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Short communication

Bone marrow-origin stem/progenitor cells in the mammary gland of heifers

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Abstract

The aim of the study was to estimate the size of bone marrow-origin stem/progenitor population in 2-year old nonpregnant Holstein-Friesian heifers. Quantitative and qualitative analysis was done using scanning cytometry and confocal microscopy of mammary tissue slices labelled with the combination of two markers: Sca-1 (marker of stem-progenitor cells) and CD45 (marker of hematopoietic cells). The average (\pm SEM) percentage of Sca-1^{POS} CD45^{POS} cells was 0.89 ± 0.21 . They were localized mainly outside of mammary ducts, in the stroma and sometimes intraluminally. Our results indicate that the subpopulation of Sca-1^{POS} cells bearing CD45 antigen may enrich the niche of mammary stem/progenitor cells from the bone marrow and participate in the growth of the mammary gland in post-pubertal heifers.

Key words: stem/progenitor cells, bone marrow, mammary gland, heifers

Introduction

Mammary stem cells (MaSCs) play a crucial role in respect to cycle of mammary growth, differentiation, lactation and involution in farm and companion animals (Borena et al. 2013). Research done on mouse and human mammary gland models has revealed that stem cell antigen-1 (Sca-1) can serve as a good marker of the mammary stem cell population (Welm et al. 2002, Deugnier et al. 2006). Our previous study has revealed that cells expressing Sca-1 protein comprise about 2% of the total cell number in mammary tissue sections of 2-year-old heifers (Motyl et al. 2011). Transcriptome analysis suggest that genes committed in biological processes, such as signal transduction, development, protein metabolism and modifica-

tions, cell structure, motility, immunity and defence are differently expressed in Sca-1^{POS} and Sca-1^{NEG} cells. However, more detailed analysis has revealed that a group of these genes is typically expressed in cells of hematopoietic origin. This may suggest that the part of Sca-1^{POS} population can be formed by cells that do not originate from the epithelial lineage, but may enrich the niche of MaSCs from the bone marrow (BM) and participate in the self-renewal of the mammary gland during lactation cycles. The present study was undertaken in order to confirm this hypothesis by analysis of BM-origin subpopulation of mammary stem/progenitor cells in 2 year-old nonpregnant Holstein-Friesian heifers. To identify and quantify this subpopulation confocal microscopy and scanning cytometry were used with combination of two

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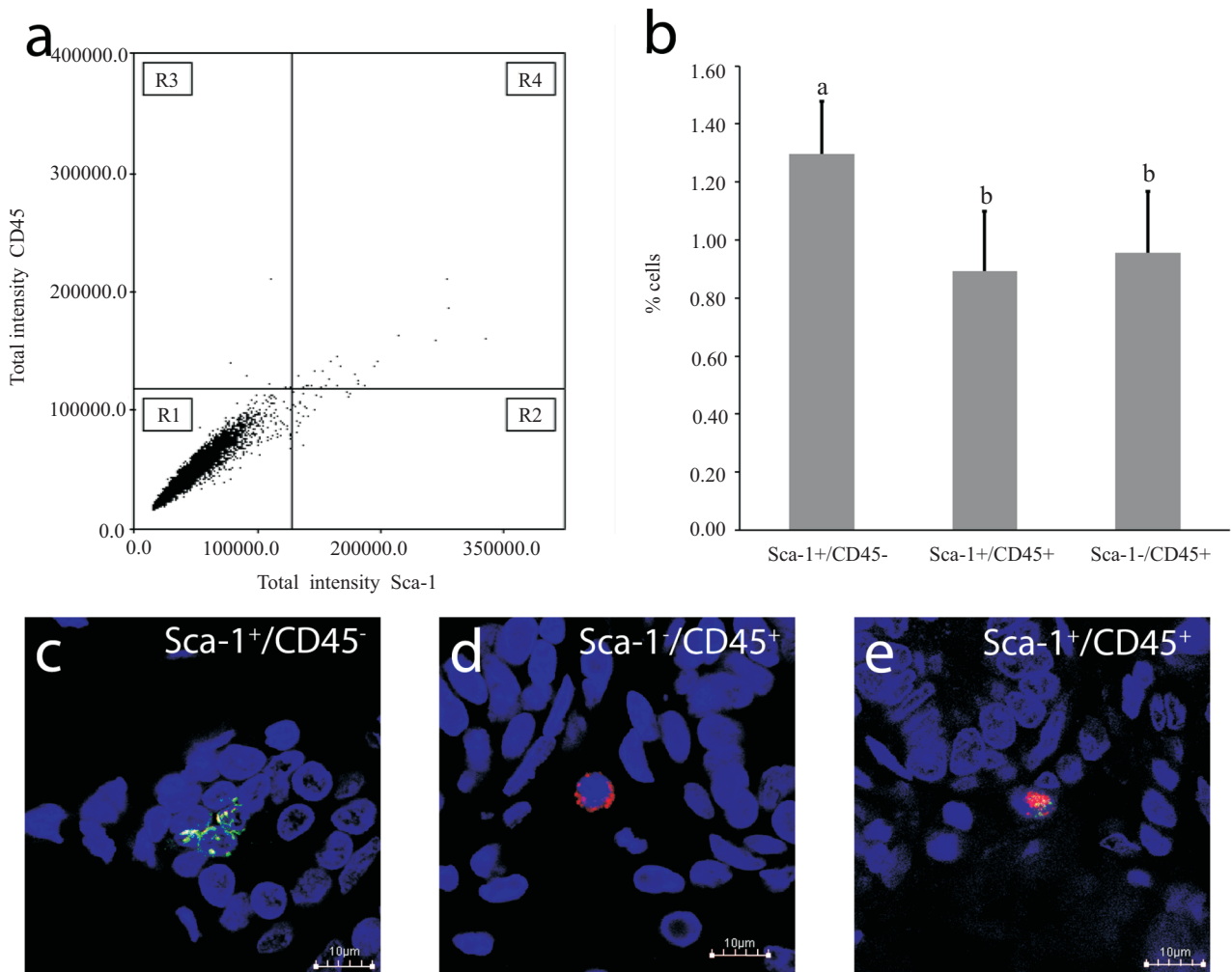


