DIFFERENTIATION POTENTIALITY OF GOAT PLACENTA DERIVED STEM CELLS

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Abstract - Stem cells now days hold the promise to cure a couple of diseases by differentiation, paracrine effect, exosome and vesicle effect or cell fusion effects. Goat is regarded as an optimal animal model for investigation and exploration of human diseases. In addition, placenta can be derived non-invasively and was reported to be the abundant source of stem cells. Hence, this study is to harvest the stem cells from goat placenta and attempt to give rise to these cells to different cell types and characterize if these cells express stem cells markers. The results demonstrated that goat placenta derived stem cells (GPSCs) are able to express stem cells markers such as CD44 and GPSCs are capable of giving rise to osteoblast, adipocyte, chondrocyte which are confirmed by staining of Alizarin Red, Oil Red O, and Toluidine Blue O respectively. Taken together, GPSCs are capable of expressing stem cells markers and differentiate to mesoderm lineage cells. This outcome is beneficial to offer a reference for future regenerative medicine and pre-clinic trial.

Keywords - Placenta, Stem Cells, Goat, Differentiation

I. INTRODUCTION

Stem cells possess the self-renewal and differentiation capabilities and play a role in repairing tissue damages for maintaining the function in our body, and are under investigation rapidly in recent years. However, the abundant source of stem cells need to be explored. To our knowledge, placenta from trophoblast is a connection between mother and fetus which can exchange the nutrition and waste for helping fetus growing. After delivery of fetus, placenta becomes a medical waste which can be collected non-invasively and without ethical concern. In previous studies, placenta derived stem cells (PSCs) were established and showed the stem cell properties in several species, such as bovine (Peng et al., 2017), human (Cao et al., 2012; Lankford et al., 2017), and dog (Yu et al., 2013). In addition, the PSCs shared higher curing potential on hind limb ischemic than other mesenchymal stem cells (MSCs) from bone marrow and adipose tissue (Jeon et al., 2016), and also possess the therapeutic effect on skin wound (Abd-Allah et al., 2015), ischemic limb injury (Xie et al., 2016), cardiac ischemia (Francis et al., 2017), and spinal cord injury (Tan et al., 2015). To now, the PSCs from goat (GPSCs) have not been published yet. Thus, this study aims to establish and characterize the GPSCs for future experiments.

II. MATERIALS AND METHODS

Isolation of GPSCs

The chorionic plate from goat placenta was collected and cleaned by washing saline with 1% penicillin/ streptomycin (P/S, Gibco™, 15140122, E.U. Approved, South American). After that, the whole tissue was disinfected in 75% alcohol for 10 seconds and washed with DPBS. The tissue was minced with cell culture medium (α-MEM (Sigma, M0894, 3050 Spruce street, SL Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Gibco™, 10270106, E.U. Approved, South American) and 1% P/S), then transferred into 100 mm^2 cell culture dishes (TPP, 93100, Trasadingen, Switzerland) with 10 mL fresh medium and cultured in 38.5°C incubator (Thermo Scientific™ Forma™ Steri-Cycl™ CO_2 Incubators) containing 5% CO_2 for 3 days. The GPSCs were cultured to passage 5 (P5) for conducting all the experiments.

Flow cytometry analysis of GPSCs

The GPSCs were analyzed the surface antigen following procedure. Briefly, these cells were suspended by adding 0.25% trypsin-EDTA (Gibco™, 25200072, Grand Island, New York, USA), and the supernatant was removed by centrifuging under 500 xg for 5 minutes. After re-suspending the GPSCs with DPBS (Gibco®, 21600010, E.U. Approved, South American), these cells were stained with the antibodies for 30 minutes. Finally, the stained GPSCs were detected by flow cytometry (BECKMAN COULTER EPICS ALTRA).

