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Long-term maintenance of human Pluripotent Stem Cells in defined media and feeder-free condition using recombinant protein

Conventional human pluripotent stem cell (hPSC) cultures require high concentrations of expensive human fibroblast growth factor 2 (hFGF-2) for hPSC self-renewal and pluripotency in defined media for long-term culture. The hPSC culture media need to be changed every day partly due to the hFGF-2 thermal instability in solution at 37°C. It has been known that the binding site of human DJ-1 (hDJ-1), also known as PARK-7 is FGF receptor-1. In the present study, for the first time, we have demonstrated that recombinant protein human FGF-2 can replace hDJ-1 in the essential eight media to maintain the pluripotency of H9 human embryonic stem cells (hESCs) under feeder-free condition. After more than ten passages, H9 hESCs cultured with human FGF-2 or human DJ-1 successfully sustained the distinctive hESC morphology. Furthermore, H9 hESCs revealed high expression levels of pluripotency markers including SSEA4, Tra1-60, Oct4, Nanog, and Alkaline phosphatase. DNA microarray revealed that more than 97% of the 21,448 tested genes, including the pluripotency markers, Sox2, Nanog, Klf4, Lin28A, Lin28B, and Myc, have similar mRNA levels between the two groups. Karyotyping revealed no chromosome abnormalities in both groups. They also differentiated sufficiently into three germ layers by forming in vitro embryoid bodies and in vivo teratomas. The moderate difference in both groups was shown in the real-time PCR assay using several pluripotency markers and three germ layer markers. The proliferation rate measured at different concentration of growth factors and the structural analysis of mitochondria using transmission electron microscopy demonstrated the distinguishable feature of H9 hESCs in two groups, namely hFGF2 and hDJ-1. On the whole, in-house made recombinant protein hDJ-1 can maintain the self-renewal and the pluripotency of H9 hESCs in a feeder-free system for long-term without alteration of their characteristics.

Recent Publications

1. DJ-1 Can Replace FGF-2 for Long-Term Culture of Human Pluripotent Stem Cells in Defined Media and Feeder-Free Condition Kim J, Baek S, Hong YJ, de Paula MN, Jahan Prima M, Oh YM, Cha SS, Do JT, Jang YJ, Choe H. *Int J Mol Sci*. 2021 May 31; 22(11):5954.
2. Prokaryotic soluble overexpression and purification of oncostatin M using a fusion approach and genetically engineered E. coli strains. Nguyen MT, Prima MJ, Song JA, Kim J, Do BH, Yoo J, Park S, Jang J, Lee S, Lee E, Novais MP, Seo HB, Lee SY, Cho ML, Kim CJ, Jang YJ, Choe H. *Sci Rep*. 2019 Sep 23; 9(1):13706.2
3. A chemical conjugate between HER2-targeting antibody fragment and Pseudomonas exotoxin A fragment demonstrates cytotoxic effects on HER2-expressing breast cancer cells. Lee S, Park S, Nguyen MT, Lee E, Kim J, Baek S, Kim CJ, Jang YJ, Choe H. *BMB Rep*. 2019 Aug; 52(8):496-501.