Fenaux, P., Chomienne, C., and Degos, L. (2001). Semin. Hematol. *38*, 13–25.

Hölzel, M., Huang, S., Koster, J., Øra, I., Lakeman, A., Caron, H., Nijkamp, W., Xie, J., Callens, T., Asgharzadeh, S., et al. (2010). Cell *142*, 218–229.

Huang, S., Laoukili, J., Epping, M.T., Koster, J., Hölzel, M., Westerman, B.A., Nijkamp, W., Hata, A., Asgharzadeh, S., Seeger, R.C., et al. (2009). Cancer Cell *15*, 328–340.

Maris, J.M. (2010). N. Engl. J. Med. *362*, 2202–2211.

Matthay, K.K., Villablanca, J.G., Seeger, R.C., Stram, D.O., Harris, R.E., Ramsay, N.K., Swift, P., Shimada, H., Black, C.T., Brodeur, G.M., et al; Children's Cancer Group. (1999). N. Engl. J. Med. *341*, 1165–1173.

Matthay, K.K., Reynolds, C.P., Seeger, R.C., Shimada, H., Adkins, E.S., Haas-Kogan, D., Gerbing, R.B., London, W.B., and Villablanca, J.G. (2009). J. Clin. Oncol. *27*, 1007–1013.

The, I., Murthy, A.E., Hannigan, G.E., Jacoby, L.B., Menon, A.G., Gusella, J.F., and Bernards, A. (1993). Nat. Genet. *3*, 62–66.

Villablanca, J.G., Khan, A.A., Avramis, V.I., Seeger, R.C., Matthay, K.K., Ramsay, N.K., and Reynolds, C.P. (1995). J. Clin. Oncol. *13*, 894–901.

EZH2 Mutations: Mutating the Epigenetic Machinery in Myeloid Malignancies

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Two recent studies identified loss-of-function mutations in the histone H3 methyltransferase EZH2 in myelodysplastic syndromes and myeloproliferative neoplasms, provided further demonstration of mutations in epigenetic modifiers in myeloid malignancies, and suggest *EZH2* functions as a tumor-suppressor gene in these malignancies rather than an oncogene as in some other malignancies.

Laboratory studies have implicated epigenetic dysregulation as a common pathogenetic mechanism in myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPNs), and acute myeloid leukemia (AML). Notably, genome-wide methylation studies have identified a set of genes recurrently targeted by aberrant promoter hypermethylation in AML (Figueroa et al., 2010). Recent studies have shown that chromosome translocations involving *MLL* and *JARID1A* found in myeloid malignancies result in dysregulation of chromatin state and resultant activation of genes that contribute to leukemogenesis (Krivtsov et al., 2008; Wang et al., 2009). In addition, hypomethylating agents (5-azacytidine and decitabine) are approved for the treatment MDS and AML, illustrating the therapeutic relevance of epigenetic alterations in myeloid malignancies. However, until recently, somatic mutations that directly dysregulate the epigenetic state of leukemic cells but are not chromosomal rearrangements had not been reported.

Two groups led by Nicholas Cross and Joop Jansen recently reported identification of recurrent somatic *EZH2* mutations in MDS, MPNs, and MDS/MPN overlap disorders (Ernst et al., 2010; Nikoloski et al., 2010). Alterations in the long arm of chromosome 7 are common in MDS and AML and are associated with adverse outcome of these patients. Both groups first used high-resolution SNP arrays to characterize regions of acquired uniparental disomy (aUPD) and microdeletions involving chromosomal locus 7q36, which includes the *EZH2* gene. They then performed gene resequencing of *EZH2* and identified somatic frameshift, nonsense, and missense mutations in MDS, MDS/MPN, and MPN, with and without concomitant chromosome 7 alterations. The identified missense mutations most commonly affected the CXC-SET domain, which is required for histone methyltransferase activity, or domain II, which is necessary for binding to SUZ12, of EZH2. Importantly, they did not identify *EZH2* mutations in AML with chromosome 7 abnormalities, including those with chromosome 7 aUPD. These results suggest the existence of an additional, not yet identified, tumor suppressor gene for MDS, MPN, and AML on chromosome 7. This is consistent with previous cytogenetic studies of chromosome 7 deletions in MDS and AML that have delineated several regions of common deletion on chromosome 7, only one of which includes the *EZH2* locus (Le Beau et al., 1996). In addition, both current studies suggest that patients having MDS with *EZH2* mutations have worsened overall survival compared to those having MDS without *EZH2* mutations, independent of the presence or absence of cytogenetic abnormalities involving chromosome 7.

