Targeting MYC in cancer therapy: RNA processing offers new opportunities

Cheryl M. Koh[1)*], *Arianna Sabò*[2)*] *and Ernesto Guccione*[1)3)4)*]

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

DOI 10.1002/bies.201500134

[1)] Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), Singapore
[2)] Center for Genomic Science of IIT@SEMM, Fondazione Istituto Italiano di Tecnologia (IIT), Milan, Italy
[3)] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[4)] National Cancer Centre Singapore, Singapore

***Corresponding authors:**
Cheryl M. Koh
E-mail: mykoh@imcb.a-star.edu.sg
Arianna Sabò
E-mail: arianna.sabo@iit.it
Ernesto Guccione
E-mail: eguccione@imcb.a-star.edu.sg

Abbreviations:
eIF, eukaryotic initiation factor; **IRES,** internal ribosome entry site; **NMD,** nonsense mediated RNA decay; **SAGA,** Spt-Ada-Gcn5-acetyltransferase.

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.

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.

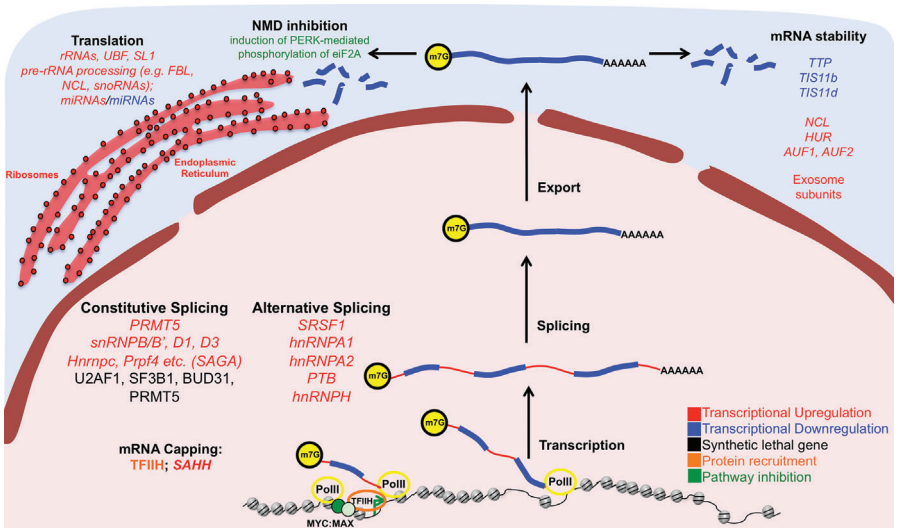

Figure 1. MYC: regulator of post-transcriptional mechanisms. MYC directly promotes cap addition at its target genes by recruiting TFIIH and by indirectly upregulating S-Adenyl-L-homocysteine hydrolase (SAHH). MYC upregulates several components of the alternative and constitutive splicing machinery. MYC directly upregulates the expression of SAGA components, which are then recruited to several MYC target genes involved in splicing (e.g. Hnrnpc and Prpf4). U2AF1, SF3B1, BUD31 (core components of the U2 snRNP), and PRMT5, an arginine methyltransferase that is essential for snRNPs maturation, are MYC-synthetic lethal genes. MYC indirectly regulates RNA degradation by modulating the expression of AU-binding proteins (AUBPs) and components of the exosome machinery. On the other hand, MYC inhibits the Nonsense Mediated Decay (NMD) pathway. MYC upregulates the transcription of ribosomal RNAs by RNA polymerase I and III, enhances pre-rRNA processing and rRNA post transcriptional processing (e.g. snRNAs upregulation). MYC also regulates the expression and the maturation of several miRNAs, broadly affecting translation efficiency of multiple targets.

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, which then phosphorylates the RNA Pol II carboxy-terminal domain (CTD), a step that is required for the recruitment of the capping machinery [12]. It can also act indirectly on the same process, as demonstrated by the ability of the MYC transactivation domain alone (without DNA binding capacity) to increase capping of CDK transcripts [13]. Moreover, MYC transcriptionally up-regulates S-Adenyl-L-homocysteine 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 the 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 hnRNPA1 [17], hnRNPA2, and PTB (polypyrimidine tract binding protein) [18]. These proteins regulate, among several other events, the alternative splicing of pyruvate kinase (PKM), 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 suppress 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 splicing [26], as well as MYC and EMT [27, 28].

