OPEN ACCESS
Freely Available Online

JOURNAL OF EVOLVING STEM CELL RESEARCH
ISSN NO: 2574-4372

RESEARCH ARTICLE
DOI : 10.14302/issn.2574-4372.jesr-15-768

Microrna-1285 might Potentially Regulate *OCT4* Gene Expression by Direct Targeting of its Promoter

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Abstract

The human *OCT4* gene encodes a transcription factor that maintains pluripotency and self-renewal in Embryonic Stem (ES) cells. This gene generates several known transcripts by alternative promoter and alternative splicing (OCT4A, OCT4B and OCT4B1). Even though OCT4A is the main isoform responsible for stemness properties, several recent controversial studies claimed that this isoform is expressed in cancer cell lines and differentiated cells, in addition to the ES cells. Our in silico studies revealed that OCT4A promoter has a completely match binding site for hsa-miR-1285. This microRNA was detected in the human embryonic stem cells for the first time and further studies showed that miR-1285 can target some tumor suppressor genes, (TSGs), such as *p53*, and oncogenic genes, such as *TGM2.*

Additional bioinformatics analysis of short RNA sequencing data from ENCODE cell lines showed that miR-1285 is expressed in different cancer cell lines and differentiated cells. In this study, we supposed that miR-1285 potentially can bind to the *OCT4* promoter and might regulate transcription of the *OCT4* in the human cancer cell lines and differentiated cells.

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Keywords: *OCT4* gene, OCT4A, microRNA, hsa-miR-1285, cancer cells, promoter.

Received: Oct 05, 2015; **Accepted**: Dec 31, 2015; **Published**: Jan 16, 2016;

Introduction

OCT4 (known as *POU5F1* and *OCT3/4*) is a key transcription factor in maintaining pluripotency and self -renewal in human and mouse embryonic stem (ES) cells [1-4]. This gene encodes at least three transcripts, designated as OCT4A, OCT4B and OCT4B1, by alternative splicing and alternative promoter mechanisms [5-8]. The Transcription Start Site (TSS) of OCT4A is located in upstream of exon1, but the OCT4B and OCT4B1 variants are transcribed from an alternative promoter in interon1 of *oct4* gene [8]. The OCT4A is localized in the nucleus of ES cells and is the main variant responsible for stemness properties in these cells furthermore, its overexpression in fibroblast cells produces induced pluripotent stem (iPS) cells [9-12].

Up until now, several studies have shown that the expression of *OCT4* gene can be regulated by microRNAs, and also some microRNA genes can be regulated by *OCT4* protein. Greer Card et al. (2008) showed that miR-302 is transcriptionally activated by Oct4/Sox2 which binds to the promoter region of the miR-302 cluster in human ES cells [13]. In 2009, Xu et al. demonstrated that miR-145 promoter is bound and repressed by *OCT4* in ES cells and surprisingly, *OCT4* 3′-UTR can be targeted and down-regulated by miR-145 [14].

MicroRNAs are a group of non-coding RNAs (nc-RNAs), which are ☐ 21 nucleotides long and were introduced for the first time by Ambros group (1993), and play important roles in numerous biological process such as proliferation, cell cycle control, apoptosis, differentiation, migration and metabolism by regulating gene expression in post transcriptional manner. These nc-RNAs are transcribed by RNA polymerase II and then processed by Drosha and DGCR8 into microRNA precursors, which are nearly 60–110 nucleotides in length with stem loop structure and generate 20-25 nucleotides mature microRNA in the cytoplasm by Dicer protein. MicroRNAs that are perfectly matched with their target mRNAs (often in 3′-UTR) can degrade their targets, but those which are matched with their targets imperfectly inhibit their translation into the protein.

Recent studies have shown that microRNAs might regulate gene expression by potential miRNA target sites within gene promoters. These microRNAs target promoters and consequently inhibit or induce transcription from those promoters. A single microRNA could regulate several mRNAs and also the same mRNA might be targeted by multiple microRNAs. Recent studies have shown that some microRNAs can act as tumor suppressors or oncogenes. The other reports verified that stemness and differentiation could be influenced by microRNAs [15-19].

