Cell Research (2008) 18:s121.

www.nature.com/cr

The comparison of human embryonic stem cells cultured in feeder-containing culture system and feeder-free culture system

Shuwei Luo[1, 2], Ge Lin[1, 2], Guangxiu Lu[1, 2]

[1]Institute of Reproductive & Stem Cell Engineering, Central South University; [2]National Engineering & Research Center of Human Stem Cells, Changsha, Hunan, China

Human embryonic stem cells (hESCs) are routinely maintained in an undifferentiated state by co-culture with feeder cells. Recently, a new feeder-free culture system modified from neural cell culture medium (N2B27) was successfully established. But no extensive comparative study on the differences between hESCs cultured in N2B27 system and in traditional feeder-containing system was conducted. In this study, various aspects for identifying hESCs cultured in both systems, including hESCs molecular markers expressions, cell population distribution, apoptosis, cell proliferation, *in vitro* and *in vivo* differentiation capabilities were compared. Our results showed that hESCs in both systems maintained their pluripotency, expressing pluripotent genes Oct4, Nanog and Sox2 as well as hESCs markers Oct4, SSEA4 and TRA-1-60. FACS analysis showed that the percentage of SSEA-4+ and TRA-1-60+ hESCs in N2B27 system was 97.3 and 93% respectively, which was much higher than that in feeder-containing system (81.5% and 71.6% respectively). This indicated that hESCs was purer in N2B27 system than feeder-containing system. The apoptosis rate of hESCs in N2B27 system was about 8.44%, which was lower than that in feeder-containing system (10.8%). Moreover, nearly all hESCs clones in N2B27 system remained undifferentiated, and grew more rapidly than those in feeder-containing system, the cell number after 6 days' culture reaching about twice of that in feeder-containing system. For differentiation capabilities, during the formation of embryoid bodies hESCs in N2B27 system highly expressed multipotent genes Nanog and Oct4 and hardly expressed neural development related genes Pax6 and Sox1, which indicated their restricted capacity for differentiating into neuroectoderm compared with the hESCs in the feeder-containing system. However, when retinoid acid was added, both systems showed a similar expression pattern of endoderm and neuroectoderm genes. As for vivo differentiation, the ranks of teratocarcinomas formed by hESCs in both system were III. We had also found that high concentration of FGF2 (40ng/ml and 100ng/ml) had no influence to the expression of pluripotent genes, but could decrease hESCs apoptosis. Thus, we conclude that N2B27 system was suitable for hESCs culture *in vitro* and that 4ng/ml FGF2 added in N2B27 system was sufficient to maintain hESCs undifferentiated state..

***Keywords*:** feeder cells, FGF2

Cell Research (2008) **18**:s121. doi: 10.1038/cr.2008.211; published online 4 August 2008

Correspondence: Guangxiu Lu
E-mail: luobosing@hotmail.com