Targeting MYC in cancer therapy: RNA processing offers new opportunities

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INTRODUCTION

MYC is a transcription factor, which not only directly modulates multiple aspects of transcription and co-transcriptional processing (e.g., RNA-Polymerase II initiation, elongation, and mRNA capping), but also indirectly influences several steps of RNA metabolism, including both constitutive and alternative splicing, mRNA stability, and translation efficiency. As MYC is an oncoprotein whose expression is deregulated in multiple human cancers, identifying its critical downstream activities in tumors is of key importance for designing effective therapeutic strategies. With this knowledge and recent technological advances, we now have multiple angles to reach the goal of targeting MYC in tumors, ranging from the direct reduction of MYC levels, to the dampening of selected house-keeping functions in MYC-overexpressing cells, to more targeted approaches based on MYC-induced secondary effects.

KEYWORDS:
cancer therapy; MYC; post-transcriptional regulation; RNA; splicing; synthetic lethality; transcription

Introduction

MYC is an attractive target in cancer therapy

The abnormal activation of the c-Myc (Myc) oncogene, either due to transcriptional overexpression (gene amplification, translocation, alterations in upstream signaling pathways) and/or protein stabilization, is one of the most common features of cancer cells. Indeed, high MYC protein levels are not only able to drive tumor initiation and progression, but are also essential for tumor maintenance: sustained MYC overexpression is required by cancer cells, and growth arrest, apoptosis and differentiation occur upon reduction in MYC levels. This has not only been described for MYC-driven mouse tumor models, but also in tumors driven by other oncogenes (reviewed in [1]), making c-MYC a highly attractive target for anti-cancer therapy. Unfortunately, MYC itself is not an easily “druggable” protein, due to lack of enzymatic activity or any deep pocket, which could be traditionally targeted by small molecule inhibitors. For this reason, one of the priorities in the field is the inhibition of MYC co-factors and/or downstream effectors that might play critical roles in tumor development or maintenance.

MYC functions as a transcription factor

MYC is a transcription factor of the basic helix loop helix leucine zipper (bHLH-LZ) family. Together with its partner protein, MAX [2, 3], MYC can bind to target DNA sequences, the E-boxes (including the canonical CACGTG and other non-canonical sites), when they are embedded in a euchromatic context [4]. Once bound to chromatin, MYC is able to transcriptionally regulate protein coding and non-coding RNAs [5] that are produced by RNA Pol I, RNA Pol II, and RNA Pol III [6]. Notwithstanding the vast amount of gene expression analysis performed in the last decades with the aim of finding a core of MYC regulated genes, identifying the relationship between MYC binding to chromatin and the consequent transcriptional output is still an open debate. With the advent of genome-wide profiles, and the realization that MYC not only binds every active promoter, but also active enhancers, this long-standing issue has become even more complex.

Abbreviations:
eIF, eukaryotic initiation factor; IRES, internal ribosome entry site; NMD, nonsense mediated RNA decay; SAGA, Spt-Ada-Gcn5-acetyltransferase.
In the last few years, two opposite views have emerged. One suggests that MYC amplifies the transcriptional output of all active promoters [7, 8]; the other that MYC regulates, either positively or negatively, discrete sets of genes mainly involved in proliferation, cell growth, metabolic reprogramming, and RNA biogenesis [9]. We will not go into the details of this debate here, because it has been extensively covered elsewhere very recently [5]. Instead, we will focus our attention on the plethora of equally important post-transcriptional mechanisms that are regulated, both directly and indirectly, by MYC (Fig. 1). Because these contribute to shaping the transcriptomic and the proteomic landscape of MYC overexpressing cells, we will then propose how this knowledge can be used to target MYC’s oncogenic function.

**MYC regulates post-transcriptional mechanisms**

**MYC promotes mRNA capping**

RNA Pol II transcripts are subject to a specific post-transcriptional modification that consists of the addition of a 7-methylguanosine to the first transcribed nucleotide (5’ cap). This cap structure not only guarantees RNA stability, but also proper pre-mRNA downstream processing. For example, CBC (cap binding complex) and eIF4E (eukaryotic initiation factor 4E) are recruited to the mRNA by interaction with the cap structure, and subsequently mediate processing and translation initiation, respectively [10, 11].

