Molecular Pathways: Targeting MYC-induced Metabolic Reprogramming and Oncogenic Stress in Cancer

Bo Li and M. Celeste Simon

Abstract

MYC is a multifunctional transcription factor that is deregulated in many human cancers. MYC impacts a collaborative genetic program that orchestrates cell proliferation, metabolism, and stress responses. Although the progression of MYC-amplified tumors shows robust dependence on MYC activity, directly targeting MYC as a therapeutic method has proven to be technically difficult. Therefore, alternative approaches are currently under development with a focus on interference with MYC-mediated downstream effects. To fuel rapid cell growth, MYC reprograms cancer cell metabolism in a way that is substantially different from normal cells. The MYC-induced metabolic signature is characterized by enhanced glucose and glutamine uptake, increased lactate production, and altered amino acid metabolism. Targeting MYC-reprogrammed cancer cell metabolism is considered to be promising based on multiple preclinical studies. In addition, the increased biosynthetic demand of MYC-driven tumors coupled with limited nutrient access within tumor microenvironments create multiple levels of oncogenic stress, which can also be used as tumor-specific targets for pharmacologic intervention. Presumably, the best therapeutic strategy for treating MYC-amplified tumors is combined targeting of multiple MYC-mediated pathways, especially those involved in regulating cell proliferation, metabolism, and oncogenic stress. Clin Cancer Res; 19(21); 5835–41. ©2013 AACR.

Background

MYC is a pleiotropic transcription factor that participates in many cellular processes, including cell proliferation, apoptosis, differentiation, metabolism, and genome stability (1). Upon activation, MYC recognizes CACGTG sequences termed “E Boxes”, as a heterodimer paired with its binding partner MAX. MYC cofactors such as TRRAP and GCN5 are then recruited to E Boxes within chromatin and contribute to MYC-driven transcriptional events (2). Depending on the specific cellular and signaling contexts, MYC is able to induce or suppress the expression of up to 15% of all known genes, partly because of the prevalence of MYC cognate sites within the genome (1). Interestingly, it was recently revealed that MYC is particularly enriched in the promoter regions of active genes, therefore functioning to amplify existing transcriptional signals (3, 4). This breakthrough finding revolutionizes our understanding of MYC, expanding the horizon beyond traditional MYC target genes to virtually all actively transcribed genes. The MYC gene family comprises 3 major members: c-MYC, MYCN, and MYCL. c-MYC is the only isoform expressed ubiquitously in a broad range of tissues and organs, especially during early embryogenesis. In contrast, MYCN and MYCL are expressed in a more tissue-restricted manner, such as in the central nervous system and lung epithelium (2). Nevertheless, it is generally believed that MYC family members can be functionally interchangeable, which is partially supported by the fact that knockin expression of MYCN into the c-MYC locus does not interfere with normal mouse development and reproduction (5).

Given the pleiotropic role of MYC in multiple cellular functions, MYC expression and activity need to be tightly regulated. MYC protein is frequently induced in proliferating cells to orchestrate the signaling and metabolic events that drive cell-cycle progression and is quickly targeted for proteasomal degradation after proliferation signals regress (1, 6). In normal differentiated tissues, MYC expression levels remain low except in those bearing a high turnover rate. Meanwhile, deregulated MYC resulting from genomic amplification or translocation contributes to neoplastic transformation and tumor progression (1, 7). Not surprisingly, high levels of MYC expression were found to be hallmark of many cancer types, including Burkitt lymphoma, neuroblastoma, medulloblastoma, breast cancer, and multiple myeloma (2, 8). Early studies using transgenic mice suggested that tumors with elevated MYC expression are frequently addicted to MYC, as tumor progression was severely impaired if MYC was silenced (1). Moreover, a systematic investigation of possible side effects of inhibiting global MYC expression was recently conducted in the...
KRAS\(^{G12D}\) lung cancer model, where a dominant negative form of MYC (OmoMYC) was ubiquitously expressed under the CMV promoter in these mice (9). While KRAS-driven tumor progression was significantly inhibited by the presence of OmoMYC, there were no obvious physiologic changes observed in most organs and tissues except for a reversible growth retardation found in skin, testis, and intestinal epithelium (9). These observations provide solid basis for targeting MYC as an efficient and tolerable therapy for treating cancers harboring-amplified MYC.

