A SORTING STRATEGY FOR BOVINE MAMMARY PROGENITORS

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Introduction

The mammary gland is a bilayered system organized into a series of branching ducts that terminate in secretory alveoli. Their epithelium is composed of an inner layer of cytokeratin(CK)18$^+$ luminal cells, which secrete milk, and an outer layer of contractile CK14$^+$ myoepithelial cells, which contribute to milk ejection during lactation[1]. The existence of mammary renewable stem cells (MaSCs) has been deduced from the profound regenerative ability of the mammary epithelium[2]. At puberty and during subsequent pregnancies, the mammary gland undergoes extensive cycles of proliferation and apoptosis that result in dramatic morphologic changes. These waves of proliferations are sustained by a population of adult stem cells that reside in this tissue for the entire animal lifespan[3]. This subpopulation is able to generate different types of committed progenitors (bipotent, luminal-restricted and myoepithelial-restricted).

The aim of our study is to characterize the MaSCs population of the bovine species and set up a sorting strategy to purify different primitive populations.

To this end, we used antibodies directed against integrin α6 (CD49f) and P-cadherin which are expressed in the basal compartment, where MaSCs reside. Functional assays were used to assess the progenitor and stem cell content in the different mammary subfractions.

Methods

Bovine mammary tissue samples were collected at a local abattoir and tissue was mechanically and enzymatically dissociated in order to obtain a single cell suspension [Fig.1A].

Cell phenotypes were then analyzed with an Attune® cytometer in order to assess the expression of CD49f and P-cadherin. Mammary CD45$^-$ cells were then subsequently sorted with a BD FACSAria™ III according to expression levels of CD49f and P-cadherin. Cells from each subfraction were used to perform Colony Forming Cell (CFC) assay in which cells are seeded at clonal density to detect progenitor frequencies and types [Fig.1B]. Cells from the same sorted fractions were also transplanted in immunodeficient mice to detect more primitive cells with the potential to regenerate a mammary tree. The gels were then recovered 3 to 4 weeks after transplantaton.