We recently found that MYC directly binds to the promoters and upregulates the expression of the core snRNP assembly genes [29]. In particular, we showed that depletion of PRMT5 results in apoptosis and cell cycle arrest, and that it was accompanied by the aberrant splicing of pre mRNAs with weak 5′ splice sites. The phenotypic and molecular changes following PRMT5 depletion were significantly more pronounced in B cells from Eμ-*myc* mice than those from wild-type mice, suggesting that MYC overexpressing cells have an increased dependence on a functional core splicing machinery. We hypothesize that given that MYC-overexpressing cells are actively transcribing mRNAs, this places an increased demand on the splicing machinery to maintain the splicing fidelity necessary to generate properly spliced transcripts, which can subsequently be translated into functional proteins. Along similar lines, in MYC-hyperactive cells, the 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 Tristetraprolin (TTP/Tis11/Zfp36) and its family members, Tis11b (Brf1/ Zfp36l1) or Tis11d (Brf2/Zfp36l2), 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 antiapoptotic, 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

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, Mknk2, Bim*	[22–24]
SAGA components, e.g. Gcn5, Cdc101, Taf12, and Atxn7l3	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. *Dnajb5*, *Sl1*, *Mcm7*, *Skp2*, etc.	[30]

themselves can undergo alternative splicing events that introduce PTCs, which subsequently signal for NMD [37, 38]. NMD also post-transcriptionally regulates several normal transcripts, in particular, genes encoding proteins involved in the unfolded protein response (UPR) and stress-related genes [39, 40]. In contrast, endoplasmic reticulum (ER)-stress, along with other cellular stresses (such as reactive oxygen species, hypoxia, nutrient deprivation, etc.), inhibits the NMD response [41] in a complex regulatory circuit. MYC overexpression has been shown to inhibit NMD in B lymphocytes via the activation of the UPR, and in particular, the induction of PERK-mediated phosphorylation of eIF2A [42]. MYC is also required for the inhibition of NMD by 5-azacytidine, in an eIF2A-independent manner [43]. One possibility would be that this occurs via miRNAs, since they are widely regulated by MYC [44] and have been shown to act on NMD [45].

(3) A different RNA quality surveillance pathway is represented by the exosome, a complex containing nuclease activity that is involved in the degradation of aberrant RNA, the maturation of ribosomal RNA and sn/snoRNAs and the turnover of the products of RNA maturation [46]. This complex is also recruited by AUBPs to degrade ARE containing RNAs [47]. The expression of several subunits of this complex is positively regulated in a MYC-dependent manner in fibroblasts [48] and B cells [9]. Whether the catalytic activity of the complex is also increased by MYC is still an open question.

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 is part 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 TFIIIB, 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 rRNA 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-130a [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).

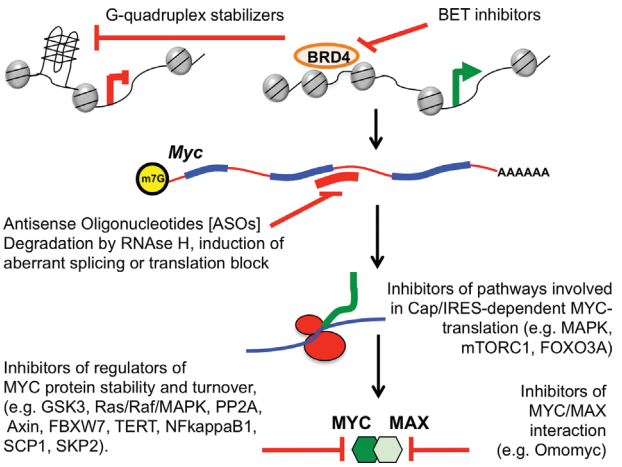

Figure 2. Strategies to directly target MYC. Multiple strategies can be used to directly reduce MYC levels in cells. Reducing MYC transcription can be achieved using G-quadruplex stabilizers and BET inhibitors. Furthermore, antisense oligonucleotides can induce RNAse H-based degradation of MYC mRNA or the aberrant splicing of the MYC pre-mRNA. Translation of MYC mRNA to protein can be blocked by antisense oligonucleotides or by inhibiting pathways involved in its Cap/IRES-dependent translation, such as the MAPK, mTORC1, and FOXO3a pathways. Regulators of MYC protein stability and turnover can also be inhibited to promote MYC protein degradation. Additionally, because MYC function is tightly linked to its dimerization with its binding partner, MAX, targeting this interaction can alter the transcriptional output downstream of MYC.

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 the major underlying cause of its overexpression. An additional consideration is that BET proteins modulate the expression of numerous genes in addition to MYC [73] and accordingly, bromodomain inhibition is by no means MYC-specific.

