MYC and TFEB Control DNA Methylation and Differentiation in AML

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Summary: Although the MYC transcription factor has been consistently implicated in acute myeloid leukemia (AML), its gene targets and precise role in leukemogenesis remain unknown. In this issue of Blood Cancer Discovery, Yun and colleagues provide evidence that MYC directly suppresses the expression of TFEB, an mTORC1-regulated transcription factor. They show that, in the context of the myelocytic/granulocytic lineage, TFEB acts as a tumor suppressor by inducing the IDH1/2–TET pathway, which in turn, leads to altered DNA methylation and increased expression of genes involved in myeloid differentiation and apoptosis. Therefore, high levels of MYC suppress an epigenetic pathway that should normally act to attenuate leukemic progression. Identification of the components of this pathway is likely to inform new therapeutic tactics for AML and possibly other cancers.

See related article by Yun et al., p. 162 (3).

The bHLH-Z transcription factor MYC is known to be an extraordinarily well-connected protein: It not only directly interacts with MAX and transcriptional coregulator complexes but also interacts functionally with other members of what is known as the proximal MYC network (e.g., MXD proteins, MondoA). These latter interactions are indirect in that they are independent of evident protein–protein association among network members. The members of the MYC network can all be considered to be members of the MYC superfamily—related through paralogous bHLH-Z domains (1). Moreover, there is increasing evidence that MYC functionally interacts with the wider universe of more distantly related bHLH-Z transcription factors. For example, MYC has been reported to influence the activity of the transcription factors that control circadian rhythm (the bHLH-Z proteins BMAL1/CLOCK) through both indirect and direct interactions (2).

The article by Yun and colleagues (3) in this issue of Blood Cancer Discovery and another recent report (4) indicate that MYC is profoundly involved in the activity of TFEB, a bHLH-Z factor and member of the MiT–TFE family (comprising TFE3, TFE4, and MITF). Under non-stressed conditions, TFEB is sequestered in the cytoplasm and some cellular mechanisms, including TFEB phosphorylation by mTORC1 on the lysosomal surface. By contrast, mTORC1 inhibition or nutrient starvation promotes TFEB release, nuclear transport and specific genomic binding to loci that are involved in lysosomal functions and autophagy (5). In this way, it is nutrient sensing by mTORC1 that, under conditions of amino acid starvation for example, triggers a switch to autophagy and promotes survival.

In renal cell carcinomas, TFEB is highly expressed due to chromosome translocation or gene amplification and promotes oncogenesis (5). However, in human acute myeloid leukemia (AML), chromosome regions containing autophagy genes are often deleted. Moreover, single copy loss of Atg5, a key autophagy gene, is able to drive leukemia progression in mice (6), supporting a tumor-suppressive role of autophagy in AML.

As shown in the current study, MYC is highly expressed in a subset of AML, and its expression positively correlates with increased numbers of immature myeloblasts and elevated expression of stem cell markers in The Cancer Genome Atlas and several other AML patient cohorts. Not surprisingly, knockout of MYC promotes myeloid differentiation and inhibits proliferation in AML cells as well as myeloid progenitors, whereas MYC overexpression showed the opposite effects. Therefore, MYC is essential in maintaining normal myeloid progenitor characteristics as well as survival and proliferation of AML cells.

Given the seemingly opposing functions of MYC and autophagy in myeloid development and leukemogenesis, Yun and colleagues set out to examine potential connections between MYC and TFEB. The levels of TFEB and its target genes are inversely correlated with that of MYC in AML cell lines as well as in multiple AML patient cohorts. Consistent with this, doxycycline (dox)-induced expression of MYC in myeloid and leukemia cells decreases the levels of TFEB and its target genes and reduces lysosome biogenesis. Genetic and pharmacologic inhibition of MYC, on the other hand, significantly activates TFEB expression and increases autophagy. Given the seemingly opposing functions of MYC and autophagy in myeloid development and leukemogenesis, Yun and colleagues set out to examine potential connections between MYC and TFEB. The levels of TFEB and its target genes are inversely correlated with that of MYC in AML cell lines as well as in multiple AML patient cohorts. 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TFEB and other MiT–TFE transcription factor family members. In addition, MYC can also bind to the promoters of TFEB target genes, thereby precluding TFEB binding and transcription activation (4). Consistent with the previous finding, Yun and colleagues show that MYC suppresses TFEB promoter activity, potentially by directly binding to the E-box region upstream of the transcriptional start site. Interestingly, in AML, as distinct from other cancer types, MYC itself does not occupy the promoters of TFEB target genes or affect the binding of TFEB to its target promoters. Therefore, MYC suppresses autophagy and downregulates TFEB target genes by directly inhibiting TFEB expression in AML (Fig. 1).

To directly test if TFEB functions as a tumor suppressor in AML, Yun and colleagues (3) inducibly expressed TFEBS211A, a mutant form of TFEB that constitutively localizes to the nucleus, in myeloid progenitors and AML cells in the presence of dox. Upon dox treatment, cells expressing TFEBS211A showed elevated levels of TFEB target gene expression and lysosome biogenesis. Moreover, short-term TFEBS211A expression promoted monocytic and granulocytic differentiation, whereas long-term TFEBS211A expression leads to apoptosis accompanied by higher levels of apoptotic markers. To further test the role of TFEB in vivo, the authors performed mouse xenograft experiments using AML cells with dox-inducible TFEBS211A. Consistent with ex vivo results, dox-induced expression of TFEBS211A delayed leukemia development and improved survival in recipient mice compared with control groups. Collectively, these data indicate that TFEB acts as a tumor suppressor in AML.

