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RESEARCH ARTICLE

Characterization and Differentiation into Adipocytes and Myocytes of Porcine Bone Marrow Mesenchymal Stem Cells

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Abstract

Bone marrow mesenchymal stem cells (BMSCs) could differentiate into various cell types including adipocytes and myocytes, which had important scientific significance not only in the field of tissue regeneration, but also in the field of agricultural science. In an attempt to exhibit the characterization and differentiation into adipocytes and myocytes of porcine BMSCs, we isolated and purified porcine BMSCs by red blood cell lysis method and percoll gradient centrifugation. The purified cells presented a stretched fibroblast-like phenotype when adhered to the culture plate. The results of flow cytometry analysis and immunofluorescence staining demonstrated that the isolated cells were positive for mesenchymal surface markers CD29, CD44 and negative for hematopoietic markers CD45 and the adhesion molecules CD31. Cells were induced to differentiate into adipocytes with adipogenic medium containing insulin, dexamethasone, oleate and octanoate. Oil Red O staining demonstrated that the porcine BMSCs successfully differentiated to adipocytes. Moreover, the findings of real-time PCR and Western blotting indicated that the induced cells expressed adipogenic marker genes (PPAR-γ, C/EBP-α, perilipin, aP2) mRNA or proteins (PPAR-γ, perilipin, aP2). On the other hand, porcine BMSCs were induced into myoctyes with myogenic medium supplemented with 5-azacytidine, basic fibroblast growth factor, chick embryo extract and horse serum. Morphological observation by hochest 33342 staining showed that the induced cells presented as multi-nucleus muscular tube structure. And myogenic marker genes (Myf5, desmin) mRNA or proteins (Myf5, MyoD, myogenin, desmin) were found in the induced cells. In addition, the results of immunofluorescence staining revealed that myogenic marker (Myf5, MyoD, myogenin, desmin, S-MyHC) proteins was positive in the induced cells. Above all, these results suggested that the isolated porcine BMSCs were not only consistent with the characterization of mesenchymal stem cells, but also exhibited the multipotential capacity to form adipocytes and myocytes, which provided the basis to investigate the regulation mechanism involved in the selective differentiation of porcine BMSCs.

Key words: porcine, bone marrow mesenchymal stem cell, adipocyte, myocyte

INTRODUCTION

Mesenchymal stem cells (MSCs) were multipotential capabilities to differentiate into various mesenchymal tissue cells including osteogenic cells, chondrocytes, adipocytes and myocytes (Pittenger *et al*. 1999). The multiple differentiation potential of MSCs *in vitro* and *in vivo* had prompted great interest in the treatment of important metabolic tissue diseases

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relatively to adipose tissue and muscle tissue, such as obesity and muscular dystrophies (Dani and Billon 2012). Bone marrow mesenchymal stem cells (BMSCs) were considered the gold standard for use in tissue regeneration among MSCs (Monaco *et al*. 2012). Due to similarities to human in physiological mechanism, pig was introduced as a valuable model to study these tissues engineering (Zhang *et al*. 2011). Remarkably, porcine BMSCs were potential to differentiate into multiple lineages *in vitro* (Bosch *et al*. 2006) like BMSCs from other species, such as human (Pittenger *et al*. 1999), rats (Karaoz *et al*. 2009), dogs (Spencer *et al*. 2012) and sheep (McCarty *et al*. 2009). Although some studies reported a little characterization of porcine BMSCs, the data about isolation, purification and characterization of porcine BMSCs was still undertaken poorly.

On the other hand, in the field of agricultural science, researchers had great interest in how to enlarge skeletal muscle mass, simultaneously increase the number of intramuscular adipose and reduce the number of abdominal adipose, which increased the quantity of pock production and eating quality. So it was important to deeply understand the development of the fat cells and skeletal muscle cells in pock. Since it had been well established that fat cells and skeletal muscle cells were derived from MSCs (Du *et al*. 2010), it was thought that porcine BMSCs were utilized as models to study cell differentiation such as adipogenesis and myogenesis. Therefore, it was essential to further study the differentiation of porcine BMSCs.

Several studies reported that BMSCs, mainly conducted on human and mouse, were induced into adipocytes in adipogenic medium composing of insulin, dexamethasone, indomethacin, 3-isobutyl-1-methylxanthine (Pittenger *et al*. 1999; Ivanova-Todorova *et al*. 2009; Celebi *et al*. 2010). And other factors, such as hydrocortisone (Janke *et al*. 2002), triiodothyronine (Flynn 2010), oleate and octanoate (Sanosaka *et al*. 2008), had been also used to induce into adipocytes from other species. As to porcine BMSCs, some studies demonstrated that almost 30-50% of the cells were induced into adipocytes in adipogenic medium filled with lipid droplets at day 14-20 (Ringe *et al*. 2002; Vacanti *et al*. 2005; Bosch *et al*. 2006). However, since it taken the long period of time (2-4 wk) for BMSCs to induce into adipocytes, which had hampered the study of adipogenesis, for example, in the search for adipogenic inhibitory factors or inhibitory compounds. So it was required to develop the methods to induce porcine BMSCs differentiation into adipocytes faster and more effectively.

In addition, recently most researchers had focused on the differentiation into cardiomyocyte, with few attentions on skeletal muscle. A few studies reported that human or mice stem cells could differentiate into myocytes by 5-azacytidine (Wakitani *et al*. 1995; de Bari *et al*. 2001; Burlacu *et al*. 2008). Gang (2004) found that human umbilical cord blood MSCs were successfully induced into skeletal muscle cells by the differentiation medium containing dexamethasone, hydrocortisone and horse serum (Gang *et al*. 2004). However, there was little study on the method of inducing primary porcine BMSCs differentiation into myocytes to date.

Thus, the aims of present study were to optimize the method for the isolation and purification of a more homogeneous population of porcine BMSCs, and to establish models that porcine BMSCs were inducted into adipocytes and myocytes, which provided the basis to investigate the regulation mechanism involved in the selective differentiation of porcine BMSCs.

RESULTS

Isolation, culture and morphology of porcine BMSCs

In the present study, the mononuclear cells were obtained from the marrow of femur and tibia bones of 5-d-old landrace boar by Percoll density gradient centrifugation. The number of mononuclear cells isolated from total bone marrow aspirate (about 80 mL) was $(3.8\pm0.5)\times10^7$. These cells were enough to be plated in two 75-cm^2 flasks. By day 2, cells began to adhere (Fig. 1-A), and the nonadherent cells were removed by medium change. By day 4, the number and size of cells gradually increased and cells presented a stretched fibroblast-like phenotype (Fig. 1-B). By four passages of culture, the adherent cells became a population comprised mainly of