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Short communication

Comparison of mesenchymal stem cells and leukocytes from Large White and Göttingen Minipigs: Clues for stem cell-based immunomodulatory therapies

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ABSTRACT

The mesenchymal stem cells (MSCs) are one of the most promising cell types for human and veterinary use and their therapeutic effect is associated with their immunomodulatory properties. Farm animal models, such as pigs, have become a valuable tool to evaluate the safety and efficacy of adoptively transferred MSCs in the setting of veterinary medicine. In order to evaluate the immunomodulatory effect of stem cell-based therapies in porcine breeds, a deep analysis and comparison of MSCs and leukocyte subsets are absolutely necessary. Here we provide a detailed analysis of bone-marrow derived MSCs and leukocyte subsets from Large White pigs and Göttingen Minipigs. Significant differences were observed between the two pig breeds in terms of T cell subsets that need to be considered for immune monitoring of stem cell-based therapies.

1. Introduction

Mesenchymal Stem Cells (MSCs) have an important therapeutic potential in the treatment of numerous diseases. However, many clinical trials in animals and humans have shown disappointing results that could be attributed to suboptimal culture conditions or inaccurate preclinical testing (Sharma et al., 2014). In veterinary medicine, MSCs have been used in joint diseases such as arthritis, tendinitis, bone fractures or cartilage injuries in horses and dogs (Black et al., 2007; Smith et al., 2003). These cells secrete cytokines and growth factors which have been shown to regulate inflammatory-related diseases (Caplan and Dennis, 2006; Arnhold and Wenisch, 2015; Barrachina et al., 2016; Zhou et al., 2016). A deeper understanding of those mechanisms by which MSCs modulate inflammation and contribute to tissue remodeling will expand the clinical applicability of adult stem cells. For this purpose, farm animals such as pigs or goats (particularly for cardiovascular and orthopedic lesions) have served as clinically relevant animal models to evaluate MSCs-based therapies in preclinical settings (Murphy et al., 2003; Crisostomo et al., 2015).

Porcine derived-MSCs have been previously isolated from adipose tissue, bone marrow or peripheral blood and fully characterized by molecular, phenotypic and functional analysis. These MSCs faithfully mimic the behavior and characteristics of human MSCs being a valuable tool to evaluate the safety and efficacy of adoptively-transferred cells in preclinical settings (Casado et al., 2012). In the case of preclinical studies in pigs, the Large White breed has been previously used in myocardial infarction studies, renal ischemia-reperfusion and transplantation studies (Giraud et al., 2011; Koudstaal et al., 2014). Regarding mini-pigs, the Göttingen Minipig is gaining popularity and is widely accepted by regulatory authorities for preclinical evaluations. These animals have a strictly managed genetic background and they are better suited for laboratory work than Large White pigs. In comparison to Large White pigs, the Göttingen Minipigs are more suitable for long-term studies because of their small size and ease of handling. Göttingen Minipigs have become increasingly important in toxi-

Abbreviations: MSCs, mesenchymal stem cells; BM-MSCs, bone marrow-derived mesenchymal stem cells; MFI, mean fluorescent intensity; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; FBS, fetal bovine serum; ACTB, beta-actin; NK, natural killer cells; NKT, natural killer T cells.

