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The ins and outs of calcium signalling in lactation and involution: Implications for breast cancer treatment

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ABSTRACT

The mammary epithelium is highly responsive to hormonal and non-hormonal signalling cues for physiological growth, function and tissue remodelling. Whilst steroid hormones freely diffuse across the cell membrane to bind to intracellular hormone receptors, cell-impermeable ligands, including many peptide hormones, growth factors and cytokines, bind to receptors on the plasma membrane and relay their message via the specific activation of intracellular signal transduction pathways. A signalling pathway that is indispensable for decoding many extracellular signals into cellular responses is calcium (Ca^{2+}). Changes in the expression of specific Ca^{2+} channels, pumps and binding proteins may therefore greatly alter the nature of the cellular response to various growth, morphogenetic and cell death stimuli. This review summarises changes in the expression, localisation and function of key Ca^{2+} channels and pumps in mammary epithelial cells during lactation and discusses how this altered Ca^{2+} handling may later expose these cells to targeted cell death during post-lactational involution. A greater understanding of the processes regulating the growth, death and regeneration of the mammary epithelium under physiological conditions may provide important insights into the proliferation and survival mechanisms underpinning malignant growth. The therapeutic manipulation of specific calcium signalling pathways in breast cancer cells to control aberrant cell proliferation and/or turnover represents an aim for the future.

1. Introduction

The adult mammary gland is composed of a bifurcating and bilayered ductal tree embedded within an adipocyte-rich stroma. Two distinct epithelial cell types are present in the mammary gland: luminal epithelial cells line the lumen of each duct and are surrounded by a layer of basal (myoepithelial) cells in contact with the basement membrane [1,2]. After puberty, these cells remain largely quiescent, with some proliferation producing small alveolar buds in response to cyclical ovarian stimulation [3]. However, during pregnancy mammary epithelial cells rapidly proliferate to form lobuloalveolar structures that are able to produce and expel milk for neonatal nourishment. Once lactation is complete, the mammary gland regresses to a nulliparous-like state, capable of sustaining further cycles of growth, secretion and cell death with subsequent pregnancies [1,4]. The complete elimination of the alveolar epithelium during post-lactational involution is a major cell death event in mammals [5], and it is not yet understood how ductal epithelial cells and putative alveolar stem cells evade this cell death programme. The remarkable capacity of the mammary epithelium to reinstate a proliferative programme and to selectively regulate cell death under physiological conditions may provide important insights into the proliferation and survival mechanisms that support malignant growth.

Post-lactational involution of the mammary gland occurs in two distinct phases: an early, reversible phase and a late, remodelling phase [5,6]. The first phase of involution is characterised by milk stasis and the STAT3-dependent conversion of luminal alveolar cells from a secretory to a phagocytic phenotype [7,8]. The reuptake of milk fat globules (MFGs) and their delivery to and degradation by lysosomes results in lysosomal membrane permeabilisation and the subsequent leakage of cathepsin proteases into the cytosol [7]. In addition to the release of these executioner proteases, lysosomal membrane permeabilisation in phagocytic mammary epithelial cells could also dramatically elevate cytosolic Ca^{2+} levels. Resting cytosolic Ca^{2+} concentrations are tightly regulated at submicromolar levels [9]. By comparison, milk contains approximately 8 mM and 60 mM Ca^{2+} in humans and mice, respectively [10]. Thus, the reabsorption of secreted milk and the uncontrolled release of milk Ca^{2+} into the cytoplasm of luminal epithelial cells may deliver a secondary cell death signal (Fig. 1). Moreover, Ca^{2+} may also play a

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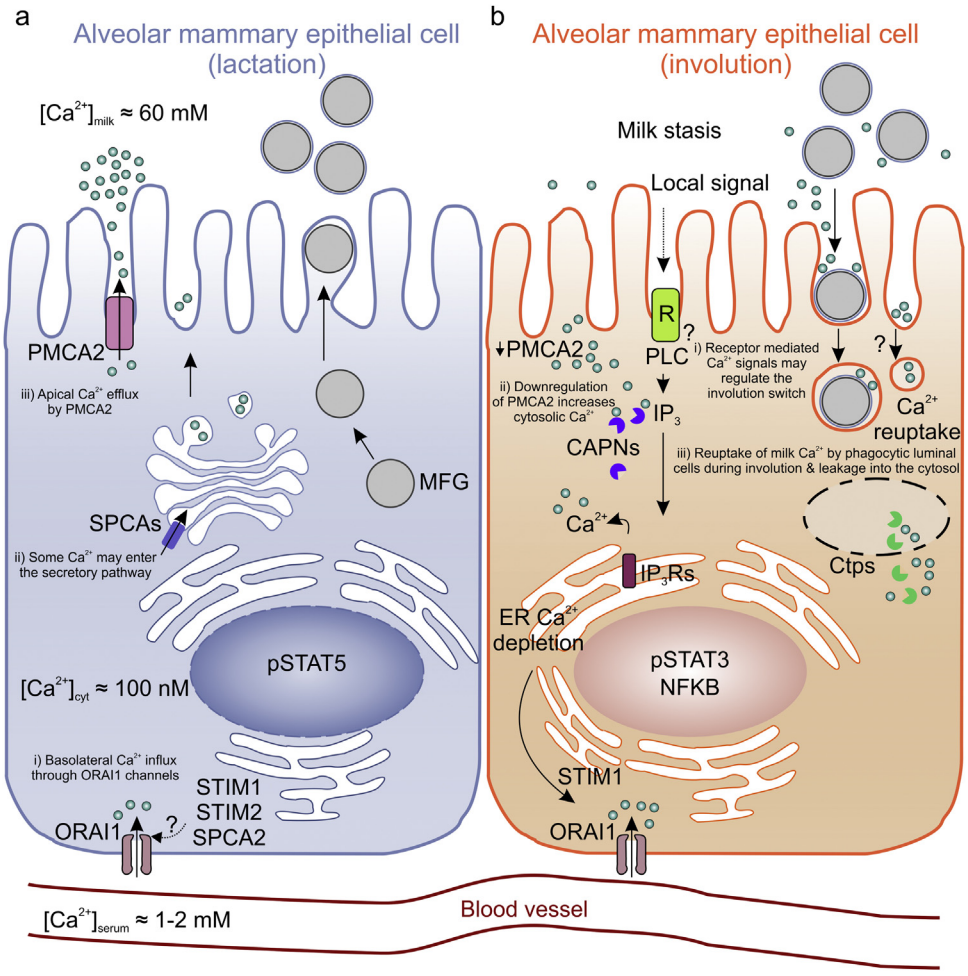

