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New Insights on the Roles of miR-21 in Cancer Development and Proliferation

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Abstract: MicroRNAs (miRNAs) are small noncoding RNAs strongly implicated in the control of gene expression post-transcriptionally. They account for a wide range of functions recognized as characteristic cancer signatures, including apoptosis, invasion, metastasis, and proliferation. MicroRNA-21 (miR-21) is well-recognized for its crucial role in various solid tumors, such as glioblastoma, ovarian cancer, non-small cell lung cancer, and many others. In numerous malignancies, miR-21 selectively targets multiple key components and influences a broad spectrum of cellular processes, including cancer stemness and cell death. The miR-21 gene exhibits both cancer-promoting and cancer-suppressing properties, though most research highlights its facilitative role in cancer development. MiR-21 mediates PTEN reduction to enhance PI3K/Akt signaling in cancer progression. Its overexpression inhibits apoptosis and significantly promotes pro-survival autophagy. The notable upregulation of miR-21 in cancerous tissues positions it as a promising cancer biomarker with considerable diagnostic and prognostic potential. This study aims to define the functional roles of miR-21 as a crucial regulator in various cancers and its potential as a therapeutic target.

Keywords: miR-21; Cancer; Molecular mechanisms; Development

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1. Introduction

Approximately 22 nucleotides long, microRNAs (miRNAs) are small noncoding RNAs essential for various cellular functions, including cell growth, suppression of cell death, neovascularization, DNA damage response, stress response, immune-mediated responses, and notably, cancer progression. An estimated 2,000 genes are encoded in the human genome. MicroRNAs can control gene expression in multiple coding transcripts by binding to target mRNAs through specific complementary nucleotides with a length of 7.2 base pairs. Thus, a single miRNA has the capacity to selectively target multiple cellular mRNA transcripts [1]. RNA polymerase II/III transcribes the initial coding region to generate a precursor miRNA, characterized by a hairpin-like structure with a 5′-7-methylguanosine (m7G) cap and a poly(A) tail. Splicing by the RNase III endonuclease

facilitates the intronic excision of primary miRNA. The DGCR8 protein, involved in excision, aids Drosha in hydrolyzing the target site, releasing a 70-nucleotide base pair stem-loop structure, known as pre-miRNA, with a 3′ overhang. The DNA molecule then undergoes an endoribonucleolytic process mediated by Dicer, creating a 3′ overhang on both ends [2]. The selection of a miRNA "guide strand" depends on the thermal stability of the 5′ end of the double strand. This "guide strand" binds to the Argonaute protein 2 (Ago2) complex, while the unbound strand, called the "passenger strand (miRNA*)," is degraded. Mature miRNAs are typically generated through this mechanism [3]. At the final maturation stage, poly(A)-specific ribonuclease (PARN) and other 3′-5′ exoribonucleases further trim or remove these extra nucleotides [4].

Dysregulated miRNA expression is frequently observed in cancer, suggesting a potential link between abnormal miRNA expression and carcinogenesis. Extensive research has highlighted the impact of miRNA regulators on various cancers. For instance, the ubiquitin ligase TRIM71 inhibits tumor growth by regulating let-7 in the Lin28B-let-7-HMGA2 pathway. Additionally, essential miRNA processors and associated transcription factors, such as Drosha, Dicer, TRBP, and Ago2, play a significant role in cancer development. Besides miRNA regulators and miRNAs, many other non-coding RNA types have also been identified as important in gene translation control [5]. Empirical evidence suggests that interactions between long non-coding RNAs (lncRNAs) and miRNAs can act as regulators in various diseases, particularly cancers, indicating that miRNA regulatory mechanisms are more complex than previously understood. Furthermore, although miRNAs were traditionally viewed as intracellular gene regulators, recent discoveries of miRNAs in bodily fluids suggest they may also act as systemic signaling messengers [6].

