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# Suppression of dsRNA response genes and innate immunity following Oct4, Stella, and Nanos2 overexpression in mouse embryonic fibroblasts

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ABSTRACT

The self-renewal capacity of germline derived stem cells (GSCs) makes them an ideal source for research and use in clinics. Despite the presence of active gene network similarities between embryonic stem cells (ESCs) and GSCs, there are unanswered questions regarding the roles of evolutionary conserved genes in GSCs. To determine the reprogramming potential of germ cell- specific genes, we designed a polycistronic gene cassette expressing Stella, Oct4 and Nanos2 in a lentiviral-based vector. Deep transcriptome analysis showed the activation of a set of pluripotency and germ-cell-specific markers and the downregulation of innate immune system. The global shut down of antiviral genes included MHC class I, interferon response genes and dsRNA 2′-5′-oligoadenylate synthetase are critical pathways that has been affected . Individual expression of each factor highlighted suppressive effect of *Nanos2* on genes such as *Isg15* and *Oasl2*. Collectively, to our knowledge this is the first report showing that *Nanos2* could be considered as an immunosuppressive factor. Furthermore, our results demonstrate suppression of endogenous retrotransposons that harbor immune response but further analysis require to uncover the correlation between transposon suppression and immune response in germ cell development.

## 1. Introduction

Regulation of gene expression and global (epi) genomics modification in germ cell progenitor cells serves them as an interesting topic in molecular genetics. Primordial germ cells (PGCs) as sole progenitors of spermatogonia and oocytes apply exclusive genetic programs to guarantee the safe genetic information transfer to the next generation. Scrutinizing the similarities and differences of gene expression patterns, regulatory mechanisms and surveillance machinery between PGCs and other stem cells uncovered several critical features dealing with maintenance of pluripotency [7]. Once sexual differentiation begins, PGCs follow different paths in the female and male gonads. At approximately E13.5, meiosis starts in female PGCs, whereas the male germ cells (called “gonocytes”) remain quiescent until birth and form germline stem cells (GSCs) as male germ cells. However, the short period of time between E10.5 to E13.5 is a unique state that will be discussed more.

During a short phase of development, from E10.5 to E13.5 germline progenitor cells express pluripotency and germ cell specific factors. In

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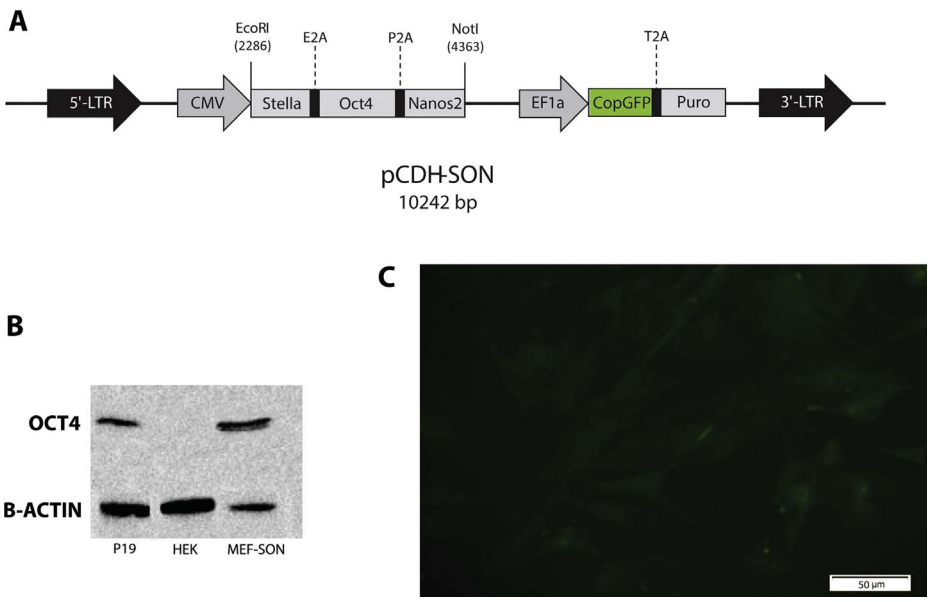


**Fig. 1.** Polycistronic lentiviral vector expressing STELLA, OCT4 and NANOS2 (SON). (A) Map of the polycistronic lentivirus expressing 3 selected genes; hydrolyzing 2A peptides separate genes in the construct. (B) Western blotting of OCT4 shows appropriate cleavage of the gene cassette. P19 and HEK293T cells are the positive and negative control, respectively. Whole-cell lysates were derived from $1 \times 10^7$ cells per sample. (C) Florescent microscopy of MEFs transduced with SON lentivirus.

this critical step gamete progenitor cells show a highly similar (epi) genetics pattern to embryonic stem cells. Several sequences of remarkable events take place in this short period of time, make a globally hypomethylated genome for PGCs with erased imprinting regions and rearranged histone markers. This uncommon circumstance is exclusively observed in PGCs and oocytes. Despite the considerable amount of spatiotemporal gene expression, there are many unanswered questions about this process. This state has exclusive transcriptional and translational features that can be used as a model for study dark sides of gene expression regulation in germ cell reprogramming.

