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Review

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Calreticulin – a multifaced protein

Kalretikulina – białko o wielu twarzach

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Summary

Calreticulin (CALR) is a highly conserved multi-function protein that primarily localizes within the lumen of the endoplasmic reticulum (ER). It participates in various processes in the cells, including glycoprotein chaperoning, regulation of Ca^{2+} homeostasis, antigen processing and presentation for adaptive immune response, cell adhesion/migration, cell proliferation, immunogenic cell death, gene expression and RNA stability. The role of CALR in the assembly, retrieval and cell surface expression of MHC class I molecules is well known. A fraction of the total cellular CALR is localized in the cytosol, following its retro-translocation from the ER. In the cell stress conditions, CALR is also expressed on the cell surface via an interaction with phosphatidylserine localized on the inner leaflet of the plasma membrane. The above-mentioned mechanism is relevant for the recognition of the cells, as well as immunogenicity and phagocytic uptake of proapoptotic and apoptotic cells.

Lastly, the presence of *CALR* exon 9 gene mutations was confirmed in patients with myeloproliferative neoplasms. Their presence results in an abnormal CALR structure due to the loss of its ER-retention sequence, CALR extra-ER localisation, the formation of a complex with thrombopoietin receptor, and oncogenic transformation of hematopoietic stem cells. It is also known that *CALR* exon 9 mutants are highly immunogenic and induce T cell response. Despite this fact, *CALR* mutant positive hematopoietic cells emerge. The last phenomenon is probably the result of the inhibition of phagocytosis of the cancer cells exposing CALR mutant protein by dendritic cells.

Keywords: **Calreticulin, oncogenic transformation, immune system escape, myeloproliferative neoplasms**

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Abbreviations: **AML** – acute myeloid leukemia, **CALR** – calreticulin, **ER** – endoplasmic reticulum, **ET** – essential thrombocythemia, **ICD** – immunogenic cell death, **MPL** – thrombopoietin receptor, **MPN** – myeloproliferative neoplasms, **PMF** – primary myelofibrosis.

INTRODUCTION

Calreticulin (CALR) was first identified in 1974 as a calcium-binding protein in the endoplasmic reticulum (ER) in rabbit skeletal muscles [51]. CALR is a 46 kDa protein containing 417 amino acids and is encoded by the CALR gene located on chromosome 19p13.13 with 9 exons. It is a highly conserved multi-function protein that primarily localizes within the lumen of the ER where it functions as a glycoprotein-folding chaperone and a Ca^{2+} storage molecule [40]. CALR is made up of three protein domains: (I) an amino (N)-terminus lectin binding domain containing an ER targeting signal sequence (residues 18–197); (II) a proline-rich P-domain containing high-affinity binding sites for Ca^{2+} (residues 198–308); and (III) a C-domain containing multiple low-affinity Ca^{2+}-binding sites and an ER retention signal (KDEL) (residues 309–417) [19, 63]. The structure of the CALR C-domain is highly unstable and it has been proposed that its spatial conformation may be closely related to its Ca^{2+} buffering activity and its protein binding functions [47]. The linear representation of human wild type and mutant calreticulin domains is presented in Fig. 1.

CALR participates in various processes in the cells, including protein chaperoning, regulation of Ca^{2+} homeostasis, antigen processing and presentation for adaptive immune response, cell adhesion/migration, cell proliferation, immunogenic cell death, gene expression and RNA stability. CALR changes its own localization in the cell depending on the process it is involved in [14, 40, 46].

Within the ER system, it is involved in the quality control process during protein synthesis. As a chaperone, calreticulin regulates the folding of glycoproteins, including integrin surface receptors, and transporters in the ER [40]. CALR interacts with a glycosylated protein until it is folded correctly. Misfolded proteins which do not fold into the correct three-dimensional structure (native conformation) are recognized by CALR and targeted to the ER-associated degradation (ERAD) pathway. In the structure of CALR, the "folding checking unit" is represented by its N- and P-domains [46].

