Fighting AML with its own weapons

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In this issue of Blood, Mill et al report that acquired loss-of-function mutations of RUNX1 impair ribosomal biogenesis, rendering myeloid progenitor cells in acute myeloid leukemia (AML) susceptible to apoptotic elimination following combined inhibition of protein translation and BCL-2 (see figure). Harnessing AML-specific adaptations in the protein translation machinery for therapeutic exploitation to enhance susceptibility to apoptosis has recently been tested preclinically. This study demonstrates that for patients with RUNX1-mutant AML this approach allows for a more efficient impairment of leukemic cell growth.

RUNX1 (AML1) is a DNA sequence-specific transcriptional master regulator governing gene expression programs of hematopoietic development and differentiation. Its encoding gene is subject to recurrent germline or somatic hotspot mutations that are associated with several hematologic malignancies and leukemia predisposition syndromes. Underscoring their clinical uniqueness, patients bearing RUNX1 lesions are separate entities in the standing World Health Organization classification of AML.

Broadly, two classes of gene alterations have been identified with distinct molecular and hematologic disease phenotypes: monoallelic chromosomal translocations and mono- or biallelic somatic mutations. Translocations, such as t(8;21)(q22;q22) and t(12;21)(p13;q22), largely retain RUNX1’s DNA-binding capacity and exert neomorphic activities, as opposed to fusion lesions, which have a favorable prognosis, deletions, missense, splicing, frameshift, and nonsense mutations have a loss-of-function or dominant negative activity. The latter mutations are associated with poor prognosis, which may be due to the resistance of RUNX1-mutant (mutRUNX1) AML to mainstay

Loss-of-function mutations in RUNX1 (mutRUNX1) impair gene regulation of ribosomal biogenesis and protein translation, c-MYC, as well as myeloid differentiation regulators (left, lineage-specific transcription factor [LSTF]). Pharmacologic inhibition of protein synthesis using homoharringtonine or omacetaxine (HTT/OM)-induced myeloid differentiation despite also reducing RUNX1 and PU.1, as well as lowering BCL2 and MCL1 levels, which renders RUNX1mut AML cells more sensitive to apoptotic elimination following venetoclax or BET inhibition (OTX015) (VEN/OTX) (right). Figure created with BioRender.com.
chemotherapies, albeit mechanistic insights have been sparse.

To elucidate clinically relevant molecular consequences of RUNX1 loss-of-function mutations, Mill et al developed a set of preclinical models of human AML, including AML cell lines, patient-derived primary cells, as well as 2 novel isogenic AML cell lines bearing homozygous or heterozygous RUNX1R174* mutations. The authors show that homozygous and heterozygous RUNX1R174*-mutant AML cells had compromised myeloid differentiation potential at the morphological and molecular levels compared with RUNX1 competent controls (see figure). This is consistent with the known role of RUNX1 in regulating transcription of and in concert with key hematopoietic transcription factors through chromatin remodeling activity and enhancer activation to drive hematopoietic remodelling.

Interestingly, monoallelic RUNX1R174* was sufficient to also decrease c-MYC and perturb c-MYC dependent gene expression. c-MYC is a global regulator of transcription, chromatin structure, DNA replication, and protein synthesis. Accordingly, the authors found decreased total RNA levels and molecular perturbations consistent with reduced ribosomal biogenesis and protein translation in monoallelic RUNX1R174*-mutant AML cells. Protein synthesis is tightly regulated and governed by a complex machinery; modulation of protein translation rates can confer unique cellular properties to stem cells and therapy resistance in cancer. An elegant preclinical study from Speck and colleagues has uncovered substantially increased resistance of Runx1-deficient mouse nonleukemic hematopoietic stem and multipotent progenitor cells to genotoxic stress-induced ablation. The investigators attributed this resistance to reduced protein translation rates and altered susceptibility to apoptosis induction.

