Review

MicroRNA-221 and MicroRNA-222 in Common Human Cancers: Expression, Function, and Triggering of Tumor Progression as a Key Modulator

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ABSTRACT

MicroRNAs (miRNAs) are a class of short (~22 nucleotides [nt]), single-stranded RNA oligonucleotides that are regulatory in nature and are often dysregulated in various diseases, including cancer. miRNAs can act as *oncomiRs* (miRNAs associated with cancer) or tumor suppressor miRNAs and have the potential to be a diagnostic, prognostic, noninvasive biomarker for these diseases. MicroRNA-221 (miR-221) and microRNA-222 (miR-222) are homologous miRNAs, located on the human chromosome Xp11.3, which factored significantly in impairment in the regulation of a wide range of cancers. In this review, we have highlighted the most consistently reported dysregulated miRNAs that trigger human tissues to express cancerous features and surveyed the role of those miRNAs in metastasis, apoptosis, angiogenesis, and tumor prognosis. Also, we applied the causes of drug resistance and the role of coordinated actions of these miRNAs to epigenetic changes and selected miRNAs as a potential type of cancer treatment.

Keywords: microRNA, miR-221/-222, invasion, metastasis, apoptosis, angiogenesis, drug resistance, epigenetic change

Abbreviations

miRNAs, microRNAs; nt, nucleotides; 3′-UTR, 3′ untranslated region; mRNAs, messenger RNAs; pre-miRNA, precursor microRNA; RNase, ribonuclease; DGCR8, DeGeorge Critical Region 8; RISC, RNA-induced silencing complex; miR-221, microRNA-221; miRNA-222, microRNA-222; NSCLC, non–small-cell lung carcinoma; HCC, hepatocellular carcinoma; BLBC, basal-like breast cancer; TNBC, triple-negative breast cancer; ER, estrogen receptor, PR, progesterone receptor; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand; p27Kip1, cyclin-dependent kinase inhibitor 1B; PI3K/AKT, phosphatidylinositol-3-kinase and protein kinase B; LNCaP, lymph-node carcinoma of the prostate; CDKs, cyclin-dependent kinases; G1 phase, Gap1 phase; CDKN, cyclin-dependent kinase inhibitor; CPEB, cytoplasmic polyadenylation element binding protein 1; AT, atypical teratoid; RT, rhabdoid tumor; CNS, central nervous system; PC, pancreatic cancer; MMPs, matrix metalloproteinases; STAT, signal transducer and activator of transcription; ADAM17, disintegrin and metalloproteinase domain containing protein 17; TIMP2, TIMP metallopeptidase inhibitor 2; LNA, locked nucleic acid; MM, multiple myeloma; EMT, epithelial-mesenchymal transition; TRPS1, transcriptional repressor GATA binding 1; ZEB2, zinc finger E-box-binding homeobox 2; MET, mesenchymal to epithelial transition; ADIPOR1, adiponectin receptor 1; NFκB, nuclear factor kappa B; IL-6, interleukin 6; PUMA, p53-upregulated modulator of apoptosis; BAD, B-cell lymphoma 2–associated death promoter; PBAD, phosphorylated B-cell lymphoma 2–associated death promoter; BMF, B-cell lymphoma 2–modifying factor; PTEN, phosphatase and tensin homolog; OSCC, oral squamous-cell carcinoma; TIMP3, TIMP metallopeptidase inhibitor 3; TGF-β, transforming growth factor β; FZD5, frizzled class receptor 5; RCC, renal-cell carcinoma; SCF, stem-cell factor; HUVECs, human umbilical-vein endothelial cells; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; MEOX2, mesenchyme homeobox 2; FOSL1, FOS-like 1 activator protein 1 transcription factor subunit; MEKi, MEK inhibitor; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; PLZF, promyelocytic leukemia zinc finger; HMGB1, high mobility group box 1; RAGE, receptor for advanced glycation end product; BMSCs, bone-marrow stromal cells; ALL, acute lymphoblastic leukemia; DNMT, DNA methyltransferase; HDAC, histone deacetylase; BRG1, Brahma-related gene 1; SWI/SNF, switch/sucrose nonfermentable; ATXN1, ataxin 1; PS, phosphorothioate; PO, phosphodiester; Chol, cholesterol; ARH1, aplysia RAS homology member I; NA, nonapplicable; ER-α, estrogen receptor alpha; SOCS1, suppressor of cytokine signaling 1; MGMT, O-6-methylguanine-DNA methyltransferase; TMZ, temozolomide; PTPμ, protein tyrosine phosphatase; DDIT, DNA damage inducible transcript; MDM2, mouse double minute 2 homolog; BBC3, B-cell lymphoma 2–binding component 3; ANGPTL, angiopoietin-like; ECs, endothelial cells; ETS1, ETS proto-oncogene 1, transcription factor; APAF-1, apoptotic peptidase activating factor 1; RECK, reversion-inducing cysteine-rich protein with kazal motifs; RelA, RelA proto-oncogene; PDLIM2, PDZ-LIM domain 2; EGFP, enhanced green fluorescent protein; PCPH, cetylated polyethyleneimine; PLC/PRF/5, a human hepatocellular carcinoma cell line; r, recombinant; Ad, adenovirus; AAV, adenoassociated virus; i, inhibitor; Dox, doxorubicin; R8, arginine octamer; PNA, peptide nucleic acid; Fl, fluorescein; Rpep, arginine peptide; AEEA, 2-(2-aminoethoxy) ethoxyacetyl spacer; BC, breast cancer; AP-1, activator protein 1

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MicroRNAs (MiRNAs) are a class of small (19–25 nucleotides [nt] in length) single-stranded, noncoding RNA sequences that play central roles in modulating key biological processes by adjusting the posttranscriptional regulation of gene expression through base pairing with the complementary sequences in the 3′ untranslated region (3′-UTR) of their target messenger RNAs (mRNAs). We note that miRNAs are aberrantly expressed within cancerous tissues and can be detected in biological body fluids containing serum, plasma, and urine.[1,2] The great interest in the scientific community regarding miRNAs reflects their central role in the regulation of numerous biological and pathological procedures, including proliferation, differentiation, development, metastasis, invasion, and apoptosis.

One-third of eukaryotic genes are directly regulated through these noncoding RNAs. Also, half of human miRNA genes are situated in susceptible regions against cancer, emphasizing their significant role in tumor expansion.Thus, the anomalous expression of miRNAs can be a sign of multiple diseases, including cancers.[3–7]

Figure 1 indicates the biogenesis of miRNAs, which contains the maturation of miRNA precursors, assembly of the mature miRNA into microprocessor complexes, and the adjustment of expression of protein-coding genes by destroying or blocking translation of mRNA targets, which can be a complex procedure. Similar to other human genes, miRNA is transcribed through RNA polymerase II in the nucleus. The next stage is the generation of a precursor microRNA (pre-miRNA) through the ribonuclease (RNase) III Drosha enzyme and the processing of the double-stranded DNA-binding protein DeGeorge Critical Region 8 (DGCR8) in the nucleus; then, it is transported to the cytoplasm by Exportin 5.[8,9] In the following sequence, pre-miRNA is processed in the miRNA duplex (~18–25 nt) by the activity of the RNase III endonuclease Dicer protein. One strand of this duplex is matured miRNA that forms a complex with Argonaute proteins, called RNA-induced silencing complex (RISC).

