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MicroRNA-196 inhibits HOXB8 expression in myeloid differentiation of HL60 cells

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ABSTRACT

MicroRNAs (miRNAs) are endogenous small RNAs (~21-25 nts) that can regulate gene expression at a post-transcriptional level. Recently a large number of miRNAs have been identified from plants and animals. In addition, it has been reported that these small RNAs can participate in cell differentiation of developmental timing. In this study, we show that microRNA-196 (miR-196) inhibits HOXB8 expression in myeloid differentiation of HL60 cells. MiR-196 has perfect complementarity with target sequence of 3'-UTR in *HOXB8* mRNA. Exogenous expressed miR-196 repressed expression of HOXB8 via cleaving mRNAs. Moreover, miR-196 enhanced myeloid differentiation of HL60 cells. These results suggest that miR-196 participates in myeloid differentiation of HL60 cells via regulation of HOXB8 expression.

INTRODUCTION

MicroRNAs are small non-coding RNAs that have an ability to regulate gene expression at a post-transcriptional level (1,2). These RNAs associate with several endogenous proteins and form ribonucleoprotein complex (miRNP) that can act inhibition of mRNA translation by partial base-pairing to the 3'-UTR of target mRNAs. In addition, miRNAs can also function as small-interfering RNAs (siRNAs) when they bind to target mRNAs by almost perfect base-pairing in mammals and plants (1,2). These functions of miRNAs indicate that miRNA-mediated gene silencing is very similar to RNA interference (RNAi) pathway (3,4).

The role of miRNAs is well studied in *C.elegans*. *Lin-4* and *let-7* are identified from the genetic analysis of developmental timing in the nematode (5). *Lin-4* is complementary to sequences in the 3'-UTR of *lin-14* and *lin-28* mRNAs. The synthesis of LIN-14 and LIN-28 proteins is repressed by *lin-4* during the early larval stages of *C. elegans* developmet. In addition, *lin-4* or *let-7* mutant worms fail to execute certain developmental switches, resulting in the abnormal repetition of certain larval stages. These observations suggest that miRNAs participate in cell differentiation of developmental timing.

In mammals, although more than 200 miRNAs have been found, identification of target genes and functional analysis of miRNAs has been remained. We have previously demonstrated that human miRNA 23 (miR-23) can regulates expression of Hairy/enhancer of split protein (HES1) that participates in Notch-signaling pathway at a post-transcriptional level during retinoic acid-induced neuronal differentiation (6). In addition, exogenous miRNA expression in hematopoietic stem/progenitor cells led to an increased fraction of B-lineage cells (7). Moreover, it has been reported that microRNA-196 (miR-196) cleaves chimera lusiferase mRNA that fused 3'-UTR sequence of *HOXB8* gene *in vitro* and *in vivo* (8).

In this study, we show that miR-196 inhibits HOXB8 expression in myeloid differentiation of HL60 cells.

RESULTS AND DISCUSSION

At first, we searched target genes of miR-196 using Blast search program. As a result, we could find *HoxB8* mRNA as a target gene of miR-196. Target sequence of miR-196 was found with perfect complementarity in 3'-UTR of *HOXB8* mRNA (Fig. 1).

HOXB8 is a member of the mammalian HOX complex, a group of 39 transcription factors best known for

their roles during early development in providing positional information along the anteroposterior axis (9). In addition, this protein is transcriptionally activated in AML myeloid leukemia cells. Since HOXB8 prevents differentiation of factor-dependent myloid progenitors (10), we investigated the role of miR-196 and HOXB8 in vitamin D3-induced monocytic differentiation of HL60 cells.

Then we examined the level of HOXB8 in un- and differentiated HL60 cells by Western blotting analysis with HOXB8 specific polyclonal antibody. As results, the level of HOXB8 was decreased during vitamin D3-induced monocytic differentiation of HL60 cells. We next checked the both levels of *HOXB8* mRNA and miR-196 by Northern blotting analysis. The level of *HOXB8* mRNA was decreased during the differentiation. By contrast, the level of miR-196 was increased during the differentiation. These results suggest that expression of HOXB8 is regulated at a transcriptional or a post-transcriptional level in vitamin D3-induced differentiation.

To examine whether miR-196 can inhibits expression of HOXB8, we constructed miR-196 expression vector that is controlled by pol II promoter. Then miR-196 expression vector was introduced into HL60 cells. Levels of HOXB8 and *HOXB8* mRNA were determined by Western blotting and Northern blotting analysis, respectively. As results, the level of HOXB8 in cells that expressed miR-196 was decreased compared with that in cells that expressed mutant miR-196. In addition, the level of *HOXB8* mRNA was also decreased in the presence of exogenous miR-196. These results suggest that miR-196 can regulate expression of HOXB8 at a post-transcriptional level.

Next, to investigate a target specificity of miR-196, we constructed plasmids for expression of a chimeric gene for luciferase that contains 3'-UTR of *HOXB8* mRNA under the sequence of luciferase gene (Luc-HOXB8). Then the plasmid was introduced into HL60 cells that expressed miR-196 or mutant miR-196. As results, we detected luciferase activity in undifferentiated HL60 cells that expressed the gene for Luc-HOXB8. By contrast, the luciferase activity in differentiated cells that expressed the gene for Luc-HOXB8 was lower than that in undifferentiated cells. Additionally, the luciferase activity of Luc-HOXB8 in undifferentiated HL60 cells that expressed miR-196 was lower than that in untreated WT HL60 cells. Mutant miR-196 did not affect the luciferase activity in cells that expressed Luc-HOXB8.

Moreover, to examine whether exogenous miR-196 expression affects vitamin D3-induced monocytic differentiation, miR-196 expression plasmid was introduced into HL60 cells in the presence or absence of vitamin D3. As results, exogenous miR-196 led to an increase of monocytic cell population in the presence of vitamin D3. By contrast, exogenous mutant miR-196 did not affect the differentiation in the presence or absence of vitamin D3. These results suggested that miR-196 has a critical role in vitamin D3-induced monocytic differentiation of HL60 cells.

In conclusion, our results indicate that miR-196 regulates expression of *HOXB8* gene in vitamin D3-induced monocytic differentiation of HL60 cells.

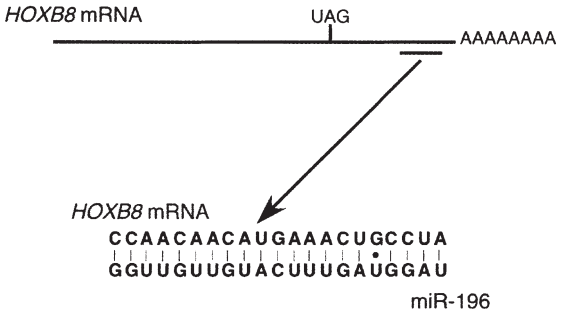

Fig. 1. The secondary structure between *HOXB8* mRNA and miR-196

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