A DNMT3A mutation common in AML exhibits dominant-negative effects in murine ES cells

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We investigated the effects of a DNMT3A mutation common in acute myeloid leukemia (AML) on DNA methylation in mouse embryonic stem (ES) cells. Consistent with previous reports, mutation of Arg882 (R882) in DNMT3A results in reduced DNMT3A activity in vitro. However, we show that exogenously expressed R882-mutant DNMT3A proteins inhibit wild-type DNMT3A and DNMT3B, whereas DNMT1 maintains DNA methylation patterns. Furthermore, expression of DNMT3A R882H in ES cells containing endogenous DNMT3A or DNMT3B induces hypomethylation. These results suggest that DNMT3A R882 mutations, in addition to being hypomorphic, have dominant-negative effects.

Key Points

- Mouse Dnmt3a R878H (human R882H) mutant protein inhibits wild-type Dnmt3a/Dnmt3b in murine ES cells, suggesting dominant-negative effects.

Introduction

Recent studies identified somatic mutations of the DNA methyltransferase gene DNMT3A in ~20% of patients with acute myeloid leukemia (AML) and, with lower frequencies, in other hematological malignancies. DNMT3A functions cooperatively with its homolog DNMT3B to initiate de novo DNA methylation, whereas DNMT1 maintains DNA methylation patterns. Although multiple DNMT3A mutations have been identified in AML, the majority (~60%) affect a single amino acid in the catalytic domain, resulting in substitution of Arg882 (R882) with histidine (most common) or other residues. R882-mutant DNMT3A proteins have decreased methyltransferase activity in vitro, which led to the notion that these are primarily loss-of-function mutations. Indeed, heterozygous Dnmt3a mice die at ~4 weeks of age (cause of lethality unknown). Ablation of Dnmt3a in hematopoietic stem cells (HSCs) results in progressive impairment of HSC differentiation and expansion of the HSC pool, but the animals were not reported to develop leukemia. In this study, we investigated the effects of DNMT3A R882 mutations in cells using mouse Dnmt3a-mutant proteins. We showed that the mutant proteins are capable of interacting with wild-type Dnmt3a and Dnmt3b and, when ectopically expressed in murine embryonic stem (ES) cells, induce hypomethylation, suggesting dominant-negative effects.

Study design

Plasmids

Plasmid vectors and oligonucleotides used to generate them are listed in supplemental Tables 1 and 2, respectively, on the Blood Web site.

Transfection, immunoprecipitation, and immunoblotting

Dnmt3a-mutant ES cells were used to generate stable clones. COS-7 cells were used for coimmunoprecipitation experiments. Transfection was performed using lipofectamine (Invitrogen). Immunoprecipitation and immunoblotting were performed using standard protocols. Antibodies used are listed in supplemental Table 3.

DNA methylation analysis

Methylation of the major satellite repeats (MSRs) and the intracisternal A-particle (IAP) retrotransposon was analyzed by methylation-sensitive restriction digestions and Southern hybridization. Methylation of MSRs was quantified by bisulfite sequencing.

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4086  BLOOD, 12 DECEMBER 2013 • VOLUME 122, NUMBER 25
Results and discussion

Human DNMT3A and mouse Dnmt3a show high sequence identity (>96% overall, 100% in catalytic domain), and human R882 corresponds to murine R878 (supplemental Figure 1). Mutation of this residue impairs Dnmt3a methyltransferase activity in vitro.2,3,10 To confirm the effect in vivo, we performed a remethylation assay in [Dnmt3a;Dnmt3b] double knockout (7aabb) ES cells. These cells, after continuous culture for ~5 months (~80 passages), show substantial depletion of DNA methylation, which can be differentially remethylated by transfected Dnmt3 isoforms.11 Myc-tagged WT Dnmt3a/Dnmt3a2 or mutant proteins with R878 substituted with histidine (R878H), cysteine (R878C), or serine (R878S) were stably expressed in 7aabb cells. The expression of Myc-Dnmt3a/Dnmt3a2 or mutant proteins with R878 substituted with anti-Myc antibody, and the precipitated proteins (Myc IP), as well as total cell lysates (TCLs), were analyzed by immunoblotting with anti-Flag and anti-Myc antibodies. (C) Similar experiments as in B except that Flag-Dnmt3b1 or Dnmt3b2 was used instead of Flag-Dnmt3a2. The Flag- and Myc-tagged proteins and the immunoglobulin G (IgG) heavy chain are indicated.

