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REVIEW

MicroRNA-based screens for synthetic lethal interactions with c-Myc

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microRNAs (miRs) are small, non-coding RNAs, which play crucial roles in the development and progression of human cancer. Given that miRs are stable, easy to synthetize and readily introduced into cells, they have been viewed as having potential therapeutic benefit in cancer. c-Myc (Myc) is one of the most commonly deregulated oncogenic transcription factors and has important roles in the pathogenesis of cancer, thus making it an important, albeit elusive therapeutic target. Here we review the miRs that have been identified as being both positive and negative targets for Myc and how these participate in the complex phenotypes that arise as a result of Myc-driven transformation. We also discussseveral recent reports of Myc-synthetic lethal interactions with miRs.These highlight the importance and complexity of miRs in Myc-mediated biological functions and the opportunities for Myc-driven human cancer therapies.

Keywords: microRNAs;c-Myc; synthetic lethality; cancer

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Introduction

microRNAs (miRs) are a small class of endogenous non-coding RNA molecules that are first transcribed into primary miR (pri-miR) products by RNA polymerase II. The pri-miRs are then cleaved into precursor miR (pre-miR) by the type III RNase Drosha. Following their transport from the nucleus to the cytoplasm by Exportin-5/Ran-GTP, pre-miRs are processed to double-stranded RNAs by Dicer, another type III RNase. After the double-stranded RNAs are unwound, individual mature miRs, which are 19-21 nt in length, are incorporated into an RNA-induced silencing complex (RISC) and bind to the 3'-untranslated region (3'UTR) of target mRNAs. These then affect gene expression either by inhibiting translation or by promoting target degradation[1-3]. It is noteworthy that in most cases, the complementarity of miRs to their target sequences is less than 100%. As a result, individual miRs can bind imperfectly to multiple transcripts with slightly different target sequences and affinities and thereby affect multiple cellular phenotypes[1-3].

Although many aspects of miR biosynthesis and function are understood, there remain a number of questions concerning the precise means by which miRs affect gene expression.There are several ways by which this is accomplished depending on whether their match to 3'UTR target gene sequences are perfect or imperfect. These include induction of target mRNA degradation, translation-coupled protein degradation, inhibition of translation initiation, inhibition of translation elongation and termination of translation[1-3]. Some miRs can also inhibit mRNA processing by interfering with splicing [1-3].The imprecise binding of miRs can make the absolute prediction of true physiologic target sites challenging. Indeed, target mRNAs with otherwise identical miR binding sites can possess widely differing degrees of miR binding in an actual cellular context and even those targets with similar degrees of miR binding can show large variations in response. miRBase, a data base for miRs, contains the sequences of more than 2000 human pri-miR genes and 2588 mature human miR sequences; however, the functions for many of these remain to be defined. miRs are involved in various normal biological processes, such as development, cell growth and metabolic programming[3-5]. Loss of control of miR expression can contribute to the development of many diseases, including cancer[3-6]. Because miRs are very stable, easy to synthetize and easily introduced into cells, they have been viewed as having therapeutic potential for a wide range of diseases including cancer[6-7].

Differental miR expression patterns between tumors and adjacent normal tissues provide useful information for tumor classification, prognosis and management[3, 5-7]. Specific miRs may display tumor suppressor or oncogenic function. For example, the enforced expression of let-7, miR-26 and miR-34a can inhibit malignant transformation and tumorigenesis whereas enforcing expression ofmiR-21 promotes B cell lymphomagenesis[3, 5-10]. Altered miR expression in cancers canresult from both genetic and epigenetic changes. An example of the former is seen with deletion of 13q14, which inactivates the tumor suppressors miR-15a and miR-16 in chronic lymphocytic leukemia. An example of the latter occurs with hypermethylation of CpG islands in the promoters of miR-127and miR-124, which leads to loss of their tumor suppressorfunction in human bladder cancer and hepatocellular carcinoma, respectively[3, 5-10].