Altering the topology of the DNA upstream of the MYC gene, such as by the stabilization of the MYC G-quadruplex with small molecules [74, 75], has also been shown to be effective in reducing MYC transcription.

Can we target MYC translation and protein turnover/stability?

Once transcribed, the MYC mRNA can be bound by RNA binding proteins that regulate its stability [76] or 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 hnRPA1 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].

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

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 Co^{III} 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, Sudemycin C1 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.

Acknowledgments

We are grateful to S. Campaner for critical reading of the manuscript. CMK and EG acknowledge funding from JCO grants #1234c00017, #11/03/FG/07/04, and #13302RG061, and BMRC Young Investigators Grant #13/1/01/YA/002. AS acknowledges funding from AIRC-Cariplo TRIDEO #15610 and WWCR #15-1260.

The authors have declared no conflict of interest.

References

1. **Gabay M, Li Y, Felsher DW**. 2014. MYC activation is a hallmark of cancer initiation and maintenance. *Cold Spring Harb Perspect Med* **4**: pii: a014241.

2. **Blackwood EM, Eisenman RN**. 1991. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* **251**: 1211–7.
3. **Amati B, Brooks MW, Levy N, Littlewood TD**, et al. 1993. Oncogenic activity of the c-Myc protein requires dimerization with Max. *Cell* **72**: 233–45.
4. **Guccione E, Martinato F, Finocchiaro G, Luzi L**, et al. 2006. Myc-binding-site recognition in the human genome is determined by chromatin context. *Nat Cell Biol* **8**: 764–70.
5. **Kress TR, Sabo A, Amati B**. 2015. MYC: connecting selective transcriptional control to global RNA production. *Nat Rev Cancer* **15**: 593–607.
6. **Gomez-Roman N, Felton-Edkins ZA, Kenneth NS, Goodfellow SJ**, et al. 2006. Activation by c-Myc of transcription by RNA polymerases I, II and III. *Biochem Soc Symp* **2006**: 141–54.
7. **Lin CY, Loven J, Rahl PB, Paranal RM**, et al. 2012. Transcriptional amplification in tumor cells with elevated c-Myc. *Cell* **151**: 56–67.
8. **Nie Z, Hu G, Wei G, Cui K**, et al. 2012. C-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. *Cell* **151**: 68–79.
9. **Sabo A, Kress TR, Pelizzola M, de Pretis S**, et al. 2014. Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. *Nature* **511**: 488–92.
10. **Gonatopoulos-Pournatzis T, Cowling VH**. 2014. Cap-binding complex (CBC). *Biochem J* **457**: 231–42.
11. **Montanaro L, Pandolfi PP**. 2004. Initiation of mRNA translation in oncogenesis: the role of eIF4E. *Cell Cycle* **3**: 1387–9.
12. **Cole MD, Cowling VH**. 2009. Specific regulation of mRNA cap methylation by the c-Myc and E2F1 transcription factors. *Oncogene* **28**: 1169–75.
13. **Cowling VH, Cole MD**. 2007. The Myc transactivation domain promotes global phosphorylation of the RNA polymerase II carboxy-terminal domain independently of direct DNA binding. *Mol Cell Biol* **27**: 2059–73.
14. **Fernandez-Sanchez ME, Gonatopoulos-Pournatzis T, Preston G, Lawlor MA**, et al. 2009. S-adenosyl homocysteine hydrolase is required for Myc-induced mRNA cap methylation, protein synthesis, and cell proliferation. *Mol Cell Biol* **29**: 6182–91.
15. **Braunstein S, Karpisheva K, Pola C, Goldberg J**, et al. 2007. A hypoxia-controlled cap-dependent to cap-independent translation switch in breast cancer. *Mol Cell* **28**: 501–12.
16. **Pourdehnad M, Truitt ML, Siddiqi IN, Ducker GS**, et al. 2013. Myc and mTOR converge on a common node in protein synthesis control that confers synthetic lethality in Myc-driven cancers. *Proc Natl Acad Sci USA* **110**: 11988–93.
17. **Biamonti G, Bassi MT, Cartegni L, Mechta F**, et al. 1993. Human hnRNP protein A1 gene expression. Structural and functional characterization of the promoter. *J Mol Biol* **230**: 77–89.
18. **Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A**, et al. 2008. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* **452**: 230–3.
19. **David CJ, Chen M, Assanah M, Canoll P**, et al. 2010. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature* **463**: 364–8.
20. **Nadiminty N, Tummala R, Liu C, Lou W**, et al. 2015. NF-kappaB2/p52: c-Myc:hnRNPA1 pathway regulates expression of androgen receptor splice variants and enzalutamide sensitivity in prostate cancer. *Mol Cancer Ther* **14**: 1884–95.
21. **Rauch J, Moran-Jones K, Albrecht V, Schwarzl T**, et al. 2011. C-Myc regulates RNA splicing of the A-Raf kinase and its activation of the ERK pathway. *Cancer Res* **71**: 4664–74.
22. **Das S, Anczukow O, Akerman M, Krainer AR**. 2012. Oncogenic splicing factor SRSF1 is a critical transcriptional target of MYC. *Cell Rep* **1**: 110–7.
23. **Anczukow O, Rosenberg AZ, Akerman M, Das S**, et al. 2012. The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nat Struct Mol Biol* **19**: 220–8.