Tri-lineage differentiation

In adipogenic and osteogenic differentiation, the 1 x 10^5 GPSCs were seeded into 6 well dishes, and cultured under differentiation medium for 21 days. (Adipocytes inducing medium: α-MEM supplemented with 10% FBS, 0.5 mM isobutyl-methylxanthine (Sigma, I5879, Eschenstraße 5, 82024 Taufkirchen, Germany), 100 μM indomethacin (U. S. pharmacopeia, 1341001, 12601 twinbrook parkway, Rockville, MD, USA), 10 μg/mL beef insulin (U. S. pharmacopeia, 1342208, 12601 twinbrook parkway,

Rockville, MD, USA) and 1 µM dexamethasone (Sigma, 31381, Eschenstraße 5, 82024 Taufkirchen, Germany); Osteocytes inducing medium: α-MEM supplemented with 10% FBS, 10 mM glycerol phosphate (Sigma, 17766, Eschenstraße 5, 82024 Taufkirchen, Germany), 50 µM ascorbic acid (Sigma, 33034, Eschenstraße 5, 82024 Taufkirchen, Germany) and 0.1 µM dexamethasone).

In chondrogenic differentiation, 2.5 x 10^5 GPSCs were centrifuged in 15 mL centrifuge tube, and the chondrocytes inducing medium (α-MEM supplemented with 1% FBS, 50 µM ascorbic acid, 10 ng/mL transforming growth factor-β2 (Sigma, T5300, Eschenstraße 5, 82024 Taufkirchen, Germany) and 6.25 µg/mL beef insulin) was added for culturing 21 days.

After 21 days inducing, the differentiated GPSCs were confirmed by the staining of Oil red O, Alizarin red S, and Toluidine blue O respectively.

III. RESULTS AND DISCUSSION

The goat placenta was collected from the goat farm within 2 hours. To harvest the single cell (Figure 1), the placenta fragments were cultured for 3 days, and filtered by 100 µm cell strainer. These cells showed a strong proliferative potential, and the expression of CD44, CD105, and CD4 was 94.2%, 11.2%, and 1.4% respectively (Figure 2) which demonstrated the stem cell related surface antigens but not T-lymphocytes antigen. For further investigation of differentiation potential, GPSCs were induced under appropriate condition and successfully differentiated into adipocytes (Figure 3), osteocytes (Figure 4), and chondrocytes (Figure 5) which showed the oil generation, calcium depositions, and matrix following Oil red O, Alizarin red S, and Toluidine blue O staining respectively.

Comparing with previous studies (Cao et al., 2012; Beeravolu et al., 2017; Wu et al., 2018), the differentiation potential of GPSCs is similar, but the low expression of CD105 is dissimilar. CD105 also called endoglin is a stem cell marker and relates to the regulation of transforming growth factor-β (TGF-β) signaling (Wong et al., 2000), which could regulate the cell growth by the down-regulation of c-Myc (Malliri et al., 1996; Warner et al., 1999). Interestingly, CD105 positive expression is considered as a indicator to obtain the greater chondrogenic potential in synovium-derived mesenchymal stem cells (SSCs) (Fan et al., 2016), but not in bone marrow-derived mesenchymal stem cell (BMSCs) (Cleary et al., 2016). CD105 negative expression possess greater osteogenic and chondrogenic differentiation from human amniotic membrane mesenchymal stem cells (AM-hMSCs) (Leyva-Leyva et al., 2013) and from injured tendon-derived progenitor cells (inTPCs) (Asai et al., 2014) respectively. Furthermore, Anderson et al., 2013 had demonstrated that the expression of CD105 would be affected by passage and cell confluency on $CD105^+$ but not $CD105^-$ MSCs, and the osteogenic and adipogenic differentiation potential on $CD105^-$ MSCs was greater. The CD105 seems to have dissimilar effect in different cells. The cell derived from tissue may have different subpopulation on CD105 expression (Leyva-Leyva et al., 2013; Asai et al., 2014)). Above all, GPSCs owns the stem cell properties and can be applied to subsequent experiments in the future.

