

**Abstract:**

The piwi family genes are highly conserved during evolution and play a crucial role in stem cell self-renewal, gametogenesis, and RNA interference in diverse organisms ranging from *Arabidopsis* to humans. Piwil2, also known as Hili, is one of the four human homologues of piwi. Piwil2 was found in germ cells of adult testis, suggesting that this gene functions in spermatogonial stem cell (SSCs) self-renewal and formation. Therefore, as a bid for infertility treatment, we examined the piwil2 potential to Trans-differentiate human fibroblasts, as terminally differentiated cells, to germ cell lineage. Primary Human Neonatal Foreskin Fibroblasts (Nff) were transfected by a plasmid overexpressing hpiwil2 under the control of CMV promoter. After establishing stable cell line, semi-quantitative RT-PCR analysis was then applied to assess the expression level of our target genes including those related to pre-meiotic and late meiotic germ cell lineage. Our semi-quantitative gene expression analysis on the human fibroblasts ectopically expressing hpiwil2 demonstrated an increase in the expression level of many premeiotic germ cell markers including HSP90, CD49f, C-kit, Stella, Fragilis and Stra8. However, the expression of late meiotic markers such as Scp3, pgk2 were not detectable under the standard cell growth conditions which were applied in our study. Notably, in line with the pluripotent characteristics of premeiotic spermatogonial stem cells (SSCs), the hpiwil2 expressing human fibroblasts demonstrated an elevated level of pluripotency markers including Oct4, C-myc, Klf4, Nanog and Stat3 as well. Our results clearly indicated the capability of hpiwil2 gene in reprogramming of human fibroblast to premeiotic germ cell lineage like SSCs, providing novel approach for treatment of male infertility.

**Key words:** piwil2, spermatogonial stem cell, male infertility, pluripotency



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# **The role of Piwil2 protein in expression of human spermatogonial stem cell markers**

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