BASIC SCIENCE: OBSTETRICS

Overexpression of miR-152 leads to reduced expression of human leukocyte antigen-G and increased natural killer cell mediated cytolysis in JEG-3 cells

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OBJECTIVE: The purpose of this study was to gain a further understanding of the relationship between miR-152 and human leukocyte antigen (HLA)-G in human trophoblast cell line (JEG-3).

STUDY DESIGN: The JEG-3 cells were transfected with pre–miR-152. The effect of the overexpressed miR-152 on HLA-G expression, trophoblast invasion, and natural killer (NK) cell–mediated cytolysis were assessed by reverse-transcription polymerase chain reaction (RT-PCR) and Western blot analysis, transwell invasion assay, and NK cell cytotoxicity assay, respectively.

RESULTS: The miR-152 repressed HLA-G expression but exerted no effect on JEG-3 cell invasion, and overexpression of miR-152 led to increased NK cell–mediated cytolysis in JEG-3 cells.

CONCLUSION: The data indicate that miR-152 may function as an immune system enhancer through up-regulating NK cell–mediated cytolysis of host cells.

Key words: cytolysis, human leukocyte antigen-G, JEG-3 cell, miR-152

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Human leukocyte antigen (HLA)-G is a nonclassical HLA class Ib molecule that is mainly expressed at the maternal-fetal interface during pregnancy as well as in tumors, infected tissues, transplanted organs, etc.[1] Evidence shows that HLA-G is involved in inducing and maintaining immune tolerance via interaction with inhibitory receptors present on natural killer (NK) cells, T and B lymphocytes, and antigen-presenting cells.[2]

HLA-G expression is necessary for a successful pregnancy. The abnormal expression of HLA-G may alter the maternal-fetal immune tolerance and thus be associated with failed pregnancy, such as preeclampsia and miscarriage. Moreover, HLA-G plays an important role in many human diseases, including cancer, viral infection, and inflammatory diseases as well as organ transplantation.[1-3]

The fact that HLA-G exerts immunomodulatory effects during physiological and pathophysiological processes suggests that its expression is under tight regulation. An understanding of the regulation of HLA-G expression is essential to fully appreciate the function of this protein. However, the exact mechanisms underlying the regulation of HLA-G expression are still poorly understood. It has been reported previously that microRNAs (miRNAs) participate in the regulation of the HLA-G gene expression.[4]

The miRNAs are noncoding ribonucleic acids (RNAs) of 21-24 nucleotides that function as negative regulators of gene expression by binding to complementary sequences in the 3'-untranslated region (3'-UTR) of their target gene messenger RNAs (mRNAs) and subsequent induction of translation inhibition, which can also be associated with transcript destabilization.[5-7] The miRNAs play a fundamental role in diverse biological and pathological processes, which include cell proliferation, apoptosis, and carcinogenesis.[8,9]

Recent data indicate that miR-152 targets HLA-G 3'-UTR in the human bronchial epithelial cell line.[4] It is well known that the 2-8 nt (seed region) of miRNA is suggested to be the most important for target recognition.[10-12] The HLA-G mRNA 3'-UTR of the human trophoblast cell line (JEG-3 choriocarcinoma cell line) contains a binding site that is reverse complementary with the miR-152 seed region (Figure 1). Furthermore, previous work from our laboratory has demonstrated that miR-152 was overexpressed in preeclamptic placentas,[13] in

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TABLE
The primer sequences and reaction conditions for RT-PCR

Gene	Primer sequences (F: forward; R: reverse)	Annealing temperature (°C)	Cycle (n)
HLA-G	F: CTGACCCTGACCGAGACCT R: CTCGCTCTGGTTGTAGTAGCC	56	29
β-Actin	F: TGCGCAGAAAACAAGATGAGATT R: TGGGGGACAAAAAGGGGGAAGG	55	25
miR-152	RT: GTCGTATCCAGTGCAGGGTCCGAG GTATTCGCACTGGATACGACcccaag F: GTCGTCAGTGCATGACAGAACTT R: GTGCAGGGTCCGAGGT	60	35
U6	R: AACGCTTCACGAATTTGCGT F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT	60	30
HLA-G-UTR	F: GACTAGTAGAAAGAAGAGCTCAGATTGA R: CCCAAGCTTCAGAAGTAAGTTATAGCTCAG	58	36

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which low expression of HLA-G was confirmed.[14]

These results of previous studies suggest that miR-152 may be involved in the pathogenesis of failed pregnancy through negatively regulation of HLA-G. To gain a further understanding of the relationship between miR-152 and HLA-G in the human trophoblast cells, we analyzed the effect of the overexpressed miR-152 on HLA-G expression, trophoblast invasion, and NK cell–mediated cytolysis in JEG-3 cells.

