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MiR-128 and miR-125 regulate expression of coagulation Factor IX gene with nonsense mutation by repressing nonsense-mediated mRNA decay

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ABSTRACT

Hemophilia could be caused by a nonsense mutation of the Factor IX gene, leading to a deficiency of Factor IX (F9). The nonsense mutation frequency of F9 is more than 10% according to the database. Nonsense-mediated mRNA decay (NMD) is a defined cellular response that can potentially prevent the production of such deleterious C-terminal truncated proteins from aberrant mRNA. Here, we constructed a mini-gene of Factor IX (Mini-hF9) and some nonsense mutants and characterized the mini-gene splicing pattern. We discovered that NMD regulated mini-hF9 expression in two nonsense mutants: E7a (nt 34 G > T in exon 7) and E7b (nt 52 G > T in exon 7), but not in another nonsense mutants: E7c (nt 85 G > T in exon 7) and E8 (nt 42 C > T in exon 8). In addition, mini-hF9 transcripts were accumulated after transfection with miR-128 or miR-125 mimics in E7a and E7b. Our results suggest that PTC (premature termination codon) location is a key determination for triggering NMD; miR-128 and miR-125 could help to increase the nonsense-mutant F9 levels by repressing NMD.

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1. Introduction

The coagulation Factor IX (F9) gene encodes a serine protease that is critical for blood clotting and coagulation. Individuals with severe hemophilia B have functional F9 levels that are less than 1% of normal values and have frequent bleeding events, which are commonly associated with crippling arthropathy and early death [1,2]. Restoration to ~5% activity converts severe hemophilia B to a mild form [3]. There are 87 nonsense mutations in all 799 point mutations, according to the Hemophilia B Mutation Database (http://www.factorix.org/).

Transcripts containing premature termination codons (PTCs) are a serious problem for cells, as they may encode C-terminal truncated proteins with dominant-negative or deleterious gain-of-function activity [4,5]. Nonsense-mediated decay (NMD), a pathway that degrades mRNAs containing nonsense codons, has been defined that can potentially prevent the production of such deleterious C-terminal truncated proteins from PTC-containing mRNA. In eukaryotic cells, NMD requires both active mRNA translation and NMD-specific *trans*-acting factors [6]. Phosphorylation/dephosphorylation cycles of UPF1 are essential for NMD [7]. The SMG1 protein mediates the phosphorylation of UPF1, and the SMG5, SMG6 and SMG7 proteins mediate the dephosphorylation of UPF1 [7–9]. Mammalian tissue culture studies have established the splicing-dependent exon–exon junction complex (EJC) as another important *trans*-acting component for NMD [8,10,11]. In mammals, NMD cannot distinguish PTCs in the penultimate exons that are located less than ~55 base pairs (bp) from the final intron [4].

MicroRNAs (miRNAs) are small (~23 nt), regulatory, noncoding RNA molecules that control the expression of their target mRNAs predominantly by binding to the 3′ untranslated region (UTR) [12,13]. MiR-128 was recently reported to repress NMD function by targeting UPF1 and the EJC core component MLN51 (also known as CASC3 or BTZ) in mammals [14]. Specifically, induction of the brain (and thymus) –restricted miRNA-128 early in brain development reduces NMD efficiency by targeting mRNAs encoding UPF1 and

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MLN51, thereby reducing the functional levels of these proteins [15,16]. Another miRNA that may contribute to this regulation is miR-125 (including miR-125a and miR-125b), a neurally expressed miRNA that recently shown to repress the expression of another NMD factor SMG1 [16,17]. These data suggest the existence of a conserved mRNA circuit that links the microRNA and NMD pathways and results in the induction of cell type-specific transcripts during development.

In this work, we constructed a mini-gene of Factor IX (Mini-hF9) and some nonsense mutants and characterized the mini-gene splicing pattern. In two nonsense mutants, E7a (nt 34 G > T in exon 7) and E7b (nt 52 G > T in exon 7), transcripts were observed to be regulated by NMD, which had a premature termination codon (PTC) located >55 nucleotides upstream of the last exon–exon junction. In another mutants, E7c (nt 85 G > T in exon 7) and E8 (nt 42 C > T in exon 8), the levels of F9 transcripts did not decrease compared with wild type, which had a PTC located less than 55 nucleotides upstream of the last exon–exon junction or in the last exon. In addition, we discovered that mini-hF9 transcripts were accumulated after transfection with miR-128 or miR-125 mimics in E7a and E7b. Our results suggest that PTC (premature termination codon) location is a key determination for triggering NMD; miR-128 and miR-125 could help to increase the nonsense-mutant F9 levels by repressing NMD.

