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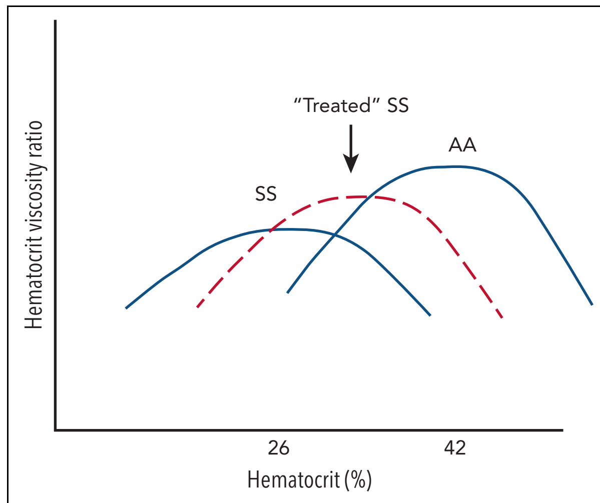

Schematic illustrating the HVR as a function of hematocrit for patients with SS hemoglobin (untreated), SS hemoglobin (treated with hydroxyurea or chronic transfusions), and AA hemoglobin. HVR is a metric of the microvascular oxygen-delivery potential and balances the positive effects of increased oxygen delivery vs the negative effects of increased viscosity. The hematocrit that optimizes HVR, as well as the peak HVR obtained, is lower in patients with SS compared with those with AA hemoglobin. In SCD, effective therapies must not only increase hemoglobin but improve red cell rheology.

oxygen-carrying capacity. As the number of approved therapies increases, combination therapies may allow hemoglobin levels closer to the lower limits of normal. This work also suggests that current concepts regarding the acceptability of moderate anemia (hemoglobin levels <10 g/dL) need to be reconsidered in the context of cerebral oxygen delivery and brain damage.

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LYMPHOID NEOPLASIA

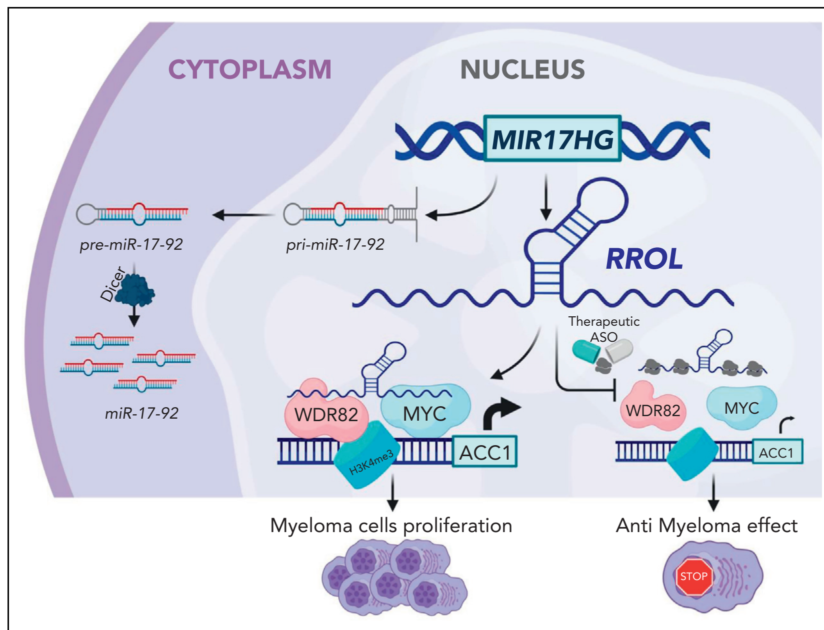
Comment on Morelli et al*, page 391*

RROL lncRNA role in multiple myeloma

Xabier Agirre | Universidad de Navarra; and Centro de Investigación Biomédica en Red de Cáncer

In this issue of *Blood*, Morelli et al[1] show that the novel long noncoding RNA (lncRNA) named *RROL* (RNA Regulator of Lipogenesis), derived from the *MIR17HG* gene, acts as a chromatin scaffold for protein interaction that results in the growth of multiple myeloma cells. Their study shows that *RROL* inhibition exhibits a potent antimyeloma activity both in vitro and in different in vivo models, suggesting a therapeutic potential.