Table 1. A summary of miRNA types, roles, functions, and mechanisms in cancer

miRNA type	Role	Functions	Mechanism of action	Molecular targets	Cancer types	References
OncomiRs	Tumor-promoting	Promote cell proliferation, invasion, and metastasis	Inhibit tumor-suppressor genes	Cell cycle regulators, apoptosis genes	Breast, lung, colon, prostate	[7]
Tumor-suppressor miRNAs	Tumor-suppressing	Inhibit cell proliferation, invasion, and metastasis	Target oncogenes	Oncogenes, cell cycle regulators	Lung, prostate, breast, pancreatic	[8]
Let-7 family	Tumor-suppressing	Regulate cell differentiation, proliferation, and apoptosis	Target RAS oncogenes	RAS family	Lung, breast, colon	[9]
miR-34 family	Tumor-suppressing	Regulate cell cycle, apoptosis, and senescence	Target *p53* and *c-MYC*	*p53*, *c-MYC*, *BCL2*	Liver, lung, colon	[10]
miR-21 family	OncomiRs	Causes proliferation, invasion, and metastasis	Inhibit tumor-suppressor genes	*PTEN*, *PDCD4*	Breast, lung, colon, prostate	[11]
miR-17-92 cluster	OncomiRs	Increase cell proliferation, invasion, and metastasis	Inhibit tumor-suppressor genes	*PTEN*, *TP53*, *E2F1*	Lung, breast, colon, prostate	[12]
miR-155	Dual role (oncogenic or tumor-suppressive)	Regulate immune response, cell proliferation, and apoptosis	Target various genes	Various genes	Breast, lung, colon, prostate	[13]

Numerous cancer types have principal driver genes and their associated mutations, which frequently occupy key roles in various cellular pathways and have the potential to disrupt or be influenced by miR-21.

Bailey and colleagues identified at least 15 cancer types with similar genetic alterations in genes such as *P53* and *PTEN*. Many of these mutations show a strong association with miR-21 and the specific proteins it targets. To explore the impact of miR-21 overexpression on tumor development, Hatley and colleagues used G12D-mutant KRAS mouse models of NSCLC. Their research demonstrated that the removal of miR-21 significantly decreased tumor size compared to healthy lung tissue [14]. Long-term pulmonary cancer patients with epidermal growth factor receptor (EGFR) mutations also showed increased miR-21 activity levels compared to those without such mutations. EGFR's effect on miRNA development via Ago2 modification post-translation highlights a significant link between genetic alterations and miR-21 functionality. Multiple independent studies have demonstrated elevated miR-21 levels in pancreatic cancer, breast carcinoma, and colorectal cancer (CRC). A comprehensive analysis of 540 human samples revealed frequent miR-21 overproduction in breast, lung, gastrointestinal, prostate, colon, and pancreatic cancers. Furthermore, an analysis of TCGA pan-cancer patient datasets shows a consistent pattern across various cancers [15].

Although the precise mechanisms linking miR-21 overexpression with other oncogenic factors remain unclear, miR-21's role in cancer progression has been documented in several studies. In glioma, for example, the β-catenin pathway regulates miR-21 via STAT3, a well-known oncogenic transcription factor that promotes tumor growth and aggressiveness. miR-21 overexpression modulates EGFR/Akt signaling, selectively targeting the VHL (von Hippel–Lindau) and PPAR-α (peroxisome proliferator-activated receptor alpha) pathways. Additionally, increased miR-21 levels appear to disrupt the MAPK signaling pathway by reducing Sprouty 2 (Spry2) expression, leading to accelerated glioma progression. In pancreatic ductal adenocarcinoma (PDAC), miR-21 directly modulates Spry2, promoting EGF-stimulated cell proliferation. Experimental evidence has shown that miR-21 effectively suppresses the tumor suppressor PTEN and enhances growth and invasion in non-small cell lung cancer (NSCLC). Similarly, miR-21 directly downregulates PDCD4 in breast malignancies [16]. Notably, certain cancers are dependent on one or more oncogenic genes, such as *miR-21*, a phenomenon termed "oncomiR addiction." Using Tet-off and Cre recombinase technologies, researchers demonstrated that upregulating miR-21 in a crossbred mouse model was essential for the malignant phenotype of pre-B-cell lymphoma, confirming their hypothesis. Crucially, when miR-21 was deactivated, tumors regressed, affirming the therapeutic potential of targeting miR-21 [17].

2. miR-21 regulations

In cancer, certain miRNAs are not adequately regulated, and the gene transcripts of primary miRNAs do not indicate an increased expression level of their mature counterparts. Researchers have proposed that the mechanism of miRNA production, particularly the conversion of primary-to-precursor transcripts, influences cancer. Furthermore, RNA helicases from the DEAD-box family, such as DDX5, DDX18, and DDX23, have been shown to impact the expression of miR-21 in various carcinoma types. Research has also demonstrated that ligand stimulation directs small mothers against decapentaplegic (SMAD) signaling transducers, specifically SMAD1/5 and SMAD2/3. This recruitment is facilitated by RNA helicase DDX5, which selectively recognizes primary miR-21, thereby expediting miR-21's rapid production [18].

