From cell surface to nucleus: CCRL2 regulates response to hypomethylating agents in myelodysplastic syndromes

Caner Saygin

Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, IL, USA

Correspondence: C. Saygin
caner.saygin@uchicagomedicine.org

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Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis with varying degrees of dysplasia and peripheral cytopenias.[1] The treatment of MDS focuses on improving the cytopenias and alleviating transfusion requirements, while preventing progression to secondary acute myeloid leukemia (AML).[2] Hypomethylating agents (HMA) are the standard of care for treatment of high-risk MDS, and several studies looking at combination therapies with newer agents failed to show survival benefit.[3] Patients with high-risk MDS or secondary AML have a dismal prognosis, especially those in whom HMA fail. New therapies are urgently needed to improve outcomes for these patients.

In this issue of *Haematologica*, Karantanos *et al.* report the role of CCRL2 in driving resistance to HMA therapy in cell lines and mouse models of MDS and secondary AML.[4] Previous work from the authors showed that the chemokine receptor CCRL2 was highly expressed in $CD34^+$ stem/progenitor cells from patients with MDS and secondary AML.[5] High CCRL2 expression was associated with poor survival in these patients, and the authors' mechanistic studies revealed that JAK-STAT pathway activation was the downstream mediator of the effect of CCRL2. They also found that $CCRL2^{high}$ cells had increased expression of DNA methyltransferase 1 (DNMT1) when compared to their $CCRL2^{low}$ counterparts. Given the adverse prognosis associated with high CCRL2 levels, Karantanos *et al.* tested the hypothesis that CCRL2 might be a driver of HMA resistance in MDS and AML, and could also serve as a biomarker of response to HMA.

In an unbiased RNA-sequencing analysis comparing control *versus* CCRL2 knockdown (KD) TF-1 cells (a human erythroleukemia cell line), the authors observed downregulation of genes involved in polycomb repressive complex 2 (PRC2) (e.g., *AEBP2*, *EED*, and *SUZ12*), DNA methylation (e.g., *DNMT*), DNA damage repair and retinoblastoma pathways. They validated these results in the MDS-L cell line and the publicly available BloodSpot database, which includes data from 228 MDS cases. Given the association between CCRL2 expression and genes involved in DNA methylation and histone modification, the authors investigated the efficacy of HMA therapy (i.e., azacitidine) in CCRL2 KD cells (Figure 1). When compared to the control cells, CCRL2 KD cells had decreased self-renewal capacity upon azacitidine treatment, as assessed using *in vitro* serial transplant experiments. CCRL2 KD enhanced the cytotoxicity of azacitidine therapy, and CCRL2 KD cells showed evidence of differentiation by surface immunophenotyping after azacitidine treatment. In contrast, CCRL2 overexpression decreased the clonogenicity inhibition effect of azacitidine, and increased resistance to HMA therapy. An *in vivo* model using the MDS-L cell line showed reduced tumor burden in CCRL2 KD mice treated with azacitidine compared to that in mice engrafted with CCRL2 wildtype cells and treated with azacitidine. The former had higher CD11b expression as well, which may suggest increased differentiation of malignant cells. Finally, in a cohort of 20 MDS patients, there was no correlation between CCRL2 expression and age, revised International Prognostic Scoring System score, cytogenetics and number of mutations. Interestingly, CCRL2 levels were higher in male patients, and in patients with MDS/myeloproliferative neoplasm overlap or *SETBP1* mutations. Patients with high CCRL2 expression had lower rates of complete remission after HMA therapy.

This study provides several important points for reflection. First, the authors identified a high-risk subset of MDS patients with high CCRL2 expression, which might be a predictor of response to HMA therapy. They also provide critical preclinical data showing synergy between low CCRL2 levels and azacitidine treatment. This might be due to the impact of CCRL2 signaling on the expression of DNA methyltransferases and members of the PRC2 complex. As a seven transmembrane protein on the cell surface, CCRL2 may be targeted by antibody-based approaches (such as antibody-drug conjugates) or chimeric antigen receptor T-cell immunotherapy. The results of the study by Karantanos *et al.* do, therefore, have immediate clinical and translational applications.

