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Research Article

The Regulatory Effect of lncRNA PSORS1C3 on Different Variants of OCT4 in non-Pluripotent Cells

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Abstract

OCT4 is the major regulator of pluripotency in embryonic stem cells and its association with tumorigenesis, cellular stress response, and homeostatic multifactorial diseases have been recently reported. To serve the versatility in its function, OCT4 generates several transcript variants which their expression levels are tightly regulated through different mechanisms. PSORS1C3 is a long non-coding RNA with overlapping genomic location with OCT4 gene. Here, we investigated the effect of PSORS1C3 overexpression on OCT4 expression in different cell lines. Our data revealed that ectopic expression of PSORS1C3 did not affect OCT4 transcripts abundance in NT2 cells, as a model of pluripotent cells. However, in HEK293T cells, PSORS1C3 overexpression led to an increase in OCT4B as a homeostatic isoform and a decrease in OCT4A transcript level. We also observed that manipulating PSORS1C3 in HeLa cells, as a model of epithelial carcinoma line, caused an upregulation in OCT4A, OCT4C which could regulate stemness and proliferation and OCT4B transcripts at different time points. Our findings indicated that PSORS1C3 could affect the expression level of OCT4 spliced variants, according to their functions and the cells molecular context as well as genetic background. Considering these diverse regulatory effects and co-expression of OCT4 and PSORS1C3 in some cell lines, it is safe to consider PSORS1C3 as a modulator of OCT4 expression in non-pluripotent cells and in association with homeostatic pathways.

***Keywords*:** OCT4, PSORS1C3, expression regulation, lncRNA

Introduction

Oct4 is an octamer-binding transcription factor that regulates stemness, pluripotency and development (Campbell et al., 2007). As many stemness regulators play additional roles in tumorigenesis, OCT4 is also associated with cellular transformation, tumor invasion and drug resistance, and its expression alterations have been reported in many cancers (Atlasi et al., 2007; Du et al., 2009). Aside from its association with cancer, OCT4 is also linked with multifactorial diseases like psoriasis (Chang et al., 2007), inflammatory bowel diseases (Maragkoudaki et al., 2015), cardiovascular disorders (Lin et al., 2015) and major depressive disorder (Murphy et al., 2017) that share a common ground of deregulation in homeostatic pathways.

OCT4 has multiple spliced variants that each serves different functions at both RNA and protein levels (Gao et al., 2010; Li et al., 2015). OCT4A functions as a transcription factor and regulates stemness, cellular transformation and cell cycle. OCT4B and B1 show anti-apoptotic functions and are related to stress response and survival pathways (Asadi et al., 2011; Farashahi Yazd et al., 2011). The expression pattern of OCT4 transcripts varies in different cells, as well as during different biological events, where their expression levels are precisely regulated through different mechanisms (Wang and Dai, 2010; Rijlaarsdam et al., 2011).

Long non-coding RNAs (lncRNAs) are known as major regulatory factors capable of controlling gene expression at both transcriptional and post-transcriptional stages (Bhat et al., 2016). LncRNAs orchestrate the expression of their target genes through interactions with transcription machinery, chromatin remodelers and by acting as a guide, decoy or distributor of regulatory proteins or microRNAs (Engreitz et al., 2016). There are several reports on OCT4 expression regulation by lncRNAs in stem cells (Wang et al., 2013; Bai et al., 2015), however, the non-coding regulatory circuits that orchestrate OCT4 splicing in non-stem cells is not clearly identified.

PSORS1C3 is a lncRNA located upstream of OCT4 in the HLA-C locus which was first discovered in a

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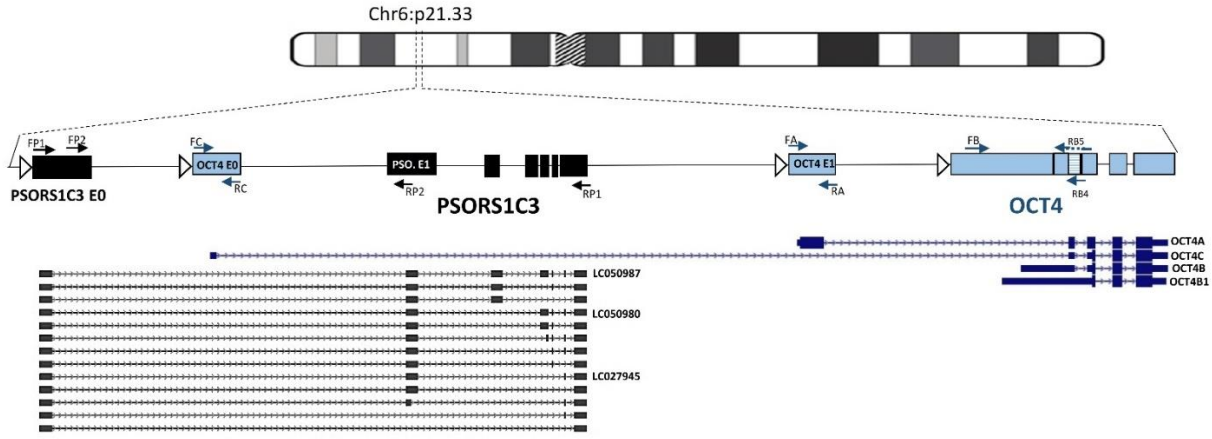