Finally, mRNA decay is caused by a complete linkage between miRNA and the target mRNA; also, the process of translation is hindered by partial base matching.[5,10,11] MiRNAs have a notable role in triggering all biologic processes. Also, because a single miRNA can target hundreds of mRNAs, improper miRNA expression contributes to the progression of many diseases that involve human cancers.

In this review, we focused on the most systematically reported dysregulation of microRNA-221 (miR-221) and microRNA-222 (miRNA-222) in multiple kinds of human tissues, which trigger those cells to develop cancerous features. By reviewing the results of recent studies, we evaluated the represented essential contribution of miR-221/-222 to tumorigenesis and cancer progression. In **Table 1**, we show data regarding the role of miR-221/-222 and the target genes of those entitites in various cell lines and cancerous tissues.

Effective Role of MiR-221/-222 in Triggering Malignant Tumors

MiR-221/-222 are overexpressed in different types of malignant neoplasms, including non–small-cell lung carcinoma (NSCLC), colorectal carcinoma, breast cancer, hepatocellular carcinoma (HCC), glioblastoma, bladder cancer, prostate cancer, melanoma, and ovarian cancer.[31,47–50] In a report,[12] Stinson et al reported a greater amount of miR-221 and miR-222 in basal-like breast cancer (BLBC), compared with the luminal type. They identified that these miRNAs were abundant in basal B, which is more aggressive than basal A. Also, through this research, the researchers found that these miRNAs were more abundant in triple-negative breast cancer (TNBC) than estrogen receptor (ER)–/progesterone receptor (PR)–positive ones.

Also, Garofalo et al,[31] reported that miR-222 is widely overexpressed in HCC and NSCLC, compared with less-invasive and healthy lung and liver cells. In addition, 4 NSCLC cell lines were recently assessed[51] in terms of miR-221 and miR-222, which illustrates the high expression of miR-221 and miR-222 in tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)–resistant and TRAIL-semiresistant cell lines, compared with TRAIL-sensitive cells.

Another study[52] provided evidence that miR-222 exosomal expression frequently reflected its abundance in melanoma-origination cells, precisely paralleled through suppression of its target genes, such as cyclin-dependent kinase inhibitor 1B (p27Kip1), and induction of the phosphatidylinositol-3-kinase and protein kinase B (PI3K/AKT) pathway. Therefore, expression of miR-222 exosomal expression can confirm its functional implication and its ability to increase

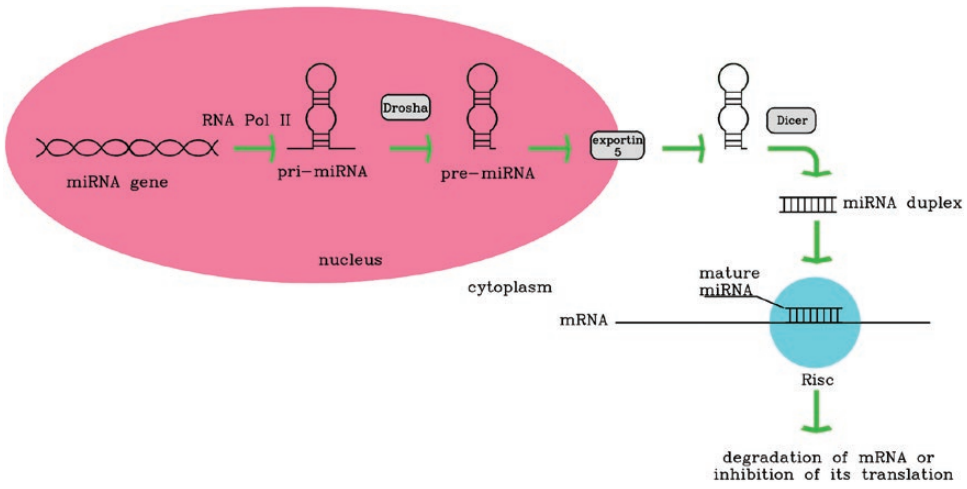

Figure 1

The biogenesis of microRNAs (miRNAs). Primary miRNA cleaved to precursor miRNA (~60–70 nucleotides [nt]) by RNase III endonuclease Drosha in the nucleus. It is transported to the cytoplasm by Exportin5. Precursor microRNA (pre-miRNA) is processed in miRNA duplex (~22 nt) through the activity of the Dicer enzyme. One strand of this duplex is mature miRNA that forms a complex with Argonaute proteins, called RNA-induced silencing complex (RISC). Also, messenger RNA (mRNA) decay is caused by a complete linkage between miRNA and the target mRNA, and the process of translation is hindered by partial base matching.

tumor malignancy in melanoma in humans. Another research report[19] shows the reverse relationship among the expression of miR-221/-222 and the cell-cycle inhibitor p27Kip1 which, through ectopic overexpression of these miRNAs directly, can lead to downregulation of p27 in lymph-node carcinoma of the prostate (LNCaP) cells.

Impact of miR-221/-222 in Tumor Progression

Cell-cycle progression controlled by various factors, such as cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors that are broadly adjusted through these noncoding RNAs. p27, a CDK inhibitor that can suppress cell-cycle progression, has been shown[26] to be a considerable direct target of miR-221/-222. Also, Lu et al[53] found that miR-221 can promote proliferation in human glioma by targeting p27 and initiating cancerous processes in brain tissue.

Another study[54] reported that these 2 miRNAs can regulate melanoma progression by downregulation of p27/cyclin-dependent kinase inhibitor 1B (p27Kip1; also known as *CDKN1B*), and C-KIT target process genes. Also, a recent report[56] divulged that the inhibitory impact of miR-221 and miR-222 on p27 could be disrupted through the cytoplasmic element binding protein 1 (*CPEB1*) gene. *CPEB1* belongs to the *CPEB* family, which modulates translation by attaching to the 3′UTR of mRNAs. P27 is a target of miR-221/-222 and *CPEB1*; unlike miRNA-221 and miRNA-222, which inhibits the expression of p27, *CPEB1* encourages it. Due to the competition occuring between miRNA-221/-222 and *CPEB1* on contiguity of the p27 overlapping area, the repressing mechanism of *CPEB1* is specific to the tumor cells.

