Spliceosome gene mutations are among the 50–60 driver mutations underlying myelodysplastic syndromes (MDSs). U2AF1 mutations for example have been reported to occur in up to 16% of primary myelofibrosis (PMF), and was found to be associated with anemia and thrombocytopenia in PMF. We could show that spliceosome gene mutations are already present in early stages of PMF before fibrosis and cytopenia become manifest. Recently, a negative association between mutations of calreticulin (CALR) and spliceosome genes has been described.

CALR is a Ca2+-binding protein, which was found in 2013 to be mutated in JAK2- or MPL-unmutated PMF and essential thrombocytemia. Mutations were mutually exclusive of JAK2 or MPL mutations. JAK2-mutated and triple-negative patients were shown to have significantly shorter survival periods in comparison to those with somatic frameshift mutations in the CALR gene. Tefferi et al. described significantly lower frequency of spliceosome mutations in CALR-mutated cases and attributed the lower incidence of anemia to the lower frequency of U2AF1 mutations.

Up to now allogeneic hematopoietic stem cell transplantation (AHSCT) represents the only curative treatment modus for patients with PMF. Selection of patients suitable for this kind of treatment is performed according to prognostic scoring and tolerable risks of individual patients. Data of Heuser et al. suggest a better overall survival for CALR-mutated PMF patients after AHSCT.

In this study, we analyzed 69 patients with PMF grades of fibrosis 2–3 (Table 1) who have undergone allogeneic stem cell transplantation for JAK2, MPL, CALR and spliceosome gene mutations (SRSF2, U2AF1 and SF3B1) using bone marrow trephines and pyrosequencing as described. CALR was rarely combined with splice factor gene mutations (10.5% of all CALR-mutated cases; negative correlation, \(P = 0.0418\)) and these combinations were restricted to SF3B1. Combined mutations with U2AF1 and SRSF2 could not be found at all. The frequency of accompanying splice factor gene mutations in CALR-mutated patients was significantly lower than that in patients without a CALR mutation (21/50, 42%; \(P = 0.04\)) or in those with a JAK2 mutation (18/41, 44%; \(P = 0.04\)). U2AF1 was the most frequent splice factor gene mutation associated with JAK2. In PMF, splice factor gene mutations were associated significantly more often with a JAK2 mutation than with a CALR mutation (\(P < 0.00005\); Fisher's exact tests).

In our cohort 7 patients (10%) revealed neither JAK2 nor MPL or CALR mutation. In the ‘triple-negative’ subgroup of PMF, exclusively mutations of SRSF2 occurred (n=2), but no alterations of U2AF1 and SF3B1 could be observed (Table 1). Because of the low number of MPL-mutated cases in this series additional samples of PMF with bone marrow fibrosis grade 2–3 and known MPL mutation (n=20, all JAK2 exon 14 wild type) were investigated for combination with splice factor gene mutations.
proliferating in parallel as well as clonal evolution with stepwise acquisition of different mutations by a single neoplastic clone. Molecular monitoring of patients having undergone AHSCT for PMF should not be restricted to JAK2, MPL, or CALR, but all mutations present in the primary fibrotic neoplastic myeloproliferation should be included to interpret abnormal blood values after AHSCT. The apparently better prognosis of CALR-mutated PMF including cases treated with AHSCT may at least in part be attributable to a less likely association with splice factor gene mutations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The study was supported by a grant of the Deutsche Krebshilfe to HK and GB (grant number 1097154, TP AD).

S Bartels1, U Lehmann1, G Büsche1, J Schlue1, M Mozer1, J Stadler1, I Trivial1, H Alchalby2, N Kröger2 and H Kreipe1

1Institute of Pathology, Medizinische Hochschule Hannover, Hannover, Germany and 2Department of Stem Cell Transplantation, University Medical Center, Hamburg-Eppendorf, Hamburg, Germany E-mail: Kreipe.Hans@MH-Hannover.de