2. Materials and methods

2.1. Construction of the mini gene and nonsense mutants

A mini gene of Factor IX (Mini-hF9) was cloned into the pcDNA3.1 (−) vector. 6 × Myc and 6 × HA tags were added at the 5′- and 3′-ends of the mini gene, respectively, to analyze the expression of the mini gene. The Mini-hF9 gene contains three parts: the first part contains the whole exon 1 and the 5′ partial intron 1; the second part contains the 3′ partial intron 5, the whole exon 6 and the 5′ partial intron 6; and the last part contains the 3′ partial intron 6, the whole exon 7, intron 7, and exon 8 followed by termination codon (Fig. 1A). We then obtained the nonsense mutants E7a (nt 34 G > T in exon 7), E7b (nt 52 G > T in exon 7), E7c (nt 85 G > T in exon 7) and E8 (nt 42 C > T in exon 8) using PCR

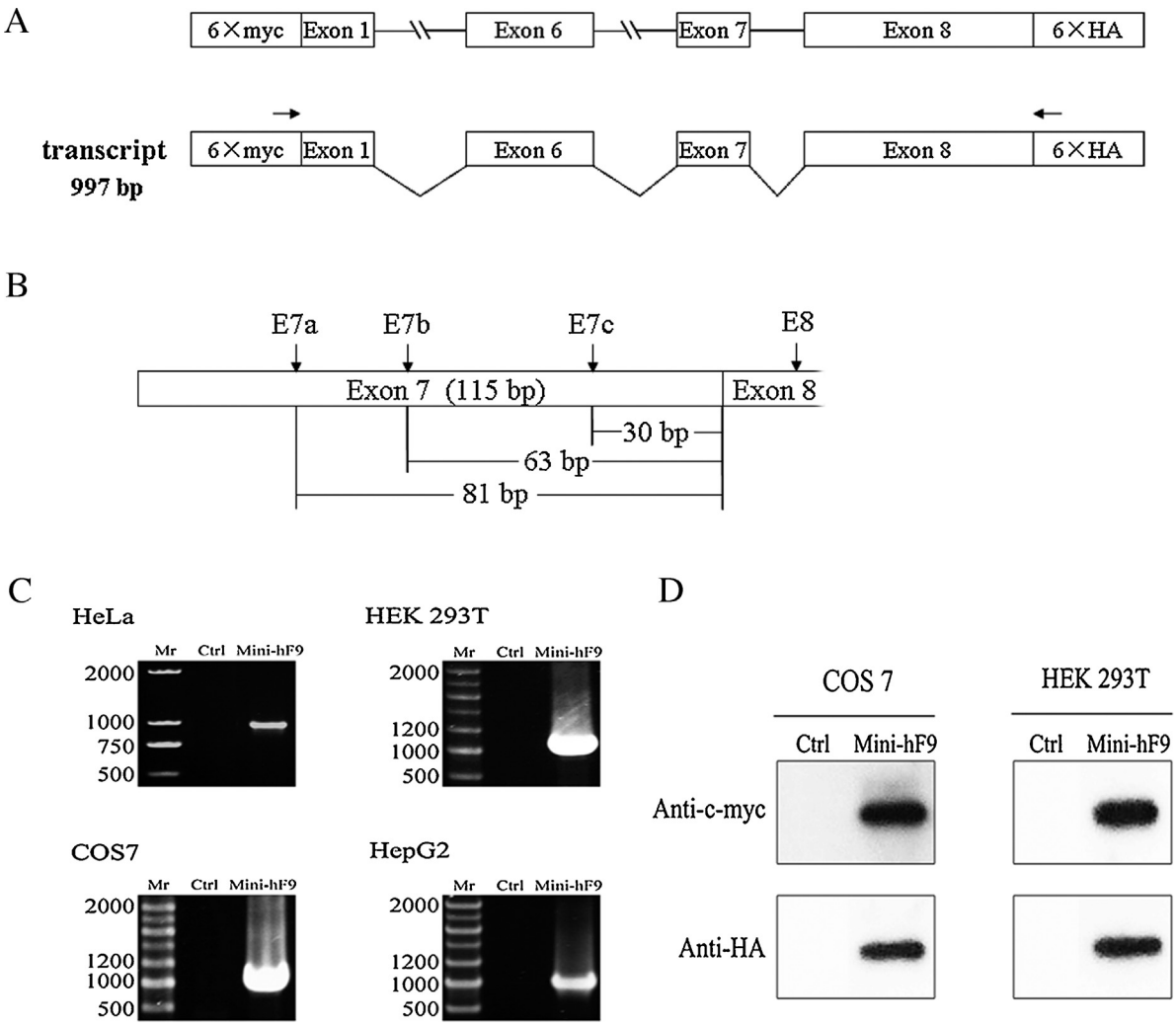

Fig. 1. Construction and expression of Mini-hF9 gene. (A) Diagram of the full-length Mini-hF9 precursor mRNA and normally spliced mRNAs derived from this mRNA. (B) Diagram of the PTC location in E7a, E7b, E7c and E8 mutants. (C) RT-PCR products obtained with the primer couple (BF319 and BF320, shown as arrows in A) on a 1.2% agarose gel. Lane Mr indicates molecular weight marker. Lane Ctrl indicates RT-PCR products from cells transfected with pcDNA3.1 (−) expression vectors. No altered splicing events were detected in cells transfected with the Mini-hF9 gene expression vector. The only RT-PCR products were found and confirmed by sequencing. (D) Western blotting analysis of myc/HA-tagged Mini-hF9 protein in COS-7 and HEK293T cells. Lane Ctrl indicates proteins from cells transfected with pcDNA3.1 (−) expression vectors. The only full length protein was detected in cells transfected with the Mini-hF9 gene expression vector.

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