NOBOX BINDS THE PROMOTER OF PEPTIDYLARGININE DEIMINASE 6 THROUGH NOBOX DNA BINDING ELEMENTS

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Purpose: Nobox, a homeobox transcription factor, is expressed preferentially in oocytes and germ cells within mouse ovary. Nobox knockout female are infertile due to losing oocytes rapidly by 14 days of postnatal life. In addition Nobox deficiency disrupts the expression of oocyte-specific genes including peptidylarginine deiminase 6 (Pad6). We examined weather or not Nobox directly regulate the expression of Pad6 gene.

Materials and methods: Total cellular RNA was isolated from ovaries of wild type or Nobox –/– mice. In situ hybridizations were performed on ovarian sections derived from three different animals. Electrophoretic Mobility Shift Assays were conducted by incubating 32P-labeled probes with purified Nobox homeodomain containing GST fusion proteins in the absence or presence of anti-GST antibody.

Results: We examined Pad6 expression in multi-tissues using RT-PCR. Pad6 was preferentially expressed in ovaries. In addition Pad6 transcripts are detected as early as embryonic day 16.5. In 6 weeks-old wild type ovaries, Pad6 mRNA expression was detected in oocytes from primordial through antral follicles but was excluded from granulosa cells, theca cells, and corpora lutea. Pad6 is drastically down-regulated in Nobox–/–newborn ovaries. Pad6 transcripts are not detected in Nobox–/– newborn ovaries. The Pad6 promoter contains four putative Nobox DNA binding elements (position –1433, –2451, -2463, and -2466) relative to the translational start site (+1). Interestingly, these putative NBEs contain one TAATTG sequence (-2466) and three TAATTA sequence (-2462, -2451 and -1433). These two NBEs formed DNA-protein complexes with purified Nobox homeobox domain.

Conclusion: Our findings indicate that Nobox is a critical regulator that orchestrates oocyte-specific genes including Pad6 during folliculogenesis. Nobox may directly regulate the expression of Pad6 through four Nobox DNA binding elements.