이학석사학위논문

P19 배아줄기암세포가 신경세포 분화하는 과정에서 *Lefty1* 유전자의 전사 조절 기전 연구

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Eunsong Choi

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ABSTRACT

Studies on the transcriptional regulation of *Lefty1* during neuronal differentiation of P19 embryonic carcinoma cells

Eunsong Choi

Stem cell division should be tightly controlled for a proper balance between self-renewal and differentiation. It is recently known that some types of stem cells have to undergo minimal rounds of cell division in order to turn on expression of a set of genes for differentiation. Here, I searched for genes of which the expression is controlled in a cell-division-dependent manner during neuronal differentiation of P19 embryonic carcinoma cells. I identified three candidate genes, *Lefty1*, *Msi2*, and *Prtg* of which the expressions were upregulated during the retinoic acid-induced neurogenesis in P19 cells. Their expressions were induced by RA in a delayed manner, and such induction was

significantly dampened by cell cycle blocking drugs such as thymidine and RO3306. I defined a domain within the *Lefty1* promoter sequence that is responsible for cell-division-dependent activation. These results provide a clue on mechanisms how gene expression is controlled by cell division for neuronal differentiation.

Key Words: Cell division, P19 cell, Neurogenesis, Differentiation, Transcription.

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INTRODUCTION

Cell division is frequently linked to cell fate switches, in which the transition through mitosis and G1 cell cycle phase is crucial for establishing a window of opportunity for pluripotency exit and the initiation of differentiation (Dalton et al., 2015; Soufi et al., 2016). Previous investigations reported that cell cycle regulators, such as cyclin D, control cell fate decisions by recruiting transcriptional co-repressors and co-activator complexes onto neuroectoderm, mesoderm, and endoderm genes, showing the cell cycle controlled transcriptional complexes. These findings support the importance of the cell cycle in orchestrating transcriptional networks to instruct cell fate decisions (Paulkin et al., 2015). Even though researches about cell cycle components and cell fate determination are highly active, the mechanism concerning how cell division round numbers affect the change in the set of expressed genes still remains uninvestigated.

In order to onset cell differentiation, cells must modify their transcriptome and their cell cycle character in a highly coordinated way. In this way, the identity of a cell can be defined by its cell type specific patterns of transcription (Hsieh et al., 2012). The cell division is believed to be required for a cell to differentiate properly into a specific cell type, such as the case of the

mitotic clonal expansion in adipocytes. In adipocyte differentiation, a concerted transcriptional and cellular program is required for proper cell specification, including growth arrest of confluent adipocytes, re-entry to the cell cycle and additional two rounds of cell division, termed to be the mitotic clonal expansion (Yashomati et al., 2000). It was revealed that blocking the cell division of 3T3-L1 pre-adipocytes decreased adipocyte differentiation markers as a consequence of prevented mitotic clonal expansion (Tang et al., 2002). These data support that cell division in adipogenesis is a prerequisite for the expression of genes that produce the adipocyte phenotype. As the case of the adipogenesis, the cell division round number is thought to be involved in the cell specification into different cell types.

Mouse P19 embryonal carcinoma cells can be reproducibly differentiated into neurons upon treatment with high concentration of retinoic acid (Chambon et al., 1995). Due to its easy manipulation, P19 cell is considered an advantageous model system for investigating the cross talk between cell division and differentiation. Here, I sought to determine the importance of cell division in differentiation using retinoic acid induced neurogenesis of P19 embryonic carcinoma cell system. In this study, I observed the cell-division-dependent change in the set of expressed genes at transcriptional level. *Lefty1, Msi2* and *Prtg* were screened to be genes of which

their transcriptions are activated in a cell-division-dependent manner. Studying their transcriptional control would provide a clue for the understanding of how cell division rounds can affect the cell differentiation. Candidate regions at the *Lefty1* promoter responsible for the conditional transcriptional activation were studied, remarking the cell division progression as a requisite for the proper establishment of differentiation.

MATERIALS AND METHODS

Cell culture and cell synchronization

P19 embryonic carcinoma cells were cultured at 37℃ and 5% CO_2 in DMEM supplemented with 10% fetal bovine serum. Cells were synchronized at S cell cycle phase by the 2mM thymidine block and at G2/M cell cycle phase by the 10nM RO3306 treatment for 12hrs.

Fluorescence-activated cell sorting

P19 cells were harvested and washed in PBS. Cold 70% ethanol were dropped to pellet while vortexing for fixation and stored at a minimum of 30 minutes at 4°C. Cells were washed three times in PBS. Propidium iodide (PI) with RNase was used for DNA staining. 10000 cells were counted for each sample to draw the propidium iodide DNA histogram plot. The cells at each cell cycle phase were measured by using markers set within the plot representing the percentage for each cell cycle phases.

Quantitative Real time-PCR

P19 cells were homogenized using Trizol reagent for RNA isolation. Isolated

RNAs were reverse transcribed in order to synthesize cDNA. SYBR Green dye was detected in the extension step of the real-time PCR, so that signal intensity increases with increasing cycle number due to the accumulation of PCR product. This measurement enabled the analysis of relative mRNA expression between the samples.

Luciferase assay

Lefty1 promoter sequences were sub-cloned into the Pgl3-luciferase vector for luciferase assay. Transfected cells and stably expressing P19 cells were lysed and luciferin was injected for enzymatic reaction. Luminescence was measured by a microplate luminometer. Values were normalized by measuring co-transfected beta galactosidase values and total protein amount.

Western blot

Cultured P19 cells were lysed in 1XSDS sample buffer. Samples were loaded in 8% polyacrylamide gels for electrophoresis and then transferred into nitrocellulose membranes. The membranes were blocked in 5% skim milk in TBS-T for 1hr, incubated with anti-lefty1 (1:500), anti-α-tubulin (1:10000) antibodies overnight at 4°C. After washing 4 times with TBST for 5min, the membranes were incubated with mouse (1:20000) and rabbit secondary

antibodies (1:15000) for 1hr. After the membranes were washed three times with TBST for 5min, the peroxidase activity was detected using ECL solutions.