CALR also functions as an intracellular Ca^{2+} homeostasis regulator, as it contains two calcium binding sites in the P-domain (high-affinity, low-capacity) and C-domain (low-affinity, high-capacity). More than half of calcium ions stored in the ER lumen binds with CALR. Higher levels of CALR may lead to increased intracellular Ca^{2+} storage. In contrast, CALR-deficient cells have a lower capacity for Ca^{2+} storage in the ER lumen. Elimination of the CALR gene in mice (knockout mice for calreticulin) resulted in defects in the heart development and function. Moreover, calreticulin-deficient cells inhibited bradykinin-induced Ca^{2+} release from the ER and impaired nuclear import of the NF-AT3 transcription factor [45, 46, 48].

In the cytoplasm, CALR plays a role in the control of cell adhesion through the regulation of fibronectin expression and matrix deposition. This process is mediated via a c-Src-regulated pathway and the fact that c-Src activity is inversely related to calreticulin abundance [53]. The alternation of CALR levels affects cell adhesion on the extracellular matrix. CALR specifically binds to the cytoplasmic KXGFFKR motif of the integrin α-subunit, mediating integrin activation and transducing Ca^{2+} signalling between the integrins and Ca^{2+} channels [12]. In the extracellular matrix, CALR is involved in wound healing [46].

Calreticulin plays an important role in the immune system, especially in terms of the peptide loading complex (PLC) dynamics, major histocompatibility complex class I molecules (MHC I) cell surface expression, and adoptive immune response [10]. PLC facilitates the assembly and folding of MHC class I molecules with antigenic peptides within the ER. The PLC comprises of a transporter associated with protein processing (TAP), thiol oxidoreductase of the endoplasmic reticulum (ERp57), MHC class I molecules and tapasin (transmembrane glycoprotein, mediating the interaction between newly assembled MHC class I molecules and TAP). Peptides that bind to MHC class I heterodimers typically derive from the cytosol, and are transported by TAP into the ER lumen. CALR protein is a constituent of the PLC, but its association depends on the presence of MHC class I molecules. ATP is a key factor that modulates the chaperone activity of CALR and CALR interactions with MHC class I molecules [10, 58]. The calreticulin ATP–binding site is localized in the lectin binding domain. High-affinity calcium binding by CALR is required for optimal nucleotide binding. Therefore, the CALR mutants affecting the ATP or high-affinity calcium binding exhibit prolonged associations with monoglucosylated forms of cellular MHC class I molecules, delaying the MHC class I dissociation from the PLC and their transit through the secretory pathway [71]. Reduced surface MHC class I levels in the cells lacking CALR confirm the role of calreticulin in the process of MHC I molecules recruitment [4].

Calreticulin may promote the phagocytosis of apoptotic stressed cells and certain cancer cells [65].

TRANSCRIPTIONAL REGULATION OF THE CALRETICULIN GENE

Taking into account the differential expression of CALR in many tissues and cell types, it is not surprising that the *CALR* gene is under tight control of several specific transcription factors. Although the *CALR* gene promoter contains a number of putative transcription-factor-binding sites, only a few factors were experimentally confirmed to regulate *CALR* gene transcription in a direct manner during cardiogenesis and in general. Nkx2.5 (NK2 transcription factor related locus 5), GATA-6 (GATA-binding protein 6) and MEF2C (myocyte enhancer factor 2C) which are critical regulators of the developing heart, and PPARγ (peroxisome-proliferator-activated receptor γ) – critical for adipogenesis, act as *CALR* gene activators. COUP-TF1 (chicken ovalbumin upstream promoter transcription factor 1) – highly expressed during embryonic development