Unidentified miRNA regulatory elements may play a role in tumor development. Elevated expression of miR-21 isomers (isomiRs) in colorectal and breast carcinoma suggests that these non-canonical variants may significantly contribute to the progression of both cancers. Notably, recent findings suggest that heterogeneous

nuclear ribonucleoprotein C (hnRNPC) can interact directly with primary miR-21, resulting in the alteration of the miR-21 isoform in hepatic cancer cells [19]. These generated isomiR-21s inhibit growth hormone receptors and promote tumor formation. The miR-21-5p strand acts as the leading strand, while the miR-21-3p strand is cleaved as the passenger strand, outlining the production process of microRNA-21. In CRC cell lines and PC9 lung adenocarcinoma cells, recent studies indicate that miR-21-3p levels are significantly elevated. Additionally, different variants of miR-21-3p exhibit distinct effects on cellular functions in CRC [20].

3. miR-21 and apoptosis

MiRNA-21 has been extensively shown to participate in various cellular death processes, including ferroptosis, autophagy, apoptosis, and necroptosis. miR-21 is believed to impact tumor suppressors PTEN and PDCD4, which are essential in initiating apoptosis by modifying the PI3K/Akt/mTOR signaling cascade. Increased expression of PTEN and PDCD4 resulting from miR-21 downregulation leads to growth inhibition and cell death. This study demonstrates that miR-21 directly interacts with and inhibits Sprouty1 (Spry1), a suppressor of the Ras/MEK/ERK cascade. This interaction reduces apoptosis in cardiac fibroblasts. In response to glioblastoma-induced downregulation of miR-21, enzyme matrix metalloproteinase inhibitors such as RECK and TIMP3 are upregulated, which in turn promotes caspase activation and reduces glioma cell motility [21].

The primary inducers of necroptosis—RIP1, RIP3, and MLKL—are indirectly influenced by miR-21 through the alteration of cyclin-dependent kinase 2-associated protein (CDK2AP). Evidence has shown that miR-21 suppresses tumor suppressor genes *PTEN* and *FasL*, and inhibiting miR-21 may lessen the severity of acute pancreatitis [22].

miR-21 has a strong association with autophagy and has proven significant in various malignancies, including colorectal cancer, hepatic cancer, and glioma. In colorectal cancer, miR-21 appears to facilitate autophagy control by interacting with the PTEN/Akt/transcription factor EB (TFEB) pathway. Phosphorylation of TFEB, a crucial component in autophagosome formation, results in the suppression of VMP1 expression. Hepatocellular carcinoma (HCC) cell lines resistant to sorafenib exhibit decreased autophagy and elevated miR-21 levels. In this system, the activation of PTEN and Akt is also restricted, indicating that miR-21 inhibits autophagy induced by sorafenib through the PI3K/Akt pathway. Increased autophagy and heightened radiosensitivity were observed in glioma cell lines with miR-21 inhibition, underscoring miR-21's role in radioresistance by regulating autophagy [23].

Ferroptosis is a recently characterized, regulated form of necrotic cell death marked by the progressive accumulation of iron and lipid peroxidation, leading to oxidative cell death. In many cancers, miR-21 upregulation appears linked to elevated levels of reactive oxygen species (ROS) by influencing downstream targets such as STAT3, proline oxidase, and PDCD4, thereby inducing oxidative stress. These findings suggest a possible role for miR-21 in ferroptosis. In melanoma cell lines, the application of miR-21-3p notably enhanced interferon-gamma (IFN-γ)-induced ferroptosis by targeting thioredoxin reductase 1 and increasing ROS production [24].

An intriguing connection in cancer biology is the relationship between lncRNAs and miRNAs. LncRNAs are untranslated RNA molecules over 200 nucleotides in length, sharing similarities with miRNAs, such as the absence of protein production. In cancer, they are often dysregulated. LncRNAs play regulatory roles in cells, including the ability to reduce the production of macroRNAs by sequestering them. In cancer, the lncRNA/miR-

21 axis mediates the regulation of cell death mechanisms. The lncRNA OTUD6B-AS1, for example, inhibits cell growth and survival while promoting programmed cell death. OTUD6B-AS1-mediated degradation of miR-21–5p leads to increased PNRC2 production and subsequent apoptosis, thereby impeding disease progression. Additionally, lncRNA MCM3AP-AS1 inhibits miR-21 expression, activates PTEN signaling, and promotes apoptosis, leading to suppressed cell growth [25].

