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Mutational Landscape in Myeloproliferative Neoplasms (MPN) and Eosinophilia: Diagnostic and Treatment – Section 12

CALR Mutations in MPN

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Take home messages:

- Mutant CALR protein interacts with and activates TpoR, traffics via the secretory pathway bringing TpoR with immature N-glycosylation to the cell-surface, which is required for full-oncogenic effects, the mutant CALR-TpoR being potential therapeutic targets for MPN treatment
- Mutant CALR proteins are secreted and might play immunosuppressive roles, as well as potentially pro-oncogenic roles
- JAK2-dependent signaling pathways are persistently activated in a TpoR-dependent manner while calcium homeostasis is perturbed in a TpoR-independent manner

Introduction

The BCR/ABL-negative myeloproliferative neoplasms (MPNs) are clonal hematological malignancies characterized by the acquisition of somatic mutations in stem/progenitor cells leading to overproduction of myeloid blood cells. Recurrent somatic mutations in the gene coding for the chaperone protein calreticulin (CALR) were identified in 2013 in roughly 25% of ET and 35% of PMF cases.[1] Over 60 different CALR mutants resulting from a −1/+2 frameshifting mutation of the *CALR* exon 9 are known, characterized by a de novo C-terminal sequence harboring positively and hydrophobic residues and the loss of the ER-retention signal KDEL. The 2 prevalent mutations in *CALR* are a 52-base deletion (type-1: del52, c.1092_1143del) or a 5-base insertion (type-2: ins5, c.1154_1155insTTGTC). Several studies have demonstrated an essential role for the thrombopoietin receptor (TpoR/Mpl) in the oncogenic effects exerted by CALR mutants. These oncoproteins interact with and activate TpoR leading to persistent JAK2-STAT5/STAT3 signaling.[2–4]

Current states of the art

TpoR and mutant CALR interaction

CALR mutants are directly binding immature forms of receptor and forces their traffic through the secretory pathway to the cell surface,[5,*6] a prerequisite localization necessary for its full oncogenic activity.[*6] Mutant CALRs require oligomerization[*7] in order to activate TpoR by forming TpoR-CALR mutant complexes, which need the most distal N-glycosylation site of TpoR (Asn117) and an intact lectin-binding site on CALR. Aside from the Asn117, a hydrophobic patch in the extracellular domain of TpoR is required for stabilization and activation[*6] and a threshold of positively charged residues in the tail.[8]

CALR and its rogue chaperone activity

The CALR mutants act as rogue chaperones allowing transport to the cell-surface of immature TpoR and also rescue of receptors that are known to be blocked in the endoplasmic reticulum, such as TpoR R102P, which in homozygous state induces congenital amegakaryocytic thrombocytopenia due to inaccessibility to ligand.[*6]

In vivo models

Several in vivo knock-in models are available for CALR del52.[*9,*11] In heterozygous state the phenotype is thrombocytosis, and a slow dominance detected after 120 days post-transplantation.[*11] Not all type I CALR mutants appear to induce the same effect in vivo, as CALR del61 knock-in is induces a much weaker effect than CALR del52 knock-in and only the latter phenotype is transplantable.[*11]

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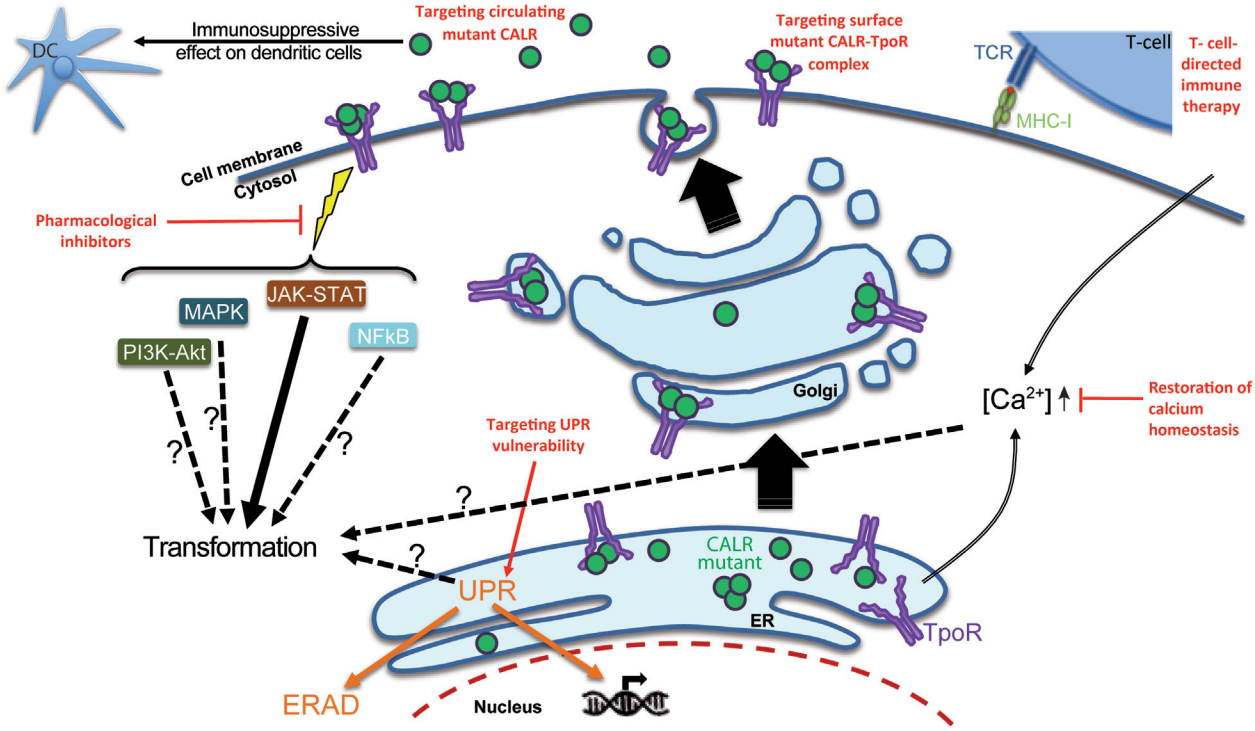