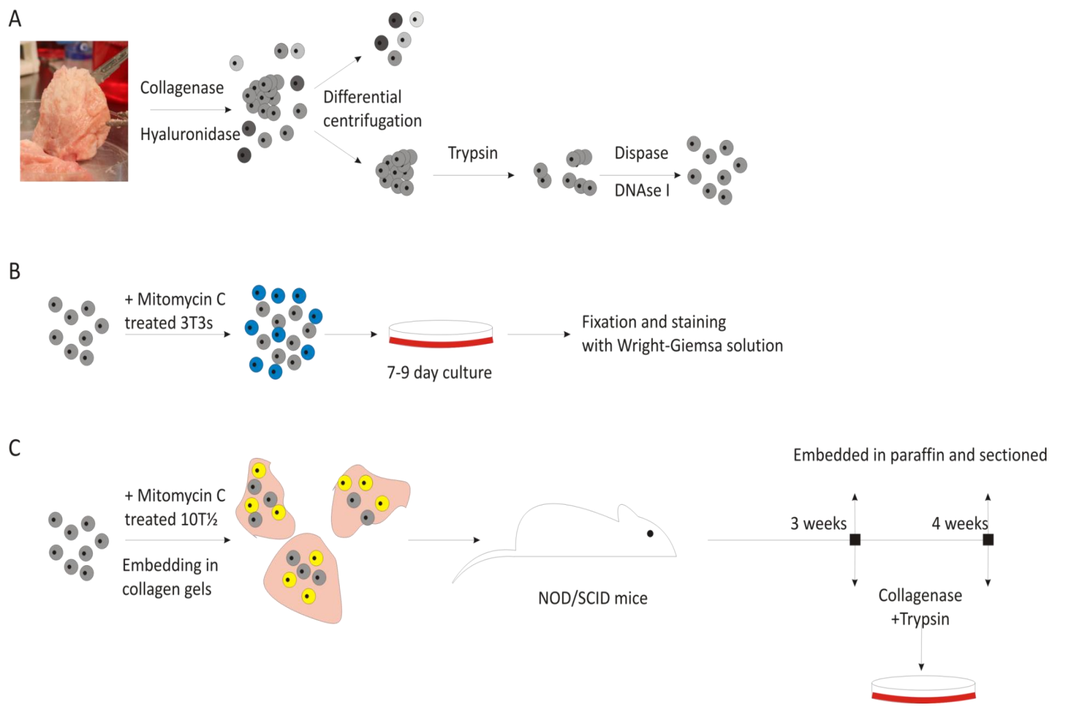

Figure 1. Schematic representation of the dissociation protocol for isolation of bovine mammary epithelial cells (A) and outlines of the Colony Forming Cell (CFC) assay adopted for the detection of progenitors (B) and of the xenotransplantation approach for the functional identification of bovine mammary stem cells.

