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Remodeling mechanisms of the mammary gland during involution

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ABSTRACT The process of post-lactational regression, or involution, of the mammary gland is a complex event characterised by extensive death of the secretory epithelium coupled with remodelling of the extracellular matrix and adipogenesis to regenerate the fat pad. Associated with these events is an inflammatory cascade and acute phase response. The critical signalling pathways that regulated involution have been defined and a wide variety of genes have been shown to modulate the various processes involved, including cell death, phagocytosis, tissue remodelling and innate immune response.

KEY WORDS: *cell death, apoptosis, Stat, lysosome, inflammation*

Introduction

The primary function of the mammary gland is to provide nutrition, in the form of milk, to newborn mammals. Milk also provides passive protection from infection and in higher mammals, suckling promotes bonding (Oftedal, 2002). However, this life-giving role has a flip side as the breast is highly susceptible to cancer (Bray *et al*., 2004). Although it has been established that an early full-term pregnancy provides some protection from breast cancer (Albrektsen *et al*., 1994; Lambe *et al*., 1994), there is compelling epidemiological evidence that pregnancy increases the risk of breast cancer in the short term (Lambe *et al*., 1994) and more recently, experimental evidence has suggested that the process of post-lactational regression provides a tumour promotional environment (McDaniel *et al*., 2006). In this article, I will review the process of post-lactational involution, the signalling pathways involved and the role of different cell types in this complex event.

The development of the mammary gland occurs in three distinct phases: during embryogenesis, puberty and pregnancy. A rudimentary structure is generated in the embryo and it is not until puberty that extensive ductal elongation occurs culminating in the filling of the fat pad with an extensively arbourised epithelium by about 8-10 weeks of age. In response to pregnancy, tertiary branching and formation of lobuloalveolar structures is initiated in concert with de-differentiation of the adipocytes in the fat pad. The mammary gland then becomes a milk-producing organ upon birth of the offspring and following their weaning, the now superfluous lobuloalveolar cells die in an exquisitely controlled process of cell death coupled with tissue remodelling and re-emergence of lipid-filled adipocytes. The gland is returned to an almost pre-pregnant state and this cycle of gestation, lactation and involution repeats itself with each successive pregnancy.

The process of involution

The process of involution is complex requiring not only the initiation of extensive cell death to remove the milk-producing epithelial cells but also the controlled influx of macrophages and other immune cell types to remove the dead cells, residual milk and debris. These events are coupled with breakdown of extracellular matrix, remodelling of blood vessels and re-differentiation of adipocytes to regenerate the fad pad.

In the past decade, our understanding of the molecular and cellular events that underlie involution in the mouse mammary gland has greatly increased primarily due to investigations with genetically modified mice and global transcriptional profiling studies which have provided a detailed analysis of the first 4 - 6 days of involution. In most of these studies a synchronous involution is induced by either removal of pups at the peak of lactation or by milk stasis using teat sealing. Interestingly, involution takes place in two discrete phases called first phase and second phase. The first phase lasts for approximately 48 hours in the mouse and is reversible, while in the second phase a remodelling programme is initiated that returns the gland to a pre-pregnant state (Lund *et al*., 1996). It seems likely that this early reversibility is required to

Abbreviations used in this paper: LMP, lysosomal membrane permeabilisation; MMP, matrix metalloproteinase; ROS, reactive oxygen species.

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allow offspring to re-initiate lactation by suckling if required after a prolonged period away from the mother. Notably, in fur seals lactation can recommence after a period of three weeks while the mother is offshore feeding (Sharp *et al.*, 2006). First phase involution is characterised by the appearance of shed, dying cells within the lumena of the alveoli which become expanded by the accumulation of milk. At this time, infiltration by neutrophils also occurs. Using teat-sealing in mice, it was demonstrated that the first phase is regulated by local factors and not by circulating hormones (Li *et al.*, 1997; Marti *et al.*, 1997).

In the second phase, cell death is accompanied by upregulation of matrix metalloproteinases (MMPs) which are important in the remodelling of the surrounding stroma which is accompanied by adipocyte refilling, influx of macrophages and active plasma kallikrein that regulates adipocyte differentiation and stromal remodelling (Lilla *et al.*, 2009). This phase is dependent on circulating factors and can be halted by the administration of glucocorticoid (Feng *et al.*, 1995; Lund *et al.*, 1996), which may act through the maintenance of tight junctions (Zettl *et al.*, 1992). MMP function is blocked in the first phase by expression of tissue inhibitors of metalloproteinases (TIMPs)(Green and Lund, 2005).