Transcription factor binding site prediction.

ALGGEN-PROMO online software was used to identify potential transcription factors that can bind to *Lefty1* promoter sequences at -1300 to -1350 positions respective to the transcription start site. All potential transcription factor motifs were analyzed using the MotifMap online database. Set of transcription factors was listed in order of matching relevance between the ALGGEN PROMO prediction data and the motif found from MotifMap.

RESULTS

Cell division control of P19 cells

In order to study importance of cell division for cell specification, I synchronized P19 cells using cell cycle blocking drugs. Doubling time of P19 cells is known as 14.3 h (Yoneda et al., 2012). Therefore, thymidine and RO3306 was treated for 12 h. FACS analysis revealed that the cells were arrested at S and G2 phase by thymidine and RO3306, respectively (Figure 1). I tried to synchronize the cell cycle progression of P19 cells with thymidine block and release. The results showed that cell cycle was synchronized up to 8 h after the release, but returned to asynchronous state in 24 h, regardless of the presence or absence of retinoic acid (RA) (Figure 2). These results indicate that cell cycle of P19 cells can be manipulated with treatment of cell cycle blocking drugs. I used thymidine and RO3306 useful tools for following studies how cell division progression affects the change in specific gene transcription during the differentiation onset in P19 cells.

Neuronal differentiation of P19 cells is induced by retinoic acid

It is well known that the P19 embryonic carcinoma cells can be differentiated into neuronal cells by RA (McBurney et al., 1983). The RA

treatment is known to reduce expression of selected genes for stem-ness and enhance expression of genes for neuronal differentiation (Miyazawa et al., 2014). Therefore, I determined expression of *Oct4*, a stem cell marker, and *Ascl1*, a pro-neural marker, in RA treated P19 cells. The *Oct4* mRNA level decreased after 24 h of RA treatment, suggesting the loss of stem-ness of P19 cells. On the other hand, expression of *Ascl1* increased with time, suggesting induction of neural differentiation of P19 cells (Figure 3). These results suggest that RA induces neuronal differentiation of P19 cells by controlling the expression of both genes for stemness and differentiation.

Identification of candidate genes with cell-division-dependent expression upon RA-treated P19 cells.

Observing the gene expression change between the cells that undergo cell division normally and cells in which cell division is blocked, can give a clue about the importance of cell division for a cell to differentiate into a certain type of cell. Therefore, I screened genes with cell-division-dependent expression upon RA-treated P19 cells along several criteria. From a review paper, a list of 30 genes was identified to be induced by RA in mouse cell line (Balmer et al., 2002). Their transcriptional expressions upon RA in P19 cell were confirmed with PCR analyses. Among 30 genes, 13 genes were

upregulated at relatively early time point of 24 h in RA-treated P19 cells (Table 1). When thymidine was treated to block the cell division, three genes, *Lefty1*, *Msi2* and *Prtg* showed a decrease in their expression, suggesting that their expression depends on cell division during RA-induced neurogenesis in P19 cell (Figures 4 and 5A).

I determined mRNA levels of *Lefty1*, *Msi2* and *Prtg* in RA-treated P19 cells. Expression of all three genes was induced in a delayed manner so that the highest levels are reached near 24 h after the RA treatment (Figure 5B). In case of *Lefty1*, the protein level also reached its higher level at 24hr after RA treatment (Figure 5C) I also determined expression of the genes in two different culture conditions, such as an adherent culture and a suspension culture. In P19 cell adherent condition for monolayer culture, all three genes showed upregulation upon retinoic acid and these expressions were dampened when cell division were blocked by thymidine or RO3306 (Figure 6A). On the other hand, only *Lefty1* showed cell-division-dependent upregulation upon retinoic acid in suspension culture condition allowing the formation of the P19 cell embryonic bodies. The suspension culture of P19 cell is reported to be important for the induction of neurogenesis since it provides the physical environment for the formation of embryonic bodies necessary for further differentiation steps of neurogenesis allowing the formation of mature neurons (Jing et al., 2006).

Therefore, in this study, *Lefty1*, expressed in RA-treated P19 cell suspension culture, was chosen to be the most adequate gene to study of the underlying transcriptional mechanism providing a clue to reveal the importance of cell division in P19 cell neurogenesis.

Definition of the *Lefty1* promoter sequence for the cell-division-dependent activation

Cell differentiation and commitment to a certain cell status are driven by the expression of a numerous specific genes. Among many different steps of gene regulation, the study of transcriptional regulation of gene expression can provide an insight about how a certain cell can decide its fate and differentiate into a certain type of cell (Chen and Rajewsky, 2007).

To study the transcriptional control of *Lefty1* expression, we determined the cis-element responsible for cell-division-dependent induction. *Lefty1* promoter sequences were isolated from P19 cell genome DNA with specific primers used in previously reported positions (Niwa et al., 2006). The -1400 to +80 positions of *Lefty1* promoter respective to the transcription start site was obtained for the analysis of the transcriptional regulation. *Lefty1* promoter sequences differing in length were fused with *luciferase* gene to be subjected to luciferase assay. The *SV40-luciferase* and *pGL3-luciferase* fusion

genes were used as positive and negative controls. As expected, the SV40 promoter activity was not affected by RA. By the luciferase assay, the -1400 ~ +80 sequence of *Lefty1* promoter, containing the sequences at -1400 ~ -1300 positions, was observed to be upregulated upon RA treatment. Moreover, this induction was suppressed when thymidine was treated for cell division block (Figure 7).

