Purification of ruminant mammary stem cell population and uses thereof for production of transgenic proteins *in vivo*

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INTRODUCTION: while strong evidence has been gathered on adult mammary stem cells in mice and humans, only circumstantial evidence has been presented about their counterparts in the ruminant species. Given the economical interest revolving around dairy species and the histological similarities between the human and bovine mammary gland, the hypothesis of the existence of a bovine mammary stem cell population is certainly intriguing [1]. Given the high milk yield that can be obtained from a dairy breed like the Holstein-Frisian, the opportunity to use the mammary gland as a high efficiency bioreactor for the production of heterologous proteins has been quite appealing in recent years [2]. Thus weisolated a cell population enriched in stem cells which are able to (re)generate polarized and functional structures *in vivo* when transplanted in an animal model. We focused on engineering these cells for transgene production in milk since they represent a preferential target for *in vitro* manipulation.

METHODS:

Primary epithelial mammary cells or induced-pluripotent mammary stem cells (IPCs) [3] were used as source of mammary stem cells. Colony forming cell assay (CFC) and xenograft in NOD/SCID mice were used as models for testing functional properties in vitro and in vivo. Immunofluorescent and flow cytometry analyses were performed to characterized the regenerated structures.

RESULTS:

We report that in addition to a CD49f^{+} phenotype, stem cells are ALDH^{-} and p-cadherin^{-}. Furthermore we give evidence that stem cells come from the basal layer of the lumen (K14 $^{+}$ and K18^{-}). These cells can be maintained in culture for up to 35 days but no expansion was observed. IPCs can be a continuous source of mammary stem cells but commitment to a mammary phenotype is hard to achieve.. Progesterone and estradiol administration induces a partial differentiation towards the mammary phenotype.

The complete regeneration of functional alveolar tissue in vivo was obtained only through the use of epithelial purified cells, therefore we obtained production of exogenous proteins by transfecting primary cells.

DISCUSSION & CONCLUSIONS: We were able to demonstrate that bovine mammary tissue contains lineage-restricted progenitors with in vitro clonogenic activity as well as more primitive uncommitted cells that regenerate bilayered multilineage milk-producing mammary structures when transplanted in immunodeficient mice. We reported the ability of isolated ruminant mammary cells to regenerate mammary tissue following their transplantation into a histocompatible recipient and also a proof concept that it is possible to engineer these cells to produce human beta casein, a major component of human milk [1].We also propose that the cells responsible to secrete endogenous and exogenous proteins in the lumen of alveolar-like structures retain peculiar features: they lack aldehyde dehydrogenase activity and p-cadherin expression while expressing high levels of CD49f. We propose that basal myoepithelial cells contain cells with stemness and that only luminal progenitors in the mammary gland are ALDH^{+}. A great milestone to achieve is to purify a large amount of these cells for further manipulation. Long term culture of primary mammary cells and new IPCs derived from mammary epithelial cells are far to be used for field applications.

REFERENCES: [1]A. Capuco et al (2013) *Bovine mammary stem cells: cell biology meets production agriculture*, Animal **6:**382-93. [2]M. Martignani et al (2010) *Human milk protein production in xenografts of genetically engineered bovine mammary epithelial stem cells*, PloSOne **19**, 5:e13372. [3] D. Cravero et al (2015) *Generation of Induced Pluripotent Stem Cells from Bovine Epithelial Cells and Partial Redirection Toward a Mammary Phenotype In Vitro* Cell Reprogram **17**: 211-20.

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