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The genetic dissection of myeloproliferative neoplasms

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Habilitationsschrift

The genetic dissection of myeloproliferative neoplasms

Zur Erlangung der Venia Legendi der Universität Zürich

Alexandre Pierre André Theocharides

Freitag, den 2. September 2016

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1. Introduction

Myeloproliferative neoplasms (MPNs) are hematopoietic stem cell (HSC) disorders characterized by increased proliferation of one or more hematopoietic cell lineages with the highest incidence in the elderly population.[2] Chronic myeloid leukemia (CML) is defined by the presence of the *BCR-ABL1* gene fusion transcript. Polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) are distinguished from CML by the absence of *BCR-ABL1*. More than 50 years ago William Damashek postulated that PV, ET and MF (i.e. *BCR-ABL1* negative MPNs, henceforth abbreviated MPNs) originate from a common clonal ancestor since they share a common clinical phenotype.[4] *BCR-ABL1* negative MPN patients are at increased risk for cardiovascular events, thromboembolic complications, bleedings, and infections which cause significant morbidity and mortality.[5] In addition, MPNs can progress to acute myeloid leukemia (AML).
In 2005, with the discovery of a recurrent unique somatic point mutation in Exon 14 of the Januskinase 2 (*JAK2*) in *BCR-ABL1* negative MPNs, a common clonal origin to PV, ET and MF was identified and confirmed the hypothesis postulated by Damashek earlier.[1,6-10] The discovery of the *JAK2* mutation revolutionized the field of MPNs and related disorders in basic and clinical research. In the last decade new sequencing technologies have uncovered a plethora of somatic mutation in these diseases, which contribute to the better understanding of their pathogenesis. In this endeavor the mutations in the ER resident chaperone Calreticulin (CALR) were first described two years ago in ET and MF patients with unmutated *JAK2*.[1,10] Today, *JAK* inhibitors are available and Ruxolitinib, the first in class *JAK* inhibitor, has been approved by the food and drug administration for the treatment of advanced stage MPN.[11,12]

The focus of my research is the investigation of mechanisms that contribute to the pathogenesis of MPNs. In the last years I have uncovered processes that contribute to the leukemic transformation of MPN and recently described how *CALR* mutations can affect the chaperone function of CALR. [13-15]

The leukemic transformation process in MPN

MPNs can progress to AML, which is clinically indistinguishable from *de novo* AML (i.e. an AML that does not arise from an antecedent hematopoietic neoplasm). However, the risk of AML transformation significantly varies between MPNs.[2] While transformation to AML is a rare event in ET (1-5% of patients), leukemic transformation occurs in 20-30% of MF patients (20-30%).[2]
The process of leukemic transformation in MPN is not well understood. To identify somatic mutations that may contribute to the leukemic transformation process in MPNs investigations have focused on the identification of mutations in samples harvested before and after AML transformation using whole exome or targeted sequencing approaches. The comparison of the genetic profile of the two disease phases allows the identification of genetic aberrations that were acquired during the leukemic transformation process. However, to determine whether these genetic aberrations actively contribute to disease progression, their functional validation *in vitro* and *in vivo* is required.
The spectrum of somatic mutations significantly varies between *de novo* AML and AML secondary to a MPN (post-MPN AML) suggesting a distinct pathogenic mechanism.[16] The acquisition of somatic mutations in the tumor suppressor *TP53* in MPNs is significantly associated with AML transformation

and correlates with reduced overall survival in retrospective studies.[16] Accordingly, the expression of *JAK2*-V617F in mouse bone marrow *TP53* knockout cells leads to the development of AML in mice and demonstrates that *TP53* mutations functionally contribute to the leukemic transformation process in *JAK2* mutant MPN.[17] In addition to mutations in *TP53*, amplification of chromosome 1q containing the gene encoding for *MDM4* has been associated with AML transformation. *MDM4* is a known inhibitor of *TP53* and upregulation of MDM4 in AML secondary to MPN is mutually exclusive to *TP53* mutations. Together mutations in *TP53* and gain of chromosome 1q are found in almost 50% of post-MPN AML making the *TP53* axis, the most frequently affected genetic pathway.[18]

Other somatic mutations that are associated with increased risk of leukemic transformation and potentially contribute to the progression to post-MPN AML include mutations in the epigenetic regulators *DNMT3a*, *ASXL1*, *EZH2*, *TET2* and the B-cell transcription factor *IKZF1*.[19] While the evidence for the involvement of *TP53* in transformation to MPN blast phase is solid (see above) the functional contribution of these other somatic mutations remains to be determined.

