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Topaz1 suppression disrupts the expression of testicular long non-coding RNAs during murine spermatogenesis.

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Background of Topaz1: Topaz1, for Testis and Ovary specific PAZ domain gene 1, has been characterized in our lab as a new germ cell specific gene highly conserved in vertebrates with a role during gametogenesis of mammals. To study the role of this gene, a line of Topaz1-depleted mice has been generated. Thus, whereas female mice are fertile, homozygous male mutants are infertile. Abnormal meiosis occurs in testes Topaz1+/- mice causing cell death and an absence of spermatozoa. Previous transcriptional study of Topaz1+/+ mice by microarray comparative analysis highlighted the significant proportion of deregulated long non-coding RNA (lncRNA) in mutant testis. The aim of this work is to study (1) the whole transcriptome of Topaz1+/+ mouse testes and (2) the involvement of IncRNA in murine spermatogenesis.

Methodologies:
• RNA sequencing (RNA Seq) to identify all deregulated genes in Topaz1+/+ testis whose IncRNA.
• Localization by In Situ hybridization (ISH) of some deregulated IncRNA.
• Generation of a new line of knockout mice (CrispR/Cas9 technology) without one of these IncRNA → Studies of the phenotype.

Fig. 1: Overview of 2748 deregulated transcripts in P18.5 testes lacking Topaz1. 91% (2491) are down-regulated; 9% (257) are up-regulated. A large proportion (27%) of deregulated genes are lncRNAs.

Fig. 2: A-C: ISH (RNAscope® Brown) of different deregulated IncRNAs: LncRNA-Chr7(A), LncRNA-Chr6 (B) and LncRNA-Chr10 (C) in 2 months-old WT testis section. D-F: Merge images showing ISH with lncRNA (red) probes, immunodetection of VASA (DDX4) (green) and DAPI staining (blue).

→ LncRNAs are expressed in different spermatocytes depending on the stage of the seminiferous epithelium: LncRNA-Chr10 seems nuclear whereas IncRNA-Chr6 cytoplasmic. LncRNA-Chr7 looks as if located in both cytoplasm and nuclei. Scale bar = 20μm

Fig. 3: Histological analysis of 2 month-old testis showed the presence of spermatozoids in the lumen of the seminiferous tube and in the cauda of epididymis in both WT and IncRNA-Chr10+/− mice. → LncRNA-Chr10+/− mice are fertile with a normal progression of spermatogenesis. Scale bar = 50 μm

Fig. 4: Evaluation of epididymal spermatozoa concentration (A) and motility (B) show significantly differences between WT and IncRNA-Chr10+/− mice.

→ LncRNA-Chr10+/− mice have a 57% decrease of spermatozoa concentration and motility was significantly higher.

** : pvalue<0.01

Fig. 5: Functional annotation clustering using DAVID analysis with 206/258 deregulated genes in IncRNA-Chr10+/− testes compared to WT (from RNAseq data of P18.5 testes; data not show).

→ Despite the absence of noticeable phenotype in IncRNA-Chr10+/− mouse line, transcriptomic analysis of P18.5 testes indicated that 6 gene clusters are significantly deregulated (ES >1.3).

This highlighted the biological process deregulated in absence of the IncRNA-Chr10 with the most interesting referred to microtubule (MT) base process (cluster 1 and 6) and spermatogenesis (cluster 3).

Conclusion:
Number of LncRNA expressed in the testes is significant. In Topaz1 null mice, with male meiotic arrest, a large number of testicular IncRNA are significantly deregulated (Fig1). Three of them were observed by ISH showing different localisations into spermatocyte cells, suggesting distinct roles during spermatogenesis (Fig2). To determine a potential spermatogenetic role, a new mouse line deleted of one of them (LncRNA-Chr10, expressed in nuclear germ cells) has been created. These mice are fertile in both sexes (Table 1) and spermatogenesis of mutant takes place normally (Fig3). However, they have a decrease of epididymal spermatozoa concentration and an increase of spermatozoa motility (Fig5). Moreover, transcriptomic analysis of P18.5 LncRNA-Chr10+/− testes (by RNAseq) shows more than 250 deregulated genes involved particularly in cytoskeleton and spermatogenesis (Fig5).

→ Thus, although LncRNA-Chr10, expressed exclusively in the testes with an expression linked with Topaz1 expression, its depletion does not appear crucial for fertility in mice. The LncRNA-Chr10 seems to play role in spermatogenesis motility and concentration. This lncRNA may regulate protein coding gene expression especially genes in relation to the cytoskeleton.