Figure 1. Schematic view of PSORS1C3-OCT4 locus and localization of primers which were used for cloning and gene expression quantification.

linkage analysis study on psoriasis (Holm et al., 2005). In our previous work, we reported a co-expression between PSORS1C3 canonical transcripts and OCT4 in different cells lines. By identifying a novel starting exon (exon 0) for both PSORS1C3 and OCT4, we demonstrated that these neighboring genes were physically entangled (Figure 1) (Malakootian et al., 2017). However, the expression screening indicated that PSORS1C3 longer variants which contained the transposon derived exon 0 had a fair co-expression with OCT4A mostly in non-pluripotent cells. Considering the significance of exonised transposon elements in biological pathways, we decided to investigate a possible regulatory effect of ectopic expression of PSORS1C3 long variants on OCT4 transcript level.

Materials and Methods

Constructing PSORS1C3 expressing vectors

PSORS1C3 long variants expressing vectors were constructed by cloning 3 of PSORS1C3 transcripts (LC050986, LC050987 and LC027945 (Figure1)) in pTracer-SV40 vector (Invitrogen, USA). Briefly, HepG2 and AGS cells (PSORS1C3 positive cell lines) were cultured to 70% confluency, as described before (Malakootian et al., 2017) and were lysed for RNA extraction with TRIzol reagent (Thermo Fisher Scientific, USA). Total RNA (2 µg) was treated with DNase I (Thermo Fisher Scientific, USA), then reversely transcribed using PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa, Japan), according to the manufacturer's protocol. PSORS1C3 long transcripts were amplified using FP1 and RP1 primers (Figure1, Table 1) by Pfu polymerase (GeneAll, South Korea) following the company's suggested protocol. Amplicons were purified using ExpinTM Combo GP kit (GeneAll, South Korea) and were directly cloned into pTracer-SV40 vector (Invitrogen, USA). The accuracy of the cloning and the identity of transcripts were confirmed by DNA sequencing (Macrogen, South Korea).

Cell transfection and targets expression analysis

HEK293T and HeLa cell lines were obtained from the Iranian biological resource center (IBRC, Iran). NTERA2c.D1 (NT2) cells were gifted by Dr. Peter Andrews. Cells were cultured in specific media as described before (Malakootian et al., 2017). Cells were seeded at 70% confluency and then transfection was done using Lipofectamine LTX (Thermo Fisher Scientific, USA) according to the manufacturer's instruction. In order to mimic PSORS1C3 natural expression, vectors containing 3 different variants were mixed for transfection. To determine the efficacy of transfection, cells were monitored for GFP signal emission, by fluorescent microscopy. Transfected cells were then lysed for RNA extraction 24 and 48 hours after transfection. After DNase I treatment and cDNA synthesis, real-time PCR was performed using target-specific primers (Table 1, Figure 1) and BioFACT™ 2X Real-Time PCR Master Mix (BioFACT, South Korea) to evaluate expression alterations in OCT4A, B, B1 and C transcript variants.

Results

PSORS1C3 was successfully overexpressed in different cell lines

24 hours after transfecting different cell lines with recombinant vectors containing three different isoforms of PSORS1C3, transfected cells were inspected by a fluorescent microscope and the