The contribution of CALR mutations to the pathogenesis of MPN

In contrast to *JAK2*, more than 50 mutations have been described in *CALR,* which all lead to a mutant-specific reading frame.[1,10] *CALR* type 1 (52 base pair (bp) deletion) and *CALR* type 2 (5 bp insertion) are the two most frequently detected *CALR* mutations and cover >80% of the mutational spectrum of *CALR*. *CALR* mutations affect the C-terminal, which contains a lysine, aspartic acid, glutamic acid, leucine (KDEL) motif and a Ca^{2+}-binding domain **(Figure 1)**.

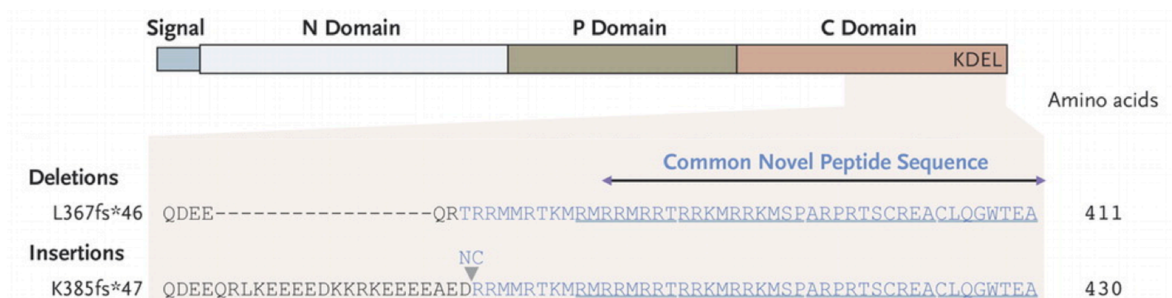

Figure 1. Functional CALR domains and most frequent *CALR* mutations (adapted from Nangalia et al.).[1] The CALR protein is composed of a N-terminal, a Proline-rich and a C-terminal domain, which contains a KDEL motif. The two most frequent *CALR* mutations are shown below. Both, type 1 (L367fs*46, 52 bp deletion) and type 2 (K385fs*47, 5 bp insertion), lead to a novel common peptide sequence, which lacks the KDEL motif.

The discovery of *CALR* mutations in ET and MF patients is particularly intriguing since *CALR* mutations have not been described in other cancers. Furthermore, unlike *JAK2* and *ABL*, CALR is not kinase, a tumor suppressor or a known oncogene. CALR is a molecular chaperone for *N*-glycoproteins (GPs), modulates calcium homeostasis and acts as a prophagocytic signal on the cell surface. Moreover, CALR is essential for cardiac development and homozygous *CALR* knockout embryos die before day 14.5 due to a severe defect in early cardiogenesis.[20]

The unique association of *CALR* mutations with ET and MF suggests an association between *CALR* mutations and megakaryocyte development since both disorders are characterized by pathologic proliferation and/or differentiation of the megakaryocytic lineage.[2] Of note, the *CALR* type 1 mutation is predominantly associated with MF, while the type 2 mutation is more prevalent in ET suggesting functional differences between the two mutants.[21]

MPN patients with *CALR* mutations respond equally well to *JAK*-inhibitor therapy as patients with *JAK2* mutations, which supports the involvement of the *JAK*-STAT pathway in the pathogenesis of *CALR* mutated MPNs.[11,22] In line with these observations, recent studies demonstrate that the mutated CALR protein, but not the wild-type protein, binds and leads to pathological activation of the thrombopoietin receptor (MPL) thereby inducing *JAK*-STAT signaling.[3,21,23]

2. Results

Theocharides A, Boissinot M, Girodon F, Garand R, Teo S-S, Lippert E, Tichelli A, Hermouet S, Skoda RC.
Leukemic blasts in transformed JAK2-V617F positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation
Blood. 2007 Jul 1;110(1):375-9. Epub 2007 Mar 15.

