The Tribble with APL; a new road to therapy

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Summary
The t(15;17) translocation generates a PML-RARα fusion protein causative for acute promyelocytic leukemia (APL). Li et al. identify the pseudokinase stress protein TRIB3 as an important factor in APL disease progression and therapy resistance. Targeting the interaction of TRIB3 and PML-RARα using peptide technology provides a novel therapeutic approach.

Main Text
Acute promyelocytic leukemia (APL) is a distinct subtype (5-15%) of acute myeloid leukemia (AML) characterized by a t(15:17) chromosomal translocation in myeloid precursor cells, leading to the expression of the PML-RARα oncofusion gene. PML-RARα expression blocks promyelocyte differentiation leading to the proliferation of leukemia blasts. The molecular basis for the effects of PML-RARα lies in its activity as a constitutive repressor of RARα and RXR target genes and in its dominant negative effects on PML, a protein that is necessary for the formation of nuclear bodies that have extensive influence on the activity of many transcription factors, including p53. Current therapies for APL include treatment with pharmacological doses of all-trans-retinoic-acid (ATRA) and arsenic trioxide (As_2O_3), both of which lead to the SUMOylation and ubiquitination dependent degradation of PML-RARα, and APL cell differentiation. These treatments are effective with ~75% of patients cured of the disease, however therapy resistance and disease relapse remains an unmet clinical need.

In this issue of *Cancer Cell,* Li and colleagues identify the pseudokinase stress protein TRIB3 among the most up-regulated genes in $CD34^+$ cells from APL patients (Li et al., 2017). In a series of elegant experiments utilizing transgenic mice with enhanced or deficient Trib3 expression, this study demonstrates that TRIB3 promotes APL by interacting with and stabilizing PML-RARα. TRIB3 binds to the SUMOylation motifs of PML-RARα to inhibit the SUMOylation, ubiquitination and degradation of PML-RARα. TRIB3 depletion induced p53-mediated senescence and differentiation in APL cells, and inhibited APL progression *in vitro* and *in vivo*. Disturbing the TRIB3/PML-RARα interaction using a peptide targeting the SUMOylation motifs in PML attenuated APL progression *in vivo* by promoting PML-RARα degradation. The study also demonstrates that targeting TRIB3/PML-RARα interaction has therapeutic potential (Figure 1). The authors could show anti-APL effects of this peptide when used alone, and also combined efficacy when used in combination with ATRA or As_2O_3. Intriguingly, TRIB3 depletion in APL cells induced the expression of PML but not PML-RARα, and selectively induced the degradation of PML-RARα but not PML. The mechanism responsible for the dichotomous effect of TRIB3 on PML and PML-RARα remains one of the open questions from this study, the elucidation of which is likely to give important insights into the pathobiology of PML-RARα.

The three TRIB protein pseudokinases (TRIB1, TRIB2 and TRIB3) have important roles in cancer and inflammation, and respond to a diverse array of cellular stresses. The formation of regulated multiprotein complexes that drive cellular signaling is a recurring theme in TRIB biology. TRIB proteins have been shown to act as molecular scaffolds for the assembly and regulation of signaling pathways e.g. through the MEK/MAPK module, and degradation via ubiquitination of TRIB 'substrates' that interact with the pseudokinase domain e.g. CDC25C, Acetyl CoA carboxylase (ACC), and C/EBPs. However, key differences between TRIB family members are important when considering their therapeutic targeting in leukemia. TRIB pseudokinases are classified based on sequence homology as serine/threonine pseudokinases that either lack (TRIB1), or exhibit low (TRIB2 and TRIB3) vestigial ATP affinity and phosphotransferase capacity *(*Bailey et al., 2015, Murphy et al., 2015). In addition, TRIB1 and TRIB2 are more similar (possessing a sequence homology of ~71%) compared to the most recently evolved family member TRIB3 (Eyers et al., 2017), whose homology with both TRIB1 and TRIB2 is only ~55%, suggesting mechanistic and functional divergence. Indeed

earlier studies showed that when highly expressed, TRIB1 and TRIB2 but not TRIB3, degrade the myeloid transcription factor C/EBPα, inhibit myeloid differentiation, and drive AML (Keeshan et al., 2006, Dedhia et al., 2010). The role of TRIB3 in leukemia has therefore been elusive.
Whilst TRIB3 has not been shown to be a driver of leukemia, its expression has been associated with different subtypes of leukaemia. *In silico* analysis revealed high TRIB3 expression in the erythrocyte lineage of haemopoiesis, and significantly increased expression in AML with t(8;21), trisomy 8 and 11, and t(15;17) and in FAB M2 and M3 subtypes (Liang et al., 2013). The relevance of this high expression in erythropoiesis had not been elucidated until studies using TRIB3 knockout mice (Dev et al., 2017), and now in AML using myeloid specific knockout and knockin mice by Li et al in this issue of *Cancer Cell*. This work identifies for the first time, a novel mechanism involving TRIB3 in chemotherapy resistance, distinct from known TRIB1 and TRIB2 oncogenic activity. TRIB1 and TRIB2 have been shown to have a role in resistance to ATRA-mediated therapy, mediated via their degradative activity on the myeloid differentiation factors C/EBPα and β (Keeshan et al., 2016). The study by Li and colleagues represents an important milestone in the understanding of TRIB3 oncogenicity in APL pathogenesis that distinguishes TRIB3 from the other TRIB family members. The authors generated myeloid-specific transgenic knockin and knockout mice, and utilized mouse primary APL cells and primary patient material, to identify PML and PML-RARα as novel targets for TRIB3 activity, which results in the regulation of both their expression thus contributing to APL disease progression and response to chemotherapy. In fact, targeting TRIB3 in APL has exciting potential in patients that do not respond well to ATRA/ As_2O_3 based therapy. It is also very interesting that all TRIB proteins have potent oncogenic and therapy resistance functions in AML through independent and specific mechanisms. This most recent study along with others strongly suggests that targeting TRIB proteins and their protein-protein interactions in AML will be of therapeutic benefit.

This latest study identifies a novel target for developing improved therapeutic regimes in AML. Indeed, there is also great potential for targeting TRIB proteins in other subtypes of AML associated with high TRIB expression. It remains to be explored how TRIB proteins function in subtypes of AML driven by other common AML oncogenes such as AML1-ETO and MLL rearrangements. One possibility for future therapeutic consideration is the link between TRIB3 and the inhibition of NF-κB and p53, two transcription factors that are also inhibited by PML-RARα (Ahmed et al., 2017). In summary, insight into the function of TRIB proteins rather than mere assessment of their mRNA expression levels holds the key to understanding their role and therapeutic potential in leukemia and cancer, as exemplified by Li et al.

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Figure Legend

Figure 1. TRIB3 stabilizes PML-RARα to promote drug resistance in APL. TRIB3 binds to PML-RARα and PML to inhibit all-trans retinoic acid (ATRA) and As2O3-induced SUMOylation (s), ubiquitination (s) and proteasomal degradation of PML-RARα. Depletion of TRIB3 or inhibition of TRIB3 and PML-RARα interaction results in the re-formation of PML nuclear bodies, p53 dependent senescence and differentiation of APL cells.