one type of cancer. Further large-scale validation studies are needed before its clinical application in each type of cancer.

miR-21 as a Prognostic Biomarker

Although miR-21 has been found to be dysregulated in many types of cancer, its role as a prognostic biomarker has still not been elucidated, as the results have been inconsistent.¹⁰¹ Zhou et al.¹⁰⁹ published an article that aimed to investigate the correlation between miR-21 and overall survival (OS) in general cancers. This work demonstrated that miR-21 correlated with poor survival in general carcinomas, with a pool hazard ratio (HR) of 1.91 for OS, 1.42 for disease-free survival (DFS), and 2.2 for recurrence-free survival (RFS) and cancer-specific survival. Interestingly, when divided into subgroups, miR-21 could predict poor OS in gastrointestinal tumors (HR = 1.68), pancreatic cancer (HR = 2.53), lung cancer (HR = 1.59), breast cancer (HR = 2.55), and liver cancer (HR = 1.93). Moreover, poor DFS was observed when miR-21 was elevated in pancreatic cancer (HR of 2.87)^{109,110} and lung cancer (HR = 2.05),^{97,109} and poor RFS and cancer-specific survival were detected in gastrointestinal tumors (HR = 2.5), lung cancer (HR = 2.25), and prostate cancer (HR = 2.04).¹⁰⁹ These results prove that miR-21 plays an important role in general carcinomas, regardless of its origin. More studies have been conducted regarding the role of miR-21 as a prognostic biomarker for colorectal cancer, in which high expression of this miRNA in tissues correlates with poor OS and DFS.¹⁰⁵

Although this miRNA has been linked to the prognosis of many cancers, specific studies are needed to confirm this general association

**Table 1. miR-21 as a Diagnostic, Prognostic, and Predictive Biomarker in Several Cancers**

Cancer	Expression	Function	Targets	Biomarker Type	References
Gastric cancer	downregulated in gastric juices but upregulated in gastric cancer tissues	promotes cell proliferation and invasion	PTEN PDCD4 RECK	early diagnosis and prognosis of lymph node metastasis	134,135
Colorectal cancer	upregulated	carcinoma-associated fibroblast formation, tumor formation, metastasis	SMAD7 SMAD 6 ITGβ4 PDCD4	early diagnosis (circulating miR-21), prognosis adjuvant therapy (tissue miR-21), predictor of tumor relapse	84,136
Glioma	upregulated	cell growth, apoptosis cell proliferation, cancer stem cell differentiation	PTEN RECK FasL PDCD4 Bcl2	diagnosis (screening)	93
Breast cancer	upregulated	cell survival, apoptosis, resistance to systemic therapy	PTEN PDCD4 TPM1 RTN4	diagnosis triple-negative lymph metastasis and grade III	137
Lung cancer	upregulated	apoptosis, cell proliferation, survival, angiogenesis, inhibition of nuclear factor κB (NF-κB) activation	PTEN PDCD4 Smad7 Bcl2 EGFR Cas8 TGF-β	prognosis and diagnosis (OS)	95,138
Prostate cancer	upregulated	motility, invasion, apoptosis, androgen-independent growth	RECK MARCKS PDCD4 TPM1 PTEN	early diagnosis	107

and to offer additional information for each type of cancer through non-invasive but highly accurate testing.^{111–113}

miR-21 as a Predictive Biomarker

The use of miR-21 as a biomarker is not limited to diagnosis and prognosis, as several studies have uncovered its usefulness as a predictive biomarker in breast, lung, and ovarian cancers, among others. This is particularly important due to the resistance to treatments that some cancers develop. Increased miR-21 levels were linked to resistance to platinum-based chemotherapy in patient samples with NSCLC (stages I–III).¹¹⁴ In this study, resistant responders had increased levels of miR-21 compared with sensitive responders. The resistance to platinum due to an increase in miR-21 was attributed to *PTEN* and *BCL2* expression in this case. Although the molecular mechanism remains unclear, it is known that the loss of *PTEN* leads to increased activity of the AKT and mammalian target of rapamycin (mTOR) pathways. *BCL2*, which is thought to be indirectly regulated

by miR-21, promotes a chemoresistant phenotype in regard to cisplatin.¹¹⁵

Additionally, miR-21 was also found to play a role in the sensitivity to gemcitabine treatment in patients with advanced pancreatic cancer (stages III and IV).¹¹⁶ A high level of serum miR-21 was associated with a poor response to gemcitabine in 177 patients because the time to progression after treatment was of only 80 days, compared with 161 days in patients whose expression of miR-21 was low. The authors of the research linked the decreased sensitivity to gemcitabine to apoptosis resistance after treatment, as miR-21 targets Fas/FasL. Moreover, serum miR-21 levels were also found to predict the outcome of HER2-positive breast cancer patients who had received neoadjuvant chemotherapy and trastuzumab.¹¹⁷ Increased levels of miR-21 led to worse clinical responses to chemotherapy during neoadjuvant treatment when combined with trastuzumab. In this way, it was found that miRNA levels distinguished between clinical and