MYC directly promotes cap addition at its target genes by recruiting TFIIH and by indirectly upregulating S-Adenylylhomocysteine hydrolase (SAHH), which is required to metabolize SAH, an inhibitory byproduct of the capping reaction, thus antagonizing a potential negative feedback loop [14].

Aside from cap-dependent translation, selected mRNAs can also be translated in a cap-independent manner from internal ribosome entry sites (IRES). The overexpression of translation initiation factors, 4EBP1 and eIF4G, has been observed in various cancer types, and under hypoxic conditions, they promote the IRES-mediated translation of mRNAs that confer survival advantages [15]. Interestingly, mTOR inhibitors that block the phosphorylation of 4EBP1 have been shown to be synthetic lethal in MYC-driven cancers (discussed later) [16].

**MYC regulates the abundance of splicing factors**

MYC has been shown to directly modulate the transcription of various splicing factors, such as hnRNP A1 [17], hnRNP A2, and PTB (polypyrimidine tract binding protein) [18]. These proteins regulate, among several other events, the alternative splicing of pyruvate kinase (PKM) isoforms, specifically, generating more of the embryonic/tumor isoform, PKM2, which promotes aerobic glycolysis, and less of the adult isoform, PKM1, which favors oxidative phosphorylation [18, 19]. Additionally, these splicing factors have been implicated in the generation of constitutively active androgen receptor splice variants, which may contribute to the development of castration-resistant prostate cancer [20].

Similarly, MYC regulates hnRNPH, which is necessary for the correct splicing of oncogenic a-Raf pre-mRNA [21]. In normal cells, low MYC and hnRNPH expression allows the production of a short isoform of a-Raf, which encodes a truncated A-Raf protein that suppresses Ras activation and transformation. Conversely, in cancer cells, full-length A-Raf can be produced, due to the high levels of MYC and hnRNPH, and in this form, it can effectively inhibit the activity of the MST2 pro-apoptotic kinase.
The promoter region of arginine/serine-rich splicing factor Srsf1 has been shown to contain two non-canonical E-boxes, through which MYC can directly activate its transcription [22]. Srsf1 and MYC have also been shown to cooperate in the transformation of mammary epithelial cells, possibly by synergistically activating eIF4E phosphorylation [23]. Srsf1 is not only involved in splicing, but also in several other aspect of RNA metabolism, such as nuclear export, nonsense-mediated RNA decay (NMD), and translation (reviewed in [24]).

During somatic reprogramming, MYC directly upregulates the expression of Spt-Ada-Gcn5-acetyltransferase (SAGA) components, which are then recruited on several MYC target genes, activating their transcription [25]. Many of these genes encode proteins that are involved in RNA splicing, and are essential both during the early stages of reprogramming, as well as for the maintenance of the established pluripotent stem cell state. Consequently, MYC and SAGA indirectly modulate alternative splicing, primarily promoting exon inclusion, during cell reprogramming. Interestingly, many of the alternatively spliced transcripts are involved in cell migration, transcriptional regulation, or RNA processing [25]. This supports the possibility that in other contexts, such as during the initiation of cancer metastasis, the regulation of various splicing factors by MYC may contribute to EMT/MET. This notion is supported by the well-established link between MYC and SAGA components, e.g. Gcn5, Cdc101, Mnk2, Tead1, Ron, Bin1, Mknk2, Srsf1 Alternative exon usage, e.g. A-raf HnRNPH Intron inclusion, e.g. Slain2, Plod2, Fat1, Pcm1 References [17–20] [21] [22–24] [25] [25]

Table 1. MYC-regulated and MYC-synthetic lethal splicing factors are involved in constitutive and alternative splicing