Furthermore, lncRNA/miR-21 interactions play a critical role in modulating tumor cell death in various cancers. Recent studies have also investigated the role of circular RNAs (circRNAs) in miRNA regulation. The closed-loop structure of circRNAs enhances their stability by making them resistant to enzymatic degradation. In cancer, circRNAs regulate miRNA expression through sponging, resulting in dysregulation. Exosomal circEPB41L2, for instance, binds to miR-21–5p and miR-942–5p, increasing PTEN expression and inhibiting Akt, thereby suppressing colorectal cancer progression. Several studies confirm that miR-21 acts as a regulator of apoptosis in cancer, influencing tumor progression and responses to clinical therapies. Thus, further research is needed to explore the precise targeting of this non-coding RNA for cancer treatment [26].

4. Role of miR-21 as a biomarker

MiRNAs are often detectable in plasma and serum, making them promising candidates for use as molecular biomarkers in cancer diagnosis and prognosis through minimally invasive methods. miRNAs can be found in biofluids either within extracellular vesicles (EVs) or as standalone ribonucleoprotein complexes outside of vesicles. These miRNAs can help differentiate diseases at distinct clinical stages. Given miR-21's prevalence in multiple cancer types and its association with various pathogenic characteristics, it has been studied as a potential diagnostic and prognostic biomarker for cancer [27].

Patients with adenomas and CRC show significantly elevated serum levels of miRNA-21. Higher levels of miR-21 in circulation and tissues may also correlate with tumor progression, metastasis, and patient survival. A study comparing exosomal miRNAs in ovarian cancer patients identified significant increases in several miRNA types, allowing distinction between those with and without the disease. miR-21 could be particularly effective as a biomarker for rare cancers with limited biomarker expression. An analysis of extracellular vesicles (EVs) from cerebrospinal fluid (CSF) found a link between abnormal miR-21 expression and patient survival with leptomeningeal metastases (LM) [28].

The predictive tool miRcode can identify potential lncRNA-miRNA interactions. However, these findings require further experimental validation to confirm the existence of complex mRNA-miRNA-lncRNA networks in disease progression. Improvements in highly sensitive and immediate detection techniques for specific miRNAs, such as miRDREL, could enhance understanding of the mechanisms by which oncogenic RNAs, like miR-21, contribute to cancer [29].

5. Significance of miR-21 in various types of cancers

5.1. Brain tumors

The interplay of epigenetic components is crucial in determining the malignant and aggressive nature of brain tumors. Attenuation of lncRNA DGCR5 expression in glioma, along with the overexpression of DGCR5 protein, can promote or inhibit malignant cell growth. The lncRNA DGCR5 inhibits glioma cell motility and

invasion by upregulating E-cadherin expression while downregulating the transcription factors Twist and Snai2. DGCR5 reduces miR-21 expression, which in turn suppresses glioma growth. Previous research in laboratory and animal models has shown a correlation between increased levels of miR-21-5p and glioma progression, where suppressing miR-21-5p upregulates TET1 expression, inhibiting glioma growth [30]. By suppressing TET1, miR-21-5p enhances glioma progression.

Elevated miR-21 concentrations have also been associated with chemotherapy resistance in glioblastoma by downregulating Spry2, leading glioma cells to display malignant characteristics. A covalent interaction between the long non-coding RNA LINC00294 and miR-21-5p increases CASKIN1 expression, which in turn triggers apoptosis in glioma cells. With a diagnostic sensitivity of 90% and specificity of 100%, miR-21 is a critical biomarker for distinguishing glioma patients from non-patients. Recently, the discovery of an aggressive brain tumor type highlighted miR-21's crucial role in controlling tumor proliferation. By suppressing FASLG, miR-21 enhances structural characteristics and cell division. In glioblastoma, reduced expression of PWRN1, an upstream regulator of miR-21–5p, has been observed. PWRN1 downregulates miR-21-5p transcription, helping to inhibit malignant cell proliferation and spread in glioblastoma.

Downregulation of miR-21 contributes to the emergence of radiation resistance in both pre- and post-operative glioblastoma samples. Elevated miR-21 levels can serve as reliable biomarkers for glioblastoma. Therapeutic inhibition of miR-21 is effective in slowing glioblastoma growth, emphasizing its crucial role in disease progression. To enhance gene delivery, scientists have developed polymeric constructs specifically engineered to transport anti-miR-21, impeding glioblastoma advancement in both laboratory and animal models [31].