c-Myc and miRs

c-Myc (Myc), a helix-loop-helix leucine zipper (HLH-ZIP) oncogenic transcription factor, is de-regulated and/or over-expressed in a large fractions of human malignancies[11-14]. Myc dimerizes with Max, another HLH-ZIP protein, and binds to E-box sequences to activate transcription of target genes, including somemiRs[11-14]. Myc also acts as a transcriptional repressor by interacting with and suppressing other transcription factors and/or by modulating chromatin status [11-14].Through a myriad of downstream targets, Myc supervises a variety of biological processes, including cell proliferation, survival, metabolism and transformation[11-19]. Over-expressing Myc invarious mouse models of cancer causes tumors of a variety of types whereas homozygous deletion of Myc is associated with developmental abnormalities and embryonic lethality [13,17-19]. Homozygous deletion of Myc in rat fibroblasts cells prolongs doubling time, indicating that it plays a central role in regulating cell proliferation[11-14]. The basis for this involves a profound impairment of mitochondrial structure and function, which lead to chronic ATP depletion and the up-regulation of AMP-activated protein kinase, which futilely attempts to restore a normal energy balance by suppressing energy-consuming and anabolic processessuch as protein translation and proliferation[20].

Myc is one of the most frequently altered oncogenes in human cancers[15]. Its over-expression occurs as a consequence of DNA amplification, chromosomal translocation, or protein stabilization either by direct point mutations in the Myc protein itself or, more commonly by activation of pathways that promote Myc's phosphorylation-dependent stabilization[11-15]. Myc has been shown to be necessary for cancer progression and maintenance and its up-regulation is often correlated with poor clinical outcomes and aggressive cancer phenotypes in human neoplasms[21-25].

Myc transcriptionally regulates the expression of mRNAs, long non-coding RNAs and miRs[12, 26-35]. The latter in particular represent an important subset of Myc's transcriptional targets and significantly contribute tothe complexity of its function and biological readoutby virtue of their post-transcriptional effects on mRNA stability and translation[12, 25-35]. Myc can both activate and suppress miR expression, thereby regulating a variety of functions pertaining to cell growth, apoptosis, metabolic re-programming, tumorigenesis, angiogenesis and metastasis[25-37]. Myc may also affect the maturation of some pri-miRs. In the case of let-7, this occurs indirectly as a result of Myc's up-regulation of the RNA binding proteins Lin-28 and Lin-28B, which then negatively regulates let-7 [38]. Myc and miRs can also reciprocally regulate one another's expression. miRs have been shown to regulate a variety of Myc-induced phenotypes[29, 30,34,39].

Table 1. Myc-upregulated miRs

No.	miR	Location	Targets	Function	Reference(s)
1	miR-9	1q22	E-cadherin, E2F1	Proliferation, EMT, survival, metastasis	[40-47]
2	miR-17	13q31.3	E2F1,TGFBR2, CHEK2, TSP1,CTGF	Migration, tumor growth	[27,49-54]
3	miR-18a	13q31.3	PIAS3, TSP1,CTGF	Proliferation, apoptosis	[49-55]
4	miR-19a	13q31.3	PTEN, BIM, TSP1,CTGF	EMT, angiogenesis	[49-54,56]
5	miR-19b	13q31.3	MXD1, TSP1,CTGF	Angiogenesis, cell cycle	[49-54,56]
6	miR-20a	13q31.3	E2F1, PTEN, TSP1,CTGF	Proliferation, metastasis, cell cycle	[49-54,57]
7	miR-20b	Xq26.2	PTEN, NCOA3, CAPRIN2	Lymphoma progression	[49-57]
8	miR-25	7q22.1	BIM, DR4	Proliferation, apoptosis	[58-59]
9	miR-92a	13q31.3	P57, PTEN	Proliferation, cell cycle	[49-57]
10	miR-93	7q22.1	P21	Proliferation, apoptosis	[60-63]
11	miR-106a	Xq26.2	PTEN, Hcyp19A1,IRS-2	Growth, metastasis,	[63,64]
12	miR-106b	7q22.1	P21, PTEN	Tumorigenesis	[58,65]
13	miR-221	Xp11.3	SOCS3	Migration, invasion	[66-67]
14	miR-378a	5q32	TOB2	Cell growth	[30,48]