| MYC-regulated/ MYC-synthetic lethal splicing factors | Type of splicing event upon perturbation of the splicing factor and examples of downstream mRNAs affected | References |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------|------------|
| HnRNPA1, HnRNPA2, and PTB                         | Alternative exon usage, e.g. PKM1/2, Androgen receptor                                        | [17–20]    |
| HnRNPH                                             | Intron inclusion, e.g. A-raf                                                                       | [21]       |
| Srsf1                                              | Alternative exon usage, e.g. Mnk2, Tead1, Ron, Bin1, Mnk2, Bim                                    | [22–24]    |
| SAGA components, e.g. Gcn5, Cdc101, Taf12, and Atxn7B | Exon inclusion, e.g. Slain2, Plod2, Fat1, Pcm1                                                  | [25]       |
| Core splicing proteins, e.g. Prmt5, Snrnp B, Snrnp D1, and Snrnp D3 | Intron retention and exon skipping of pre-mRNAs with weak 5’ splice sites, e.g. Atr, Ep400, Dvl1, Mdm4, etc. | [29]       |
| Bud31                                              | Intron retention of numerous pre-mRNAs involved in gene expression, splicing, unfolded protein response, and DNA replication and repair, e.g. Dnab5, S11, Mcm7, Skp2, etc. | [30]       |

Inhibition of another core spliceosome protein, BUD31, resulted in global intron retention and aberrant pre mRNA processing [30]. Indeed, as we will discuss later, MYC overexpressing cells are more sensitive to splicing inhibition [30, 31].

Table 1 summarizes MYC-regulated and MYC-synthetic lethal splicing factors involved in alternative and constitutive splicing.

**MYC indirectly regulates several pathways of RNA degradation**

(1) The AU-binding proteins (AUBPs) regulate RNA stability by binding to AU-rich elements (AREs), which are found in up to 16% of human protein-coding transcripts [32]. Among the AUBPs, MYC represses Tristetrapolin (TTP/Tis11/Zfp36) and its family members, Tis11b (Brf1/Zfp361) or Tis11d (Brf2/Zfp362), which are involved in RNA degradation. In contrast, MYC positively regulates HuR, AUf1, AUf2, and Nucleolin (Ncl), which stabilize ARE-containing transcripts. In this way, MYC may promote a general increase in the stability of short-lived RNAs [33]. The targets of TTP-induced degradation are mainly transcripts encoding genes involved in cancer and inflammation. Since many other RBPs are also subject to TTP-induced degradation [34], the effects of MYC on the RNA processing machinery are thus amplified. Interestingly, the down-regulation of TTP by MYC has been shown to be a critical step in MYC-induced lymphomagenesis. Since the forced overexpression of TTP in Eμ–myc lymphomas delays tumor onset, TTP has been ascribed as a tumor suppressor [33]. Similarly, an increase in Nucleolin levels can ensure the stabilization of the antia apoptotic, pro-oncogenic BCL-XL factor [35], which is essential for MYC-driven cancer progression [36].

(2) Nonsense mediated RNA decay (NMD) is a safeguard mechanism that mediates the degradation of transcripts with premature termination codons (PTCs), which are often produced by incorrectly spliced RNA. This prevents their translation into truncated proteins. Of note, many genes encoding regulatory and basal splicing factors...
MYC-regulated proteins, such as Nucleolin, nucleophosmin, rRNA is processed into the 18S, 5.8S, and 28S rRNA by several must be processed and post-transcriptionally modified. The pre-acetyltransferases, GCN5 and TRRAP, and the selective increased recruitment of TFIIIB, RNA polymerase III, the transcription of tRNA and 5S rRNA [6, 54]. This occurs via basal RNA polymerase I transcriptional complex.

Thus, on one hand, MYC augments the RNA processing capacity of the cells (by increasing the level of capping and splicing factors), while on the other hand, it selectively modulates the stability of certain oncogenic mRNAs (by upregulating/downregulating AUBPs and inhibiting the NMD pathway), while ensuring overall fidelity via the upregulation of exosome components. These concerted MYC-driven actions lead to the (often) observed increase in total mRNA levels and consequently, proteins and cell size [49].

MYC promotes translation/ribosome biogenesis
MYC regulates ribosomal biogenesis and mRNA translation in a highly orchestrated manner. First, it stimulates the transcription of ribosomal RNAs by RNA polymerase I [50–52] by binding to E-boxes in the promoters of the rDNA clusters and recruiting TRRAP, further remodeling the chromatin structure to a more permissive state. Additionally, MYC positively regulates the expression and/or recruitment of RNA polymerase I co-factors. These include UBF [53], which enhances promoter escape, and SL1 [51], which part is of the basal RNA polymerase I transcriptional complex.

