Mutant *CALR* functions: gains and losses

Daniel Prins and Anthony R. Green, Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge

In this issue of *Blood*, Shide et al. separate the roles of loss of a normal *CALR* allele and gain of a mutant *CALR* allele in *CALR*-driven essential thrombocythaemia (ET)[1].

Approximately one in four patients with ET, a blood cancer characterized by over-production of platelets, has a frameshift mutation in the gene encoding calreticulin (*CALR)*[2,3]. Research to date has demonstrated a gain-of-function role for the frameshifted CALR protein in binding to the thrombopoietin receptor (TpoR), thus acting as a rogue ligand and triggering proliferation and megakaryopoiesis. However, there has been less investigation of the effects of the loss of a normal *CALR* allele: *CALR*$^{-/-}$ mice are embryonic lethal due to malformation of the heart, preventing study of haematopoiesis in these mice[4].

Shide et al. generated a mouse with haematopoietic-specific deletion of the *CALR* allele to allow separation of gain- and loss-of-function effects of the *CALR* mutation. Loss of one or both *CALR* alleles in haematopoietic cells had little effect on bone marrow haematopoiesis and did not cause ET, though loss of both alleles increased extramedullary haematopoiesis. In transplantation experiments, loss of one or both alleles increased bone marrow repopulation in primary recipients, but only loss of a single allele maintained this advantage in secondary transplants, indicating that *CALR* haploinsufficiency yields a competitive advantage. The authors further demonstrate that a *CALR*del52 transgene can only drive a MPN phenotype (thrombocytosis) in transplants when WT CALR is haploinsufficient. Finally, they use transcriptomic data to investigate potential mechanisms underlying the advantage of *CALR*$^{+/-}$ cells. Compared to WT stem/progenitor cells, they find that *CALR*$^{+/-}$ cells have increased expression of E2F targets, suggestive of higher cell cycling. When comparing WT and *CALR*$^{+/-}$ cells also expressing transgenic *CALR*del52, the authors again show upregulation of E2F target genes, in addition to an increase in stem cell self-renewal pathways and decreases in pathways responsive to the pro-inflammatory cytokines TNF-α and IFN-γ.

These results advance the field in several ways, not least by demonstrating a previously unknown role for normal CALR in haematopoiesis. Additionally, they shed light on important questions about differences between mouse models and human patients: while human patients typically show clonal disease, mouse models to date have shown either no[5,6] or a slow-rising[7] competitive advantage. The results of Shide et al. suggest that an approach wherein two WT *CALR* alleles are maintained[6] may

show no competitive advantage, as this requires loss of a normal *CALR* allele. Therefore, knock-in approaches may be more informative, as they more closely resemble patients. One mouse model with knock-in of a humanized *CALR*del52 allele gave rise to a strong ET phenotype but no advantage within transplants[5]. A separate CRISPR-Cas9-based mouse model of the *CALR*del52 mutation within the mouse *CALR* gene exhibited a lower platelet phenotype and a slow-rising competitive advantage in primary transplants, consistent with the requirement for *CALR* haploinsufficiency to yield a stem cell advantage[7]. The differences between the models remain unresolved, but may reflect differences in how murine and human frameshifted CALR protein bind to TpoR[7] or other differences in the generation of the models. Furthermore, the results of Shide et al. emphasize that the level of *CALR*del52 expression is crucial: progression to myelofibrosis in mouse models is only seen when *CALR*del52 is highly expressed, as via retroviral expression[6] or homozygosity of the knocked-in *CALR*del52 allele[5]. Together, these results underscore the importance of ensuring that model systems resemble the situation in human patients, who have one mutant and one wild-type allele. Similarly, while results from transplant experiments can give insight into stem cell function, it is important to remember that these are artificial settings that do not resemble steady state haematopoiesis in patients.

Going forward, more research will be necessary to understand why *CALR* haploinsufficiency is so critical to bestow a competitive advantage on stem cells: while there are indications of increased cell cycling and self-renewal, no direct mechanism has been proposed. It remains to be seen if this mechanism will rely on one of the canonical functions of CALR, such as protein chaperoning or calcium signalling[8], or if the mechanism will be as surprising as the discovery of TpoR activation by frameshifted CALR protein. As the transcriptomic data were obtained from stem/progenitor cells from transplant recipients, they may be confounded by the stresses of the transplantation protocol on both donor cells and the recipient bone marrow niche. Overall, this study is an important advance in our understanding mutant *CALR*-driven ET: in addition to the well-studied role of mutant CALR as a rogue ligand for TpoR, Shide et al. show a role for the loss of a wild-type CALR allele that warrants further investigation.

References

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