The genetic data implicating *EZH2* in the pathogenesis of myeloid malignancies is in contrast to that observed in other malignancies. A recent study employed whole transcriptome resequencing to identify somatic *EZH2* mutations in lymphoma and found that all mutations affected a specific residue within the EZH2 SET domain, tyrosine 641 (Morin et al., 2010). However, tyrosine 641 was not identified as a mutational hotspot in MDS and MPN by Cross, Jansen, and colleagues. Moreover, previous studies

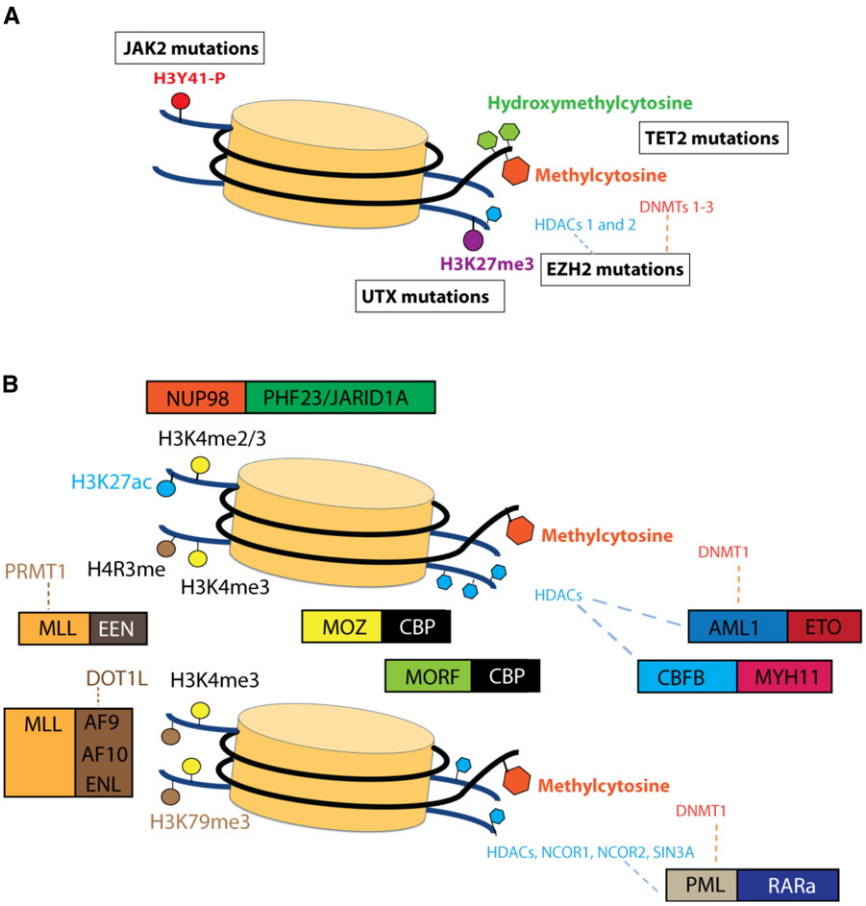

Figure 1. Simple Mutations and Chromosomal Translocations in the Epigenetic Machinery in Myeloid Malignancies

(A) Somatic mutations that affect the epigenetic machinery include gain-of-function mutations in JAK2 (which can phosphorylate histone 3 tyrosine 41) and loss-of-function mutations in the H3K27 demethylase UTX, in the H3K27 methyltransferase EZH2, and in the methyl-hydoxylase TET2.

(B) Chromosomal translocations that affect the epigenetic machinery include MLL fusions that loss H3K4 methyltransferase and gain H3K79 methyltransferase activity, JARID1A/PHF23 fusions that regulate H3K4 di/tri-methylation, and a spectrum of leukemogenic fusions (including PML-RARA, core binding factor fusions, and MOZ/MORF fusions) that interact with histone deacetylases and influence chromatin state.

reported that *EZH2* is overexpressed in prostate and breast carcinomas and that *EZH2* overexpression is associated with metastatic progression of epithelial malignancies (Varambally et al., 2002). Taken together, the genetic data suggest that *EZH2* can function as an oncogene or a tumor suppressor gene in different oncogenic contexts, a hypothesis that requires investigation in subsequent functional studies. The contradicting effects of EZH2 in different tissues is of immediate therapeutic relevance, because inhibition of EZH2 is being investigated as a potential therapy for hematopoietic and epithelial malignancies but inhibition of EZH2 might enhance malignant transformation in some tissues.

An important question is how inactivating mutations in *EZH2* contribute to hematopoietic transformation. EZH2 is a histone H3 lysine 27 (H3K27) methytransferase, and the H3K27 trimethyl mark placed by EZH2 is associated with transcriptional repression. Cross and colleagues demonstrated that MDS/MPN-associated EZH2 mutant proteins had markedly reduced methyltransferase activity. Although these studies demonstrate that EZH2 mutations are loss of function with respect to H3K27 methyltransferase activity, the mechanism by which loss of EZH2 contributes to hematopoietic transformation has not been established. By contrast, overexpression of *EZH2* in epithelial malignancies is associated with transcriptional repression of a discrete set of genes including *INK4B-ARF-INK4A*, *E-cadherin*, *p57*, and *p27* (Varambally et al., 2002). It will therefore be important in subsequent studies to use genome-wide techniques, including microarray and chromatin immunoprecipitation sequencing (ChIP-seq), to investigate gene expression and chromatin state in *EZH2* wild-type and mutant cells to delineate whether inactivating EZH2 mutations contribute to hematopoietic transformation through alterations in chromatin state at specific target genes. Previous work demonstrated that conditional inactivation of *Ezh2* in the mouse hematopoietic system results in defective B lymphocyte development without demonstrable effects on normal myelopoiesis (Su et al., 2003). It will therefore be important to better delineate the effects of EZH2 loss on hematopoietic stem/progenitor function and to develop models of MPN/MDS by combining *Ezh2* loss with other somatic alterations found in *EZH2*-mutant MPN/MDS patient samples.