Morin et al. (2008) detected mir-1285, for the first time in the embryonic stem cells [20]. The subsequent study indicated that miR-1285 can target 3′-UTR of P53 mRNA and directly inhibits its expression [21]. Further studies showed that miR-1285 targets oncogenic genes, including *TGM2* in renal cell carcinoma (RCC) development [22]. Our in silico analysis indicated that miR-1285 has a binding site on the *OCT4* promoter and might regulate the *OCT4* gene expression in cancer and non-pluripotent cells.

Whereas *OCT4* is a transcription factor responsible for stemness properties of embryonic stem cells, some studies claimed that *OCT4* is expressed in

cancer and differentiated cells. Thus, there are many controversies about the expression of *OCT4* in the cancer and differentiated cells. In this study, using in silico and experimental data, we speculated that miR-1285 could regulate *OCT4* expression in differentiated and cancer cells. More experimental studies are needed to confirm the exact influence of miR-1285 on *OCT4* expression in cancer and differentiated cell lines.

Material and Methods

In Silico analysis for *OCT4* gene

Promoter and 3'-UTR of the human *OCT4* gene were scanned using miRBase (www.mirbase.org) and mirEval databases (http://mimirna.centenary.org.au/mireval) for finding microRNA target sites. These bioinformatics analyses indicate a 21-nts perfectly matched target site for hsa-miR-1285-3p at position of -316 to -295 upstream of *OCT4* start codon (ATG), and there was no target site for this microRNA in the whole *OCT4* gene sequence (Fig1).