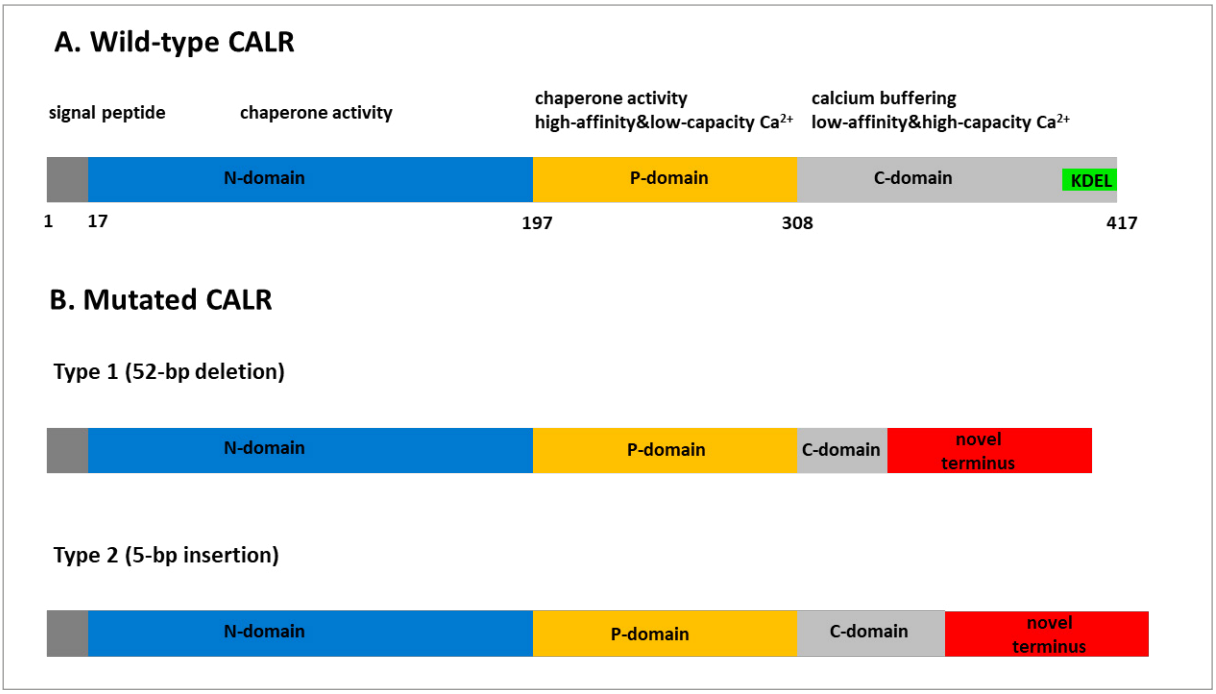

Fig. 1. Linear representation of human wild type (A) and mutant type 1 and 2 (B) calreticulin domains

and Evi-1 (ecotropic viral integration site 1) which may decline CALR gene activity in the postnatal heart, act as *CALR* gene repressors. In addition, Ca^{2+} depletion and ER stress have also been identified as important transcription factors regulating the calreticulin gene expression [25, 42, 46, 50, 57].

CALR PROTEIN AND NEOPLASMS

Several studies have shown that calreticulin is involved both in the development (tumor generation) and progression of different neoplasms. The impact of CALR on tumor formation and progression may depend on different cell types and clinical stages. The tissues of many tumor types express significant higher levels of CALR compared to normal tissues, and CALR expression levels are positively correlated with tumorigenesis [73].

CALR expression levels have shown to be significantly upregulated in oral [9], breast [6, 41], colorectal [2], prostate [1], pancreas [60], oesophageal [15], vaginal [28] and gastric cancer [9]. Elevated CALR levels in the tumor tissue correlate with worse clinical outcome in oesophageal, breast, pancreas, gastric and bladder cancer [40]. The concentration of urinary CALR has the tendency to increase in high grade tumors, and the use of CALR dosage has been proposed as a biomarker for the urothelial cancer marker [34]. In neuroblastoma, increased CALR expression is found to be associated with tissue histotype and better prognosis [7, 32].

CALR mRNA expression has been revealed to be significantly higher in acute myeloid leukemia (AML) compared to other hematologic malignancies. The CALR overexpression blocks the translation of CEBPA, a myeloid key transcription factor, which affects myeloid differentiation [54]. Chao et al. identified calreticulin as a pro-phagocytic signal highly expressed on the surface of several human neoplasms, including AML and lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), non-Hodgkin lymphoma (NHL), bladder cancer, glioblastoma, and ovarian cancer, but minimally expressed on most normal cells. Cell surface calreticulin is required for anti-CD47 antibody-mediated phagocytosis. Increased integrin associated protein (CD47) expression, which is the "receptor" for thrombospondin-1 and the "ligand" for SHP-1-recruiting inhibitory immunoreceptor signal regulatory protein (SIRPα), correlates with high calreticulin levels on tumor cells. It is also necessary for the protection from calreticulin-mediated phagocytosis [8]. Located on the cell surface, CALR serves as a damage-associated molecular pattern and affects both innate and adaptive immunity [17], and has been attributed prognostic values in several cohorts of patients with neoplasms [22]. The secretion of soluble CALR by malignant cells has a strong immunosuppressive effect [38].