We were intrigued by the findings of Hsieh et al[55] that miR-221/-222 can promote tumor growth via targeting nuclear protein SUN2 in atypical teratoid (AT)/rhabdoid tumor (RT), which is a kind of central nervous system (CNS) tumor. Targeting miR-221, miR-222, and SUN2 may represent a new therapeutic strategy for AT/RT remedy. Also, Xu Q et al[57] show that these 2 miRNAs promote pancreatic cancer (PC) progression by controlling matrix metalloproteinases (MMPs). Treatment of PC with cells that mimic miR-221-/-222 leads to downregulation of TIMP metalloproteinase inhibitor-2 (TIMP2, which is the target gene of miR-221/-222) and an increase in MMP-2 and MMP-9, the two components of the MMP family that contribute to invasive characteristic of cancer.

Table 1. Summary of Results Regarding the Functions of MiR-221/-222 and Their Target Genes in Human Cancer Tissues and Cell Lines

Cancer Type	Cell Lines	Target Gene(s)	Function(s)	Reference
Breast cancer	CAL85-1, MDA-MB-231, MCF-10a	*TRPS1*	Promoted EMT	12
	MCF-7, MDA-MB36, T47D	β*4* integrin, *STAT5A*, *ADAM-17*	Led to proliferation and invasion	13
	MCF-7, MDA-MB-231, MCF-12A, SK-BR-3	*ARH1*	miR-221 induced proliferation and invasion and inhibited apoptosis in the cell by targeting *ARH1*	14
	NA	*ADIPOR1*	Induced EMT	15
	MDA-MB-231, MDA-MB-453, T47D, MCF-7, HS578T	*CDKN1B*, *SOCS1*	Promoted cell-cycle progression, migration, and invasion	16
	MCF-7, T47D, HCC1954, MDA-MB-468, MDA-MB-231	*ATXN1*	miR-221 regulated hierarchy in breast cancer and promoted stemness in luminal cells	17
Breast cancer and lung cancer	MCF-7, A549, HEK293	*PUMA*	Downregulation of miR-221/-222 resulted in an increase in *PUMA*, which has an apoptotic effect in the cell	18
Prostate carcinoma	PC3, LNCaP	*p27*	Tumor progression	19
Glioma	NA	*TIMP2*	Promoted cell invasion and angiogenesis	20
	U251	*p27Kip1*	Promoted growth	21
	U87MG, T98G, LN428, LN308, A172, HEK239	*MGMT*	Genetic damage could not be repaired; miR-222 increased response to TMZ	22
Glioblastoma	U87MG, T98G, LN-308, LN-319, A172, LN-428, LN18, LN-229,	*PTP*μ	Regulated cell motility and invasion	23
	A172, U251, H4, LN229	*PUMA*	Inhibited apoptosis	24
HCC	NA	*BMF*	Tumor progression	25
	HEP3B	*CDKN1C/p57*, *CDKN1B/p27*	miR-221 controlled cell growth and cell cycle in HCC cell lines	26
	HEPG2	NA	miR-222 modulated sensitivity to sorafenib via PI3K/AKT pathway	27
	104 tissue specimens, 35 HCC cell lines	*DDIT4*	Promote tumorigenesis	28
	HEPG2, HEP3B, SNU449, Huh-7, 47 tissue specimens	*MDM2*	miR-221 modulated the response to doxorubicin damage and apoptosis	29
	SK-HEP-1, HepG2, SMMC-7721	*BBC3*, *ANGPTL2*	Knockdown of miR-221 reduced tumor progression	30
Lung and liver cancer (NSCLC and HCC)		*PTEN*, *TIMP3*	Led to migration, invasion, and apoptosis resistance	31
Atherosclerotic disease	ECs	*STAT5A*	miR-222 had an antiangiogenic role by regulating STAT5A	
MM	U266	*p27*, *p57*, *PUMA*, *PTEN*	miR-221/-222 inhibitors had antitumor roles in MM	33
Pancreatic cancer	Capan-2	*p57*	miR-222 regulated proliferation in Capan-2 cells	34
Acute leukemia	NA	*ETS1*	Overexpression of miR-222 reduced proliferation and induced apoptosis in leukemic cells	35
OSCC	UM1	*PUMA*	Reduced sensitivity to cisplatin	36
Laryngeal cancer	Hep2	*APAF-1*	Knockdown of miR-221 promoted apoptosis and inhibited proliferation	37
Colorectal carcinoma	Caco2	*CDKN1C/p57*	Knockdown of miR-221 inhibited growth of cancer cells	38
	SW480, HCT116, HT29, LoVo, SW620	*RECK*	miR-221 induced invasion and metastasis	39
	HCT116, RKO, Lovo, DLD-1, HCT15, HT29, H508, SW1116, SW480	*RelA*, *PDLIM2*	Positive feedback loop: miR-221/-222 stabilized *RelA* mRNA by directly binding to it, and upregulated RelA and STAT3 proteins by binding to *PDLIM2*; also, miR-221/-222 were upregulated by *RelA* and *STAT3*	40

Table 1. Continued

Cancer Type	Cell Lines	Target Gene(s)	Function(s)	Reference
Osteosarcoma	MG-63	*PTEN*	Promoted proliferation, migration, and invasion	41
Gastric cancer	SGC7901	*PTEN*	Knockdown of miR-221/-222 decreased invasion and growth and increased radiosensitivity	42
	MGC-803	*HAI-1*	Induced cell growth	43
	BGC-823, SGC-7901	*RECK*	Induced growth and invasion in cancer cells	44
RCC	ACHN	NA	miR-221/-222 increased proliferation of ACHN cells	45
	786-O, ACHN, Caki-1, Caki-2	*TIMP2*	miR-221 induced cell proliferation and invasion	46

Abbreviations: miR, microRNA; TRPS1, *transcriptional repressor GATA binding 1; EMT, epithelial-mesenchymal transition;* STAT, *signal transducer and activator of transcription; ADAM-17, disintegrin and metalloproteinase domain 17;* ARH1, *nicotinamide adenine dinucleotide phosphate-adrenodoxin reductase; NA, nonapplicable;* ADIPOR1, *adiponectin receptor 1; ER-α, estrogen receptor alpha; TNBC, triple-negative breast cancer;* CDKN, *cyclin-dependent kinase inhibitor;* SOCS1, *suppressor of cytokine signaling 1;* ATXN1, *ataxin 1;* PUMA, *p53-upregulated modulator of apoptosis; LNCaP, lymph-node carcinoma of the prostate;* TIMP3, *metalloproteinase inhibitor 3; p27Kip1, cyclin-dependent kinase inhibitor 1B (p27, Kip1);* MGMT, *O-6-methylguanine-DNA methyltransferase; TMZ, temozolomide;* PTPμ, *protein tyrosine phosphatase; HCC, hepatocellular carcinoma; BMF, B-cell lymphoma 2–modifying factor; PI3K/AKT, phosphatidylinositol-3-kinase and protein kinase B; DDIT, DNA damage inducible transcript; MDM2, mouse double minute 2 homolog;* BBC3, *B-cell lymphoma 2–binding component 3; ANGPTL, angiopoietin-like; NSCLC, non–small-cell lung cancer;* PTEN, *phosphatase and tensin homolog; ECs, endothelial cells; MM, multiple myeloma;* ETS1, *ETS proto-oncogene 1, transcription factor; OSCC, oral squamous-cell carcinoma;* APAF-1, *apoptotic peptidase activating factor 1;* RECK, *reversion-inducing cysteine-rich protein with kazal motifs;* RelA, *RelA proto-oncogene, nuclear factor kappa betaNF-κB subunit;* PDLIM2, *PDZ-LIM domain 2; mRNA, messenger RNA; HAI-1, hepatocyte growth factor activator inhibitor 1; RCC, renal-cell carcinoma;* TIMP2, *metalloproteinase inhibitor 2.*