Transcriptional profiling data have further subdivided these two functional phases into four discrete transcriptional events (Clarkson *et al.*, 2004; Stein *et al.*, 2004), marked by four clusters of genes that have distinct temporal patterns of expression. A subset of genes is dramatically induced within 12 hours of forced involution with expression declining rapidly thereafter. This could suggest that a different mechanisms is triggering cell death in the first phase of involution than in the second. Whether there are four distinct phases to the involution process, rather than the two described morphologically, is not yet clear. Therefore, in any study of involution, it is important to consider these early signalling events and the fact that cell death appears to be a stochastic process. It is also essential to consider the nonepithelial cells of the gland, as both the adipocytes and the vasculature are dramatically modified during involution, and phagocytes (both professional and nonprofessional) are essential for clearing the gland of cell debris and milk components.

The essential signalling pathways that initiate involution have been identified using sophisticated genetic approaches. Although a considerable number of genes are implicated in cell death regulation during involution, many of these might be downstream components of these signalling pathways, and thus may have a minor role to play in the overall process. It is clear that involution is a highly complex process in which all aspects are tightly regulated. In this review we will consider in detail the mechanism and control of cell death and the role of the innate immune system in remodelling events.

Cell death during involution – how is it initiated?

Milk stasis seems to be the primary trigger of involution and cell death (Li *et al.*, 1997). There have been several suggestions as to how milk stasis might initiate involution including mechanical stretch of the alveolar epithelium due to milk retention in the lumen (Quaglino *et al.*, 2009) or the build up of secreted factors in the milk. The plasma membrane calcium-ATPase 2 (PMCA2),which transports 60–70% of milk calcium, is dramatically upregulated during lactation to be just as dramatically downregulated during involution. Recently, PMCA2 was shown to be regulated by changes in the shape of mammary epithelial cells (VanHouten *et al.*, 2010). Furthermore, mice harbouring a null mutation in the *Atp2b2* gene (encoding PMCA2) exhibited precocious cell death at day 18 of pregnancy and this was accompanied by activation of Stat3. It seems likely that secreted factors such as LIF (Kritikou *et al.*, 2003; Schere-Levy *et al.*, 2003), serotonin (Matsuda *et al.*, 2004), and TGFβ-3 (Nguyen and Pollard, 2000) are also important in inducing cell death as mice deficient for LIF exhibited delayed involution (Kritikou *et al.*, 2003) while directed expression of TGFβ-3 in the alveolar epithelium of lactating mice induced apoptosis without tissue remodelling. Serotonin suppresses β-casein gene expression and causes mammary alveoli to shrink.

A considerable number of genes have been implicated in involution. These are summarised in Table 1. Much of these data have been derived from genetically altered mice and the involution phenotypes are often subtle, suggesting that crosstalk between pathways is important. The most dramatic delay in involution is seen when the transcription factor Stat3 is conditionally deleted in mammary epithelium. Cell death and tissue remodelling are completely abrogated in the absence of Stat3 (Chapman *et al.*, 1999) and the reversible phase of involution is extended until at least 6 days post forced involution (Humphreys *et al.*, 2002). A number of

