

Review

Macro-management of microRNAs in cell cycle progression of tumor cells and its implications in anti-cancer therapy

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The cell cycle, which is precisely controlled by a number of regulators, including cyclins and cyclin-dependent kinases (CDKs), is crucial for the life cycle of mammals. Cell cycle dysregulation is implicated in many diseases, including cancer. Recently, compelling evidence has been found that microRNAs play important roles in the regulation of cell cycle progression by modulating the expression of cyclins, CDKs and other cell cycle regulators. Herein, the recent findings on the regulation of the cell cycle by microRNAs are summarized, and the potential implications of miRNAs in anti-cancer therapies are discussed.

Keywords: MicroRNA; cell cycle progression; cancer therapy

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Introduction

The cell cycle, which is involved in many cellular processes such as proliferation, development and differentiation, is a crucial process in mammals. In a normal cell, the maintenance of cell cycle fidelity is strictly controlled by surveillance proteins, including cyclins and cyclin-dependent kinases (CDKs). After receiving mitogenic signals, the cells exit from the G₀ phase and enter the G₁ phase. Subsequently, cyclin D binds to CDK4 and CDK6, leading to the partial inactivation of Rb family proteins. Inactivation of Rb proteins results in the transcription of E2F1-related genes. Cyclin E1, which is encoded by an early E2F1 responsive gene, then binds to CDK2 and completely inactivates Rb protein, driving the cells to enter the S phase. When the cells enter the S phase, cyclin A binds to CDK2 and promotes the phosphorylation of many proteins involved in DNA synthesis. Prophase is then initiated after cyclin A binds to CDK1. At the end of interphase, breakdown of the nuclear envelope leads to the interaction of cyclin B with CDK1, which in turn allows the cells to undergo mitosis. In addition to the positive regulation by cyclins/CDKs, the cell cycle is also controlled by negative regulatory proteins, such as CDK inhibitors, which mainly include two families: the INK4 and Cip/Kip families^[1]. Additionally, cell cycle regulation is

monitored by many checkpoints, such as the ATM/ATR pathway^[2]. As summarized above, proper progression through the cell cycle is strictly monitored by various positive and negative surveillance factors. Improper progression through the cell cycle results in many diseases, including cancer. Indeed, the disruption of normal cell cycle progression is a very important event in the initiation and development of cancer. Cancer cells often show genomic instability, which is usually caused by cell cycle dysfunction and leads to unscheduled proliferation^[3]. Moreover, genomic instability predisposes cancer cells to acquire more genetic alterations, which contributes to a more aggressive cancer phenotype. Thus, understanding cell cycle dysregulation in cancers will facilitate the development of improved and novel anti-cancer therapies.

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression mainly by binding to the 3' untranslated regions (3'-UTRs) of target genes^[4]. After miRNAs bind to a target gene, the target protein is down-regulated owing to mRNA degradation or translation termination. Currently, there are 1424 known human microRNAs, and it is predicted that approximately 30% of human protein-coding genes are regulated by miRNAs^[5]. In the past five years, compelling studies have demonstrated that miRNAs are involved in many physiological and pathological processes, such as cancer. In 2005, He L and co-workers found that expression of the miR-17-92 cluster was increased in B-cell lymphoma and cooperated with c-myc to accelerate tumor formation *in vivo*^[6].

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Additionally, Cimmino A *et al* demonstrated that miR-15 and miR-16 induced apoptosis by targeting BCL-2^[7]. These studies indicated that microRNAs can act as potent oncogenes or tumor suppressors and can regulate the progression of cancer. Since then, an increasing number of studies have clarified the role of miRNAs in cell cycle control, proliferation and tumor metastasis. In this review, we will focus on the regulation of tumor cell cycle progression by miRNAs, how miRNAs alter cell cycle regulatory networks and the implications of this regulation in anti-cancer therapy.