24. **Das S, Krainer AR**. 2014. Emerging functions of SRSF1, splicing factor and oncoprotein, in RNA metabolism and cancer. *Mol Cancer Res* **12**: 1195–204.
25. **Hirsch CL, Coban Akdemir Z, Wang L, Jayakumaran G**, et al. 2015. Myc and SAGA rewire an alternative splicing network during early somatic cell reprogramming. *Genes Dev* **29**: 803–16.
26. **Wu CY, Tsai YP, Wu MZ, Teng SC**, et al. 2012. Epigenetic reprogramming and post-transcriptional regulation during the epithelial-mesenchymal transition. *Trends Genet* **28**: 454–63.
27. **Cowling VH, Cole MD**. 2007. E-cadherin repression contributes to c-Myc-induced epithelial cell transformation. *Oncogene* **26**: 3582–6.
28. **Cho MH, Park JH, Choi HJ, Park MK**, et al. 2015. DOT1L cooperates with the c-Myc-p300 complex to epigenetically derepress CDH1 transcription factors in breast cancer progression. *Nat Commun* **6**: 7821.
29. **Koh CM, Bezzi M, Low DH, Ang WX**, et al. 2015. MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. *Nature* **523**: 96–100.
30. **Hsu TY, Simon LM, Neill NJ, Marcotte R**, et al. 2015. The spliceosome is a therapeutic vulnerability in MYC-driven cancer. *Nature* **525**: 384–8.
31. **Hubert CG, Bradley RK, Ding Y, Toledo CM**, et al. 2013. Genome-wide RNAi screens in human brain tumor isolates reveal a novel viability requirement for PHF5A. *Genes Dev* **27**: 1032–45.
32. **Gruber AR, Fallmann J, Kratochvill F, Kovarik P**, et al. 2011. AREsite: a database for the comprehensive investigation of AU-rich elements. *Nucleic Acids Res* **39**: D66–9.
33. **Rounbehler RJ, Fallahi M, Yang C, Steeves MA**, et al. 2012. Tristetraprolin impairs myc-induced lymphoma and abolishes the malignant state. *Cell* **150**: 563–74.
34. **Mukherjee N, Jacobs NC, Hafner M, Kennington EA**, et al. 2014. Global target mRNA specification and regulation by the RNA-binding protein Z FP36. *Genome Biol* **15**: R12.
35. **Zhang J, Tsaprailis G, Bowden GT**. 2008. Nucleolin stabilizes Bcl-X L messenger RNA in response to UVA irradiation. *Cancer Res* **68**: 1046–54.
36. **Kelly PN, Grabow S, Delbridge AR, Strasser A**, et al. 2011. Endogenous Bcl-xL is essential for Myc-driven lymphomagenesis in mice. *Blood* **118**: 6380–6.
37. **Lareau LF, Inada M, Green RE, Wengrod JC**, et al. 2007. Unproductive splicing of SR genes associated with highly conserved and ultra-conserved DNA elements. *Nature* **446**: 926–9.
38. **Ni JZ, Grate L, Donohue JP, Preston C**, et al. 2007. Ultraconserved elements are associated with homeostatic control of splicing regulators by alternative splicing and nonsense-mediated decay. *Genes Dev* **21**: 708–18.
39. **Karam R, Lou CH, Kroeger H, Huang L**, et al. 2015. The unfolded protein response is shaped by the NMD pathway. *EMBO Rep* **16**: 599–609.
40. **Gardner LB**. 2008. Hypoxic inhibition of nonsense-mediated RNA decay regulates gene expression and the integrated stress response. *Mol Cell Biol* **28**: 3729–41.
41. **Popp MW, Maquat LE**. 2013. Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu Rev Genet* **47**: 139–65.
42. **Wang D, Wengrod J, Gardner LB**. 2011. Overexpression of the c-myc oncogene inhibits nonsense-mediated RNA decay in B lymphocytes. *J Biol Chem* **286**: 40038–43.
43. **Shao A, Wilkinson MF**. 2014. Suppressing nonsense-a surprising function for 5-azacytidine. *EMBO Mol Med* **6**: 1518–20.
44. **Chang TC, Yu D, Lee YS, Wentzel EA**, et al. 2008. Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat Genet* **40**: 43–50.
45. **Bruno IG, Karam R, Huang L, Bhardwaj A**, et al. 2011. Identification of a microRNA that activates gene expression by repressing nonsense-mediated RNA decay. *Mol Cell* **42**: 500–10.
46. **Januszyk K, Lima CD**. 2014. The eukaryotic RNA exosome. *Curr Opin Struct Biol* **24**: 132–40.
47. **Chen CY, Gherzi R, Ong SE, Chan EL**, et al. 2001. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell* **107**: 451–64.
48. **Perna D, Faga G, Verrecchia A, Gorski MM**, et al. 2012. Genome-wide mapping of Myc binding and gene regulation in serum-stimulated fibroblasts. *Oncogene* **31**: 1695–709.
49. **Iritani BM, Eisenman RN**. 1999. C-Myc enhances protein synthesis and cell size during B lymphocyte development. *Proc Natl Acad Sci USA* **96**: 13180–5.
50. **Arabi A, Wu S, Ridderstrale K, Bierhoff H**, et al. 2005. C-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. *Nat Cell Biol* **7**: 303–10.
51. **Grandori C, Gomez-Roman N, Felton-Edkins ZA, Ngouenet C**, et al. 2005. C-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nat Cell Biol* **7**: 311–8.
52. **Grewal SS, Li L, Orian A, Eisenman RN**, et al. 2005. Myc-dependent regulation of ribosomal RNA synthesis during Drosophila development. *Nat Cell Biol* **7**: 295–302.
53. **Poortinga G, Hannan KM, Snelling H, Walkley CR**, et al. 2004. MAD1 and c-MYC regulate UBF and rDNA transcription during granulocyte differentiation. *EMBO J* **23**: 3325–35.
54. **Gomez-Roman N, Grandori C, Eisenman RN, White RJ**. 2003. Direct activation of RNA polymerase III transcription by c-Myc. *Nature* **421**: 290–4.