While CCRL2 is being recognized as an important protein

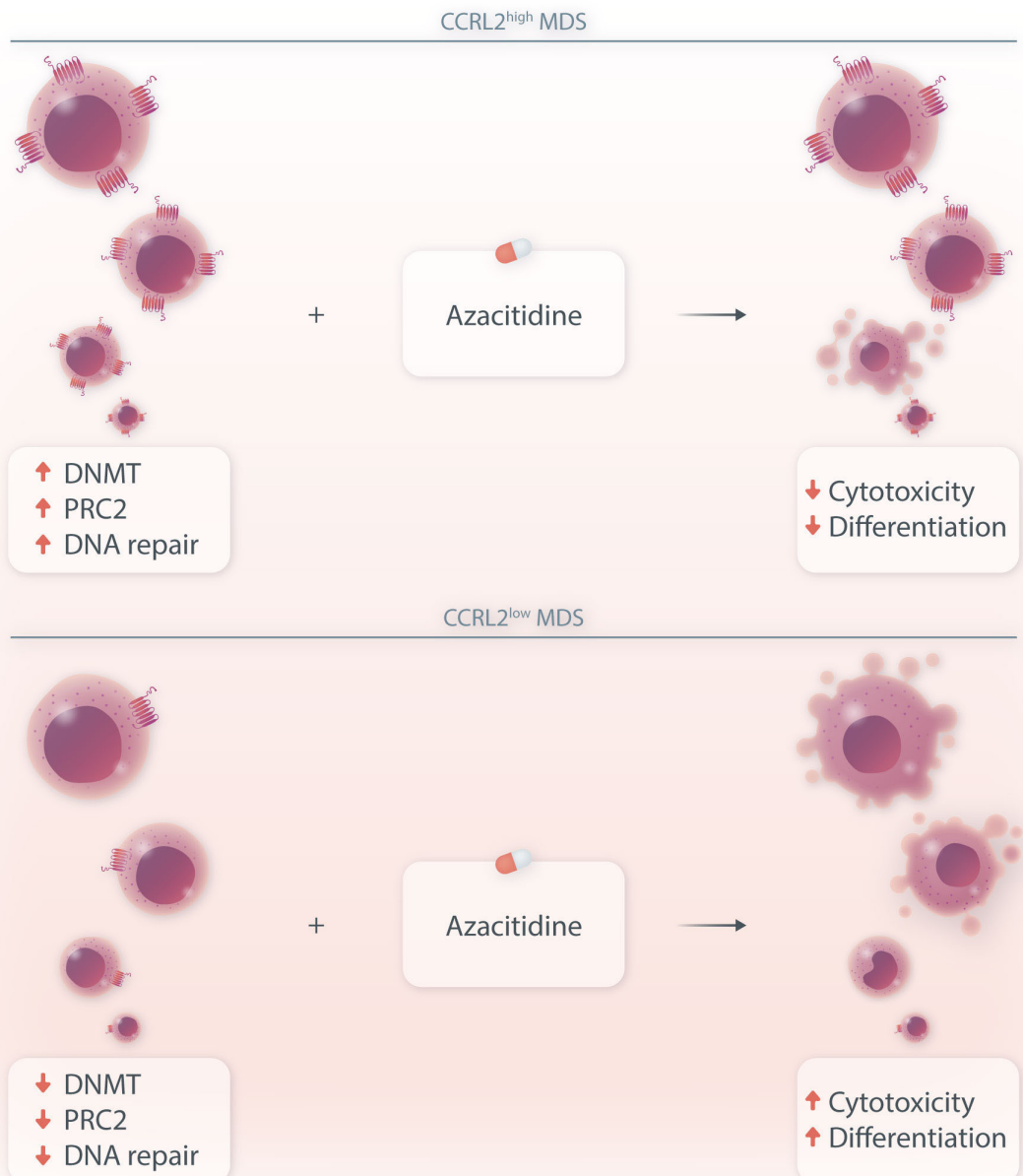

Figure 1. CCRL2^{high} myelodysplastic syndrome cells are characterized by increased expression of DNA methyltransferase and polycomb repressive complex 2, resulting in decreased response to azacitidine therapy. MDS: myelodysplastic syndrome; DNMT: DNA methyltransferase; PRC2: polycomb repressive complex 2.

in myeloid neoplasms, several questions remain unanswered. What are the mechanisms by which CCRL2 regulates the expression of *DNMT* and PRC2 complex genes? Does CCRL2 interact with a ligand on MDS and AML cells? Can CCRL2-targeted therapies rescue HMA-refractory MDS cases? Recent studies have also shown the role of CCRL2 in augmenting anti-tumor T-cell immunity in solid tumors.[6,7] It will, therefore, be interesting to investigate the role of CCRL2 in the MDS microenvironment, as well as its impact on therapies targeting innate immune checkpoints such as CD47.

Disclosures

No conflicts of interest to disclose.

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References

1. Saygin C, Godley LA. Genetics of myelodysplastic syndromes. Cancers (Basel). 2021;13(14):3380.
2. Brunner AM, Leitch HA, van de Loosdrecht AA, Bonadies N. Management of patients with lower-risk myelodysplastic syndromes. Blood Cancer J. 2022;12(12):166.
3. Sekeres MA. Improving clinical trials in higher-risk myelodysplastic syndromes. Best Pract Res Clin Haematol. 2022;35(4):101406.
4. Karantanos T, Teodorescu P, Arvanitis M, et al. CCRL2 affects the sensitivity of myelodysplastic syndrome and secondary acute myeloid leukemia cells to azacitidine. Haematologica. 2023;108(7):1886-1899.
5. Karantanos T, Teodorescu P, Perkins B, et al. The role of the atypical chemokine receptor CCRL2 in myelodysplastic syndrome and secondary acute myeloid leukemia. Sci Adv. 2022;8(7):eabl8952.
6. Del Prete A, Sozio F, Schioppa T, et al. The atypical receptor CCRL2 is essential for lung cancer immune surveillance. Cancer Immunol Res. 2019;7(11):1775-1788.
7. Yin W, Li Y, Song Y, et al. CCRL2 promotes antitumor T-cell immunity via amplifying TLR4-mediated immunostimulatory macrophage activation. Proc Natl Acad Sci U S A. 2021;118(16):e2024171118.