The macro-management of miRNAs in cell cycle progression

Evidence for the importance of miRNAs in regulating the cell cycle originated in studies of the miRNA target gene *lin-4*^[8], which acts to modify latent reiterative cell lineages and cell division in *C elegans*. In the past five years, increasing evidence has indicated that miRNAs directly control cell cycle progression by targeting cell cycle regulators. Additionally, miRNAs indirectly control cell cycle progression by targeting signal transduction pathways. Therefore, miRNAs affect cell cycle progression by targeting various genes that span the cell cycle regulatory network.

Let-7 family

The let-7 miRNA was first discovered to be essential for development in *C elegans*, possibly by targeting the *lin-41* gene^[8]. It was reported that let-7 is downregulated in human lung cancer, and enforced expression of let-7 in lung cancer cells inhibits proliferation^[9]. A subsequent study indicated that let-7 not only inhibits proliferation but also alters the cell cycle progression of cancer cells^[10]. In that study, Johnson *et al* demonstrated that let-7 overexpression retains cells in the G₁ phase and alters cell cycle-related pathways. CDK6, CDC25A, and cyclin D2 have been validated as direct downstream targets of let-7 in lung cancer cells. Schultz *et al* found reduced expression of let-7b in melanoma cells, and let-7b also delays the G₁/S transition by down-regulating cyclin D1, cyclin D3, cyclin A, and CDK4^[11]. Another let-7 family member, let-7a, is down-regulated in prostate cancer and induces G₁ arrest by reducing the levels of the downstream targets E2F2 and cyclin D2^[12]. In addition to regulating the G₁/S transition, the let-7 family also affects the G₂/M phase. In human primary fibroblasts, let-7 expression is reduced in the dividing cells. Ectopic expression of let-7 in fibroblast cells decreases proliferation and increases the cell fraction in the G₂/M phase. Let-7 induces G₂/M arrest by down-regulating the CDC34 protein, which then increases wee1 protein levels and subsequently increases the inhibitory phosphorylation of CDC2^[13]. In summary, the let-7 family members negatively control cell cycle regulators, which results in reduced proliferation and other cell-type specific phenotypes.

miR-17-92 and miR-106b/25 clusters

Another versatile miRNA family that controls the cell cycle includes the miR-17-92 and miR-106b/25 clusters. This

miRNA family is composed of three miRNA clusters located on three different chromosomes. The miRNAs include miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1, which are encoded on chromosome 13; miR-106b, miR-93, and miR-25, encoded on chromosome 7; and miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2, and miR-363, encoded on the X chromosome. The miR-17-92 cluster is amplified in B-cell lymphoma, and enforced expression of miR-17-92 antagonizes c-myc-induced apoptosis and acts with c-myc to increase tumor development in a B-cell lymphoma animal model. These results indicate that the miR-17-92 cluster functions as an oncogene^[6]. Expression of the miR-17-92 cluster is also elevated in lung cancer and promotes the proliferation of lung cancer cells^[14]. Recently, miR-17-92 was found to be induced by c-myc and to promote tumor angiogenesis^[15]. In addition to c-myc, miR-17-92 is induced by E2F transcription factors and forms a negative regulatory loop by directly targeting E2Fs^[16, 17]. As a potential mechanism of cancer cell cycle regulation by miR-17-92, it was reported that c-myc-induced miR-17 and miR-20a cooperate with the RB/E2F signaling pathway to control the accurate timing of the G₁/S transition^[18]. Cloonan *et al* reported that miR-17-5p regulates more than 20 G₁/S transition-related genes^[19], and RBL2 was identified as a functional downstream gene of miR-17-5p^[20, 21]. It should be noted that the targets of miR-17-92 depended on the cellular context, which results in diverse functions of the miR-17-92 cluster in different cells. For example, in c-myc-overexpressing Raji cells, miR-17-19b-1 reduces the expression of Bim. In BCL-2-overexpressing SUDHL4 cells, miR-17-19b-1 decreases the expression of CDKN1A/p21 and facilitates the G₁/S transition^[22]. In contrast to the function of miR-17-19b-1 in SUDHL4 cells, miR-17/20a inhibits the G₁/S transition in breast cancer cells by reducing the expression of cyclin D1^[23]. It has been reported that miR-17-92 regulates a set of G₁/S transition-related genes, including pro-proliferation genes, such as cyclin D1 and E2F1, and anti-proliferation genes, such as CDKN1A (p21), PTEN, RB1, RBL1 (p107), and RBL2 (p130)^[24]. The dual regulation of pro-proliferation and anti-proliferation target genes by miR-17-92 may explain the variation in the reported functions of miR-17-92.

