J. Dairy Sci. 99:1–13
http://dx.doi.org/10.3168/jds.2015-10486

Primary cilia distribution and orientation during involution of the bovine mammary gland

J. Biet,*†[1] C. A. Poole,‡[2] K. Stelwagen,§ J. K. Margerison,# and K. Singh*[3,4]
*AgResearch Ltd., Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand
†Institute of Agriculture and Environment, College of Sciences, Massey University, PB 11222, Palmerston North 4442, New Zealand
‡Department of Medicine, Dunedin School of Medicine, University of Otago, PO Box 913, Dunedin 9054, New Zealand
§SciLactis Ltd., Waikato Innovation Park, Ruakura Road, Hamilton 3240, New Zealand
#Bioscience and Animal Science, University of Nottingham, Sutton Bonington, Leicestershire, LE12 5RD, United Kingdom

ABSTRACT

The regulation of mammary gland involution occurs through multiple levels including environmental factors, hormones, and local intramammary signals. Primary cilia (PC) are signaling organelles that sense biochemical and biophysical extracellular stimuli and are vital for cellular and tissue function. The aim of this study was to examine the distribution, incidence, and orientation of PC. Furthermore, we determined changes in expression levels of the signal transducer and activator of transcription (STAT)6 at the onset of bovine mammary gland involution. Mammary tissue was collected from pasture-fed, primiparous, nonpregnant Friesian dairy cows at mid lactation (n = 5 per group) killed 6-h after milking (lactating controls) and during involution after 7 and 28 d of nonmilking (NM). Fluorescent immunohistochemistry and confocal microscopy of tissue sections showed that PC were present on luminal secretory epithelial cells (SEC), myoepithelial cells (MEC), and stromal fibroblast cells (SFC). Furthermore, in all 3 experimental groups, different PC positions or orientations relative to the cell surface were identified on SEC and MEC, which projected toward the lumen and were either straight, bent, or deflected against the apical cell surface, whereas PC in SFC were confined to the interalveolar space. However, by 28-d NM, fewer PC projected into the luminal space and most appeared deflected or projected toward the interalveolar space. Furthermore, by 28-d NM, with the increase in stromal connective tissue, more PC were detected within the interalveolar and interlobular stroma. At 28-d NM, we observed a decrease in luminal cilia relative to the total number of cilia. The number of ciliated cells in the total fraction (SEC, MEC, and SFC) was the same for all 3 groups, although in the luminal fraction (SEC and MEC), PC per nuclei increased by 28-d NM relative to lactation. At all 3 stages, we detected variations in shape and orientation of PC within the same alveolus, with some PC projecting directly into lumen, whereas others appeared to be bent or deflected flat against the cell surface. Within each treatment, the average number of bent cilia was low, whereas the average number of deflected cilia was higher than the average number of cilia projecting directly into the lumen. Quantitative real-time reverse transcription PCR analysis showed that expression levels of milk protein genes (α_{S1}-casein, α-lactalbumin, and κ-casein) declined and that of lactoferrin increased in the involuted mammary tissue following NM, compared with lactating controls. Although STAT6 mRNA levels did not change following NM, STAT6 protein levels did increase following 28-d NM compared with the control lactation group. In conclusion, PC were detected in all cell types in the mammary gland, and changes in orientation during involution suggest the potential for PC to play a role in signal transduction through both mechanosensation and chemosensation. Furthermore, the STAT6-mediated signaling pathway may have a role during involution of the mammary gland.

Key words: primary cilia, bovine mammary gland, mechanotransduction, involution, signal transducer and activator of transcription 6 (STAT6)

INTRODUCTION

Milk production in dairy cattle declines following peak lactation primarily due to increased loss of secretory epithelial cells (**SEC**) via apoptosis (Wilde et al., 1997). Mammary function is regulated not only at the hormonal level (Wilde et al., 1999)—local intramammary signals also play a role in initiating SEC apop-