55. **Kenneth NS, Ramsbottom BA, Gomez-Roman N, Marshall L**, et al. 2007. TRRAP and GCN5 are used by c-Myc to activate RNA polymerase III transcription. *Proc Natl Acad Sci USA* **104**: 14917–22.
56. **Filipowicz W, Pogacic V**. 2002. Biogenesis of small nucleolar ribonucleoproteins. *Curr Opin Cell Biol* **14**: 319–27.
57. **Herter EK, Stauch M, Gallant M, Wolf E**, et al. 2015. SnoRNAs are a novel class of biologically relevant Myc targets. *BMC Biol* **13**: 25.
58. **Ji H, Wu G, Zhan X, Nolan A**, et al. 2011. Cell-type independent MYC target genes reveal a primordial signature involved in biomass accumulation. *PLoS ONE* **6**: e26057.
59. **Rosenwald IB, Rhoads DB, Callanan LD, Isselbacher KJ**, et al. 1993. Increased expression of eukaryotic translation initiation factors eIF-4E and eIF-2 alpha in response to growth induction by c-myc. *Proc Natl Acad Sci USA* **90**: 6175–8.
60. **Jones RM, Branda J, Johnston KA, Polymenis M**, et al. 1996. An essential E box in the promoter of the gene encoding the mRNA cap-binding protein (eukaryotic initiation factor 4E) is a target for activation by c-myc. *Mol Cell Biol* **16**: 4754–64.
61. **Psathas JN, Thomas-Tikhonenko A**. 2014. MYC and the art of microRNA maintenance. *Cold Spring Harb Perspect Med* **4**: pii: a014175.
62. **Deng K, Guo X, Wang H, Xia J**. 2014. The lncRNA-MYC regulatory network in cancer. *Tumour Biol* **35**: 9497–503.
63. **Frenzel A, Loven J, Henriksson MA**. 2010. Targeting MYC-Regulated miRNAs to Combat Cancer. *Genes Cancer* **1**: 660–7.
64. **Ma MZ, Li CX, Zhang Y, Weng MZ**, et al. 2014. Long non-coding RNA HOTAIR, a c-Myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer. *Mol Cancer* **13**: 156.
65. **Wang T, Wang G, Hao D, Liu X**, et al. 2015. Aberrant regulation of the LIN28A/LIN28B and let-7 loop in human malignant tumors and its effects on the hallmarks of cancer. *Mol Cancer* **14**: 125.
66. **Wang X, Zhao X, Gao P, Wu M**. 2013. C-Myc modulates microRNA processing via the transcriptional regulation of Drosha. *Sci Rep* **3**.
67. **Levens D**. 2008. How the c-myc promoter works and why it sometimes does not. *J Natl Cancer Inst Monogr* **2008**: 41–3.
68. **Hnisz D, Abraham BJ, Lee TI, Lau A**, et al. 2013. Super-enhancers in the control of cell identity and disease. *Cell* **155**: 934–47.
69. **Zuber J, Shi J, Wang E, Rappaport AR**, et al. 2011. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* **478**: 524–8.
70. **Mertz JA, Conery AR, Bryant BM, Sandy P**, et al. 2011. Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc Natl Acad Sci USA* **108**: 16669–74.
71. **Delmore JE, Issa GC, Lemieux ME, Rahl PB**, et al. 2011. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* **146**: 904–17.
72. **Fowler T, Ghatak P, Price DH, Conaway R**, et al. 2014. Regulation of MYC expression and differential JQ1 sensitivity in cancer cells. *PLoS ONE* **9**: e87003.
73. **Filippakopoulos P, Knapp S**. 2014. Targeting bromodomains: epigenetic readers of lysine acetylation. *Nat Rev Drug Discov* **13**: 337–56.