In addition to the miR-17-92 cluster, E2F1 also regulates the expression of the miR-106b/25 cluster and is suppressed by the miR-106b/25 cluster, thereby forming a negative regulatory loop^[25]. Moreover, up-regulation of the miR-106b/25 cluster impairs the transforming growth factor (TGF)- β tumor suppressor pathway by targeting CDKN1A (p21 Waf1/Cip1) and BCL-2L11 (Bim), which in turn reverses G₁ arrest induced by the TGF-beta signal^[25]. Therefore, up-regulation of the miR-106b/25 cluster negatively modulates the TGF-beta tumor suppressor pathway and facilitates the development of tumors.

miR-34 family

The miR-34 family includes important p53-responsive miRNAs, and when upregulated, the miRNAs induce p53-mediated processes, such as cell cycle arrest and apoptosis^[26, 27].

In human A549 cells, miR-34a induces G₁ arrest by targeting cyclin D1 and CDK6, which in turn affects the phosphorylation of Rb protein^[28]. In neuroblastoma, miR-34a reduces the expression of BCL-2 and MYCN to block the G₁/S transition^[29]. E2F3 is also a target of miR-34a in neuroblastoma^[30]. However, in human glioma and medulloblastoma cells, miR-34a induces G₁ arrest by reducing c-MET, Notch1, Notch2, and CDK6^[31]; in human astrocytes, however, overexpression of miR-34a does not alter cell cycle progression^[31]. Another member of the miR-34 family, mir-34b, also regulates cell cycle regulators. In acute myeloid leukemia, miR-34b targets the CREB protein and decreases various CREB downstream proteins, such as BCL-2, cyclin A1, cyclin B1, cyclin D, nuclear factor-kappa B, Janus-activated kinase 1 and signal transducer and activator of transcription 3^[32]. miR-34 also regulates cell cycle progression independent of the p53 signal. In p53-null K562 cells, phorbol esters induce miR-34a expression by activating an alternative phorbol ester-responsive promoter to produce a longer pri-miR-34a transcript. Additionally, enforced expression of miR-34a in K562 cells inhibits proliferation and G₁/S transition by targeting CDK4 and CDK6^[33]. Another p53 family member, p63 positively regulates cell cycle progression by repressing the expression of miR34a and miR34c^[34]. In the absence of p63, miR-34a and miR-34c are increased in primary keratinocytes and embryonic skin, with concomitant G₁ arrest and inhibition of the cell cycle regulators cyclin D1 and CDK4^[34]. Taken together, these data indicate that the miR-34 family modulates the expression of various cell cycle-related genes and is therefore important in the cell cycle progression of numerous cell types.

Other miRNAs regulating cell cycle progression

Cell cycle regulators are also modulated by many other miRNAs. One example is cyclin D1, which is regulated by miR-449a^[35], miR-193b^[36], miR-15/16^[37-39], miR-19a^[40], miR-195^[41], and miR-302a^[42]. The cyclin D1 binding proteins CDK4 and CDK6 are also regulated by a number of miRNAs, including miR-107^[43], miR-449a^[44], miR-129^[45], miR-125b^[46], miR-15/16^[39], miR-24^[47], miR-195^[41], and miR-124a^[48]. Another important G₁/S regulator cyclin E, is down-regulated by miR-16^[37] and miR-195^[49]. In addition to the positive G₁/S regulators, negative G₁/S regulators are also targeted by various miRNAs. The CDK inhibitor p21 is targeted by miR-17-92 and miR-106b/93^[22, 25] in addition to many other miRNAs^[50, 51]. The p27 and p57 proteins are regulated by miR-221/222^[52, 53] and miR-92b^[54]. The INK family member p16 is regulated by miR-24^[55]. The number of miRNAs regulating the entry and progression through G₂/M is much fewer than those regulating the G₁/S transition. The G₂/M-related cyclin A is targeted by let-7^[11]. miR-132/212 reduces the expression of RB1, which leads to an increase in E2F1 levels and G₂/M arrest^[56]. Dihydrofolate reductase (DHFR), an enzyme regulating the S phase, is also regulated by miR-24^[57] and miR-192^[58]. In addition, PLK1, a critical regulator of many stages of mitosis, is targeted by miR-100^[59] and miR-593*^[60]. Ectopic expression of miR-100 and miR-593* causes G₂/M arrest and reduces proliferation. Wee

