O-08

Molecular Mechanism of Male Germ Cell Apoptosis after Busulfan Treatment

Jin-Hoi Kim

Gyeongsang National University, Chinju, Korea

Identification of spermatogonial stem cell-specific surface molecules is important in understanding the molecular mechanisms underlying the maintenance and differentiation of these cells. We have found that spermatogonia from busulfan treated mice expressed an autoantigen that distinguishes between undifferentiated and differentiated spermatogonia. Four to six weeks after busulfan treatment, germ cells located in the basal compartment of seminiferous epithelium show isotype-specific IgG deposits that form due to autoimmunity. Before busulfan treatment, the level of testicular IgG was very low but IgG levels began to increase after week 4 and peaked at week 6. When cells from the busulfan treated testis were analyzed using laser scanning cytometry (LSC), the frequency of cells positive for IgG deposits, 6-integrin, and 1-integrin were 16.5±3.8%, 11.8±2.6%, and 9.0±1.4%, respectively. Immunofluorescent staining suggested that most, if not all of the cells with IgG-deposits isolated from a laminin-coated dish, were also positive for a spermatogonial stem cell marker ?6-integrins as well as for a germ cell-specific marker TRA 98. We determined serum and intratesticular IgG levels and the soundness of seminiferous tubule basement membrane from busulfan treated mice using electron microscopy, in order to study the mechanism responsible for IgG deposits in spermatogonia. We found that the basement membranes of seminiferous tubules from busulfan treated mice were severely impaired when compared to those of normal adult, neonates and w/wv mice. Furthermore, new blood cells were observed in the surface of the damaged basement membrane along the seminiferous tubules. These results suggest that the IgG in spermatogonial stem cells accumulates from circulating blood through the impaired basement membranes induced by busulfan treatment. Taken together, our study suggests that IgG can be used as a new marker for undifferentiated

spermatogonia cells.

Nucleoside diphosphate kinases (NDPKs) are conserved through evolution and have been shown to be involved in various biological phenomena. By functional screening in yeast, we identified a new member of the NDPK family, *nm23*-M5, which encodes a 211-amino acid protein with 86% identify to the human homolog, *nm23*-H5. Northern blot analysis reveals that *nm23*-M5 encodes two transcripts of 0.8 and 0.7 kb, which are highly and specifically expressed in adult testis. Reverse transcriptase polymerase chain reaction analysis shows that *nm23*-M5 first appears in pachytene spermatocytes and increases in abundance through subsequent stages. However, a low level of *nm23*-M5 mRNA was detected by RT-PCR in other tissues such as ovary, brain, and heart. By *in situ* hybridization we localized testicular *nm23*-M5 transcripts in stage 12 to stage 16 spermatids in the neighboring lumen of seminiferous tubules, a distribution which contrasts with that of human *nm23*-H5 transcripts, which is specifically expressed in spermatogonia and early spermatcytes. In addition, the heterologous expression of *nm23*-M5 in yeast cells confers protection from cell death induced by Bax, which is due to the generation of reactive oxygen species. Taken together, these results suggest that the murine *nm23*-M5 plays an important role in late spermiogenesis by acting as a scavenger for reactive oxygen species.

Male germ cell apoptosis has been extensively explored in rodent. In contrast, very little is known about their susceptibility to apoptosis stimuli of developing germ cell stages at the time when germ cell depletion after busulfan treatment occurs. Furthermore, it is still unanswered how spermatogonial stem cells are resistant to busulfan treatment. Spontaneous apoptosis of germ cells was observed in the testis of adult mice and experimentally induced busulfan treated mice increased this apoptosis to such an extent that there was a decrease in the weight of the testis. One week after busulfan treatment, TUNEL staining resulted in selective degeneration of spermatogonia and some of early meiosis spermatocytes. RT-PCR results further validated the findings of TUNEL-stained apoptotic cells. The percentage of apoptotic-positive tubules and apoptotic cell index increased time dependently: an immediate effect on type A spermatogonia at

1 week after treatment and in another week that followed, secondary effect on haploid cells. RT-PCR results by using spermatogonia-specific biomarker showed that c-kit and Stra 8 expression was reduced, but that Gli I expression was constant, indicating the initiation of primary apoptosis of type 2 spermatogonia. Downregulation of FasL at 4 weeks after injection of busulfan and upregulation of Bax after treatment contributed to the initiation of secondary apoptosis of haploid cells. Expression of FasL was inhibited while expression of Fas increased after the 2-busulfan treatment and remained at levels about two times of the control. Our results indicate that busulfan resulted in apoptotic death of testicular germ cells and that this process occurred independently the Bcl-2 family genes and the Fas signaling system.