The TK promoter is frequently used to measure the activity of certain cis-element for transcriptional activation. Here I used the TK promoter to verify whether the found *Lefty1* promoter sequence at -1400 ~ -1300 position acts as a cis-element for the transcriptional activation. *Lefty1* promoter with the sequences in concern was fused with TK-luciferase for analysis. The *Lefty1* promoter sequence containing the -1400 ~ -1300 region was able to induce the TK promoter activation at the same range as the -1400~+80 whole *Lefty1* promoter sequence. However, the promoter sequences that do not contain those 100bp sequences at -1400 ~ -1300 position were not able to induce any activity. The activation of *Lefty1* promoter sequences farther from -1400 position, including the sequence between -2000 ~ -1400 position, was also analyzed. However, the induction was not significant. This data highlight the importance of -1400 ~ -1300 as the region of the promoter responsible for the cell division-dependent transcriptional activation in RA-treated P19 cells (figure 8A, B).

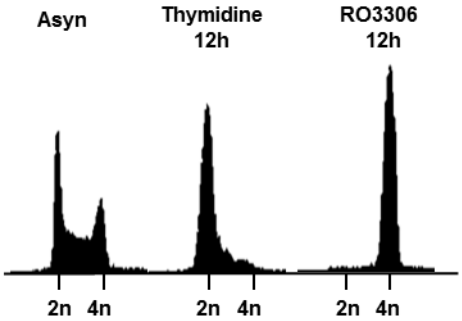
Detailed analysis was done in order to narrow-down the found promoter region. *Lefty1* promoter sequences differing in 20bp length and with internal deletions inside the range of -1400 ~ -1300 position were fused with luciferase gene for the analysis. All constructs showed activation indifferently in luciferase activity, except the *Lefty1* promoter sequence of -1300 ~ +80 position, demonstrating that any promoter sequences having the comprising sequences of -1400 ~ -1300 position can be induced by the retinoic acid (Figure 9A, B).

To sum, all these results suggest that *Lefty1* promoter sequences at -1400 ~ -1300 position are somehow affected by the retinoic acid in a cell-division-dependent manner for the transcriptional activation.

Candidate transcription factors for the cell-division-dependent activation of *Lefty1* transcription

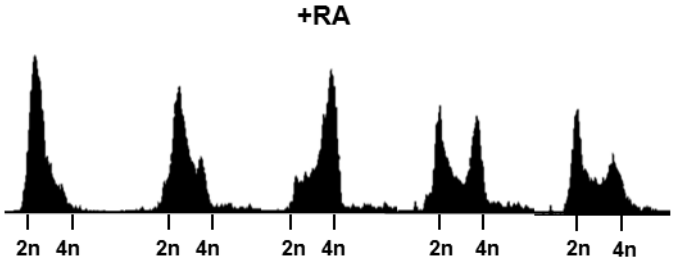
Transcription factor binding sites in the promoter are among the most important elements for the study of the transcriptional regulation in differentiation. So far, the best way to identify transcription factor binding sites in genomic sequences is by the sequence similarity searches. Finding the matching consensus binding motifs of transcription factors for given sequence is one method suitable for the prediction task (Hurtley, S, 2013). To assess what

transcription factors can bind to the found *Lefty1* promoter cis-element, *Lefty1* promoter sequences at -1350 ~ -1300 position was analyzed using the ALGGEN PROMO transcription factor binding site prediction online software. Several set of transcription factors were predicted for their binding at the analyzed sequence. Around 13 binding motifs were found through the prediction. Among these binding patterns, I could observe three different binding hotspots where most transcription factors were likely to bind (Figure 10). Observed hotspots at the analyzed promoter sequence regions are probable to act as cis-element for transcription factor binding upon retinoic acid treatment. These predictions suggest that several set of transcription factors can bind to the designated sites upon *Lefty1* promoter for cell-division-dependent expression during RA induced neurogenesis in P19 cell.

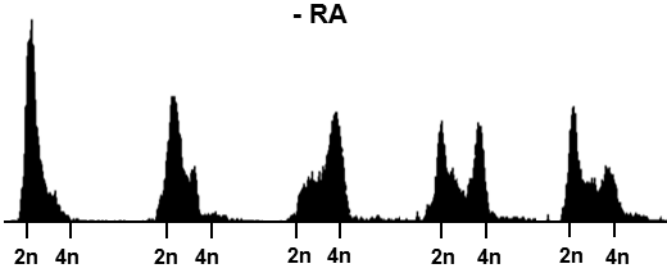


	Asynchronous	Thymidine	RO3306
SubG1	1%	3%	1%
G1	37%	78%	3%
S	27%	13%	29%
G2/M	33%	4%	51%

Figure 1. Cell division control of P19 cells. P19 cells were treated with thymidine or RO3306 for 12 h and subjected to FACS analysis. Proportion of cells at sub-G1, G1, S and G2/M phases were estimated.



Time (h)	0	2	4	8	24
SubG1	0%	0%	1%	3%	1%
G1	70%	53%	20%	29%	38%
S	19%	38%	68%	59%	27%
G2/M	2%	3%	2%	9%	28%



Time (h)	0	2	4	8	24
SubG1	2%	1%	1%	3%	1%
G1	75%	48%	18%	34%	40%
S	21%	46%	45%	25%	27%
G2/M	2%	4%	33%	36%	26%

Figure 2. Cell synchronization of P19 cells with thymidine block and release. P19 cells were arrested at S phase with thymidine treatment for 12 h and released into a fresh medium. The cells were then treated with RA and subjected to FACS analysis at indicated time points.