A study by Dentelli et al found that miR-221/-222 regulates proliferation, tumor growth, and invasion in luminal-like breast cancer by targeting signal transducer and activator of transcription (STAT)5A, disintegrin and metalloproteinase domain-containing protein 17 (ADAM17), and β4-integrin. Their results revealed that miR-221/-222 can control β4-integrin expression in luminal MCF-7 cells. Upregulation of miR-221/-222 resulted in downregulation of β4-integrin in this cell line. Downregulation of these miRNAs in MCF-10 cells did not alter β4-integrin expression. Their results revealed this mechanism is specific to tumor cells.[13]

In another report, Di Martino et al[58] investigated the anti-tumor activity of locked nucleic acid (LNA)–i-miR-221 in MM cells with or without *t*(4, 14) translocation. They proved the anti-MM function in cells carrying translocation (group A) but not in the other ones (group B), so their outcomes suggested that LNA-i-miR-221 might be useful for the treatment of group A of patients. miR-221/-222 modulates the behavior of cancer cells by regulating different target genes and some biological process in human cells. **Figure 2** refers to the direct target genes of miR-221/-222.

Role of MiR-221/-222 as a Tumor Suppressor

As we implied earlier herein, miRNAs can act as oncomiRs or tumor-suppressor miRs; miR-221 and miR-222 are no exception to this rule. The results of research by Coskun et al[35] indicated that upregulation of miR-222 in acute leukemic cells by suppressing the proto-oncogene EST1 can lead to induction of cell-cycle arrest and inhibit cell growth. Also, Goto et al[59] reported that miRNA-221 and miRNA-222 act as tumor suppressors in prostate-cancer cells, and their expression represent a decline. The researchers report ECM29 as a novel target of miR-221/-222; low expression of these miRNAs can enhance migration and invasion in this type of malignant neoplasm.

Induction of Tumor-Cell Invasion and Metastasis by MiR-221/-222

Metastasis can be defined as a complex and multistage procedure for which the epithelial-mesenchymal transition (EMT) is considered an important biological step during metastasis. Within the EMT, epithelial cells lose their cellular adhesions and represent the mesenchymal phenotype.[60,61] Some studies[62–64] reported that the expression of miR-221/-222 is associated with invasive characteristic and metastasis events in human cancers. **Figure 3B** refers to the mechanisms through which miR-221/-222 modulates EMT in breast cancer.

The findings of a study by Stinson et al[12] indicated that EMT increased through miR-221 and miR-222 via transcriptional repressor GATA binding 1 (*TRPS1*) in breast carcinoma. In general, *TRPS1* is classified in the GATA family and is an EMT inhibitor; this gene can block EMT

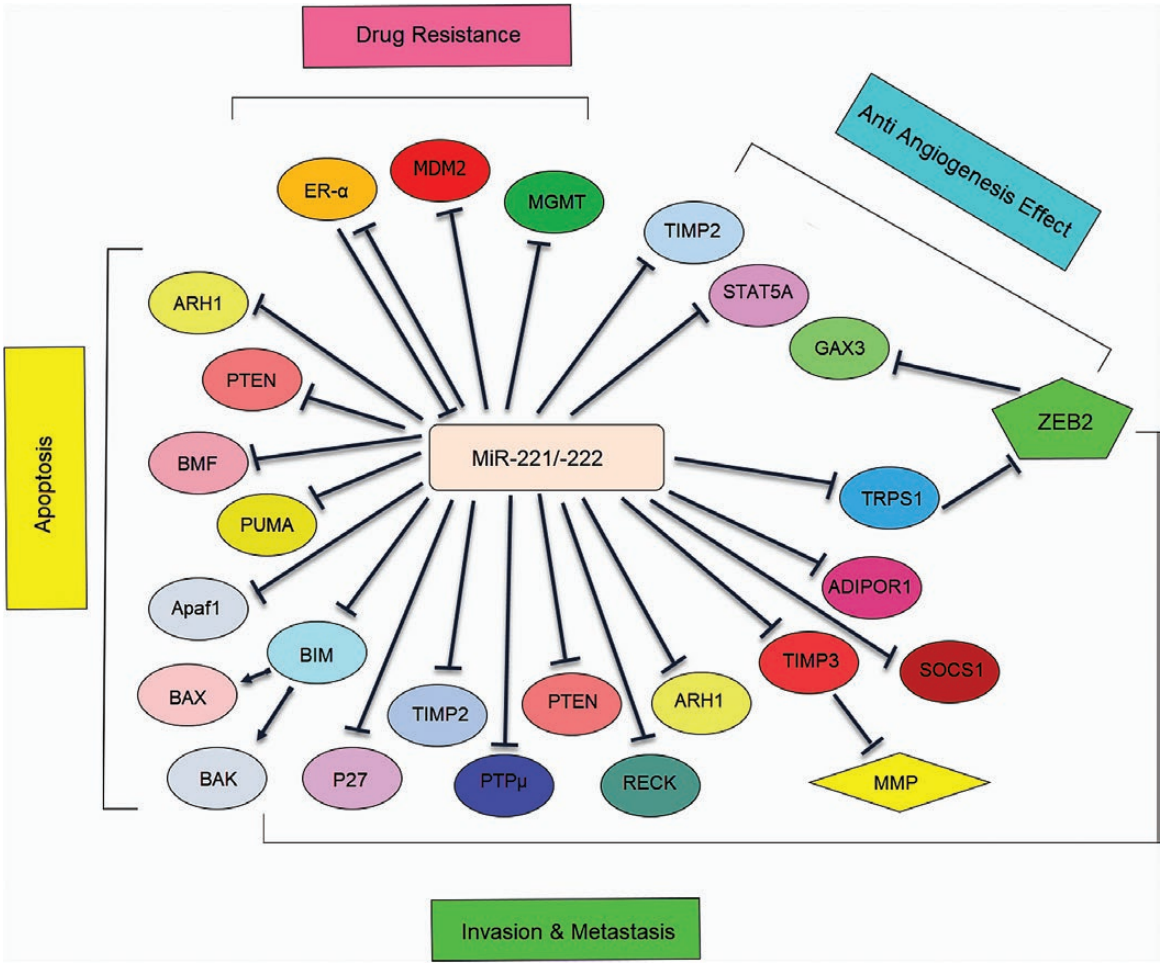

Figure 2

Schema summarizing of the different genes targeted by microRNA-221/-222 (miR–221/-222).

by downregulating zinc finger E-box-binding homeobox 2 (ZEB2); thus, *TRPS1* knockdown resulted in an increase in ZEB2. Up regulation of ZEB2 decreases E-cadherin abundance and increases vimentin levels to promote the EMT phenotype. These researchers treated MCF10A cells with entities that mimicked miR-221/-222; subsequently, they measured the abundance of E-cadherin and vimentin. A decline was observed in E-cadherin as an epithelial marker, and a rise was reported in vimentin as a marker of mesenchymal transition. Also, mesenchymal to epithelial transition (MET) phenotypes were created from inhibition of miR-221/-222 by anti–miR-221 and anti–miR-222. Therefore, the researchers showed that the expression of miR-221/-222 is associated with EMT markers and phenotypes.