For the past 2 decades, numerous attempts have been made to develop strategies to directly target MYC activity or expression. However, little success has been achieved along this route. For instance, inhibiting MYC activity using small molecules to disrupt the specific MYC–MAX interaction has proven to be quite difficult, largely because of the "flat" interface between the MYC–MAX dimer (10, 11). The best IC\(_{50}\) value of currently identified MYC inhibitors is at the micromolar level, which is not potent enough to be considered for clinical evaluation (12). Lately, another promising strategy emerged following the discovery of G-quadruplex motif, which is a transcription module located in the MYC promoter region that suppresses MYC gene expression (13). Some leading compounds stabilizing the G-quadruplex motif, such as TMPyP4, showed promising effects in inhibiting lymphoma cell proliferation (14). However, the intrinsic complexity of these structures restrains the possibility of targeting a specific gene like MYC using this method. Recently, a small-molecule bromodomain inhibitor JQ1 was found to efficiently affect MYC transcription and block MYC-driven tumorigenesis (15–17). Although, a JQ1 derivative compound GS552762 has entered clinical trials (NCT01587703), the specificity of this molecule merits close attention due to the prevalent interaction between bromodomain proteins and acetylated histones. In fact, JQ1 treatment has already been reported to cause infertility in male mice (18).

Continuous difficulties lying in directly targeting MYC led to the thoughts of developing alternative methods to circumvent this problem. It is well established that activated MYC reshapes cancer cell metabolism in a way that is coordinated with a sustained proliferative signal (ref. 6; Fig. 1). MYC-induced glucose and glutamine consumption accompanied by enhanced lactate production provide necessary energy and intermediates for the rapid construction of new daughter cells (7). MYC also stimulates ribosomal and mitochondrial biogenesis to sufficiently cover the energy and intermediates for the rapid construction accompanied by enhanced lactate production provide necrogenic stress accumulated in fast growing cancer cells (25). In this article, we will focus on several MYC-induced oncogenic and metabolic changes that could be vulnerable for molecular targeting, with an emphasis on current translational efforts aiming to develop better therapeutic strategies to treat MYC-induced malignancies.

**Clinical–Translational Advances**

### Targeting a MYC-induced Warburg effect

Cancer cells preferentially generate energy through aerobic glycolysis rather than oxidative phosphorylation, a well-established phenomenon described as the Warburg effect (26). Although glycolysis is an inefficient way of using glucose, it provides essential intermediates for the biosynthesis of macromolecules, including proteins, lipids, and nucleotides (26). Glycolysis also produces minimal oxidative stress, a survival obstacle that is often encountered by rapidly growing cancer cells (27). Activated MYC drives the expression of almost all glycolytic enzymes, particularly LDH, hexokinase 2, and enolase 1, as well as glucose transporter (GLT1; ref. 7; Fig. 1). Unfortunately, inhibiting the early steps of glycolysis often introduces nontolerable toxicity, as evidenced by the failed clinical trials of the hexokinase inhibitors 2-deoxy-o-glucose (NCT00633087) and lonidamine (NCT00435448). However, targeting lactate metabolism via a small molecule FX11 that inhibits lactate dehydrogenase (LDH) and LDH isozyme that is specifically induced in tumor tissues, blocks MYC-driven lymphoma progression without introducing any observable side effects in mice (21). It would be of great interest to follow the effect of FX11 or its optimized derivatives in future clinical trials.

In addition to suppressing LDHA activity, an alternative approach to interfere with lactate metabolism is to block the excretion of lactate from cancer cells through inhibiting monocarboxylate transporter (MCT) activity, given that high levels of intracellular lactate lower cytosolic pH and cause severe cytotoxicity (28). Currently a MCT1-specific inhibitor AZD3965 is being tested for treating prostate cancer, gastric cancer, and large B-cell lymphoma (NCT01791595).

Another attractive target within the glycolytic pathway is pyruvate dehydrogenase kinase (PDK). PDK inhibits pyruvate dehydrogenase through direct phosphorylation, which prevents central carbon metabolites from entering the tricarboxylic acid (TCA) cycle, thus ensuring consistent glycolytic flux and the Warburg effect (29). PDK expression can be robustly increased by the cooperation between MYC and hypoxia-inducible factor (HIF), a transcription factor frequently induced in solid tumors with poor vascularity (ref. 30; Fig. 1). Interestingly, MYC opposes HIF activity in the normal physiologic condition, whereas in transformed tissues it often collaborates with HIF to promote glucose–lactate conversion partially through PDK1 coactivation (30–33). Several clinical trials on brain tumors, head and neck carcinoma, and other recurrent/metastatic solid malignancies are ongoing, using a PDK1-inhibiting compound dichloroacetate as a single agent or in combination with other therapeutics (NCT00566410, NCT01386632, and NCT01111097).
Targeting MYC-induced glutamine metabolism

