Podium Presentation

Production of haploid germ cells in a two-dimensional (2D) culture of neonatal mouse spermatogonial stem cells

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Abstract

Spermatogenesis is a complex process of germ cell proliferating and differentiation that requires extensive interactions of various cell types, hormones, and growth factors, making it difficult to be replicated in vitro. There is a need to preserve the fertility potential of pre-adolescent boys undergoing gonadotoxic cancer treatments; therefore, in vitro propagation and differentiation of spermatogonial stem cells (SSC) are important goals in male reproductive medicine. The objective of the present study was to establish a 2D cell culture system to generate mature male germ cells from neonatal SSC.

Neonatal SSC were pooled from 6-9 day-old ICR mouse pups (n=14) and cultured (2.0×10e3/well, in 24-well plates) on mitomycin-C treated MEF feeder cells for 10 days in a serum-free medium (DMEM-F12+0.25%BSA+15ng/mL GDNF+3ng/mL bFGF; n=4 replications). The SSC colonies were then dissociated and sub-cultured (4.0×10e3/well, in 12-well plates, 3 plates per replicate) for an additional 20 days in a differentiation medium (DMEM+4%KSR+6%FBS+10ng/mL GDNF+10ng/mL bFGF; n=4 replications). Data were analyzed using ANOVA.

Prior to inducing differentiation (day-10), all resultant colonies expressed genes specific for undifferentiated spermatogonia (*Ngn3, Plzf*) but not those of meiotic stages (*Stra8*). After 10 days in the differentiation medium, expression levels of *Ngn3* were down-regulated, while those of *Stra8* were up-regulated. After 20 days in the differentiation medium, expression levels of *Acr* were up-regulated, suggesting the completion of meiosis. Immunofluorescence and RT-PCR analyses also confirmed the presence of haploid male germ cells, and flow cytometry showed that 4.4% of cells were haploid male germ cells.

We concluded that under these serum-free culture conditions SSC could generate colonies capable of differentiation. Using stage-specific gene and immunofluorescence analyses we found that neonatal mouse testis cells had indeed differentiated into haploid male germ cells in a 2D-culture system, with a relatively higher efficiency of haploid-cell production than organ culture systems (4.4% vs. typically <2%).