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# EDITORIAL

# C/EBPα, do not forget your TIP60

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The paper by Bararia *et al.*,[1] published in this issue of Leukemia has brought TIP60, another cellular acetyltransferase under the spotlight of myeloid leukemogenesis. Transcriptional deregulation is a recurring theme in human acute myeloid leukemia (AML).[2] This is often caused by nonrandom chromosomal translocations resulting in aberrant expression of transcription factors or the formation of chimeric transcription factors including the retinoic acid receptor (RARα), core-binding factor (either of its two subunits AML1 or CBFβ) and mixed lineage leukemia proteins (MLL).[3] Although these oncogenic transcription factors may possess different transcriptional properties, aberrant recruitment of histone modification enzymes such as acetyltransferases and deacetylases emerges as key players and potential therapeutic targets for the resultant diseases.[4] AML1-ETO and PML-RARα recruit transcriptional corepressor complexes containing histone deacetylases to suppress gene expression. MLL fusions aberrantly activate gene expression via recruitment of histone aceyltransfases such as CBP and p300. Recent studies have also revealed the downregulation of the myeloid transcription factor C/EBPα (CCAAT/enhancer-binding proteins) as a critical process for both AML1-ETO- and PML-RARα-mediated transformation.[5,6] The putative tumor suppressor function of C/EBPα in myeloid leukemogenesis is further strengthened by the identification of C/EBPα mutations in about 7–9% of AML patients.[7] To gain further insights into the functional regulation of C/EBPα, Bararia *et al*. have biochemically purified and validated another acetyltransferase, TIP60 as a C/EBPα-interacting partner.[1] They show that TIP60 directly interacts with C/EBPα, and acts as a transcriptional co-activator in an acetyltransferase-dependent manner. TIP60 complexes with C/EBPα to enhance acetylation of histones 3 and 4, and regulates expression of downstream targets including C/EBPα itself (Figure 1). Consistently, the expression of TIP60 correlates with C/EBPα expression in human leukemia samples and U937 cells upon retinoic acid-induced differentiation, suggesting TIP60 as a direct co-activator for C/EBPα in hematopoietic cells.[1]

TIP60 is a founding member of the MYST family of lysine acetyltransferases, which also include MOZ and MORF. Notably, both MOZ and MORF are directly involved in chromosomal translocations in AML to form chimeric fusion proteins with CBP and/or p300.[8] Two recurrent features of these fusions are gain of additional acetyltransferase activities, and subversion of the normal cellular functions of CBP/p300 as tumor suppressors. As this study proposes TIP60 as a co-activator for C/EBPα functions, the transcriptional activities of TIP60 seem to be context dependent. TIP60 acts as a transcriptional corepressor when complexed with TEL, which fuses with AML1 in the majority of childhood leukemias. In the latter case, TIP60 may promote transcriptional repression and thus transformation mediated by TEL-AML1.[9] In addition to histones, TIP60 also acetylates transcription factors such as the androgen receptor and c-Myc, that directly affect their DNA binding and transcriptional activities.

Besides its function in transcriptional regulation, TIP60 plays an important role in DNA damage response pathways. The activation (involving acetylation) of the DNA damage sensor ATM depends on active TIP60.[10] Cells expressing catalytically inactive TIP60 exhibit increased double-strand DNA breaks. Moreover, Tip60 itself is recruited at double-strand DNA breaks to acetylate histones involved in DNA repair in fruitfly.[11] These central functions in DNA damage responses suggested that loss of TIP60 function might contribute to cancer development. A first hint pointing in that direction came from a screen for genes that can bypass p53-mediated cell cycle arrest.[12] Specific knockdown of TIP60 inhibited p53-mediated growth arrest. This was accompanied with disturbed expression of p53 target genes and cell cycle arrest following DNA damage. A subsequent study confirmed these findings and showed that TIP60 binds p53.[13] Soon after these reports, it was shown that TIP60 can acetylate p53 at a specific lysine residue in its DNA-binding domain following DNA damage (Figure 1).[14,15] Importantly, this acetylated form of p53 specifically activated a subset of target genes that trigger apoptosis. To study TIP60 in malignant transformation, knockout mice were generated. Embryos with complete loss of *Tip60* die before implantation but *Tip60* heterozygous mice do not show developmental or tumor-prone phenotypes.[16] However, in mice that are prone to develop B-cell lymphomas due to an overactive form of the oncoprotein Myc (Eμ-Myc), loss of one *Tip60* allele results in a significantly enhanced onset and penetrance of B-cell lymphomas. In the lymphomas that arose in the heterozygous mice, the remaining wild-type allele was not lost. Instead, it was duplicated at the expense of the targeted null allele resulting in restored Tip60 expression. Thus, Tip60 is a haploinsufficient tumor suppressor at an early stage of tumor development in Eμ-Myc mice.[16] Loss of one *Tip60* allele did not impair Myc-induced proliferation and apoptosis. Instead, it significantly impaired Myc-induced DNA damage responses. Remarkably, the expression of Myc and p53 target genes was not affected in Eμ-Myc mice lacking one *Tip60* gene locus. Based on these results, primary human tumors were analyzed for TIP60 status revealing lowered TIP60 RNA expression in a significant subset of breast carcinomas and lymphomas. The majority of the tumors exhibiting diminished TIP60 RNA expression showed loss of heterozygosity at the *TIP60* gene locus. Thus, loss of one *TIP60* allele is frequently observed in various primary human tumors. Analysis of the p53 status showed that cases with *TIP60* loss of heterozygosity often had mutated rather than wild-type p53. This suggests that diminished TIP60 expression acts complementary to disturbed p53 pathways in malignant transformation.