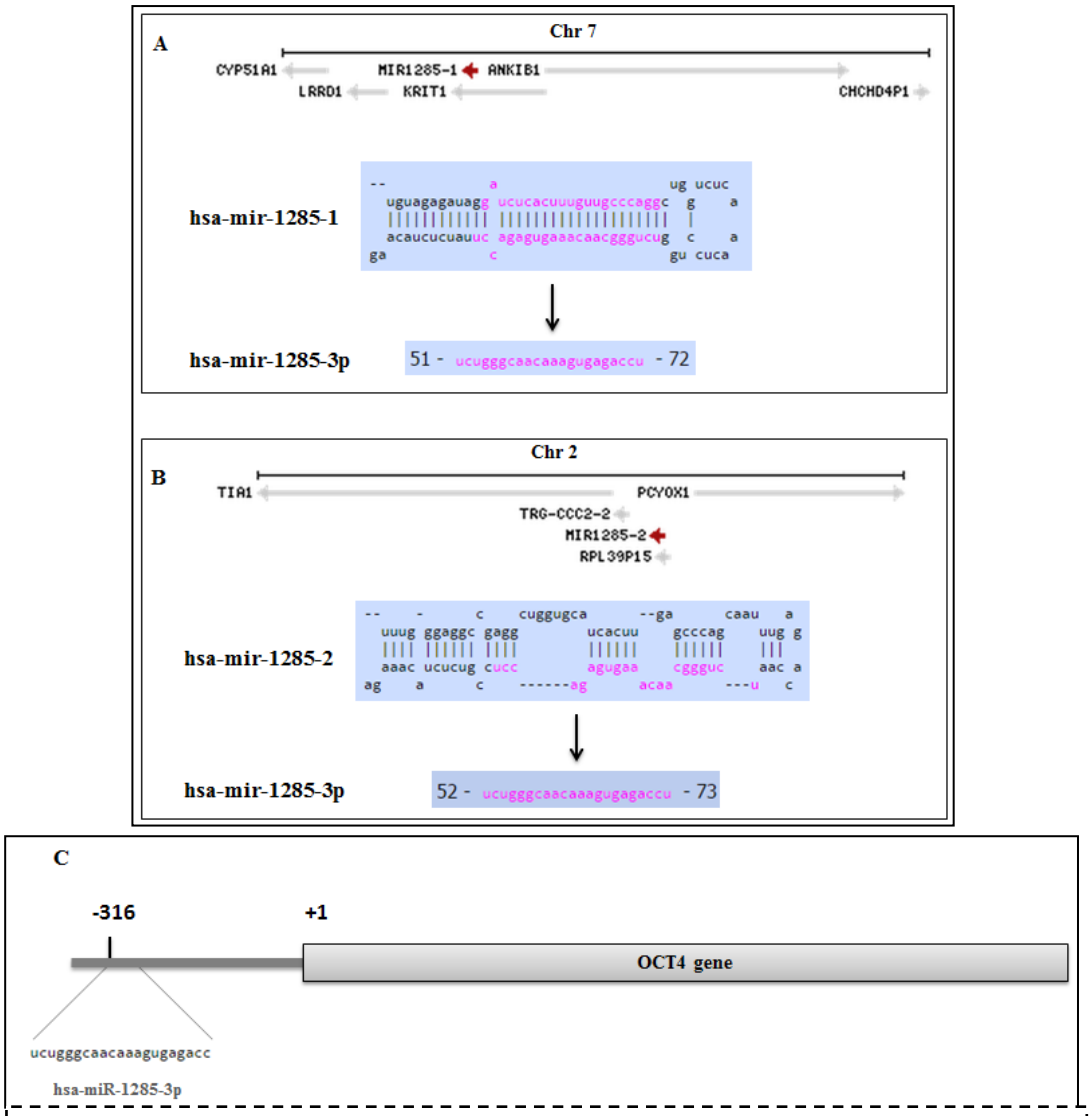

Figure 1. Schematic structure of hsa-miR-1285-1 (A) and hsa-miR-1285-2 (B). hsa-miR-1285-1 transcribes from chromosome 7 and hsa-miR-1285-2 transcribes from chromosome 2. Both microRNAs can produce hsa-miR-1285-3p. (C) Binding site location of microRNA-1285 on the OCT4 promoter. MiR-1285-3p can bind to the upstream of Transcription Start Site (TSS) of OCT4A (position of -316).

In Silico Analysis for hsa-miR-1285

The expression of hsa-miR-1285 in various cancer cell lines and differentiated cells was considered by data obtained from ENCODE project in UCSC genome browser. Also, target genes and signaling pathways, which might be influenced by miR-1285, were predicted by DIANA microT and DAVID 6.7 databases, respectively.

Cell Culture

5637 cells (obtained from the Pasteur Institute, Iran), was cultured in RPMI-1640 media (Invitrogen), supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 100 U/ml penicillin and 100mg/ml streptomycin (Sigma), and incubated at 37°C with 5% CO2. NT2, 1321N1, Daoy and A172 cells (obtained from the Pasteur Institute, Iran) were cultured in DMEM-HG containing 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin.

RNA Extraction and cDNA Synthesis

For each sample, Total RNA was extracted using TRIzol reagent (Invitrogen) (10^7 cells per milliliter). To remove any DNA contamination, RNAs was treated with TURBO DNase (Ambion, Austin, TX) at 37°C for 30 min, followed by heating and EDTA inactivation and quantified by spectrophotometry and electrophoresis on 1% agarose gel. The first strand of cDNA was synthesized using 100 pmol of anchored oligo dT primer (MWG Biotech, Ebersberg, Germany), 200 units of RevertAid H Minus MMuLV Reverse Transcriptase (MBI Fermentas, St. Leon-Rot, Germany), and 1 µg of total RNA according to the manufacturer's instructions. For each sample, a no-reverse transcription (No-RT) control was used in parallel with the DNase-treated RNA to detect any potential nonspecific amplification of genomic DNA.

Semi-quantitative RT-PCR Analysis

PCR was performed using 0.5 µl of cDNAs and No-RT samples with 12.5 µl of 2x PCR master mix red (Amplicon), 0.4 µM of each primer and 11.2 µl ddH2O in a final volume of 25 µl. The PCR amplification was performed for 28 cycles for GAPDH as an internal control and 35 cycles for miR-1285. PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized under the UV light (data not shown). PCR products were cloned in T-cloning vector (Vivantis) and direct DNA sequencing (MACROGEN) was confirmed the authenticity of amplicons.