Myc-upregulated miRs

The first reported effect of Myc on miR expression was its up-regulation of the polycistronic miR-17-92 cluster[26, 27]. Myc induces transcription of pri-17-92 via its direct binding to an E-box within the first intron of thepri-17-92 gene. In humans, the miR-17-92 cluster is located in an intron of the MIR17HG gene located on chromosome 13q31.3. The primary pri-17-92 transcript is processed into seven different mature miRs: miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a which are widely expressed in different tissues and are essential for many developmental and pathogenic processes [26, 27]. miR-17-92 deletion in mice results in perinatal death due to a combination of severe pulmonary hypoplasia and ventricular septal defects. In humans, homozygous germ-line deletion of MIR17HG significantly reduces mature miR-17-92 levels and is associated with a syndrome characterized by microcephaly, short stature, and digital defects[40,41]. miR-17-92 acts as an oncogene "complex" that regulates multiple cellular processes which promote malignant transformation, increased survival, rapid proliferation, and angiogenesis[28-30,42,43]. miR-17-92 was reported to be involved in some human epithelial and hematopoietic malignancies. In the latter case, these include neoplasms as diverse as diffuse large B-cell lymphoma, Burkitt's lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia[28-30,42,43]. A direct role for miR-17-92 in lymphomagenesis has been demonstrated by virtue of the ability of Eμ-drivenmiR-17-92 in transgenic mice to promote the development of B-cell malignancies and massive splenomegaly with a high degree of penetrance[30, 42]. miR-17-92 appears to promote tumorigenesis by antagonizing tumor-suppressing mechanisms, notably apoptosis and senescence, through the activities of different miR components encoded by this cluster[30, 42].

miR-9 expression is also activated by Myc as well as by Myc's close relative N-Myc, both of which bind to a miR-9 promoter E-box. miR-9 is up-regulated in breast cancer cells and directly targets E-cadherin so as to increase cell motility and invasiveness[44-47].

Interestingly, miR-378 was reported to be a Myc-activated miR that can cooperate with activated Ras or HER2/neu to promote cellular transformation by directly inhibiting the anti-proliferative BTG family member, TOB2, which transcriptionally represses the proto-oncogene cyclin D1[48]. Additional Myc-upregulated miRs and some of their known functions are summarized in Table 1.

Myc-downregulated miRs

Although protein-coding gene activation tends to occur more commonly than does repression in response to Myc activation, the reverse seems to be true for miRs[12,26-37,68]. For example, Burkitt's lymphoma is a highly aggressive B-cell neoplasm, which originates from germinal center B cells and harbors chromosomal translocations involving Myc and immunoglobulin promoter-enhancer loci[69]. Through the analysis of human and mouse B cell lymphoma models, Chang *et al*. found Myc to be responsible for the repression of multiple miRs, including members of the let-7 and miR-30 familiesas well as, miR-15a/16, miR-22, miR-26a/b, miR-29a/b/c, miR-34a, miR-99a/b, mir-146a, miR-150 and miR-195. They found that Myc directly bound to these miR promoters, thereby attesting to their direct suppression, and that enforced re-expression of the repressed miRs diminished the tumorigenic potential of lymphoma cells. This suggested that widespread miR repression by Myc contributes to tumorigenesis and can be reversed by normalizing the expression of at least some miRs[31].

In γ-irradiation-induced lymphomas, miR-15a, miR-22, miR-23b, miR-125b, miR-26a/b, miR-29a/b and several let-7 family members have been reported to be repressed in

