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Glial cell derived neurotrophic factor induces spermatogonial stem cell marker genes in chicken mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) are known with the potential of multi-lineage differentiation. Advances in differentiation technology have also resulted in the conversion of MSCs to other kinds of stem cells. MSCs are considered as a suitable source of cells for biotechnology purposes because they are abundant, easily accessible and well characterized cells. Nowadays small molecules are introduced as novel and efficient factors to differentiate stem cells. In this work, we examined the potential of glial cell derived neurotrophic factor (GDNF) for differentiating chicken MSCs toward spermatogonial stem cells. MSCs were isolated and characterized from chicken and cultured under treatment with all-trans retinoic acid (RA) or glial cell derived neurotrophic factor. Expression analysis of specific genes after 7 days of RA treatment, as examined by RT-PCR, proved positive for some germ cell markers such as *CVH*, *STRA8*, *PLZF* and some genes involved in spermatogonial stem cell maintenance like *BCL6b* and *c-KIT*. On the other hand, GDNF could additionally induce expression of *POU5F1*, and *NANOG* as well as other genes which were induced after RA treatment. These data illustrated that GDNF is relatively more effective in diverting chicken MSCs towards Spermatogonial stem cell –like cells in chickens and suggests GDNF as a new agent to obtain transgenic poultry, nevertheless, exploitability of these cells should be verified by more experiments.

1. Introduction

Stem cells have the potential to differentiate into various cell types and could be categorized according to their differentiation potency. Spermatogonial stem cells (SSCs) have been considered as pluripotent stem cells because they are capable of differentiating into almost all cell types in the body (Kanatsu-Shinohara et al., 2004). SSCs have great applications in many fields including developmental biology, germ cell related disorders like male infertility, transgenic technologies in industrial animals like poultries and survival of rare or extinct species.

Thus a priority in stem cell research is to establish optimal conditions for derivation and maintenance of SSCs *in vitro*. Isolation and manipulation of these cells is difficult (Tegelenbosch and de Rooij, 1993; Hofmann, 2008). Although, several studies have reported culturing SSCs in the last few years but their maintenance remains a difficult task (Kanatsu-Shinohara et al., 2003; Momeni-Moghaddam et al., 2013).

Breakthroughs in stem cell research have shown that stem cells from one type could be transdifferentiated into another type. Several reports illustrated that mesenchymal stem cells could be differentiated into cells expressing the molecular markers of primordial germ cells (PGCs), spermatogonial stem cells and sper-

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Table 1
Specification of primers used for gene expression analyses.

Amplicon lengh	Sequence	Accession No	Name
154bp	F-5'-AATGAGGCAGAGAACACGGACAAC-3' R-5'-GGGACTGGGCTTCACACATTTGC-3'	NM_001110178.1	POU5F1
195bp	F-5'-CTCCAGCAGCAGACCTCTCCTTG-3' R-5'-CCTTCCTTGTCCCACTCTCACCTT-3'	NM_001146142.1	NANOG
166bp	F-5'-GATGAGTTGCGGGAGATGAG-3' R-5'-TTGGAGAATAGATGGTGGCGT-3'	NM_001012930.1	BCL6B
169bp	F-5'-AGGGATGGACATGGGCAATACAAC-3' R-5'-GCGATTCTGACTGCGGTGGATG-3'	D13225.1	c-KIT
441bp	F-5'-TGAAAAACAAACAATGGAAGAAGA-3' R-5'-CTAGACAATCCCTGAGTCTCGTTT-3'	XM_416179.3	STRA-8
274 bp	F-5'-CAGGCGTGGATGGCTAACTC-3' R-5'-CAGAACTCCTCCCTCTACCAAATC-3'	AB004836.1	CVH
262bp	F-5'-ATCCTCTTCCACCGCAACAGTCAG-3' R-5'-TGTCCTCCTCTTCATCGGCACCTC-3'	XM_417898.2	PLZF
304bp	F-5'-ATACAACTATCAGGCTCCACCACA-3' R-5'-TGCTCTTCCTTTTCTGAAGTGATG-3'	NM_204218.1	DAZL
154bp	F-5'-TGCTTGCTCTGTTACCCTCCTG-3' R-5'-ATGACGGCTTGCCCTGACC-3'	EF467327.1	SPRY-1
265bp	F-5'-CCTTCATCGATCTGAACTACATGG-3' R-5'-GGAGCTGAGATGATAACACGCTTA-3'	NM_204305.1	GAPDH

matogonia (Nayernia et al., 2006; Lue et al., 2007; Heo et al., 2011). Moreover, immunomodulatory effects of mesenchymal stem cells and their great potential to differentiate into many types of cells including osteocytes, cardiomyocytes, neurons and epithelial cells (Benayahu et al., 1989; Makino et al., 1999; Woodbury et al., 2000; Paunescu et al., 2007) make them good candidates for generation of germ cell lineages such as spermatogonial stem cells.

