Journal of Reproduction Engineering
Vol.7, Supplement, 328-329, 2005

Establishment of the long term culture systems in mouse spermatogonial stem cells; Promissing possibility brought about ,by using the GS cell , to the progress in the study of germline manipulation

Takashi Shinohara

Reproductive Biology Unit, Division of Developmental Biology and Health Science, Horizontal Medical Research Organization, Graduate School of Medicine, Kyoto University Yoshida-Konoe, Sakyo, Kyoto 606-8501, Japan.

Spermatogonial stem cells are the only stem cells in the body that can transmit genetic information to the offspring. These stem cells, by through self-replication, support the spermatogenesis continuously in the testis throughout the life cycle. We have recently succeeded in the long-term culture of mouse spermatogonial stem cells. Due to their unique morphology, we named them germline stem (GS) cells. Mouse genocyte, which were isolated from the testis of neonatal offspring, were cultured in the presence of glial cell line-derived neurotrophic factor (GDNF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and leukemia inhibitory factor (LIF). We found the formation of germ cell colony showing a characteristic morphology in the culture. The GS colony can grow exponentially in vitro for more than 5 months. Upon transplantation into infertile animals, GS cells can produce normal fertile offspring, indicating that they are real stem cells. GS cells have several advantages over ES cells. First, although ES cells are only available during the embryonic period, GS cells can be derived from postnatal animals. Second, they are not tumorigenic and committed to the germline lineage.

REFERENCES

1) Shinohara, T., Avarbock, M. R. and Brinster, R. L. β1- and α6-integrin are surface markers on spermatogonial stem cells. *Proc. Natl. Acad. Sci. USA* 96, 5504-5509, 1999.

2) Shinohara, T., Orwig, K. E., Avarbock, M. R. and Brinster, R. L. Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells. *Proc. Natl. Acad. Sci. USA* 97, 8346-8351, 2000.

3) Shinohara, T., Orwig, K. E., Avarbock, M. R. and Brinster, R. L. Remodeling of the postnatal mouse testis is accompanied by dramatic changes in stem cell number and niche accessibility. *Proc. Natl. Acad. Sci. USA* 98, 6186-6191, 2001.

4) Kanatsu-Shinohara, M. Ogura, A., Ikegawa, M., Inoue, K., Ogonuki, N., Tashiro, K., Toyokuni, S., Honjo, T. and Shinohara, T. Adenovirus-mediated gene delivery and in vitro microinsemination produce offspring from infertile male mice. *Proc. Natl. Acad. Sci. USA* 99, 1383-1388, 2002.

5) Kanatsu-Shinohara, M., Ogonuki, N., Inoue, K., Miki, H., Ogura, A., Toyokuni, S. and Shinohara, T. Long-term proliferation in culture and germline transmission of mouse male germline stem cells. *Biol. Reprod.* 69, 612-616, 2003.