

ABSTRACT

In the mammalian ovary, the oocyte is surrounded by a zona pellucida layer (ZP), consisting of four glycoproteins in humans (ZP1-ZP4) and three in mice (ZP1-ZP3). The main functions of the zona pellucida are to participate in the fertilization process and prevent polyspermy, as well as its involvement in embryo development. Zona pellucida has already been successfully used as a target antigen for active immunization of certain wild-life populations as a contraceptive method, as ZP3-specific antibodies initiate a loss of ovarian function due to autoimmune oophoritis mediated by autoreactive T cells. Classically, ZP3 is expressed in the ovary exclusively but was found also in several cancer tissues (ovarian, prostate, colorectal, and lung cancers). This finding expanded the opportunity to use ZP3 in active or passive cancer immunization. Very recently, surprising ZP3 expression in mouse and human testis was shown.

The aim of the present study was to characterize the ZP3 expression in healthy human and mouse tissues, with a special emphasis on the testis, and to analyze its functional implication during the testicular ontogeny and spermatogenesis process in mice.

In this research study, mouse (n=6) and human tissues (n=14) preserved in RNAlater and/or 4% paraformaldehyde (PFA) and a commercially available mouse spermatocyte cell line GC-2spd(ts) were used. For the ontogenesis study, mouse testis from 7 groups: embryonic day (ED) 18, postnatal day (PND) 1, PND 7, PND 15, PND 21, PND 35, 2-months-old (n=6/each group). ZP3 expression was examined using the RT-PCR analysis. Using RNAscope in situ hybridization and immunohistochemistry methods, mRNA transcripts and protein localization of ZP3 were checked, respectively. The whole-mount immunostaining of seminiferous tubule staining was performed to assess the ZP3 expression in stem and progenitor cells (SSPCs) and visualized by confocal microscopy. ZP3 expression at the protein level was analyzed in luteinizing hormone (LH) receptor (R) knockout mice testis (LuRKO), which are completely devoid of spermatogenesis. To analyze the hormonal effects that may regulate the ZP3 action, mouse spermatocyte GC-2spd(ts) cells were treated with estradiol, testosterone, progesterone, FSH, LH, and hCG. Subsequently, ZP3 expression at the mRNA level and its protein localization using immunofluorescence assay were performed.

Immunohistochemical analysis showed ZP3 protein and RNAScope ZP3 mRNA transcripts localization in human and mouse testis, namely in the germ cells, spermatogonia, spermatocytes, and spermatids, as well as in mouse spermatocyte GC-2spd(ts) cells. ZP3 was absent in normal humans and mice (n=14/species) testicular Leydig and Sertoli somatic cells, SSPCs, and differentiating progenitor spermatogonia. During the mouse testis ontogenesis, ZP3 expression appeared on PND21 (during puberty). Younger PND1, PND7, PND15, and fetal ED18 mice did not express ZP3 in the testis. Additionally, no ZP3 could be detected in LuRKO mice. Moreover, estradiol, testosterone, progesterone, FSH, LH, or hCG treatments had no effects on the ZP3 expression level in GC-2spd(ts) cells.

In conclusion, the presented data showed novel ZP3 expression at molecular levels in human and mouse testis, which enhances the potential for ZP3 as a target antigen for reversible male contraception. Ontogenesis study and no ZP3 expression in LuRKO mice emphasized the functional impact of ZP3 in the testis during spermatogenesis. Finally, negative ZP3 expression in any other healthy tissues (besides the ovary and testis) excludes potential off-target effects of ZP3 cancer immunotherapy.