MYC also stimulates the RNA polymerase III-driven transcription of tRNA and 5S rRNA [6, 54]. This occurs via increased recruitment of TFIIB, RNA polymerase III, the acetyltransferases, GCN5 and TRRAP, and the selective acetylation of histone H3 at the corresponding promoters [55].

Additionally, in order to obtain mature rRNAs, the RNA must be processed and post-transcriptionally modified. The pre-rRNA is processed into the 18S, 5.8S, and 28S rRNA by several MYC-regulated proteins, such as Nucleolin, nucleophosmin, fibrillarin, Nop56, and Bop1. snoRNPs play an essential role in the rRNA modification process, by providing target specificity to methylation and pseudouridylation reactions [56]. Both the RNA (snoRNAs) and protein components of these complexes are also regulated by MYC [9, 57].

Finally, MYC directly activates the expression of ribosomal components such as RPL and RPS family members [58], as well as eIF family members that are involved in translational initiation [59, 60].

Thus, MYC plays an essential role in ensuring not only transcription, but also the proper maturation of the translation machinery.

MYC transcriptionally modulates microRNAs and long non-coding RNAs
Many microRNAs [61], which are small (~22 nucleotide) non-coding RNAs, and long non-coding RNAs [62], which are defined as being longer than 200 nucleotides, have been identified as direct MYC targets. MYC-regulated miRNAs may be present in the intronic sequences of protein-coding genes or within non-protein-coding loci, and are subject to both positive and negative regulation by MYC.

MYC has been reported to directly induce various oncogenic miRNAs (e.g. the mir-17-92 cluster), as well as repress tumor-suppressive miRNAs (e.g. miR-26a and miR-34a), thereby favoring cancer cell proliferation (reviewed in [63]). MYC also indirectly modulates miRNA expression, for example, via the induction of the long-noncoding RNA, HOTAIR, which in turn, represses the tumor-suppressive miR-190a [64].

Furthermore, MYC controls the expression of proteins involved in miRNA biogenesis: among them, lin28B is an RNA-binding protein that binds to the stem-loops of precursor miRNAs, like let7 and miR-150, and inhibits their Dicer- and Drosha-mediated processing, accelerating their decay [65]. On the other hand, MYC has also been reported to promote pri-miRNA processing by transcriptionally regulating Drosha [66].

By regulating miRNA biogenesis and processing, that in turn regulate the expression of several mRNAs, MYC is thus able to greatly expand the number of its indirect targets.

What are the implications for targeting MYC in cancer therapy? How to directly target MYC (oncogene addition)
Multiple mechanisms account for the overexpression of MYC that is observed in numerous cancers. These include: translocation or amplification of the MYC locus, or its increased transcription; alterations in MYC protein stability caused by mutations in key residues (such as T58 and S62) or by the altered expression of proteins involved in its post-translational modifications (phosphorylation, ubiquitination, etc.). Additionally, MYC function is highly context specific, and depends on its binding partners, as well as chromatin context. Accordingly, any of these angles can be exploited to ultimately reduce MYC-dependent oncogenic signaling (Fig. 2).
Problems & Paradigms

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Can we directly target MYC transcription?

Transcription of the MYC gene is controlled in a complex manner, by the action of numerous transcriptional regulators [67], as well as enhancers [68]. BET bromodomain proteins, which bind acetylated lysine residues, in conjunction with the mediator co-activator complex, can also regulate MYC transcription. Blocking their function with small molecule inhibitors, such as JQ1, i-BET, and MMS417, have been shown to downregulate MYC transcription and consequently, the expression of MYC target genes [69–71]. However, a caveat regarding the efficacy of decreasing the transcription of MYC via Brd4 inhibition is that this strategy is limited to cases in which Brd4 is the predominant regulator of MYC transcription [72]. The strategy may not be as effective, for example, if gene amplification or protein stabilization is involved in the Cap/IRES-dependent translation of Myc [82]. Alternative strategies have used morpholino oligonucleotides either designed to block Myc cap-dependent translation [85, 86] or alter its correct splicing, thereby reducing functional MYC protein levels [87].

Can we target Myc interactions?

