# IN THE SPOTLIGHT

# Targeting *MYC* Translation in Colorectal Cancer

Alina Castell and Lars-Gunnar Larsson

**Summary:** There is a great interest in finding ways to inhibit the expression or activity of the "undruggable" MYC, a master regulator of transcription and one of the most deadly oncoproteins in human cancer. In this issue of *Cancer Discovery*, Wiegering and colleagues find a way of inhibiting translation of *MYC* in colorectal cancer cells by directly targeting the translation initiation factor eIF4A, resulting in inhibition of MYC-dependent proliferation of colorectal tumor cells *in vitro* and *in vivo. Cancer Discov; 5(7); 701–3. ©2015 AACR.*

*See related article by Wiegering et al., p. 768 (7).*

The transcription factor MYC is among the most attractive but also most elusive targets for cancer therapy, and for many years has been believed to be "undruggable." MYC activates or represses transcription of a large group of genes involved in cellular processes such as cell growth, metabolism, apoptosis, and protein synthesis (1). Deregulation of *MYC* occurs in more than half of all human tumors and often correlates with aggressive disease, resistance to therapy, and poor prognosis, emphasizing the urgent need to find ways of targeting this pathway. Evidence from mouse models has shown that elimination of MYC function using genetic tools often causes complete and irreversible tumor regression, suggesting that MYC is a promising, although challenging, target for cancer therapy (2).

Several strategies are being pursued to target MYC directly or indirectly (Fig. 1). These include targeting MYC–cofactor interactions essential for MYC function (3), targeting MYC activity, for instance by inhibiting kinase-dependent activation of MYC (3, 4), targeting druggable key downstream target gene products of MYC (1, 3), or exploiting synthetic lethal interactions involving MYC (3). Another approach has been to target MYC expression at different levels. For instance, inhibitors of bromodomain–histone interactions were shown to downregulate *MYC* transcription and inhibit tumor growth in hematopoietic and other tumors (3). MYC normally turns over rapidly via the ubiquitin–proteasome pathway. After phosphorylation of MYC by GSK3β, the E3 ubiquitin ligase SCF[FBXW7] binds and targets MYC for degradation. However, in many tumors, MYC is stabilized by loss of FBXW7 or through PI3K/AKT–mediated inhibitory phosphorylation of GSK3. In the latter case, pharmacologic inhibition of PI3K has been shown to increase MYC turnover (1, 3).

Targeting *MYC* mRNA translation is yet another option. Translation initiation is regulated by eukaryotic initiation factors (eIF), including the cap-binding protein eIF4E, the RNA helicase eIF4A, and the scaffolding protein eIF4G (which are all MYC target genes), forming the eIF4F complex that recruits ribosomes to the 5′-cap of mRNA (Fig. 1; ref. 5). For some mRNAs, translation can be initiated by an internal ribosomal entry site (IRES) localized in the 5′ untranslated region (5′-UTR) of the mRNAs. The 5′-UTR of *MYC* mRNA allows for both cap- and IRES-mediated translation, of which the latter is dependent on eIF4A, but not on eIF4E (6). 4EBP1 and PDCD4 are negative regulators of translation that bind and sequester eIF4E and eIF4A, respectively. These inhibitors are inactivated by phosphorylation by the mTORC1 mTOR complex upon growth signaling, resulting in release of eIF4E and eIF4A, after which the eIF4F complex can be formed, followed by translation initiation. Inhibition of the mTOR pathway is therefore a plausible way to inhibit *MYC* translation (5).

In this issue of *Cancer Discovery*, Wiegering and colleagues (7) explore the possibility of targeting the PI3K and mTOR pathways simultaneously using the dual PI3K and mTOR inhibitor BEZ235 as a possible strategy to combat MYC in colorectal cancer cells. Previous work from some of the coauthors of the article has demonstrated that colorectal cancer induced by APC loss in transgenic mice depends on *Myc* (8). Combined targeting of the PI3K and mTOR pathways by BEZ235 would potentially induce MYC protein degradation and inhibit *MYC* mRNA translation simultaneously, thereby "killing two birds with one stone." Contrary to what was expected, BEZ235 treatment affected neither turnover nor translation but instead increased MYC expression despite efficient inhibition of PI3K and mTOR. This was due to loss of a negative feedback loop involving FOXO3A, previously described in breast cancer (7), resulting in increased expression of several growth factor receptors and activation of the MAPK pathway. Consequently, *MYC* mRNA expression was upregulated. Another surprise was that PI3K inhibition did not reduce AKT phosphorylation and activity, possibly due to other stimulatory kinases, and, consequently, did not affect MYC turnover. A further disappointment was that the inhibition of the mTOR pathway by BEZ235 did not affect *MYC* mRNA translation. Possible explanations for this were the downregulated expression of PDCD4 found in colorectal cancer cells, and that 4EBP1, which indeed was dephosphorylated and stabilized by BEZ235, was not expressed at a high enough level in these cells to efficiently sequester eIF4E. However, even