1 kinase, a nuclear protein that delays mitosis by negatively regulating the cyclinB-CDK1 complex, has been identified as a target of miR-128a, miR-155, and miR-516a-3p^[61]. A summary of the miRNAs regulating the cell cycle is given in Table 1 and Figure 1.

MicroRNAs add to the complexity of the cell cycle regulatory network

As mentioned above, the cell cycle is monitored by an intricate network of positive and negative regulatory signaling pathways. An imbalance between the positive and negative regulatory pathways is common in cancers. With the addition of miRNAs as cell cycle regulators, new pathways have been discovered, and the context of the network has been remarkably enriched. Additionally, crosstalk between miRNAs and signaling pathways essential to cell cycle progression adds complexity to the cell cycle regulatory network.

RAS/Raf/MEK/Erk signaling pathway

When cells receive exogenous mitogenic signals, such as growth factor stimulation, the receptor tyrosine kinases (RTKs) will activate RAS and its downstream signaling pathways. One important signaling pathway induced by RAS activation is the Raf/MEK/Erk pathway, which leads to the activation of many cyclin/CDK complexes related to the G₁/S transition. The hyperactivation of RAS is common in many tumors. In addition to mutation and constitutive mitogenic stimulation, many studies have indicated that tumor suppressor miRNAs targeting RAS are down-regulated in many cancers, which may contribute to the abnormal RAS activation. Let-7 family miRNAs^[62, 63], miR-18*^[64], miR-143^[65], miR-181^[66, 67], miR-96^[68], and miR-214^[69] directly target RAS and inhibit the proliferation and cell cycle progression of cancer cells, which are down-regulated in various cancers. The down-regulation of these miRNAs may be caused in part by the activation of the RAS signaling pathway, which forms a positive feedback loop. In pancreatic cancer cell lines, the expression of the miR-143/145 cluster is frequently lost. Molecular analysis indicated that RREB1, a transcriptional factor downstream of RAS, can directly bind to the promoter of miR-143/145 and repress their expression^[70]. Interestingly, RAS and RREB1 were found to be targets of miR-143 and miR-145, respectively. Thus, in pancreatic cancer, activation of RAS activates RREB1, which in turn represses the expression of the miR-143/145 cluster. The repression of miR-143/145 in turn potentiates RAS signaling, which forms a feed forward circuit. Moreover, some molecules downstream of RAS/MAPK/Erk, such as c-myc, cyclin D2, and CDK6, are also repressed by the miR-143/145 cluster^[71]. In addition to tumor suppressive miRNAs, RAS signaling also controls the expression of tumor-promoting miRNAs to facilitate its biological functions. miR-21 is a direct target of AP-1, a transcription factor downstream of the RAS/MAPK/Erk pathway. miR-21 inhibits the expression of several molecules that negatively regulate the RAS/MAPK/Erk pathway, such as Btg2, Spry1, and spry2^[72].