According to a study by Fucikova et al., CALR exposure on the plasma membrane of AML blastic cells is associated with significantly better overall survival rates and could be considered a novel powerful prognostic biomarker in patients with AML. It may be associated with an observation that high levels of ecto-CALR are correlated with an increased proportion of circulating T cells specific for leukemia-associated antigens, effector memory $CD4^+$ and $CD8^+$ T cells, and enhanced natural killer cell cytotoxic and secretory functions. The latter findings strongly support the hypothesis that *CALR* exposure could favor the

initiation of anti-cancer immunity in patients with AML [21, 23, 66]. High expression of CALR levels or CALR exposure on neoplastic cells has been linked to improved disease outcome in patients with neuroblastoma, non-small cell lung, ovarian and colorectal carcinoma, as well [20, 32, 55, 68].

CALR MUTATIONS AND PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

Until 2013, no driver mutation was known for the Philadelphia negative myeloproliferative neoplasms (MPN Ph-) patients who did not carry *JAK2*V617F or thrombopoietin receptor gene (*MPL*) genetic aberrations. In 2013, recurrent somatic mutations in the CALR gene were reported in patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) [36, 49]. Describing *CALR* as a novel MPN Ph- driver mutation was initially surprising, regarding the finding that an ER chaperone was involved in the development of ET and PMF. In contrast to *JAK2* and *MPL* mutations, *CALR* defects were not directlylinked to a deregulated JAK/STAT signalling pathway. Therefore, since 2013, several efforts have been made to establish the link between *CALR* mutations, deregulated signalling network and MPN Ph- neoplasms development [31]. *CALR* mutants occur predominantly in ET and PMF, two malignancies that are characterized by aberrant megakaryopoiesis. *JAK2, MPL* and *CALR* mutation are mutually exclusive in MPN Ph- characterized by megakaryocyte hyperplasia, suggesting that they activate common oncogenic pathways [3, 5, 18].

All of the *CALR* mutations described in ET and PMF are frameshift mutations in exon 9 and affect a highly conserved amino acid sequence at the end of the C-terminus domain. They lead to +1-bp frameshift and losing of the KDEL sequence (ER retention signal) and the original CALR stop codon [49]. The CALR C-terminus domain plays an important role in the correct functioning of the CALR protein, as it contains major Ca^{2+} binding sites within the protein. In the presence of the mutation, the CALR protein lacks the C-terminus containing ER retention signal, and the subcellular localization of CALR is impaired. Moreover, CALR mutations change the nature of the C-domain by altering its chemical and physical characteristics (positive isoelectric points), and can lead to abnormal CALR behaviour within the cell [16, 62].

CALR frameshift mutations are classified according to the length of the somatic deletion or insertion in exon 9 of the *CALR* gene. *Type 1 52-bp* deletion (p.Leu367fs*46), and type 2 5-bp TTGTC insertion (p.Lys385fs*47) are the most common variants and constitute more than 80% of all *CALR* mutations [65].

Generally, *CALR* exon 9 insertions or deletions are unique for myeloproliferative neoplasms. Single-nucleotide mutations within exon 9 are not observed in MPN. The reason is that they are unable to activate the thrombopoietin receptor (MPL). *CALR* variants, such a single nucleotide polymorphism/mutation, mutations of the splicing site, as well as indel mutations, are present in at least 20 different types of hematologic malignancies and solid cancers. They are likely to increase the susceptibility of normal cells to develop driver mutations and contribute to local immunosuppression [18, 39, 69].

Germline mutations in calreticulin are also described and are distinct from classical somatic mutations that define MPN Ph-. All germline mutations identified by Szuber et al. involved indels occurring as multiples of 3 bp, and preserved both the original reading frame and KDEL retention signal. Germline CALR mutants, like wild type CALR, present acidic pH values, in contrast to type 1 and type 2 *CALR* mutants which express highly basic charged isoelectric points. Szuber et al. indicate that *CALR* could be a candidate gene in schizophrenia, as germline mutations are associated with this disorder [64].

