Taking on Challenging Targets: Making MYC Druggable

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OVERVIEW

The transcription factor proto-oncogene c-MYC (hereafter MYC) was first identified more than 3 decades ago and has since been found deregulated in a wide variety of the most aggressive human malignancies. As a pleiotropic transcription factor, MYC directly or indirectly controls expression of hundreds of coding and noncoding genes, which affect cell cycle entry, proliferation, differentiation, metabolism, and death/survival decisions of normal and cancer cells. Tumors with elevated MYC expression often exhibit highly proliferative, aggressive phenotypes, and elevated MYC expression has been correlated with diminished disease-free survival for a variety of human cancers. The use of MYC overexpression or MYC-dependent transcriptional gene signatures as clinical biomarkers is currently being investigated. Furthermore, preclinical animal and cell-based model systems have been extensively utilized in an effort to uncover the mechanisms of MYC-dependent tumorigenesis and tumor maintenance. Despite our ever-growing understanding of MYC biology, currently no targeted therapeutic strategy is clinically available to treat tumors that have acquired elevated MYC expression. This article summarizes the progresses being made to discover and implement new therapies to kill MYC over-expressing tumors—a target that was once deemed undruggable.

MYC or the highly related MYCN proteins are estimated to be deregulated in approximately 50% of all human malignancies, including but not limited to lymphomas, neuroblastomas, melanomas, breast, ovarian, prostate, and liver cancers. Unlike another notorious oncoprotein RAS—a small GTPase that always harbors oncogenic point mutations—the mechanisms of MYC deregulation rarely involve mutational changes in its protein coding sequence. Instead, MYC can be deregulated through chromosomal translocation, gene amplification, and post-translational modifications, all of which result in elevated MYC protein expression and deregulated activities of MYC-dependent pathways.[1] MYC is a pleiotropic transcription factor that affects both up- and downregulation of target genes, including both mRNA and miRNA genes.[2] Recent developments in gene expression analyses have demonstrated that the mRNA expressions of roughly 300 – 400 coding genes and about a dozen miRNAs can be significantly altered, both up and down, on acute MYC activation in mammalian cells or tissues.[3,4] Recent evidence suggests that MYC can act as an enhancer or amplifier of existing activated gene transcription, which may contribute to the seemingly ubiquitous effects of MYC activity.[5,6] Regardless, those genes rapidly upregulated following MYC activation are often pro-cell proliferation and they regulate nutrient metabolism and alter survival genes. On the other hand, MYC downregulates genes involved in control of cell cycle progression—such as endogenous cell cycle inhibitors—some of which are considered tumor suppressors. Thus, MYC activation can precisely orchestrate a cellular context in which cell proliferation is favored and enhanced, while intrinsic surveillance programs that do not tolerate such a shift in nontumorigenic cells are disabled. How can we therapeutically inhibit the transforming capabilities of MYC?

DIRECT INHIBITON OF MYC-DEPENDENT TRANSCRIPTION

Difficulties in Directly Inhibiting MYC

MYC has proven to be a highly potent oncoprotein when it is overexpressed, but is also a pleiotropic transcription factor essential for normal cell cycle progression and mammalian development. For example, germ-line deletion of the MYC gene results in embryonic lethality because of developmental defects in multiple organs.[7] In normal and tumor cells, MYC-dependent signaling is particularly important for cell cycle progression from G1 to S cell cycle phases. These overlapping functions in normal and cancer cells present challenges to inhibiting MYC as a therapy for cancer.

