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Characterization of Bovine Mammary Stem Cells: A Brief Update

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Abstract

Adult stem cells have immense potential to regenerate the damaged tissue and regaining the activity of the particular organ. The stem cells present in the mammary gland have wide plasticity maintaining the dynamic function of mammary gland. Bovine mammary stem cells (MaSCs) are least studied so far, although substantial studies have been done on MaSCs of mouse and human mammary gland. The hierarchy of the bovine mammary epithelial cells is yet to be elucidated in molecular details although studies reveal the crucial role of MaSCs as the source of epithelial and myoepithelial cells. Various cell surface markers (CD-24, CD-49f, EpCAM, Sca-1 etc.) have been identified to distinguish the MaSCs from other cells present in the mammary gland. Researches are going on to understand the physiological phenomena occurring in the stem cell proliferation, differentiation and recruitment in the mammary gland as their manipulation can enhance the lactation yield, can regenerate the mammary gland cell population lost after suffering from the acute inflammatory disease of mammary gland i.e. mastitis. This review focuses on the recent studies on bovine MaSCs and their characterization, highlighting the different surface markers validated so far and the potential of these stem cells for persistent lactation and regenerative therapy.

Key words: Bovine, Mammary gland, Stem cell, and Lactation

Introduction

The mammary gland is developed from ectoderm layer and has lobules and alveoli which are made up of mainly 3 cell types such as alveolar epithelial (milk synthesizing units), ductal epithelial (lining the ducts), and myoepithelial cells forming the ductal and alveolar basal layer [1-3]. These differentiated cell types are derived from adult mammary stem cells present in the mammary gland. Till now, substantial studies have been done on the mammary epithelial stem cells of human and mouse mammary gland, whereas in-depth studies on bovine mammary stem cells is yet to be performed [1]. The primary goal to explore the potential of bovine mammary stem cells is to augment milk production in dairy animals [4]. The differentiated alveolar epithelial cells are mainly responsible for milk secretion and persistent lactation, where the

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differentiation status decides the secretory activity [4]. It was found that intermediate and fully differentiated cells are secretory whereas epithelial cells with poor differentiation are non-secretory cells [5, 6]. The regeneration of the mammary epithelial cells mainly depends on the mammary progenitor cells (progenitor cells are derived from mammary stem cells (MaSCs)), although the differentiation status is guided by steroidal hormones (estrogen and progesterone), and prolactin [4]. Milk yield of dairy animals can be enhanced by proper herd management, good nutrition, frequent milking, appropriate calving interval, use of bovine somatotrophic hormone etc. [7, 8]. Besides, MaSCs might have tremendous potential for persistent lactation and their manipulation may enhance milk production as studied so far in dairy animals [9, 10]. Besides study on bovine MaSCs can give clues on why there is rare occurrence of mammary gland cancer in livestock animals. The prevalence of mammary gland cancer is rare in cattle, pigs, horses, sheep etc. although it is common in humans and canines [11, 12]. Adding on to this, there is possibility of manipulating the genetic makeup of MaSCs to produce milk components of desired composition and producing the human milk protein in milk of cattle [13]. The complexity of production of transgenic cows can be overcome by producing transgenic MaSCs and regenerating mammary structures and ultimately milk production. This strategy was evidenced by producing human milk proteins in xenografts of genetically manipulated bovine mammary epithelial stem cells [13]. The study on MaSCs is supposed to have value in human medicine as well besides their application in veterinary sector [14].

This review focuses on the recent development in the MaSC study of bovine mammary gland, their characterization and their manipulation to enhance milk yield for persistent lactation.

Identification of bovine MaSCs

A study through genetic lineage tracing and clonal analysis in mouse mammary gland revealed that mammary gland structure is maintained by two populations of unipotent progenitor cells [15]. The presence of self-renewing adult stem cells in mammary tissue was determined for the first time in mouse by limiting dilution transplantation experiments [16, 17]. Later on, it was observed that the whole mammary gland can be regenerated by progenies of a single cell through tissue fragment transplantation into a fat pad-cleared mammary gland [18].Ellis and Capuco for the first time reported the presence of putative stem cells in bovine mammary gland which are heavily proliferative in nature [19]. They identified the lightly stained parenchymatous cells comprising of 10 % of the total mammary gland parenchyma to be putative MaSCs. Later on, Capuco identified the MaSCs by their property of retention of labeled DNA strand which were further characterized by studying the expression of nuclear proliferation antigen Ki67 and estrogen receptor[ER] expression [20]. The $Ki67^{+}ER^{-}$ cells were proposed to be MaSCs and the $Ki67^{+}ER^{+}$ cells were their progenitor cells.

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Characterization of bovine MaSCs