By constitutive activation of the MPL receptor driven by CALR mutant proteins, CALR oncogenicity is a novel mechanism of oncogenic transformation in MPN Ph- [18]. Both *JAK2*V617F and *CALR* mutations cause JAK/STAT pathway dysregulation; however, it is mediated by distinct mechanisms. This helps to explain the specific phenotypes associated with each type of mutation. *CALR* mutation activates the JAK/STAT signalling pathway in megakaryocytic and granulocytic progenitor and precursor cells by interacting with the MPL and increasing the MAPK activity, conferring autonomy of the cell from interleukin (IL)-3-related cellular growth [59]. According to the results of retroviral [44] and transgenic studies, *CALR* mutations are sufficient to generate specific MPN Ph- phenotype in mice [61]. Moreover, CALR mutant mice harbouring a deleted *CALR* exon 9 did not show any disease phenotype, demonstrating that the novel C-terminus domain of CALR mutants was essential for disease induction [44].

It has been postulated that oncogenic transformation of progenitor hematopoietic cells depended on MPL and its N-glycosylation [5, 44]. The hypothesis was formulated on the basis of the results of retroviral mouse model study of CALR del52 and CALR ins5 positive hematopoietic stem cells. This confirmed post-transplant ET-like disease phenotype (independently of the type of the mutants) and fibrotic disease transformation in the case of the CALR del52 mutant in studied animals [44]. The results of the studies also documented physical interaction between CALR mutant protein and MPL, due to the positive electrostatic charge of the modified C-terminus of the CALR protein [3, 18]. The mechanism of interaction between CALR and MPL was elucidated by Araki et al. Briefly, in normal conditions, P-domain of CALR cannot interact with the MPL receptor (the inhibitory function of the P-domain). This inhibitory effect of the P-domain is, however, abolished by the novel C-terminus in mutant CALR, thus enabling the N-domain to interact with the extracellular domain of MPL, and leading to its dimerization and activation [3].