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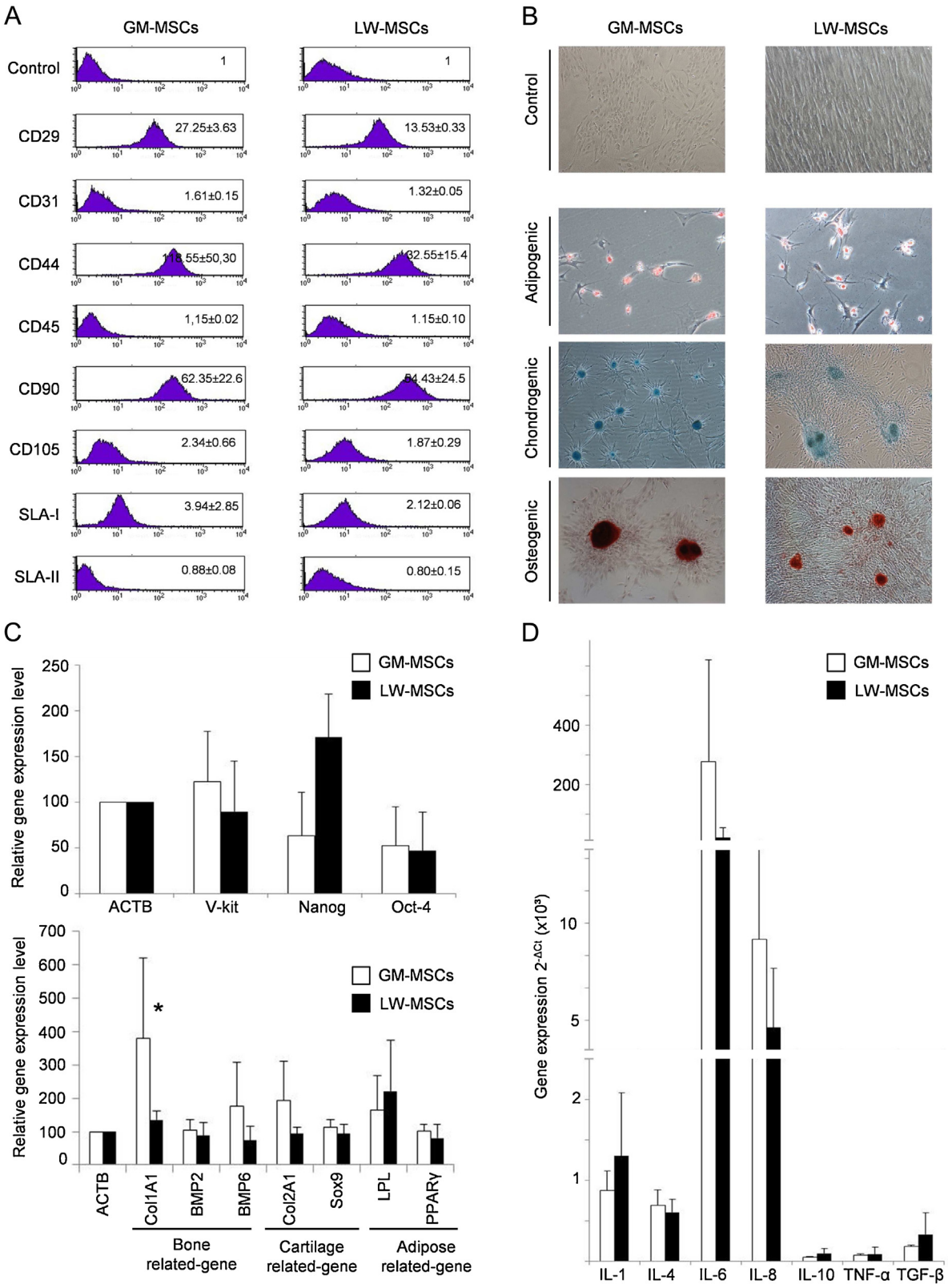

Fig. 1. Characterization of bone marrow-derived mesenchymal stem cells from Göttingen Minipigs and Large White pigs.
(A) Phenotypic analysis of bone marrow-derived mesenchymal stem cells harvested from the iliac crest of Göttingen Minipigs (GM-MSCs) and Large White pigs (LW-MSCs). Representative histograms together with the expression levels (mean ± SD of three different cell lines) are shown. The expression level of cell surface markers is represented as Mean Relative Fluorescence Intensity (MRFI), which is calculated by dividing the mean fluorescent intensity (MFI) by its negative control; (B) Differentiation potential of Göttingen Minipig (left) and Large White (right) MSCs. Cells were maintained for 21 days with standard medium (control) or with specific differentiation media for adipogenic, chondrogenic and osteogenic lineages. Differentiation was evidenced by specific stainings: Oil Red O for adipocytes, Alcian Blue for chondrocytes and Alizarin Red S for osteocytes; (C) Gene expression analysis by conventional RT-PCR. Data are expressed as expression percentage referred to ACTB as housekeeping gene. The relative quantification was performed by measuring the brightness intensity of each band with GeneSnap software. Mean ± SD of three different performed experiments are shown. $^{*}p \leq 0.05$. (D) Gene expression analysis by real time quantitative PCR. PCR products were quantified by the $2^{-\Delta Ct}$ method using ACTB as housekeeping gene. Mean ± SD of three different performed experiments are shown.