5.2. Gastric cancer

While hospitalization and mortality rates for gastric cancer have declined in recent years, it remains one of the most common cancers and a leading cause of death for both men and women worldwide. Two primary global objectives are the early detection and effective treatment of gastric cancer. Cancer stem cells (CSCs) are known for their self-renewal abilities and play a critical role in cancer progression. In gastric cancer, the gene *CBX7* regulates miR-21 expression and impacts cancer stem cell function. *CBX7* activates the Akt/NF-κB pathway, increasing miR-21 expression and enhancing characteristics associated with gastric cancer stem cells. MiRNA-21-5p plays a significant role in accelerating the proliferation and spread of gastric cancer [32].

Exosomal miR-21–5p promotes mesothelial-to-mesenchymal cell transformation by activating the TGF-β/Smad signaling pathway, with upregulated expression observed in gastric cancer. Through activation of the PI3K/Akt/Wnt/β-catenin axis and downregulation of 15-PGDH expression, miR-21 supports the formation and proliferation of gastrointestinal cancer cells. Hyperproliferation and programmed cell death are defining features of gastric cancer, and miR-21 tightly controls these processes by upregulating Bcl-2 to prevent apoptosis and drive cancer progression. Suppression of miR-21 leads to increased PTEN expression, which blocks the PI3K/mTOR axis, thereby hindering gastric cancer advancement.

As in other cancers, miR-21 has the potential as a biomarker in patients diagnosed with gastric cancer. This potential is supported by a growing body of evidence showing significantly elevated levels of miR-21 in both the blood and urine of affected individuals [33].

5.3. Liver cancer and hepatocellular carcinoma

Increased levels of miR-21–3p positively impact the long-term viability of hepatocellular cancer stem cells.

Conversely, reduced levels of miR-21–3p lead to increased PTEN expression, which in turn inhibits the PI3K/Akt signaling pathway responsible for the death of liver cancer stem cells induced by TRAIL. Similar to other malignant cell types, miR-21 exerts its effects by reducing PTEN expression, thus promoting the proliferation and invasion of liver cancer cells.

miR-21 is a promising candidate for use as a biomarker in the identification of hepatocellular diseases. The combined expression of miR-130b and miR-21 provides superior diagnostic accuracy compared to the individual expression of either miR-21 or miR-130b. Disruption of PTEN expression by exosomal miR-21 promotes the initiation of disease progression. Elevated miR-21 expression in patients has been associated with a more unfavorable prognosis and shorter survival [34].

The downregulation of SMAD7 by miR-21–3p results in overexpression of cancer-promoting factors, thereby accelerating cancer development. Administration of anti-cancer medications can impact miR-21 expression in the treatment of hepatocellular carcinoma. The root and rhizome of Longa produce polyphenols that exhibit biocompatibility with healthy cells and possess antioxidant and anti-inflammatory properties. These compounds activate programmed cell death and suppress the growth and spread of hepatocellular cancer cell lines. The concurrent administration of curcumin enhances the absorption of the medication, inhibiting the progression of hepatocellular carcinoma.

In laboratory studies, curcumin efficiently inhibits the progression of hepatocellular carcinoma in living organisms and induces programmed cell death in a dose-dependent manner. Curcumin also inhibits hepatocellular carcinoma development by reducing miR-21 expression, which in turn increases and controls the TGF-β1/Smad3 interaction. The downregulation and inhibition of PTEN by miR-21 reduction decreases cell proliferation, thereby constraining the progression of hepatocellular carcinoma [35].

5.4. Colorectal cancer

CRC remained the most common malignancy in both males and females in 2019, presenting significant challenges in terms of precise diagnosis, successful therapy (including the emergence of drug resistance), and the spread of metastases. MiRNA-21-5p plays a key role in the process of CRC tumor differentiation. The downregulation of miR-21-5p is associated with the advancement of colorectal cancer, while its upregulation leads to a concomitant increase in the release of inflammatory molecules, including IL-1β and IL-18. The findings of this study provide novel insights into the role of miR-21 in cancer and highlight the intrinsic anti-cancer characteristics of this non-coding RNA. Further investigation offers strong evidence supporting the cancer-promoting function of miR-21 in CRC. The exosomal release of miR-21-5p by carcinoma cells suppresses β-catenin signaling and facilitates the progression of colorectal cancer. In CRC, the expression of MEG2 is reduced, and it functions as a regulator of apoptosis, proliferation, and metastasis development. Through the downregulation of MEG2 expression, miR-21 facilitates the progression of colorectal cancer [11].