At the time of the first study presented in the *Habilitationsschrift* the relevance of the *JAK2*-V617F mutation in transformation from MPN to AML was unclear. To study the role of the *JAK2*-V617F mutation in leukemic transformation, we examined 27 patients with MPNs who transformed to AML.[13] DNA was isolated from MPN samples at MPN diagnosis and from samples after transformation to AML. At MPN diagnosis, *JAK2*-V617F was detectable in 17 of 27 patients. Surprisingly, only 5 of 17 patients developed *JAK2*-V617F-positive AML, whereas 9 of 17 patients transformed to *JAK2*-V617F-negative AML. Acquired loss of heterozygosity (LOH), which arises through mitotic recombination, is a frequent genetic phenomenon in hematopoietic cancers.[24] We hypothesized that *JAK2*-V617F-negative AML may arise from a HSC which has lost the *JAK2*-V617F mutation through LOH, followed by the expansion of the AML clone with both *JAK2* wild-type alleles. However, microsatellite analysis in a female patient in our study showed that LOH was not responsible for the transition from *JAK2*-V617F-positive MPN to *JAK2*-V617F-negative AML, and clonality determined by the *MPP1* polymorphism demonstrated that the granulocytes and leukemic blasts inactivated the same parental X chromosome. This was independently confirmed by another study and excluded LOH as possible mechanism for transformation to *JAK2*-V617F-negative AML.[25]
We then assessed whether samples at MPN and at AML diagnosis carried common cytogenetic aberrations. Detection of genetic aberrations at both stages of the disease suggests that both, MPN and AML, have a common clonal ancestor. In one patient positive for *JAK2*-V617F in neutrophil granulocytes at AML transformation, but with *JAK2*-V617F-negative leukemic blasts (i.e. very immature leukemic cells), we found a deletion of chromosome 11q in neutrophils and the leukemic blasts. This observation suggests that both, the *JAK2*-V617F positive granulocytes and the *JAK2*-V617F leukemic blasts have a common clonal ancestor. In that case the *JAK2*-V617F mutation may have been chronologically acquired in a hematopoietic stem or progenitor cell after the acquisition of the deletion in chromosome 11q. Presumably the HSC, which carries the deletion of chromosome 11q, but not the *JAK2*-V617F mutation acquired other somatic mutations that led to AML transformation.
From our study we concluded that *JAK2*-V617F-positive MPN frequently yield *JAK2*-V617F-negative AML, and that AML transformation of a common *JAK2*-V617F-negative ancestor represents a possible mechanism.

Theocharides A, Dobson S, Laurenti E, Notta F, Voisin V, Cheng P, Yuan J, Guidos CJ, Minden M, Mullighan C, Torlakovic E, Dick JE.
Dominant-negative Ikaros cooperates with BCR-ABL1 to induce human acute myeloid leukemia in xenografts.
Leukemia. 2015 Jan;29(1):177-87. Epub 2014 May 5.

Historically, our understanding of mechanisms underlying human leukemogenesis are inferred from genetically engineered mouse models. Relatively, few models that use primary human cells recapitulate the full leukemic transformation as assayed in xenografts.[26-28] In the second project presented in the *Habilitationsschrift* we aimed at developing a model for *BCR-ABL1* positive B lymphoblastic leukemia (B-ALL).[29] In *BCR-ABL1* positive B-ALL mutations in the B-cell transcription factor IKAROS (encoded by *IKZF1*) are detected in >80% of patients and lead to the formation of a dominant negative isoform (Ik6), which suppresses the function of wild-type IKAROS.[30,31]
We expressed *BCR-ABL1* and Ik6 simultaneously in human cord blood cells enriched for hematopoietic stem and progenitors. In contrast to our prediction mice did not develop B-ALL, but developed aggressive AML with disseminated myeloid sarcomas (i.e. solid tumors composed of leukemic cells) within 4 weeks following transplantation of cord blood transduced with vectors expressing *BCR-ABL1* and Ik6. *BCR-ABL*-Ik6 cells also showed a competitive advantage *in vitro*. We performed gene expression profiling and compared cord blood cells transduced with Ik6, *BCR-ABL1* and both *BCR-ABL1* and Ik6. Our data shows that Ik6 induces transcriptional programs in *BCR-ABL1*-transduced progenitors that contained repressed B-cell progenitor programs, along with strong stemness, proliferation and granulocyte-monocytic progenitor (GMP) signatures, a novel combination not induced in control groups. Interestingly, our model also uncovered an active interleukin 6 (IL-6) pathway in cells expressing *BCR-ABL1* and Ik6 pointing towards a central role of IL-6 in AML transformation, which parallels findings that come from clinical observations predicting a functional role for IL-6 in AML.
From our study we concluded that wild-type IKAROS restrains stemness properties and has tumor suppressor activity in *BCR-ABL1*-initiated leukemia. Interestingly, although IKAROS mutations/deletions are common in lymphoid transformation, they are found also at low frequency in AML that progress from a prior MPN state.[32] Our experimental system therefore provides an excellent functional model to gain insight into these cases of AML transformation. IKAROS loss of function may act as a secondary mutation in the transformation of MPN to AML as discussed in the first project described above. More generally, the data points to the importance of deregulated stemness/lineage commitment programs in human myeloid leukemogenesis.