One of the novel intriguing field is study of "immunologic homeostasis" in germ cells. Interestingly, the negative regulation of immune response in germ cell development has been highlighted [1]. The notion that immune response to exogenous nucleic acids is suppressed started from competent nature of oocytes to accept sperm genome during fertilization and have been surveyed recently [1].

*Stella* or *Dppa3* (NM_139218) is a low molecular weight, nucleocytoplasmic protein. Its expression begins at E7.0–E7.25 and continues during the migrating phase of PGCs as well as in *in vitro* culture of GSCs and SSCs [10]. *Oct4* (NM_013633) is a master regulator of pluripotency. It has been studied most extensively in ES cells and has crucial role in evoking and maintaining pluripotency [20]. *Nanos2* (NM_194064) is a GSC inducer gene from RNA-binding protein family that promotes GSC development.

To obtain insight into the mysterious interactive cooperation of germ cell-specific factors, we evaluated the transcriptome of mouse embryonic fibroblasts (MEFs) by concurrent overexpression of three specific germ cell factors: *Stella*, *Oct4* and *Nanos2*. We designed a polycistronic gene cassette to overexpress these three GSC-specific genes (*Stella-Oct4-Nanos2*, or "SON", for their order in the expression cassette) in mouse embryonic fibroblast (MEF) cells to investigate whether this combination of factors is sufficient to induce differentiated cells to a GSC-like state and to determine what transcriptome changes accompany this reprogramming. Our RNA sequencing (RNA-seq) results indicate that many genes related to self-renewal and germ cell programs are activated in MEF-SON cells. Surprisingly, innate immune response genes with specific retrotransposon transcripts were among the downregulated genes.

## 2. Material and methods

### 2.1. Mice

Pregnant BALB/c mice were purchased from Razi Institute of Mashhad and kept in the animal facility at the faculty of Science of Ferdowsi University of Mashhad. Animal ethics committees of Ferdowsi university of Mashhad authorized the animal procedures described here. All animal procedures, protocols and experiments were carried out in accordance with the approved guidelines for care and use of animals for research project.

### 2.2. Cell lines and culture conditions

For MEF cell preparation, E12.5–14.5 mouse embryos were dissected in a 10-cm dish with 1X phosphate buffered saline (PBS Buffer). After removing the brain, limbs, and internal organs, the embryos were minced with a sterile razor blade and trypsinized for 10 min at 37 °C. The suspension was divided in several equal volumes and placed in 10-cm dishes coated with 0.1% gelatin (Sigma-Aldrich, Germany). MEFs were cultured in Dulbecco's Modified Eagle Medium with 4.5 g/l glucose (Gibco, Life Technologies, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1X penicillin/streptomycin, and 1X non-essential amino acids (all from Gibco) and incubated at 37 °C in a humidified 5% $CO_2$ atmosphere. All MEFs at passages 2 and 3 were used for viral transduction. HEK293T (ATCC CRL-3216) and P19 (ATCC CRL-1825) cells were obtained from the Pasteur Institute of Iran (Tehran, Iran) and were cultured in Dulbecco's Modified Eagle Medium and a-MEM (Sigma-Aldrich, Munich, Germany) respectively, with 10% heat- inactivated FBS.

### 2.3. Constructs

Our previously reported construct, FUM-FD [5], containing the *Stella*, *Oct4*, *Nanos2* genes in a translational fusion, was used as a template of PCR for sub-cloning of the Stella-2A-Oct4-2A-Nanos*2* (SON) cassette. It was amplified by PCR with proofreading capable TaKaRa *Ex Taq* DNA Polymerase (TaKaRa, Japan) and *Eco*RI-Stella (5′-AATATTA CTGAA **GAATTC** ATGGAGGAACCATCAGAGAAAG-3′) and *Not*I-Nanos2 (5′-AATATATCTTAA **GCGGCCGC** CTATCGCTTGACTCTGCGACCAG-3′) primers were used from the FUM-FD construct. The resulting PCR fragment was cloned into the pCDH-513b lentiviral vector (System Biosciences, Palo Alto, CA, USA) using the *Eco*RI-*Not*I sites as shown