In addition to the regulation of specific miRNAs, the RAS/

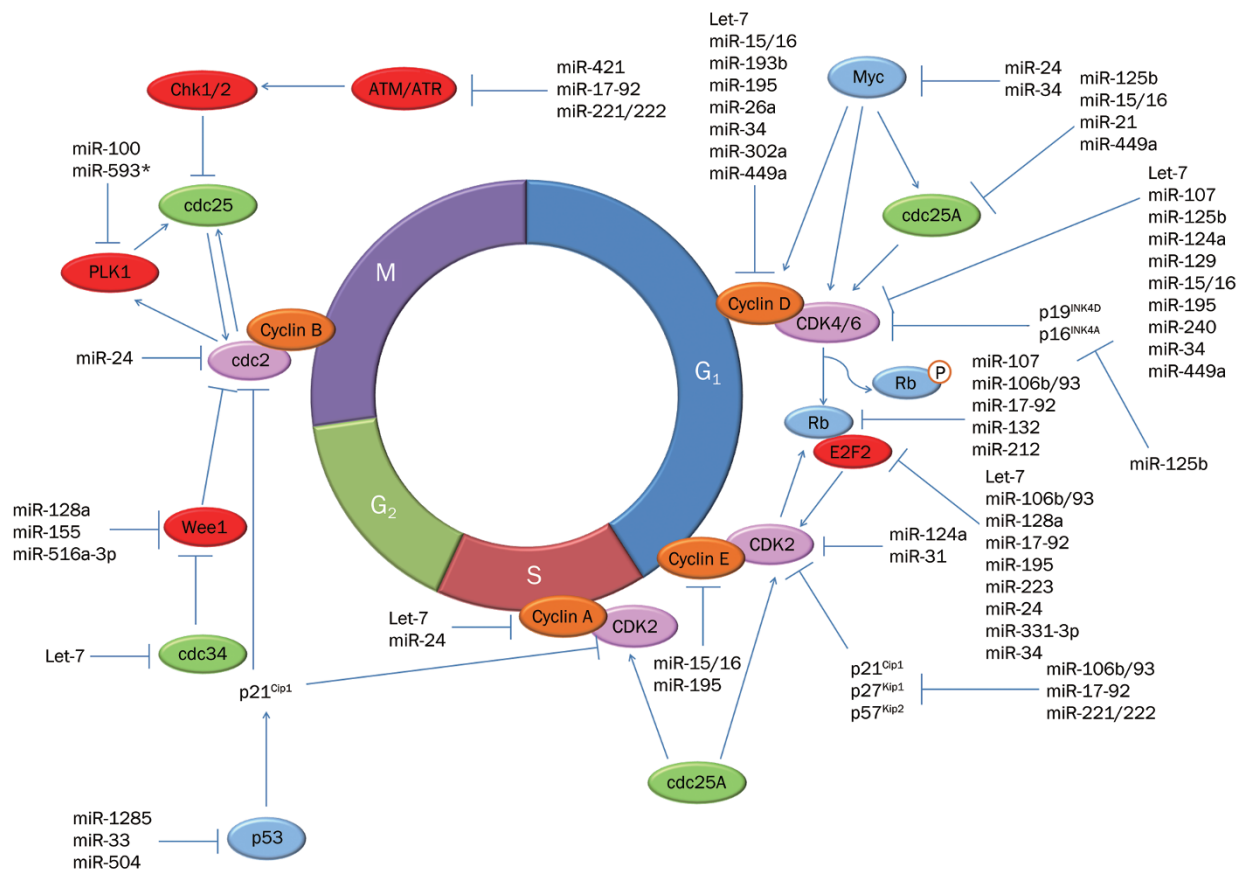


Figure 1. MicroRNAs in the control of cell cycle progression.

MAPK/Erk pathway also can control the expression of all miRNAs through phosphorylation of the HIV TAR RNA binding protein (TRBP), a critical protein of the miRNA generating complex^[73]. The phosphorylation of TRBP by MAPK/Erk signaling enhances the expression of proliferation-promoting miRNAs and represses the expression of the let-7 tumor suppressor family. Together, these studies suggest that the RAS pathway can interact with the miRNA network to exert its biological functions.