TABLE 1

FACTORS THAT REGULATE INVOLUTION

Gene	Function	Method of assessment
Factors that inhibit involution and/or cell death		
IRF-1	Transcription factor	Gene deletion
Stat5	Transcription factor	Overexpressing transgenic
TIMP-3	MMP inhibitor	Gene deletion
SOCS-3	Cytokine signalling regulator	Conditional gene deletion
Akt	Serine/threonine kinase	Overexpressing transgenic
Sim2s	Transcription factor	Overexpressing transgenic
Bcl2	Apoptosis regulator	Overexpressing transgenic
Bcl-x	Apoptosis regulator	Conditional gene deletion
IGF-I	Growth factor	Overexpressing transgenic
IGF-II	Growth factor	Overexpressing transgenic
JAK2	Kinase	Constitutively active mutant
Factors that promote involution and/or cell death		
Stat3	Transcription factor	Conditional gene deletion
c/ebpδ	Transcription factor	Gene deletion
IKK2/β	Regulatory kinase	Conditional gene deletion
p53	Transcription factor	Gene deletion
PTEN	lipid phosphatase	Overexpressing transgenic
IL-6	Cytokine	Gene deletion
LIF	Cytokine	Gene deletion
FasL	Cytokine	Gene deletion
IL-10	Cytokine	Gene deletion
Bax	Apoptosis regulator	Gene deletion
Cathepsin L	Cysteine protease	Specific inhibitor
ATF4	Transcription factor	Overexpressing transgenic
Smad3	TGF-β signalling mediator	Gene deletion
IGFBP-5	IGF inhibitor	Overexpressing transgenic
α-lactalbumin multimers	Milk protein	Culture studies
Mnt	Transcriptional repressor	Conditional gene deletion
Factors that affect remodeling and/or biosynthesis		
plasmin	Serine protease	Gene deletion
Mfge8	Phagocytosis regulator	Gene deletion
kallikrein	Plasminogen activator	Inhibitor studies
MMP3	protease	Gene deletion

genes that are regulated by Stat3, such as c-ebpu61540, OSMR and IGFBP-5, are also regulators of cell death and involution (Thangaraju *et al*., 2005). A similar delay is observed when the upstream regulator of the NF-κB pathways, IKKβ, is conditionally deleted (Baxter *et al*., 2006) suggesting that crosstalk between these signalling pathways is required for the process of involution to occur and that each pathway is necessary, but neither is sufficient, to trigger cell death. Components of apoptosis pathways have also been implicated including death receptor ligands such as FasL (Song *et al*., 2000) which is important for apoptosis at 24 h involution and TRAIL, which is upregulated by IL-10 during involution (Sohn *et al*., 2001) in addition to members of the Bcl2 family of apoptosis regulators such as Bax, which promotes cell death as deletion of Bax results in a modest decrease in apoptosis in first phase and Bcl2, which promotes survival of the epithelium in a gain-of-function transgenic model (Schorr *et al*., 1999). Loss of the anti-apoptotic Bcl-xL accelerates involution (Walton *et al*., 2001). During second phase involution, cell death occurs alongside tissue remodelling and it is more difficult to discern delayed involution phenotypes. However, removal of dead cells and residual milk is critical for involution to proceed and subsequent successful lactation as reduced phagocytosis in the absence of the bridging molecule MFGE8 results in inflammation and tissue scarring and subsequent failed lactation (Atabai *et al*., 2005; Hanayama and Nagata, 2005).

What is the mechanism of cell death during involution?

Until recently, the mechanism responsible for the destruction of the secretory epithelium during involution was presumed to be apoptosis, a form of programmed cell death that occurs in all multicellular animals. The term apoptosis (Greek for 'falling leaves') was coined by Kerr, Wyllie and Currie in 1972 (Kerr *et al*., 1972). The genetics and biochemistry of apoptosis have been extensively studied (Danial and Korsmeyer, 2004). However, it is now apparent that there are multiple cell death pathways and as many as ten genetically programmed cell death pathways have been defined that occur in different situations and in response to diverse stimuli (Melino *et al*., 2005).

The morphology of the dying cells that are detached from the alveolar wall and shed into the lumen is not classically apoptotic (Fig. 1). Shed cells appear to be swollen, have two hypercondensed nuclei, and a complete lack of membrane blebbing. They show no features of entosis, a recently described form of programmed cell death that has been described in mammary epithelial cells *in vitro* (Overholtzer *et al*., 2007). Furthermore, TdT-mediated dUTP nick end labelled (TUNEL) positive cells could not be detected in the first 24 h of involution although these are apparent at 72 h involution. However, executioner caspases are still activated during involution and indeed cleavage of caspase 3 if often used as a measure of cell death during involution. Recently, the reason for this unusual morphology was discovered. Stat3 was shown to induce a lysosomal pathway of cell death (LM-PCD) that is independent of executioner caspases, which are dispensible for involution (Kreuzaler *et al*.). Interestingly, Stat3 upregulates the expression of the lysosomal enzymes cathepsin B and cathepsin L while downregulating the expression of their endogenous inhibitor Spi2a. Cathepsins leak from the lysosomes during involution and initiate cell death, resulting in the shedding of cells into the lumen where caspases 3 and 6 are cleaved, probably in response to anoikis resulting from detachment from neighbouring alveolar cells. This was the first demonstration that a lysosomal pathway of cell death occurs in a normal physiological context *in vivo*. It will be interesting to determine whether other signalling pathways contribute to LM-PCD during involution. This discovery requires a re-appraisal of previous data on involution. Mediators of LMP are depicted in Fig. 2.