In tumors, MYC protein expression can be elevated as a consequence of gene amplification, increased MYC transcription, or increased MYC protein stability and activity through post-translational regulation. Currently, there is no

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consensus for identifying MYC-elevated tumors. Various published studies have used genomic amplication, immunohistochemical staining, mRNA expression, or MYC-regulated gene signature expression to identify MYC-elevated tumors in humans. A major challenge in directly inhibiting MYC activity has been its structure and function as a transcription factor. Modulating protein-protein or protein-DNA interactions of transcription factors with cell permeable small molecule inhibitors has proven to be a major challenge for chemists and structural biologists. No primary sequences that identify active sites—found in other enzymes such as kinases— have been identified in MYC, limiting the development of small molecule antagonists of MYC function. However, to abrogate MYC-dependent transcriptional activity, potentially promising MYC inhibition strategies have been sought based on interrupting direct protein-protein interactions involving MYC and its coactivator MAX.

MYC belongs to a family of proteins containing the basic helix-loop-helix and leucine zipper (bHLH-LZ) domains.[1] Structurally, these two functional domains are located adjacent to each other toward the carboxyl-terminal end of MYC. The basic region contributes to DNA binding, while the leucine-zipper (LZ) domain forms a heterodimer with another family member MAX, which has been characterized as a cofactor for MYC. MYC-MAX heterodimerization is required for MYC localization to its target consensus DNA sequence CACGTG, known as the enhancer box (E-box). E-box binding mediates the transcriptional and transforming capabilities of MYC. On the other hand, MAX- and E-box-independent functions of MYC in transcriptional regulation have also been proposed.[8] MAX is also a heterodimerization partner of the MXD family of proteins. The MXD family of proteins belong to another group of the bHLH-LZ proteins, which function as transcriptional repressors.[1] Thus, the absolute oncogenic potential of MYC may, at least in part, depend on the availability of MAX for MYC binding and on an intricate balance between MYC and MXD proteins.

KEY POINTS

- Despite MYC's widespread amplification or increased expression in many cancer types, no clinically approved therapies that target it currently exist.
- The major obstacle in directly inhibiting MYC function is that it is an essential pleiotropic transcription factor that controls the expression of hundreds of genes.
- Several small molecule nonkinase inhibitors that can modulate MYC transcriptional activity are currently under preclinical development and evaluation.
- MYC-driven tumors are dependent on various downstream signaling pathways, including those that regulate the cell cycle, stress-responses, and metabolic pathways, increasing the possibilities of targeting these pathways.
- Synthetic lethal approaches that target essential signaling pathways downstream of MYC activity—such as cell cycle and metabolism—can provide new therapeutic opportunities to selectively kill MYC-driven tumors.

Targeting MYC Activation

Given the requirement for heterodimerization between MYC and MAX, a variety of extensive screening efforts were made to identify small molecules that specifically disrupt protein-protein binding. One such small molecule is 10058-F4. It recognizes the MYC amino acid residues 402– 412, which reside within the HLH-LZ domain.[9] 10058-F4 attracted a considerable amount of attention particularly because it was shown to be rather specific to the sequence in the MYC HLH-LZ domain and it did not interfere with either MAX-MXD or MAX-MAX binding. For these reasons, 10058-F4 has been widely used particularly in cell-based assays to inhibit MYC-dependent transcription, and it has been demonstrated to induce antitumorigenic effects such as cell cycle arrest and apoptosis in a variety of established cancer cell lines.[10] Despite its success in vitro, 10058-F4 did not prove to be effective in in vivo animal studies primarily because of its limiting PK/PD properties. Improved versions are currently under development.[11]

