

mechanisms of spermatogenesis. Nodal could promote proliferation of mouse spermatogonial stem/progenitor cells via Smad2/3 and oct-4 activation. The objective of this study was to determine the role of Nodal signaling in the differentiation of iPS cells to male germ cells.

**Design:** Comparative and controlled study.

**Materials and methods:** In this study, embryoid body (EB) formation and the exposure of Nodal induction were applied to induce the male germ cells from mouse iPS *in vitro*. Germ cell-specific genes and proteins were assessed using real-time PCR, immunoblotting and flow cytometry. The moleculars of Nodal signaling pathway were detected by immunoblotting.

**Results:** We found that Nodal and its receptors *Alk4*, *ActR-IIB* except *Alk7* were expressed in the mouse iPS cells, whereas both Nodal and its receptors were detected in the EBs. Nodal could promote the propagation of iPS cells and Nodal RNAi disrupted the proliferation of iPS cells. The results of real-time PCR and western blots showed that Nodal could up-regulate the expression of germ-cell marker genes and proteins in iPS-derived EBs. Moreover, the level of Smad2/3 phosphorylation, *Oct4* and *Foxb1* transcription, and cyclin D1 and E were increased with graded Nodal signaling.

**Conclusions:** Collectively, the above results suggest that Nodal promotes the generation of male germ cells from iPS cells via the activation of Smad2/3 and *Oct4* and *Foxb1* transcription. This study offers novel insights into molecular mechanisms of male germ cell development.

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**Keywords:** Induced pluripotent stem cells; Nodal; Smad2/3 pathway; male germ cells

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## AB199. Human germ cell secreting factor Nodal regulates Sertoli cell functions

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**Objectives:** To explore the regulatory effects of germ cells and germ cells secreting factor Nodal on the function of Sertoli cells derived from obstructive azoospermia and non-obstructive azoospermia patients.

**Design:** Comparative and controlled study.

**Materials and methods:** Human Sertoli cells and germ cells were isolated using two-steps enzymatic digestions from the testes of obstructive azoospermia and non-obstructive azoospermia patients respectively. Expressions of Nodal signaling components in human Sertoli cells and germ cells were identified by PCR and immunochemistry. Human germ cells and Sertoli cells were cocultured *in vitro* to evaluate their effects on Sertoli cells. Human recombinant nodal and its receptor inhibitor SB431542 were added in the Sertoli cells culture medium to study their effects on Sertoli cell functions. CCK8 measurement was used to evaluate the proliferative activity. Q-PCR and western blot were applied to assess the expression of functional Sertoli cell genes.

**Results:** Human germ cells down-regulated blood-testis-barrier associated genes (*CLDN11*, *OCN*) expressions of Sertoli cells in co-culture system. Nodal was expressed in germ cells but not in Sertoli cells, whereas its receptors *ALK4*, *ALK7*, and *ActR-IIB* were detected on Sertoli cells, which indicated Nodal signaling pathway, may play roles in the regulation of germ cells to Sertoli cells. Human recombinant nodal could promote the proliferation of human Sertoli cells, while the proliferative activity was inhibited by SB431542. Nodal could enhance the expressions of functional Sertoli cell genes (*GDNF*, *SCF*, *BMP4*, and *ABP*), while SB431542 decreased their expressions. In contrast, Nodal decreased the expression of blood-testis-barrier associated genes (*CLDN11*, *OCN*), while SB431542 increase their expressions.

**Conclusions:** Human Sertoli cell functions could be regulated by germ cells via paracrine pathway. Human germ cells secrete Nodal which could regulate Sertoli cell functions.

**Keywords:** Nodal signaling; human Sertoli cells; human germ cells; azoospermia

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## AB200. Association of rs3129878 and rs498422 in the *HLA* region with non-obstructive azoospermia in the Han Chinese population

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**Objective:** The previous genome-wide association study (GWAS) of non-obstructive azoospermia (NOA) in the Han Chinese populations identified two NOA-risk loci (rs498422 and rs3129878) within the *HLA* region, and provided strong evidence for the genetic influence of male infertility. A further case-control study found that only rs3129878 remained to be significantly associated with NOA in the Japanese population. Therefore, we conducted the association study to further validate whether the risk of NOA caused by these two SNPs was still existed in an independent Han Chinese male population, consisting of 550 NOA cases and 555 normal controls.

**Design:** A case-control study of the NOA susceptibility genes within the *HLA* region associations.

**Materials and methods:** These two SNPs were analyzed in 550 NOA patients and 555 controls of Chinese origin using direct sequencing. Then, the genotype and allele distributions of them were further analyzed using the online software SHEsis (<http://analysis.bio-x.cn>).

**Results:** The association studies strongly supported the significant association of rs498422 and rs3129878 with NOA for both genotype and allele distributions ( $P=0.047$  and  $P=1.87 \times 10^{-10}$ , respectively).

**Conclusions:** In our replication study of Chinese samples, we provided genetic evidence for the contribution of these two NOA-risk SNPs within the *HLA* genes region in predicting males at high risk of NOA in Han Chinese population. Considering genetic differences among populations, future validating studies in independent samples are suggested.

**Keywords:** Association study; non-obstructive azoospermia (NOA); single nucleotide polymorphism (SNP)

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