Table 2. Myc-downregulated miRs

No.	miR	Location	Targets	Function	Reference(s)
1	let7 family member	9q22.32 22q13.31 19q13.41	Myc,Bcl-2,Bcl-Xl,CCND2,HMGA2, SLC5A5	Growth, migration,invasion, EMT	[68,69,76]
2	miR-15a	13q14.2	CCND1,Bcl-2,Bcl-xL, YAP1,FOXP3	Autophagy, proliferation	[30,77]
3	miR-16	13q14.2	CCND1,CCND2, HSP70	Apoptosis, growth, proliferation	[30,76,78]
4	miR-23a	19p13.13	GLS, ABCF1	Metabolism, Chemoresistance	[16,79-81]
5	miR-23b	9q22.32	GLS, Smads,HMGB2	Metabolism, Chemoresistance	[16,79,81]
6	miR-24	9q22.32	OCT4, Smads, DHFR	Development, cancer progression	[79,80]
7	miR-26a	3p22.2	EZH2,Lin28B,IL6, HMGA1,EphA2	Proliferation, tumorigenesis,	[70, 82]
8	miR-27a	19p13.13	FOXO1,Apaf-1,DPD	Differentiation, metastasis	[30,80]
9	miR-27b	9q22.32	ST14, Smads	Differentiation,apoptosis	[79,80]
10	miR-28	3q28	Nrf2, MPL, CCND1, HOXB3, NM23-H1	Proliferation, migration	[72]
11	miR-29a/b	7q32.3	CDK6,IGF1R,LAMC1, MCL1,AKT2	Growth,migration	[71,76]
12	miR-30a	6q13	IGF1R, HP1gamma, SOX4, Drp-1	Migration, invasion	[83]
13	miR-34a	1p36.22	IL6R,HNF4G,cyclin E1	Proliferation, migration, invasion	[30,73]
14	miR-122	18q21.31	IL-1α,DLX4,OCLN	Fibrosis, proliferation, metabolism	[74]
15	miR-129-2	11p11.2	PDK4, SOX4,BDKRB2	Migration,chemoresistance	[75]
16	miR-148a	7p15.2	Myc,Wnt1, Dnmt1, IKKβ, LDLR, PKM2	Differentiation, cell cycle	[25,30]
17	miR-185	22q11.21	DNMT1,Myc,SOCS3,VEGFA, E2F6,HIF2a	Proliferation	[84]
18	miR-363	Xq26.2	USP28,S1PR1,E2F3, MBP-1,Mcl-1	Cell cycle,cisplatin-induced apoptosis	[25,30]
19	miR-449a	5q11.2	N-Myc,E2F3,BCL2, CDC25A,NOTCH1	Survival, cisplatin-induced cytotoxicity, invasion, proliferation	[85]

response to Myc activation[69]. Restoration of miR-26a expression in the cells attenuated cycle progression and proliferation by inhibiting the Polycomb complex protein EZH2[70]. EZH2 is a histone-lysine N-methyltransferase enzyme, which catalyzes the methylation of histone H3 and thereby serves to epigenetically repress transcription. Presumably, EZH2 down-regulates genes that would otherwise prevent transformation and/or proliferation in response to Myc de-regulation.

Along similar lines, Zhang *et al*. found that miR-29 is repressed by Myc through a co-repressor complex comprised of EZH2 and histone deacetylase 3 (HDAC3). They showed that dual inhibition of HDAC3 and EZH2 disrupted theMyc-EZH2-miR-29 axis, leading to restoration of miR-29 expression, down-regulation of miR-29-targeted genes, and lymphoma suppression[33,35,71].

miR-28 was also found to be significantly repressed by Myc in Burkitt's lymphoma. Restoring miR-28 expression impaired cell proliferation and clonogenicity by inhibiting spindle checkpoint proteins including MAD2L1[72].

In human P-493 B lymphoma and PC3 prostate cells, Myc transcriptionally represses miR-23a/b, which can directly target mitochondrial glutaminase. This increases glutaminase mRNA and protein levels, which promotes glutamine catabolism. Glutaminase catalyzes the conversion of glutamine to glutamate, which in turn in converted into α-ketoglutarate, a key TCA cycle substrate that, is also needed for nucleotide and amino acid biosynthesis and maintenance of redox homeostasis[16]. Increasing the supply of α-ketoglutarate represents a form of metabolic re-programming that may serve to provide substrates to mitochondria other than those derived from glucose which are increasingly used in support of the Warburg effect [12,16, 20].

Inhibition of miR-34a by Myc is also essential for tumorigenesis and survival. Repression of miR-34a was shown to be required for IL-6-induced epithelial-to-mesenchymal transition (EMT) and invasion. In miR-34a-deficient mice, colitis-associated intestinal tumors induced by azoxymethane/dextran sodium sulfate showed up-regulation of the EMT facilitators p-STAT3, IL-6R and SNAIL and progressed to invasive carcinomas [73].