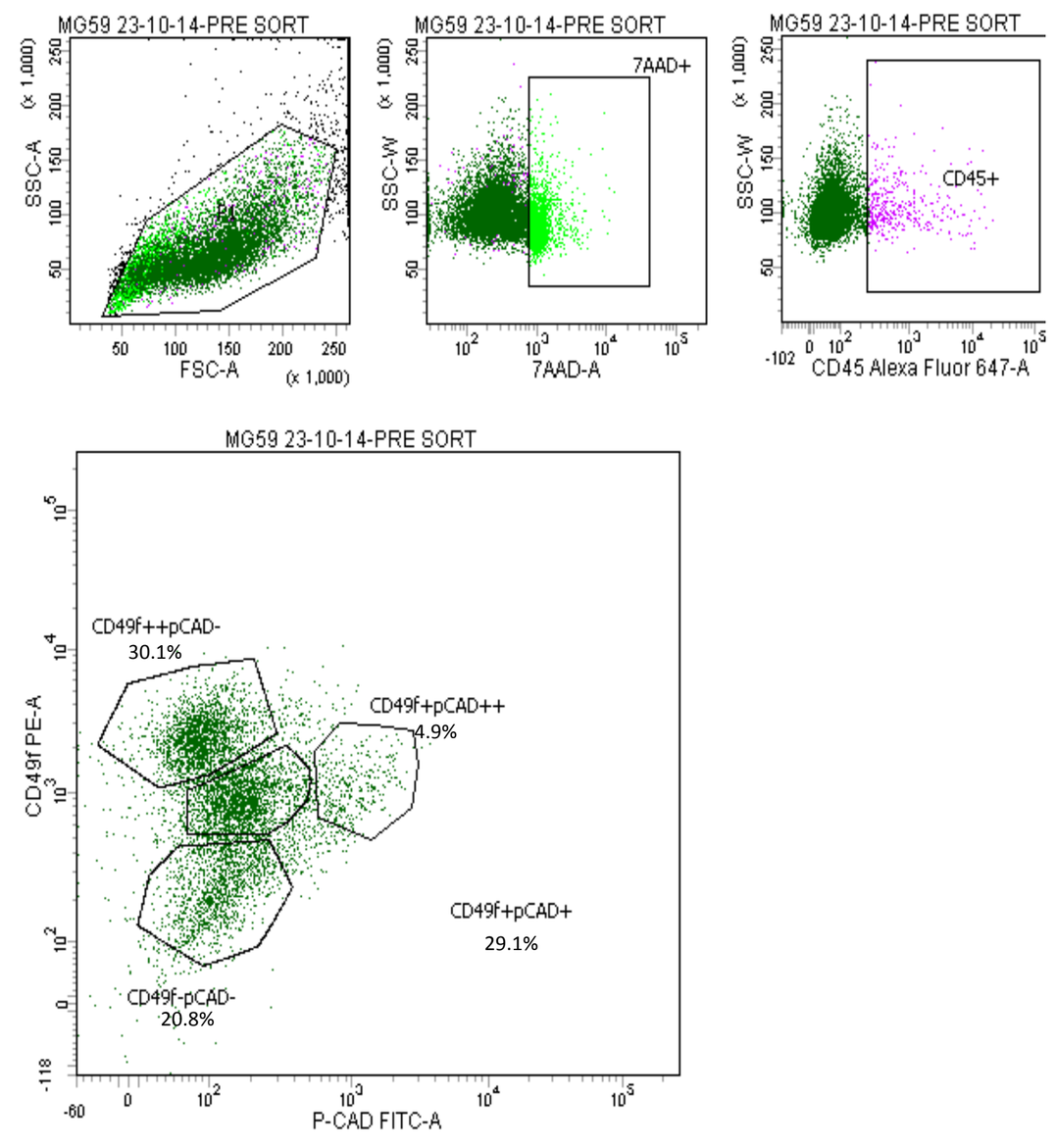

Figure 2. Gating strategy for cell sorting. Dead cells (7AAD$^+$) and immune cells (CD45$^+$) were excluded from the sorting process. Four different populations were detected and they were sorted in different tubes.

	Progenitor frequency	Myoepithelial	Luminal
CD49f$^-$/P-cad$^-$	1 in 2000	0.1±0.1%	99.9±0.1%
CD49f$^+$/P-cad$^+$	1 in 74	0.1±0.1%	99.9±0.1%
CD49f^{++}/P-cad$^-$	1 in 57	64.3±6.4%	35.7±6.4%
CD49f$^+$/P-cad^{++}	1 in 49	0.1±0.1%	99.9±0.1%

Table 1. After sorting, cells from the 4 different subpopulations were used to perform CFC assays (n=3). Since mammary progenitors retain a high proliferative potential, they are able to give rise to colonies that are clonal in nature, when seeded at low density. Progenitor frequency is calculated as number of colonies on total cell seeded. We were able to detect colonies made by luminal or myoepithelial cells (based on morphology and expression of markers such as CK14 and CK18) with a different distribution in the cell fractions.

