IN THE SPOTLIGHT

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 bHLHZ 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 bHLHZ domains (1). Moreover, there is increasing evidence that MYC functionally interacts with the wider universe of more distantly related bHLHZ transcription factors. For example, MYC has been reported to influence the activity of the transcription factors that control circadian rhythm (the bHLHZ 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 bHLHZ factor and member of the MiT–TFE family (comprising TFEB, TFE3, TFEC, and MITF). Under non-stressed conditions, TFEB is sequestered in the cytoplasm following 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-lysosome formation and autophagic flux. Moreover, such regulation is also observed in vivo, where MYC overexpression in fetal liver cells suppresses TFEB and its target gene expression after transplantation, suggesting that downregulation of TFEB may be linked to MYC’s capacity to promote myeloid and AML cell proliferation.

It was previously reported that in human solid tumor cell lines, MYC overexpression suppresses expression of...
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 TFEB S211A, 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 TFEB S211A showed elevated levels of TFEB target gene expression and lysosome biogenesis. Moreover, short-term TFEB S211A expression promoted monocyctic and granulocytic differentiation, whereas long-term TFEB S211A 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 TFEB S211A. Consistent with ex vivo results, dox-induced expression of TFEB S211A 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 monocyctic and granulocytic differentiation. In that cellular context, such regulation is at least partially achieved by changes in DNA methylation. TFEB induces expression of isocitrate dehydrogenases 1 and 2 (IDH1, IDH2) 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 neomorphic mutations in IDH (e.g., IDH1R132H) or TET inhibition 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 regulate 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 agonist 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 azacitidine, a DNA methyltransferase inhibitor used for AML treatment. Myeloid progenitor and AML cells with both TFEB overexpression and azacitidine treatment exhibited significant increases in global 5hmC levels and cell death compared with cells with either treatment alone. Similarly, induced expression of TFEBS211A in AML xenografts, combined 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 combination 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 progenitor 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 addition, 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 feasible 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 sufficient 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 studies on this front will provide more insights into the tumor-suppressive functions of TFEB.

Authors’ Disclosures

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REFERENCES

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