Delineating the MaSC/progenitors committed to the development in the bovine mammary gland at puberty

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BACKGROUND

During puberty and gestation, **Mammary Stem Cells (MaSC)** and their progeny **drive the development of the mammary gland**. In this study, we investigated the epithelial MaSC/progenitor populations within the bovine mammary tissue at puberty, a key physiological stage during which MaSCs expand and differentiate into mature cells.

RESULTS

Xenotransplantation of Holstein heifers mammary explant in mouse mammary gland resulted in the development of an epithelium *in vivo*

We observed the development of epithelial outgrowths in 6 out of 8 transplanted mice (figure 1a), **highlighting the presence of MaSC in the bovine mammary explant**.

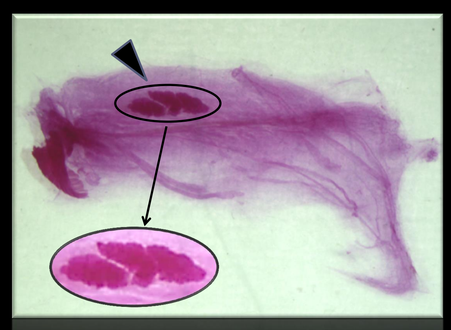


Figure 1a : mammary gland whole mount ⇨

Immunofluorescence analysis of outgrowths **revealed bovine-specific epithelial structures** containing basal and luminal cells, expressing keratin (KRT)14 and KRT7, respectively, **in a dense stromal tissue** producing collagen (figure 1b).

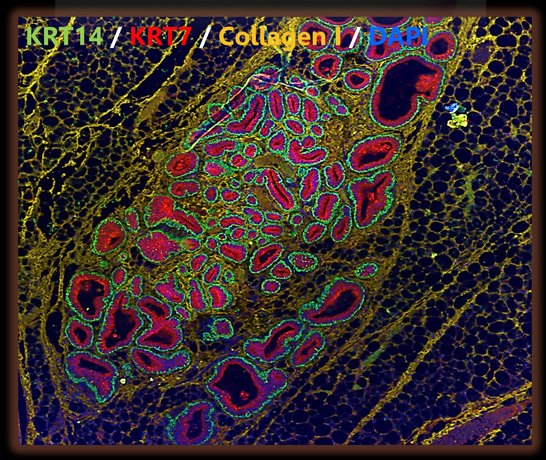


Figure 1b : mammary gland IHC ⇨

Distinct epithelial populations were present during the developmental phase of the mammary ductal-alveolar architecture at puberty.

Flow cytometry highlighted 4 cell populations belonging to the epithelial lineage expressing CD49f and CD24 or not (figure 2a). The mean percentage of the cell population is indicated in red (n=3).

Within these 4 epithelial populations, only the $CD49_f^{high}CD24^{pos}$ and the $CD49_f^{high}CD24^{neg}$ cells were able to form mammosphere *in vitro* (figure 2b)

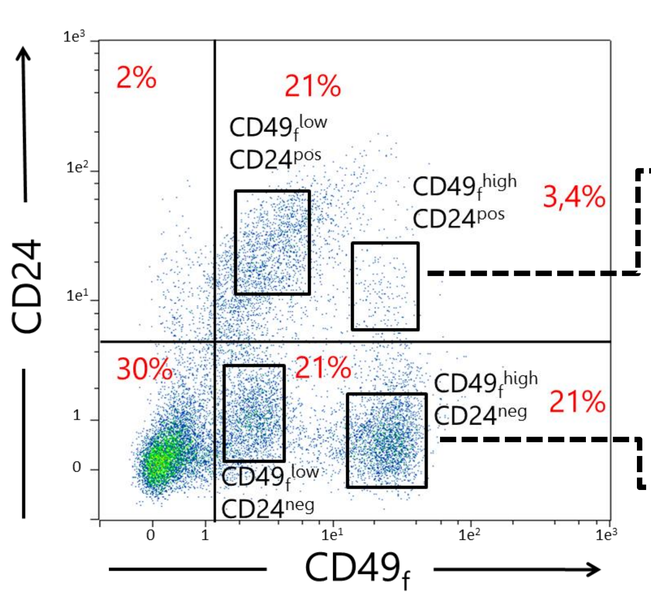


Figure 2a : flow cytometry plot

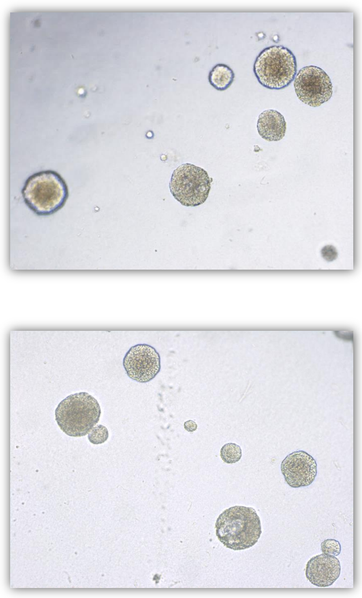


Figure 2b : mammosphere assay

Most of the MaSC cells ($CD49_f^{high}CD24^{pos}$ population) exhibited an ALDH1 activity (70%), a characteristic of the luminal restricted MaSC/progenitor. ALDH1 negative MaSC (30%) would represent a quiescent MaSC pool