Importantly, the antiproliferative effects of TFEB do not appear to be mediated through regulation of autophagy, as inhibition of autophagy in myeloid progenitor and AML cells does not affect cell fate or survival. Instead, based on RNA-sequencing analysis, TFEB activates genes essential for monocytic and granulocytic differentiation. In that cellular context, such regulation is at least partially achieved by TFEB’s transcriptional activation of IDH1/2 and/or TET, which, in turn, leads to changes in DNA methylation on these genes. TFEB transcription can be directly suppressed by MYC, which is highly expressed in subsets of AML. The precise mechanism by which MYC suppresses TFEB expression is unknown.

Figure 1. The MYC–TFEB–IDH1/2–TET axis in AML. Under nutrient-replete conditions, TFEB is phosphorylated by mTORC1 and other kinases and, as a result, is sequestered in the cytoplasm. When mTORC1 is inhibited upon starvation or AMPK activation (e.g., by GSK-621), unphosphorylated TFEB translocates to the nucleus and activates the transcription of genes involved in autophagy and lysosome biogenesis. In the current study, Yun and colleagues show that TFEB functions as a tumor suppressor in AML by regulating the expression of genes controlling myeloid lineage differentiation and cell death in myeloid progenitor and AML cells. This is partially achieved by TFEB’s transcriptional activation of IDH1/2 and/or TET, which, in turn, leads to changes in DNA methylation on these genes. TFEB transcription can be directly suppressed by MYC, which is highly expressed in subsets of AML. The precise mechanism by which MYC suppresses TFEB expression is unknown.
in active demethylation (for review, see ref. 7). Specific neo-
morphic mutations in IDH (e.g., IDH1R132H) or TET inhibit-
ion impairs DNA demethylation and results in aberrant 
proliferation and a block to differentiation. Such mutations 
are associated with a subset of AML as well as other tumor 
types such as glioblastomas and gliomas (8), and selective 
small-molecule mutant IDH inhibitors are showing promise 
in treatment of AML (9).

Many of the differentially regulated genes identified by 
Yun and colleagues (3) in TFEBS211A-expressing AML cells,
including genes controlling myeloid lineage differentiation 
and cell death, showed exclusive gain of 5hmC as well as both 
gain and loss of 5mC. Consistent with a MYC-TFEB-IDH–
TET demethylation circuit, the authors find that inducible 
expression of MYC, or coexpression of IDH1R132H, suppresses 
5hmC levels and myeloid differentiation induced by TFEB in 
myeloid progenitor and AML cells. Together, these findings 
suggest that MYC control of TFEB can epigenetically regu-
late cell differentiation and survival via the IDH1/2–TET 
pathway (Fig. 1).

Lastly, the authors explore the potential therapeutic value 
of TFEB activation in treating AML. GSK-621, a specific 
analyst of AMPK, induces TFEB nuclear localization, target 
gene activation, myeloid differentiation, and cell death in 
AML cells. Similar effects were also observed when primary 
AML patient samples were treated with this compound. 
Given the effect of TFEB on DNA methylation, the authors 
also tested if TFEB activation could act synergistically with 
azaclitidine, a DNA methyltransferase inhibitor used for 
AML treatment. Myeloid progenitor and AML cells with 
both TFEB overexpression and azacitidine treatment exhib-
ited significant increases in global 5hmC levels and cell death 
compared with cells with either treatment alone. Similarly, 
induced expression of TFEBS211A in AML xenografts, com-
bined with azacitidine, modestly improved overall survival 
of the recipient mice compared with those with single agent 
or no treatment, suggesting that TFEB activation and DNA 
hypomethylating agents may serve as a potential combina-
tion treatment for AML.

Taken together, the current study presents a novel regulatory 
pathway where TFEB induces the expression of IDH1/2 and 
TET2, which in turn leads to a global increase in 5hmC and 
the subsequent differentiation and apoptosis of myeloid pro-
genitor and AML cells (Fig. 1). TFEB expression is directly 
suppressed by MYC, which is often present at high levels in AML 
cells. As noted by the authors, the inverse correlation between 
MYC and TFEB expression appears to occur specifically in 
AML cells, but not in other leukemia or lymphoma cell lines. 
Therefore, whether the MYC-TFEB-IDH1/2–TET axis also 
functions in other tumor types awaits further study. In addi-
tion, the mechanism through which MYC, generally thought 
of as an activator of expression, suppresses TFEB expression 
remains unclear. Previous work has shown that, in HeLa cells, 
MYC inhibits the expression of TFEB and other MiT–TFE

family members by co-occupying their promoters with HDAC2 
(4). It would be interesting to see if a similar mechanism also 
applies in AML. In addition, Miz-1, a POZ domain-containing 
transcriptional activator, is known to interact with MYC–MAX 
heterodimers and repress gene expression under MYC-high 
conditions (10). Given the high levels of MYC in AML, it is fea-
stable that MYC could also suppress TFEB expression via Miz-1.
Finally, not all genes that are differently regulated upon TFEB 
expression displayed changes in DNA methylation. Moreover, 
as noted by the authors, expression of IDH1 alone is not suf-
ficient to induce AML cell differentiation or death, indicating 
other potential mechanisms may be at play, including some 
that may be independent of epigenetic regulation. Future 
udies on this front will provide more insights into the tumor-
suppressive functions of TFEB.

Authors’ Disclosures

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