It is distinctly possible that macroautophagy also occurs during involution. This is a survival mechanism, usually initiated by cells during starvation to recycle cellular components (Kroemer and Levine, 2008). Autophagy would provide a mechanism that ensures the reversibility of first phase involution. To date, there is scant evidence of autophagy during involution although genetic models are now available and a definitive answer to this question may become available in the bear future. It is worth noting in this context that fully functional lysosomes are required for autophagy.

How is the mammary gland remodelled during involution?

Although there is extensive cell death during involution, the architecture of the gland does not change notably during the first 48 h. However, the switch to second phase triggers extensive remodelling resulting in removal of the remaining epithelium concomitantly with redifferentiation of adipocytes. This phase is marked by protease activity, which remodels the extracellular matrix. It is not clear what the trigger is for the switch to second

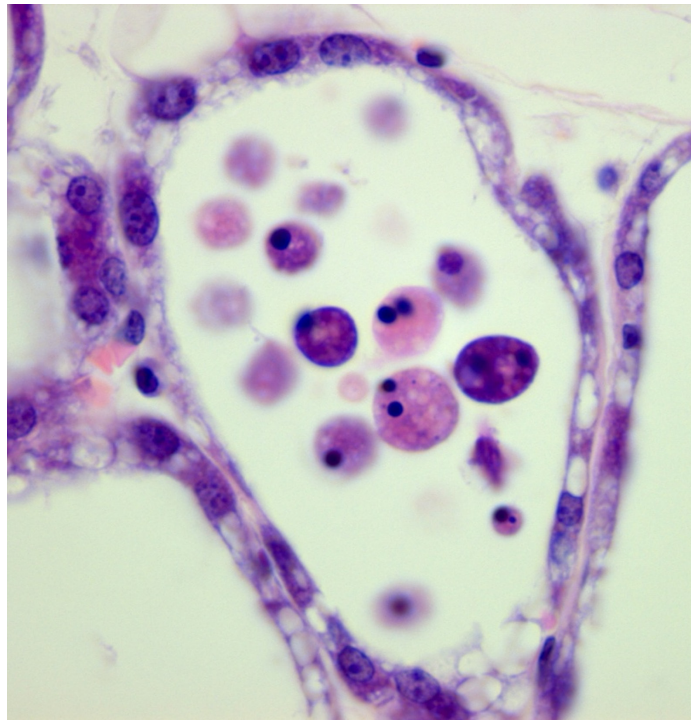

Fig. 1. Cell death during mammary gland involution does not resemble apoptosis. *A single alveolus from a 24 h involution mammary gland tissue section showing numerous cells shed into the alveolar lumen. Note the highly condensed nuclei and swollen appearance of the cells with no evidence of membrane blebbing.*

