The complex relationship of Tribbles pseudokinase 1, PML/RARA and C/EBPα in leukemia: two possible couples but not a trio

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Tribbles gene was firstly identified in Drosophila where it was shown to be involved in cell cycle regulation.[1-3] The name Tribbles was proposed by Seher and Leptin because the proliferating mesodermal cells observed in the tribbles-deficient drosophila embryos reminded them of the rapidly-dividing science fiction alien organism named "Tribbles" that appeared in the TV series "Star Trek" in December 29, 1967.[3] There are three human orthologs of the drosophila Tribbles gene named Tribbles pseudokinase 1, 2 and 3 (*TRIB1*, *TRIB2* and *TRIB3*).[4] As indicated by their names, Tribbles genes encode "pseudokinases": i.e. proteins that have a kinase domain lacking some of the amino acids that are essential for kinase enzymatic activity.[5] In addition to the pseudokinase domain, Tribbles proteins also contain a C terminal E3 ligase-binding domain, that is involved in the regulation of the ubiquitination of several target proteins.[4] Mammalian Tribbles play an important role on the control of different physiological functions including the regulation of lipid metabolism, inflammation and innate immunity.[4] Likewise, Tribbles proteins have been implicated in both the regulation of tumorigenesis and the mechanism of action of several anticancer agents.[6,7]

TRIB1 and TRIB2 but not TRIB3 seem to play a relevant role in leukemia.[6,8,9] Thus, *TRIB1* (which was initially identified as a gene placed at a retroviral integration site[10]) has been shown to be over-expressed in patients with acute myeloid leukemia (AML).[6,8,9] Moreover, the human *TRIB1* gene is located in the same chromosome region as *MYC*, a gene that is also frequently amplified in AML.[6,8,9] In line with these observations, MYC and TRIB1 have been shown to co-operate in AML.[6,8,9] The oncogenic role of TRIB1 in AML relies at least in part on the ability of TRIB1 to regulate the stability of the transcription factor C/EBPα *via* the E3 ligase COP-1.[11] C/EBPα plays an important role in the control of myeloid differentiation and its (TRIB1-COP1-dependent) degradation blocks this process thereby facilitating the transcription of leukemia promoting genes.[6,8,9]

Now, in an article included in this issue of Haematologica,[12] Keeshan *et al.* further explore the role played by TRIB1, MYC, C/EBPα and PML/RARA in AML and acute promyelocytic leukemia (APL) shedding light (and adding also some layers of complexity) on the role played by this pseudokinase on these malignancies. The most frequent type of APL derives from a balanced chromosomal translocation [t(15;17)(q22;q12)] that leads to the fusion of the N-terminus of the promyelocytic leukemia protein (PML) with the C terminus of the retinoic acid receptor-alpha (RARA) transcription factor to produce the PML/RARA fusion protein.[13] In the absence of its ligand (retinoic acid, RA) the nuclear receptor RARA represses the transcription of genes involved in myeloid differentiation whereas in the presence of physiological levels of RA, RARA promotes the expression of these genes. In contrast, PML/RARA does not respond to RA and therefore cannot drive differentiation. In addition, PML/RARA also prevents the formation of the PML nuclear bodies (nuclear structures that are involved in the regulation of p53 and other important signaling mechanisms.[13]) The combination of the two events seems to be responsible for the accumulation of promyelocyte characteristic of the disease.

In this context, Keeshan *et al.* investigated whether TRIB1 co-operates with PML/RARA and MYC in the development of AML and APL. Using an elegant approach, bone marrow cells derived from wild-type (WT) animals or from transgenic mice over-expressing PML/RARA were transduced with retroviral vectors encoding MYC and TRIB1. These cells were subsequently transplanted into lethally-irradiated animals and finally, the genotypes of the clones that produced leukemias in these animals analyzed. Interestingly, leukemias derived from WT-bone marrow cells expressed both TRIB1 and MYC whereas those derived from PML/RARA cells in most cases expressed MYC but not TRIB1. These observations suggest that TRIB1 co-operates with MYC (but not with PML/RARA) to induce leukemias. One of the reasons for this lack of co-operation between TRIB1 and PML/RARA could be that both proteins promote leukemia through the same mechanism and that therefore leukemia development in the mice is not facilitated by the common selection of the