The wide plasticity of the cells of mammary gland is somehow linked to the MaSC population of the mammary gland. Mouse MaSCs have been identified having combinations of different surface markers like CD-24, CD-29 (β-1 integrin), CD-49f (α-6 integrin), Sca-1 (stem cell antigen-1), and CD-61 (β-3 integrin) [21-23]. The characterization of bovine MaSCs is highly necessary to distinguish them from other cells. The flow cytometry study using mammary gland biopsy from primiparousmilching cows at day 30, day 90, day 150, and day 250 of lactation, revealed different cell lineages based on the cell surface markers CD-49f, CD-24, EpCAM (Epithelial cell adhesion molecule), and CD-10 [24]. The specific population of stem cells were characterized by $CD\text{-}24^{+}/CD\text{-}49f^{+}$ phenotype. Besides, 4 populations of progenitor cells were identified including bi-potent luminal progenitor cells ($CD\text{-}24^{-}/CD\text{-}49f^{+}$, luminoalveolar progenitor cells ($CD\text{-}24^{-}/EpCAM^{+}$), myoepithelial progenitor cells ($CD\text{-}24^{+}/CD\text{-}10^{-}$) and lumino ductal progenitor cells ($CD\text{-}49f^{-}/EpCAM^{+}$). There was significant decrease of bipotent luminal progenitor cells during lactation stage.FACS analysis of the cells of bovine mammary gland revealed four types of cells based on surface marker CD-24 and CD-49f expression [25]. The putative stem cells with $CD\text{-}24^{med}CD\text{-}49^{+}$ markers are located in basal region, the putative progenitor cells had $CD\text{-}24^{high}CD\text{-}49f^{-}$ phenotype, basal cells had $CD\text{-}24^{-}CD\text{-}49f^{+}$ phenotype, may be derived from putative stem cells in the hierarchy. The fourth category was the luminal cells with the $CD\text{-}24^{med}CD\text{-}49f^{-}$ phenotype. Another approach [26] showed putative stem cells to be Sca-1 positive comprising of 2% of total cell number in mammary tissue, and localized in stem/progenitor cell niche and are ER-α^{-} (Estrogen receptor-α) phenotype. These estrogen non-responsive cells are indicative of the stem/progenitor cells responsible for tissue regeneration during mammary gland development, lactation, and involution process. Bovine MaSCs were characterized by their ability to retain the bromodeoxyuridine (BrDU) label for extended time [20, 9]. The MaSCs retained labeled DNA due to selective segregation of template strands during mitosis. The MaSCs retaining the BrDU label are termed label-retaining epithelial cells (LRECs) and are found to be 0.4 % of total cell population in mammary gland [9].Recent study in mammary tissues of buffalo mammary gland showed the presence of hepatocyte nuclear factor 4-alpha (HNF4A, a marker for hepatocyte progenitor cells) in the putative mammary stem/progenitor cells adding to the list of existing stem cell markers [27]. Study on immortalized bovine MECs in the in vitro culture system [28] revealed expression of stem/progenitor cell marker (CD44 and p63) besides the epithelial (cytokeratin 7, 8, 18, and 19), and mesenchymal (vimentin) markers.

Successful transplantation of bovine MEC population (sorted as per CD24 and CD49f expression) into cleared fat pad of immune compromised mice [29] showed outgrowths composed of myoepithelial cells (indicates presence of unipotent basal stem cell. Luminal cell sorting (according to presence of E-cadherin revealed three distinct cell populations such as luminal progenitor cells, early differentiating cells, and late differentiating cells. These findings provide the evidence of regenerative cells existing in the bovine mammary gland.

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Induction of bovine MaSCs for persistent lactation

Study showed that MaSC populations and their regulations induce a favorable environment for self-renewal, and maintenance of mammary gland besides the established endocrine regulations [30]. Xanthosine (a purine nucleoside) is found to induce symmetrical division in stem cells undergoing asymmetric division. The symmetric division leads to stem cell proliferation, thus enhancing their number. The asymmetric division suppression by xanthosine is controlled in a p53 – dependent manner through the enzyme inosine monophosphate dehydrogenase [31]. Experiments conducted using xanthosine (intramammary infusion) showed enhanced stem cell population in mammary gland and it is predicted to increase the milk yield [9]. Xanthosine treatment in cultured bovine mammary epithelial cells showed enhanced cell number and promoted symmetric cell division [32]. Study on effect of xanthosine in the gene expression in mammary cells of early lactating Beetal goats through RNA-Seq technique revealed promising results [33]. Global gene expression profiling of mammary epithelia obtained from milk fat globules showed down regulation of genes involved in inflammation signaling pathway, and down regulation of cell adhesion molecules, whereas up regulation of anti-bacterial genes were found indicating the support to maintain healthy mammary gland (Choudhry et al. 2018). Study by Rauner and Barash (2014) showed contradictory results about xanthosine treatment [34]. They found xanthosine infusion did not affect the number of LRECs as experimented by transplantation of heifer mammary parenchyma tissue in cleared mammary fat pad tissue of immune deficient mouse. Rather, a 50 % decrease in proliferation rate of bovine MECs was observed 11 week after xanthosine infusion. It is predicted that this reduction in proliferation may be due to decreased expression of IMPDH in guanine synthesis.

Future perspectives of bovine MaSCs

Mastitis causes huge loss to the dairy industry as the animals donot gain back the optimal potential of milk yield after suffering from this disease. Sharma and Jeong (2013) showed that bovine mammary stem cells have potential for regeneration of the damaged tissues and least risk of rejection is involved [35]. The huge loss occurred to the number of mammary epithelial cells can be regained if the MaSCs can be induced in vivo to differentiate into these types of cells and becoming functionally active for lactogenesis. Furthermore, the MaSCs can be manipulated to be transgenic by introducing the target gene in those cells and availing the expressed proteins in the milk. Successful introduction of lactoferrin transgene into the bovine MaSCs has led the possibility of stem cell manipulation to get the desired protein in milk, and can overcome the issue of low transgenic efficiency and lower level expression of foreign proteins [36, 37].An in vitro approach [38]attempting to culture the mammary stem cells revealed that bovine mammary epithelial cells retain stem cell – like phenotype in prolonged culture (25 days) having regenerative property. Regenerated structures were developed by transplantation in immune deficient mice and progenitor cell content was analyzed by colony-forming cell assay (CFC assay) with convincing findings. Cravero et al. (2015) have generated induced pluripotent stem

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cells (iPSCs)from bovine mammary epithelial cells [39]. Further differentiation of the pluripotent cells to cells of mammary tissue in the in vitro condition revealed the potential of iPSCs.

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