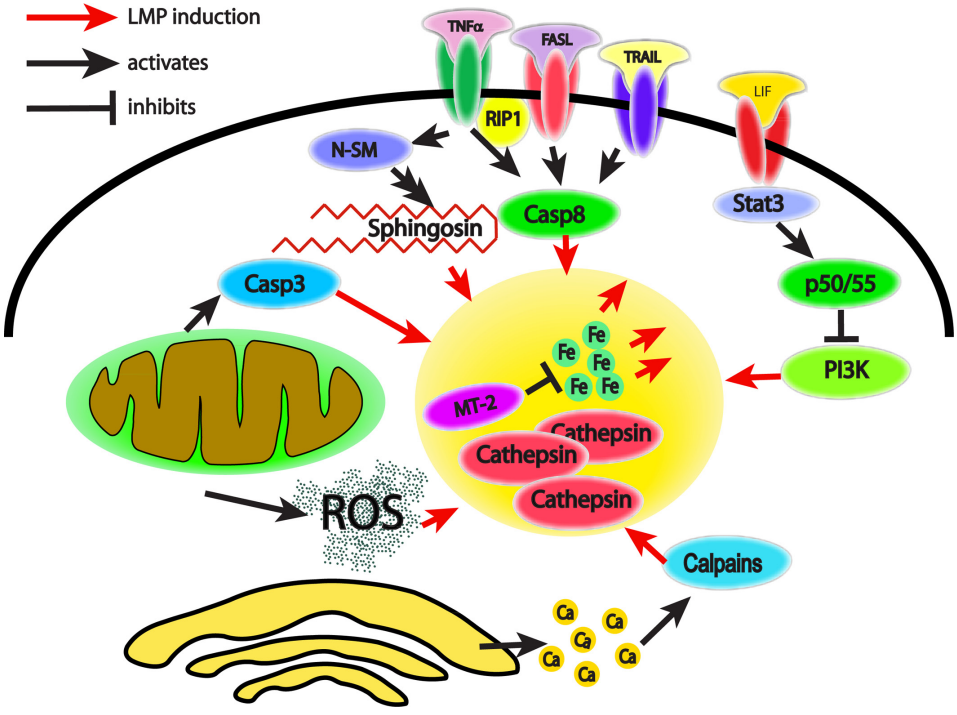

Fig. 2. Inducers of Lysosomal Membrane Permeabilisation (LMP). *Schematic overview of selected inducers of LMP relevant to mammary gland involution. LMP can be induced downstream of death receptors either directly or through the involvement of Caspase 8. Furthermore TNF can activate neutral sphingomyelinase, which ultimately leads to the production of sphingosine and LMP. Damaged mitochondria produce ROS and activate caspase 3, both of which are implicated in LMP. Calcium activated calpains, lysosomal cathepsins and the PI3K pathway can all affect LMP. Metallothionein 2 can protect lysosomes from excessive free iron.*

phase but it could be that changes in systemic hormones lead to the induction of the expression of proteases such as MMPs that degrade extracellular matrix (ECM), and serine proteases that activate plasminogen.

The primary source of MMPs is the stromal cells, and expression of MMP-2, MMP-3, MMP-9 and MMP-11 is upregulated during involution. MMPs cleave a variety of substrates such as collagen IV, chemokines and E-cadherin, which will facilitate cell detachment. Thus, cells that have survived first phase involution can be induced to die by anoikis (detachment-induced cell death) provided MMPs are expressed and activated. The corollary of this is that MMPs must be inhibited during first phase involution and this is achieved by TIMPs. The inhibitor of MMP-2, TIMP-3, is of particular importance since involution proceeds more rapidly in TIMP-3 deficient mammary glands and the reversibility of first phase is lost (Fata *et al.*, 2001). Overexpression of TIMP-1, or loss of stromelysin, results in accelerated differentiation of adipocytes, while having no effect on cell death (Alexander *et al.*, 2001). This suggests that MMPs do not directly affect cell death but are primarily involved in remodelling the stromal environment.

Inflammatory signalling and immune cells in involution

The activation of Stat3 and NF-κB signalling pathways suggests that inflammatory signalling may also be important during involution. This notion is supported by data from the microarray studies mentioned above that identified an immune cascade and acute phase response during involution (Clarkson *et al.*, 2004; Stein *et al.*, 2004). Importantly, both pro- and anti-inflammatory genes are expressed, probably in order to ensure that overt inflammation does not occur. An influx of immune cells has been observed, starting with neutrophils at day 1 involution followed by macrophages, eosinophils, plasma cells and B-lymphocytes around day 4 (Stein *et al.*, 2004). These cells are often found in a peri-ductal location but immune cells have been found in the milk of sheep and cows during involution, suggesting that they can traverse into the lumens of ducts (Tatarczuch *et al.*, 2000).

Phagocytes are critically important to the involution process as mentioned above. In early involution, this role is undertaken by the viable epithelium utilising the same recognition molecules as professional phagocytes. The scavenger receptor, CD14, is strongly induced in involution in a Stat3-dependent manner and mammary epithelial cells have been shown to express calreticulin, CD91 and CD36 (Monks *et al.*, 2005). Engulfment induces production of anti-inflammatory cytokines such as TGFβ (Monks *et al.*, 2002), essential for ensuring involution proceeds without inflammation.