As an alternative to the depletion of MYC levels, the interaction between MYC and its oligodimerization partner, MAX, can also be targeted in order to inhibit MYC function [88]. Omomyc is a version of MYC mutated in the residues of the leucine zipper that are critical for its dimerization specificity. As a result, whereas MYC does not homodimerize, Omomyc can form both dimers with MYC, as well as with MAX. Importantly, it does not bind MAD or other HLH proteins. The MYC/Omomyc dimers bind DNA with low affinity, and thus Omomyc acts in a dominant negative fashion by sequestering MYC away from MAX and DNA. As a consequence, MYC-mediated transcriptional activation is affected [88]. However, MIZ-1-mediated MYC binding to promoters and trans-repression are not affected by Omomyc. Consequently, Omomyc maintains the repression of genes that are usually negatively regulated by MYC, while enabling the dampening of MYC-dependent transcriptional activation [89]. Not surprisingly, MYC inhibition with Omomyc translation [77, 78], for example, CELF1 and HuR. Following the depletion of polyamines by inhibition of ornithine decarboxylase, the increase in CELF1 reduces MYC levels. Interestingly, ornithine decarboxylase heterozygosity has also been shown to delay MYC-driven lymphomagenesis [79], and the inhibition of Srm, which is also involved in polyamine biosynthesis, is chemopreventative in B-cell lymphomas [80].

Myc mRNA can be translated both by 5‘ cap- and internal ribosome entry site (IRES)- dependent mechanisms [81]. In multiple myeloma cells, increased Myc IRES activity, which is dependent on hnRNP A1 and Rps25, has been observed following ER stress [78]. In these cells, a compound that blocked the binding of hnRNPA1 to the Myc IRES was only toxic in the presence of ER stress. Furthermore, a small molecule inhibitor of the translation initiation factor eIF4a, silvestrol, was recently shown to reduce tumor growth in vivo in a mouse model of colorectal carcinogenesis by suppressing both cap- and IRES-dependent translation of Myc [82].

The stability and turnover of the MYC protein is regulated by a series of post-translational modifications that are controlled by a myriad of proteins [83]. As the expression of many of these proteins is also often deregulated in cancer, their inhibition with small molecules could reduce the stability of MYC, resulting in its degradation, and could, therefore, have therapeutic potential.

Can we design Antisense/ASO approaches to reduce Myc mRNA or protein abundance?

Antisense phosphorothioate oligonucleotides targeting Myc mRNA have demonstrated efficacy in multiple cancer types. These highly stable oligonucleotides bind to mRNA and trigger RNase H activity, resulting in the hydrolysis of the DNA/RNA duplex [84]. Alternative strategies have used morpholino oligonucleotides either designed to block Myc translation [85, 86] or alter its correct splicing, thereby reducing functional MYC protein levels [87].
results in reduced proliferation and increased apoptosis. Interestingly, the apoptotic phenotype was more pronounced in cells expressing particularly high levels of MYC [90]. Notably, the systemic inhibition of MYC in vivo with Omomyc was well tolerated by normal regenerating tissues, and the effects of MYC inhibition could be reversed completely and quickly [91].

Several small molecule inhibitors of MYC/MAX dimerization have also been developed. These compounds bind to and stabilize the monomeric form of MAX and prevent the association of MYC and MAX, thereby reducing MYC function in cells (reviewed in [92]). More recently, alpha-helix mimetics that prevent the binding of MYC/MAX heterodimers to DNA, but do not cause the dissociation of MYC and MAX, have also been developed [93].

Other transcription factors have been targeted by similar strategies based on directly or indirectly preventing protein:protein or protein:DNA interactions. For example, S3I-201 prevents the dimerization of Stat3 and the binding of Stat3 to DNA [94], and CoH Schiff base complexes can alter the structure of zinc fingers, which are a common feature of transcription factors, and selectively disrupt their DNA-binding ability [95].

How to indirectly target MYC (non-oncogene addiction)

Synthetic lethal screens can be performed to identify the Achilles heel of MYC-overexpressing cells

In addition to targeting the “oncogene addiction” to MYC observed in many cancer types by the approaches described above, strategies aimed at exploiting “non-oncogene addiction” in cancer cells with elevated MYC levels have also proven useful. These studies are based on the concept of synthetic lethality, whereby the perturbation of two or more genes in combination, but not of either gene singly, results in a significant deleterious phenotype, such as decreased proliferation or increased cell death [96]. In this respect, synthetic lethal screens have been useful in identifying critical MYC effectors that may be potential therapeutic targets [97]. These screens have employed functional genomics approaches with siRNA or shRNA-based knockdown, and compared the phenotypic and molecular responses of cells with “normal” MYC levels with cells with elevated MYC levels (and therefore hyperactive MYC-induced transcriptional programs), in order to identify vulnerabilities in particular pathways that are hyperactive or compromised, specifically in MYC overexpressing cells.