PI3K/AKT pathway

The PI3K/AKT pathway is another important signaling cascade induced in response to mitogenic stimulation. PI3K/AKT can transduce multiple extracellular signals during G₁ and induce the cell to commit to DNA replication and division. In cancers, the aberrant activation of the PI3K/AKT pathway results in the up-regulation of cyclin D1, activation of the CDK2/cyclin E complex and down-regulation of p21 and p27, coordinately promoting cell cycle progression and the uncontrolled proliferation of cancer cells. PTEN and SHIP are negative regulators of the PI3K/AKT pathway. Many members of the PI3K/AKT pathway are regulated by miRNAs. miR-29a and miR-126 target the p85 α and p85 β subunits of PI3K^[74, 75], respectively. The down-regulation of miR-126 in colon cancer is associated with the up-regulation of the p85 β subunit, which

induces the phosphorylation of AKT and promotes the proliferation of colon cancer cells^[75]. The AKT family is also regulated by miRNAs. For example, AKT1 is suppressed by miR-149*^[76], AKT2 is suppressed by miR-184 and miR-150^[77, 78], and AKT3 is suppressed by the miR-15/16 cluster^[79]. PTEN is an important tumor suppressor that antagonizes PI3K activity by inhibiting the transformation of PIP₂ to PIP₃. In cancers, the expression of PTEN is repressed by many oncogenic miRNAs, including miR-21^[80, 81], miR-221/2^[82], miR-301^[83], miR-144^[84], miR-136^[84], and miR-19^[85]. SHIP is another lipid phosphatase that inhibits the generation of PIP₃. The expression of SHIP can be suppressed by miR-155 in leukemia and lymphoma^[86]. The down-regulation of SHIP and up-regulation of miR-155 initiates a series of events that lead to leukemia/lymphoma in a transgenic mouse model. Furthermore, the reduction of SHIP by miR-155 activates PI3K/AKT activation^[87]. mTOR is a direct target of PI3K/AKT activation, which is activated by AKT phosphorylation. The activation of mTOR promotes the translation of cell cycle-related proteins, such as c-myc, and represses the expression of p21 and p27. In src-transformed tumor cells, miR-99a is down-regulated, which results in the up-regulation of mTOR^[88]. Moreover, the expression of miR-99a is inversely correlated with mTOR expression in lung cancer, and the re-expression of miR-99a in cancer cells inhibited the growth of tumors. Two other miRNAs that target mTOR

Table 1. MicroRNAs and targeted cell cycle regulators.

MicroRNAs	Cell cycle regulators	Reference
miR-124a	CDK2, CDK6	48
miR-132/212	RB1	56
miR-107	CDK6, RBL2	43
miR-137	CDC42	121
miR-26a	Cyclin D1, Cyclin D2	112
miR-593*	PLK1	60
miR-410/650	CDK1	122
miR-449a	Cyclin D1, CDK6, CDC25A	35, 44
miR-33	p53	123
miR-193b	Cyclin D1	36
miR-221/2	p27, p57, PTEN	52, 53
miR-331-3p	E2F1	124
miR-29	CDC42	125
Let-7	E2F2, CyclinD1, Cyclin D2, Cyclin D3, Cyclin A, CDK4, CDC34	9-13
miR-17-92, miR-106b/93	p21, p63, p57, E2F1, E2F2, E2F3, RBL2	15-17, 19-25, 54
miR-129	CDK6	45
miR-125b	CDKN2A, CDK6, CDC25A	46
miR-34	c-myc, Cyclin D1, CDK4, CDK6, E2F3, MYCN	28-34
miR-421	ATM	126
miR-223	E2F1	94
miR-15/16	Cyclin E1, Cyclin D1, Cyclin D2, Cyclin D3, CDC25A, c-myb, CDK6	37-39
miR-21	CDC25A	127
miR-24	E2F2, MYC, CCNA2, CDC2, CDK4	47, 57
miR-100	PLK1	59
miR-195	CDK6, Cyclin D1, E2F3, Cyclin E1	41
miR-128a	E2F3a, Wee 1	61, 128
miR-302a	Cyclin D1	42
miR-1285	p53	129
miR-504	p53	130
miR-155, miR-516a-3p	Wee 1	61

are miR-101 and miR-199a-3p, which suppress the expression of mTOR in lymphoma^[89] and hepatocarcinoma^[90], respectively.