Expression of acute phase response genes is another characteristic of involution, perhaps not surprisingly as many of these genes are regulated by Stat3 and/or NF-κB. One of these is uterocalin, a lipocalin that induces apoptosis of neutrophils and other leukocytes. Uterocalin could protect mammary epithelial cells from damaging free radicals and proteases released from leukocytes and macrophages. Among the most strikingly upregulated genes are orosomucoids 1 and 2 which act as carriers of basic and neutrally charged lipophilic compounds including steroids and protease inhibitors. Serum amyloid A proteins, the precursors of inflammation-associated reactive amyloidosis, can bind to high density lipoproteins, calcium, laminin and heparin/heparan-sulfate, and have been implicated in inducing leukocyte migration.

Conclusion

Steady progress has been made in investigating the mechanisms of mammary gland involution. Analyses of changes in gene expression during the involution process have highlighted roles for immune cells and the inflammatory/acute phase responses. A role for mammary epithelia cells as phagocytes has highlighted the plasticity of these cells. Recent insights have been obtained into the mechanism of cell death which will provide the basis for much future research. However, several outstanding questions remain and could be the focus for future work. Is the initiating signal for apoptosis mechanical or biological? How is the transition from the reversible to irreversible phase controlled? How are the stem cells protected from cell death? These are challenging questions

to address and, despite our progress, we still lack a detailed understanding of the involution process.

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References

ALBREKTSEN, G., HEUCH, I., TRETLI, S., and KVALE, G. (1994). Breast cancer incidence before age 55 in relation to parity and age at first and last births: a prospective study of one million Norwegian women. *Epidemiology* 5: 604-611.

ALEXANDER, C.M., SELVARAJAN, S., MUDGETT, J., and WERB, Z. (2001). Stromelysin-1 regulates adipogenesis during mammary gland involution. *J Cell Biol* 152: 693-703.

ATABAI, K., FERNANDEZ, R., HUANG, X., UEKI, I., KLINE, A., LI, Y., SADATMAN-SOORI, S., SMITH-STEINHART, C., ZHU, W., PYTELA, R., *ET Al.,* (2005). Mfge8 is critical for mammary gland remodeling during involution. *Mol Biol Cell* 16: 5528-5537.

BAXTER, F.O., CAME, P.J., ABELL, K., KEDJOUAR, B., HUTH, M., RAJEWSKY, K., PASPARAKIS, M., and WATSON, C.J. (2006). IKKbeta/2 induces TWEAK and apoptosis in mammary epithelial cells. *Development* 133: 3485-3494.

BRAY, F., MCCARRON, P., and PARKIN, D.M. (2004). The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res* 6: 229-239.

CHAPMAN, R.S., LOURENCO, P.C., TONNER, E., FLINT, D.J., SELBERT, S., TAKEDA, K., AKIRA, S., CLARKE, A.R., and WATSON, C.J. (1999). Suppression of epithelial apoptosis and delayed mammary gland involution in mice with a conditional knockout of Stat3. *Genes Dev* 13: 2604-2616.

CLARKSON, R.W., WAYLAND, M.T., LEE, J., FREEMAN, T., and WATSON, C.J. (2004). Gene expression profiling of mammary gland development reveals putative roles for death receptors and immune mediators in post-lactational regression. *Breast Cancer Res* 6, R92-109.

DANIAL, N.N., and KORSMEYER, S.J. (2004). Cell death: critical control points. *Cell* 116: 205-219.

FATA, J.E., LECO, K.J., VOURA, E.B., YU, H.Y., WATERHOUSE, P., MURPHY, G., MOOREHEAD, R.A., and KHOKHA, R. (2001). Accelerated apoptosis in the Timp-3-deficient mammary gland. *J Clin Invest* 108: 831-841.

FENG, Z., MARTI, A., JEHN, B., ALTERMATT, H.J., CHICAIZA, G., and JAGGI, R. (1995). Glucocorticoid and progesterone inhibit involution and programmed cell death in the mouse mammary gland. *J Cell Biol* 131: 1095-1103.

GREEN, K.A., and LUND, L.R. (2005). ECM degrading proteases and tissue remodelling in the mammary gland. *Bioessays* 27: 894-903.

HANAYAMA, R., and NAGATA, S. (2005). Impaired involution of mammary glands in the absence of milk fat globule EGF factor 8. *Proc Natl Acad Sci USA* 102: 16886-16891.

