Data Article

Data on analysis of OCC-1 transcript levels in pluripotent and differentiated states of P19 cells

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ABSTRACT

We investigated the expression of OCC-1 at mRNA level during retinoic acid (RA) induced differentiation of mouse embryonic carcinoma P19 pluripotent cancer cells by quantitative real time PCR (qPCR). By employing four-fold serial dilutions of P19 cDNA, standard curves were generated for the reference gene (L37) and the gene of interest (OCC-1). PCR efficiencies for L37 and OCC-1 were calculated. Since the amplification efficiencies of these two genes were unequal, the standard curve method was used for the relative quantification of OCC-1. Data analysis revealed that the expression of OCC-1 was reduced by about 69\% after 4-day treatment with RA, when significant down-regulation of key pluripotency factors, including OCT4 and Nanog was observed [1].

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1. Data description

In mouse, the Ensembl database reports three isoforms including two coding (1500009L16Rik-201 and 1500009L16Rik-203) and one non-coding RNA on the chromosome 10 (1500009L16Rik-202) (Fig. 1). The expression of OCC-1 in EC P19 cells was analyzed by RT-PCR. PCR reaction with OCC-1 specific primers yielded a 100-bp fragment which was verified by Sanger sequencing (Fig. 1, and Table 1). In order to quantify and compare the relative mRNA levels of OCC-1 before and after RA-induced differentiation of P19 cells, three biological replicates for both untreated (control) and RA-treated (test) P19 cells were subjected to RNA extraction and cDNA synthesis. Then, quantitative real-time PCR (RT-qPCR) was performed in triplicates (Fig. 2). Fourfold serial dilutions of P19 cDNA was used to generate standard curves for OCC-1 and L37. Slope of standard curves was used to calculate amplification efficiencies of L37 and OCC-1 (Fig. 3 and Table 2). Data analysis using standard curve method showed that transcript level of OCC-1 was reduced to approximately 69% in 4 day- RA-treated P19 cells in comparison with undifferentiated P19 cells (Fig. 2 and Table 3).

2. Experimental design, materials, and methods

2.1. Cell culture and RA-induced differentiation

P19 cells were obtained from Pasteur Institute (Tehran, Iran). They were subjected to differentiation by seeding at a density of 0.2 \times 10^6 in 8-cm diameter bacteriological petri dishes at the presence of α-MEM medium supplemented with 0.5µM RA. After 4 days of RA treatment, differentiated aggregates (EB4) were formed and used for RNA extraction.

2.2. Quantitative real-time PCR (RT-qPCR)

Total RNA was isolated using the Total RNA Isolation Kit (DENAziast Asia Co., Mashhad, Iran) according to the manufacture’s protocol. The quality and quantity of RNA were evaluated by agarose gel
electrophoresis and Nanodrop spectrophotometry. First strand cDNA was generated in the presence of 1mM dNTPs, 0.5 µg Oligo(dT)18 primer, 1x reverse transcription buffer, and 200 U of reverse transcriptase in a total volume of 20 µl. The reaction was incubated for 60 min at 42 °C followed by enzyme inactivation at 72 °C for 10 min.

The primers and hydrolysis probes for RT-qPCR were designed using “Beacon Designer” software (Table 1). qPCR reaction was performed in a total volume of 25 µl which contained 1 µl cDNA, 1x PCR buffer, 0.4 mM dNTPs, 1.5 mM MgCl2, 200 nM probe, 200 nM of each primer, and 0.04 U of Taq DNA polymerase in a Rotor Gene Q real time thermocycler (Qiagen, USA). All reaction components were purchased from Genet Bio (Daejeon, South Korea). The thermocycler program for OCC-1 and L37 was an initial step of 94 °C (3 min), followed by 40 cycles of 94 °C (30 s), 58 °C (30 s), and 72 °C (30 s).

In order to calculate the PCR efficiency, standard curves for OCC-1 and L37 were generated by employing fourfold serial dilutions of P19 cDNA. The PCR reactions at each serial dilution were performed in triplicates. It is estimated that there are $1.9 \times 10^{12}$ mRNA molecules with an average size of 1000 base in 1 µg of total RNA [3]. Accordingly, we estimated the copy number data of amplicons in 1 µg total RNA for each gene (Table 2). The standard curves were plotted with the data of Ct values on the "Y"
axis and the log_{10} copy number of each transcript on the “X” axis (Fig. 3). The slope (S) of the lines were used to calculate PCR efficiencies. The correlation (R^2) was 0.99 for OCC-1 and L37 and the amplification efficiencies for OCC-1 and L37 were 89.8% and 100%, respectively. Since the amplification efficiencies of the reference gene (L37) and the gene of interest (OCC-1) were unequal, the standard curve method for relative quantification was used [4].