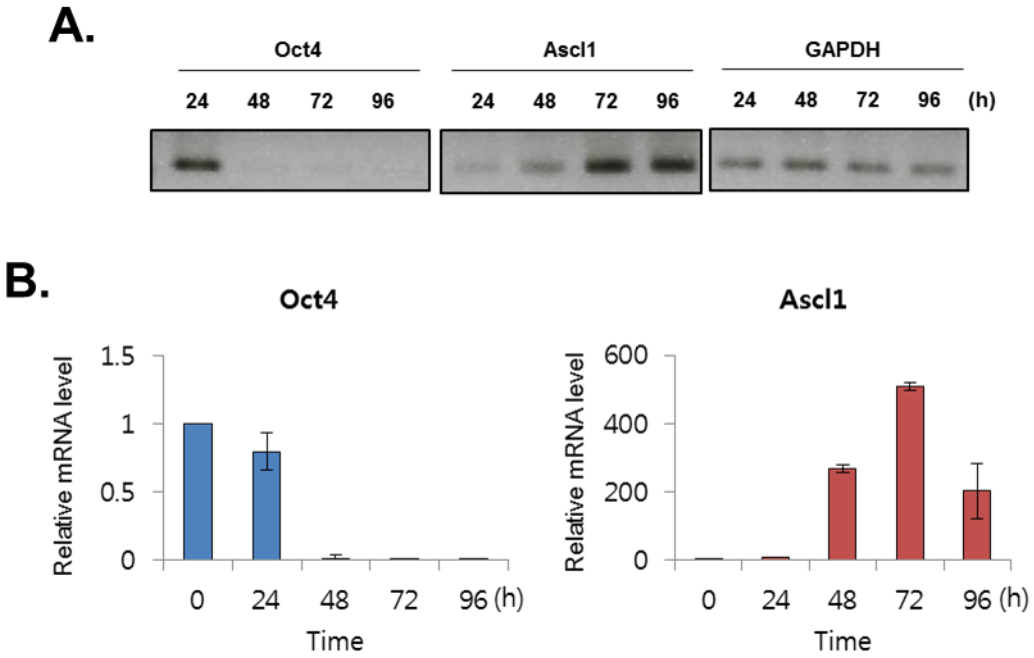


Figure 3. Expression of *Oct4* and *Ascl1* during RA-induced differentiation of P19 cells. (A) P19 cells were treated with RA and cultured for up to 4 days. At indicated time points, the cells were harvested and subjected to PCR analysis with primers specific to *Oct4*, *Ascl1* and *Gapdh*. (B) The same set of RNA samples were subjected to qPCR analysis. The experiments were repeated three times. Values are presented as the mean ± standard error.

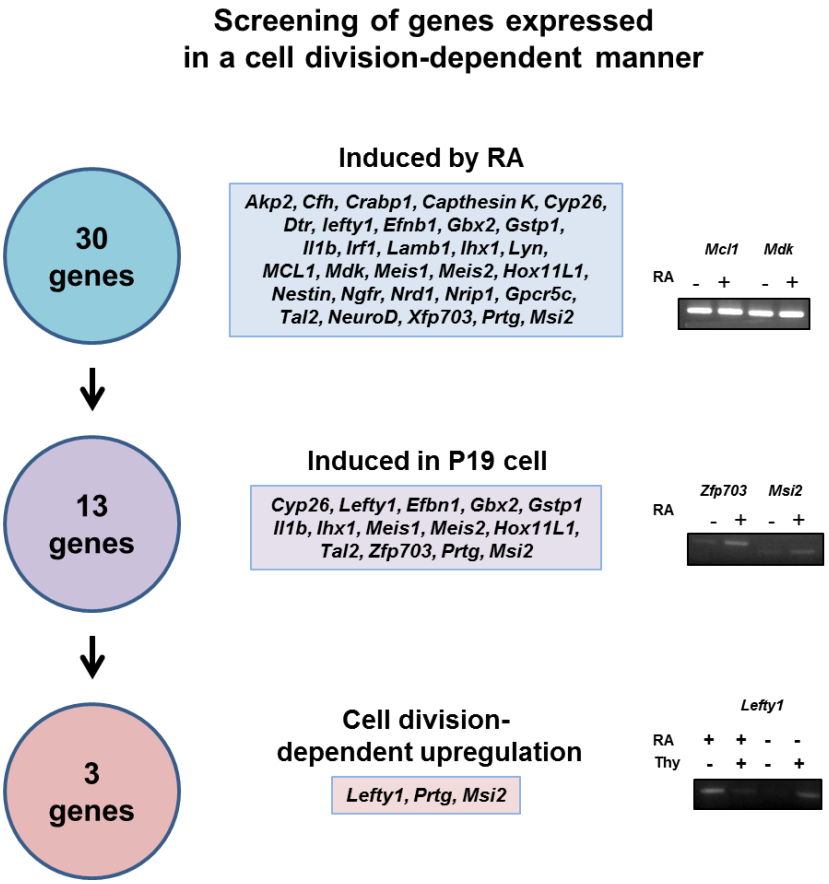


Figure 4. Screening of genes whose expression depends on cell division during RA-induced neurogenesis of P19 cells. The number of genes at each screening steps is indicated at the left panel. The genes of each group are listed in the middle panel. Representative PCR analyses are shown at the right panel. Among 30 candidate genes, 13 genes were confirmed to be induced by RA in P19 cells. We finally identified *Lefty1*, *Msi2*, and *Prtg* whose expression was upregulated in a cell division-dependent manner.

Table1. Summary of screening of genes whose expression depend on cell division during RA-induced differentiation of P19 cells