Targeting MYC-Associated Chromatin Modifications

More recent efforts to pharmacologically inhibit MYC pathway activation have targeted chromatin modifications associated with the process of MYC-mediated transcriptional activation. Gene transactivation upon MYC binding to the E-box is associated with covalent, post-translational acetylation on lysine residues on nearby histone proteins. Such acetylated lysine resides are recognized and bound by the bromodomain (acetyl-lysine recognition domain) and extra-terminal (BET) family of transcriptional coactivator proteins, which in turn recruit components of the transcription initiation complex. A prototype small molecule, named JQ1, was designed to BRD4 the first bromodomain of the BET family member BRD4. BRD4 is overexpressed in and its expression is correlated with disease progression of multiple myeloma (MM), in which MYC is deregulated.[12] JQ1 was intended to competitively bind the BET family bromodomains and sequester them from lysine-acetylated histones, thus indirectly repressing MYC-dependent transcription. In vitro, JQ1 induced G1 cell cycle arrest and cellular senescence in a panel of established MM cell lines in which MYC is overexpressed. Surprisingly, treatment of these cells with JQ1 was accompanied by diminished MYC protein expression suggesting that MYC transcription itself is regulated by BRD4.[12] In multiple mouse models of MM and other MYC-driven malignancies—including patient-derived xenografts—JQ1 was effective in controlling tumor burden and extending animal survival.[12] Interestingly, JQ1 was found to alter transcription of only 113 genes in the MM cells.[12] The underlying mechanism of BRD4 activity and its modulation by JQ1 in MM cells have been actively pursued and attributed to the existence of specific enhancer elements upstream of target genes.[12,13] Thus, further preclinical efficacy studies on BRD4 inhibition against a wider range of cancers with elevated MYC expression via different mechanisms will be highly in-

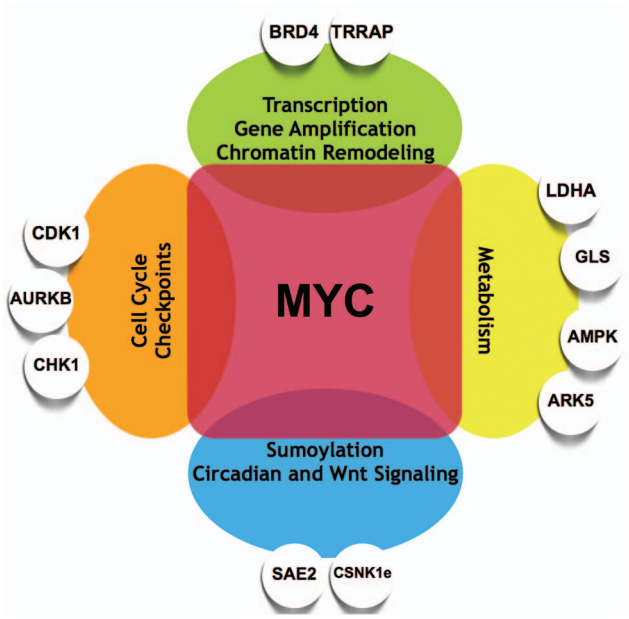

FIG 1. Summary of identified MYC synthetic-lethal interactions.

formative. JQ1 derivatives are currently being further developed for potential clinical use.

EXPLOITING MYC-DEPENDENT SYNTHETIC LETHAL INTERACTIONS

Concept of Synthetic Lethality

MYC's essential role in both cancer and normal tissue development and homeostasis raises the concern that even if direct MYC inhibitors could be developed, they might be too toxic for clinical use. An alternative approach is to identify and target signaling pathways activated by MYC selectively in tumor cells but not in nontumorigenic cells. This form of genetic interaction is referred to as synthetic lethality.[14] The term "synthetic lethality"—first employed in genetic studies using the model genetic organism *Drosophila*—refers to a genetic context in which a mutation in a gene that does not cause lethality itself can cause lethality when combined with a mutation in another gene that is also not lethal by itself.[15] Use of this term in describing cancer therapy has since evolved to include contexts in which one genetic defect may be not only a classical loss-of-function or gain-of-function point mutation but also an overexpressed oncoprotein or loss of enzymatic activity in a kinase via small molecule inhibition. Thus, this therapeutic concept may allow for targeting classically nondruggable targets such as nonenzyme oncoproteins that have acquired activating mutations or are overexpressed, or tumor suppressors with loss-of-function mutations or genetic loss. The concept of synthetic lethality has been vigorously pursued in targeting tumors with RAS mutations;[16-18] small molecule inhibitors of polo-like kinase, in particular, are currently being evaluated in clinical trials. The synthetic lethal combination that has been most successful both in preclinical and clinical settings is the use of small molecule poly(ADP-ribose) polymerase (PARP) inhibition—an enzyme required for DNA repair—for patients whose tumors harbor mutated BRCA1 or BRCA2: genes required for activity of the homologous DNA repair pathway. The mechanism of synthetic lethality relies on the inability of the BRCA-deficient cancer cells to perform homologous recombination, while PARP inhibition prevents an alternative DNA repair pathway.[19,20] Several PARP inhibitors are currently being evaluated in late phase clinical trials. PARP inhibitors thus serve as an important proof of concept that synthetic lethal approaches are clinically relevant and exploitable.

