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Lentiviral vector-mediated transduction of goat undifferentiated spermatogonia☆

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ABSTRACT

Recent studies show that spermatogonial stem cells (SSCs) are able to colonize and form mature spermatozoa following transplantation into germ cell depleted testes of recipient males. Therefore, efficient ways for enrichment and gene transfer into SSCs provides a powerful tool for production of transgenic animals. In order to adapt the technique to goats, three issues were addressed: (i) enrichment of the undifferentiated spermatogonia including SSCs using magnetic activated cell sorting (MACS), (ii) lentiviral vector-mediated transduction of an enhanced green fluorescent protein (EGFP) transgene into enriched cells, and (iii) transplantation of transduced undifferentiated spermatogonia into the germ cell depleted testes of immune-suppressed mice to assess for migration and colony formation ability. Enriched cells were transduced by lentiviral vectors and subsequently analyzed for expression of *THY1*, *PLZF*, *VASA*, *UCHL1* and *BCL6B* genes. Cells were also analyzed for GFP and PLZF by flow cytometry. Enriched transduced cells were transplanted into germ cell depleted mice testis. Quantitative analysis of transcripts revealed that MACS-enrichment significantly increased the expression of SSC-characteristic genes *THY1*, *PLZF*, *VASA*, *UCHL1* and *BCL6B* compared to non-enriched population ($P \leq 0.05$). EGFP transduction did not affect the expression levels of SSC-characteristic genes. Flow cytometry revealed that 72% of transduced-enriched cells were positive for EGFP. Finally, transduced-enriched goat SSCs could colonize within the cells into the seminiferous tubules of germ cell depleted recipient mice at higher frequency than non-enriched cells. The results indicated that enrichment of goat undifferentiated spermatogonia by magnetic-activated cell sorting for THY1 antibody combined with lentiviral vector-mediated transduction has the potential to be used for production of transgenic goats.

Abbreviations: SSC, spermatogonial stem cell; MACS, magnetic activated cell sorting; PBS, phosphate buffer saline; HBSS, Hank's buffer saline solution; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; TU, transducing units; BT, before transduction; AT, after transduction.

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1. Introduction

Despite low number of spermatogonial stem cells (SSCs) in the neonatal testis (less than 0.1% of all germ cells) (Tegelenbosch and de Rooij, 1993), these cells are unique as they will be enable to continuously generate large numbers of differentiating spermatogonia following puberty. Accordingly, the initial minute number SSCs produces millions of sperm each day to provide a means to transfer the paternal genetic information to the next generation (Miao, 2011). These characteristics make SSC an excellent tool for genetic modification to efficiently generate transgenic animals in mammalian species (Ryu et al., 2007; Kim et al., 2012). Recent studies show that SSCs are able to colonize and form mature spermatozoa following transplantation into germ cell depleted testes of recipient males. Therefore, efficient ways for enrichment and gene transfer into SSCs provides a powerful tool for production of transgenic animals.

Transgenic farm animals have been considered as a noteworthy alternative for production of pharmaceutical products (Rudolph, 1999). Development of efficient ways for enrichment, and gene modification of SCCs are necessary pre-requisites for transgenesis via male germ cells (de Barros et al., 2012). We previously verified that THY1 is a characteristic surface marker of goat undifferentiated spermatogonia including SSCs (Bahadorani et al., 2011) and can be used for efficient enrichment of these cells by using magnetic-activated cell sorting (MACS) technique (Abbasi et al., 2013). However, because of the rarity of SSCs and because SSCs proliferation is slow not only in vitro but in vivo (Ryu et al., 2007; Kim et al., 2010), attempts for in vitro genetic modification of SSCs is difficult and has raised the need for an efficient genetic modification method (Nagano et al., 2000). In this regard, the efficiency of viral vectors for introducing stable genetic modification into SSCs has been evaluated by a number of studies. (Akkina et al., 1996). Initially, Nagano et al. (2000) used retroviral vectors to introduce transgene into male germ line stem cells in mice. Since then, lentiviral (LV) vectors have been intensively used for gene therapy and transgenesis (Hofmann et al., 2003), particularly in non-dividing cells (Kootstra et al., 2003). For example, Rodriguez-Sosa et al. (2009) transduced ram spermatogonia using LV vectors. Also, Liu et al. (2010) used LV vectors to introduce MxA gene, Myxovirus-resistant A gene, into the Chicken SSCs. Hamra et al. (2002) used LV vectors for production of transgenic rats. For simple evaluation of the efficiency of viral mediate gene transfer into SSCs, use of LV vectors driving the expression of visible reporter genes such as green fluorescent protein (GFP) is common in research-purposed studies (Rodriguez-Sosa et al., 2009).