Received October 3, 2015.
Accepted December 20, 2015.
[1]Present address: Auckland 0624, New Zealand.
[2]Present address: Wanaka, Central Otago 9305, New Zealand.
[3]Present address: Hamilton 3200, New Zealand.
[4]Corresponding author: kuljeet.singhparhar@outlook.com

tosis and involution (Quarrie et al., 1994; Wilde et al., 1997). The primary signal that initiates the involution process is unknown. However, several mechanisms have been postulated, including the accumulation in milk of regulatory factors such as a putative feedback inhibitory factor (Wilde et al., 1999), milk casein fractions (Shamay et al., 2003), and serotonin (Collier et al., 2012). Alternatively, physical distension of the mammary gland, which results in changes in cell shape, may activate mechanotransduction pathways, resulting in loss of tight junction integrity and cell-extracellular survival signaling (Davis et al., 1999; Stelwagen, 2001). In support, cell–cell communications via tight junction proteins (Cooper et al., 2004) and cell–extracellular matrix communication (Singh et al., 2005) are disrupted following milk engorgement at the onset of involution of the bovine mammary gland. Furthermore, mammary cell stretch in vitro was associated with the induction of apoptotic pathways (Quaglino et al., 2009) and acute physical distension of rat mammary glands in vivo accelerated the onset of apoptosis of SEC, activation of the pro-apoptotic marker signal transducer and activator of transcription (**STAT**)3, and the loss of extracellular matrix β1-integrin adhesion receptors and the tight junction factor occludin (Phyn et al., 2007).

Cells sense and transform the various forms of external stimuli they receive through interactions with the extracellular matrix, neighboring cells, and soluble cues from the microenvironment into a cascade of cellular and molecular events (DuFort et al., 2011). Important differences exist between force-generated or mechanical signaling [e.g., bending of the primary cilium (**PC**)] and chemical signaling (e.g., soluble signals, such as growth factors; Wells, 2013). Mechanical signals can be highly directional and thereby convey and transmit complex information in 3 dimensions. Soluble factors, on the other hand, diffuse radially, provide limited directional information, and require translation into secondary messengers. Consequently, unlike soluble signals, force-mediated signals can begin and end rapidly (Wells, 2013) and are at least 40 times faster than growth factor-induced signal transduction (Na et al., 2008).

The transduction of physical forces appears to occur through changes in protein conformation (Schwartz, 2010). These force-induced effects on conformational change represent a general mechanism that may regulate enzymatic activity, enable new molecular interactions, open mechanosensitive protein channels, or liberate soluble bound factors, which in turn may activate signaling pathways in an autocrine or paracrine fashion (Hoey et al., 2012; Jones and Nauli, 2012). Therefore, mechanical and chemical signals are often interdependent (Wells, 2013). Hence, mechanical forces can act directly and indirectly on chemical signals but seem to exceed the speed of signaling through soluble factors by several orders of magnitude (DuFort et al., 2011; Wells, 2013). Nonetheless, the mechanical forces experienced by cells are generally small. Therefore, the force sensor must either be much more sensitive than the typical protein complex, or highly elongated and projected into the environment to sense and amplify the force experienced (Janmey and McCulloch, 2007).

The PC differ significantly from motile cilia (Barnes, 1961); their singularity, universality, and structural characteristics indicate a role in sensing and transducing several biochemical and biophysical extracellular stimuli in different tissues and organs. Protrusion of a PC into the extracellular space enables access to the cell's external milieu, its elongated geometry provides a high surface-to-volume ratio, and the regulated entry of proteins into the cilium leads to specialization and compartmentalization (Singla and Reiter, 2006; Hoey et al., 2012). Sensory modalities such as mechanical stimulation (bending of the cilium), chemosensation (detection of a specific ligand, growth factor, hormone, or morphogen), or in some cases stimulation by light, temperature, osmolality, or gravity are achieved by a bilayered lipid membrane that is continuous with the plasma membrane of the cell body but contains a distinct subset of receptors and other proteins involved in signaling (Veland et al., 2009; Jones and Nauli, 2012)

The occurrence of the PC is a cell cycle-dependent and dynamic process of assembly and resorption (Nguyen and Jacobs, 2013). During interphase, assembly of the PC will occur on nonproliferating G_0-G_1 cells. Primary cilia arise from a basal body, derived from the mother centriole, anchoring the cilium to the cell's microtubular cytoskeleton, whereas intraflagellar transport mechanisms controls its length (Satir and Christensen, 2007). Furthermore, a close structural interrelationship between PC, the secretory organelles, and the extracellular matrix has been identified that indicates a potential role for PC to act as a multifunctional, cellular probe mediating the interaction between connective tissue cells and their biomechanically functional extracellular matrix (Poole et al., 1985). The analysis of PC of various established kidney cell lines demonstrated that PC bend in response to fluid flow (Roth et al., 1988) and thus, PC have a mechanosensory role via detection of fluid flow across kidney cells (Schwartz et al., 1997). Further experimental evidence indicates that mechanically stimulated cilia are part of a calcium signaling system (Praetorius and Spring, 2001). Additionally, a study with chondrocytes provided evidence that PC are required for ATP-induced Ca^{2+}signaling (Wann et