Targeting Cell Cycle Kinases

Cancer cells with elevated MYC expression often exhibit highly proliferative and poorly differentiated phenotypes, suggesting that the MYC-driven cells are poised to continuously drive the cell cycle. It may also suggest that other cellular processes have had to adjust to accommodate such significant changes in cell physiology. What if such MYC-driven cancer cells suddenly lost their homeostasis by losing one of the major components of the cell cycle? A number of articles from our group and others have addressed this question.

Inhibition of the mitotic kinase CDK1 with an experimental small molecule purvalanol A induced apoptosis in model epithelial and fibroblast cell lines engineered to overexpress MYC.[21] The cell death observed was independent of p53 function. Purvalanol A also induced cell death in MYC-driven lymphoma cells and extended survival in a mouse model of MYC-driven hepatoblastomas.[21] This concept of synthetic lethality between MYC overexpression and CDK1 inhibition was further tested against an aggressive subset of

TABLE 1. Proposed Pharmacological Targets to Inhibit in MYC-dependent Tumors

Targets	Biological Processes	Agent	Clinical status
MYC	MYC-MAX binding, transcription	10058-F4 and its derivatives	Pre-clinical
BRD4	Chromatin modification, transcription	JQ1	Pre-clinical
CDK1	Cell cycle	dinaciclib	Phase I-III
Aurora A, B	Cell cycle	alisertib, and others	Phase I-II
Chk1	Stress response, cell cycle	LY2603618, and others	Phase I-II
SAE1/2	SUMOylation, post-translational modification	ginkgolic acid, spectomycin B1	Pre-clinical
Glutaminase	Glutamine uptake and utilization	BPTES, compound 968	Pre-clinical
AMPK, ARK5	Energy homeostasis	BML-275	Pre-clinical

hormone and HER2 receptor triple-negative breast cancer, in which MYC signaling is elevated.[22] Purvalanol A, CDK1-specific siRNA, and dinaciclib—a CDK inhibitor compound in clinical trials—all induced apoptosis in a panel of triple-negative breast cancer cell lines. Dinaciclib was also effective at inducing apoptosis and tumor regression in mouse xenograft models.[22] The mechanism of synthetic lethality involved an acute upregulation of a pro-apoptotic Bcl-2 family member BIM, which may break the MYC-calibrated balance between the overall activities of pro-apoptotic and pro-survival members of the Bcl-2 family proteins.[22] Based on these observations, a dinaciclib phase I trial using MYC expression and signaling as a clinical correlate biomarker of response has been initiated (ClinicalTrials.gov Identifier: NCT01676753). This is among the first trials in which a small molecule CDK inhibitor is used to determine whether MYC overexpressing cancers are selectively targeted.