No.	Gene	NCBI gene ID	Forward (5' -> 3')	Reverse (5' -> 3')	Upregulation(24h)	Cycle dependency
1	Lefty1	13590	CCAACCGCACTGCCCTTAT	CGCGAAACGAACCAACTTGT	Y	Y
2	msi2	76626	GGCTACCCCAACTTTGTGG	CCCCGTACACCTTCCTACC	Y	Y
3	prtg	235472	GGATGCTGGGAATTACCGCT	CTGGCTATAATGGTTGGCATGT	Y	Y
4	Cyp26	13082	TGGGACCTGTACTGTGTGAG	CCAAACAGATGCGTCTTGTAGA	Y	N
5	Efbn	13641	TGTGGCTATGGTCGTGCTG	CCAAGCCCTTCCCACTTAGG	Y	N
6	Gbx2	14472	CAACTTCGACAAAGCCGAGG	ACTCGTCTTTCCCTTGCCCT	Y	N
7	Gstp1	14870	ATGCCACCATACACCATTGTC	GGGAGCTGCCCATACAGAC	Y	N
8	Hox11l1	21909	CAGAACCGACGCACCAAGT	GACACCGAAGCCACCTTGTT	Y	N
9	Ihx1	16869	CCCATCCTGGACCGTTTCC	CGCTTGGAGAGATGCCCTG	Y	N
10	Il1b	16176	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG	Y	N
11	Meis1	17268	TCAGCAAATCTAACTGACCAGC	AGCTACACTGTTGTCCAAGCC	Y	N
12	Meis2	17536	CTGGCGAGATCACGATGACG	AAGCTACGCTGTTGTCTAACC	Y	N
13	Tal2	21350	AGAGTGTCAACAATGCCTTTGC	ACTCCTGTTTGATGCAGGCTT	Y	N
14	Zfp703	353310	GCTGGATCTAACCCAAGGACA	GACAGCGGTTGCAGGTACTC	Y	N
15	Akp2	11647	CCAACTCTTTTGTGCCAGAGA	GGCTACATTGGTGTTGAGCTTTT	N	N
16	Capthesin K	13038	AATACCTCCCTCTCGATCCTACA	TGGTTCTTGACTGGAGTAACGTA	N	N
17	Cfh	12628	CAGGCTACCTACAAATGCCG	TCCGACATATCCTGGATGGGT	N	N
18	Crabp1	12903	GCAGCAGCGAGAATTTCGAC	CGCACAGTAGTGGATGTCTTGA	N	N
19	Dtr	15200	CGGGGAGTGCAGATACCTG	TTCTCCACTGGTAGAGTCAGC	N	N
20	Gprc5c	70355	CACTCACAGAACCTTGCTGAT	CTGCCTCCGAGACAATGCC	N	N
21	Gstp1	14870	ATGCCACCATACACCATTGTC	GGGAGCTGCCCATACAGAC	N	N
22	IRF1	16362	ATGCCAATCACTCGAATGCG	TTGTATCGGCCTGTGTGAATG	N	N
23	Lamb1	16777	GAAAGGAAGACCCGAAGAAAAGA	CCATAGGGCTAGGACACCAAA	N	N
24	Lyn	17096	GTGACATTGTGGTGGCCTTAT	ACCATTCCCCATGCTCTTCTA	N	N
25	Mcl1	17210	CAAAGATGGCGTAACAAACTGG	CCGTTTCGTCCTTACAAGAACA	N	N
26	Mdk	17242	TGGAGCCGACTGCAAATACAA	GGCTTAGTCACGCGGATGG	N	N
27	Nestin	18008	CCCTGAAGTCGAGGAGCTG	CTGCTGCACCTCTAAGCGA	N	N
28	Ngfr	18053	CTAGGGGTGTCCTTTGGAGGT	CAGGGTTCACACACGGTCT	N	N
29	Nrd1	230598	GGGGTCTGTGCGAAGAATCAT	CGCCCAAGTCCTGTCCATT	N	N
30	Nrip	268903	AGACCAGAACTTTAACCTCTCGG	CGATGGAATCAGACAGCCTCT	N	N

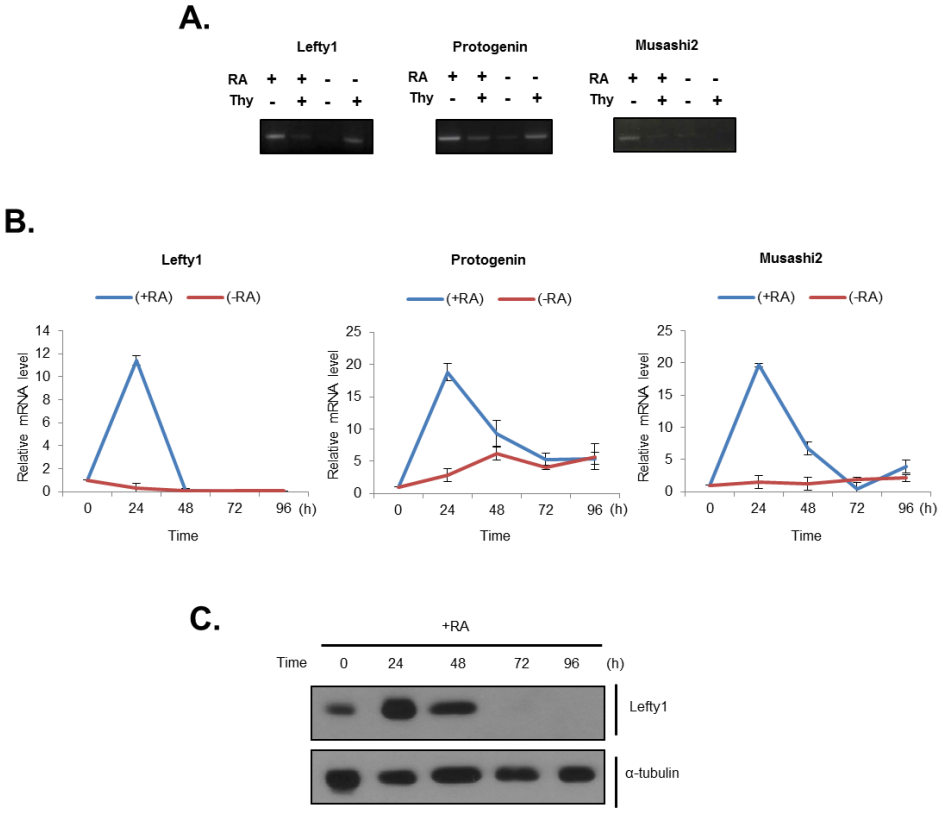


Figure 5. RA induction of *Lefty1*, *Msi2*, and *Prtg* expression in differentiated P19 cells. (A) Total RNA was prepared from P19 cells treated with RA and/or thymidine for 24 h, then subjected to PCR analysis with specific primers of *Lefty1*, *Msi2*, and *Prtg*. (B) P19 cells were treated with RA and cultured for up to 4 days. At indicated time points, cells were subjected to PCR analysis with primers specific to *Lefty1*, *Msi2* and *Prtg*. Experiments were repeated three times. Values are means and standard errors. (C) Cell lysates were prepared at indicated time points during 4 days of RA treatment. Immunoblot analyses were performed with the antibodies specific to Lefty1 and α-tubulin.