Myc is pathologically activated in and essential for promoting human hepatocellular carcinoma (HCC)[18, 23-25]. In these tumors, Myc represses miR-122 expression directly by binding its promoter. Over-expression of miR-122 in miR-122-/- hepatocytes was shown to reduce c-Myc protein levels, while its depletion in miR-122+/+ hepatocytes led to an increase. miR-122 indirectly inhibited Myc transcription by targeting Tfdp2 and E2f1 in HCC, both of which bind to the Myc promoter and increase transcription. These suggest a double-negative feedback loop between miR-122 and Myc[74].

Similarly, Myc was also found to bind directly to

conserved regions in the promoters of miR-148a and miR-363 and repress their expression. miR-148a-5p directly binds Myc's 3'UTR and inhibits its expression whereas miR-363-3p destabilizes Myc indirectly by inhibiting expression of USP28, a Myc deubiquitinase. Together, these two miRs provide for a novel and elegant form of positive feedback control by which Myc indirectly promotes the stabilization of both its own mRNA and protein.The importance of these interactions is underscored by the fact that inhibition of either miR-148a-5p or miR-363-3p induces hepatocarcinogenesis, whereas activation of them has the opposite effects[25].

miR-129-5p is another miR that is transcriptionally repressed by Myc. miR-129-5p levels negatively correlate with the clinical stage of human HCC and thus with survival. Restoring miR-129-5p expression suppresses diethylnitrosamine (DEN)-induced hepatocarcinogenesis in mice by targeting and down-regulating the activity of pyruvate dehydrogenase kinase 4 (PDK4)[75]. PDK4 is a critical negative regulator of pyruvate dehydrogenase (PDH), a rate-limiting enzyme that connects glycolysis and oxidative phosphorylation by converting pyruvate to acetyl coenzyme A. PDH's inhibition via PDK4-mediated phosphorylation should allow for the accumulation of glycolytic substrates, and their diversion into the anabolic pathways needed to support tumor growth. PDK4 inhibition via miR-129-5p normalizes the flow of pyruvate into the TCA cycle and an overall inhibition of the Warburg effect by depriving tumor cells of these critical anabolic substrates. This, along with the changes in glutamine flow discussed above, represents an additional Myc-regulated pathway that is responsible for the metabolic re-programming so commonly associated with cancer cells[75]. We summarize all the above Myc-suppressed miRs as well as additional ones in Table 2.

Regulation of Myc expression by miRs

Myc expression is tightly controlled, both at the transcriptional and post-translational level and miRs play critical roles in these processes[11-15,28-31]. Some of the mechanisms underlying these varied forms of control have already been discussed above.

The first reported Myc-targeting miR was let-7a, which down-regulates Myc and reverts Myc-induced growth in Burkitt's lymphoma cells[86,87]. miR-145 was reported to be transcriptionally activated by p53 interacting with its response element in the miR-145 promoter. Myc is a direct target for miR-145. Enforced expression miR-145 silences Myc expression while anti-miR-145 enhances its expression. miR-145 silencing of Myc expression accounts at least in part for the miR-145-mediated tumor cell growth inhibition both in vitro and in vivo. miR-145 inhibitionis also able to reverse p53-mediated Myc repression[39,88].

miR-34a, a positive target of tumor suppressor p53, has been reported to function as a tumor suppressor that binds to Myc's 3'UTR and decreases its expression in prostate cancer and renal cell carcinoma[89]. In addition to this negative regulation of Myc, miR-34a was also found to target FoxM1, which, together with Myc, is involved in the activation of telomerase reverse transcriptase, which represents the critical step in immortalization [90, 91]. It has been shown that miR-34b/c's targeting of Myc requires mitogen-activated protein kinase-activated protein kinase 5 (MK5). MK5 activates miR-34b/c expression via phosphorylation of FoxO3a, thereby promoting nuclear localization of FoxO3a and enabling it to induce miR-34b/c expression. miR-34b/c binds to the 3'UTR of Myc and inhibits its expression. Myc in turn directly activates MK5 expression, forming a negative feedback loop that is dysregulated in colorectal tumorigenesis[92].