MATERIALS AND METHODS

Cell lines and culture conditions

JEG-3 choriocarcinoma cells (HBT-36) and NK-92MI cells (CRL-2408) were obtained from American Type Culture Collection (Manassas, VA). The JEG-3 cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, BRL, Gaithersburg, MD), containing 10% fetal bovine serum (FBS). The NK-92MI cells were maintained in alpha minimum essential medium (α-MEM, Gibco) supplemented with 2 mM L-glutamine, 0.2 mM i-inositol, 0.02 mM folic acid, 0.1 mM 2-mercaptoethanol, 12.5% FBS, and 12.5% horse serum. All cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Transient overexpression of hsa-miR-152

The miR-152 precursor (pre-miR-152) and Cy3 dye labeled pre-miR negative control #1 (pre-miR-control) were purchased from Ambion (Austin, TX). The JEG-3 cells were transfected with pre-miR-152 using siPORT NeoFx reagent (Ambion) according to the manufacturer's protocol.

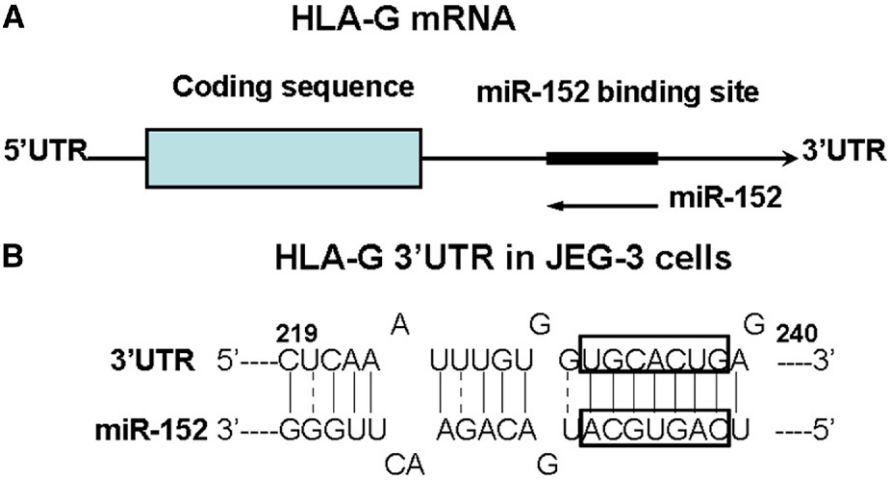
To confirm the efficiency of transfection, the Cy3-labeled control #1 was also transfected. The medium was replaced with fresh growth medium after 12 hours. Forty-eight to 72 hours after transfection, cells were used for reverse-transcription polymerase chain reaction (RT-PCR) analysis, Western blot analysis, transwell invasion assay, and NK cell cytotoxicity assay.

RT-PCR analysis

Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA). RNA integrity was confirmed by electrophoresis in a 1.5% agarose denaturing gel. For the detection of HLA-G gene, 1 μg of the total RNA was subsequently reverse transcribed into complementary deoxyribonucleic acid (cDNA) primed by oligo (dT) with the use of the RevertAid first-strand cDNA synthesis kit (MBI; Fermentas, Vilnius, Lithuania). β-Actin was used as an endogenous control.

For the detection of mature miRNA, 4 μg of the total RNA was reverse transcribed to cDNA with gene-specific RT primer, which could fold to a stem-loop structure. The highly conserved and universally expressed small nuclear RNA U6 was used as an endogenous control. All primer sequences and the reaction con-

FIGURE 1
The target sequence of miR-152 in the 3'-UTR of HLA-G



A, Schematic of HLA-G mRNA and miR-152. **B,** The sequence of miR-152 and its potential matching site in the HLA-G mRNA 3'-UTR of JEG-3 cells. The HLA-G 3'-UTR contains a binding site that is reverse complementary with the miR-152 seed region (2-8 nt).

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