Probing whether reduced ribosomal biogenesis may render mutRUNX1 AML cells vulnerable to further inhibition of protein translation using homoharringtonine (HTT), a natural cytotoxic alkaloid from the evergreen tree known to interfere with chain elongation, or its analog omacetaxine (OM), the authors discovered more severely compromised protein synthesis at various levels in mutant compared with RUNX1 wild-type AML cells. This was accompanied by reduced levels of short-lived proteins, including c-MYC and antiapoptotic MCL1 and BCL-xL. Mechanistically, the authors attributed the effects of HTT to epigenetic inactivation and impaired expression of MYC targets, specifically genes encoding transfer RNAs and others facilitating RNA translation in mutRUNX1 AML cells.

Notably, key myeloid differentiation instructing regulators, such as RUNX1 itself and one of its most critical targets, PU.1, were also reduced at the protein level in HTT-treated RUNX1R174*-WT AML cells compared with wild-type counterparts. Despite this, HTT-exposed mutRUNX1 AML cells displayed morphological and molecular signs of myeloid maturation and decreased viability, indicating the restoration of differentiation programs either independent of, or otherwise suppressed by the two master regulators. Whether and how much of the gene regulatory differences observed in treatment-naive and HTT-exposed mutRUNX1 AML cells is attributable to loss of RUNX1 alone, a combination of impaired RUNX1 and PU.1 protein dosage, or potentially perturbed recruitment dynamics of these master regulators and cooperating lineage-specific transcription factors is an emerging question with relevance to potentially extending this approach to targeting aberrant myeloid cell growth in the absence of genetic RUNX1 lesions.

The authors further explored whether reduced levels of key antiapoptotic proteins, MCL1 and BCL-xL, found in HTT-treated mutRUNX1 AML cells, increased the cell’s susceptibility to apoptotic cell death. And indeed, impairment of protein synthesis using HTT or OM rendered AML cells more susceptible to pharmacologic BCL-2 inhibition by venetoclax or BRD2/3/4 BET inhibitor (OTX015) (known to inhibit expression of apoptosis inhibitors), HTT/OM synergized with venetoclax and BET inhibition in reducing cell viability of mutRUNX1 and wild-type AML cells, while only moderately compromising nonleukemic hematopoietic stem and progenitor cells ex vivo, and curtailing mutRUNX1 AML cell growth in patient-derived xenotransplantation models in vivo.

Insights gained from this work provide a rationale for the clinical application of combined protein translation and BCL-2 inhibitors in mutRUNX1 AML. This paper also poses critical follow-up questions. Perhaps the most pressing being: Will this combination treatment allow for the elimination of (pre) leukemic stem cells and reduce the risk of not eliminating all disease-relevant cancer cells that drive AML relapse and progression? Although restoration of apoptosis signaling may not be sufficient for the eradication of leukemic stem cells, it is possible that inhibition of protein synthesis, especially mitochondrial protein translation, may curtail metabolic adaptations driving venetoclax resistance of leukemic stem cells and allow for the effective eradication of all malignant cells in patients. Further investigations following this important work will undoubtedly continue leading our way for harnessing AML’s defenses as our offense.

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REFERENCES

Comment on Ambrosio et al, page 922

COM Manding platelet α-granule cargo

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In this issue of Blood, Ambrosio et al1 identify 3 proteins and complexes that contribute to α-granule biogenesis, yielding insights into how the myriad of cargo is packaged into this abundant class of platelet granules.

Studies of gray platelet syndrome (MIM 139090) and arthrogryposis–renal dysfunction–cholestasis syndrome (MIM 208085) identified NBEAL2 and VPS33B/VPS16B, respectively, as gene products important for α-granule production in megakaryocytes (MKs). Ambrosio et al expanded those findings, identifying syntaxin 12 (STX12; aka syntaxin 13) as interacting with VPS33B/VPS16B. They further showed how STX12 and a sorting complex called the COMMD (copper metabolism MURR1 domain)–CCDC22 (coiled-coil domain-containing 22)–CCDC93 (CCC) complex compete for VPS33B/VPS16B binding, suggesting a progressive hand-off mechanism for cargo sorting. Deletion of STX12, CCDC22, or COMMD3 reduced α-granule numbers and cargo levels in immortalized megakaryocyte progenitor cell lines (imMKCLs). These deletions also increased the number of multivesicular bodies (MVBs), which are thought to be an intermediate in α-granule biogenesis. Selective deletion of these proteins, as well as sorting nexin 17 (SNX17), delineated the trafficking route for P-selectin, showing that the α-granule membrane protein is retrieved from the plasma membrane and sorted, in the endosomes, to nascent granules. These observations increase our list of players in α-granule biogenesis and solidify the importance of endosomes in packaging de novo synthesized and endocytosed cargo into the same granules.

