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Establishment and Characterization of Neonatal Mouse Sertoli Cell Lines

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Sertoli cells isolated from 6-day postpartum mouse testes were conditionally immortalized with the simian virus 40 large tumor antigen gene (SV40-LTAg) under the control of a promoter inducible with ponasterone A, an analog of ecdysone. This strategy produced 2 cell lines, which exhibited mixed phenotypes. We first tested the conditional expression of the LTAg gene in the presence or absence of ponasterone A. The results showed that both cell lines expressed LTAg when the inducer was present in the culture media. When ponasterone A was removed, the majority of the cells died. After 60 generations, however, the continued expression of LTAg in the absence of the hormone indicated that unknown changes may have occurred in the genome of the cells. One of the cell lines was further subcloned, resulting in 7 new lines exhibiting a morphology resembling that of Sertoli cells in tissue culture. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed on RNA collected from each cell line in order to determine which cells were phenotypically similar to Sertoli cells in vivo. All cell lines expressed the products of the Sertoli cell—specific genes stem cell factor (SCF) and sulfated glycoprotein-2 (SGP-2), in addition to α-inhibin, GATA-1, and steroidogenic factor-1. Further, the lines express growth and differentiation factors known to act upon germ cells in vivo and in vitro such as leukemia inhibitory factor (LIF), transforming growth factor beta (TGF-ß), and basic fibroblast growth factor (bFGF). Moreover, when used as feeder layers in cocultures, at least 2 of these lines are able to maintain the viability of type A spermatogonia for at least 7 days and to support the first steps of spermatogonial differentiation.

Key words: Testis, immortalization, simian virus large T antigen, ecdysone, ponasterone A, growth factors

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