LncRNAs are genes composed of more than 200 nucleotides that lead only to RNA transcripts, which do not translate into proteins, and have tissue- and cell-type–specific expression. The functional mechanism of the vast majority of identified lncRNAs is unknown or unexplored, but owing to the results obtained in diverse studies carried out in recent years, it has been possible to elucidate that lncRNAs, like coding genes, are involved in all crucial human cell functions. Although we still have much to discover, we also know that lncRNAs can be expressed from different genome regions (introns, intergenic) or chromatin states (promoters, enhancers), which can be very important in defining their likely function and can suffer various aberrations owing to both genetic and epigenetic alterations. Consequently, the deregulation of lncRNA expression can



RROL is transcribed from *MIR17HG* lncRNA and mediates the assembly of the MYC-WDR82 protein complex and promotes its occupancy in the promoter region of the *ACC1* gene, leading to the expression of *ACC1* and to the proliferation of MM cells. Therapeutic antisense oligonucleotides (ASOs) against *RROL* expression dismantle the *RROL*-MYC-WDR82-*ACC1* complex, resulting in an antiproliferation effect for MM cells. Created with BioRender.com.

affect all biological cell functions, contributing to human carcinogenesis, metastasis, and even resistance to chemotherapy. In this sense, in the case of multiple myeloma (MM), the altered expression of lncRNAs has been associated with the development and progression of the disease, which affects patient survival.[2,3] This body of knowledge places us in a highly promising novel scenario in which carrying out thoughtful studies about the role of lncRNAs in relation to human tumors has great potential. LncRNAs may be the key to better stratification of patients with cancer, ie, as a biomarker for survival, and may provide essential targets for the development of innovative therapeutic strategies to improve the response to current chemotherapy treatments and improve patients' quality of life.

To identify lncRNAs involved directly in MM survival and proliferation, Morelli et al performed systematic targeted viability screening in MM cell lines using a CRISPR interference library against 913 lncRNAs expressed in MM patient samples and cell lines. The authors determined that the inhibition of *MIR17HG* lncRNA expression led to the greatest decrease in MM cell growth, showing similar potential to inhibition of key MM genes such as MYC or IRF4, suggesting that *MIR17HG* lncRNA is the most critical lncRNA in terms of dependency in MM. *MIR17HG* is known as the precursor of microRNA (miRNA) cluster *miR-17-92*, and these miRNAs have been shown to be involved in different types of human tumors including MM, as previously shown by the authors.[4] In addition to the miRNAs, in this study the authors observed that in MM cells, this *MIR17HG* gene produced a nuclear lncRNA transcript that they named *RROL* (see figure). They observed that high *RROL* expression was associated with shorter event-free and overall survival in 3 large cohorts of patients with newly diagnosed MM. These results demonstrated the potential of *RROL* as a biomarker for MM. Interestingly, the authors found that the inhibition of the *RROL* expression was the main factor responsible, regardless of the action of *miR-17-92*, for the essentialness of *MIR17HG* observed in MM and was, therefore, the principal mediator of MM dependence on this *MIR17HG* gene.

Morelli et al also demonstrated that *RROL* bound MYC and WDR82 proteins, facilitating their assembly and promoting their occupancy especially in the promoter region of *ACC1* gene. They showed that WDR82, a regulatory component of the SET1 methyltransferase complex, was necessary to increase the 3HK4me3 levels in the promoter region of *ACC1*, which was the fundamental epigenetic modification for MYC to bind to the chromatin of this promoter region and generate transcriptional activity by increasing the expression of the last piece of this complex, which is the *ACC1* gene. *ACC1* (or *ACACA*, acetyl-CoA carboxylase alpha) is the first and rate-limiting enzyme in the fatty acid biosynthesis or de novo lipogenesis pathway, promoting tumorigenesis or, in this case, proliferation of MM cells (see figure). These results indicate that the aberrant expression of *RROL* has a direct impact on the metabolic adaptation of MM cells, which is absolutely necessary for their malignant growth and for continuous production of monoclonal immunoglobulins. Furthermore, this suggests that in order to have the most complete metabolic information, we need to take into account the expression of lncRNAs, as well as the coding genes, in the configuration of human metabolic pathways and the construction of genome-scale human metabolic models, which is being carried out with great effort and dedication by the human genome-scale metabolic network, known as HUMAN1.[5]

Finally, one of the most interesting points in the study by Morelli et al is that they developed 2 therapeutic ASOs against *RROL* lncRNA expression, showing their inhibition of the *RROL*-MYC-WDR82-*ACC1* complex and their antimyeloma efficacy in 3 different in vivo MM models. Remarkably, this study reemphasizes the importance of RNA medicine or RNA-targeting therapeutics and suggests the urgent need to develop strategies that target the expression of disease-relevant RNA molecules or their structures and delivery technologies to enable these RNA-based therapies to reach their target cells.[6,7] This will surely lead to great advances in the treatment of patients with cancer, here MM, and of other human diseases.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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PLATELETS AND THROMBOPOIESIS