74. **Brown RV, Danford FL, Gokhale V, Hurley LH**, et al. 2011. Demonstration that drug-targeted down-regulation of MYC in non-Hodgkins lymphoma is directly mediated through the promoter G-quadruplex. *J Biol Chem* **286**: 41018–27.
75. **Ou TM, Lu YJ, Zhang C, Huang ZS**, et al. 2007. Stabilization of G-quadruplex DNA and down-regulation of oncogene c-myc by quindoline derivatives. *J Med Chem* **50**: 1465–74.
76. **Weidensdorfer D, Stohr N, Baude A, Lederer M**, et al. 2009. Control of c-myc mRNA stability by IGF2BP1-associated cytoplasmic RNPs. *RNA* **15**: 104–15.
77. **Liu L, Ouyang M, Rao JN, Zou T**, et al. 2015. Competition between RNA-binding proteins CELF1 and HuR modulates MYC translation and intestinal epithelium renewal. *Mol Biol Cell* **26**: 1797–810.
78. **Shi Y, Yang Y, Hoang B, Bardeleben C**, et al. 2015. Therapeutic potential of targeting IRES-dependent c-myc translation in multiple myeloma cells during ER stress. *Oncogene*, in press. DOI: 10.1038/onc.2015.156.
79. **Nilsson JA, Keller UB, Baudino TA, Yang C**, et al. 2005. Targeting ornithine decarboxylase in Myc-induced lymphomagenesis prevents tumor formation. *Cancer Cell* **7**: 433–44.
80. **Forshell TP, Rimpi S, Nilsson JA**. 2010. Chemoprevention of B-cell lymphomas by inhibition of the Myc target spermidine synthase. *Cancer Prev Res* **3**: 140–7.
81. **Nanbru C, Lafon I, Audigier S, Gensac MC**, et al. 1997. Alternative translation of the proto-oncogene c-myc by an internal ribosome entry site. *J Biol Chem* **272**: 32061–6.
82. **Wiegering A, Uthe FW, Jamieson T, Ruoss Y**, et al. 2015. Targeting translation initiation bypasses signaling crosstalk mechanisms that maintain high MYC levels in colorectal cancer. *Cancer Discov* **5**: 768–81.
83. **Farrell AS, Sears RC**. 2014. MYC degradation. *Cold Spring Harb Perspect Med* **4**: pii: a014365.
84. **Balaji KC, Koul H, Mitra S, Maramag C**, et al. 1997. Antiproliferative effects of c-myc antisense oligonucleotide in prostate cancer cells: a novel therapy in prostate cancer. *Urology* **50**: 1007–15.
85. **Iversen PL, Arora V, Acker AJ, Mason DH**, et al. 2003. Efficacy of antisense morpholino oligomer targeted to c-myc in prostate cancer xenograft murine model and a Phase I safety study in humans. *Clin Cancer Res* **9**: 2510–9.
86. **Sekhon HS, London CA, Sekhon M, Iversen PL**, et al. 2008. C-MYC antisense phosphosphorodiamidate morpholino oligomer inhibits lung metastasis in a murine tumor model. *Lung Cancer* **60**: 347–54.
87. **Giles RV, Spiller DG, Clark RE, Tidd DM**. 1999. Antisense morpholino oligonucleotide analog induces missplicing of C-myc mRNA. *Antisense Nucleic Acid Drug Dev* **9**: 213–20.
88. **Soucek L, Helmer-Citterich M, Sacco A, Jucker R**, et al. 1998. Design and properties of a Myc derivative that efficiently homodimerizes. *Oncogene* **17**: 2463–72.
89. **Savino M, Annibali D, Carucci N, Favuzzi E**, et al. 2011. The action mechanism of the Myc inhibitor termed Omomyc may give clues on how to target Myc for cancer therapy. *PLoS ONE* **6**: e22284.
90. **Soucek L, Jucker R, Panacchia L, Ricordy R**, et al. 2002. Omomyc, a potential Myc dominant negative, enhances Myc-induced apoptosis. *Cancer Res* **62**: 3507–10.