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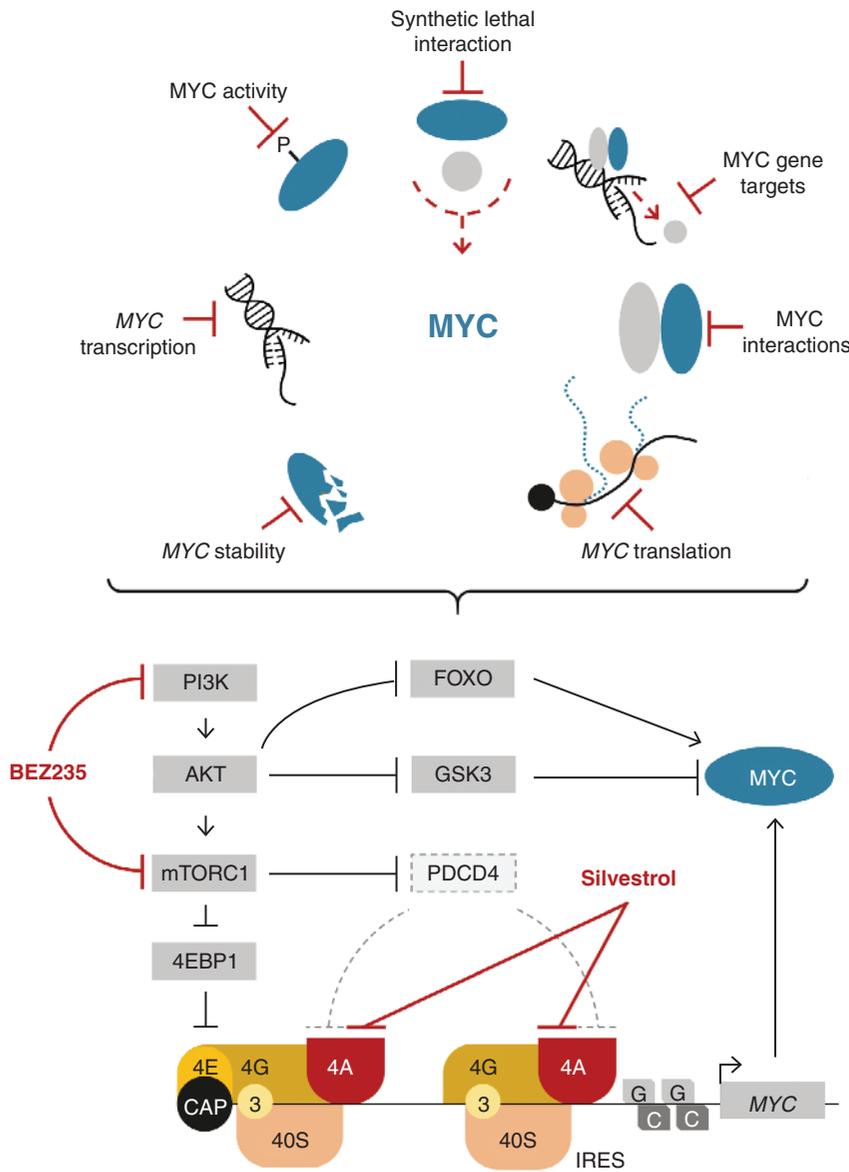
**Figure 1.** A new strategy to combat MYC: inhibition of MYC translation by direct targeting of eIF4A. Top, different ways of targeting MYC. Middle, regulation of MYC protein turnover and translation in colorectal cancer cells via the PI3K–mTOR pathway, including the FOXO-mediated negative feedback loop, is depicted in more detail. Bottom, the cap-binding translation complex consisting of eIF4E (4E), eIF4G (4G), and eIF4A (4A), and the IRES-binding complex containing 4A and 4G, but not 4E, are depicted at the 5′ end of the *MYC* mRNA. These complexes recruit the eIF3 (3) and the 40S ribosome subunit to the mRNA. 4EBP1 and PDCD4 inhibit 4E and 4A, respectively. PDCD4 is downregulated in colorectal cancer. BEZ235 is a dual PI3K and mTOR kinase inhibitor, whereas silvestrol is a direct inhibitor of eIF4A.

overexpression of a 4EBP1 mutant that cannot be inactivated by mTOR only partially blocked translation of *MYC* mRNA. This is likely due to the activity of *MYC*'s IRES, which works independent of eIF4E (6).