Curcumol, a naturally derived organic compound from Rhizoma Curcumae, has shown beneficial properties in suppressing the progression of certain cancers. Curcumol has been recognized as a suppressor of malignant colorectal cancer cell proliferation. It suppresses the excessive expression of PTEN, resulting in a decrease in miR-21 expression and the inhibition of the PI3K/Akt pathway during the progression of colorectal cancer. Elevated production of IL-6 is subsequently associated with the accelerated development and spread of colorectal cancer. CRC cells exhibit increased accumulation of IL-6, alongside elevated levels of miR-21 and miR-29b, which play a crucial role in promoting the release of IL-6 to facilitate tumor progression. The

growth of colorectal cancer cells is inhibited by the downregulation of miR-21, a specific target of lncRNAs, by DGRC5. Through the inhibition of miR-21 expression and stimulation of PTEN signaling, LINC00312 hinders the advancement of colorectal cancer. Additionally, novel non-invasive techniques have been developed to identify miR-21 in plasma and saliva. Meta-analysis studies further validate the diagnostic importance of miR-21 in colorectal cancer [36].

5.5. Prostate cancer

Prostate cancer is the malignant and carcinomal enlargement of the prostate gland in males. This heterogeneous condition has the potential to manifest in severe symptoms that are resistant to medical treatment. Increased expression of miR-21 and Wnt-11 in prostate cancer results in enhanced colony formation and proliferation of malignant cells. Furthermore, the progression from epithelial to mesenchymal lineage is facilitated by elevated levels of miR-21 and Wnt-11, leading to a highly aggressive variant of prostate tissue cancer. The inhibition of PTEN signaling promotes the proliferation and migration of prostate cancer cells by upregulating miR-21. CircSLC8A1 inhibits the migration and progression of prostate cancer by sequestering and reducing miR-21 expression. Additionally, miR-21 has the potential to serve as both a biomarker and a therapeutic target. The rapid identification of prostate cancer requires the development of effective electrochemical sensors for miR-21. Moreover, miR-21 can be regarded as a reliable biomarker for identifying prostate malignancies, with elevated plasma expression levels. However, current research efforts regarding the functions of miR-21 in the regulation of biological pathways in prostate cancer are somewhat limited [37].

5.6. Bladder cancer

The significance of miR-21 as an indicator of disease progression and as a regulator of physiological processes in bladder cancer cells has been examined. However, to fully elucidate the precise function of this miRNA in bladder cancer and its correlation with molecular pathways and processes, additional systematic studies are necessary, as is the case with prostate cancer. A pharmaceutical agent used in the treatment of certain solid tumors, formononetin, has been shown to demonstrate synergistic effects in suppressing breast cancer cell progression and proliferation under regulated laboratory conditions. Furthermore, formononetin efficiently inhibits PI3K/Akt signaling, leading to a decrease in the gene expression levels of *MMP-2* and *MMP-9*, thus limiting cancer cell proliferation. It functions as a regulator of microRNA expression within the framework of cancer therapy protocols. By stimulating the PTEN signaling pathway, formononetin decreases the expression of miR-21 in bladder cancer. This additional activity results in the suppression of PI3K/Akt and a decrease in disease progression. The administration of ovatodiolide has been shown to suppress bladder cancer by inhibiting the secretion of exosomal miR-21 generated by M2-polarized macrophages. The long non-coding RNA NBAT1 regulates the production of SOCS6 by reducing the expression of miR-21, thereby decelerating the progression of the cell cycle. The significant identification of miR-21 in urine samples obtained from bladder cancer patients suggests its potential use as a biomarker [38].

5.7. Breast cancer

The role of miR-21 in breast cancer cells has been investigated as a regulator of physiological processes and its significance as an indicator of disease progression. However, similar to prostate cancer, further systematic studies are required to clarify the exact function of this miRNA in breast cancer and its relationship with

molecular pathways and processes. A pharmacological agent used in the treatment of certain solid tumors, formononetin, demonstrates synergistic effects in suppressing breast cancer cell progression and proliferation under regulated laboratory conditions. Furthermore, formononetin efficiently inhibits PI3K/Akt signaling, resulting in a decrease in the gene expression levels of *MMP-2* and *MMP-9*, thus limiting cancer cell proliferation. It functions as a regulator of microRNA expression within the framework of cancer therapy protocols. Through stimulation of the PTEN signaling pathway, formononetin decreases the expression of miR-21 in bladder cancer, leading to the suppression of PI3K/Akt signaling and a reduction in disease progression. The administration of ovatodiolide also provides benefits in suppressing bladder cancer by inhibiting the secretion of exosomal miR-21 generated by M2-polarized macrophages. The long non-coding RNA NBAT1 regulates the production of SOCS6 by reducing the expression of miR-21, thereby decelerating cell cycle progression. The significant identification of miR-21 in urine samples from bladder cancer patients suggests its potential use as a biomarker [39].