91. **Soucek L, Whitfield J, Martins CP, Finch AJ**, et al. 2008. Modelling Myc inhibition as a cancer therapy. *Nature* **455**: 679–83.
92. **Berg T**. 2011. Small-molecule modulators of c-Myc/Max and Max/Max interactions. *Curr Topics Microbiol Immunol* **348**: 139–49.
93. **Jung KY, Wang H, Teriete P, Yap JL**, et al. 2015. Perturbation of the c-Myc-Max protein-protein interaction via synthetic alpha-helix mimetics. *J Med Chem* **58**: 3002–24.
94. **Siddiquee K, Zhang S, Guida WC, Blaskovich MA**, et al. 2007. Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proc Natl Acad Sci USA* **104**: 7391–6.
95. **Heffern MC, Kurutz JW, Meade TJ**. 2013. Spectroscopic elucidation of the inhibitory mechanism of Cys2His2 zinc finger transcription factors by cobalt(III) Schiff base complexes. *Chemistry* **19**: 17043–53.
96. **McLornan DP, List A, Mufti GJ**. 2014. Applying synthetic lethality for the selective targeting of cancer. *New Engl J Med* **371**: 1725–35.
97. **Cermelli S, Jang IS, Bernard B, Grandori C**. 2014. Synthetic lethal screens as a means to understand and treat MYC-driven cancers. *Cold Spring Harb Perspect Med* **4**: pii: a014209.
98. **Toyoshima M, Howie HL, Imakura M, Walsh RM**, et al. 2012. Functional genomics identifies therapeutic targets for MYC-driven cancer. *Proc Natl Acad Sci USA* **109**: 9545–50.
99. **Huang CH, Lujambio A, Zuber J, Tschaharganeh DF**, et al. 2014. CDK9-mediated transcription elongation is required for MYC addiction in hepatocellular carcinoma. *Genes Dev* **28**: 1800–14.
100. **Kessler JD, Kahle KT, Sun T, Meerbrey KL**, et al. 2012. A SUMOylation-dependent transcriptional subprogram is required for Myc-driven tumorigenesis. *Science* **335**: 348–53.
101. **Zhou Z, Patel M, Ng N, Hsieh MH**, et al. 2014. Identification of synthetic lethality of PRKDC in MYC-dependent human cancers by pooled shRNA screening. *BMC Cancer* **14**: 944.
102. **Liu L, Ulbrich J, Muller J, Wustefeld T**, et al. 2012. Deregulated MYC expression induces dependence upon AMPK-related kinase 5. *Nature* **483**: 608–12.
103. **Rottmann S, Wang Y, Nasoff M, Deveraux QL**, et al. 2005. A TRAIL receptor-dependent synthetic lethal relationship between MYC activation and GSK3beta/FBW7 loss of function. *Proc Natl Acad Sci USA* **102**: 15195–200.
104. **Murga M, Campaner S, Lopez-Contreras AJ, Toledo LI**, et al. 2011. Exploiting oncogene-induced replicative stress for the selective killing of Myc-driven tumors. *Nat Struct Mol Biol* **18**: 1331–5.
105. **Hoglund A, Nilsson LM, Forshell LP, Maclean KH**, et al. 2009. Myc sensitizes p53-deficient cancer cells to the DNA-damaging effects of the DNA methyltransferase inhibitor decitabine. *Blood* **113**: 4281–8.
106. **den Hollander J, Rimpi S, Doherty JR, Rudelius M**, et al. 2010. Aurora kinases A and B are up-regulated by Myc and are essential for maintenance of the malignant state. *Blood* **116**: 1498–505.
107. **Hook KE, Garza SJ, Lira ME, Ching KA**, et al. 2012. An integrated genomic approach to identify predictive biomarkers of response to the aurora kinase inhibitor PF-03814735. *Mol Cancer Therapeut* **11**: 710–9.