HUMPHREYS, R.C., BIERIE, B., ZHAO, L., RAZ, R., LEVY, D., and HENNIGHAUSEN, L. (2002). Deletion of Stat3 blocks mammary gland involution and extends functional competence of the secretory epithelium in the absence of lactogenic stimuli. *Endocrinology* 143: 3641-3650.

KERR, J.F., WYLLIE, A.H., and CURRIE, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26: 239-257.

Kreuzaler, P.A., Staniszewska, A.D., Li, W., Omidvar, N., Kedjouar, B., Turkson, J., Poli, V., Flavell, R.A., Clarkson, R.W., and Watson, C.J. (2011) Stat3 controls lysosomal-mediated cell death in vivo. *Nat Cell Biol* 13: 303-309.

KRITIKOU, E.A., SHARKEY, A., ABELL, K., CAME, P.J., ANDERSON, E., CLARKSON, R.W., and WATSON, C.J. (2003). A dual, non-redundant, role for LIF as a regulator of development and STAT3-mediated cell death in mammary gland. *Development* 130: 3459-3468.

KROEMER, G., and LEVINE, B. (2008). Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* 9: 1004-1010.

LAMBE, M., HSIEH, C., TRICHOPOULOS, D., EKBOM, A., PAVIA, M., and ADAMI, H.O. (1994). Transient increase in the risk of breast cancer after giving birth. *N Engl J Med* 331: 5-9.

LI, M., LIU, X., ROBINSON, G., BAR-PELED, U., WAGNER, K.U., YOUNG, W.S., HENNIGHAUSEN, L., and FURTH, P.A. (1997). Mammary-derived signals activate programmed cell death during the first stage of mammary gland involution. *Proc Natl Acad Sci USA* 94: 3425-3430.

LILLA, J.N., JOSHI, R.V., CRAIK, C.S., and WERB, Z. (2009). Active plasma kallikrein localizes to mast cells and regulates epithelial cell apoptosis, adipocyte differentiation, and stromal remodeling during mammary gland involution. *J Biol Chem* 284: 13792-13803.

LUND, L.R., ROMER, J., THOMASSET, N., SOLBERG, H., PYKE, C., BISSELL, M.J., DANO, K., and WERB, Z. (1996). Two distinct phases of apoptosis in mammary gland involution: proteinase-independent and -dependent pathways. *Development* 122: 181-193.

MARTI, A., FENG, Z., ALTERMATT, H.J., and JAGGI, R. (1997). Milk accumulation triggers apoptosis of mammary epithelial cells. *Eur J Cell Biol* 73: 158-165.

MATSUDA, M., IMAOKA, T., VOMACHKA, A.J., GUDELSKY, G.A., HOU, Z., MISTRY, M., BAILEY, J.P., NIEPORT, K.M., WALTHER, D.J., BADER, M., *ET AL.,* (2004). Serotonin regulates mammary gland development via an autocrine-paracrine loop. *Dev Cell* 6: 193-203.

MCDANIEL, S.M., RUMER, K.K., BIROC, S.L., METZ, R.P., SINGH, M., PORTER, W., and SCHEDIN, P. (2006). Remodeling of the mammary microenvironment after lactation promotes breast tumor cell metastasis. *Am J Pathol* 168: 608-620.

MELINO, G., KNIGHT, R.A., and NICOTERA, P. (2005). How many ways to die? How many different models of cell death? *Cell Death Differ* 12 Suppl 2: 1457-1462.

MONKS, J., GESKE, F.J., LEHMAN, L., and FADOK, V.A. (2002). Do inflammatory cells participate in mammary gland involution? *J Mammary Gland Biol Neoplasia* 7: 163-176.

MONKS, J., ROSNER, D., GESKE, F.J., LEHMAN, L., HANSON, L., NEVILLE, M.C., and FADOK, V.A. (2005). Epithelial cells as phagocytes: apoptotic epithelial cells are engulfed by mammary alveolar epithelial cells and repress inflammatory mediator release. *Cell Death Differ* 12: 107-114.

NGUYEN, A.V., and POLLARD, J.W. (2000). Transforming growth factor beta3 induces cell death during the first stage of mammary gland involution. *Development* 127: 3107-3118.

OFTEDAL, O.T. (2002). The mammary gland and its origin during synapsid evolution. *J Mammary Gland Biol Neoplasia* 7: 225-252.