Myc and E2F family

Myc is a transcription factor that integrates various extracellular signals and initiates transcriptional events to control proliferation, apoptosis and cell cycle progression. The dysregulation of myc is a common event in cancer development. In recent years, studies on miRNAs in the myc regulatory network have contributed to the understanding of the mechanism of myc activities. As a transcription factor, myc regulates the expression of many miRNAs^[91]. It has been determined that myc-induced miRNAs directly participate in the down-regulation of myc-repressed genes. For instance, myc activation induces the expression of miR-221 and miR-222, which target p27 and p57. p27 and p57 are known to be repressed by myc. Thus, myc-induced miRNAs can interact with myc target genes to regulate cell cycle progression. In addition to regulating the miRNAs, myc is also directly targeted by miRNAs. In neuroblastoma, let-7e and miR-101 directly target the 3'-UTR,

repress the expression of myc and inhibit the growth of tumor cells^[92]. In non-small cell lung cancer, miR-145 inhibits proliferation and the G₁/S transition by directly targeting c-myc^[93].

The E2F family members are direct targets of myc and regulate the expression of many genes controlling cell cycle progression. As mentioned above, the expression of the E2F family members is tightly controlled by the miR-17-92 family. The interaction between the miR-17-92 and E2F family members is complex. E2F1, E2F2, and E2F3 directly bind to the promoter of miR-20a and activate its transcription; however, miR-20a modulates the translation of E2F2 and E2F3, which suggests an autoregulatory feedback loop between the E2F family members and miR-20a^[16]. The rest of the members of the miR-17-92 and miR-106b/93 clusters also interact with the E2F family members and form autoregulatory feedback loops^[17, 25]. In addition to the miR-17-92 family members, E2F1 also binds to the promoter of miR-223 and represses its transcription, and E2F1 is also targeted by miR-223^[94].

p53 network

p53 is one of the central players in the response to DNA dam-

age and other cellular stresses. p53 is a tumor suppressor that is frequently deleted and/or mutated in tumors. When cells encounter genotoxic insults, p53 controls the transcription of many target genes, among which p21 is the major response molecule that induces cell cycle arrest. The miR-34 family members are direct targets of p53^[26, 27] and coordinate with p53 to suppress cell cycle progression and tumor growth. miR-107 is another target of p53^[95]. p53 can bind to the promoter of PANK1, the host gene of miR-107, and activate the transcription of PANK1 and miR-107. Interestingly, miR-107 suppresses the expression of two important G₁/S regulators, CDK6 and RBL2. These studies show that miRNAs act as important players in the p53 network.

Implications of cell cycle-related miRNAs in anti-cancer therapies

Cell cycle-related miRNAs can act as oncogenes or tumor suppressors

Cell cycle deregulation and the resulting uncontrolled proliferation is one of the hallmarks of cancer. miRNAs have been shown to be crucial regulators of cell cycle progression, and aberrant expression of miRNAs is universal in human cancer. The first evidence of an aberrantly expressed miRNA was the miR-15/16 cluster, which is frequently deleted and/or down-regulated in chronic lymphocytic leukemia (CLL)^[96]. Through microarray technology, the miRNA expression profiles of lung^[97], liver^[98, 99], breast^[100], colon^[101, 102] and gastric cancer^[103] have been reported, and the results indicate that miRNAs are abnormally expressed in various cancers. Many cell cycle-related miRNAs are specifically deregulated in cancer and function as oncogenes or tumor suppressors. It has been reported that the miR-17-92 cluster is frequently amplified^[14, 15], and let-7 is frequently down-regulated^[9, 104] in various cancers. miR-17-92 exerts oncogenic functions through positively regulating cell cycle-related proteins, including p21, p63, p57, and RBL2^[18-21], whereas let-7 exerts its tumor suppressive function by down-regulating RAS, cyclins and CDK4^[8-11]. miR-21 is activated by RAS signaling and suppressed by several negative regulators of RAS signaling and the PI3K/AKT pathway, which in turn potentiates RAS activation and PI3K/AKT activities^[72]. The crosstalk between miR-21 and cell cycle regulation contributes to the development of a cancer phenotype. In summary, the cell cycle-related miRNAs can act as oncogenes or tumor suppressors by affecting cell cycle progression, which causes uncontrolled proliferation and more aggressive cancer phenotypes.

