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Both sides now: losses and gains of mutant CALR

Ann Mullally | Harvard Medical School

In this issue of *Blood*, Di Buduo et al report on the consequences of loss of the interaction between mutant calreticulin (CALR) and endoplasmic reticulum (ER) resident protein 57 (ERp57) as it pertains to altered calcium signaling in myeloproliferative neoplasms (MPNs).[1]

"Something's lost but something's gained in living every day" sang Joni Mitchell in 1969 in what would become one of her most popular and widely recorded songs of all time, "Both Sides Now." In the context of mutant CALR (an ER chaperone protein that causes approximately 20% to 25% of MPNs[2,3]), almost everything that has been published to date in understanding its mechanism of oncogenicity has focused on the aberrant protein interactions mutant CALR gains. Motivated by earlier reports that mutant CALR also loses protein-protein interactions found in wild-type CALR,[4,5] Di Buduo et al focused on investigating the consequences of the loss of the interaction between mutant CALR and ERp57, a member of the protein disulfide isomerase family.[1]

In MPNs, mutations in *CALR* occur as heterozygous insertions and/or deletions in the last coding exon, the end result of which is the generation of a novel, mutant-specific C-terminal peptide that is common across all mutations.[2,3] *CALR* mutations are classified as type 1 or type 2, depending on the number of negatively charged amino acids eliminated (more removed in type 1 than in type 2).[6] The mutant C terminus of mutant CALR differs in 2 main ways from the wild-type C terminus: (1) the mutant has a positive electrostatic charge unlike the wild-type, which is negatively charged, and (2) there is a loss of calcium binding sites in the mutant, in particular in the type 1 mutation. A key mechanism of mutant CALR oncogenicity is gain of a pathogenic binding interaction with the thrombopoietin receptor MPL, which results in the activation of MPL-JAK/STAT signaling in a ligand-independent manner.[7-9] It has been shown that binding to MPL[7-9] and the positive charge of the mutant CALR C terminus[7] are both required for the oncogenic activity of mutant CALR, but the role of perturbed calcium signaling has had limited study to date.[6]

The article by Di Buduo et al investigates calcium signaling in human megakaryocytes in both normal and *CALR*-mutant MPN contexts. In the ER of human megakaryocytes, CALR binds ERp57, which is known to interact with stromal interaction molecule 1 (STIM1), an ER calcium sensor, to modulate the activity of store-operated calcium (Ca^{2+}) entry (SOCE), a regulator of calcium flux into the cell. When MPL signaling is activated in response to thrombopoietin in megakaryocytes, this results in calcium release from the ER to the cytosol in an inositol triphosphate (IP3)–dependent manner, which in turn activates SOCE, triggering calcium influx into the cell across the plasma membrane. In megakaryocytes from patients with *CALR*-mutant MPNs, the authors demonstrate diminished interactions between mutant CALR, ERp57, and STIM1, resulting in activation of SOCE, which generates spontaneous cytosolic calcium flows. Importantly, cellular proliferation has previously been linked to calcium signaling inside the cell,[10] and the higher in vitro proliferation rate of *CALR*-mutant megakaryocytes (compared with normal megakaryocytes) in response to thrombopoietin stimulation is diminished in the presence of a SOCE inhibitor. In summary, the authors conclude that *CALR*-mutant megakaryocytes are more proliferative at least in part as a consequence of activated SOCE signaling, which occurs as a result of the loss of normal CALR protein binding interactions in the ER. Accordingly, inhibiting SOCE and/or other calcium regulatory pathways may represent a therapeutic vulnerability in mutant CALR-driven MPNs.

Strengths of the work by Di Buduo et al include the novelty of the concept and the use of primary human megakaryocytes for several of the experiments. A limitation of the study is that all of the work was performed in vitro. Although this is reasonable for intricate studies of intracellular calcium flux, it would be valuable to test the impact of inhibiting calcium regulatory pathways in vivo by using preclinical mutant CALR MPN models, in particular to determine whether MPL-expressing disease-initiating *CALR*-mutant MPN stem cells are preferentially sensitive to SOCE inhibition. Notably, an earlier study demonstrated abnormal cytosolic calcium signaling in cultured megakaryocytes from *CALR*-mutant MPN patients with a type 1 mutation, but not those with a type 2 mutation.[6] The study by Di Buduo et al did not detect substantial differences between type 1 and type 2 *CALR* mutations with respect to calcium signaling or sensitivity to SOCE inhibition, which is somewhat surprising given that the type 1 mutation results in the loss of several calcium binding sites. Although type 1 and type 2 *CALR* mutations generate a common mutant-specific CALR C-terminal peptide, they demonstrate differences in MPN disease phenotype.[6] Additional studies may reveal more subtle differences in calcium signaling between type 1 and type 2 *CALR* mutations.

In each refrain of "Both Sides Now," Joni Mitchell concludes that illusions are what she recalls; regarding clouds, love and life, she really doesn't know these at all. Thankfully, with respect to mutant CALR, we could not be further from illusions, given the rapid pace in advancing the understanding of the mechanism that underlies how a mutated chaperone protein causes MPNs. The study by Di Buduo et al is an important contribution to the field and opens up a novel avenue of investigation focused on the role of calcium regulatory pathways. As with all studies of mutant CALR biology, the responsibility now rests with us to endeavor to exploit new mechanistic insights for therapeutic benefit to help our patients with MPN live many, many days.

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