Table 1. Sequence of primers which were used in this study

Target name	Application	Primer name	Sequence
OCT4A	Real time PCR	FA	F:TCGCAAGCCCTCATTTC
		RA	R:CCATCACCTCCACCACCT
OCTB	Real time PCR	FB	F:AGACTATTCCTTGGGGCCACAC
		RB5	R:GGCTGAATACCTTCCCAAATAGA
OCT4B1	Real time PCR	FB	F:AGACTATTCCTTGGGGCCACAC
		RB4	R:CTTAGAGGGGAGATGCGGTCA
OCT4C	Real time PCR	FC	F:TGAGCGAGAAGCACGATCC
		RC	R:GGAACGAACCGTCGC C
PSORS1C3	Real time PCR	FP2	F:CCAGAGCAGCACGTAGCAG
		RP2	R:CCCTCCTTGCAGCATCATAAG
PSORS1C3	Transcript cloning	FP1	F: GTTTTGTCTGGGGCTCGTC
		RP1	R: CTTCACACACACCTTTATTATTAC

observed GFP signal indicated the efficacy of transfection (Figure 2A). Furthermore, we used qPCR (primers: FP2 and RP2) to evaluate the scale of overexpression in each cell line. Our analysis confirmed that PSORS1C3 was successfully overexpressed in transfected cells (Figure 2B).

PSORS1C3 ectopic expression had cell type specific effects on OCT4 transcripts

Ectopic expression of PSORS1C3 differentially affected each of the used cell lines. Accordingly, PSORS1C3 overexpression in NT2 cells did not affect OCT4 expression level significantly (Figure 3A), but the manipulation in HEK293T cells caused a downregulation in OCT4A expression (*p* value=0.008) and upregulation in OCT4B transcript (*p* value=0.042) since 24 hours post transfection (Figure 3B). In Hela cells, however, OCT4B level was increased only 48 hours after transfection (*p* value=0.023). The expression level of OCT4A and OCT4C were also significantly elevated (*p* value=0.041 and 0.02, respectively) after PSORS1C3 overexpression in Hela cells (Figure 3C).

Discussion

Cell type-specific expression of a gene is necessary for accurate functioning of signaling/homeostatic pathways and is commonly preserved by regulation at transcription level (Arvey et al., 2012). In particular, genes with several transcript variants show distinct patterns of expression for their variants in diverse cell types as well as during various biological events (Shi et al., 2017). The complex network of molecular regulators that manage the specificity of expression is different for each cell (Arvey et al., 2012), thus manipulating one of them could skew the network and differentially affect the target genes expression in each cell. According to our data PSORS1C3 ectopic expression could affect OCT4 expression, but the manifestation of the change in each OCT4 transcript variant was different in each tested cell line.

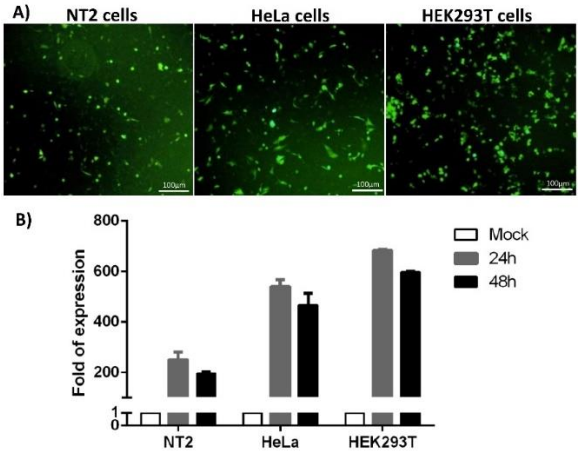

Figure 2. The efficacy and accuracy of transfection. A) The success of transfection was evaluated by visualizing GFP signal using a fluorescent microscopy, 24 h post transfection. B) Real-time PCR data confirmed the over expression of PSORS1C3 transcripts at 24 and 48 h post transfection.

As a teratocarcinoma cell line, NT2 needs and expresses high levels of OCT4A, B1(Atlasi et al., 2008) and C (Malakootian et al., 2017) that are needed for maintaining stemness state. The stemness-related regulatory pathways are strictly