Interestingly miR-29a was reported to be a tumor suppressor that is induced by PRIMA-1Met, a small molecule with anti-tumor activity. miR-29a overexpression or exposure to PRIMA-1Met reduced multiple myeloma cell proliferation by targeting Myc. On the other hand, over-expression of Myc at least partially reverted the inhibitory effects caused by PRIMA-1Met or miR-29a overexpression suggesting that the miR-29a/Myc axis mediates anti-myeloma effects of PRIMA-1Met. Importantly, intratumoral delivery of miR-29a mimics induced regression of tumors in mouse xenograft model of MM and this effect synergized with PRIMA-1Met[93].

miR-33b is down-regulated in osteosarcoma tumors and cell lines and its overexpression significantly inhibits proliferation, migration, and invasion of and by osteosarcoma cells. Mechanically miR-33b negatively regulates Myc at the posttranscriptional level, via a specific target site within the 3'UTR. Over-expression of Myc impaired miR-33b-induced inhibition of proliferation and invasion in osteosarcoma cells. The expression of Myc was inversely correlated with miR-33b expression in osteosarcoma tumors and cells. Taken together, these findings suggest that miR-33b inhibits osteosarcoma cell migration and invasion and serves as a tumor suppressor by targeting Myc expression [94].

miR-130a has also been shown to be a Myc-targeting miR. In these studies, ribosomal protein L11 promoted miR-130a's targeting of the 3'-UTR of Myc mRNA thus repressing Myc

Table 3. Myc-targeting miRs

No.	miR	Location	Function	Cancer type	Reference(s)
1	let7 family member	9q22.32, 19q13.41, 22q13.31	Growth, transformation, cisplatin resistance, radio-sensitivity, invasion, Differentiation	Lymphoma, HCC,melanoma,ovarian carcinoma, breast cancer, lung cancer,medulloblastoma	[30,69,86,87]
2	miR-24	9q22.32	Development, cancer progression	HCC,lymphoma	[96]
3	miR-29a	7q32.3	Growth, migration, invasion	Leukemia, lymphoma, lung cancer	[93]
4	miR-33b	17p11.2	EMT,migration, invasion	Melanoma,osteosarcoma	[94]
5	miR-34a	1p36.22	Proliferation, migration, invasion	Bladder cancer,sarcomas	[30,89-91]
6	miR-34b/c	11q23.1	Metastasis, EMTgrowth, apoptosis, chemo-resistance,self-renewal,	Colon cancer, breast cancer,HCC,renalcancer,lung cancer	[30,92,97]
7	miR-125b	11q24.1	Tumor growth, proliferation	Breast cancer,pancreaticcancer,melanoma,HCC	[69]
8	miR-130a	11q12.1	Proliferation,migration,invasion, gefitinib resistance	Breast cancer,HCC, lung cancer	[95]
9	miR-132	17p13.3	Growth, migration, invasion	Glioma,lung cancer	[69]
10	miR-135a	3p21.1	Migration, invasion,metastasis	Prostate cancer,HCC	[98]
11	miR-145	5q32	Metastasis,invasion	Bladder cancer,HCC,prostate cancer	[39,88]
12	miR-148a	7p15.2	Cell cycle, Differentiation	HCC, Squamous cell carcinoma	[25]
13	miR-154	14q32.31	Growth, EMT	Colorectal cancer,lung cancer, prostate cancer.	[69]
14	miR-184	15q25.1	Proliferation, invasion	Ovarian cancer, glioma,lung cancers	[99,100]
15	miR-320b	1q42.1	Proliferation, invasion	Colorectal cancer,	[101]
16	miR-451	17q11.2	Radio-resistance, proliferation	Lung, head and neck cancer	[102-104]
17	miR-494	14q32.31	Growth, proliferation, cell cycle	Gastric carcinoma, lymphoma, HCC, ovarian cancer	[105-107]
18	miR-744	17p12	Proliferation	HCC	[108]

Expressionin response to UV irradiation. miR-130a overexpression promoted Ago2 binding to Myc mRNA, significantly reduced the levels of both Myc mRNA and protein and inhibited cell proliferation. UV treatment markedly enhanced the binding of L11 to miR-130a, Myc mRNA and Ago2 in cells. Inhibiting miR-130a significantly suppressed UV-mediated Myc reduction. L11 was re-localized from the nucleolus to the cytoplasm where it associated with Myc mRNA upon UV treatment. These findings reveal a novel mechanism underlying Myc down-regulation in response to UV-mediated DNA damage[95]. Interestingly miR-24 inhibited Myc expression by recognizing seedless but highly complementary 3'-UTR sequences[96]. All known Myc-targeting miRs are listed in Table 3.