Fig. 1. An overview of Ca^{2+} signalling in lactation and involution. (a) Ca^{2+} transport in mouse alveolar luminal cells during lactation. Ca^{2+} is transported across the basolateral membrane by ORAI1 Ca^{2+} channels (i). Whilst some Ca^{2+} may enter the secretory pathway (ii), the majority of milk Ca^{2+} is transported across the apical membrane by PMCA2 (iii). (b) Proposed mechanisms by which altered Ca^{2+} handling in alveolar luminal cells may participate in post-lactational involution. Ca^{2+} signalling may be a key signal transduction pathway in the involution signalling cascade, potentially acting downstream of PLC-coupled cell surface receptors (i). The reduction in PMCA2 expression during the early phase of involution may elevate intracellular Ca^{2+} levels and activate CAPNs (ii). Luminal epithelial cells switch to a phagocytic phenotype during involution. The reuptake of milk, a Ca^{2+}-rich fluid, and its trafficking to the lysosomal compartment may drastically increase cytosolic Ca^{2+} levels following involution-associated lysosomal membrane permeabilisation (iii). Ca^{2+}, calcium; $[Ca^{2+}]_{milk}$, milk Ca^{2+} concentration; $[Ca^{2+}]_{cyt}$, cytosolic Ca^{2+} concentration; $[Ca^{2+}]_{serum}$, serum Ca^{2+} concentration; Ctps, cathepsin proteases; ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; MFG, milk fat globule; NFKB, nuclear factor kappa B; PLC, phospholipase C; PMCA2, plasma membrane Ca^{2+} ATPase 2; R, receptor; pSTAT, phosphorylated signal transducer and activator of transcription; SPCA, secretory pathway Ca^{2+} ATPase; STIM, stromal interaction molecule.

key role in the signalling events that instigate the switch from lactation to involution. This review discusses key basolateral and apical pathways involved in the fortification of milk with Ca^{2+} during lactation and investigates how this Ca^{2+} signalling toolkit may later expose these cells to programmed cell death, providing a basis for the targeted removal of the alveolar epithelium during mammary gland involution.

2. Basolateral Ca^{2+} entry

The dramatic expansion of the mammary epithelium during pregnancy is accompanied by marked angiogenic growth, such that by parturition networks of capillaries envelope each alveolus, providing luminal cells with an ample source of Ca^{2+} and other nutrients for milk production (Fig. 2) [11]. Until recently, the identity of the Ca^{2+} channels responsible for Ca^{2+} entry across the basolateral (blood-facing) membrane of luminal secretory cells during lactation was not known [12–15]. In 2011, McAndrew et al. demonstrated that the then newly-identified store-operated Ca^{2+} channel pore-forming subunit *Orai1* was significantly up-regulated

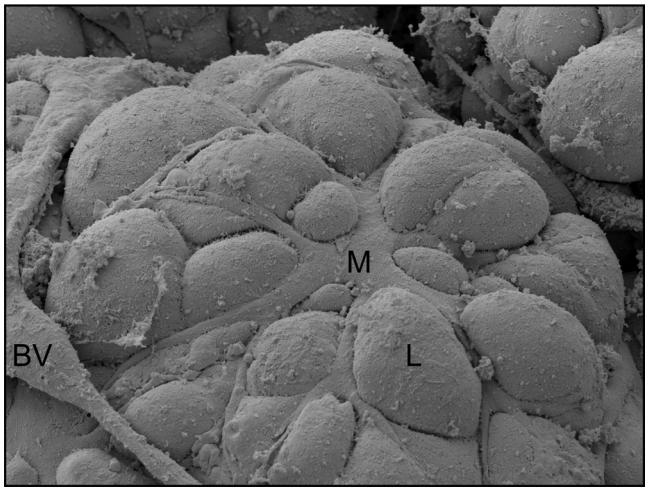

Fig. 2. Scanning electron microscopy (SEM) imaging of an alveoli from a lactating mouse mammary. L, luminal epithelial cell; M, myoepithelial cell; BV, blood vessel.