VSP33B and VSP16B (aka C14orf133, VIPAR, SPE-39) have several potential binding partners.2 Because VSP33B is a member of the Sec1/Munc18 family of Qa-soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor chaperones, Ambrosio et al first asked which of the 13 human syntaxin (STX) gene products bound recombinant VPS33B/VSP16B complexes. Pull-down and coimmunoprecipitation experiments confirmed robust interactions between STX12 and VPS33B/VSP16B. Interestingly, a phosphomimetic mutation in the Habc domain of STX12 enhanced binding, suggesting regulation of the "closed" configuration of STX12 to control this interaction. Modulation of STX12 levels by small interfering RNA or clustered regularly interspaced short palindromic repeats decreased the number of α-granules and the levels of 3 α-granule cargoes (von Willebrand factor [vWF], platelet factor 4 [PF4], and P-selectin). STX12 was localized to endosomes and partially colocalized with the key sorting protein, SNX17, which also recognizes P-selectin.3

Previous proteomic studies identified CCDC22 as a VSP33B/VSP16B interactor.2 Ambrosio et al expanded those observations, showing that STX12 and CCDC22 competed for binding to a specific site on VSP33B. This interaction was shown to be functionally important for α-granule biogenesis using rescue experiments in VPS33B−/− cells. They further showed that deletion of CCDC22 in imMKCLs reduced α-granule numbers, as well as PF4 and P-selectin levels. Together with a COMMD family member and CCDC93, CCDC22 forms a larger complex (called the CCC complex), which is important for endosome cargo sorting. Ambrosio et al found that 3 of the 13 COMMD genes (COMMD3, COMMD5, COMMD7) were upregulated as imMKCLs differentiate. Deletion of COMMD3 reduced α-granule numbers and PF4 and P-selectin levels. Treating the cells with the vacuolar H+ ATPase inhibitor, bafilomycin A1, reversed this reduction, suggesting that the loss of COMMD3 leads to a missorting of cargo to the lysosome for degradation. Interestingly, COMMD3 and STX12 show partial colocalization on a tubular organelle that could be the tubular recycling endosome, which is known to be a hub of anterior and retrograde protein trafficking.4 Such partial colocalization might be expected if sequential interactions in membrane microdomains of this compartment are required for cargo sorting.

This study represents a landmark advance in our understanding of α-granule biogenesis and opens the field to further dissection of the process. These studies reveal much about how cargo gets to an α-granule. STX12, SNX17, and CCC complexes are part of the endosomal sorting machinery. They interact with the cytoplasmic tails of endocytosed membrane proteins and direct them to various compartments (ie, the plasma membrane, Golgi apparatus; see figure). As shown by Ambrosio et al, loss of these elements leads to cargo degradation in an acidic compartment, presumably the lysosome. Does this imply that many α-granule proteins are secreted first and then recovered from the extracellular space via endocytosis? For PF4 and P-selectin, such a pathway is consistent with the data presented, as well as that from other groups.5,6 Because PF4 is almost exclusively expressed by MKs, a significant portion of it, and perhaps other factors, must not be immediately stored in α-granules post-Golgi but instead undergo exocytosis before repackaging. Several proteins (eg, vWF and TSP1) produced by the MK are found in early endosome (RAB5+) structures alongside cargo that is known to be endocytosed (eg, FGN).7 This pathway might give MKs


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