Comment on Marín-Quílez et al, page 406

GALE force in platelet production

Attila Braun and Elmina Mammadova-Bach | Ludwig Maximilians University

In this issue of *Blood*, Marín-Quílez et al identify novel functions of UDP-galactose-4-epimerase (GALE) that play a critical role in the glycosylation of maturated megakaryocytes and platelets.[1]

Growing experimental evidence suggests that genetic mutations affecting glycosylation and/or sialylation may contribute to abnormal platelet production and function. There are 2 types of glycosylation found in mammals: N-linked glycosylation transfers a mannose oligosaccharide to the asparagine residue within the sequon, whereas O-linked glycosylation occurs between N-acetylgalactosamine and serine or threonine residues during posttranslational modification. Hypoglycosylation results in congenital disorders of glycosylation (CDG) in humans. CDG patients frequently have altered hemostasis, such as thrombosis or bleeding complications, but the role of platelet glycosylation has not been rigorously explored.[2] The biosynthesis of glycans (sugar trees) is usually terminated by the addition of sialic acid "cap" to prevent further chain elongation. Sialic acids are exposed on the platelet surface and regulate protein interactions, platelet aggregation, and adhesion. Aging platelets lose sialic acids from glycoproteins located on the membrane surface, forming desialylated platelets.[3] The hepatic endocytic complex regulated by the Ashwell-Morell receptor removes these desialylated platelets from circulation.[3] The abolished function of the sialyltransferase gene (*St3gal4*$^{-/-}$) in mice results in thrombocytopenia, due to decreased sialylation and the exposure of terminal galactose residues on the platelet glycan surface.[4] The *GNE* gene encodes a bifunctional enzyme, which controls sialic acid biosynthesis, and mutation of *GNE* causes thrombocytopenia. The *β4GalT1* gene encodes a key enzyme involved in the addition of galactose moieties to glycoproteins. Mice transplanted with fetal liver-derived *β4GalT1* knockout hematopoietic cells have profound thrombocytopenia, and in vitro cultivated mutant megakaryocytes are unable to produce proplatelets.[5] Abolished *Slc35a1* gene function also reduces sialylation in megakaryocytes and platelets, thereby inducing thrombocytopenia.[6] Altogether, these results suggest that altered glycosylation, reduced sialic acid biosynthesis, or transfer to the sugar trees increases the number of desialylated platelets in the circulation, which is likely to be the major cause of reduced platelet life span and consequent thrombocytopenia in many CDG patients.

GALE encodes the UDP-galactose-4-epimerase enzyme, which regulates the final step of the Leloir galactose metabolic pathway, converting UDP-galactose to UDP-glucose. Abolished or dysregulated GALE functions result in an autosomal recessive form of type III galactosemia, leading to the accumulation of galactose and galactose-1-phosphate in the cell. CRISP-Cas9–mediated GALE deficiency in human cells triggers imbalanced nucleotide sugar content, reducing basal levels of UDP-N-acetylgalactosamine, thereby dramatically changing the process of glycoprotein and glycolipid biosynthesis. GALE deficiency also results in Fas hypoglycosylation and hypersensitivity to Fas ligand–induced cell death.[7]

Prior studies had already described rare GALE mutations with resultant hematologic defects in patients, including thrombocytopenia with dysplastic megakaryocytes.[8,9] Reduced GALE expression with anti-GALE short hairpin RNA in CD34$^+$-derived megakaryocyte precursors results in impaired megakaryocyte differentiation in vitro, suggesting that GALE plays a regulatory role in megakaryopoiesis and platelet production. Although GALE deficiency has been described in human patients, the exact in vivo pathologic mechanisms remain unclear, owing to the limited number of cases and the lack of humanized mouse model.

Marín-Quílez et al show that GALE is localized in the endoplasmic reticulum (ER) and that its expression is increased in mature megakaryocytes but reduced in platelet-like particles, indicating a restricted, time-dependent function of GALE during late steps of megakaryopoiesis (see figure). They identify 3 new GALE mutations in human patients with macrothrombocytopenia, associated with moderate to severe bleeding tendency. GALE-mutant patients had giant or gray platelets with aggregation and degranulation defects, which account for their bleeding diathesis. The enzymatic activity of UDP-galactose-4-epimerase and N-acetyl-lactosamine levels were strongly reduced, indicating abnormal glycosylation in megakaryocytes and platelets. GPIbα and β1 integrin were hypoglycosylated in GALE-mutant megakaryocytes, both proteins were mainly accumulated in the ER and cytoplasm, and externalization to the plasma membrane was strongly impaired. Defective glycosylation of GPIbα has been described in patients with Bernard-Soulier syndrome with consequent accumulation of GPIbα in the ER,[2] and similar to GALE mutant platelets, only residual GPIbα protein was externalized. FLNA and GPIbα interaction regulates posttranslational assembly and trafficking of the GPIb-IX-V

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