MYC-induced aerobic glycolysis directs central carbon metabolites away from the mitochondria, resulting in a significant depletion of TCA cycle intermediates (6). To compensate for substrate loss that hinders rapid cell growth, alternative bioenergetic resources need to be supplemented. Glutamine, the most abundant nonessential amino acid in the human body, represents a critical replenishment for mitochondrial respiration. In addition to its role in protein synthesis, glutamine can also be catabolized to a-ketoglutarate and feed into the TCA cycle, a process termed "anapleurosis" (34). This is accomplished by a two-step glutaminolytic reaction: first, glutamine is converted to glutamate via GLS-mediated deamination; then glutamate dehydrogenase (GDH) or glutamate-dependent transaminases remove the amino group from glutamate to generate a-ketoglutarate (6, 34). Cancer cells harboring amplified MYC exhibit MYC-dependent glutamine addiction, as glutamine depletion or glutaminolysis inhibition triggers dramatic apoptotic responses in MYC-overexpressing fibroblasts, lymphoma cells, and neuroblastoma cells, but not in MYC-nonexpressing counterparts (35–39). The glutamine dependence induced by MYC amplification is partially mediated by MYC's ability to upregulate a few glutamine transporters (ASCT2, SN2, etc.) and GLSs (Fig. 1), which opens a therapeutic window for drug targeting (6, 39).
Indeed, several glutaminolysis inhibitors have been developed and tested preclinically. 6-diazo-5-oxo-L-norleucine (DON) is a nonspecific GLS inhibitor that showed antitumor activities in both cell culture and animal experiments (40, 41). However, its severe side effects such as neuronal toxicity prevented its bedside application (42). BPTES is a specific inhibitor of GLS1, the kidney isoform of GLS that is frequently activated by c-MYC (43). The in vitro and in vivo proliferation of P493 lymphoma cells overexpressing c-MYC are significantly inhibited by BPTES treatment, a phenotype that is even more dramatic in hypoxic conditions (37). It’s worth noting that the amplified MYCN associated with neuroblastoma predominantly induces the expression of GLS2, the liver isoform of GLS that cannot be inhibited by BPTES (36, 43). Therefore, caution is needed when treating cancers driven by different MYC isoforms. Compound 968 is another GLS1 inhibitor identified by screening for molecules that block Rho-GTPase–induced transformation (44). 968 selectively targets a short-splicing isoform of GLS1 named GAC which is presumably accounting for the elevated basal GLS activity detected in transformed cells. Consistent with this finding, 968 administration significantly decreases the growth rate of human breast cancer and lymphoma cells without affecting normal cell growth (44). Although these preclinical results are encouraging, neither BPTES nor 968 have been transferred to clinical examination, also special attention needs to be paid to the neuronal side effects that might be caused by these GLS inhibitors. Meanwhile, glutaminolysis can also be disrupted by inhibition of GDH or transaminases, 2 groups of enzymes catalyzing the second step of glutaminolysis (ref. 34; Fig. 1). It has been reported that even short-term treatment with aminooxyacetate (AOA), a pan-transaminase inhibitor, was sufficient to delay autochthonous neuroblastoma growth in the TH-MYCN+/- mouse model (31). Unfortunately, the intolerable side effects of AOA prevent it from being approved to treat human patients (45). More specific and potent transaminase inhibitors are necessary. Moreover, MYC-amplified neuroblastoma and glioblastoma cells are also sensitive to GDH inhibition, especially in glucose-depleted conditions (36, 46). Administration of a nonspecific GDH inhibitor epigallocatechin gallate (EGCG), which is a green tea extract, triggers dramatic apoptosis in these cells and inhibits neuroblastoma xenograft tumor growth (36). EGCG is currently in phase II trials on colorectal cancer (NCT01360320) and multiple myeloma (NCT00942422).