**Table 2. miR-21 in Clinical Trials and RNA-Based Therapeutics**

Method	Disease	Experimental Models	Status	Reference
Anti-miR-21	Alport syndrome	Alport syndrome patients (18–65 years old)	clinical trial phase 1 multicenter study	125
Anti-miR-21	bladder cancer	mouse xenograft	preclinical	126
Anti-miR-21	cardiovascular disease	C57BL/6 male mice	preclinical	139–141
Anti-miR-21	liver injury	bile duct ligation mouse model	preclinical	142
Anti-miR-21	pancreatic cancer	Mia PaCa-2 Lucia F1 cells in mice (xenograft model)	preclinical	124
qRT-PCR	colon cancer stage II	patients with stage II colon cancer (18–65 years old)	interventional clinical trial	127
Anti-miR-21	prostate cancer	DU145 cells in nude mice (xenograft model)	preclinical	143

non-clinical responders; however, they could not distinguish between pathologic complete response and non-pathologic complete response in the same study. Therefore, more information and validation of miR-21 as a predictive biomarker for chemotherapy is needed, and multicenter studies will be convenient for this purpose.

miR-21 as a Therapeutic Target in Clinical Trials

In addition to its use as a potential biomarker in a diverse range of cancers, miR-21 has become of great interest in the field of miRNA therapeutics, an area that intends to use miRNA replacement therapy through miRNA mimics and the inhibition of miRNA function through antimiRs.¹¹⁸ The ability of miRNAs to target multiple genes that might be altered in a disease gives them an advantage as therapeutic molecules, either by the use of miRNA mimics or antimiRs. In the case of miR-21, antimiRs offer a new therapeutic approach for liver injury¹¹⁹ and cardiovascular disease.¹²⁰ In particular, mouse models with kidney injury have shown that anti-miR-21 reduced reactive oxygen species (ROS) accumulation by rescuing the expression of peroxisome proliferator-activated receptor α (*PPAR α*),¹²¹ and this effect resulted in decreased liver steatosis in mice. Nonetheless, these results are not conclusive, as *PPAR α* might not be the only miR-21 target that has an impact on this network. Although miR-21 appears to be a promising alternative as an anticancer therapy, there are still some obstacles to address before its clinical application. One of the main concerns is the delivery of miRNA therapeutic molecules, as anti-miR-21 has not overcome the tumor microenvironment, and, as a consequence, *in vivo* targeting of this miRNA has not been completely successful in all cancers.¹²²

In cancer therapeutics, miR-21 is an appealing candidate because of all of the carcinogenic processes it has been traced to. In particular, in breast cancer, Mei et al.⁹¹ demonstrated that the combination of paclitaxel (Taxol) and a miR-21 inhibitor enhanced chemothera-

peutic effects in breast cancer cells (MCF7). However, this is not the only case where a miR-21 inhibitor has been shown to be efficient; anti-miR-21 has also been shown to reduce tumor size in xenograft mouse models of prostate cancer with an androgen-independent cell line, DU-145.¹²³ Furthermore, in pancreatic cancer, miR-21 inhibition has been shown to stop tumor growth in an aggressive xenograft mouse model; additionally, in combination with gemcitabine, it can induce tumor regression. The authors hypothesized that an increase in angiogenesis due to the inhibition of miR-21 could enhance the delivery of gemcitabine to the tumor.¹²⁴ In view of this information, miR-21 was introduced as a potential therapeutic target in breast cancer and other cancer types, such as prostate and pancreatic cancers, where the regulation of these genes plays an important role in cancer progression.

In fact, clinical trials for suppressing miR-21 are currently being conducted (Table 2). For example, Genzyme is conducting a phase 1 multicenter study where RG-012, a chemically modified oligonucleotide that can bind to miR-21, is being tested in patients with Alport syndrome (ClinicalTrials.gov: NCT03373786). The molecule has proven to decrease the rate of progression of renal fibrosis by inhibiting miR-21.¹²⁵ Alternatively, ADM-21, a miR-21 inhibitor, is under assessment in a xenograft mouse model of bladder cancer.¹²⁶ Results have shown that ADM-21 effectively decreases bladder cancer growth *in vitro* and *in vivo* by inhibiting the miR-21 regulation of the protein phosphatase 2 regulatory subunit $B\alpha$ (*PPP2R2A*), a regulator of the AKT/mTOR pathway. After toxicity studies have concluded, testing this modified oligonucleotide in clinical phase 1 trials is needed.¹²⁶ Finally, an interventional clinical trial is currently being conducted that involves the study of six miRNAs (including miR-21) to determine whether a patient with stage II colon cancer should not receive adjuvant chemotherapy (ClinicalTrials.gov: NCT02466113) according to OS and DFS measurements.¹²⁷ Although trials are currently being conducted on miR-21 therapeutics, there are still many therapeutic and biomarker options that have not yet been explored, especially in cancer, where it is one of the most dysregulated miRNAs.