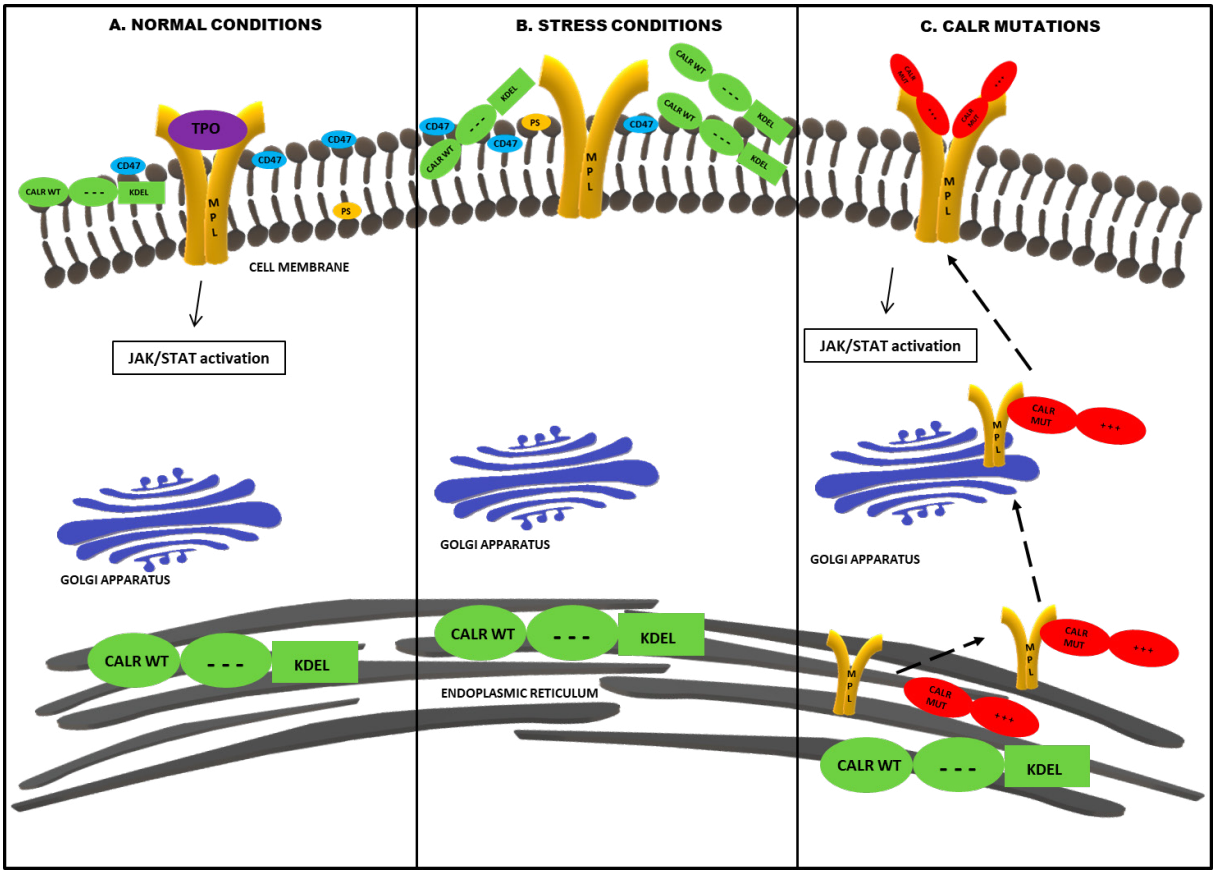

Fig. 1. The function of calreticulin (CALR) in normal and stress conditions, and the mechanism of mutant CALR (CALR MUT) interaction with the MPL protein in the secretion pathway leading to neoplastic transformation of hematopoietic stem cell. A. In normal conditions, CALR participates in various processes in the cells, including glycoprotein chaperoning, the regulation of Ca^{2+} homeostasis, antigen processing and presentation for adaptive immune response. Wild type CALR presents acidic pH values (preserved KDEL retention signal), in contrast to CALR-s mutant protein. Normal cells express low amounts of the CALR 'eat-me' signal, and high amounts of the integrin associated protein (IAP, CD47) 'don't eat-me' signal on the surface. B. In stress conditions, upon the induction of apoptosis, phosphatidylserine (PS) and CALR are translocated to the outer leaflet of the cell membrane. Some proapoptotic stimuli (i.e. UVB irradiation) may initiate the mobilization of CALR to the outer cell membrane even before the PS translocation. CALR molecules on the surface of apoptotic cells are able to trans-activate low density lipoprotein receptor-related protein 1 (LRP1, CD91) on the phagocytes surface, promoting phagocytosis of apoptotic cells. C. In normal conditions, the P-domain of CALR cannot interact with the MPL receptor (the inhibitory function of the P-domain). The inhibitory effect of the P-domain is abolished by the novel C-terminus in KDEL-deleted CALR. In the case of CALR MUT, after synthesis, abnormal protein is moved through the ER to bind to immature MPL. Thereafter, the CALR MUT-MPL complex is stabilized and translocated to the cell surface in the pre-activated form which results in the induction of signalling via the JAK-STAT pathway; **CALR WT** – wild type calreticulin, **KDEL** – endoplasmic reticulum retention signal, --- – negatively charged isoelectric points, **CALR MUT** – mutant calreticulin, +++ positively charged isoelectric points, **MPL** – thrombopoietin receptor, **CD47** – integrin associated protein (IAP), **PS** – phosphatidylserine, **TPO** – thrombopoietin

The CALR mutant has been shown to interact with the MPL receptor, leading to aberrant cell signalling. It is known that the MPL receptor is essential for the CALR mutant MPN progression, as CALR mutants directly interact with this receptor leading to its activation. The activation of the MPL receptor by the CALR mutant binding is dependent on the N-glycosylation residues located in the extracellular domain of this receptor and the glycan binding site of the N-terminus domain of the CALR mutant [3, 5]. Mutant CALR physically interacts with the MPL receptor, and the positively charged amino acids localized within the novel C-terminus domain are essential for this direct interaction [18].

CALR mutants may also prime hematopoietic stem cells for megakaryocyte differentiation by alternation of the transcriptional program. The latter hypothesis is based on the observation that CALR mutants upregulate the key megakaryocytic factor NF-E2, the megakaryocytic surface marker CD41 (integrin alpha-IIb), and the thrombopoietin receptor [27].

Pietra et al. found that type 1 mutations had greater changes in cytosolic calcium signals in cultured megakaryocytes than both normal controls and patients with either *JAK2* or type 2 *CALR* mutation. It may be explained by the difference in the positively-charged C-terminus between type 1 and type 2 mutations. Namely, in type 1 mutations, the negatively charged amino acids are almost completely replaced by positively or neutrally charged amino acids; whereas in type 2, there is a partial replacement. Altered calcium flux within the cell affected

by these different mutant C-termini may explain the propensity to a myelofibrotic phenotype associated with type 1 mutation [56].

