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JOURNAL ARTICLE

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# Changes in the expression of junctional and nonjunctional complex component genes when inter-sertoli tight junctions are formed in vitro

C. C. Wong, S. S. Chung, J. Grima, L. J. Zhu, D. Mruk, W. M. Lee and C. Y. Cheng Population Council, The Rockefeller University, New York, New York 10021, USA.

Throughout spermatogenesis, germ cells move progressively from the basal to the adluminal compartment, which is accompanied by continual disassembly and reassembly of intercellular junctions suggesting germ cell movement is composed of intermittent phases of junction disassembly and reassembly. A study was performed to correlate the

expression of junctional-complex components (such as zonula occludens-1 [Z0-1], a tight-junction component protein) and nonjunctional complex components (such as urokinase-type plasminogen activator [uPA], a serine protease; cathepsin L, a cysteine protease; alpha2-macroglobulin, a nonspecific protease inhibitor; and cystatin C, a cysteine protease inhibitor) at the time when inter-Sertoli tight junctions were established in vitro. This is an attempt to investigate whether the expression of nonjunctional component genes also correlates with the formation of inter-Sertoli tight junctions in vitro. This is part of an effort to understand the physiologic elements of germ cell movement in the epithelium. Sertoli cells cultured in vitro are known to undergo programmed cell death. To ensure that the changes in target gene expression were not the result of apoptosis, Sertoli cells were cultured in vitro at densities of 0.25, 0.75, and 3 x 10(6) cells/cm2 for up to 7 days on bicameral culture units coated with Matrigel (Collaborative Research) and were assessed by morphologic analysis and agarose gel electrophoresis. It was noted that many of the Sertoli cells cultured at 3 x 10(6) cells/cm2 underwent apoptosis by day 7, in contrast to cultures at 0.25 and 0.75 x 10(6) cells/cm2 illustrating the Sertoli cell number per unit of area may be an important parameter to be considered when studying Sertoli cell function in vitro. Also, it was shown that the expression of ZO-1 increased significantly between days 2 and 3 prior to the establishment of inter-Sertoli tight junctions assessed by transepithelial resistance measurement (TER), which illustrates that ZO-1 can be used as a marker to monitor this cellular event. More interestingly, there was also a transient increase in the expression of uPA and cathepsin L between days 2 and 3 at the time preceding the formation of tight junctions. In Sertoli cells cultured at low density (2 x 10(4) cells/cm2), when a confluent monolayer of cells could not form, there were no changes in the expression of either ZO-1, uPA, or cathepsin L throughout the 7-day culture period. These results show that the establishment of specialized junctions, such as tight junctions between Sertoli cells in vitro, may require the participation of both junctional and nonjunctional complex components.

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