Also, Hwang et al[15] discovered another mechanism through which miR-221/-222 can regulate the EMT in breast cancer. They identified adiponectin receptor 1 (*ADIPOR1*) as a target of miR-221/-222 that inhibits the EMT process and reported that *ADIPOR1* can negatively regulate nuclear factor kappa beta (NFκβ), interleukin 6 (IL-6), and JAK2/STAT3 pathways to suppress EMT in breast cancer.

In another work of research, Li et al[16] evaluated the influence of miR-221/-222 on how cells invade or migrate to other tissues. They treated basal-like MDA-MB-231 cells with entities that mimicked miR-221/-222 and also performed wound-healing migration and transwell invasion assay. The results showed that wound healing and invasion

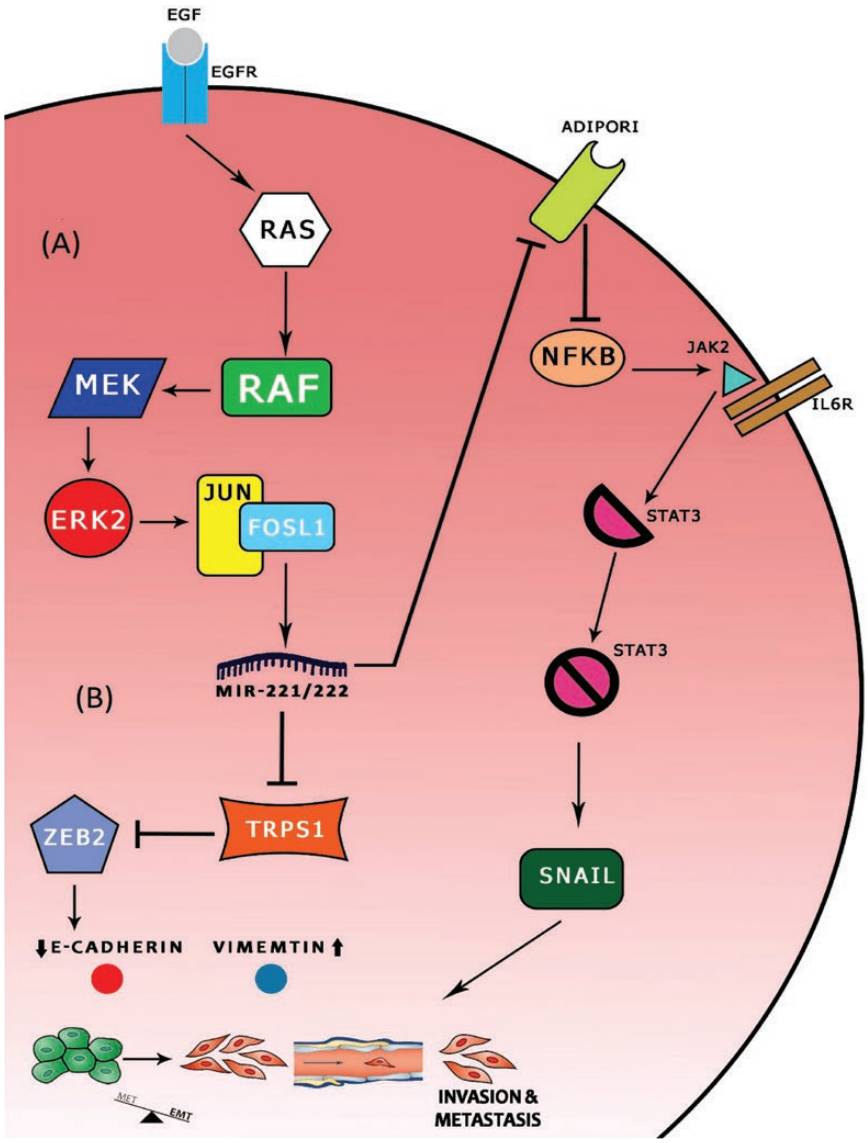

Figure 3

Regulation mechanism of miR-221/222 and EMT progression in breast cancer. **A**, A model of regulating miR-221/-222 in breast cancer. The RAS-RAF–mitogen-activated protein kinase kinase (MEK)–FOS-like 1 activator protein 1 transcription factor subunit (FOSL1) signaling axis regulates the way miR-221 and miR-222 are expressed. FOSL1 heterodimerizes with a member of the JUN family to build the activator protein 1 (AP-1) complex. There is an AP-1 binding region, 12 kb upstream of miR-221/-222. FOSL1 is a positive regulator of these microRNAs. **B**, The mechanisms through which these microRNAs modulate the EMT in BC. MiR-221/-222 is targeted by transcriptional repressor GATA binding 1 (*TRPS1*), the inhibition of zinc finger E-box-binding homeobox 2 (ZEB2) by *TRPS1* is removed, and ZEB2 is released and promotes EMT. MiR-221/-222 also induces EMT by targeting adiponectin receptor 1 (*ADIPOR1*). *ADIPOR1* regulates nuclear factor kappa B (NF$\kappa\beta$), interleukin 6 (IL-6), *JAK2*/signal transducer and activator of transcription (*STAT3*) pathway to suppress EMT in BC.

were increased in these cells. The authors also treated non-invasive luminal MCF-7 cells with entities that mimic miR-221/-222; however, the aforementioned results were not observed. Anti-miR-221/-222 were treated in 3 BLBC cell lines—MDA-MB-231, SUM159, and Hs578t—then, a migration and invasion assay was applied. The results illustrated

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that wound closure and invasion were reduced in all 3 cell lines. In addition, the MTT assay clarified that MDA-MB-231 growth could be prevented by miR-221/-222 inhibitors.

MiR-221/-222 as Regulators of Apoptosis Mechanisms in Cancer

Tumor cells can adopt various strategies to override apoptosis containment, amplification of the antiapoptotic machinery, downregulation of the proapoptotic program, or both of these tactics. Proapoptotic factors are widely adjusted and controlled via miRNAs. To suppress apoptosis in glioma, miR-221/-222 targets a proapoptotic gene named *PUMA* (p53-upregulated modulator of apoptosis).[24]

In another study, Zhang et al[18] reported that in epithelial carcinoma, miR-221/-222 targets *PUMA*. Their study results revealed that downregulation of miR-221/-222 by oligonucleotides increased caspase 3/7 expression and led to apoptosis in A549 and MCF-7 cells. To investigate the effect of miR-221 knockdown on apoptosis, they treated cell lines with anti–miR-221 and then measured apoptosis markers; cleaved caspase 3 and B-cell lymphoma 2–associated death (BAD) phosphorylation. They observed that downregulation of miR-221 caused an increase in cleaved caspase 3 and a decrease in B-cell lymphoma 2–associated death promoter (PBAD) in TNBC cell lines.