Challenges for miR-21 as a Biomarker and Therapeutic

Ironically, one of the greatest advantages of miR-21 for its use as a biomarker is also its most difficult challenge: miR-21 has been found to be dysregulated in more than one condition, including several types of cancer.¹²⁸ Then, how can this plausible biomarker be specific enough to detect a condition and differentiate among others? This is particularly important because miR-21 has been proposed as a non-invasive circulating biomarker; however, if its dysregulation is so common in many different diseases, its expression pattern might follow broad stress responses in a cell instead of specific conditions. In other words, if miR-21 is involved in many types of cancer, it might confer robustness to the carcinogenic process by dampening the protein production of its targets. If this is the case, miR-21 would not be the best biomarker for a specific cancer. It would instead describe a carcinogenic process occurring in any organ or tissue showing elevated levels of miR-21 in cancer. Therefore, it would be more convenient to use miR-21 as part of a biomarker panel specific for



each condition in combination with tissue-specific miRNAs to validate the occurring carcinogenic process and locate the affected tissue.

Another issue that has to be solved in order for miR-21 to become a biomarker is the standardization of methods. Recently, inconsistencies between the expression of several miRNAs measured under the same conditions have been found,¹²⁹ such as the analysis made by Gao et al.¹⁰¹ for miR-21 in breast cancer. This finding was not understood, as miRNAs have become of great interest as biomarkers due to their stability. However, it has been proposed that preprocessing steps make a difference when measuring their expression.¹³⁰

In addition, miR-21-based therapeutics also present challenges. The broad regulation of miRNAs within a cell has been discussed, and miRNAs can have many targets related to different functions. This can have a positive aspect when most targets follow the functions of oncomiRs or tumor suppressors, but when a target gene has functions that can either promote or suppress the carcinogenic process,¹³¹ such as the miR-21 target *BCL2*,¹³² the usefulness of a miRNA-based therapeutic is questioned. Furthermore, only some of the targets of a miRNA have been validated, and cancer is a complex disease. How can we be certain that miRNA therapies will have the results we expect from models? Finally, some other challenges faced by miRNA therapeutics are toxicity and long-term effects, both of which address the fact that anti-miRs have to avoid the action of nucleases, but little is known about the toxic metabolites produced by the oligonucleotide that is degraded.¹³³

Although miR-21-based biomarkers and therapeutics seem very plausible for use in the near future, there are still challenges to address before approving them. If these challenges are overcome, miR-21 could become one of the first miRNA biomarkers and therapy molecules used for complex and sometimes fatal diseases, such as cancer.

Conclusions

Experimental and clinical evidence points to miR-21 as a key element in cancer development, as it is involved in invasion, metastasis, cell proliferation, and apoptosis. The identification of some of its target genes and their downregulation effects in cancer remains to be fully elucidated. Despite this fact, miR-21 is a promising biomarker for diagnosis, prognosis, and prediction, although its broad function and dysregulation have been linked to more than one type of cancer. Furthermore, *in vitro* and *in vivo* studies have shown that its inhibition has positive effects on cancer therapy, which enhances its role as a plausible therapeutic target. Currently, clinical trials and toxicity pharmacokinetic evaluations are being conducted for miR-21 inhibitors, such as ADM-21, as therapeutic molecules.

However, there still exist some limitations for its use, such as the lack of knowledge about dose requirements and its administration. Nevertheless, work on these limitations is currently underway, and important issues, such as the administration and delivery of miRNA mimics and anti-miRs, are being solved. These findings will bring a new era for therapeutics, RNA-based therapeutics, which is especially promising

for miRNAs in cancer, where they are especially dysregulated. Low-cost therapy and diagnosis with almost no immune response complications could enhance the use of personalized therapies that could provide all cancer patients with the most effective and specific combination for their sickness. Therefore, to reach the era of RNA therapeutics, two main goals must be achieved: improve the delivery strategies and identify the side effects that these molecules could have on complex diseases.

In conclusion, further information and basic research are required before miR-21 can be used extensively as an approved biomarker and therapeutic target, but its upregulation in a large number of cancers, as well as its relationship with many processes that regulate cancer progression, make it a promising molecule in cancer and in RNA-based therapies.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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