Retinoic acid has been mostly used for differentiating mesenchymal stem cells towards SSCs. The action of this small molecule to differentiate stem cells is believed to be related to induction of *Stra8* gene (Li et al., 2007; Zhou et al., 2008). On the other hand, molecular mechanisms for regulation of self-renewal and maintenance of SSCs are governed by GDNF and associated factors like Bcl6, Lhx1 and Spry1 (Oatley et al., 2006; Hofmann 2008; Costantini 2010). In this study, we compared the effects of GDNF and RA as inducers to test the possibility for switching the chicken MSCs towards SSC-like cells. Treated cells were characterized by expression analysis of some germ cell molecular markers such as *STRA8*, *CVH*, *PLZF*, *DAZL* and factors required for self-renewal and maintenance of SSCs such as *POU5F1*, *NANOG*, *c-KIT* and *BCL6*b (Phillips et al., 2010) at RNA level and germ cell surface markers, TRA-1-60 and TRA-1-81 at protein level.

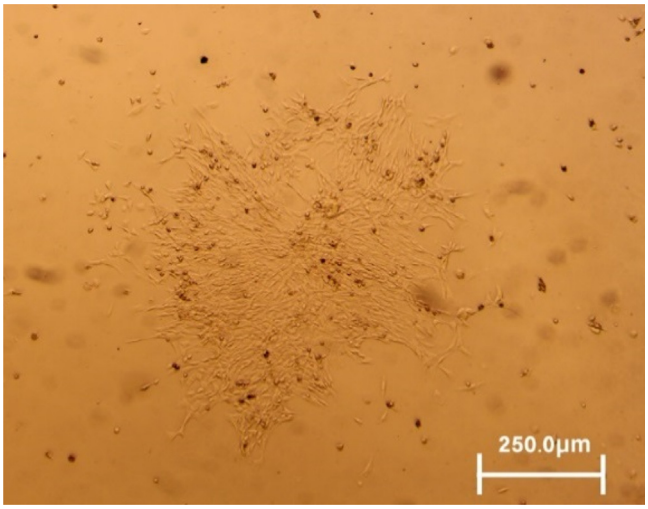

Fig. 1. Primary culture of MSCs isolated from chicken bone marrow. The spindle like morphology and colonial aggregation of cells can be observed.

2. Materials and methods

2.1. Isolation and expansion of cMSCs

cMSCs were isolated from the bone marrow of a 1–10 day old Hy-line chicken. Femur and tibia were soaked in phosphate buffered saline (PBS) supplemented with 2× penicillin/streptomycin. The pieces of broken bones were put on a 70 μm cell strainer and then washed thoroughly by PBS containing the antibiotics remove cell debris. Cells passing through the strainer were washed with PBS twice and cultured in Low glucose-DMEM containing 12.5% FBS (Gibco), 2 mM L-glutamine (Sigma) and 200 μ/ml penicillin/streptomycin, incubated at 37 °C and 5% CO_2. During the first 24 h, the medium was changed every 8 h to get more purified cMSCs and 4 ng/ml basic fibroblast growth factor (bFGF) (Chemicon) was added after 24 h (expansion medium). When the colonies of MSCs were formed, they were first passaged 1:1 using 0.25% trypsin. After that, when cells reached confluency, they were passaged at a density of 1×10^4 cells per cm^2.

2.2. Characterization of cMSCs

2.2.1. Differentiation assays

2.2.1.1. Adipogenic differentiation. In order to verify the adipogenic differentiation potency of cMSCs, cells were cultured (~2×10^4 cells/cm^2) using expansion medium in 6-well plates. After reaching 80–90% confluency, medium was exchanged with adipogenic induction medium (DMEM supplemented with 10% FBS, 100 μ/ml penicillin/streptomycin, 1 μM dexamethasone, 10 mM β-glycerol phosphate and 100 μM indomethacin) for 21 days. The medium was replaced every 3–4 days and the adipogenesis potential was assessed by Oil Red O staining. To do so, cells were fixed with 4% paraformaldehyde (PFA) and incubated at room temperature for at least 30 min. Fixed cells were washed with distilled water and incubated with 60% isopropanol for 5 min at room temperature. Finally, cells were stained with 0.3% Oil Red O staining solution (Sigma–Aldrich, Germany) and incubated at room temperature for 15 min. In order to visualize the nuclei, cells were stained with hematoxylin.