KNOCKOUT ANALYSIS OF OOCYTE-SPECIFIC MULTI-COPY GENE, *OOG1*

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Sex-specific genes in germ cells are necessary to keep the sex of germ cells. The cascade of sex-specific gene control over the down-stream genes supports proper germ cell differentiation. *Oog1* is an oocyte-specific gene and seems to function as a transcription factor during meiosis. In knock down (KD) analysis, it has been suggested that *Oog1* suppresses sperm-specific genes and activates oocyte-specific genes in oocytes to maintain the female germ cell sex. In *Oog1*-KD oocytes, sperm-specific genes such as Tektin2 (*Tekt2),* Tudor Domain Containing 6 (*Tdrd6),* Kelch Like Family Member 5 (*Klhl5)* and Transition Protein 2 (*Tnp2)* were upregulated and oocyte-specific linker histone *H1foo* was downregulated. Significant phenotype, however, is not observed in *Oog1*-knockdown mice. We thought that knock out (KO) analysis is more effective because any mRNA is not produced. We tried to produce *Oog1*-KO mice using CRISPR/Cas9 system. It is challenging because *Oog1* has 5 copies. The two copies locate on chromosome 4 and the others locate on chromosome 12. We used gRNAs shared by all 5 copies, which of two are on the exon 3 and the other is on the exon 4. Genetically modified (GM) mice produced using three gRNAs are 8 males and 10 females. GM mice produced using one gRNA are 5 males and 2 females. To perform mutation analysis, five pairs of PCR primers specific to each copy were designed to amplify only targeted copies. In one GM mouse line, one copy in chromosome 4 is defected largely enough to be recognized by the size of DNA amplified by PCR.

TA cloning shows that another copy in chromosome 4 is also defected in both alleles although the mutation can not be recognized by the size of DNA amplified by PCR. In another GM mouse line, 3 copies of *Oog1* in chromosome 12 are all mutated. DNA size amplified by PCR indicates that one copy is defected largely and another copy has extra DNA insertion. TA cloning showed that the remaining one copy has a few bases deletions in both alleles. We next intend to generate complete KO mice by mating these two CRISPR/Cas9 GM mouse lines and examine the detail function of *Oog1*.