Fig. 1a. Representative cytogram of mammary cells labelled with anti-Sca-1 and CD45 antibodies (see materials and methods). Region: R1-Sca-1^{neg} CD45^{neg}, R2-Sca-1^{pos} CD45^{neg}, R3-Sca-1^{neg} CD45^{pos}, R4-Sca-1^{pos} CD45^{pos}. 1b The percentage of cells expressing Sca-1 and CD45 antigens in the mammary tissue of HF heifers. Results are presented as means \pm SEM, n=40. Bars described by different superscript letters differ significantly ($p \leq 0.05$). 1c,d,e Representative confocal images of mammary cells stained with anti-Sca-1 FITC-conjugated antibody (green), primary polyclonal anti-CD45 antibody and secondary Alexa Fluor 633-conjugated antibody (red); DNA was counterstained with HOECHST 33342 (blue). Bar = 10 μ m.

markers: Sca-1 (marker of stem/progenitor cells) and CD45 (marker of hematopoietic cells).

Materials and Methods

The mammary tissue was obtained at a slaughterhouse from 2 year-old non-pregnant Holstein-Friesian heifers (n = 40), free of clinical signs of mastitis. Since the number of MaSCs can be dependant on hormonal status of the ovary, mammary tissue samples were taken from the animals being in luteal phase. Udders were removed and the mammary tissue was collected and fixed for confocal microscopy and scanning cytometry. For analyses 5 μ m-thick tissue slices were labelled with primary CD45 mouse anti-bovine (AbD

Serotec) and secondary Alexa 633 goat anti-mouse antibodies (Life Technologies, Invitrogen Carlsbad, CA USA) and mouse anti-Sca-1-FITC-conjugated antibody (BD Pharmingen, USA). Nuclei were counterstained with HOECHST 33342. Negative control of immune-staining was performed by omitting primary antibodies. No fluorescence related to unspecific binding was detected. Scanning cytometry analysis was performed using Olympus SCAN^R screening station (Olympus Polska, Sp. z o.o., Warsaw, Poland), and combined analysis software (SCAN^R Analysis v. 1.3.0.3). The results were statistically evaluated using Microsoft Excel 2003 software (Microsoft Corporation, Redmond, WA, USA). Representative microphotographs of cells of interest were taken with an FV-500 laser scanning confocal microscope (Olympus).

Results and Discussion

Co-expression study of Sca-1 and CD45 antigens in the mammary gland tissue of examined heifers revealed four cell subpopulations: Sca-1^{neg} CD45^{neg}, Sca-1^{pos} CD45^{neg}, Sca-1^{neg} CD45^{pos} and Sca-1^{pos} CD45^{pos} (Fig. 1a). The largest subpopulation of Sca-1^{neg} CD45^{neg} was represented by fully differentiated epithelial, myoepithelial and stromal conjunctive tissue cells. Sca-1^{pos} CD45^{neg} cells, mainly located in the basal layers of the epithelium, were probably stem/progenitor cells of mammary origin (Fig. 1c). Their average percentage \pm SEM was: 1.30 ± 0.18 . Sca-1^{neg} CD45^{pos} cells ($0.94 \pm 0.21\%$) were found in the lumen of blood vessels, ducts and infiltrating connective tissue (Fig. 1d). These were most probably fully differentiated cells of blood origin. Their size and nuclei morphology reminded those attributed to leukocytes. The last subpopulation of Sca-1^{pos} CD45^{pos} cells ($0.89 \pm 0.21\%$), was localized mainly outside of mammary ducts, in the stroma and sometimes intraluminally (Fig. 1e). This subpopulation of nondifferentiated cells was most probably stem/progenitor cells of BM-origin. Many other reports indicate that BM can be the source of progenitor cells for different types of tissue, i.e. skeletal muscle, heart and liver. It has been also found that the BM-derived cells are able to rescue postnatal mammary gland development of sub-lethally irradiated mice (Gouon-Evans et al. 2000). Sangai et al. (2006) have revealed a potential role of BM-derived, circulating cells in the development of mouse mammary gland, where BM-derived cells could serve as progenitors for myoepithelial cells and periductal fibroblasts of the mammary gland.

Our results suggest that the small population of Sca-1^{pos} cells bearing CD45 antigen in the mammary

tissue of post-pubertal heifers can be formed by cells that do not originate from the epithelial lineage, but from BM. Their predominant role is to enrich the niche of mammary stem cells where they participate in the growth and development of the mammary gland.

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