Theocharides A, Lundberg P, Lakkaraju A, Lysenko V, Myburgh V, Aguzzi A, Skoda R, Manz M
Homozygous *Calreticulin* mutations in patients with myelofibrosis lead to acquired myeloperoxidase deficiency
Blood. 2016 Jun 23;127(25):3253-9. Epub 2016 Mar 24.

The pathogenesis of acquired myeloperoxidase (MPO) deficiency, a rare phenomenon observed in patients with Philadelphia chromosome-negative MPNs, is unknown.[33] MPO is a lysosomal heme GP expressed exclusively in myeloid cells that is folded by CALR in the ER.[34,35] It catalyzes the conversion from hydrogen peroxide to hypochlorous acid and is essential for optimal oxygen-dependent anti-microbial activity.[34,35] Whether *CALR* mutations affect the chaperone function of CALR remains to be determined.

In the third study presented in the *Habilitationsschrift* we hypothesized that acquired MPO deficiency in MPN could be associated with the presence of *CALR* mutations.[15] A cohort of 317 MPN patients (142 PV, 94 ET and 81 MF) was screened for MPO deficiency using an automated ADVIA blood cell counter. The ADVIA cell counter uses MPO activity to generate a differential white blood cell count and neutrophil granulocytes are identified by their high expression of MPO.[36] As a consequence patients with MPO deficiency lack neutrophil granulocytes in the automated, but not in the manual cell count performed by light microscopy.

In our cohort MPO deficiency was observed in 6/81 MF patients (7.4%), but not in PV or ET patients. Since MPO is essential for anti-microbial activity we assessed the history of infections in MPO deficient patients. Susceptibility to infections had been documented in 2/6 (33%) MPO deficient patients. Five out of six patients with MPO deficiency carried a homozygous *CALR* mutation and were also deficient in eosinophilic peroxidase (EPX), another GP folded by CALR in the ER. In contrast, one MF patient with a *JAK2*-V617F mutation and MPO deficiency carried two previously reported MPO mutations in inherited MPO deficiency and showed normal EPX activity.[37] Patients with homozygous *CALR* mutations had reduced MPO protein, but normal *MPO* mRNA levels supporting a post-transcriptional defect in MPO production. This data suggests that patients with homozygous *CALR* mutations develop a defect in the chaperone function of CALR that leads to MPO and EPX deficiency. In contrast, MPO deficiency in the patient with a *JAK2* mutation is the consequence of an inherited *MPO* gene mutation and is coincidental with the MPN.

To determine whether the absence functional CALR in the presence of homozygous *CALR* mutations leads to MPO deficiency we expressed human MPO in *CALR* knockout mouse embryonic fibroblasts. These experiments showed that in the absence of CALR immature MPO protein precursors are produced, but then degraded in the proteasome. Therefore acquired MPO deficiency in MPN is the consequence of degradation of immature, presumably misfolded MPO precursors and in contrast to inherited MPN deficiency is not due to a transcriptional defect.

In summary, four decades after the first description of acquired MPO deficiency in MPN our study provides the molecular correlate associated with this phenomenon and evidence that *CALR* mutations can affect the biosynthesis of GPs.