In breast cancer, increased concentrations of miR-21–5p suppress the expression of ZNF367, reducing the invasion and spread of cancer cells. Given its ability to increase miR-21 expression and decrease its efficacy, dendrosomal curcumin shows great promise as a chemotherapeutic adjunct in breast cancer. The reduction of miR-21 levels induced by the excessive expression of BRE-AS1 in breast cancer provides advantages by interfering with mechanisms of cell division and metastasis. Furthermore, a signal indicating breast cancer in the patient's blood could be the considerably high levels of exosomal miR-21-5p. These results vividly illustrate the substantial involvement of miR-21 in breast cancer [40].

5.8. Ovarian cancer

Ovarian cancer is highly prevalent among gynecological malignancies in the United States. A significant increase in miR-21 levels decreases the susceptibility of ovarian cancer to medical treatments, leading to reduced PDCD4 expression and triggering a cascade of cell death. The elevated expression of miR-21 accelerates the advancement and spread of ovarian cancer. MiRNA-21 potentiates ovarian cancer progression by activating the PI3K/Akt pathway through the suppression of PTEN signaling. Suppression of miR-21-3p hinders the proliferation and spread of ovarian cancer cells, while its inhibition enhances their susceptibility to treatment. This makes miR-21 an exceptionally promising therapeutic target for ovarian cancer. The stimulation of the Wnt/β-catenin signaling pathway by the AHNAK protein promotes the proliferation and invasion of ovarian cancer cells. Additionally, the presence of the UBE2S mutation accelerates the development of ovarian cancer and enhances resistance to treatment [41].

By inhibiting miR-532-3p, LINC01094 increases the expression of Wnt and facilitates the progression of ovarian cancer. The upregulation of CD44v6 expression by miRNA-21 induces the Wnt signaling pathway, thereby facilitating the proliferation of colonies and the progression of ovarian cancer. Elevated miR-21 expression also enhances tumor resistance in ovarian cancer tissues. An endogenous chemical, when coupled with specific treatments, promotes apoptosis and can be used for ovarian cancer therapy. The downregulation of miR-21 expression suppresses the progression and aggressive features of ovarian cancer. Berberine, a compound known for its anticancer effects, downregulates the expression of hexokinase 2 (HK2), inhibiting glycolysis in ovarian cancer cells. Treatment with berberine decreases miR-21 expression, resulting in upregulation of PDCD4 expression and enhancing sensitivity to the cisplatin receptor in ovarian cancer. Increased miR-21 expression leads to elevated levels of P-gp and HIF-1α, promoting resistance in ovarian cancer by inhibiting the

JNK-1/c-Jun axis and decreasing PDCD4 transcription. The findings of this study offer empirical support for miR-21's role in regulating signaling networks and highlight its potential use as a diagnostic biomarker [42].

5.9. Cervical cancer

The development of cervical cancer results from dysregulated colony formation and the aggressive proliferation of cervical cells. The inhibition of miR-21 has been empirically shown to result in the upregulation of PTEN and PDCD4, while simultaneously downregulating Akt. In addition to reducing the progression and growth of tumors, this approach also improves sensitivity to the treatment of cervical cancer. Extensive evidence suggests that PTEN plays essential biological roles in cervical cancer, and its upregulation inhibits tumor development. Increased levels of miR-21 in human cervical cancer result in the inhibition of PTEN, thereby promoting the growth and dissemination of malignant cells. LncRNAs are recognized as crucial modulators of miR-21 in cervical cancer [43].

Through the inhibition of miR-21, the lncRNA CASC2 regulates PTEN expression, reducing tumor growth and enhancing the sensitivity of cervical cancer cells to cisplatin therapy. A major factor contributing to cisplatin resistance in cervical cancer is the inhibition of apoptotic responses. A lncRNA decreases the expression of miR-21 to prevent the phosphorylation of E2F3, thus inducing apoptosis and increasing the vulnerability of cervical cancer cells to cisplatin. Apoptosis and suppression of cervical cancer cell proliferation can be induced by lncRNA through the reduction of miR-21 synthesis. An increasing body of data suggests that miR-21 is dysregulated during the progression of cervical cancer. Therefore, directly targeting miR-21 is a viable strategy in the management of cervical cancer [44].