Populations	% of cells expressing **CD10**	% of cells with **ALDH1 activity**
$CD49_f^{low}$ $CD24^{neg}$	7%	87%
$CD49_f^{low}$ $CD24^{pos}$	75%	78%
$CD49_f^{high}$ $CD24^{neg}$	93%	0.3%
$CD49_f^{high}$ $CD24^{pos}$	**92%**	**68%**

In-depth phenotyping highlighted that MaSC express basal marks and suggested that two subpopulations would mingle in the MaSC pool (in Finot et *al*, 2018; submitted)

MATERIEL AND METHODS

Explants (0,2 mm^2) **were sampled from the mammary gland of Holstein heifers** slaughtered at 17 months of age (n=3)

Xenotransplantation assay

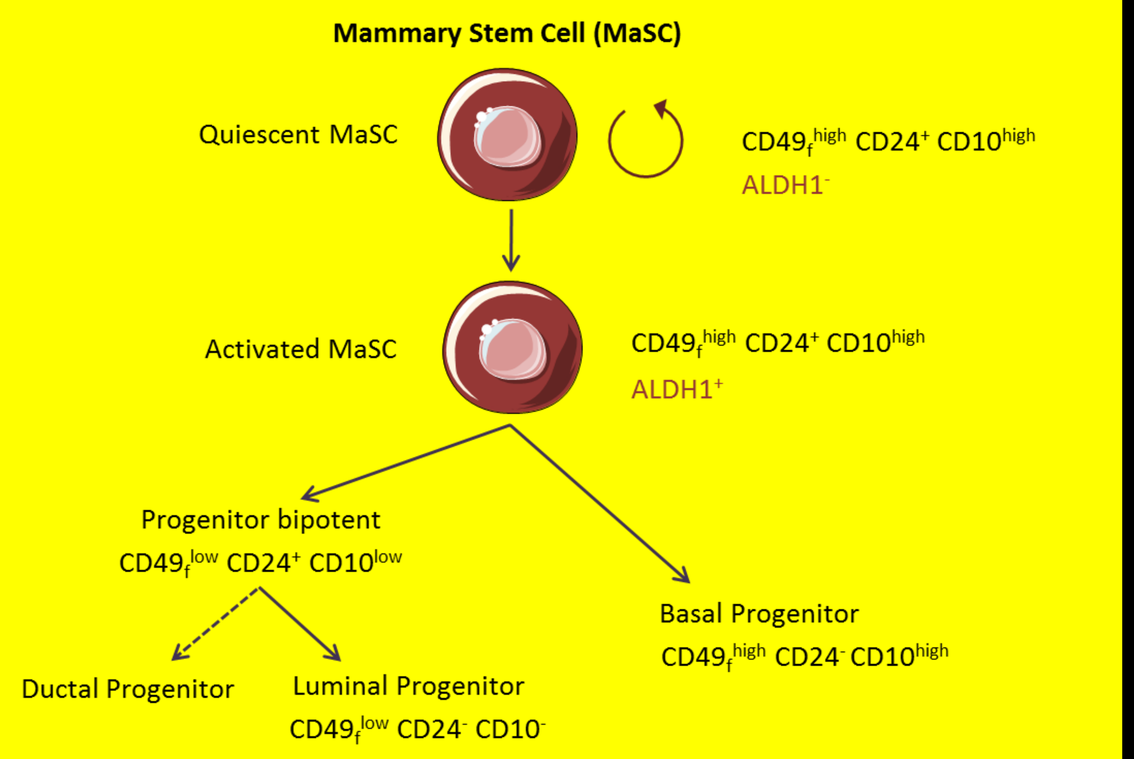
- Bovine **mammary gland explants** (0,05 mm^2) **were transplanted in 3-weeks nude mice** (Balb/c AnNRj-Foxn1$^{nu/nu}$). Mouse mammary glands were collected two months later and processed for **whole mount** (Carmine Alun staining) **and immunofluorescence** (IHC) **analysis**.

Phenotyping assays

- **Bovine mammary gland explants were dissociated to single cells** using an enzymatic (collagenase/hyaluronidase/trypsin) dissociation protocol.
- **Single cells were stained with anti-$CD49_f$ and anti-CD24 antibodies and co-expression of these markers was assessed by flow cytometry.**
- **The expression of CD10** (a basal lineage marker) **and the activity of ALDH1** (a MaSC/progenitor marker) **were assessed** on the $CD49_f$ / CD24 populations **by flow cytometry**.
- **The cell populations sorted on the basis of $CD49_f$ and CD24 staining were cultured with Matrigel** during 7 days to monitor the formation of **mammospheres**

CONCLUSIONS

This study led us to define the molecular signature of bovine MaSC and progenitors at puberty



Proposed epithelial cell lineage scheme in the bovine mammary gland at puberty

PERSPECTIVES

We aim at providing new insights into the fate of bovine MaSC and progenitors (both in their proportion and their molecular signature) at key physiological stages of development, including lactation and dried off. In an agronomic context as dairy, understanding the fundamentals of the mammary epithelial development and turnover is of importance to improve animal robustness through the enhancement of lactation efficiency.