Three biological replicates were considered for both untreated (control) and RA-treated (test) P19 cells. The value data of Ct for each triplicate was extrapolated onto the standard curve to calculate the copy number data of the transcripts in each group (Table 3). Here, L37 was used as the internal reference gene because its transcription did not change after RA-treatment [1]. To determine changes in the transcript levels of the target gene (OCC-1) after RA treatment, the data were analyzed by the following equations [5]:

\[
\text{Normalized target (test sample)} = \frac{\text{Copy number of OCC} - 1}{\text{Copy number of L37}}
\]

\[
\text{Normalized target (control sample)} = \frac{\text{copy number of OCC} - 1}{\text{copy number of L37}}
\]

**Table 1**
Sequences of primers and probes used in the study.

| Gene                                      | Primer and probe sequence (5’–3’)                      | Product length (bp) |
|-------------------------------------------|--------------------------------------------------------|---------------------|
| Overexpressed in Colon Carcinoma-1 protein (OCC-1) NM_001145198.1 | OCC-1_F: ATGGCGTCTAGTCAAACA  
                    OCC-1_R: CCAAGTTCTTCCAATTC  
                    OCC-1 Probe: FAM-ATCCAGCGTCTAGTATCTCCT-TAMRA | 100 bp              |
| Ribosomal protein L37 (Rpl37) NM_026069.3 | L37_F: GCAGATTCAGACATGGATTC  
                    L37_R: GGAAGAAGCGTAGGATCC  
                    L37 Probe: HEX-TCATATAACCGAACTCTGACCAGTG-BHQ1 | 200 bp              |

**Fig. 2. OCC-1 copy number before and after RA treatment.** Quantitative RT-PCR analysis showed significant down-regulation of OCC-1 copy number after 4 days of RA treatment of P19 cells. The aggregated P19 cells after 4 days of treatment with RA are named EB4. The OCC-1 copy number was normalized by L37 as a reference gene.
Fold difference in the level of target transcript = \frac{\text{Normalized target (test sample)}}{\text{Normalized target (control sample)}}

As the data in Table 3 indicated, a 69% reduction for OCC-1 was acquired.

2.3. Statistical analysis

A minimum of three replicate experiments were performed for all data. Analysis using Mann Whitney test demonstrated a statistical difference between the control and test groups at a p-value of less than 0.05.

Table 2

| RNA (Nanogram) | \^Copy number of mRNA | Average size (bp) |
|----------------|------------------------|------------------|
| (Bustin et al., 1999) | 1000 | 1.9*10^{12} | 1000 |
| OCC-1 | 1000 | 1.9*10^{13} | 100 |
| L37 | 1000 | 0.38*10^{12} | 200 |

\(^a\) The copy number of amplicons in 1 \(\mu\)g total RNA for each gene is calculated based on the estimation that there are \(1.9 \times 10^{12}\) mRNA molecules with an average size of 1000 base in 1 \(\mu\)g of total RNA (explained in Experimental Design, Materials, and Methods).
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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2020.105367.

Table 3
Quantification of OCC-1 transcript changes following RA treatment using the standard curve method of relative quantification.