Table 2. miR-21 roles in various cancer types

Cancer type	Roles and functions	Molecular targets	References
Breast cancer	Promotes cell proliferation, invasion, and metastasis; inhibits apoptosis	*PTEN*, *PDCD4*, *TPM1*	[45]
Lung cancer	Contributes to tumor growth, invasion, and metastasis	*PTEN*, *PDCD4*, *RECK*	[46]
Colorectal cancer	Promotes cell proliferation, invasion, and metastasis; confers chemoresistance	*PTEN*, *PDCD4*, *MMP-2/9*	[47]
Pancreatic cancer	Promotes tumor growth, invasion, and metastasis; confers chemoresistance	*PTEN*, *PDCD4*, *MMP-2/9*	[48]
Gastric cancer, ovarian cancer, and prostate cancer	Promotes cell proliferation, invasion, and metastasis; confers chemoresistance	*PTEN*, *PDCD4*, *MMP-2/9*	[49-51]

6. Conclusions and future perspectives

Notwithstanding the advancements in the identification, use, and inhibition of miR-21 over the last decade, several aspects remain unresolved, including the maturation process of miR-21 and the factors that govern the development of canonical versus noncanonical isomiR variants in certain cancer types. To achieve a more comprehensive understanding of the role of miR-21 in cancer, it is necessary to deepen our knowledge of these mechanisms and their consequences. The available empirical evidence indicates that miR-21 plays a crucial role in cancer progression by participating in cell proliferation, invasion, metastasis, and programmed cell death. However, a comprehensive understanding of the specific target genes and their subsequent downregulation

effects in cancer remains incomplete. Despite its broad function and disruption linked to several unique forms of cancer, miR-21 offers significant promise as a biomarker for the detection, prediction, and prognosis of various cancer types. Furthermore, studies carried out in animal and laboratory models have demonstrated that inhibiting this mechanism has advantageous impacts on cancer therapy, strengthening its potential as a feasible therapeutic target.

The main goal of enhancing miR-21 expression is to promote the advancement of particular pathologies, as many types of cancer exhibit varying degrees of expression. While miR-21 regulates other cancer-promoting pathways, such as STAT3 and Wnt, the PTEN/PI3K/Akt axis is the most well-acknowledged downstream target. The advancement of carcinogenesis is facilitated by miR-21 through the reduction of PTEN expression, thereby inducing PI3K/Akt feedback. These findings indicate that inhibiting miR-21 has beneficial efficacy in cancer therapy. The overexpression of miR-21 leads to the suppression of apoptotic and autophagic cell death, promoting the survival and growth of cancer cells. Characterization of the regulation of miR-21 by lncRNA and circRNA demonstrates the potential to target the upstream mediators of miR-21 in cancer therapy. Moreover, the emergence of anti-cancer drugs that specifically focus on miR-21 provides proof that this non-coding RNA is highly vulnerable to targeting. The transfer of this material between cells via exosomes further enhances its potential as a diagnostic tool for diagnosing and predicting the prognosis of cancer patients. MicroRNAs are now universally acknowledged to be present and functional in both the intracellular and extracellular environments. However, previous research has not comprehensively investigated the function(s) of miR-21 in intercellular communication within the tumor microenvironment, particularly in clinical patients or tumor models derived from patients. Further investigation may reveal other functions of miR-21 in disease development, considering its ability to specifically target a wide range of pathways that both inhibit and promote malignancy.

Specific stages of miRNA production can be selectively targeted by tailoring miR-21 suppression techniques. For instance, small-molecule inhibitors like diazobenzene and oestradiol have been employed to target specific stages in miRNA synthesis, such as the transcription process. By employing small chemicals that specifically bind to the G-hairpin of the hTERT-G-quadruplex-forming sequence, the miRNA structure has been directly targeted, leading to the downregulation of gene expression. Through rodent experimentation, this approach yielded strong anticancer benefits. Nevertheless, as mentioned earlier, certain challenges remain related to the administration, effectiveness, and adverse effects of RNA treatments. Further investigation is needed to validate the effectiveness and specificity of miR-21 inhibitors.

Disclosure statement

The author declares no conflict of interest.

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