3. Discussion

The origin of post-MPN AML

In 2007, others and us demonstrated that AML in *JAK2* mutated MPN can originate from a hematopoietic stem or progenitor cell that does not carry the *JAK2* mutation.[13,25] In our work we described 9 patients with *JAK2* mutated MPN who transformed to *JAK2* unmutated AML. In one patient we found a common cytogenetic aberration in the MPN and the AML phase suggesting a common clonal origin. In line with the hematopoietic hierarchy we proposed three mechanisms compatible with the "loss" of the *JAK2* mutation in the transformation to AML (**Figure 2**).

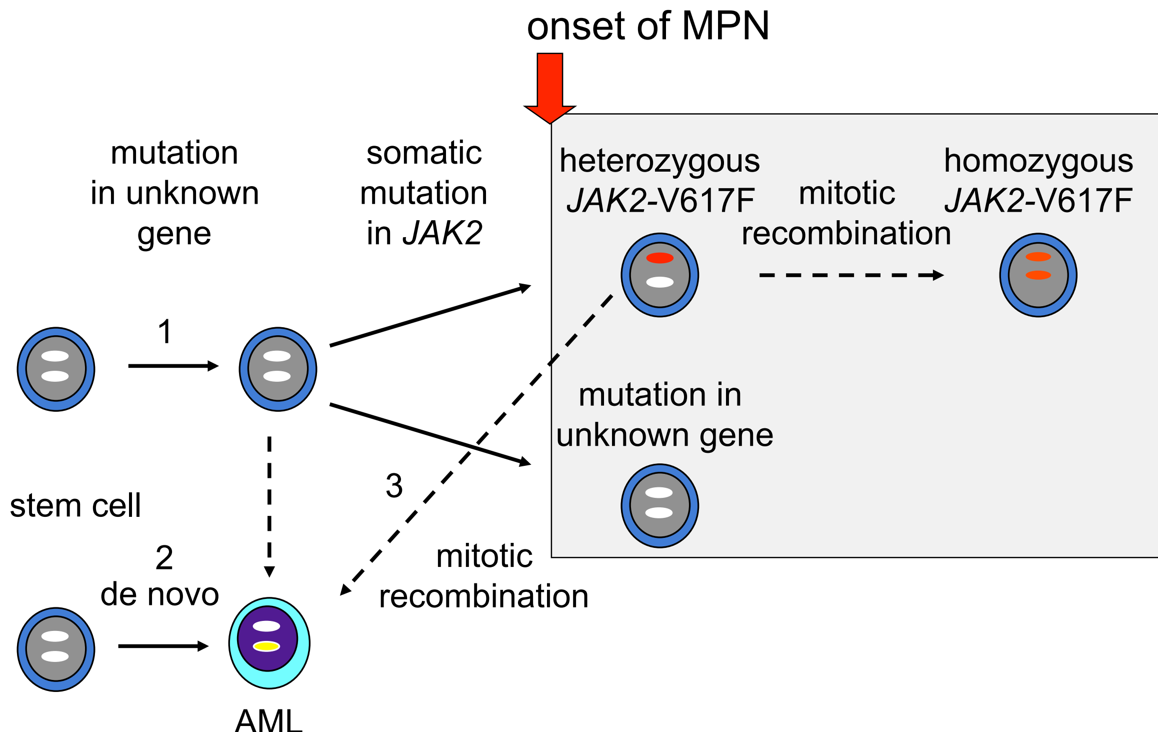

Figure 2. Hypothetical model for the transformation into *JAK2* unmutated AML proposed in 2007. Model 1: AML originates from a hematopoietic stem or progenitor cell that does not carry the *JAK2* mutation. Model 2: *JAK2* unmutated AML originates from a HSC that is independent of the *JAK2* mutated clone that gave rive to the MPN. Model 3: *JAK2* unmutated post-MPN AML originates from a *JAK2* unmutated hematopoietic stem and progenitor cell in which the *JAK2* mutation was "lost" through mitotic recombination.

