HHS Public Access

Author manuscript

Leukemia. Author manuscript; available in PMC 2015 November 10.

Published in final edited form as:

Leukemia. 2011 July ; 25(7): 1219–1220. doi:10.1038/leu.2011.82.

DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms

O Abdel-Wahab[1,2], **A Pardanani**[3], **R Rampal**[1,2], **TL Lasho**[3], **RL Levine**[1,2], and **A Tefferi**[3]

O Abdel-Wahab: abdelwao@mskcc.org

[1]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2]Leukemia Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[3]Division of Hematology, Mayo Clinic, Rochester, MN, USA

Recent reports have identified somatic mutations in *DNMT3A* in 4–22% of patients with *de novo* acute myeloid leukemia.[1, 2] These mutations, thought to result in a loss-of-function, were found to be predominantly heterozygous mutations in the methyltransferase domain, with a recurrent mutation at the R882 codon. In addition to *DNMT3A* mutations in acute myeloid leukemia, Walter *et al.*[3] have found *DNMT3A* mutations in 8% (12/150) of myelodysplastic syndromes patients as well.[3] Importantly, both Ley *et al.* and Walter *et al.* found that mutations in *DNMT3A* were associated with a worsened overall survival in both *de novo* acute myeloid leukemia and myelodysplastic syndromes, respectively.

Given these finding, we sequenced *DNMT3A* in a clinically and genetically well-annotated cohort of patients with primary myelofibrosis (PMF), the myeloproliferative neoplasm (MPN)/myelodysplastic syndromes overlap syndrome chronic myelomonocytic leukemia (CMML) and advanced phases of MPNs in order to determine the genetic and clinical implications of *DNMT3A* mutations in this class of myeloid maligancies.

Study samples from 94 patients were recruited from the Mayo Clinic (*n*=94; 46 PMF, 22 post-polycythemia vera/essential thrombocythemia MF, 11 blast-phase MPN and 15 CMML). High throughput DNA resequencing was used to seqeunce bone marrow-derived DNA for all coding regions of *DNMT3A* (NM_175629). In addition, these samples were all previously sequenced for all coding regions of *EZH2* (NM_015338), *ASXL1* (NM_004456), and *TET2* (NM_017628) and the regions of known mutations in *IDH1*, *IDH2*, *JAK2* and *MPL* as previously reported.[4] CMML samples were also screened for known *FLT3*, *K Ras* and *N Ras* mutations. Non-synonymous alterations not in dbSNP were censored from analysis.

Conflict of interest

The authors declare no conflict of interest.

We identified three somatic mutations in DNMT3A in this cohort of patients (Table 1). All three mutations were heterozygous and occurred in patients with PMF resulting in a *DNMT3A* mutational frequency of 7% (3/46) in PMF. In addition to the R882H methyltransferase domain mutation found in one PMF patient, the other patients had heterozygous nucleotide deletions outside of the known domains of DNMT3A resulting in the occurrence of premature stop codons. All three PMF patients found to have a mutation in *DNMT3A* were also found to have a co-occuring mutation in another gene known to be mutated in MPNs, including co-occurences with mutations in *JAK2, TET2* and *ASXL1* (Table 1). In addition to the three somatic *DNMT3A* mutations, we found five single nucleotide variants, which we were unable to delineate as somatic missense mutations versus unannotated SNPs. These variants, some of which were also noted by Ley *et al.*,[2] may be delineated in future studies containing more samples with paired normal tissue and are as follows: DNMT3A E30A, P99S, P569A, R659H and R899C.

None of the three PMF patients found to have *DNMT3A* mutations underwent leukemic transformation during the time of follow-up, and the patients had variable Dynamic International Prognostic Scoring System (DIPPS) prognostic scores at presentation.[5] PMF patient 1 was a 56-year-old man at time of diagnosis with a low DIPSS prognostic score at presentation and marked splenomegaly. He has been alive at 64 months without leukemic progression. In contrast, PMF patients 2 and 3 died at 13 and 27 months after presentation (neither developed leukemic transformation). PMF patient 2 was a 79-year-old man at time of diagnosis with a high DIPSS prognostic score at presentation and marked splenomegaly. PMF patient 3 was a 57-year-old woman at time of diagnosis with an intermediate-2 DIPSS prognostic score at presentation, and marked splenomegaly.

We found no mutations in *DNMT3A* in CMML, blast-phase MPN's or post-PV/ET MF suggesting that *DNMT3A* mutations may not be common events in blast-phase transformation of chronic MPNs or in the pathogenesis of CMML. However, larger patient cohorts will be needed to resolve this question more thoroughly and to determine if *DNMT3A* mutations are important in the pathogenesis of PV, ET and/or post-PV/ET MF. The recent discovery of *DNMT3A* mutations in acute myeloid leukemia, myelodysplastic syndromes and now MPNs, underscores the profound heterogeneity and promiscuity of mutations amongst the myeloid malignancies. Larger studies of patients with uniform diagnoses and comprehensive genetic analysis will hopefully allow for greater understanding of the potential clinical impact of these new mutations to MPN prognosis.

Acknowledgements

This work was supported in part by grants from the Gabrielle's Angel Foundation and from the Starr Cancer Consortium to RLL. RLL is an Early Career Award Recipient of the Howard Hughes Medical Institute and is a Geoffrey Beene Junior Faculty Chair at Memorial Sloan Kettering Cancer Center.

References

1. Yamashita Y, Yuan J, Suetake I, Suzuki H, Ishikawa Y, Choi YL, et al. Array-based genomic resequencing of human leukemia. Oncogene. 2010; 29:3723–3731. [PubMed: 20400977]
2. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010; 363:2424–2433. [PubMed: 21067377]

3. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M, et al. Recurrent *DNMT3A* mutations in patients with myelodysplastic syndromes. Leukemia. 2011 e-pub ahead of print 18 March 2011.
4. Abdel-Wahab O, Pardanani A, Patel J, Wadleigh M, Lasho T, Heguy A, et al. Concomitant analysis of ASXL1 and EZH2 mutations in myelofibrosis, chronic myelomonocytic leukemia, and blast-phase myeloproliferative neoplasms. Leukemia. 2011 e-pub ahead of print 01 April 2011.
5. Passamonti F, Cervantes F, Vannucchi AM, Morra E, Rumi E, Pereira A, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). Blood. 2010; 115:1703–1708. [PubMed: 20008785]

Table 1

Mutations in DNMT3A and co-occurring genetic abnormalities in a cohort of PMF patients[a]

Sample	DNMT3a mutation (nucleotide)	DNMT3a mutation (protein)	Karyotype	Other genetic analysis					
				JAK2	TET2	ASXL1	MPL	EZH2	IDH1/2
PMF #1	700_hetDelC	G120fsX40	20q- (all metaphases)	Mutant	WT	WT	WT	WT	WT
PMF #2	1603_hetDelT	P419fsX230	Normal	Mutant	Mutant	WT	WT	WT	WT
PMF #3	2646 G>GA	R882H	Normal	WT	WT	Mutant	WT	WT	WT

Abbreviation: PMF, primary myelofibrosis.

[a]WT: wild type.