Abstract

WW domain-containing oxidoreductase (*Wwox*) is a well-known putative tumor suppressor, highly expressed in hormonally regulated tissues and considered essentials for normal development of gonads. A Wwox deficient rat model named lethal dwarfism and epilepsy (*lde/lde*) has low gonadotropin and testosterone levels, increased apoptosis of germ cells, decreased number of Sertoli cells (SCs), and retarded growth of Leydig cells (LCs). In the present study, in order to reveal the function of Wwox in testicular development and spermatogenesis, cellular and subcellular localization of Wwox, type of germ cells that cause apoptosis, mechanism of the apoptosis, SCs and LCs differentiation, and steroidogenesis were examined by mainly *in vivo* experiment using normal (+/+) and *lde/lde* rats. Wwox was expressed in almost all testicular cells except spermatids steps 18 to 19, mature sperm and peritubular myoid cells. Wwox localized diffusely in the cytoplasm with focal intense signals (FISWs) which were gradually condensed and changed morphology in germ cells with their differentiation. Along with, Wwox was colocalized with cis-Golgi (GA) marker and resided in isolated GA enriched fractions. These subcellular localization of Wwox was also confirmed in single-cell suspension.

Delayed differentiation of spermatocytes (SPs), increased apoptosis of pachytene spermatocytes (P-SPs), and absence of post-meiotic spermatids indicated the interruption of FRS in *lde/lde* testes. Interestingly, the GA associated protein golgin-160 expression was reduced and formed cytoplasmic abnormal bright condensed signals (ABCSs) outside of GA in P-SPs of *lde/lde* testes. Similarly altered expression of golgin-160 and increased apoptosis were found in GnRH antagonist (Cetrorelix) treated testes, surgically induced cryptorchidism testes, and serum starved embryonic fibroblast cells (REFs) of *lde/lde* rats. These results indicated that Wwox deficiency caused golgin-160 alterations, increased P-SPs apoptosis and disrupted spermatogenesis in late meiosis under depletion of gonadotropins and testosterone in *lde/lde* testes. Significantly increased number of nestin positive cells, increased expression of anti-mullerian hormone and reduced expression of androgen receptor in SCs during FRS indicated that SCs of *lde/lde* were functionally immature. The retarded differentiation of SCs was likely involved with significantly reduction in proliferation and differentiation of LCs lineages in *lde/lde* testes during FRS. Taken together, these results indicated that Wwox is essential for normal SCs and LCs development, spermatogenesis and steroidogenesis in rats during FRS.