In the first model the acquisition of the *JAK2* mutation is a late genetic event and AML originates from an HSC that does not carry a *JAK2* mutation yet. The second model predicts that AML originates from an MPN-independent *JAK2* unmutated HSC clone that does not carry common genetic aberrations. Finally, *JAK2* unmuated AML could arise through mitotic recombination/LOH that leads to uniparental disomy of the *JAK2* wild-type allele, a hypothesis that we rejected by microsatellite analysis of a female patient.[13,25]

In the first model mutation(s) in HSCs chronologically precede the *JAK2* mutation. Recent studies have investigated the order of acquisition of somatic mutations in MPN. *JAK2* mutations and mutations in the epigenetic regulator Tet methylcytosine dioxygenase 2 (TET2) frequently co-occur in MPNs.[38] MPN patients with concomitant *JAK2* and *TET2* mutations can be categorized into "*JAK2*-first" and "*TET2*-first" subgroups depending on the chronological order of acquisition.[39] Although our study did

not assess *TET2* mutations in the samples investigated the “*TET2*-first” model is compatible with the first model. In this model AML could arise from a *TET2* mutated, but *JAK2* unmutated HSC. However in the discussed study the number of MPN patients with transformation to AML was not indicated, most likely due to the low number of patients.[40] In the study by Lundberg et al. the chronological acquisition of somatic mutations was assessed in 12 patients with *TET2* mutations.[38] In contrast to the study by Ortmann et al. (see above) all but one of these patients carried the *TET2* mutation already at MPN diagnosis and mutations in *TET2* occurred mostly before the *JAK2* mutation. Moreover the presence of a *TET2* mutation correlated with reduced overall survival and increased risk of leukemic transformation. No consistent pattern regarding the order of acquisition was observed for mutations in other epigenetic modifiers such as *EZH2* and *ASXL1*. In accordance with our first model outlined above this model is compatible with a leukemic clone that arises from a *JAK2* unmutated ancestral clone and leads to *JAK2* unmutated AML (**Figure 2)**.

In the second model AML arises *de novo* from an “MPN independent” *JAK2* unmutated HSC. Biclonal disease in post-MPN AML may be identified by the absence of *JAK2*-V617F and a mutational pattern that resembles *de novo* AML. The mutational pattern of *de novo* AML significantly differs from AML secondary to an antecedent MPN. While mutations in *FLT3*-ITD, *NPM1* and *NRAS* are more frequently found in the *de novo* AML, mutations in *TP53*, *RUNX1* and *CBL* are often acquired during MPN transformation to AML and not present at MPN diagnosis.[16]

The identification of somatic genetic aberrations that are acquired during leukemic transformation suggests their involvement in post-MPN AML pathogenesis. However, for most of them, their functional contribution remains to be determined. The work by Rampal et al. elegantly shows that the loss of *TP53* function in the presence of the *JAK2*-V617F mutation models post-MPN AML.[17] The second study presented in the *Habilitationsschrift* investigates the functional collaboration between *BCR-ABL1* and Ik6, a dominant-negative isoform of IKAROS, in leukemic transformation.[14] In contrast to our first hypothesis (see results section) immune-compromised mice transplanted with double-transduced cord blood cells developed AML, but not B-ALL. The model described in our study resembles AML secondary to antecedent CML. In line with our observation, mutations in IKAROS have been described in post-CML AML and AML secondary to MPNs in up to 20% of cases.[32,41] These observations suggest that the loss of IKAROS function may contribute to the pathogenesis of post-MPN AML in a subgroup of MPN patients. Importantly, IKAROS mutations have not been described in the chronic phase of CML and MPNs. This suggests that IKAROS mutations functionally promote leukemic transformation. Interestingly, our study uncovered an active role of IL-6 in AML transformation and may provide a rational for anti-IL-6 therapy in post-CML and post-MPN AML. Anti-IL-6 therapy is approved for the treatment of multicentric Castleman’s disease and the efficacy of anti-IL-6 therapy has been assessed in patients with multiple myeloma.[42] Pharmacokinetic and biosafety studies have been performed and will facilitate translation of promising experimental data into clinical trials for other disease such as post-CML/MPN AML.

Disturbed chaperone function in CALR mutated MPN

In the third study presented in the *Habilitationsschrift* we demonstrate that homozygous *CALR* mutations lead to MPO and eosinophilic peroxidase (EPX) deficiency in patients with MF.[15] MPO and

EPX are two GPs known to be chaperoned by CALR and our data represents the first evidence that *CALR* mutations can affect the chaperone function of CALR.