The CALR mutantions in the combination with additional oncogenic drivers (such as the epigenetic regulators ASXL1 or EZH2, as well as other genes involved in splicing or signalling) can modify the MPN Ph- phenotype, leading to dysplastic features with progression to myelofibrosis or, eventually, to AML [67].

ONCO- AND IMMUNOGENICITY OF CALR

CALR plays a paradoxical dual role in oncogenesis – some studies link CALR expression to tumor progression and cellular transformation, other suggest that CALR initiates an anti-tumor immune response, inhibiting tumor growth [26, 40]. The exposure of CALR on the membrane of malignant cells experiencing ER stress is well-known for its role in the activation of immune responses to dying cancer cells [35]. During the promotion of phagocytosis, CALR translocates from the ER lumen to the outer cell membrane where it interacts along with phosphatidylserine (PS). CALR binds to PS through its C-terminus acidic domain in a calcium-dependent manner, and serves as a PS-anchor factor that cooperates with other PS-binding molecules, such as C1q complement component [52, 70]. Outside the ER, CALR has immunomodulatory properties. CALR is one of the most important damage-associated molecular patterns (DAMPs) which are exposed to the cell surface during immunogenic cell death (ICD). After the induction of ICD, CALR is translocated from the lumen of the ER to the cell surface where it functions as an 'eat-me' signal for professional phagocytes [59]. Cell-surface-CALR acts as a pro-phagocytic signal and is constitutively expressed by tumor cells. Translocated to the cell surface in response to a variety of environmental stress signals that include chemotherapeutic drugs, such as anthracyclins and oxaliplatin, as well as ultraviolet C (UVC) and radiation, wild type CALR enables the recognition of cancer cells by professional phagocytes of the innate immune system [8, 21, 26].

CALR mutations that cause the removal of the C-terminus KDEL motif lead to mislocalization of the protein outside the ER, followed by CALR release from the cells. The exposure of CALR by stressed and dying cancer cells stimulates their phagocytic uptake by dendritic cells (DC) and induces DC maturation/antigen presentation which favors the induction of T cell-mediated anti-cancer immune responses [24]. Liu et al. found that excessive soluble CALR saturates the binding sites on phagocytes, thereby inhibiting phagocytosis of the CALR-exposing cells. It indicates that mutated CALR has an immunosuppressive effect and dulls responses to anti-cancer immunotherapy [39]. The function of calreticulin in normal and stress conditions, and the mechanism of mutant calreticulin interaction with MPL in the secretion pathway leading to neoplastic transformation of hematopoietic stem cells, is presented in Fig. 2.

In the case of MPN Ph-, CALR mutations fulfill a dual role: (1) they provide autocrine proliferative signal (activate MPL, causing the proliferation of hematopoietic stem cells) and (2) they subvert immune response – CALR mutant cells escape immunosurveillance by activating immunosuppression. The immunosuppressive effect of the mutant CALR may be the reason why *CALR*-mutated MPN manifest about ten years earlier than *JAK2*V617F MPN [67]. Compared to MPN Ph-, *CALR* mutations in solid tumors also result in removing the ER retention signal (KDEL), but do not create a new C-terminus. Probably, these mutations do not function as oncogenic drivers, but rather participate in local immunosuppression [39].

Liu et al. speculate that anti-CALR autoantibodies, characteristic for autoimmune diseases (primary biliary cirrhosis, rheumatoid arthritis and systemic lupus erythematosus), might quench extracellular CALR and favor autoimmune and inflammatory reactions [39]. They also revealed that CALR-secreting tumors escaped anti-cancer immune responses, because secreted mutant CALR had immunosuppressive functions. Both CALR MPL-associated mutations (del52 or ins5) found in MPN and mutations found in solid tumors (E405* or X352) caused the secretion of soluble CALR protein and significantly reduced the efficacy of immune checkpoint blockade based immunotherapy, underlining the immunosuppressive nature of the CALR secretion [38].