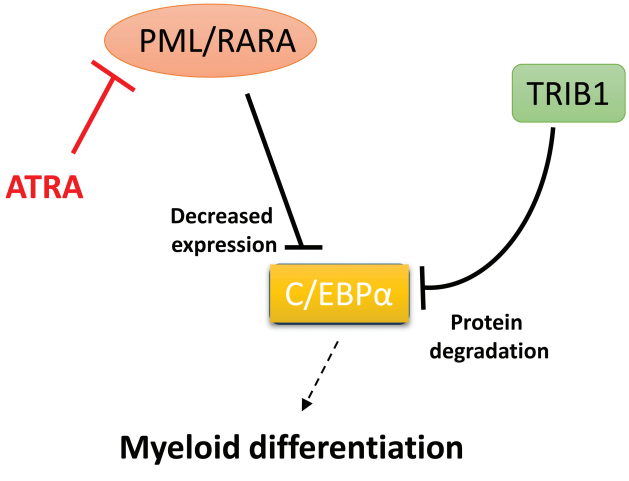

Figure 1. C/EBPα is a common target of PML/RARA and TRIB1. Proposed model (based on the work by Keesham *et al.*[12]) by which PML/RARA and TRIB1 inhibit myeloid differentiation by targeting C/EBPα. PML/RARA leads to a decreased expression of C/EBPα whereas TRIB1 promotes its ubiquitination and subsequent proteasomal degradation. In the absence of TRIB1 overexpression, ATRA treatment promotes PML/RARA degradation and modifies PML/RARA transcriptional activity, which leads in turn to increased C/EBPα expression, an event that is required to re-establish myeloid differentiation. In the presence of elevated levels of TRIB1, C/EBPα levels cannot be recovered which blocks the effect of ATRA on myeloid differentiation.

two genes. The authors hypothesized that the transcription factor C/EBPα (that plays a crucial role in the regulation of myeloid differentiation) could be the common target of TRIB1 and PML/RARA. To investigate this hypothesis, the authors used the well-established model of all-trans-RA (ATRA)-induced APL cells differentiation (ATRA treatment converts PML/RARA from a repressor to a transcriptional activator and also promotes PML/RARA degradation thereby triggering myeloid differentiation.)[13] Using this model, the authors found that TRIB1 overexpression abrogates the response to ATRA of PML/RARA-expressing APL cells. Moreover, the authors also found that overexpression of TRIB1 (but not of a TRIB1 mutant that cannot promote C/EBPα degradation) prevented ATRA-induced C/EBPα upregulation. Furthermore, an additional *in vivo* experiment showed that increased expression of TRIB1 abolishes the effect of ATRA in PML/RARA and MYC-induced leukemias. Altogether, these findings support the idea that both TRIB1 and PML/RARA negatively regulate C/EBPα (although acting through different mechanisms) and that the regulation of C/EBPα plays a relevant role in the control of myeloid differentiation and leukemogenesis (Figure 1).

In any case, several questions remain to be clarified in relation with the ideas presented in this work. For example, are TRIB1 (or TRIB2) endogenous levels down-regulated in APL patients? Initial analysis in published gene expression datasets suggest that this could be the case in APL. However, confirmation that TRIB1 protein levels, and not only mRNA levels, are affected would be important to understand the relevance in patients of the results obtained in animal models. Another point that still requires to be experimentally explored is whether PML/RARA negatively regulates TRIB1 (or TRIB2) expression. The existence of this specific regulatory mechanism is a conceivable (although still hypothetical) possibility that might explain why TRIB1 was not present in the PML/RARA leukemias investigated in this study. In addition, it would be important to understand the precise contribution of the increased expression of TRIB1 or TRIB2 to the development of AML or other leukemias. Experiments in animal models cogently support that a co-operation between MYC and TRIB1 exists. However, the authors did not find a correlation between the expression of these two genes in a human dataset obtained from patients with AML.

Therapies based on the use of ATRA and/or arsenic trioxide (ATO, another treatment used in APL patients with PML/RARA that promotes degradation of this protein as well as of normal PML)[13] have enormously improved the clinical outcome of APL patients.[13,14] However, there is still a fraction of these patients that develop resistances to ATRA and ATO therapies.[13,14] In this context, the results obtained by Keeshan and Kogan *et al.* may have interesting diagnostic/therapeutic implications. Further research should nevertheless clarify whether TRIB1 (or TRIB2) may play a role in the development of resistances in APL patients and also whether targeting TRIB1 or TRIB2 may be a therapeutic strategy to fight AML, APL or other leukemias.

Research performed during the last decade has provided evidence that the Tribbles proteins are important regulators of cell function at many different levels. The recent development of new tools for the study of Tribbles biology, including the generation of Tribbles transgenic and conditional knockout mice, together with the recent establishment of novel Tribbles collaborative networks,[15] should facilitate the development of additional studies and the acquisition of a more profound knowledge on the precise role played by these fascinating proteins in different physio-pathological conditions including cancer, and more specifically, leukemias.

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