Myc-synthetic lethal interactions

In some murine models of Myc-dependent cancers, the suppression of Myc leads to tumor regression, thus indicating that continuous Myc expression is necessary to maintain tumor growth and/or viability[11-13,18]. Myc's role in the pathogenesis of cancer and the apparent "addiction" of some cancer to continuous Myc expression makes it an enticing, albeit elusive chemotherapeutic target[11-13,18,109-113]. Strategies aimed at inhibiting Myc itself including antisense oligodeoxy nucleotides and RNAi, small molecule disruptors of the Myc-Max heterodimer and inhibitors of chromatin modifying complexes with which Myc associates have been investigated as options for attacking Myc-driven cancers[11-13,18,109-120]. However these attempts to pharmacologically inhibit Myc have remained, to various degrees, ineffective and/or non-specific[109-120].

Because Myc is essential for the growth of some normal as well as transformed cells, it has sometimes been viewed as being a poor therapeutic target given that the protein is also seldom mutated in cancer and possesses no obviously "druggable" domains or enzymatic activity[11-13,109,110,113]. Thus it has been suggested that taking advantage of Myc's synthetic lethal interactions could be exploited as an effective therapeutic strategy in Myc-driven human cancers[109,110,112,121-137].

A synthetic lethal interaction is defined as a combination of two mutations that confers lethality to a cell without either individual mutation alone having such an effect[109,110,112,121-127]. A classical example of synthetic lethality is seen with the use of poly ADP-ribose polymerase (PARP) inhibitors to treat BRCA1- and BRCA2-deficient breast cancers. The inability of these cancers to repair damaged DNA in response to PARP inhibition occurs only in tumor cells that are defective in BRCA1/2-mediated DNA repair and not in otherwise normal cells[121-125]. Because tumor cells contain numerous genetic, epigenetic and metabolic alterations, a large number of heterogenous synthetic lethal genes have been identified whose inhibition confers synthetic lethality[109,110,112,121-127]. Among the molecules that have been used to identify synthetic lethal targets, both in vitro and in vivo, are low molecular weight drug-like compounds, siRNA/shRNAs, CRISPRs and miRs[109,110,112,121-127].

Previous siRNA/shRNA screens for Myc-synthetic lethal have identified regulators of the Myc network, components

Table 4. Summary of synthetic lethal targets of Myc oncogene

No.	Genes	Location	Function	Reference
1	AURKB	17p13.1	Cell division	[134]
2	BUD31	7q22.1	RNA splicing	[128,129]
3	CDK1	10q21.1	Cell cycle	[131,132]
4	CDK9	9q34.1	Transcription elongation	[133]
5	DR5	8p22-p21	Cell death	[135]
6	EIF4F	4q23	Protein synthesis	[136]
7	MAP3K13	3q27	Protein kinase	[137]
8	miR-206	6p12.2	Tumor suppressor miR	[137]
9	SAE2	18q11.2	Sumoylation/DNA repair	[130]
10	SF3B1	2q33.1	RNA splicing	[128,129]
11	U2AF1	21q22.3	RNA splicing	[128,129]

of RNA transcription initiation and elongation, proteins involved in sumoylation and ubiquitylation and kinases with roles in DNA repair and cell cycle checkpoints[109,110,112,128-137]. More recently, the spliceosome was discoveredto be a new target of oncogenic stress in Myc-driven cancers. BUD31, a core component of the spliceosome, was identified as a Myc synthetic lethal gene in human mammary epithelial cells. Other core spliceosome factors that interact with BUD31, such U2AF1 and SF3B1, were also identified as Myc synthetic lethals. Myc hyperactivation increases the total cellular burden of precursor mRNA synthesis, thus likely placing greater demands and stress on the pre-RNA processing function of spliceosomes. In contrast to normal cells, partial inhibition of the spliceosome in Myc-hyperactivated cells was found to be associated with global intron retention, widespread defects in pre-mRNA maturation and deregulation of many essential cell processes. Genetic or pharmacological inhibition of the spliceosome in vivo also impaired survival, tumorigenicity and metastatic proclivity of breast cancers with high Myc level but had a less pronounced effect in tumors with lower Myc levels. Collectively, these findings suggest that oncogenic Myc confers a collateral stress on splicing, and that components of the spliceosome may be viable synthetic lethal therapeutic targets for Myc-driven cancers[128,129].