Although TIP60 has not been involved in chromosomal translocations in human leukemias, loss of TIP60 expression can directly compromise both the activities and expression of C/EBPα, which also has a tumor suppressor function in myeloid leukemogenesis. In spite of the putative tumor suppressor function of C/EBPα, a complete ablation of C/EBPα does not lead to leukemia in mice. Interestingly, mutations of C/EBPα found in AML patients do not result in a null mutation but produce truncated or mutant forms of C/EBPα (for example, a

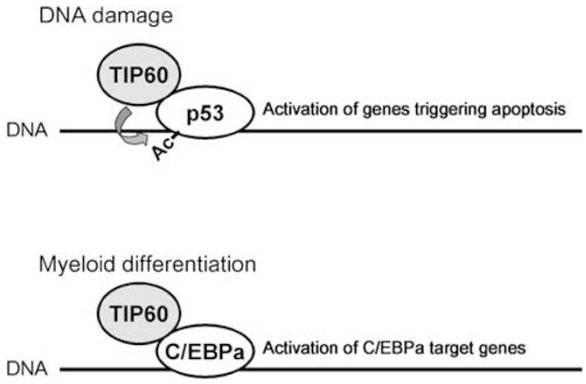


**Figure 1** TIP60 (for Tat-interactive protein 60 kDa, also known as human immunodeficiency virus Tat-interacting protein, HTATIP) is a cellular acetyltransferase that plays a role in transcriptional regulation and DNA damage response pathways. Upper panel: following DNA damage TIP60 exhibits enhanced p53 binding. TIP60 acetylates p53 (Ac) in its DNA-binding domain on lysine residue 120 resulting in the specific activation of genes that trigger apoptosis (such as *BAX* and *PUMA*). Lower panel: Bararia *et al.*[1] show that TIP60 binds C/EBPα. Gene reporter assays show that the two proteins synergize in gene activation. This requires the acetyl transferase domain of TIP60. Forced differentiation of leukemic cells by induction of C/EBPα expression results in enhanced recruitment of TIP60 to C/EBPα target genes (the *C/EBPα* gene itself and *GCSFR* gene). This is associated with a strong increase in acetylated histones at the *C/EBPα* and *GCSFR* gene promoters.[1] Whether TIP60 is responsible for this increase in histone acetylation, whether C/EBPα itself is acetylated and how the TIP60-C/EBPα interaction is regulated remain to be studied.

30 kDa N-terminal truncated C/EBPα or a basic region mutant).[7] In contrast to *C/EBPα*-null mice, mice with engineered C/EBPα that express either the 30 kDa isoform or the basic region mutant develop myeloid leukemia.[17] These results suggest that only selective loss of certain functions instead of a complete inactivation of C/EBPα functions are oncogenic. It is interesting to note that TIP60 can be recruited by either the N-terminal or the DNA-binding domain of C/EBPα, while a maximal binding will only be achieved with the wild-type full-length protein.[1] Thus, C/EBPα generated by mutated alleles such as the N-terminal truncated form will potentially have reduced ability to interact with TIP60, which will partially compromise its activities and functions. Given the tumor suppressor function of TIP60, it will be interesting to determine its interaction with different C/EBPα mutants, and the functional consequence of its reduction in normal and malignant hematopoiesis in particular in the context of C/EBPα-mediated leukemogenesis. Although functional studies are still awaiting, identification of TIP60 as an interacting partner for C/EBPα further highlights the roles of MYST lysine acetyltransferases in myeloid leukemogenesis and a potential avenue for cancer therapeutics.

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