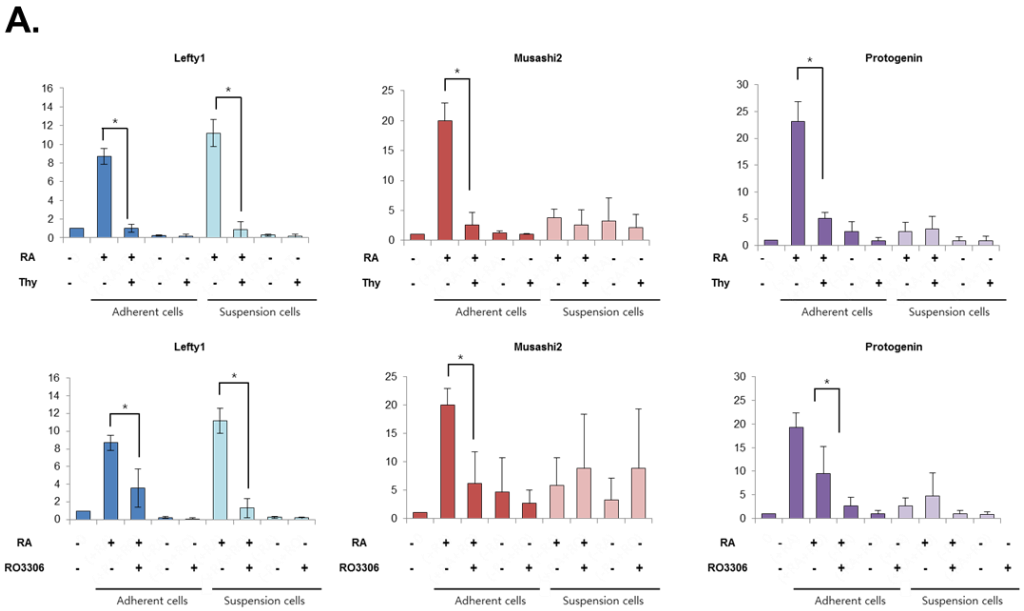


Figure 6. Cell division-dependent expression of *lefty1*, *Msi2*, and *Prtg* in differentiated P19 cells. (A) RA-induced P19 cells were treated with thymidine or RO3306 and cultured either in tissue culture plates for monolayer culture or in bacterial plates for embryonic body formation for 24 h. The cells were then subjected to qPCR analyses with primers specific to *Lefty1*, *Msi2*, and *Prtg*. The experiments were repeated three times and the results were presented as means and standard errors. *, P<0.05.

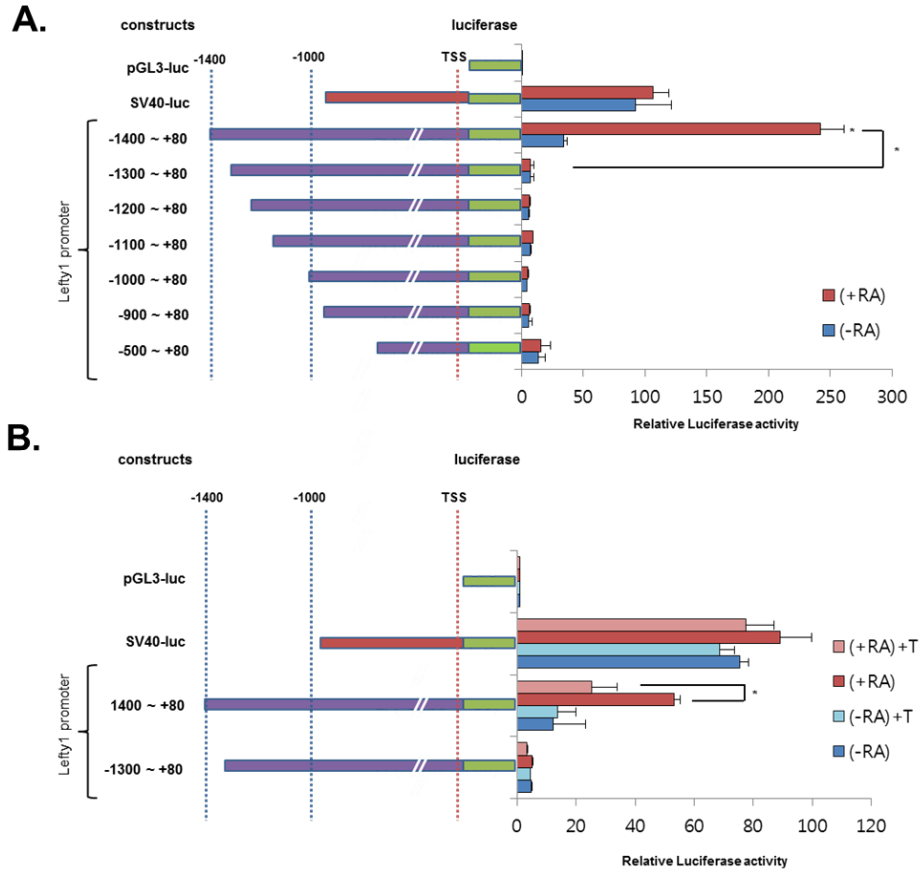


Figure 7. The *Lefty1* promoter analysis with the *Lefty1-luciferase* fusion genes in RA-treated P19 cells. (A) The P19 stable cells with the *Lefty1-luc* fusion genes were cultured in the presence of RA for 24 h. The cell lysates were subjected to luciferase assays. (B) The P19 stable cells with the *Lefty1-luc* fusion genes were cultured in the presence of RA and/or thymidine for 24 h, and subjected to luciferase assays. *SV40-luc* and *pGL3-luc* were used as positive and negative controls. The experiments were repeated three times and the results were presented as means and standard errors. *, P<0.05.