Also, Gramantieri et al[25] reported that there is a negative correlation between miR-221 and B-cell lymphoma 2–modifying factor (BMF) and a positive correlation between BMF and the apoptosis marker caspase-3 in HCC. They revealed that miR-221 inhibits apoptosis via inhibition of BMF in HCC. The study also identified that the upregulation of miR-221 resulted in a more aggressive phenotype in this type of malignant neoplasm.

In addition, Wang et al[66] showed a negative association between miR-221/-222 and caspase-10 by evaluating cases of prostate cancer. The results indicated that when miR-221/-222 is downregulated, the cancerous cells become sensitive to apoptosis caused by tumor necrosis factor–α/cycloheximide. In another study report,[67] researchers disclosed that the depletion of miR-221 and miR-222 could enhance the apoptosis process by modulation of phosphatase and tensin homolog (*PTEN*). They treated cells with an miRNA sponge and then measured the number of apoptotic cells via Annexin V/PI testing (BioLegend); their observations revealed an increase in apoptosis.

Regulation of Drug Resistance by MiR-221/-222

Recent study reports[68–70] have shown that miRNAs can regulate drug resistance in cancer through acting on multiple signaling pathways. With the detection of the role of miRNAs in drug resistance, another promising approach has emerged: the use of miRNAs encoded by the human genome as diagnostic tools to predict drug response. Thus, knowledge of all the miRNAs associated with drug resistance will be key to designing drugs with greater efficacy and safety, which will have positive health and economic ramifications.

Also, Jiang et al[36] reported that *PUMA* is directly targeted by miR-222 in oral squamous-cell carcinoma (OSCC). Their findings demonstrated that knockdown of miR-222 by AS-miR-222 elevated *PUMA* expression and caused an increase in cisplatin sensitization in UM1 cells. The authors also indicated that AS-miR-222 and cisplatin suppress proliferation and promote apoptosis in OSCC.

Regarding the fact that PI3K/AKT is an active oncogenic pathway during malignancy in liver cells, Liu et al[27] report that miR-222 enhances sorafenib resistance in HCC through the PI3K/AKT pathway. Also, Garofalo et al[31] detected that miR-221/-222 induces TRAIL resistance through *PTEN* and TIMP metallopeptidase inhibitor 3 (*TIMP3*), as targets, in NSCLC and malignant hepatoma cells. They showed that miR-221/-222 can regulate TRAIL sensitivity in NSCLC through interaction with P27 and TRAIL-induced caspase machinery.

In another study, Rao et al[50] reported that miR-221/-222 can enhance fulvestrant resistance in breast cancer by regulating multiple oncogenic pathways, such as Wnt and transforming growth factor β (TGF-β) signaling processes. Also, they observed that miR-221/-222 can increase the expression of β-catenin, frizzled class receptor 5 (FZD5), and SMADs. Overexpression of β-catenin culminated in

fulvestrant resistance in MCF7 cells. Thus, β-catenin is the main regulator of the WNT signaling cascade, and SMADs are the key regulators of the TGF-β signaling pathway.

VEGFR2 and C-KIT are 2 targets of miR-221/-222, and both of them are sunitinib receptors. Also, Khella et al[71] reported that upregulation of these 2 miRNAs associates with sunitinib resistance in renal-cell carcinoma (RCC). This occurred because of a decline in these 2 targeted proteins, so they are not available to join with sunitinib. Also, Acunzo et al[72] reported that upregulation of miR-130a could suppress miR-221/-222 by targeting MET. MiR-130a could sensitize NSCLC cells to TRAIL by decreasing miR-221/-222.

Role of MiR-221/-222 in Causing Tumor Angiogenesis

Tumor cells can create new blood vessels to supply nutrients and oxygen, which they require for their growth and progression—this process is called *angiogenesis*. The angiogenic switch depends on the balance of antiangiogenic and proangiogenic factors. Some of these factors could be modified by miRNAs, and growing evidence[73–75] suggests the regulatory role of miRNAs in angiogenesis. Herein, we focus on the roles of miR-221/-222 in the angiogenesis process during tumor-cell development. Yang et al[20] reported that miR-222 promotes angiogenesis of glioma cells by targeting *TIMP2*, which is a member of the TIMP family. Tissue inhibitors of metalloproteinase are antiangiogenic factors that can inhibit the activity of MMPs in stopping tube formation.

miR-221/-222 can display antiangiogenic effects in endothelial cells. Also, Poliseno et al[73] indicated that miR-221/-222 regulates the angiogenic activity of the stem-cell factor (SCF) by targeting the C-KIT in human umbilical-vein endothelial cells (HUVECs). SCF is identified as a ligand for the SCF-receptor C-KIT. The coauthors observed that miR-221 and miR-222 repress capillary tube formation, wound healing, survival, and migration by downregulation of the C-KIT in HUVECs.

Upregulation of miR-221/-222 indirectly decreases endothelial nitric oxide synthase (eNOS) released in endothelial cells by Dicer knockdown. The eNOS is an enzyme that produces nitric oxide (NO) in endothelial cells. NO modulates endothelial cell growth, migration, and angiogenesis. So, miR-221/-222 acts as a repressor of angiogenesis by decreasing eNOS.[76]

In another report, Chen et al[77] state another mechanism through which miR-221 represses angiogenesis. In a study on molecular and cellular biology, they demonstrated that miR-221 upregulates *GAX* by downregulating ZEB2, a repressor of *GAX*. *GAX* (mesenchyme homeobox 2 [*MEOX2*]) is a homeobox gene that has antiangiogenic effects in endothelial cells. *GAX* is downregulated by ZEB2; this process occurs via attaching to 2 ZEB2/SIP1 binding sites in GAX3. The findings by Chen et al suggest that targeting ZEB2 could be used for antiangiogenic therapy in cancer.

Also, Dentelli et al[32] reported that STAT5A is a target of miR-222 in endothelial cells. MiR-222 controls inflammation-mediated neoangiogenesis by targeting STAT5A.

Regulation of MiR-221/-222 Expression

Molecular mechanisms that regulate miR-221/-222 expression have been investigated in certain studies. Stinson et al[12] reported that FOS-like 1 activator protein 1 transcription factor subunit (FOSL1) positively regulates transcription of miR-221/-222. They demonstrated that the epidermal growth factor receptor (EGFR)–RAS-RAF–mitogen-activated protein kinase kinase (MEK)–ERK2-FOSL1 signaling axis regulates miR-221/-222 expression and subsequently promotes EMT in BLBC. To form activator protein 1 (AP-1) complexes, heterodimerization occurred between FOSL1 (as a member of the FOS family) and a member of the Jun family. Transfection of MEK inhibitor (MEKi) into BLBC cell lines led to a decrease in the abundance of miR-221/-222, showing that miR-221/-222 release is organized by the RAS-RAF-MEK axis. MEKi treatment diminished the expression level of FOSL1.

