Error-free and error-prone DNA repair gene expression data through reprogramming and passage in human iPS cells

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1. Data description

The mean RNA expression values of fibroblast and hiPSC (p31, p32) were calculated for DNA repair- and replication-related genes, as noted in our previous analysis [1]. As a result, a stable and approximately three-fold elevated expression through reprogramming was observed in all the hiPS cell (hiPSC)
lines, compared with the progenitor cells for RAD51 and BLM in HR, MSH2 and MSH6 in MMR and PARP1 and PARP2 in base excision repair (BER) which is a part of error-free repairs. RAD50, NBN and MRE11 were involved in both the HR and the non-homologous end-joining (NHEJ). MRE11 showed a slight elevation of expression, but there was no increase in expression of RAD51 or BLM, similar to our previous findings. RAD50 and NBN showed a minimal decrease in expression, consistent with the previous data [1] (Table 1).

Although expression was slightly upregulated in XRCC5 and XRCC6, that in XRCC4 was down-regulated; all their corresponding genes were involved in the NHEJ of the error-prone repair. In addition, REV3L and POLH of the polymerase representatives, thought to perform ambiguous post-replicative repairs, showed reduced expression. All these alterations in expression were the same as those shown in a series of DNA repair-related genes using microarray, with a completely separate fibroblast and third molar cell [1].

The principal component analysis (PCA) showed that progenitor fibroblast and hiPSC were greatly divided by PC1 and PC2 and that the two passage groups of (p31, p32) and (p50, p51, p53) were clearly shown by PCA. The difference between hiPSC passage group (p31, p32) and hiPSC passage group (p50, p51, p53) was indicated at cell differentiation. The mean values of each of the two groups were calculated for the FPKM values of OCT3/4(528,790),(570,815) and NANOG as indices of pluripotency. No difference was found between the two groups, but our findings demonstrated that pluripotency was maintained even in the groups of (p50, p51, p53) compared with the groups of (p31, p32) (Table 1).
2. Experimental design, materials, and methods

2.1. Cell culture

hiPSC lines [2,3] were grown in hESC serum-free human ESC (hESC) medium consisting of DMEM/F-12 (Life Technologies) supplemented with 20% knockout serum replacement (Life Technologies), 2 mM l-glutamine, 1× nonessential amino acids (Life Technologies), 0.1 mM 2-mercaptoethanol, and 5 ng/mL basic fibroblast growth factor (Katayama Chemical Industries) on Synthemax II-SC-coated tissue culture dishes (Corning). The cells were passaged using Accutase (Sigma) and seeded with the Rho kinase inhibitor Y-27632 (10 μM; LC Laboratories).

Table 1
Comparison of RNA expression levels of parental fibroblast and hiPSC passage groups of (p31, p32) and (p50, p51, p53). The average value is shown. Statistical analysis was performed between fibroblast and hiPSC passage group of (p31, p32), and between hiPSC passage group of (p31, p32) and hiPSC passage group of (p50, p51, p53) using a Student’s t test, analyzed by a Caleida Graph. Comparison between fibroblast and hiPSC passage group of (p31, p32) ** <0.01, * <0.05. Comparison between hiPSC passage group of (p31, p32) and hiPSC passage group of (p50, p51, p53) ◎◎ <0.01, ◎ <0.05. Data are expressed as the mean ± SEM.

| Symbol   | Accession No. | Activity | fibroblast P31-32 | iPSC p31-32 | p50-53 |
|----------|---------------|----------|-------------------|-------------|--------|
| PARP1    | M32721        | BER      | 36.20             | 159.82**    | 180.02 |
| PARP2    | NM_005484     | BER      | 16.83             | 22.67       | 27.94  |
| RAD51    | NM_002875     | HR       | 8.92              | 22.02**     | 22.07  |
| BLM      | NM_000057     | HR       | 2.41              | 6.84**      | 9.95   |
| MSH2     | NM_000251.2   | MMR      | 14.63             | 45.69**     | 48.64  |
| MSH6     | NM_00179.2    | MMR      | 21.58             | 53.08**     | 59.17  |
| RAD50    | NM_005732     | HR, NHEJ | 14.01             | 12.36       | 11.71  |
| MRE11    | NM_005590     | HR, NHEJ | 5.95              | 8.79        | 10.04  |
| NBN      | NM_002485     | HR, NHEJ | 22.30             | 9.21        | 14.53**|
| XRCC4    | NM_003401.5   | NHEJ     | 11.12             | 7.33        | 6.63   |
| XRCC5    | NM_021141.4   | NHEJ     | 124.88            | 172.58      | 208.53 |
| XRCC6    | NM_001469     | NHEJ     | 242.30            | 253.63      | 332.51 |
| POLH     | NM_001291970.2| TLS      | 5.73              | 4.34*       | 4.13   |
| REV3L    | NM_001286432.1| TLS      | 13.11             | 3.12*       | 330    |
| POUSF1   | NM_00173531   | pluripotency | 0.34            | 467.76**    | 397.08 |
| NANO    | AB093576      | pluripotency | 0.00           | 58.07***    | 48.08  |
| GAPDH    | NM_002046     | Housekeeping | 3691.37        | 2065.05     | 2194.58|

Fig. 1. Principal component analysis of parental fibroblast and hiPSC passage groups of (p31, p32) and (p50, p51, p53).
2.2. RNA extraction and library preparation

Total RNA was extracted from cells with an RNeasy Plus Micro Kit (Qiagen). Library preparation was performed using a TruSeq stranded mRNA sample prep kit (Illumina, San Diego, CA) according to the manufacturer’s instructions.

3. RNA sequence

Whole transcriptome sequencing was applied to the RNA samples through the Illumina HiSeq 2500 and 3000 platforms in a 75-base single-end mode. An Illumina Casava ver.1.8.2 software was used for the base calling. The sequenced reads were mapped to the human reference genome sequences (hg19) using TopHat ver. 2.0.13 in combination with Bowtie2 ver. 2.2.3 and SAMtools ver. 0.1.19. The number of fragments per kilobase of exon per million mapped fragments (FPKM) was calculated using Cufflinks ver. 2.2.1. The FPKM values were calculated from the respective sequence data, and the analyses were performed using iDEP85 (http://bioinformatics.sdstate.edu/idep/).

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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.2020.105228.

References

[1] Y. Yoshimura, Increased error-free DNA repair gene expression through reprogramming in human iPS cells, Regen. Ther. 11 (2019) 101–105, https://doi.org/10.1016/j.reth.2019.06.003.
[2] K. Igawa, C. Kokubu, K. Yusa, K. Horie, Y. Yoshimura, K. Yamauchi, et al., Removal of reprogramming transgenes improves the tissue reconstitution potential of keratinocytes generated from human induced pluripotent stem cells, Stem Cell. Transl. Med. 3 (9) (2014) 992–1001, https://doi.org/10.5966/sctm.2013-0179.
[3] Y. Yoshimura, A. Yamanishi, T. Kamitani, J.S. Kim, J. Takeda, Generation of targeted homozygosity in the genome of human induced pluripotent stem cells, PloS One 14 (12) (2019), e0225740, https://doi.org/10.1371/journal.pone.0225740.