Figure 1. Recent developments in understanding the pathogenicity of mutant CALR in MPNs. Hypothetically, therapeutic approaches could aim at : (i) disruption of mutant CALR-TpoR intracellular and especially cell-surface interaction, (ii) induction of immunological response directed against mutant CALR, TpoR or mutant CALR-TpoR complexes at cell-surface of the mutated clones, (iii) inhibition of downstream activated of pathological signaling pathways (JAK/STAT, PI3K-Akt and MAPK) by specific pharmacological inhibitors alone or in combination, (iv) targeting cells with an abnormal Unfolded Protein Response (UPR)/Endoplasmic-Reticulum-Associated protein Degradation (ERAD) pathways; (v) abnormal NF-kB signaling in hematopoietic stem cells carrying CALR mutations; (vi) targeting and (vii) restoration the normal ER and cytosolic calcium homeostasis. DC, dendritic cells, TCR, T cell receptor, MHC-I, major histocompatibility complex I. Secreted mutant CALR proteins (green) might exert immunosuppressive effects. Both secreted, and cell-surface mutant CALR in complex with TpoR could be targeted by protein therapeutics (ie, antibodies), while small molecules could penetrate cell membranes and target the mutant CALR-TpoR complexes intracellularly.

Calcium and CALR

The alteration of the calcium buffering function of mutant CALR affects cell functionality and signaling, particularly in megakaryocytes, which show constitutive activity of the stored-operated calcium entry (SOCE) machinery leading to sustained TpoR signaling downstream and Mk proliferation.[12] This is due to relocation of CALR mutants to the membrane TpoR and hence decreased formation of the CALR-ERp57-STIM1 complex.

Unfolded protein response, NF-kB signaling and mutated CALR

CALR mutated cells lose their ability to respond to ER stress, in particular the induction of apoptosis through unfolded protein response (UPR).[13] The low expression of CALR mutants observed by many groups has been shown to result from a combination of secretion and ERAD-proteasome degradation.[14] Importantly, the IRE1 arm of UPR is activated from hematopoietic stem cells to megakaryocytes in the case of CALR mutated MPN cells, as shown by a recent single cell RNA and DNA sequencing study.[*15] In contrast, only the mutated long-term hematopoietic stem cells but not later progenitors exhibit activated NF-kB signaling signature.[*15]

CALR secretion and immunosuppressive effect

Recently, secreted CALR has been found circulating in plasma from patients with *CALR* mutated MPNs and from knock-in CRISPR *Calr*^{del52} mice.[*11,16,17] Cellular CALR release has been shown to suppress antineoplastic immune responses through the blockage of dying cancer cells' phagocytosis by dendritic cells.[16] Circulating CALR mutants may stimulate TpoR-expressing cells in an autocrine or paracrine manner, as recently reported.[17] The preference of mutant CALR to oligomerize and bind TpoR with immature sugars may explain why circulating mutant CALR proteins do not induce polyclonal thrombocytosis and might favor the clone.

MHC type I assembly and CALR

As CALR is a key partner in the MHC class I peptide loading complex, the effect of *CALR* mutation has been studied. In the case of CALR frameshifted mutants, the peptide loading of MHC class I is impaired even in the presence of CALR wild type, suggesting that the heterozygous clones harboring mutant CALR are unable to present antigen at their cell surface and to induce an immune response.[18] However, immune responses of CD4+ T-cell to mutant CALR has been observed in healthy individuals, indicating that mutant CALR is immunogenic.[19]

Perspectives

Although conventional MPN therapies (interferon, JAK2 inhibitors, anagrelide, hydroxyurea) have been effective in improving quality life by decreasing symptoms, they are not curative. Particularly, IFN alpha is less active in CALR mutated MPNs than in JAK2 V617F MPNs,[20] where it can induce molecular remissions. Therapies against MPNs driven by CALR mutations could involve several approaches (Fig.1) based on the recent understanding of the

role of cell-surface TpoR-CALR mutant complexes and vulnerabilities given by abnormal UPR and calcium homeostasis.

The accessibility of CALR mutants at cell surface or free in the plasma raises the possibility to develop immunotherapeutic strategies. The inability of mice transplanted with different ratios of wild type and CALR del52 knock-in bone marrow to respond to a peptide immunogen while mice transplanted only with wild type bone marrow raises the issue of tolerance to the novel sequence, potentially due to the presence of the antigen in hematopoietic stem cells, and hence in antigen-derived presenting cells.[*15]

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