Among other CDKs, an interphase cell cycle kinase CDK2, was reported to be essential for the viability of neuroblastoma cells with MYCN amplification.[23] CDK2-specific siRNAs and seliciclib (also known as roscovitine), a small molecule CDK inhibitor with higher specificity toward CDK2, 7, and 9, induced apoptosis in a panel of established neuroblastoma cell lines. The sensitivity to CDK2 inhibition was dependent on wild-type p53 and MYCN overexpression. Seliciclib was previously evaluated in phase I and II trials. The potential clinical efficacy of CDK2 inhibition has been controversial. Earlier genetics studies demonstrated that CDK2 was not essential for mammalian embryonic development in vivo or for the cell cycle progression of nontumorigenic as well as tumorigenic cells in vitro.[24,25] Genetic ablation of CDK2 was, however, associated with compensation by other CDKs. On the other hand, employing a chemical genetic approach, it was recently reported that specific small molecule inhibition of CDK2 kinase activity diminished cell cycle progression in nontransformed and MYC-transformed epithelial cells without induction of cell death.[26,27] Interestingly, CDK2 genetic depletion via siRNA in the same system resulted in accelerated cell proliferation, which was accompanied by the upregulation of CDK1 that has been shown to be capable of functionally compensating for any of the interphase CDKs.[26] Thus, these observations suggest that small molecule inhibition of CDK2 kinase activity can exhibit antiproliferative effects. Whether CDK2 inhibitors will have a role for therapy of neuroblastomas or other MYC or MYCN-driven tumors remains to be determined.

Mitosis regulators Aurora kinases A and B, which regulate mitotic spindle attachment and dynamics, have been targeted in MYC-deregulated cancer cells. It was reported that multiple Aurora selective small molecule inhibitors caused strong antitumorigenic effects—including cell cycle arrest, apoptosis, and autophagy—in model epithelial cells in a MYC-dependent manner.[28] Small molecule Aurora kinase inhibitors were also effective in extending animal survival in multiple mouse models of MYC-induced lymphomas.[28] More recently, an Aurora kinase small molecule inhibitor, alisertib, was found to increase animal survival in a mouse model of MYCN-driven neuroblastoma, in which Aurora kinase plays a key role in maintaining MYCN protein stability that is central to its tumorigenic activity.[29] Alisertib is currently being evaluated in numerous phase I and II trials. Chk1—an essential kinase involved in DNA damage and cellular stress-responsive pathways—is another cell cycle-related kinase that has been targeted in MYC-deregulated cancer cells. The hypothesis is that highly proliferative MYC-driven cancer cells increase endogenous DNA damage from replicative stress, DNA replication fork collapse, or oxidative stress. A Chk1 checkpoint allows for repair of these insults and protects rapidly proliferating MYC-driven cells from these endogenous DNA damage insults. Interestingly, although highly proliferative MYC-induced pancreatic tumors were sensitive to Chk1 inhibition in mice, less proliferative KRAS-driven tumors were not.[30] The difference between the two potent oncogenes appeared to be the amount of DNA damage caused by each as demonstrated by the number of gammaH2AX-positive cells. Several Chk1 small molecule inhibitors are currently being evaluated in early phase clinical trials.

Non-Cell Cycle Targets

Beyond the cell cycle, MYC has also been shown to regulate numerous additional signaling pathways critical for tumor development and maintenance. A current challenge is to identify additional synthetic lethal targets in these signaling pathways downstream of MYC. To date, both hypothesis-driven targeted approaches and unbiased RNA interference-based loss-of-function screens have been undertaken to elucidate new vulnerabilities of cancer cells that exhibit elevated MYC expression.

A whole genome shRNA screen was conducted in a model human mammary epithelial cell line with conditional MYC activity (i.e., HMEC-MYC^{ER}) to identify those gene products that were essential for the viability of cells only when MYC was activated. The small ubiquitin-related modifier (SUMO)-activating enzyme 1/2 (SAE1/2, a heterodimer complex) was found to be a synthetic lethal partner of MYC.[31] SUMO proteins are small (approximately 10kDa) modifiers primarily conjugated onto nuclear proteins that can alter cellular localization and activity of their acceptor proteins.[32] Loss of SAE1/2 resulted in mitotic catastrophe in a MYC-dependent manner in vitro, which was accompanied by an alteration in the expression of a subset of MYC-responsive genes.[31] SAE1/2 function was required for the in vivo growth of MYC-overexpressing breast cancer cell lines in mouse xenograft models.[31] However, the cellular mechanisms by which the observed mitotic catastrophe occurs in a MYC-dependent manner have yet to be elucidated, and whether selective SAE1/2 inhibitors can be developed for clinical use remains to be determined.