The abundance of E-cadherin increased and vimentin decreased in these cell lines by MEKi treatment, indicating that they become more epithelial. Treatment of CAL85-1 cells with EGFR siRNA reduced extracellular signal-regulated kinase (ERK)1/-2 phosphorylation and expression of

miR-221/-222. EGFR inhibitor did not alter the abundance of miR-221/-222 in MDA-MB-231 cells with active RAS mutations. This observation demonstrated that EGFR controls the expression of miR-221/-222 through the RAS-RAF-MEK cascade.[12] **Figure 3A** indicates an axis that regulates miR-221/-222 in breast cancer.

Garofalo et al[31] reported another mechanism for miR-221/-222 activation in NSCLC and malignant hepatoma. The study results revealed that activation of miR-221/-222 is partially regulated by the c-MET oncogene and c-Jun transcription factors. The results indicated that c-Jun contributes to miR-221/-222 regulation, unlike c-Fos. They also found an AP-1 binding site in 130bp upstream of miR-221/-222. Activation of the MET oncogene leads to upregulation of miR-221/-222 through the c-Jun N-terminal kinase (JNK) cascade. MiR-221/-222 overexpression leads to downregulation of *PTEN* and *TIMP3*, which subsequently resulted in migration, invasion, and resistance to apoptosis in NSCLC and HCC cells. *PTEN* controls the PI3K/AKT pathway, which has roles in MDR and invasion. *TIMP3* induces caspase 8 and caspase 9, which are important stimulants of apoptosis.

Garofalo et al suggested that the MET, JNK, AP-1 axis regulates the expression of miR-221/-222 in these malignant neoplasms.[31] The results of another study by Felicetti et al[54] illustrated that negative control of miR-221/-222 by promyelocytic leukemia zinc finger (PLZF) occurred through direct attachment to the regulatory site. Thus, knockdown of PLZF leads to upregulation of miR-221/-222. Also, the results of research by Acunzo et al[72] showed that miR-130a is a negative regulator of miR-221/-222 in NSCLC. In 2 other studies,[78,79] it was reported that high mobility group box 1 (HMGB1) and receptor for advanced glycosylation end product (RAGE) interact with each other to regulate the expression of miR-221/-222, which subsequently leads to a decrease in *PTEN*. HMGB1 repressed *PTEN* through increasing the level of miR-221/-222. The coauthors discovered that the HMGB1/RAGE–miRNA221/-222–*PTEN* axis controls proliferation in neuroblastoma and thyroid carcinoma.

The results of another research work[80] suggested that niche cells (such as bone-marrow stromal cells [BMSCs] and human osteoblasts) control the expression of miR-221 and miR-222 in patients with acute lymphoblastic leukemia (ALL). Due to this event, an increase in p27 levels was observed, which subsequently resulted in cell-cycle arrest and inhibition of proliferation. Another consequence is the loss of sensitivity to chemotherapy drugs.

We were surprised to learn that miRNA-221/-222 could adjust other miRNAs. A recent study report[81] declared that miR-221/-222 could reduce the overall expression levels of miRNAs by downmodulation of Dicer-1. MiR-221/-222 could inhibit Dicer-1 by binding to its 3′ untranslated region in TNBCs. Dicer is a ribonuclease III enzyme that is involved in the biogenesis of miRNAs. So, miRNAs have a complex regulatory network, and further studies should be performed to discover their complicated pathways.

Epigenetics, MiR-221/-222, and Human Cancers

miRNAs have the ability to regulate genes involved in epigenetic pathways. DNA methyltransferase (DNMT) and histone modifiers, such as polycomb complex genes and histone deacetylase (HDAC), are 2 main group of epigenetic mediators.[82,83] **Table 2** refers to epigenetic modifiers that are regulated by miR-221 and/or miR-222. The recent report by Lopes et al,[84] which analyzed the expression profiles of miR-21, miR-221, miR-135b, and miR-29c in noncancerous, tumor-adjacent, and tissue specimens infected with OSCC, indicated the existence of the field cancerization result in oral tumorigenesis via an epigenetic approach. Therefore, these miRNAs have the potential of representing biomarkers for detecting field cancerization of oral cancer and contributing to pathogenic procedures, along with OSCC progression.

The results of another study[85] identified that miR-221 and miR-222 can act as regulators of the functional reprogramming of macrophages during lipopolysaccharide tolerization. Enhanced stimulation with lipopolysaccharide in mice leads to promoted expression of miR-221 and mir-222, both of which adjust Brahma-related gene 1 (*BRG1*, also known as *Smarca4*). This result promoted expression triggers the transcriptional blocking of a subset of inflammatory genes that depend on chromatin remodeling mediated by switch/sucrose nonfermentable (SWI/SNF) and STAT, which in turn elevates tolerance.

Another report[86] illustrated that HMGB1, which is released via defective cells and cancer cells, overexpressed

miR-221/-222 oncogenic clusters in human neuroblastoma–derived cell lines, indicated that the oncogenic cluster miR-221/-222 were expressed more extremely by the most undifferentiated cell line SK-N-BE (2), compared with the less-tumorigenic cell line SH-SY5Y, and that exogenous HMGB1 promotes this expression. Also, HMGB1 triggers *PTEN* expression through miR-221/-222, as identified through transiently blocking miR-221/-222 with antisense oligonucleotides. Yet another report[17] stated that the upregulation of miR-221 stimulated stem-like cells in luminal type of cancer, and the miR-221 level was associated with clinical result in patients with breast cancer, inducing EMT through upregulation of miR-221 in healthy cells and cells infected with breast cancer. The EMT-relevant gene ataxin 1 (*ATXN1*) was detected to be a miR-221 target gene regulating breast-cell hierarchy. Thus, miR-221 plays an important role in the lineage homeostasis of healthy epithelial cells and those infected with invasive breast cancer.

Targeting MiRNAs for Cancer Therapy

MiRNAs could be targeted in therapy against cancer because these small molecules can play significant roles in triggering cancerous cells. Altering the expression of miRNAs might be a useful strategy in anticancer treatment.[90,91] For oncogenic miRNAs such as miR-221/-222, the purpose of intervention is to decrease or inhibit their expression. Anti-miRs have been developed as a new therapeutic approach for deactivation of these miRNAs.