Figure 1. The R878 mutations severely impair the ability of Dnmt3a to methylate DNA but retain its ability to interact with wild-type Dnmt3a and Dnmt3b. (A) Myc-tagged WT Dnmt3a/Dnmt3b or R878 mutant (R878H [RH], R878C [RC], or R878S [RS]) was transfected into 7aabb ES cells (passage number: ~80), and stable clones were obtained. The cell lysates were analyzed by immunoblotting with anti-Myc and anti–β-actin antibodies, and genomic DNA was digested with Maeli and analyzed by Southern hybridization with a probe specific for the MSRs. Untransfected 7aabb cells and WT (J1) ES cells were used as controls. Densitometry was used to determine the relative methylation levels (methylation scores), as described in supplemental Figure 3, and bisulfite sequencing was used to quantify methylation levels in representative samples (supplemental Figure 4). NT, not tested. (B) Flag-tagged and Myc-tagged WT and/or mutant Dnmt3a2 (as indicated) were cotransfected in COS-7 cells, the cell lysates were immunoprecipitated with anti-Myc antibody, and the precipitated proteins (Myc IP), as well as total cell lysates (TCLs), were analyzed by immunoblotting with anti-Flag and anti-Myc antibodies. (C) Similar experiments as in B except that Flag-Dnmt3b1 or Dnmt3b2 was used instead of Flag-Dnmt3a2. The Flag- and Myc-tagged proteins and the immunoglobulin G (IgG) heavy chain are indicated.
Dnmt3a/Dnmt3b-mediated methylation of MSRs and IAP was inhibited in the presence of R878H mutant proteins (Figure 2A; supplemental Figures 2B, 4C, and 7A-B). To determine whether R878H mutant proteins would antagonize endogenous Dnmt3a and Dnmt3b, we transfected Myc-Dnmt3a2 or -Dnmt3a2:R878H in Dnmt3b2/2 (8bb) and Dnmt3a2/2 (6aa) ES cells, and stable clones expressing similar levels of WT Dnmt3a2 or Dnmt3a2:R878H were analyzed (Figure 2B-C). Expression of Dnmt3a2:R878H in both 8bb and 6aa cells led to hypomethylation of MSRs and IAP, whereas expression of WT Dnmt3a2 either had no obvious effect or resulted in slight increases in methylation compared with untransfected cells (Figure 2B-C; supplemental Figures 2C-D and 4D-E). A similar effect was observed when Dnmt3a:R878H was expressed in 8bb cells (supplemental Figure 7C).

It has been debated whether DNMT3A mutations contribute to leukemogenesis due to haploinsufficiency or gain-of-function/dominant-negative effects or both. Our finding that the R878H-mutant proteins would antagonize endogenous Dnmt3a and Dnmt3b, we transfected Myc-Dnmt3a2 or -Dnmt3a2:R878H in Dnmt3b2/2 (8bb) and Dnmt3a2/2 (6aa) ES cells, and stable clones expressing similar levels of WT Dnmt3a2 or Dnmt3a2:R878H were analyzed (Figure 2B-C). Expression of Dnmt3a2:R878H in both 8bb and 6aa cells led to hypomethylation of MSRs and IAP, whereas expression of WT Dnmt3a2 either had no obvious effect or resulted in slight increases in methylation compared with untransfected cells (Figure 2B-C; supplemental Figures 2C-D and 4D-E). A similar effect was observed when Dnmt3a:R878H was expressed in 8bb cells (supplemental Figure 7C).

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However, the striking effects of the mutant protein on DNA methylation in murine ES cells suggest that dominant-negative effects very likely contribute to methylation alterations in AML.

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Authorship

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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