

## The potentiality of Induced Pluripotent Stem Cells to Differentiate Into Male Germ Cells

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### Abstract

The current study tries to spot light on a novel solution to reduce the incidence of side effects resulting from chemotherapy. Therefore, the probable ameliorating action of iPSCs on testicular toxicity induced by chemotherapy was investigated. The goal of this study is to research the differentiation capability of rat induced pluripotent stem cell into male germ in vitro. samples were collected from skin biopsy of adult male albino rats were subjected to identification and quantification of male germ cells by RT-PCR analysis. A significant statistical increase in mean of SSEA1 gene expression in group I compared with group II, but there is significant statistically decrease in SSEA1 gene expression in group II compared with group III. RA can rapidly cause differentiation of IPS into male germ cells.

### 1. Introduction

“Cell therapy” can be defined as a set of strategies which use live cells with therapeutic purposes. The aim of such therapy is to repair, replace or restore the biological function of a damaged tissue or organ. Thus, the use of stem cells in cell therapy is being studied in several areas of medicine [3]. Under the right conditions, or given the right signals, stem cells can differentiate into many different mature cell types such as heart cells, skins cells or nerve cells. It holds a great potential for regenerative medicine especially in replacing cells in tissues that hardly have intrinsic renewal capacity including the heart [4].

About 15% of couples do not achieve pregnancy within one year and seek medical treatment for infertility. One in eight couples encounter problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Three percent of women remain involuntarily childless, while 6% of parous women are not able to have as many children as they would wish [1].

The current study tries to spot light on a novel solution to reduce the incidence of side effects resulting from chemotherapy. The goal of this study is to research the differentiation capability of rat induced pluripotent stem cell into male germ in vitro.

### 2. Patient and method

Samples were collected from skin biopsy of rats of average. All animal procedures were performed according to approved protocols of the Ethical Committee of the Faculty of Medicine, Benha University and in accordance with the recommendations for the proper care and use of laboratory animals.

All skin samples were subjected to identification and quantification of male germ cells by PCR analysis.

### Real time –polymerase chain reaction (RT-PCR) assessment

RT-PCR assessment was accomplished in Biochemistry Department, Faculty of Medicine, Cairo University to detect expression of SSEA1 to detect function and response of IPS differentiation.

The RNA extraction kit was provided by Thermo Fisher Scientific Inc. Germany. RT-PCR kit was

provided by Bioline, a median life science company, UK. All the components of sensifast kit and RNA extraction kit.

### 3. Results

This table shows a significant statistical increase in mean of SSEA1 gene expression in group I compared with group II, but there is significant statistically decrease in SSEA1 gene expression in group II compared with group III.

	Group I	Group II	Group III
Mean	80	17	30
SD	4.4222	2.5386	2.6667
Significance (sig.)at P < 0.01	2,3	1,3	1,2

### 4. Discussion

Infertility is associated with significant psychological distress, with levels of depression twice that of the normal population in young cancer survivors. Even for patients who may have not planned to have children, most commonly due to their very young age, the threat of infertility can result in a deep sense of loss and anger. Since post-therapy recovery of gonadal function remains unpredictable, it is important to inform patients facing infertility of this possible side effect of their treatment and all the options available to prevent it [1].

Our results shows a significant statistical increase in mean of SSEA1 gene expression in group I compared with group II, but there is significant statistically decrease in SSEA1 gene expression in group II compared with group III.

The expression profiles of male germ cell-related genes in miPSCs, given treatment with RA or progesterone in vitro. cDNA from EB cultures kept for 0, 4, and 7 days in the presence and absence of progesterone and RA was used to conduct real-time PCR and for detecting relative mRNA expression of Ddx4, AKAP3, and Stra8.

Yokonishi [4] The mean normalized expression of each gene corresponding to that of GAPDH has been depicted along the y axis. AKAP3, Akinase anchoring protein 3; C, the control group with no induction of progesterone or retinoic acid; cDNA, complementary

DNA; Ddx4, DEADbox helicase 4; EBs, embryonic bodies; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; miPSC, mice induced pluripotent stem cell; mRNA, messenger RNA; P, the progesterone induction group; PCR, polymerase

Chain reaction; RA + P, the retinoic acid and progesterone induction group; RA, the retinoic acid induction group; Stra8, stimulated by retinoic acid gene 8 protein homolog. The values of the three replicates were given as the mean  $\pm$  SEM with  $*P < .05$ ,  $**P < .01$ , and  $***P < .001$  indicated statistically significant differences in expression of mRNA between the RA- or/with progesterone in treated groups and the control [6].

They found that the expression of *Stella* gene changed on day 0 between the progesterone group and the control group ( $P < .05$ ). The expression of this gene in the RA + P group increased on this day but, there was no significant difference between them. Expression analysis of *Stella* gene in 4- and 7-day-old EBs did not show any significant difference. But, the expression of this gene in day 4 was decreased in progesterone group compared with control group [5].

In summary, we demonstrated that iPS cells may have the ability to differentiate into late-stage male germ cells. Our demonstration of the possible ability to generate male germ cells from iPS cells provides a paradigm for elucidating the mechanism of male germ cell development and has potential applications in the treatment of male infertility.

## 5. Conclusion

Previous work showing that RA can rapidly cause differentiation of IPS into male germ cells

## References

- [1] O.O. Adetoro, Ebomoyi EW, The prevalence of infertility in a rural Nigerian community. *Afr J Med Med Sci*, Vol.20, PP.23-27,2006.
- [2] A.G. Andersen, T.K. Jensen, E. Carlsen, N.Jørgensen
- [3] B. Arabadzjev, R. Petkova, A. Momchilova, S. Chakarov, R. Pankov, Of mice and men—differential mechanisms of maintaining the undifferentiated state in mESC and hESC. *BioDiscovery Human iPSCs and male infertility*, Vol.3, PP.1-193,2012.
- [4] A. Doi, I.H. Park, B. Wen, P. Murakami, M.J. Aryee, R. Irizarry, B. Herb, Ladd- C. Acosta, J. Rho, S. Loewer, Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet*. Durruthy, Vol.41, PP.1350–1353,2009.
- [5] T. Yokonishi, T. Sato, K. Katagiri, M. Komeya, Y. Kubota, T. Ogawa, In vitro reconstruction of mouse seminiferous tubules supporting germ cell differentiation. *Biol Reprod*, Vol.89, PP.15-23, 2013.
- [6] V.K. Shah, K.K. Shalia, Stem cell therapy for acute myocardial infarction-long term 24 months follow-up. *J. Clin. Trial. Cardiol*, vol.1 (2), PP.1-5, 2014.