### Targeting MYC-induced oncogenic stress and apoptosis

Upon its oncogenic activation, MYC elicits a massive transcriptional program that supports cancer cell proliferation and tumor progression. The rapid cell growth and biomass synthesis creates an unfavorable cellular environment, a situation termed "oncogenic stress" and multiple stress defensive responses are activated to overcome this issue (25). In MYC-induced lymphoma tissues, elevated protein synthesis leads to the accumulation of unfolded proteins in the ER, which initiates the unfolded protein response (UPR) through activation of the PERK/eIF2α pathway (ref. 50; Fig. 1). Loss of UPR signaling resulting from PERK deletion triggers a caspase-dependent apoptosis in MYC-overexpressing cells, which raises interest in using PERK inhibitors to treat MYC-amplified tumors. Recently, a small-molecule GSK2656157 was developed as a specific PERK inhibitor, which successfully inhibited pancreatic cancer and multiple myeloma xenograft tumor growth, although a drug-induced pancreatic damage was also observed (51). To reduce the amount of unfolded proteins in the ER, PERK/eIF2α activation blocks global protein synthesis while simultaneously increasing the expression of selective genes, such as ATF4 (50). Upon activation by PERK, ATF4 protects cells from UPR-induced apoptosis partially through its connection to autophagy (50, 52). However, amino acid deprivation, a metabolic stress that...
frequently occurs in poorly vascularized tumors, also activates ATF4 via a PERK-independent kinase GCN2. In this case, ATF4 functions as a proapoptotic factor that mediates stress-induced cell death (ref. 36; Fig. 1). It has been reported that in MYC-amplified lymphoma and neuroblastoma cells, glutamine starvation triggers apoptosis via the activation of ATF4-PUIMA/NOGA/TRB3 pathway and MYC-induced xenograft tumor growth is significantly inhibited by injection of a nonspecific ATF4 agonist 4-hydroxyphenyl retinamide (4-HPR, also known as fenretinide; ref. 36). Fenretinide is currently in clinical trials to treat neuroblastoma (NCT00646230), B-cell lymphoma (NCT00288067), and ovarian cancer (NCT01535157). Given that MYC-induced oncogenic stress sensitizes cancer cells to apoptosis, antiapoptotic factors would be another group of intriguing targets to consider. Increased glutamine flux elicited by high levels of MYC can activate mTOR (53) and transgenic activation of the mTOR downstream effector eIF4E antagonizes MYC-dependent apoptosis in vivo (ref. 54; Fig. 1). Consistent with these findings, MYC-driven lymphoma progression in mice is significantly delayed by administration of a specific mTORC1 inhibitor everolimus (55), a promising anticancer compound that is undergoing progression in mice is significantly delayed by administration of a specific mTORC1 inhibitor everolimus (53), a promising anticancer compound that is undergoing numerous clinical trials (56). Interestingly, an uncontrolled, hyperactivated mTOR signaling event is also detrimental to MYC-induced transformation. A Kinome siRNA screen revealed that AMPK-related kinase 5 (ARK5), an upstream inhibitor regulator of mTORC1, contributes to MYC-induced hepatocellular carcinoma progression (57). ARK5-restrained mTOR activity is necessary to maintain MYC-driven glutaminolysis, suggesting that ARK5 may be a potential drug target for tumors with deregulated MYC.

Conclusions and Future Perspective
Cancer cell metabolism is an "antique" research field that has recently been revisited and gained increasing momentum over the past decade. Newly developed biotechnologies and better understanding of signaling networks will elucidate the mechanisms whereby metabolic changes are induced in cancer cells. MYC is a "master regulator" oncogene that orchestrates multiple levels of cancer cell activities, including proliferation, apoptosis, stress responses, and metabolism. The multifaceted function of MYC opens up exciting possibilities for combined targeting strategies that may achieve better therapeutic responses. Consistent with this idea, coadministration of the apoptosis-inducing fenretinide and glutaminolysis inhibitor EGCG in a preclinical neuroblastoma model resulted in better outcomes than individual application (36). Fenretinide and EGCG are both clinically approved drugs and can be quickly translated into trials for testing their cooperative effect in human patients. In addition to MYC-primed apoptosis and metabolism, MYC-induced cell-cycle progression can be a third option to consider for combined therapeutic targeting. A list of proliferation-related effectors have been reported to be essential for MYC-driven tumorigenesis, including cyclin-dependent kinases 1/2, polyamine synthetic enzyme ornithine decarboxylase 1, and SUMOylation-activating enzyme 1/2 (58–62). Several compounds that selectively inhibit these enzymes have entered clinical trials, such as efomithine (NCT01586260, NCT01059071, NCT00983580), AT7519M (NCT01183949, NCT01652144, NCT01627054), and selicib (NCT00999401). We believe that the evolving knowledge about MYC will keep providing intriguing targets for treating MYC-amplified malignancies.

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No potential conflicts of interest were disclosed.

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