Holmström et al. proposed that *CALR*-mutant MPN could be a disease model of cancer immuno-editing and proved that CALR-mutant MPN displayed all three stages (elimination, equilibrium, and escape) described in this theory. Thereafter, they confirmed that T cells from *CALR* mutated MPN Ph- patients could recognize several epitopes from the mutant C-terminus, and targeted and killed autologous CALR-mutant cells. Surprisingly, they also demonstrated that healthy individuals displayed frequent and strong T cell responses to *CALR* neo-antigens, as well. They suggest that even healthy individuals acquire a *CALR* mutation. Clearing mutant cells is followed by the generation of T cell memory, so that these healthy individuals retain their CALR-mutant cells in the editing stage for several years. Moreover, the comparison of CALR neoantigen immune responses in young (ages 18–21 years) and older (ages 50–64 years) healthy individuals showed no differences in either the frequency or the amplitude in the two aged-defined groups [29, 30].

The differences in the prevalence of *JAK2*V617F and *CALR* mutations in MPN Ph-patients and in healthy individuals imply that *CALR* mutations are much more immunogenic than JAK2V617F, as individuals with a very low burden of *CALR*-mutant cells may simply clear the mutant neoplastic clone. The Danish population study of patients with MPN revealed that the JAK2V617F mutation was approximately 5.6-fold more frequent than CALR gene aberrations. At the same time, the JAK2V617F mutation was 19-fold more frequent than *CALR* mutations among healthy individuals [13].

THE ROLE OF CALR IN VIRAL AND BACTERIAL INFECTIONS

CALR has been implicated at multiple points in the life cycles of diverse viruses. Hepatitis B virus (HBV) up regulates the CALR expression at both the transcriptional and posttranscriptional levels. The increased expression of CALR antagonized the interferon pathway, leading to increased viral protein synthesis and HBV DNA replication [72]. CALR also plays a direct role in the dengue virus RNA replication by folding and assembling the dengue protein [37]. In the plant model, the co-expression of human calreticulin significantly improves the production of human immunodeficiency virus gp140 and other viral glycoproteins [43]. CALR plays an important role in opsonisation and phagocytosis processes, but its role depends on the type of interaction. When calreticulin is released from a target cell onto the target cell surface, it acts as an 'eat-me' signal; when it is released from a phagocyte and binds to a target cell, it acts as an opsonin; and finally, when it is released from a phagocyte onto the surface of a phagocyte and binds to LRP, it acts as a co-receptor. CALR released by microglia (brain-resident macrophages) acts as an opsonin, soluble protein, of *Escherichia coli* and promotes the clearance of these bacteria [11]. During *Mycobacterium tuberculosis* infection, the macrophage-surface exposure of CALR is a part of the host defence mechanism. Enhanced CALR production by macrophages induces apoptosis and thus effectively reduces the intracellular survival of mycobacteria [33].

CALR AS A TARGET FOR NOVEL TREATMENT MODALITIES

According to recent studies, extracellular CALR regulates several pathways that promote tumor cell arrest and attack by the host immune system. From this point of view, calreticulin may serve as a potential target of immunotherapy. On the other hand, increased ER-associated CALR correlates with poor prognosis in many types of cancer and that is why one of the current strategies is to favor enticing ER-CALR onto the surface of tumor cells to promote innate and adaptive immunity. Immunogenicity and protein-folding properties of calreticulin have been exploited in order to develop vaccines. In the context of patients with MPN Ph-, *CALR* exon 9 mutations, apart from being important diagnostic and prognostic markers, are becoming an important therapeutic target for cancer immune therapy as immunogenic neo-antigens. This idea is currently tested in the clinical trial „A Phase-1-first in Man Study in Patients With *CALR*-mutant Myeloproliferative Neoplasms by Vaccinating With *CALR* Exon 9 Mutant Peptide" (NCT03566446) [17, 29, 31].

CONCLUSION

To sum up, recent data revealed the multifunctional role of CALR protein in many physiological and pathological biological processes. Wild type calreticulin participates in glycoprotein chaperoning, regulation of Ca^{2+} homeostasis, antigen processing and presentation for adaptive immune response, cell adhesion/migration and immunogenic cell death. It also takes part in retaining RNA stability, gene expression and cell proliferation. Unexpectedly, wild type calreticulin facilitates spreading of some viral infections and participates in the opsonisation process of bacteria, as well. Mutated CALR protein is responsible for tumorigenesis and has an immunosuppressive effect and dulls responses to anti-cancer immunotherapy. Further studies are needed to acquire a comprehensive understanding of the role of calreticulin in these processes. On the bright side, the mutated CALR proteins are becoming a promising therapeutic target for cancer immune therapy as immunogenic neo-antigens.

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