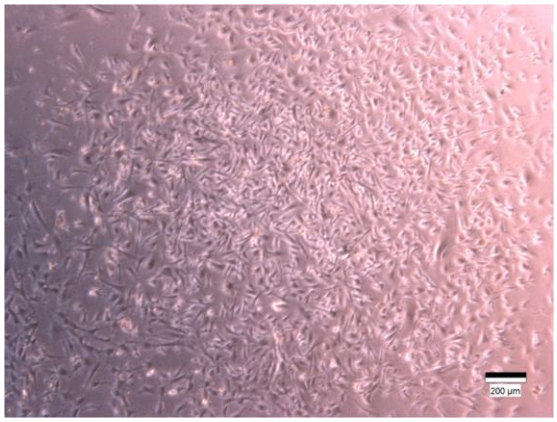


Figure 1 The GPSCs were isolated successfully from goat placenta.

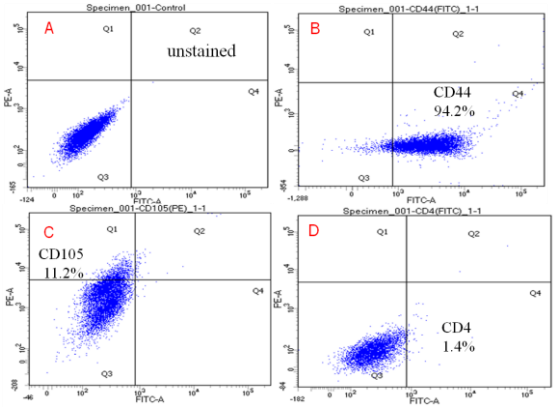


Figure 2 The GPSCs surface antigens were analyzed by flow cytometry. A) Unstained GPSCs B, C) The CD44 and CD105 were the indicators of stem cells, and the expression were 94.2% and 11.2% respectively. D) The GPSCs expressed 1.4% CD4 which was related to T-lymphocytes.

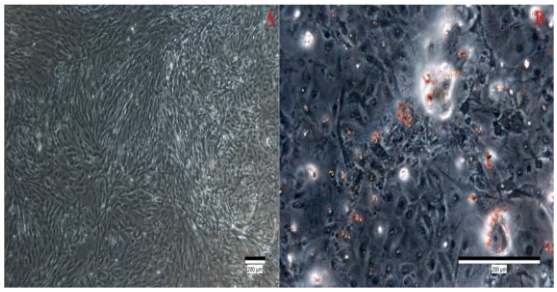


Figure 3 The adipogenic differentiation was conducted. A) GPSCs without oil droplets were cultured under normal medium. B) GPSCs with oil droplets were cultured under differentiation medium and confirmed by Oil red O staining.

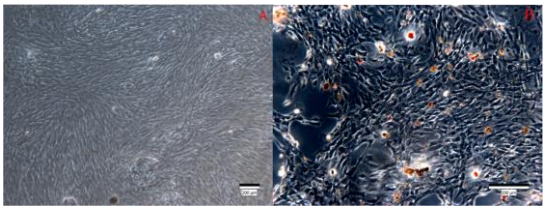


Figure 4 The osteogenic differentiation was conducted. A) GPSCs without calcium depositions were cultured under normal medium. B) GPSCs showed the calcium depositions which were cultured under differentiation medium and confirmed by Alizarin red S staining.

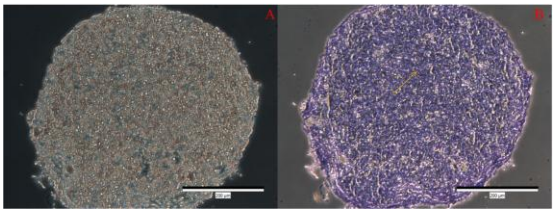


Figure 5 The chondrogenic differentiation was conducted. A) The GPSCs pellet after 21 days culturing was sliced. B) the slice showed the matrix which was confirmed by Toluidine blue O staining (yellow arrow).

IV. CONCULSION

The GPSCs were successfully isolated and expressed the stem cell properties. In the future, the GPSCs can be a alternative source to cure the goat diseases.

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