I hypothesize that *CALR* mutations affects the chaperone function of CALR through distinct mechanisms (**Figure 3**).

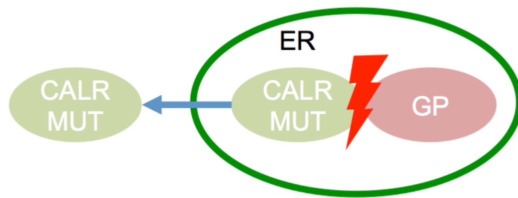

Figure 3. Possible consequences of *CALR* mutations on the interactions of CALR with GP. Alterations in the C-terminal domain of CALR may lead to a reduced retention of CALR in the ER (blue arrow) and consecutively decreased availability of CALR to fold GP. Alternatively, *CALR* mutations may affect the direct interaction with GPs (red blizzard).

Reduced availability of CALR in the ER could influence its chaperone capacity and affect the maturation of GPs. *CALR* mutations affect the C-terminal domain and lead to loss of the KDEL sequence.[1,10] The KDEL motif is important for the retention and the retrieval of CALR in and to the ER. However, whether the loss of the KDEL motif in the presence of *CALR* mutations leads to the mislocalisation of CALR in subcellular organelles remains unclear. While the study by Klampfl. et al did not find a difference in localization compared to wild-type CALR, the study by Nangalia et al. suggested that CALR mutants are not constrained to the ER.[1,10] Mislocalisation of CALR in the presence of *CALR* mutations was later described in the work by Chachoua et al. who isolated postnuclear fractions by sucrose density gradients.[21] In these experiments the wild-type, but not the mutant CALR protein equilibrated with the ER-marker GRP78 supporting a misclocalization of the mutant. This was further confirmed by high-resolution confocal microscopy, which demonstrated that the wild-type, but not the mutant CALR protein colocalize with the ER resident protein Calnexin, while the mutant protein colocalized with ERGIC53, a protein resident in the ER to Golgi intermediate compartment. In summary, the data is more supportive of an altered subcellular localization of mutated CALR proteins, which may impact on the chaperone capacity.

An alternative hypothesis suggests that the binding of GPs to CALR is altered in the presence of CALR mutants consecutively leading to GP misfolding (**Figure 3**). GPs bind to the *N*-terminal domain of CALR, which is unaffected by *CALR* mutations. However, a recent observation suggests that the interaction of CALR mutants with MPL, another GP, is abnormal (**Figure 4**).[3] The binding of MPL to wild-type CALR is inhibited by the P-domain of CALR. In contrast to the wild-type, the mutant C-terminal inhibits the inhibitory function of the P-domain and consecutively allows MPL to bind to CALR. This pathologic interaction leads to activation of *JAK*-STAT signaling downstream of MPL. It is conceivable that the binding to CALR of other GPs like MPO and EPX is affected in the presence of *CALR* mutations and leads to a maturation defect and consecutive degradation of CALR substrates. This is further supported by the study by Chachoua et al., which shows that the maturation of MPL is altered in the presence of CALR mutants and by earlier studies, which demonstrate a maturation defect of MHC class I and Tapasin, two GPs chaperoned by CALR, in CALR knockout cells.[21,43]

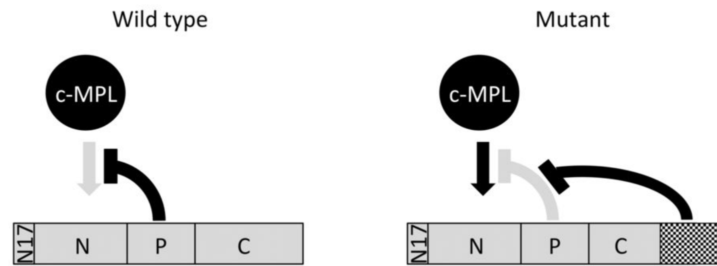

Figure 4. Mechanism of CALR-MPL interaction in the presence of CALR mutants (adapted from Araki et al.).[3] The P-domain of CALR inhibits the binding of MPL to the N-terminal domain (left). In the presence of CALR mutations the novel C-terminal domain of CALR inhibits the function of the P-domain and allows binding of MPL to CALR (right).