Transplantation studies have shown that SSCs of various mammalian species could successfully migrate and colonize in seminiferous tubules of germ cell depleted recipient male mice. This suggested that the characteristic growth factors and microenvironment required for SSCs self-renewal are probably conserved among mammalian species (Kubota and Brinster, 2006).

Among farm animals, the goat is an ideal species due to high yield of protein in the milk, relatively short generation interval and production of multiple offspring (Meade et al., 1998; Amiri et al., 2013). Like other mammals, goat spermatogonial stem cells (SSCs) have self-renewal capacity and differentiation potential (Wu et al., 2009). However, there is no report regarding in vitro gene transfer into the goat SSCs by LV vectors.

Therefore, the aims of this study were lentiviral vector-mediated transduction of EGFP transgene into the goat undifferentiated spermatogonia including SSCs enriched by using MACS for THY1 antibody. Finally, transduced and non-transduced cells were transplanted into the seminiferous tubules of germ cell depleted testes of recipient male mice.

2. Materials and methods

Unless otherwise specified, all chemicals and media were obtained from Sigma Chemical (St. Lou is, MO, USA) and Gibco (Grand Island, NY, USA) companies, respectively.

2.1. Testes preparation and SSCs enrichment by magnetic activated cell sorting

The procedures used for testes preparation and testicular cell isolation were as described previously (Abbasi et al., 2013). In brief, testes were removed from 3 to 4 weeks old goats ($n = 9$) under general anesthesia with a combination of Xylazine (0.1 mg/kg) and Ketamine (3 mg/kg). After removing the tunica vaginalis, seminiferous tissues were washed with phosphate buffer saline (PBS). All animal care and surgical procedures were undertaken in strict accordance with the approval of the Institutional Review Board and Institutional Ethical Committee of Royan Institute. Single cell suspensions of testicular cells were obtained by a previously described (Nasiri et al., 2012) modified three-step enzymatic treatment with; (i) collagenase type IV (2 mg/ml) in Dulbecco's MEM (DMEM) at 37 °C for 15 min followed by 3 rounds of washing with PBS, (ii) a mixture of collagenase type IV (1 mg/ml), hyaluronidase (1 mg/ml) in DMEM at 37 °C for 15 min, followed by washing two times with PBS, (iii) trypsin (2.5 mg/ml) in Hanks buffer saline solution (HBSS) containing DNase I (7 mg/ml; Roche, Germany) in DMEM for 5 min at 37 °C. DMEM containing 10% FBS was used to stop enzymatic reactions. Cells syrup was then filtered through a nylon mesh with pore-size of 100 and 40 μm, respectively (BD Biosciences, Durham, NC, USA) to remove undigested tissues and clumps. Filtrate was centrifuged at 500 g for 5 min and resuspended in a formulation of PBS-S containing PBS with 1% FBS, 10 mM HEPES, 1×10^4 U/ml penicillin, 1×10^4 mg/ml streptomycin, 1 mM sodium pyruvate, and 1 mg/ml glucose (de Barros et al., 2012). Prepared cells at density of 20×10^7 were incubated with mouse anti-human THY1 antibody (1:50, abcam) at 4 °C for 30 min with slow mixing every 5 min. Treated cells were washed two rounds with PBS-S by centrifugation at 500 g for 5 min each. Cells were then incubated in biotinylated anti-mouse antibody (Sanat Cruz) at 4 °C for 30 min with slow mixing every 5 min. Following two rounds of washing with PBS-S and centrifugation at 500 g for 5 min each, cells were incubated with strepavidin-conjugated antibody to magnetic microbeads

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