REFERENCES

1 Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. Blood 2013; 122: 4021–4034.
2 Tefferi A, Finke CM, Lasho TL, Wassie EA, Knudson R, Ketterling RP et al. JAK2V617F mutations in primary myelofibrosis are strongly associated with anemia and thrombocytopenia despite clustering with JAK2V617F and normal karyotype. Leukemia 2014; 28: 431–433.
3 Lehmann U, Bartels S, Hasemeier B, Geffers R, Schlue J, Büsche G et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med 2013; 369: 2379–2390.
4 Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hansson CH et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia 2014; 28: 1472–1477.
5 Klamp T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med 2013; 369: 2379–2390.
6 Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med 2013; 369: 2391–2405.
7 Kröger N, Holler E, Kobbe G, Bornhäuser M, Schwedtfechter R, Baumann H et al. Allogeneic stem cell transplantation after reduced-intensity conditioning in patients with myelofibrosis: a prospective, multicenter study of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Blood 2009; 114: 5264–5270.
8 Panagioti V, Thol F, Markus B, Fehse B, Alchalby H, Badbaran A et al. Prognostic effect of calreticulin mutations in patients with myelofibrosis after allogeneic hematopoietic stem cell transplantation. Leukemia 2014; 28: 1532–1535.
Protein kinase N3 deficiency impedes PI3-kinase pathway-driven leukemogenesis without affecting normal hematopoiesis

Leukemia (2015) 29, 255–258; doi:10.1038/leu.2014.278

Protein kinase N family genes encode AGC-type serine–threonine kinases (PKN1, PKN2 and PKN3) that interact with Rho family proteins to regulate cytoskeletal organization and gene expression.1–3 Whereas PKN1 and PKN2 are ubiquitously expressed, PKN3 expression is low in most normal human tissues but high in some malignancies.3 PKN3 catalytic activity is regulated by RhoC and by the PI3-kinase (PI3K) signaling pathway.4,5 PKN3 inactivation inhibits growth of PI3K-driven prostate and breast cancer xenografts.6,7 Its role in hematopoietic malignancies has not yet been described. Since the PI3K pathway regulates HSC function and hematopoiesis13 and the PI3-kinase (PI3K) signaling pathway is activated by RhoC and by PI3K, we hypothesized that PKN3 might be a useful target for treating leukemia patients.

We used genetically engineered mice to test whether Pkn3 deficiency impairs HSC function, MPNs, impaired HSC function and T-ALL leukemogenesis. To test whether Pkn3 regulates HSC function and leukemogenesis in vivo, we used competitive transplantation assays to test whether Pkn3 deletion rescues the HSC mobilization and leukemogenesis that occur following Pten deletion.11,12 Pten encodes a lipid phosphatase that negatively regulates the PI3K pathway.13 Pten deletion impairs HSC function, and Pten-deleted mice develop myeloproliferative neoplasms (MPN) and T-ALL.13,14 These phenotypes can be completely attenuated by simultaneously deleting Rictor to inactivate mTORC2 and its substrate, AKT.14 Since the PI3K pathway activates PKN3 in some cancers,3 we tested whether Pkn3 deletion can prevent HSC mobilization, MPNs, impaired HSC function and T-ALL in Pten-deleted mice.

We first characterized Pkn3 expression in control and Pten-deleted hematopoietic cells. Pten deletion caused a significant reduction in Pkn3 expression in HSCs and in MPN (−50 and 75%, respectively; Figure 2a). In contrast, Pten deletion did not significantly alter Pkn3 expression in thymocytes or T-ALL cells. Thus, the effects of PI3K pathway activation on Pkn3 expression are cell-type specific. This raises the question of whether genetic interactions between Pten and Pkn3 are also cell-type specific.

To test whether Pkn3 deletion rescues the HSC mobilization and MPN phenotypes, we generated Pten Δ/Δ; Pkn3 Δ/Δ; Mx1-Cre (Pten/ Pkn3 Δ/Δ) mice, as well as Cre-negative (control), Pten Δ/Δ; Pkn3 Δ/Δ, Mx1-Cre (Pten/ Pkn3 Δ/Δ) and Pten Δ/Δ; Pkn3 Δ/Δ; Mx1-Cre (Pten Δ/Δ) littermate mice. We deleted Pten and Pkn3 6 weeks after birth and analyzed spleen weights and HSC numbers 2 weeks later. We found that both Pten Δ/Δ and Pten/Pkn3 Δ/Δ mice had enlarged spleens, and spleen HSC numbers were similarly increased in both Pten Δ/Δ