Abstract citation ID: bvac150.1346

Reproductive Endocrinology

LBMON272

Chromatoid Bodies In The Regulation Of Spermatogenesis: Novel Role Of Grth

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More than 5 million couples in US are affected by infertility. Male infertility is a major cause (>30%), and understanding spermatogenesis is important to address the problem. During early spermatogenesis, mRNAs are actively transcribed, transported and stored transiently in chromatoid bodies (CBs). CBs are membrane-less perinuclear organelles which serve as storehouses of mRNAs until later stages of spermatogenesis. Gonadotropin-regulated testicular RNA helicase (GRTH/DDX25) plays critical role in spermatogenesis. In addition to its inherent helicase activity, GRTH transports mRNAs from nucleus to cytoplasm and from there to CBs for storage until translation. In Germ cells, there are two species of GRTH, the 56 kDa non-phospho and 61 kDa phospho (pGRTH) forms. Our early studies revealed a missense mutation (R242H) of GRTH in Japanese azoospermic men which resulted in the lack of pGRTH in in vitro studies. GRTH knock-in (KI) mice with the human mutant GRTH gene (R242H), show loss of the phospho-species from cytoplasm and CBs with preservation of the non-phospho form in the cytoplasm, Nucleus and CBs. GRTH-KI mice are sterile, lack elongated spermatids and spermatozoa with spermatogenic arrest at step 8 of round spermatids (RS). CBs isolated from GRTH KI mice are smaller, highly condensed, lack pGRTH and RNA-Seq analysis of isolated mRNA (from CBs) revealed differential abundance of mRNAs related to spermatid differentiation. Integrative analysis of small RNA and mRNA expression will provide cohesive outlook of molecular mechanisms of gene regulation during spermatogenesis. Hence, we proceeded with small RNAseq analysis which reveal differential expression (Padj<0. 05) of piRNAs and miRNAs. In total 226706 piRNAs upregulated and 166567 piRNAs downregulated in the round spermatids. Several miRNAs like miR32, miR7, miR184, miR335, miR140, miR141, miR1981, miR202, miR880, miR669c were downregulated. These are involved in the positive regulation of spermatid differentiation, sperm motility, mRNA processing and decay. The miRNAs, miR150, miR196a-2, miR7b, miR652, miR146, miR10b, miR379, miR122a, miR27a, miR127 and miR328 were upregulated, which negatively regulate the spermatid differentiation. Gene ontology analysis revealed that the majority of downregulated miRNAs belong to regulation of gene expression. Majority of upregulated miRNAs belong to miRNA mediated suppression of translation mostly associated with spermiogenesis and CBs. The identified miRNAs regulate essential genes like Tnp2, TSSK6, Pabpc1, Ppp1cc and UPF2 etc. These indicates that the CBs mediate highly specialized mRNA metabolism process and serve as a processing center of essential mRNAs. These results demonstrate the importance of GRTH and phospho-GRTH in the regulation of CB and their role in mRNA storage and stability of germ cell specific mRNAs during spermiogenesis and healthy sperm formation.

Presentation: Monday, June 13, 2022 12:30 p.m. - 2:30 p.m.