OVERHOLTZER, M., MAILLEUX, A.A., MOUNEIMNE, G., NORMAND, G., SCHNITT, S.J., KING, R.W., CIBAS, E.S., and BRUGGE, J.S. (2007). A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. *Cell* 131: 966-979.

QUAGLINO, A., SALIERNO, M., PELLEGROTTI, J., RUBINSTEIN, N., and KORDON, E.C. (2009). Mechanical strain induces involution-associated events in mammary epithelial cells. *BMC Cell Biol* 10: 55.

SCHERE-LEVY, C., BUGGIANO, V., QUAGLINO, A., GATTELLI, A., CIRIO, M.C., PIAZZON, I., VANZULLI, S., and KORDON, E.C. (2003). Leukemia inhibitory factor induces apoptosis of the mammary epithelial cells and participates in mouse mammary gland involution. *Exp Cell Res* 282: 35-47.

SCHORR, K., LI, M., BAR-PELED, U., LEWIS, A., HEREDIA, A., LEWIS, B., KNUDSON, C.M., KORSMEYER, S.J., JAGER, R., WEIHER, H., *et al.,* (1999). Gain of Bcl-2 is more potent than bax loss in regulating mammary epithelial cell survival in vivo. *Cancer Res* 59: 2541-2545.

SHARP, J.A., CANE, K.N., LEFEVRE, C., ARNOULD, J.P., and NICHOLAS, K.R. (2006). Fur seal adaptations to lactation: insights into mammary gland function. *Curr Top Dev Biol* 72: 275-308.

SOHN, B.H., MOON, H.B., KIM, T.Y., KANG, H.S., BAE, Y.S., LEE, K.K., and KIM, S.J. (2001). Interleukin-10 up-regulates tumour-necrosis-factor-alpha-related apoptosis-inducing ligand (TRAIL) gene expression in mammary epithelial cells at the involution stage. *Biochem J* 360: 31-38.

SONG, J., SAPI, E., BROWN, W., NILSEN, J., TARTARO, K., KACINSKI, B.M., CRAFT, J., NAFTOLIN, F., and MOR, G. (2000). Roles of Fas and Fas ligand during mammary gland remodeling. *J Clin Invest* 106: 1209-1220.

STEIN, T., MORRIS, J.S., DAVIES, C.R., WEBER-HALL, S.J., DUFFY, M.A., HEATH, V.J., BELL, A.K., FERRIER, R.K., SANDILANDS, G.P., and GUSTERSON, B.A. (2004). Involution of the mouse mammary gland is associated with an immune cascade and an acute-phase response, involving LBP, CD14 and STAT3. *Breast Cancer Res* 6, R75-91.

TATARCZUCH, L., PHILIP, C., BISCHOF, R., and LEE, C.S. (2000). Leucocyte phenotypes in involuting and fully involuted mammary glandular tissues and secretions of sheep. *J Anat* 196 (Pt 3), 313-326.

THANGARAJU, M., RUDELIUS, M., BIERIE, B., RAFFELD, M., SHARAN, S., HENNIGHAUSEN, L., HUANG, A.M., and STERNECK, E. (2005). C/EBPdelta is a crucial regulator of pro-apoptotic gene expression during mammary gland involution. *Development* 132: 4675-4685.

VANHOUTEN, J., SULLIVAN, C., BAZINET, C., RYOO, T., CAMP, R., RIMM, D.L., CHUNG, G., and WYSOLMERSKI, J. (2010) PMCA2 regulates apoptosis during mammary gland involution and predicts outcome in breast cancer. *Proc Natl Acad Sci USA* 107: 11405-11410.

WALTON, K.D., WAGNER, K.U., RUCKER, E.B., 3RD, SHILLINGFORD, J.M., MIYOSHI, K., and HENNIGHAUSEN, L. (2001). Conditional deletion of the bcl-x gene from mouse mammary epithelium results in accelerated apoptosis during involution but does not compromise cell function during lactation. *Mech Dev* 109: 281-293.

ZETTL, K.S., SJAASTAD, M.D., RISKIN, P.M., PARRY, G., MACHEN, T.E., and FIRESTONE, G.L. (1992). Glucocorticoid-induced formation of tight junctions in mouse mammary epithelial cells in vitro. *Proc Natl Acad Sci USA* 89: 9069-9073.