The authors therefore tried an alternative way of inhibiting *MYC* mRNA translation by utilizing the compound silvestrol, which targets the eIF4A helicase directly. Silvestrol is suggested to increase the affinity between eIF4A and RNA, thereby sequestering and depleting eIF4A from translation initiation complexes (5, 9). The authors showed that silvestrol indeed reduced MYC protein expression and inhibited colorectal cancer cell growth in culture. Further, silvestrol reduced the amount of *MYC* mRNA associated with polysomes and was shown to partly suppress both cap- and IRES-mediated translation of *MYC* in reporter assays. Similar results were obtained with another inhibitor of IRES-mediated translation of MYC, cymarin. The authors next compared the effect of silvestrol and BEZ235 in a mouse model of APC loss–

induced, *Myc*-dependent intestinal crypt hyperproliferation (8). BEZ235 treatment had no effect on crypt hyperproliferation and only slightly reduced MYC expression, whereas silvestrol strongly repressed both hyperproliferation and MYC protein expression. *MYC* mRNA levels were unchanged, confirming that silvestrol acts posttranscriptionally *in vivo*. Importantly, proliferation of crypt cells in wild-type mice was not affected by silvestrol treatment, suggesting that the compound has a good therapeutic window.

There are at least two important messages from this study. First, the PI3K–mTOR pathway is deregulated in a high number of tumors and is therefore highlighted as a promising therapeutic target (3, 5). The article by Wiegering and colleagues (7) indicates that a great deal of caution should be taken when considering this strategy. Dual inhibition of PI3K and mTOR did not downregulate MYC expression in colorectal cancer, and this treatment may therefore be counterproductive. Loss of the negative feedback loop leading to MYC activation seemed

mainly to stem from the PI3K-inhibitory activity of BEZ235 and, therefore, specific mTOR inhibition is still an option to be explored further in colorectal cancer. Consequently, an important task for the future will be to clarify which patient groups would benefit from targeted PI3K cancer therapy.

Second, direct inhibition of translation initiation by targeting eIF4A seems to be a promising strategy to combat MYC in the future. This view also has support from previous reports using silvestrol or other compounds targeting eIF4A, such as hippuristanol and pateamine A, in different mouse tumor models (5, 9, 10). In the colorectal tumor model, Wiegering and colleagues (7) could show that silvestrol inhibited *Myc*-dependent crypt cell hyperproliferation measured a few days after induction of APC deletion. However, the efficacy of silvestrol regarding inhibition of tumor development and improvement in overall survival needs to be evaluated further in long-term experiments in colorectal cancer and other cancers.

The effects of silvestrol on colorectal cancer cells in culture were cytostatic rather than cytotoxic (7). It remains to be shown whether the cells resume proliferation after drug withdrawal or whether they enter cellular senescence, which, in addition to apoptosis, is another documented outcome of MYC inhibition (4). Because silvestrol did not inhibit MYC translation fully, another possible explanation is that apoptosis requires more extensive MYC depletion.

As expected, silvestrol treatment had moderate global effects on translation in colorectal cancer cells. The mRNA populations affected by silvestrol have been studied in more detail by Wolfe and colleagues (10) in T-ALL cells by transcriptome-scale ribosome footprinting. They found that translational efficiency of some transcripts decreased whereas others increased by silvestrol, *MYC* belonging to the former category. The 5′-UTRs of transcripts with decreased, but not those with increased, translational efficiency contained consensus motifs potentially forming G-quadruplex structures, consistent with a role of the eIF4A RNA helicase to facilitate ribosome scanning through structurally complex regions. Such motifs as well as increased ribosome association/stalling were found at the 5′ part of the *MYC*'s 5′-UTR and within the IRES (10), which fits well with Wiegering and colleagues' findings (7) that silvestrol inhibits both cap- and IRES-mediated translation (Fig. 1). The relationship between the RNA G-quadruplex structures and *MYC* IRES function is another interesting topic for further studies.

The dependencies for canonical translational initiation factors, including eIF4A, and so-called IRES-transacting factors (ITAF) vary substantially between different IRESs and can also be cell-type specific (6). Interestingly, transcripts with eIF4A-independent IRESs showed increased translational efficiency in response to silvestrol (7, 10). IRES-mediated translation has been reported to increase upon cellular stress and often encode proteins involved in survival and tumorigenesis (6). This could potentially constitute an escape/

resistance mechanism after silvestrol treatment, and one possible explanation for why silvestrol does not cause apoptosis in colorectal cancer cells (7) and why it alone does not inhibit Eμ-*myc*–driven lymphomagenesis (9). This topic clearly needs further investigation. Another potential problem with silvestrol in a clinical setting is that it is a target for ABCB1/P-glycoprotein 1 (5).

Nevertheless, the finding that the eIF4A inhibitor silvestrol targets both cap- and IRES-mediated *MYC* translation is a significant contribution to our understanding of MYC regulation. This will definitely increase the interest in targeting *MYC* translation, efforts to improve eIF4A-targeting drugs, and potentially also the targeting of *MYC* translation at different levels in the future. Combination therapy using other types of MYC inhibitors, described above, is also conceivable. Finally, because MYC itself is a key regulator of protein synthesis, targeting translation will strike both upstream and downstream of MYC simultaneously, thereby making this strategy even more appealing.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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