Toyoshima et al. compared the effects of knocking down specific genes in isogenic control and MYC-overexpressing fibroblasts [98]. Using a high-throughput approach, their screen included siRNA designed to target ~3,300 druggable genes and 200 miRNAs. Importantly, siRNAs that had significant growth inhibitory effects on the control cells were excluded. Not surprisingly, genes that showed synthetic lethality with MYC-overexpression belonged to key cellular pathways known to be regulated by MYC. These included DNA damage repair, transcription and transcriptional elongation, senescence, ribosome biogenesis, chromatin modification, metabolism, apoptosis, and mitotic control. Further analysis of one of the MYC synthetic lethal genes that was identified, CSNK1e, verified that its knockdown slowed the growth of MYCN-amplified glioblastomas and that its expression correlated with MYC in other human cancer types.

More recently, Huang and co-workers also screened a shRNA library targeting 442 genes encoding proteins for which small molecule inhibitors exist in a murine hepatocellular carcinoma model driven by MYC overexpression and p53 loss [99]. They found that the kinase component of the p-TEFb complex, CDK9, is essential to sustain the proliferation of MYC overexpressing HCC cells.

In another synthetic lethal screen, SAE2, a SUMO-activating enzyme, was also identified as showing potent synthetic lethality in cells with hyperactive MYC [100]. SAE2 knockdown resulted in spindle defects and consequently, aneuploidy and apoptosis, but only in MYC-overexpressing cells. Mechanistically, the loss of SAE2 switched the expression pattern of several MYC dependent genes in a SUMOylation-dependent manner, causing several MYC-induced genes to become repressed. Several of these genes are known to be involved in the assembly and maintenance of the integrity of the mitotic spindle.

In RNAi-based screens focused on kinases, PRKDC [101], AMPK-related kinase 5 (see later) [102] and GSK3beta [103] were identified to exhibit synthetic lethality with MYC overexpression. Interestingly, the downregulation of the Gsk3b/Fbw7 signaling pathway potentiated TNF-related apoptosis by stabilizing MYC and increasing the expression of a death ligand receptor, which promoted cell death [103].

How to target selected MYC downstream effectors

While screens provide an unbiased method to identify genes that are synthetic lethal with MYC, many directed gain or loss-of-function studies on MYC target genes have also demonstrated similar observations of synthetic lethality. Replicative stress stimulated by hyperactive MYC is associated with the activation of the DNA-damage response mediated by Atr and Chk1. In MYC-driven tumors with high levels of replicative stress, the reduction of Atr levels prevented disease development. Additionally, Chk1 inhibitors caused the apoptosis of MYC-driven lymphomas, but not K-RAS(G12V)-driven pancreatic adenomas [104]. Chk2 deficiency and the inhibition of Chk1 and Chk2 with small molecules also promoted apoptosis and delayed lymphoma progression in vivo, and the combination of Chk2 and Parp inhibition resulted in a synergistic effect in MYC-overexpressing cells [105].

Aurora kinase A and B have been shown to be upregulated by MYC in human and murine B cell lymphomas, and their pharmacological inhibition resulted in mitotic arrest, polyploidy, and apoptosis [106]. In a diverse panel of cancer cell lines, the therapeutic efficacy of PF-03814735, an Aurora kinase A/B inhibitor, was shown to be significantly associated with MYC overexpression or amplification, and this was confirmed in vivo using small cell lung cancer models [107].
The efficacy of Aurora kinase B inhibition has been further validated in a MYC-overexpressing medulloblastoma model using the small molecule inhibitor, AZD1152-HQPA [108].

MYC-overexpressing tumors have also been shown to display increased sensitivity to inhibition of cyclin dependent kinases, including Cdk1 [109, 110] and Cdk2 [111], the activation of the death receptor pathway [112] and Pim1 inhibition [113, 114].