| Sample (1) | OCC-1 transcript | L37 transcript | Anti-log of copy number | Anti-log of copy number |
|------------|------------------|----------------|-------------------------|-------------------------|
|            | cycle threshold  | quantity (log copy number) | cycle threshold  | quantity (log copy number) | cycle threshold  | quantity (log copy number) |
|            | (ct)             |                         | (ct)             |                         | (ct)             |                         |
| **Untreated** |                  |                          |                  |                          |                  |                          |
| P19 1-1    | 22.50            | 12.60111421              | 22.86            | 13.14673291              | 23.00            | 13.20262498              |
|           | +12              | 3.9913E+12               | +12              | 1.40195E+13              | +12              | 1.40195E+13              |
| P19 1-1    | 22.67            | 12.55376045              | 22.90            | 13.13468835              | 23.50            | 13.20262498              |
|           | +12              | 3.5799E+12               | +13              | 1.3636E+13              | +12              | 1.3636E+13              |
| P19 1-1    | 22.68            | 12.55097493              | 22.43            | 13.27621198              | 23.00            | 13.20262498              |
|           | +12              | 3.5651E+12               | +13              | 1.3636E+13              | +12              | 1.3636E+13              |
| P19 1-2    | 22.28            | 12.66239554              | 25.32            | 12.40599217              | 23.50            | 13.20262498              |
|           | +12              | 4.9561E+16               | +12              | 2.5467E+13              | +12              | 2.5467E+13              |
| P19 1-2    | 21.00            | 13.0189415               | 25.21            | 12.43911472              | 24.00            | 12.80346281              |
|           | +12              | 1.0445E+18               | +12              | 2.7486E+12              | +12              | 2.7486E+12              |
| P19 1-2    | 20.92            | 13.04122563              | 25.00            | 12.50234869              | 24.00            | 12.80346281              |
|           | +13              | 1.0959E+19               | +12              | 3.1794E+13              | +12              | 3.1794E+13              |
| P19 1-3    | 20.96            | 13.03008357              | 25.25            | 12.42707016              | 24.00            | 12.80346281              |
|           | +13              | 1.0717E+19               | +12              | 2.6734E+13              | +12              | 2.6734E+13              |
| P19 1-3    | 22.07            | 12.72089136              | 23.53            | 12.94498645              | 24.00            | 12.80346281              |
|           | +12              | 5.2588E+16               | +12              | 8.8102E+12              | +12              | 8.8102E+12              |
| P19 1-3    | 21.50            | 12.87966574              | 24.00            | 12.80346281              | 24.00            | 12.80346281              |
|           | +12              | 7.5799E+16               | +12              | 6.3600E+10              | +12              | 6.3600E+10              |
| **Treated** |                  |                          |                  |                          |                  |                          |
| (B4)       |                  |                          |                  |                          |                  |                          |
| P19 4-1    | 23.36            | 12.36155989              | 23.28            | 13.02026498              | 24.00            | 12.80346281              |
|           | +12              | 2.2991E+12               | +12              | 1.0477E+13              | +12              | 1.0477E+13              |
| P19 4-1    | 22.80            | 12.51754875              | 23.50            | 12.95401987              | 25.00            | 13.20262498              |
|           | +12              | 3.2926E+12               | +12              | 8.9939E+12              | +12              | 8.9939E+12              |
| P19 4-1    | 23.00            | 12.46138344              | 23.66            | 12.90584161              | 25.00            | 13.20262498              |
|           | +12              | 2.8962E+12               | +12              | 8.0508E+15              | +12              | 8.0508E+15              |
| P19 4-2    | 21.79            | 12.79888579              | 22.10            | 13.37557964              | 24.00            | 12.80346281              |
|           | +12              | 6.2934E+11               | +12              | 2.3745E+14              | +12              | 2.3745E+14              |
| P19 4-2    | 22.33            | 12.64846797              | 21.49            | 13.55925296              | 24.00            | 12.80346281              |
|           | +12              | 4.4511E+11               | +12              | 3.6245E+12              | +12              | 3.6245E+12              |
| P19 4-2    | 22.00            | 12.74038997              | 22.21            | 13.34245709              | 24.00            | 12.80346281              |
|           | +12              | 5.5003E+11               | +12              | 2.2001E+17              | +12              | 2.2001E+17              |
| P19 4-3    | 21.65            | 12.83788301              | 24.31            | 12.71128018              | 25.00            | 13.20262498              |
|           | +12              | 6.8467E+11               | +12              | 5.1437E+15              | +12              | 5.1437E+15              |
| P19 4-3    | 22.29            | 12.65961003              | 24.18            | 12.74850653              | 25.25            | 13.20262498              |
|           | +12              | 4.5667E+11               | +12              | 5.6041E+11              | +12              | 5.6041E+11              |
| P19 4-3    | 22.10            | 12.71253482              | 24.41            | 12.68143972              | 24.00            | 12.80346281              |
|           | +12              | 5.1586E+11               | +12              | 4.8021E+19              | +12              | 4.8021E+19              |

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* Samples identified as P19 1-1, 1-2 and 1-3 are three replicates of undifferentiated P19 control cells, and samples identified as P19 4-1, 4-2 and 4-3 are three replicates of 4-day RA-treated differentiated aggregates of P19 (EB4) cells.

* Normalized OCC-1 copy numbers were acquired by dividing OCC-1 transcript copy numbers to L37 transcript copy numbers.
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