SAE2, an endonuclease that plays an important role in DNA repair and that is necessary for the growth of cancers with high Myc levels, has also been shown to interact with de-regulated Myc in a synthetic lethal manner[130].

Inhibition of some cyclin-dependent kinases was also reported to be selectively toxic in breast cancers with high Myc levels [131-133]. For example, CDK9 is a component of the Myc-regulated RNA polymerase II-directed transcriptional elongation machinery and functions to phosphorylate the C-terminal domain of RNA polymerase II's largest subunit[133]. Myc-synthetic lethal genes are summarized in Table 4.

A few reports have used miRs to identify synthetic lethal relationships in cancer cells that over-express Myc or other oncogenes. By functionally screening a miR library comprised of 1254 individual miR expression vector, we identified miR-206 as being able to impart a synthetic lethal phenotype in Myc over-expressing human cancer cells[137]. miR-206had similar growth inhibitory effects on both the in vitro and in vivo proliferation of breast cancer cells, but only when they expressed high levels of Myc as would be expected of a classic synthetic lethal relationship. This appeared to be dependent on miR-206's ability to target MAP3K13 as indicated by the ability to recapitulate similar phenotypes by suppressing MAPK3K13 through other means, including two different shRNAs. Bioinformatics-based analyses of gene expression profiles from the TCGA collection of breast cancers indicated that those tumors with the highest Myc levels tended to express the lowest levels of miR-206 and the highest levels of MAP3K13 while simultaneously being associated with more adverse clinical outcomes. The critical link between miR-206 and MAP3K13 in the development of Myc over-expressing human cancers suggested potential points of therapeutic intervention for this molecular sub-category.These results showed that MAP3K13 and perhaps downstream targets of MAP3K13 such as members of the JNK signaling pathway are Myc-synthetic lethal targets[137].

Future directions

The direct inhibition of certain"undruggable"oncoproteins, such as Myc remains an elusive goal in cancer therapy. As a way of circumventing this problem, synthetic lethal interactions could be exploited as an alternate therapeutic strategy, thus avoiding the inherent complexities of direct Myc inhibition. Given that miRs are easy to synthetize and can be readily introduced into cells, they possess significant potential for cancer therapy. Alternatively, even easier and more direct approaches could be envisioned in cases where the synthetic lethal genes identified by such screens encoded enzymes for which small molecule inhibitors could be generated. Such small molecules could then be used as substitutes for miRs or perhaps even in combination. miRs thus have potential not only as therapeutic entities but as tools to drive the ongoing discovery of new synthetic lethal

interactions. Many approaches to identify synthetic lethal interactions have been successfully developed. Currently, most screens continue to be performed in vitro using cultured cells, primarily because of the relative ease and directness of high throughput as well as the potential for multiplexed-based screens. The latter are likely to grow in popularity, given the ease with which such screens can now be combined withdeep sequencing-based approaches to identify relevant miRs or shRNAs that have been selectively depleted from complex libraries. The potential for screening in vivo using tractable organisms such as zebrafish are also high and further promises to reduce the time needed to identify promising hits, characterize their mechanisms of action and proceed to clinical trials[138, 139].

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviations

MiRs: microRNAs; Myc: c-Myc; pri-miR: primary miR; pre-miR: precursor miR; RISC: RNA-induced silencing complex; 3'UTR: 3'-untranslated region; HLH-ZIP: helix-loop-helix leucine zipper; EMT: epithelial-to-mesenchymal transition; HCC: hepatocellular carcinoma; DEN: diethylnitrosamine; PDK4: pyruvate dehydrogenase kinase 4; PDH: pyruvate dehydrogenase; MK5: mitogen-activated protein kinase-activated protein kinase 5; PARP: poly ADP-ribose polymerase.

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