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The JAK2V617F mutation disrupts the regulated association between calreticulin and the glucocorticoid receptor observed in normal erythroid cells

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Calreticulin (CALR) is a multifunctional protein normally found within the lumen of the endoplasmic reticulum that mediates the cellular response to Ca^{2+} by chaperoning other proteins to their acting sites. Somatic loss-of-function mutations in the *CALR* gene were recently discovered in 70% of patients with the Philadelphia-negative myeloproliferative neoplasm (MPN) primary myelofibrosis (PMF) who did not harbor gain-of-function mutations of *JAK2*[1,2]. Nevertheless, the JAK2 pathway is constitutively activated also in patients carrying CALR mutation and treatments with JAK2 inhibitors are effective not only in MPN patients (PMF and polycythemia vera, PV) harboring *JAK2* mutations but also in PMF patients harboring mutations in *CALR*[3].

We have previously reported that erythroid cells from PV and PMF patients express abnormal activity of the glucocorticoid receptor (GR), a nuclear receptor whose transcriptional activity plays an important role in the regulation of stress erythropoiesis[4,5]. Since GR is one of the numerous proteins regulated by CALR[6], we hypothesized that in human erythroid cells CALR regulates GR functions and that this regulation is disrupted both by *CALR* and *JAK2* mutations in MPN. In this study we tested this hypothesis by determining whether GR and CALR are associated in normal erythroid cells and whether this association is impaired in those from MPN patients. First, biochemical studies determined that human erythroblasts (Erys) expanded ex-vivo from normal stem cell sources [cord blood (CB) and adult blood (AB)] and from MPN patients contain similar levels of CALR and GR. Analyses of cell fractions indicated that in normal Erys, CALR was constitutively localized in the cytoplasm while GR was detected either in the cytoplasm or in the nucleus, depending on the growth factor (the glucocorticoid receptor agonist dexamethasone, erythropoietin or stem cell factor) to which they had been exposed. Second, robust levels of CALR and GR expression were also detected by confocal microscopy. In addition, this analyses revealed that in Erys expanded from normal sources CALR and GR are co-localized in the cytoplasm and that the cytoplasmic association between the two proteins is increased by growth factor deprivation and further enhanced by stimulation with growth factors that activate the JAK2/STAT5 signaling (dexamethasone and/or erythropoietin) while it is inhibited by stimulation with factors that do not use this pathway (stem cell factor). By contrast, in Erys expanded from MPN carrying either *CALR* or *JAK2* mutations, CALR and GR are not associated and remain not associated when the cells are exposed to dexamethasone or erythropoietin. However, in Erys from *JAK2*V17F-positive MPN patients, association between CALR and GR in the cytoplasm is restored by exposing the cells to the JAK2 inhibitor ruxolitinib. These results suggest that CALR/GR association is a downstream event induced by the JAK2/STAT5 pathway and identify for the first time that CALR functions are impaired in erythroid cells from MPN patients carrying *JAK2* mutations.

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