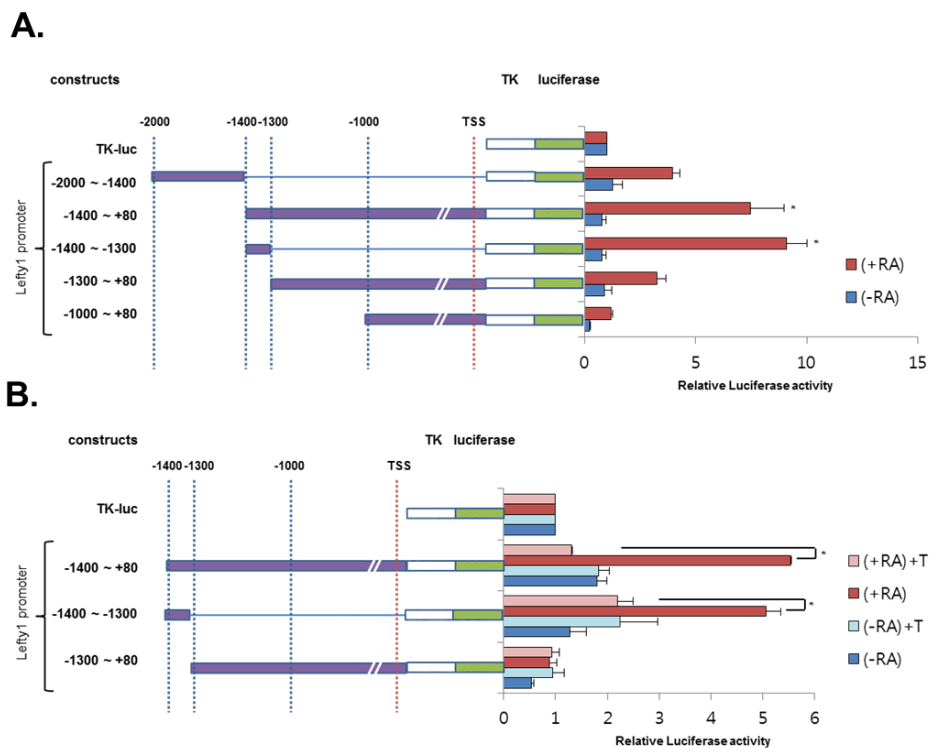


Figure 8. The cell division-dependent activation of the *Lefty1-TK-luciferase* fusion genes in RA-treated P19 cells. (A) The P19 stable cells with the *Lefty1-TK-luc* fusion genes were cultured in the presence of RA for 24 h. The cell lysates were subjected to luciferase assays. (B) The P19 stable cells with the *Lefty1-TK-luc* fusion genes were cultured in the presence of RA and/or thymidine for 24 h, and subjected to luciferase assays. *TK-luc* was used as a control. The experiments were repeated three times and the results were presented as means and standard errors. *, P<0.05.

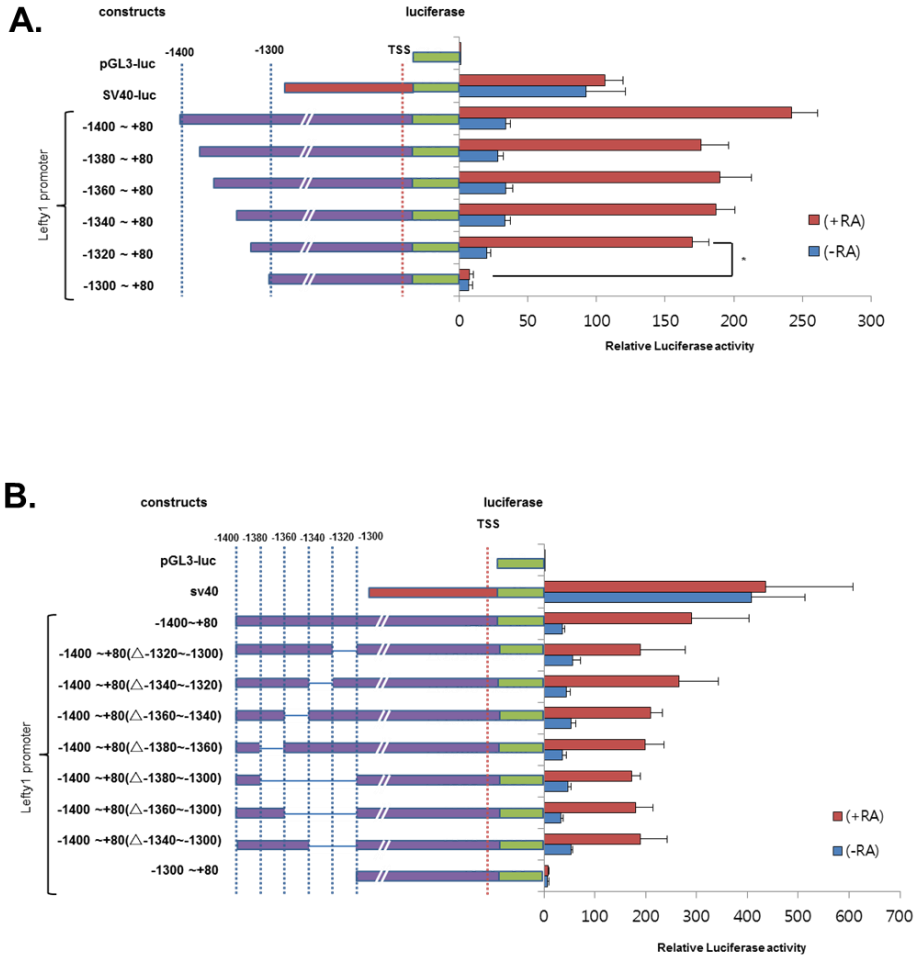
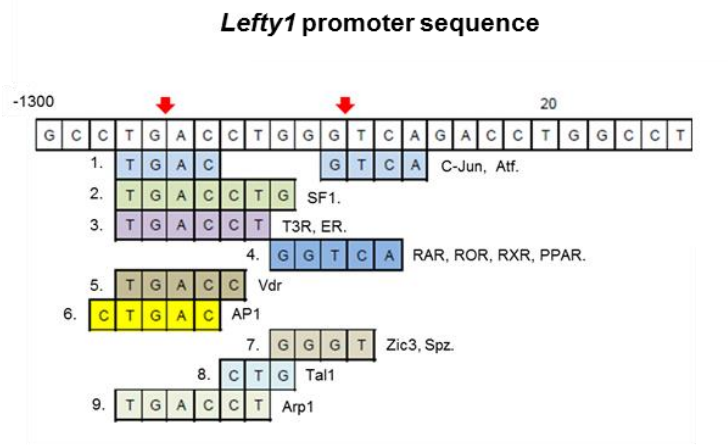