Notably, previous studies have shown that EZH2 physically interacts with DNA methyltransferases and that H3K27 trimethylation is a prerequisite for DNA promoter methylation (Vire et al., 2006). Previous studies have identified alterations in DNA promoter methylation in patients with MDS and AML. In addition, although DNA hypomethylating agents have demonstrated significant efficacy in MDS and AML patients, to date no studies have identified genetic or epigenetic alterations that predict for response, or lack of response, to these agents. Subsequent studies will therefore need to correlate *EZH2* mutational analysis with genome-wide studies of DNA methylation to determine whether *EZH2* loss in MPN/MDS cells is associated with changes in methylation-mediated regulation of gene expression, and whether *EZH2* mutational status influences the likelihood of response to epigenetically targeted therapies.

The current studies clearly indicate that inactivation of EZH2 contributes to the pathogenesis of myeloid malignancies. This work, and other recent studies reporting mutations in the H3K27 demethylase UTX and in TET2, a putative effector of DNA methylation, demonstrates that somatic mutations which dysregulate the epigenetic machinery are common in myeloid malignancies. It is likely that

alterations in the epigenetic state in myeloid malignancies can occur via distinct mechanisms (Figure 1). In some cases, somatic mutations that alter proteins that modify chromatin and methylate/demethylate DNA may result in epigenetically mediated changes in target gene expression (Figure 1A). In other cases, chromosomal translocations result in alterations in the activity of chromatin-modifying enzymes, which can directly target effectors of hematopoietic transformation (Figure 1B). It is also likely that there are heretofore-undefined mechanisms that contribute to changes in the epigenome in leukemic cells. Further studies that integrate mutational data with studies of chromatin modifications, DNA methylation, and gene expression will be crucial, and future studies probably will identify additional mutations in the epigenetic framework in leukemias and in other hematopoietic and nonhematopoietic neoplasms. Such work in combination with disease-specific in vivo models will hopefully uncover the actual biologic consequences of such newly identified mutations in epigenetic modifiers on malignant pathogenesis.

REFERENCES

Ernst, T., Chase, A.J., Score, J., Hidalgo-Curtis, C.E., Bryant, C., Jones, A.V., Waghorn, K., Zoi, K., Ross, F.M., Reiter, A., et al. (2010). Nat. Genet. *42*, 722–726.

Figueroa, M.E., Lugthart, S., Li, Y., Erpelinck-Verschueren, C., Deng, X., Christos, P.J., Schifano, E., Booth, J., van Putten, W., Skrabanek, L., et al. (2010). Cancer Cell *17*, 13–27.

Krivtsov, A.V., Feng, Z., Lemieux, M.E., Faber, J., Vempati, S., Sinha, A.U., Xia, X., Jesneck, J., Bracken, A.P., Silverman, L.B., et al. (2008). Cancer Cell *14*, 355–368.

Le Beau, M.M., Espinosa, R., 3rd, Davis, E.M., Eisenbart, J.D., Larson, R.A., and Green, E.D. (1996). Blood *88*, 1930–1935.

Morin, R.D., Johnson, N.A., Severson, T.M., Mungall, A.J., An, J., Goya, R., Paul, J.E., Boyle, M., Woolcock, B.W., Kuchenbauer, F., et al. (2010). Nat. Genet. *42*, 181–185.

Nikoloski, G., Langemeijer, S.M., Kuiper, R.P., Knops, R., Massop, M., Tonnissen, E.R., van der Heijden, A., Scheele, T.N., Vandenberghe, P., de Witte, T., et al. (2010). Nat. Genet. *42*, 665–667.

Su, I.H., Basavaraj, A., Krutchinsky, A.N., Hobert, O., Ullrich, A., Chait, B.T., and Tarakhovsky, A. (2003). Nat. Immunol. *4*, 124–131.

Varambally, S., Dhanasekaran, S.M., Zhou, M., Barrette, T.R., Kumar-Sinha, C., Sanda, M.G., Ghosh, D., Pienta, K.J., Sewalt, R.G., Otte, A.P., et al. (2002). Nature *419*, 624–629.

Vire, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., Morey, L., Van Eynde, A., Bernard, D., Vanderwinden, J.M., et al. (2006). Nature *439*, 871–874.

Wang, G.G., Song, J., Wang, Z., Dormann, H.L., Casadio, F., Li, H., Luo, J.L., Patel, D.J., and Allis, C.D. (2009). Nature *459*, 847–851.