miRNAs may serve as therapeutic targets/candidates in cancer

As oncogenes or tumor suppressors, cell cycle-related miRNAs may serve as therapeutic targets in cancers. By specific inhibition or selective re-introduction of miRNAs in cancers, miRNAs may have therapeutic effects. Approaches to the delivery of miRNAs *in vivo* include anti-miRNA oligonucleotides, antagomiRs, locked nucleic acid (LNA), miRNA sponges and nanoparticles, which each have their advantages and disad-

vantages that have been elegantly reviewed elsewhere^[105]. Importantly, some miRNAs have been shown to suppress tumor growth *in vitro* and *in vivo*. Let-7 inhibits the proliferation of hepatocellular carcinoma cells (HCC)^[106], prostate cancer cells^[12], lung cancer cells^[107] and melanoma cells^[11] *in vitro* and the tumor growth of lung cancer^[108] and breast cancer^[109] *in vivo*. Therapeutic studies on miRNAs *in vivo* have revealed promising results. miR-21 is up-regulated by RAS *in vitro* and *in vivo* and is frequently overexpressed in human cancers. LNA directed against miR-21 reduced tumor growth *in vivo*^[110], which suggests a therapeutic role for anti-miR-21 in treating cancers. In addition, miR-16, which is frequently deleted and/or down-regulated in human cancers, significantly inhibits prostate tumor growth *in vivo* when delivered by atelocollagen via tail vein injection^[111]. These results indicate that the delivery of miR-16 could be used to treat patients with advanced prostate cancer. Notably, miR-26a is down-regulated in HCC, and the restoration of miR-26a *in vitro* induces cell cycle arrest by targeting cyclin D2 and cyclin E2. Intriguingly, the delivery of miR-26a using adeno-associated virus (AAV) inhibits the proliferation of cancer cells, induces tumor-specific apoptosis and results in dramatic protection from disease progression without toxicity^[112]. Together, these *in vitro* and *in vivo* studies suggest that miRNAs can be targeted or directly delivered *in vivo* to suppress tumor growth, which may provide new strategies for developing next-generation anti-cancer therapeutics.

miRNAs are also associated with chemosensitivity and radiation sensitivity. miR-221/222 are overexpressed in fulvestrant-resistant breast cancer cells and are associated with the acquisition of fulvestrant resistance^[113]. Furthermore, up-regulation of miR-221/222 in breast cancer cells also causes tamoxifen resistance by targeting p27^[114]. miR-221/222 is a promising candidate for breast cancer with chemoresistance. Another miRNA that has been shown to be involved in chemoresistance is miR-21. miR-21-transfected HCC cells are resistant to 5-fluorouracil/interferon- α ^[115], which suggests that anti-miR-21 may increase the chemosensitivity of cancer cells. Indeed, anti-miR-21 improves the cytotoxicity induced by 5-FU in glioblastoma cells^[116]. Anti-miR-21 also increases the chemosensitivity of cells to arsenic trioxide^[117] and taxol^[118]. These findings suggest that anti-miR-21 could be used in combination with chemical reagents to increase the cytotoxic effects of cancer therapeutics.

miRNAs are also involved in the radiation sensitivity of cancers. In prostate cancer, multiple miRNAs, including miR-106b, have demonstrated altered expression in response to radiation^[119]. The up-regulation of miR-106b in prostate cancer overrides p21-activated cell cycle arrest and growth inhibition induced by radiation^[119], providing a potential target for radiation-resistant prostate cancer. In contrast to miR-106b, miR-101 sensitizes tumor cells to radiation *in vitro* and *in vivo* by targeting DNA-PKCs and ATM^[120]. Collectively, studies on the involvement of miRNAs in chemo- and radio resistance and sensitivity provide new targets and candidates for cancer treatment.

Conclusion

Herein, we summarized the current understanding of miRNAs in regulating cell cycle progression. Many cell cycle regulatory proteins are regulated by miRNAs, and these proteins also control the expression of miRNAs, which display a complex interaction network. The deregulation of this network is tightly associated with the progression of human cancers. The discovery and thorough understanding of this complicated network will open an avenue for developing novel, promising therapeutics for the treatment of cancer.

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