Anti-miRs use different modifications to achieve good cellular uptake, good efficiency, and high stability in cancer cells. The modification includes 2′O-me, 2′O-MOE, 2′F, RNA with phosphorothioate (PS) linkage, RNA with phosphodiester (PO) linkage, RNA with cholesterol (Chol) linkage, and LNA. Another strategy in miRNA-based cancer therapy is named miRNA sponge. MiRNA sponge, which contains multiple binding sites for miRNA, joins to a vector with a strong promoter. After internalization into target cells, miRNA sponge will transcript simultaneously with the vector components. High levels of these transcripts could strongly inhibit miRNA function.

Table 2. Epigenetic Modifier Regulated by MiR-221 and/or MiR-222

Epigenetic Modifier		Targeted By	Reference
DNMTs	DNMT3b	miR-221	87
HDAC	HDAC6	miR-221	88
Polycomb complex protein	Bmi-1	miR-221	89
MGMT		miR-221/-222	20

Abbreviations: miR-221, microRNA-221; DNMT, DNA methyltransferase; HDAC, histone deacetylase; MGMT, *methylguanine methyltransferase.*

Recently, miRNA zipper, an inhibitor of miRNA, was introduced; it is a promising approach in cancer treatment.[92,93] The comparison of miRNA-221 inhibitors in terms of delivery method, properties, and function are presented in **Table 3**. Also, **Table 4** compares the effects of Fl-Rpep-peptide nucleic acid (PNA)–anti-miR-221 and Fl-PNA-anti-miR-221 on miR-221 inhibition. Despite that advances has been made in miRNA-based therapy in cancer, this therapy is still in its beginning steps, and any restrictions must be resolved before this type of therapy can be used in clinics.

In a research, the dual luciferase reporter assay demonstrated *PTEN* to be a target gene of miR-221/-222. It was also illustrated that miR-221/-222 inhibition through transfection with a miR-221/-222 sponge in vitro resulted in upregulation of *PTEN*. We were surprised to discover that the proliferation and invasiveness of the miR-221/-222 sponge-transfected cells was considerably suppressed, and that apoptosis was elevated.[94]

Also, detection of viral vector activity in HCC cells illustrated their ability to diminish miR-221 endogenous levels, which was accompanied by promotion in CDKN1B/p27 protein, a known target of miR-221. The diminution of oncogenic miRNAs demonstrates a potential anticancer manner that identified novel miR-221 sponge vectors, which can decrease miR-221 activity regarding in vitro and in vivo delivery.[95]

Conclusions

In this article, we have summarized the critical function of the miR-221/-222 in various types of human cancers. Understanding the significant impact of miRNAs in human malignant neoplasms has divulged to scientists a novel starting point for cancer studies. Among the contributions

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Table 3. MiR-221 Inhibitors and Their Cellular Effects

miRNA Inhibitor	Delivery Technique	Cancer Type	Cell Line	Characteristic(s)	Result(s)	Reference
Recombinant GV249 vector (EGFP-anti-miR-221)	Ultrasound SF6 microbubbles	Hepatoma	HepG2	High safety, high stability, efficient transfection	Inhibition of cell proliferation, induction of cell apoptosis	96
PCPH/anti-miR-221	PCPH nanoparticles	Hepatoma	HepG2	Efficient transfection	Inhibition of growth	97
2′O-methyl-PS-anti-miR-221	Lipofectamine 2000 transfection	HCC	PLC/PRF/5	NA	Reduction of cell proliferation by 25% at 48-h & 72-h time points	98
r Ad-199T-miR-221 sponge, r AAV-miR-221 sponge	Viral delivery	HCC	HepG3	r Ad-199T had a stronger effect than r AAV	Increase in apoptosis; reduction in cell viability	95
LNA-i-miR-221		MM	NCI-H929, OPM2		Reduction of proliferation on t(4,14) translocated MM cells	58
LNA-miR-221 zipper	HiPerFect transfection reagent[a]	Breast cancer	MDA-MB-231	High stability, high affinity	90% knockdown of miR-221 expression, at the concentration of 50 nM of the miR-221 zipper increased the sensitivity of cells to Dox	99
R8-PNA-a221		Glioma	U251, U373, T98G		Strong inhibition of miR-221	100
2′-methoxy-modified-anti-miR-221	Liposome	Colorectal carcinoma	Caco2		Induction of apoptosis, inhibition of proliferation	38

Abbreviations: miRNA-221, microRNA-221; EGFP, enhanced green fluorescent protein; PCPH, cetylated polyethyleneimine; PS, phosphorothioate; HCC, hepatocellular carcinoma; PLC/PRF/5, a human hepatocellular carcinoma cell line; NA, nonapplicable; r, recombinant; Ad, adenovirus; AAV, adenoassociated virus; LNA, locked nucleic acid; i, inhibitor; MM, multiple myeloma; Dox, doxorubicin; R8, arginine octamer; PNA, peptide nucleic acid.
[a]*Manufactured by QIAGEN.*

Table 4. The Comparison of the Effects of Fl-Rpep-PNA-a-MiR-221 and Fl-PNA-a-MiR-221 on MiR-221 Inhibition[a]

Anti-miR Molecules	Structure	Cancer Type	Cell Line	Cellular Effect		
				Cellular Internalization	Stability and RNA Binding	Result(s)
Fl-Rpep-PNA-a 221	Fl-AEEA-(Arg)8-AAACCCAGCAGACAATGT-NH2	Breast cancer	MDA-MB-231	Efficiently cellular uptake	Constant effect without retransfection	Strong inhibition of miR-221
Fl-PNA-a 221	Fl-AEEA-AAACCCAGCAGACAATGT-NH2			Poor cellular uptake	Low stability, needed to be retransfected	Weak and temporary inhibition of miR-221

Abbreviations: Fl, fluorescein; Rpep, arginine peptide; PNA, peptide nucleic acid; miR, microRNA; AEEA, 2-(2-aminoethoxy) ethoxyacetyl spacer.
[a]*Adapted from reference [101].*

of a large number of miRNAs to cancer mechanisms, miR-221 and miR-222 are considered to be key modulators of cancer progression that are dysregulated in different types of human malignant tumors. These entities are involved in many aspects of cancer, such as invasion, metastasis, angiogenesis, apoptosis, and drug resistance. Our results highlighted the growing comprehension among the scientific community of the role of miR-221/-222 in various cancer types and the great potential of these miRNAs in cancer therapy.

However, we have still not developed standard procedures to understand the profound function of these miRNAs in the formation of tumor cells. Expanding the ways to enhance the efficiency of miRNAs in cancer remedies can be a useful approach to controlling cancer. Also, further studies should be performed to determine the miRNA pathway, involving the expression, function, target genes, and involvement of miRNAs in epigenetic change and drug resistance for cancer progression, other than in gene adjustment, which will enhance their utilization in clinical applications.

In clinical applications, miRNAs have been applied as one of the most hopeful biomarkers for cancer monitoring. Also, biopsies of bodily fluids, including plasma and urine, can be an easily accessed and noninvasive method for tracing the increase or decrease in expression levels of miR-221/-222 in multiple diseases, including cancer. Performing such biopsies may prove to be an authentic technique for the prognosis and diagnosis of various cancers. **LM**

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