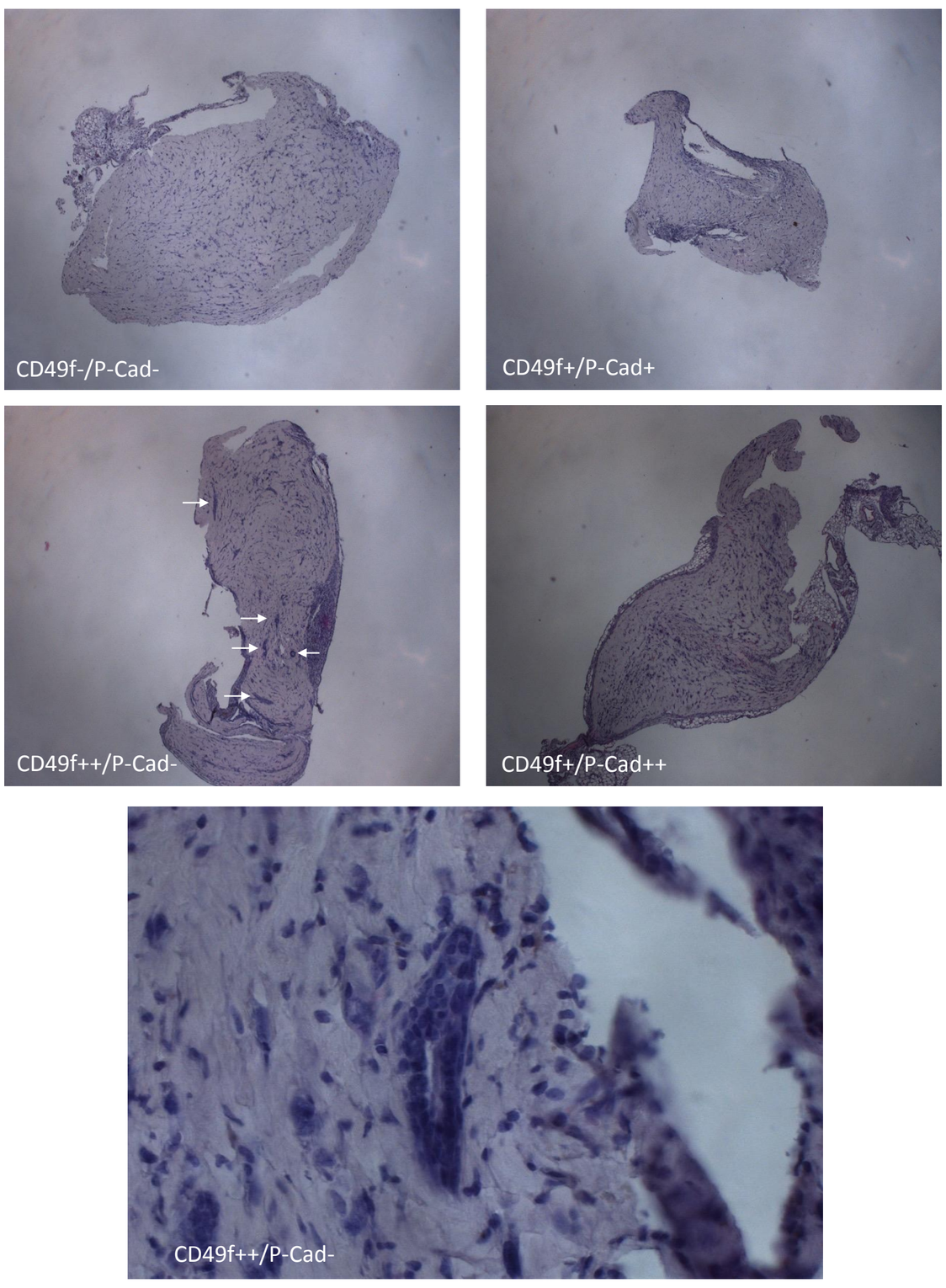

Figure 3. Representative images of sections of gels recovered from xenotransplants. Bilayered hollow structures can be detected only when CD49f^{++}/P-cad$^-$ cells are transplanted.

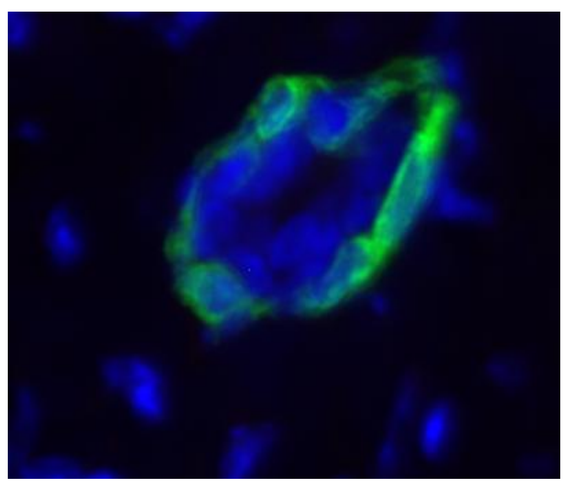

Figure 4. Immunofluorescence staining of a mammary outgrowth from the CD49f^{++}/P-cad$^-$ population. The outer layer of the pseudoalveolus expresses CK14 like the myoepithelial layer in the mammary tissue.

Discussion

By using a novel sorting strategy we show that the mammary myoepithelial cell layer can be divided in different subpopulations. Each one has a different progenitor content and only in one fraction (CD49f^{++}/P-Cad$^-$) we were able to detect a subset of adult stem cells able to regenerate a mammary epithelium. This sorting strategy might prove useful for understanding the stem cells population dynamics during the different phases that the mammary gland undergoes. Besides, given the similar organization of the mammary tissue in the bovine and the human species, the cow might represent a better animal model than the mouse.

References

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