Figure 9. Detailed analysis of the -1400 ~ -1300 sequence of the *Lefty1* promoter. (A) The *Lefty1-luc* fusion genes with (A) serial deletions or (B) internal deletions within the -1400 ~ -1300 region were generated and stably transfected into P19 cells. The cells were cultured in the presence of RA for 24 h and subjected to luciferase assays. *SV40-luc* and *pGL3-luc* were used as positive and negative controls. The experiments were repeated three times and the results were presented as means and standard errors. *, P<0.05.



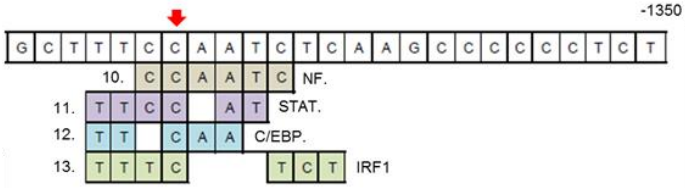


Figure10. Candidate transcription factors which can bind to -1300 ~ -1350 of the *Lefty1* promoter sequences. The *Lefty1* promoter sequences from -1300 ~ -1350 are lined in white box and colored boxes represent each potential sequences for transcription factor binding. Potential transcription factor names are listed on the right side of each binding sequences. Three positions are marked with red arrows indicating where the most transcription factors binding are likely to occur.

DISCUSSION

Here, I observed that *Lefty1, Msi2,* and *Prtg* are expressed in RA-treated P19 cells in a cell division-dependent manner. Among these found three genes, the transcriptional regulation of *Lefty1* was studied revealing the existence of cis-element responsible for the cell-division-dependent expression during RA induced neurogenesis of P19 cells.

The *Lefty1* protein is a TGFβ family protein known to be required for the left-right axis determination in developing mouse embryos. The expression of *Lefty1 in vivo* is predominantly confined to the left side of ventral side of ventral neural tube and it has been revealed that *Lefty1* deficient mice show a variety of L-R positional defects in visceral organs indicating that *Lefty1* provides a signal for the leftness (Meno et al., 1998). These functions of left-right determination for *Lefty1* gene *in vivo* is proposed to be mediated in part by different mechanisms of *Lefty1* inhibitory activity in Nodal signaling pathway, suggesting its importance in the regulation of the pluripotency exit (Chen and Shen, 2004). Moreover, it has been discovered that in human the *NPTX1,* sharing a striking homology to *Lefty1,* is one of the earliest genes expressed upon hESCs neural induction. The *NPTX1* expression accelerated the pluripotency exit and neural differentiation in human embryonic stem cells

leading to impairment of neurogenesis when *NPTX1* was deficient. In addition, it has been observed experimentally, that *NPTX1* binds to the TDGF, a co-factor for nodal signaling, in order to reduce the nodal signaling leading to the pluripotency exit, in a similar way to the *Lefty1* function of antagonistic regulation of nodal signaling in mouse (Boles et al., 2014). These results give an indirect thought that *Lefty1* is needed to be expressed upon neural induction for proper specification, which means that cell division must be held during early steps of neurogenesis. However, it remains unclear whether the presence or absence of *Lefty1* during P19 cell neurogenesis can significantly affect the cell specification. Further investigations concerning the function of *Lefty1* in early neurogenesis is necessary since there are many genes upregulated upon retinoic acid that sustain their expression even when cell division is blocked. This study has been focused on the observation of gene transcription upon induction of neuronal differentiation with or without cell division blockage, but the actual formation of neurons in later stages of the induction has not been assessed. My data suggests the existence of change in the pool of transcribed genes but whether these changed transcriptional programs can effectively perturb the differentiation or not is still unknown.

In this context, I believe that this study of the transcriptional regulation of cell-division-dependent expression of *Lefty1*, having a regulative function in

development, would provide insight into the importance of the progression of several rounds of cell division for the expression of a set of genes necessary for the onset of proper lineage specification.

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국문초록

세포의 분열은 특정 줄기세포의 재생과 분화의 적절한 균형을 위해서 엄격히 조절되어야 한다. 분화를 위한 일련의 유전자의 발현을 활성화 시키기 위해 일부의 줄기세포 유형은 최소 몇 번의 세포 분열을 거쳐야 한다는 것이 최근에 알려졌다. 본 연구에서는 P19 배아 줄기 암세포의 신경세포 분화 과정에서 세포분열에 따라 발현이 조절되는 유전자를 탐색하였다. *Lefty1*, *Msi2*, 그리고 *Prtg* 세 가지의 후보 유전자가 레티놀산으로 유도된 P19 배아 줄기 암세포의 신경세포 분화 과정에서 발현이 증가하는 것으로 선별되었다. 이 유전자들의 발현은 레티놀산이 처리 된 후, 세포주기 차단 약물인 티미딘과 RO3306을 처리하였을 때 현저히 줄어들었다. 본 연구에서 이러한 유도를 일으키는 조건의 원인이 되는 *Lefty1* 의 프로모터의 시스작용요소을 규명하였다. 본 연구의 결과들은 신경세포 분화에서 유전자의 발현이 어떠한 방식으로 세포분열에 의해서 조절되는지에 대한 단서를 제공한다.

주요어: 세포분열, P19세포, 신경세포분화, 세포분화, 전사조절.

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