Can we target core MYC-regulated cellular functions and avoid toxic effects?

As described previously, two of the core pathways regulated by MYC are protein translation and mRNA metabolism.

A key regulator of translation is the mTOR pathway [115]. As such, pharmacologically targeting the mTOR pathway with inhibitors (e.g. rapamycin), several of which are already in clinical trials, may be effective in MYC-driven tumors. For example, in a MYC-driven lymphoma model, the phosphorylation of 4EBP1 by mTOR was required for tumor initiation and maintenance [16], and oncogene-induced senescence was restored following treatment with everolimus, an mTORC1 inhibitor [116]. Additional essential components of the translation machinery that are downstream of MYC include the translation initiation factor eIF4E, a critical substrate in the mTOR pathway [117] and PRMT5, which is necessary for cap-dependent translation by eIF4E [118].

Furthermore, cells with elevated MYC show enhanced sensitivity to ARK5 inhibition [102]. ARK5 controls several aspects of cellular metabolism, regulating AMPK and inhibiting signaling downstream of mTORC1, thereby limiting protein synthesis. Additionally, it sustains mitochondrial respiration and glutamine metabolism. Accordingly, inhibition of ARK5 results in a profound depletion of ATP levels and the activation of apoptosis [102].

As previously reported, MYC is a direct transcriptional modulator of both Pol I and Pol III. The aberrantly high levels of ribosome biogenesis observed in MYC-driven cancers may contribute to oncogenesis by increasing the capacity of a cell for protein synthesis, which is necessary to support proliferative growth. Indeed, targeting ribosome biogenesis in the context of MYC overexpression has proven to be a successful strategy, both by genetically reducing the dosage of the ribosomal proteins eIF6 [119], L24 or L38 [120], or by directly targeting RNA polymerase I by small molecule inhibitors [121, 122]. The effects of Pol I inhibition was also potentiated by inhibiting ATR [122], which was previously shown to be important for Myc-driven cancers that exhibit high levels of replicative stress [104]. This combinatorial approach suggests the potential of targeting multiple MYC synthetic lethal pathways to achieve greater synergy.

The overexpression of MYC, but not other genes known to be involved in transformation, such as a dominant negative version of p53, rendered neural stem cells more sensitive to U2 snRNP splicing inhibition with the SF3b inhibitors, Sudemyacin CI and Pladeinolide B [31]. Srsf1 was also shown to cooperate with MYC, but not other oncoproteins, such as Erbb2 or Hpv16E7, in mammary cell transformation [23]. While small molecule inhibitors targeting alternative splicing factors are not available at present, in the future, antisense oligonucleotide technologies might open new therapeutic opportunities to target, for example, Srsf1 or hnRNPA1, as critical downstream effectors of MYC [123, 124].

On the other hand, there are several inhibitors of the general splicing machinery [31, 125], which may represent ideal candidates to be tested in the context of MYC overexpression. Furthermore, the inhibition of Bud31, a core spliceosomal component, has also recently been demonstrated to be synthetic lethal with MYC overexpression [30].

A unique molecule that has raised the interest of pharma companies lately is Prmt5, an enzyme at the crossroad between pre mRNA-processing [29] and translation regulation [118]. Whether Prmt5 inhibitors will prove to be more effective in MYC-overexpressing tumors over other malignancies remains to be proven.

Conclusions and prospects

In conclusion, we argue that MYC is an ideal drug target in oncology. It fits all the requirements of such a molecule, because it is one of the few proteins that is always, in one way or another, upregulated in cancer. Furthermore, it constitutes a signaling hub, which, we would predict, is absolutely essential and non-redundant, for tumor initiation and maintenance, thus making it difficult to be bypassed. Evidence from genetic models of Myc inactivation [91] has convincingly proven that it is feasible to inactivate such a central molecule with few deleterious effects on normal tissues. The current knowledge of the regulation of MYC abundance, as well as of MYC-regulated downstream pathways, coupled to novel pharmacological opportunities, provide multiple angles to inhibit this key transcription factor. In addition and quite surprisingly, two core housekeeping functions, specifically, ribosome and spliceosome biogenesis, may provide the long sought after Achilles’ heel to target: what was not so long ago thought to be the undruggable MYC, could be the target for generation of the next series of blockbuster drugs.

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