Results

hsa-mir-1285 is Expressed in Some Cancer Cell Lines and differentiated Cells

Our in silico and RT-PCR studies indicated hsa-miR-1285 is expressed in human EC cells and different human cancer cell lines such as NTERA-2, GM12878, K562, HeLa, HepG2, SK-N-SH, IMR90, HOB, HPIEPC, HSaVEC, HVMF, NHDF, etc. Moreover, the obtained data from DAVID database showed that miR-1285 might potentially target critical genes of important signaling pathways in pluripotency and carcinogenesis, such as TGF-beta, wnt, NOTCH, ErbB, PI3K, MAPK, JAK/ STAT, Hedgehog, and VEGF signaling pathways.

OCT4 gene has a Target Site for hsa-mir-1285 in its Promoter

A 21-nt-long target site for microRNA-1285 is located 262-nt upstream of exon1 in the *OCT4* gene. Whenever this microRNA binds to the *OCT4* promoter, it might regulate the expression of *OCT4.* However the exact Functional mechanism of microRNAs that target

promoters remains unclear, some studies propose that it requires complementarity to the target DNA sequences and changes chromatin remodeling associated with gene activation. A suggested model for the mechanism of gene expression induction by microRNA is direct binding to the complementary DNA within gene promoters. In this regard, microRNA functions like a transcription factor which targets complementary motifs in gene promoters [23, 24].

Discussion

OCT4 is a crucial stemness marker that maintains pluripotency and self-renewal in embryonic stem cells. The expression of *OCT4* in non-pluripotent and cancer cells is controversial in stem cell and cancer researches. Therefore, more studies are needed to unveil this complexity. In this way, we analyzed the *OCT4* gene sequence to find binding sites for microRNAs. Our bioinformatics studies revealed a target site on the OCT4 promoter for miR-1285.

In 2006, for the first time, studies revealed that small dsRNAs can induce the transcription from some gene promoters, such as E-cadherin, $p21^{WAF1/CIP1}$ and vascular endothelial growth factor (*VEGF*) [25]. Further studies by Janowski et al. (2007) indicated that promoter-targeting dsRNAs induce the expression of progesterone receptor (*PR*) and major vault protein (*MVP*) [23]. These reports indicate that small dsRNAs could regulate gene expression. Whereas these studies were performed by the synthetic dsRNAs that induced gene expression by promoter targeting, the same effect might be induced by the endogenous microRNAs, naturally. Further studies demonstrated that mir-373, which has highly complementary target sites within *E-cadherin* and *CSDC2* gene promoters, can act like small dsRNAs and regulate their expression [24].

In this way, we scanned the *OCT4* gene promoter bioinformatically, and detected a target site for new discovered hsa-mir-1285. We designed a specific primer for mir-1285 and exanimated its expression in the EC cell line (NTERA-2) and three cancer cell lines (1321N1, Daoy, A172 and 5637) by RT-PCR assay. RT-PCR data showed this microRNA was expressed in the NT2 cell line. Moreover, bioinformatics analysis showed miR-1285 is expressed in several different cancer cell lines and differentiated cells.

In conclusion, the expression of microRNA-1285 in human cancer cell lines and differentiated cells, and the presence of a completely matched binding site for this microRNA in the *OCT4* gene promoter suggests the microRNA-1285 can potentially regulate transcription of the OCT4A. We hope our study would be a stimulus for future research in response to the controversies about the expression of *OCT4* in the cancers and non-pluripotent cells.

Conflict of Interest

The authors report no conflicts of interest.

Supplementary data

Promoter and 3′-UTR of the human *OCT4* gene were scanned using miRBase database (www.mirbase.org) for finding target sites of hsa-miR-1285-3p. The bioinformatics analysis indicates a 21-nts perfectly matched target site for hsa-miR-1285-3p at position of -316 to -295 upstream of *OCT4* start codon (ATG), but 3′- UTR has no target site for this microRNA. Hsa-miR-1285 was detected for the first time in ES cells. The mature form of hsa-miR-1285-3p was produced by two distinct stem-loops which encode from MIR1285-1 and MIR1285-2 genes located on chromosome 2 and 7, respectively.

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