Metabolism is another cellular process that has gained recent attention for therapeutic targeting in cancer. Altered tumor metabolism is now recognized as a bona fide hallmark of cancer.[33] MYC has been shown to transcriptionally regulate many metabolic genes directly or indirectly via regulation

through MYC-regulated miRNAs. Two notable pathways that MYC regulates are glycolysis and glutaminolysis, both of which are important for energy production and biosynthesis.[34,35] MYC-overexpressing human cells have been shown to be particularly addicted to exogenous glutamine; thus, the glutaminolysis pathway has become a focus of therapeutic intervention in MYC-activated tumor cells.[36,37] Promising results have been found in MYC-expressing B-cell lymphoma cells treated with the glutaminase (GLS) inhibitor BPTES in both in vitro and in vivo models. GLS catalyzes the conversion of glutamine to glutamate in cells, and thus its inhibition effectively starves cells of glutamine and shuts down glutaminolysis, leading to inhibition of tumor growth.[38] In addition to BPTES, small molecule 968 has been identified as an additional GLS inhibitor, and both drugs remain in early clinical development.[39]

Although nutrient starvation is a logical treatment strategy for tumors, targeting uptake and usage of key nutrients such as glutamine could have untoward systemic side effects. Thus, it is also worthwhile to identify MYC-specific alterations in metabolic regulators that can be targeted. A kinome siRNA screen performed in an osteosarcoma cell line engineered to have conditional MYC activity (U2OS-MYC^{ER}) identified a synthetic lethal interaction in which inhibition of the 5′ AMP-activated kinase (AMPK)-related kinase 5 (ARK5) or AMPK itself induced cell death in a MYC-dependent manner.[40] Conditional in vivo knock-down of ARK5 could inhibit tumor growth in a mouse model of hepatocellular carcinomas driven by MYC and AKT, and ARK5 inhibition was also effective to extend animal survival.[40] AMPK is an essential nutrient sensor in cells and responds to low ATP/ADP ratios by activating energy conserving and downregulating energy-consuming pathways; ARK5 is an upstream regulator of AMPK. MYC appears to co-opt these metabolic regulators to maintain tumor-specific metabolic homeostasis. Specifically, AMPK activation appears to downregulate MTOR-mediated translation while maintaining electron transport chain component expression and activity. Drugs—such as metformin—that upregulate AMPK activity might therefore be predicted to protect certain MYC-driven tumors via the ARK5/AMPK pathway from cell death; this hypothesis remains to be formally proven although may be clinically important. Inhibition of metabolic regulators downstream of MYC signaling may provide a therapeutic opportunity and warrants further investigation.

CONCLUSION

MYC has been implicated in the genesis and the maintenance of numerous human cancer types. MYC has also been shown to cooperate with major driver mutations in accelerating tumor formation and progression. The hallmark of MYC function appears to be in rewiring diverse cellular signaling networks to accommodate rapid proliferation, altered metabolic demands, and the resulting cellular stresses that emanate from these demands. Despite its widespread presence in cancer, the fact that MYC is an essential gene that encodes a pleiotropic transcription factor responsible for the coordinated expression of hundreds of genes has made it a challenging therapeutic target. Nevertheless, rapid progress is being made by utilizing genomics from human primary tumor samples combined with the discovery and validation of new MYC-dependent synthetic genetic interactions (Fig. 1; Table 1). Rapid progress is being made by utilizing genomics from human primary tumor samples combined with the discovery and validation of new MYC-dependent synthetic genetic interactions to identify drugable targets. In the near future, such combined approaches are expected to yield new therapeutic approaches to treat MYC-driven tumors.

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The author(s) indicated no potential conflicts of interest.

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