108. **Diaz RJ, Golbourn B, Faria C, Picard D**, et al. 2015. Mechanism of action and therapeutic efficacy of Aurora kinase B inhibition in MYC overexpressing medulloblastoma. *Oncotarget* **6**: 3359–74.
109. **Horiuchi D, Kusdra L, Huskey NE, Chandriani S**, et al. 2012. MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. *J Exp Med* **209**: 679–96.
110. **Goga A, Yang D, Tward AD, Morgan DO**, et al. 2007. Inhibition of CDK1 as a potential therapy for tumors over-expressing MYC. *Nat Med* **13**: 820–7.
111. **Campaner S, Doni M, Hydbring P, Verrecchia A**, et al. 2010. Cdk2 suppresses cellular senescence induced by the c-myc oncogene. *Nat Cell Biol* **12**: 1–4.
112. **Wang Y, Engels IH, Knee DA, Nasoff M**, et al. 2004. Synthetic lethal targeting of MYC by activation of the DR5 death receptor pathway. *Cancer Cell* **5**: 501–12.
113. **Wang J, Anderson PD, Luo W, Gius D**, et al. 2012. Pim1 kinase is required to maintain tumorigenicity in MYC-expressing prostate cancer cells. *Oncogene* **31**: 1794–803.
114. **Kirschner AN, Wang J, van der Meer R, Anderson PD**, et al. 2015. PIM kinase inhibitor AZD1208 for treatment of MYC-driven prostate cancer. *J Natl Cancer Instit* **107**: pii: dju407.
115. **Guertin DA, Sabatini DM**. 2007. Defining the role of mTOR in cancer. *Cancer Cell* **12**: 9–22.
116. **Wall M, Poortinga G, Stanley KL, Lindemann RK**, et al. 2013. The mTORC1 inhibitor everolimus prevents and treats Emu-Myc lymphoma by restoring oncogene-induced senescence. *Cancer Discov* **3**: 82–95.
117. **Lin CJ, Nasr Z, Premsrirut PK, Porco JA, Jr.**, et al. 2012. Targeting synthetic lethal interactions between Myc and the eIF4F complex impedes tumorigenesis. *Cell Rep* **1**: 325–33.
118. **Lim JH, Lee YM, Lee G, Choi YJ**, et al. 2014. PRMT5 is essential for the eIF4E-mediated 5'-cap dependent translation. *Biochem Biophys Res Commun* **452**: 1016–21.
119. **Miluzio A, Beugnet A, Grosso S, Brina D**, et al. 2011. Impairment of cytoplasmic eIF6 activity restricts lymphomagenesis and tumor progression without affecting normal growth. *Cancer Cell* **19**: 765–75.
120. **Barna M, Pusic A, Zollo O, Costa M**, et al. 2008. Suppression of Myc oncogenic activity by ribosomal protein haploinsufficiency. *Nature* **456**: 971–5.
121. **Bywater MJ, Poortinga G, Sanij E, Hein N**, et al. 2012. Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. *Cancer Cell* **22**: 51–65.
122. **Negi SS, Brown P**. 2015. RRNA synthesis inhibitor, CX-5461, activates ATM/ATR pathway in acute lymphoblastic leukemia, arrests cells in G2 phase and induces apoptosis. *Oncotarget* **6**: 18094–104.
123. **Hua Y, Vickers TA, Okunola HL, Bennett CF**, et al. 2008. Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am J Hum Genet* **82**: 834–48.
124. **Comiskey DF, Jr., Jacob AG, Singh RK, Tapia-Santos AS**, et al. 2015. Splicing factor SRSF1 negatively regulates alternative splicing of MDM2 under damage. *Nucleic Acids Res* **43**: 4202–18.
125. **Bonnal S, Vigevani L, Valcarcel J**. 2012. The spliceosome as a target of novel antitumour drugs. *Nat Rev Drug Discov* **11**: 847–59.