regulated (Kelly and Gatie, 2017) and according to our findings,

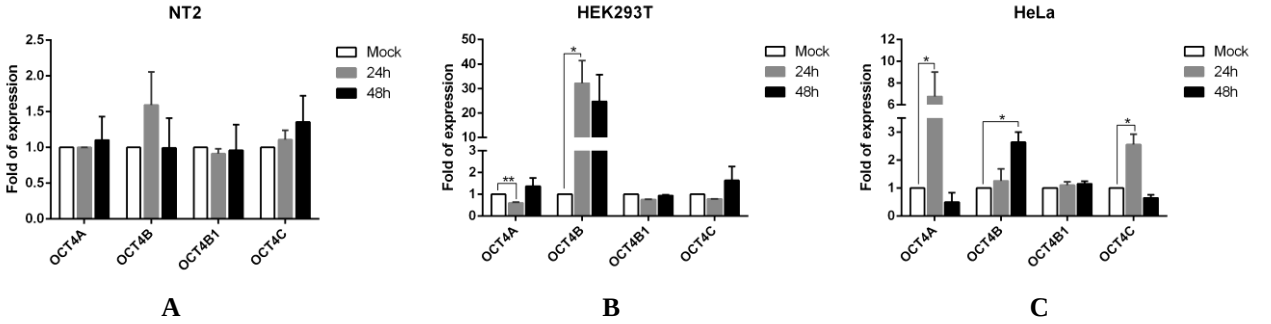

Figure 3. The effect of PSORS1C3 overexpression on OCT4 transcripts in different cells. A) Our qPCR data indicated that none of OCT4 spliced variants were affected by PSORS1C3 overexpression in NT2 cells. B) In HEK293T cells, OCT4A expression level was declined 24 h post-transfection (p value=0.008) and OCT4B transcript level was upregulated (p value=0.042) since 24 hours post transfection. OCT4B1 and OCT4C were not affected by PSORS1C3 manipulation in HEK293T cells. C) PSORS1C3 over-expression dynamically affected OCT4 transcripts in HeLa cells. OCT4B level was increased 48 h after transfection (p value: 0.023). The expression level of OCT4A and OCT4C were significantly elevated (p value=0.041 and 0.02, respectively) 24 h after PSORS1C3 over-expression. OCT4B1 was not significantly affected by the manipulation.

PSORS1C3 could not cross-talk with these pathways entry, as its over-expression did not affect OCT4 expression pattern in NT2 cells.

According to its transcriptome signature, HEK293T cell line is more close to a neuroendocrine lineage which does not express high levels of stemness markers (Stepanenko and Dmitrenko, 2015). Our data indicated that overexpressing PSORS1C3 could not affect the level of OCT4B1 and C transcripts that are commonly detected in stem cells. However, ectopic expression of PSORS1C3 caused a decline in already low level of expression of OCT4A transcript at 24 hours post transfection. We also observed that 24 hours after transfection, the expression of OCT4B sored significantly and remained elevated. Previous research found that OCT4B had cytoplasmic localization and could promote cell survival in cancer cell lines, as its knock-down using RNAi led to a down-regulation in anti-apoptotic factor Bcl2 and upregulation of pro-apoptotic factor Bax (Meng et al., 2018). Moreover, OCT4B also functions in stress management pathways since it could mediate cell response to hypoxia (Lin et al., 2019) , genotoxic stress (Gao et al., 2012) and chemical shock (Cortes-Dericks et al., 2013). These findings supported the idea that OCT4B performed a homeostatic role and its functional portrait fitted our data owing to the fact that it was the only transcript variant to be altered after PSORS1C3 manipulation in a non-pluripotent non-cancerous cell line.

Former researches demonstrated that OCT4 was weakly expressed in normal cervix and its expression level gradually increased during transformation and carcinogenesis (Wang et al., 2013). In our study, over-expression of PSORS1C3 caused an up-regulation in OCT4A and C transcripts 24 after transfection. However, their expression declined to the baseline expression 24 hours later, that indicated the persistence of cells to retain their normal transcription pattern despite the manipulation. This observation might be due to a non-canonical function of OCT4 in controlling mitotic entry in HeLa cells (Zhao et al., 2014). Zhao et al. reported that ectopic expression of OCT4 could delay cell cycle progression in HeLa cells. Hence, the observed resistance in OCT4A and C expression alterations in our study could be due to the activation of other regulatory circuits that fine-tune OCT4 expression to suit the cell homeostatic needs.

Our qPCR data also indicated that OCT4B transcript was increased 48 hours after transfection of HeLa cells with PSORS1C3 expressing vectors. The fact that there was a gap between expression responses of OCT4 different transcripts (OCT4A/C and OCT4B) after manipulation supported their different roles in cells and consequently diverse regulation on their expression level. It also proposed the idea that PSORS1C3 could modulate the expression level of different OCT4 variants in a dynamic manner and in accordance with cells regulatory network and genetic background.

Majority of lncRNAs function as modulators of other genes expression or effectors in cell regulatory pathways (Schor et al., 2018). Their expressions are cell- and state- specific and although their functions are not mostly vital for cells, but affect cell viability under stressed

conditions. lncRNAs are also necessary for optimum functioning of different signaling pathways (Nakagawa, 2016; Schor et al., 2018). As a transposon-derived OCT4 overlapping lncRNA, PSORS1C3 was not expressed in stem cells but showed a relative co-expression with its entangled gene in somatic carcinoma cell lines. Our presented data implied that PSORS1C3 could affect OCT4 expression level, according to the cells molecular context. However, uncovering the exact molecular mechanism behind this observation needs further investigations.

Conflict of interests

None.

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