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Long-term maintenance of human pluripotent stem cells in defined media using recombinant protein

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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 conditions. 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 c-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. There was the moderate difference in H9 hESCs 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 concentrations 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 the long term without alteration of their characteristics.

Recent Publications

1. Kim J, et.al. (2021), DJ-1 Can Replace FGF-2 for Long-Term Culture of Human Pluripotent Stem Cells in Defined Media and Feeder-Free Condition, Int J Mol Sci 22(11):5954

2. Kim J, et.al.(2021), Prokaryotic soluble overexpression and purification of oncostatin M using a fusion approach and genetically engineered E. coli strains, Int J Mol Sci;22(11):5954.

Biography

Julee Kim received her diploma degree in Biotechnology at the Westfaelisch Wilhelms University of Muenster in 2007. She graduated from the Westfaelisch Wilhelms University of Muenster with a Ph.D. degree in Biology at the Max Planck Institute for Molecular Biomedicine in 2013. She completed her post-doctoral training at the University of California San Francisco and at Columbia University, Irving Cancer Research Center. She worked as a research assistant professor at the University of Ulsan College of Medicine, Asan Medical Center, and as a research professor at CHA University. Currently, she is a senior researcher at the Seoul National University College of Medicine. She has 8 publications in international journals. She presented papers at more than 10 national and international conferences.

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