Although the deficiencies in MPO and EPX likely do not directly contribute to the pathogenesis of CALR mutated MPN the maturation of GPs that control cell proliferation and differentiation, such as tumor suppressors, epigenetic regulators and cell cycle checkpoints may be affected in the presence of CALR mutants. In particular, the loss of proteins involved in the negative regulation of *JAK2* including LNK, SOCS family members and CBL may contribute to MPN development. Loss of function mutations have been reported in the E3 ubiquitin ligase CBL in a wide range of myeloid malignancies. [44] CBL targets specific proteins for proteasomal degradation and acts as a negative regulator of cytokine receptor signaling.

In addition to pathological activation of *JAK*-STAT signaling, the direct or indirect activation of alternative kinases may contribute to the pathogenesis of MPN. A recent study shows that *Zfand2b* (encodes the highly conserved ER resident protein AIRAPL) knockout mice develop a myeloproliferative disease characterized by the activation of Insulin-like Growth Factor 1 Receptor (IGF1R) signaling and treatment with the IGF1R inhibitor NVP-AEW541 reverts disease.[45] Although activation of *JAK*-STAT signaling is not observed in these mice, MPN patients with *JAK2* or *CALR* mutations show downregulation of *ZFAND2B* and upregulation of IGF1R signaling probably as a consequence of microRNA 125a-3p upregulation. This data shows that dysregulated proteostasis presumably induced by *JAK2* or *CALR* mutations contributes to MPN development by activation of alternative pathways involved in cell proliferation and differentiation. Future unbiased approaches comparing the proteome and the glycoproteome of wild-type and mutant CALR will help identifying candidate proteins and lead to the identification of novel potential therapeutic targets in MPN.

Susceptibility to infections in MPN patients with acquired MPO deficiency

MPO deficiency can either be inherited or acquired. It remains to be determined whether functional differences between inherited and acquired MPO deficiency exist and whether acquired MPO deficiency leads to increased susceptibility to recurrent and opportunistic infections in patients with an underlying causative disorder such as MPN. In the third study presented in the *Habilitationsschrift* 2/6 MPN patients (33%) with acquired MPO deficiency suffered from severe recurrent infections.[15] In a large study partial MPO deficiency was detected in approximately 0.2% of healthy individuals, whereas complete MPO deficiency was found only in 55/150'000 (0.04%) study subjects.[33] In the same study, an increased incidence of serious infections and inflammatory disease was reported in patients with complete MPO deficiency.[33] All patients with homozygous *CALR* mutations in our study presented with complete MPO deficiency. The incidence of MPO deficiency reported in our study is therefore significantly higher than the one reported in subjects with the inherited form. Moreover, although the number of patients is too small to draw solid conclusions the susceptibility to infections

seems higher in acquired than in inherited MPO deficiency. An increased susceptibility to infectious complications in patients with myeloid disease may be simply related to the underlying hematological neoplasms and immunosuppressive therapy. However, an observation made more than 50 years ago by Klebanoff et al. could point towards functional differences between inherited and acquired MPO deficiency.[46] Subjects with inherited MPO deficiency may have adapted to the defect in hydrogen peroxide to hypochloric acid conversion and developed alternative molecular anti-microbial strategies.[47] These mechanisms may not be in place in subjects who develop MPO deficiency during their lifetime, leading to increased susceptibility to infections and accumulation of reactive oxygen species.
The identification of MPN patients at risk for infectious complications may be of particular importance for patients treated with *JAK*-inhibitors, which increase the risk of infections with varicella zoster and other opportunistic pathogens.[11,22]

In summary, the research findings discussed in the *Habilitationsschrift* have contributed to a better understanding of the pathogenesis of MPN. The knowledge about molecular events that contribute to leukemic transformation may allow the development of therapeutic strategies that act at an early disease stage and prevent progression to AML. Chaperone malfunction constitutes a novel mechanism in the development of hematopoietic cancer and the findings described will open up new avenues of research in related disorders like myelodysplastic syndromes and AML. Therefore the significance of the data lies not only in MPN, but may be extended to a broad range of related myeloid neoplasms.

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