DNMT3A and stem cell function: new insights into old pathways

Olga Guryanova and Ross Levine

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

doi:10.3324/haematol.2012.064410

DNA methylation represents an important epigenetic regulator of gene expression which impacts cellular developmental programs through alterations in gene expression. DNA methylation is established by highly related *de novo* DNA methyltransferases Dnmt3a and Dnmt3b, and maintained by Dnmt1.[1] Inhibition of DNA methylation by DNA methyltransferase inhibitors represents an important therapeutic option for patients with myeloid malignancies.[2] However, it can lead to de-repression of genes promoting cancer progression in certain cancer models.[3,4] Consistent with these observations, recurrent somatic nonsense and missense mutations in *DNMT3A* have been identified in acute myeloid leukemia (AML)[5,6] and myelodysplastic syndrome.[7]

A study by Challen and colleagues reported recently in Nature Genetics[8] utilized conditional *Mx-Cre*-driven excision to examine the effects of Dnmt3a loss on hematopoietic stem cell (HSC) function. *Dnmt3a*-deleted HSCs contributed normally to hematopoiesis in primary recipients. By contrast, in secondary transplants the authors observed a dramatic expansion of the stem cell compartment, which may have not been investigated in previous studies.[9] Moreover, despite a marked expansion of stem/progenitor cells, *Dnmt3a*-deficient cells did not show a parallel increase in contribution to differentiated hematopoietic lineages, suggesting a differentiation defect. Indeed, the differentiation quotient of *Dnmt3a*-deleted HSCs declined in tertiary and quaternary transplantation recipients despite preserved self-renewal potential. DNA methylation analysis using reduced representation bisulfite sequencing (RRBS) allowed the authors to identify epigenetic alterations at specific loci, which in some cases were hypomethylated and, in others, were paradoxically hypermethylated. Gene expression analysis showed that *Dnmt3a* loss leads to up-regulated expression of "HSC fingerprint" genes and lower expression of genes with a known role in HSC differentiation.

These studies provide several novel insights, and raise a number of important questions regarding the functional role of *Dnmt3a* in hematopoiesis and leukemogenesis. The lack of correlation between DNA methylation and gene expression changes in *Dnmt3a*-null cells suggests either indirect repression through methylation and diminished expression of transcription factors, or the inability of the methylation studies used in this paper to fully characterize the effects of *Dnmt3a* ablation on genome-wide methylation. Moreover, since the heterozygous missense *DNMT3A* mutations detected in AML have been shown to exhibit diminished enzymatic activity, these mutations may, at least in part, contribute to leukemogenesis through a reduction in wild-type DNMT3A enzymatic function.[6] However, the lack of an overt leukemic phenotype in these studies suggests that *Dnmt3a* loss by itself is insufficient to induce malignant disease,[8] making identification of co-operating lesions critical for the understanding of the pathogenesis of *DNMT3A* mutant leukemias. Curiously, Dnmt3a deletion in a lung cancer model promoted tumor progression but not initiation,[3] and resulted in anchorage-independent growth and expression of metastasis-associated genes in breast cancer cell lines.[4] In the light of these data, it would be valuable to investigate whether *Dnmt3a*-null HSCs are dependent on the bone marrow microenvironment or can support hematopoiesis outside this niche.

In summary, this study represents a step forward in our understanding of the relationship between Dnmt3a, epigenetic regulation and hematopoietic stem cell renewal and differentiation, and provides valuable insight into the biology of *DNMT3A* mutant hematopoietic malignancies.

References

1. Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and function of mammalian DNA methyltransferases. Chembiochem. 2011;12(2):206-22.
2. Rodriguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. Nat Med. 2011;17(3):330-9.
3. Gao Q, Steine EJ, Barrasa MI, Hockemeyer D, Pawlak M, Fu D, et al. Deletion of the de novo DNA methyltransferase Dnmt3a promotes lung tumor progression. Proc Natl Acad Sci USA. 2011;108(44):18061-6.
4. Chik F, Szyf M. Effects of specific DNMT gene depletion on cancer cell transformation and breast cancer cell invasion; toward selective DNMT inhibitors. Carcinogenesis. 2011;32(2):224-32.
5. 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(25):2424-33.
6. Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet. 2011;43(4):309-15.
7. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M, et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia. 2011;25(7):1153-8.
8. Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet. 2011;44(1):23-31.
9. Tadokoro Y, Ema H, Okano M, Li E, Nakauchi H. De novo DNA methyltransferase is essential for self-renewal, but not for differentiation, in hematopoietic stem cells. J Exp Med. 2007;204(4):715-22.