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Apoptosis and Clearance of the Secretory Mammary Epithelium

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1. Introduction

The development of the mammary gland occurs in four distinct phases: embryogenesis, puberty, pregnancy, and a post-lactational phase involving profound levels of cell death and tissue remodeling. This post-lactational phase is termed post-lactational involution. During embryogenesis, a solid epithelial bud is generated in the embryonic ectoderm. As this bud continues to grow in cell number, the epithelial bud invaginates into the underlying mesenchyme forming the nascent mammary epithelium. The mammary epithelium grows as solid epithelial cords, lengthening distally and branching to form the rudimentary epithelial network. At puberty, ductal elongation continues in a proximal-to-distal direction, and side branches appear along the ducts. The side branches also lengthen distally, and continue to branch. This pattern of distal growth and branching fills the mouse mammary fat pad with an extensively branched epithelium by the end of puberty [6]. Similar to what is seen during embryonic mammary development and patterning, the mammary ducts developing during puberty originally appear in solid epithelial cords. Apoptosis canalizes the luminal space within the ducts, allowing a patent conduit for milk to traverse through the breast epithelium [1, 7]. Ultimately, the rodent mammary epithelium is comprised of a continuous, branching network leading from the nipple to primary ducts and smaller ductules that terminate in terminal end buds (TEBs), blunt ends or alveoli. The inner luminal cells are separated from the basement membrane by an outer myoepithelial layer. Myoepithelial cells secrete basement membrane components to which the epithelium attaches, and that physically separates the epithelium from the stromal compartment.

Many morphological similarities exist between the mouse mammary gland and the human breast, although some distinctions exist. In the human breast, the cluster of epithelial acini arising from a single terminal duct, referred to as the terminal duct lobular unit (TDLU), is thought to be the milk-producing unit of the mammary gland. Therefore, the post-pubertal

human breast harbors cells capable of milk production even in the absence of pregnancy whereas the rodent mammary gland does not. However, profound expansion and differentiation of the TDLU population in the human breast is still required in order to render lactation successful.

This expansion of the alveolar epithelium during pregnancy occurs in response to both local and systemic factors that drive mammary alveolar proliferation. In rodents, the entire secretory epithelium of the mammary gland develops during the gestation period of approximately three weeks, signifying a rapid 10-fold increase in epithelial content of the mammary gland. The mammary gland produces colostrum and then milk upon parturition. However, once offspring are weaned, the milk-producing lobuloalveolar cells are no longer necessary. Rather than being maintained, these secretory cells undergo programmed cell death in an exquisitely controlled and rapid process, while leaving the ductal epithelium intact. Dying cells are cleared from the post-lactational mammary gland rapidly and without causing acute inflammation, removing up to 90% of the total mammary epithelial content within the time span of just one week in rodent models. This returns the mammary gland to an almost pre-pregnant state, so that the changes in the mammary gland that accompany pregnancy, lactation and involution may again occur with each successive pregnancy.

The process of involution is complex requiring two distinct phases, the initiation of extensive cell death to remove the milk-producing epithelial cells, followed by the controlled influx of macrophages and other immune cell types to breakdown extracellular matrix, remodel blood vessels, replenish the adipocyte population in the mammary fatpad, and to phagocytically remove dead cells, residual milk, and debris. This review will focus primarily on the events controlling cell death that occur within the first days of post-lactational involution.

2. Body

2.1. Signaling mechanisms that control post-lactational apoptosis in the breast

In recent years, molecular regulation of post-lactational involution has been studied primarily in the mouse mammary gland, due in large part to the relatively rapid gestation and nursing period in mice, and to the extensive use of genetically engineered mouse models. These models, coupled with advances in transcriptional profiling have provided a detailed analysis of the dynamic cellular and molecular events occurring during the earliest days of involution, when the majority of programmed cell death occurs.

2.2. Milk stasis

Using teat-sealing to block milk delivery in a single mouse mammary gland, investigators demonstrated that a complex multi-step process initiating massive epithelial apoptosis is triggered by local stimuli produced in the sealed mammary gland, but not by changing levels of circulating hormones that are available to the remaining nine mouse mammary glands [8, 9]. These studies revealed that milk stasis is a primary trigger of post-lactational

cell death in the mammary gland [8]. Accumulation of milk within the secretory luminal space might initiate cell death by causing a mechanical stretch of these cells, or of cell-cell junctions [10]. It is clear that mechanical stress, including cell stretching, can initiate biological responses in several epithelial and endothelial cell types, and may activate signaling pathways known to trigger cell death in the post-lactational mammary gland. For example, cell stretching induces STAT3 phosphorylation, inhibition of the survival factor AKT, and expression of Leukemia Inhibitory Factor (LIF), each of which are critical during early post-lactational involution for the induction of cell death, as discussed below. Another potential explanation of milk stasis-induced cell death is that accumulation of milk components, such as calcium, may trigger cell death [11]. In support of this hypothesis, transcription of the plasma membrane protein calcium-ATPase 2 (PMCA2), which transports 60–70% of milk calcium [12], is dramatically and rapidly reduced during involution, perhaps due to self-limiting negative feedback in an effort to control potentially toxic divalent cation levels [13]. Loss of the gene encoding PMCA2 (*Atp2b2*) in mice caused precocious alveolar cell death at lactation. Interestingly, PMCA2 expression is also regulated by enforced shape changes in mammary epithelial cells [13]. Stanniocalcin-1 (STC-1), a newly discovered mammalian hormone that accumulates nearly 3-fold upon milk stasis [14], has recently been implicated as an inducer of post-lactational involution [15].

2.3. STAT3

Transcriptional profiling studies of the mouse mammary gland at specific time points during lactation and post-lactational involution demonstrated that a specific subset of genes is dramatically induced within 12 hours of pup withdrawal, presumably in response to milk accumulation [4, 16]. It was hypothesized that this gene subset may represent potential ‘master regulators’ of programmed cell death in the post-lactational mammary gland. This idea has been largely confirmed using genetically engineered mouse models that disrupt key expression events, resulting in a delay in post-lactational programmed cell death.

The transcription factor Signal Transducer and Activator of Transcription (STAT) 3 was conditionally deleted in the mammary epithelium of genetically engineered mice, revealing its critical role in initiating the earliest events in post-lactational apoptosis [17–19]. While it has been known for some time that STAT3 regulates the expression of pro-inflammatory genes involved in the acute phase response (the early inflammatory response to tissue injury) [20, 21], and that many inflammation-related genes are expressed during post-lactational involution [4, 22, 23], these studies were the first to demonstrate the molecular similarities that exist between the involuting mammary gland and the traditional wound healing scenario [23], despite the fact that involution-induced transcriptional responses are directed primarily by epithelial cells, while wound healing-induced transcriptional responses are directed by cells of the immune system.

STAT3 is widely expressed, and is activated by tyrosine phosphorylation in response to numerous cytokines and growth factors [e.g. interleukin-6 (IL-6), IL-10, IL-17, IL-23, EGF] and tyrosine kinases (c-Src, Met, ErbB-2) [24]. Tyrosine phosphorylation of STAT3 allows

STAT3 dimers to translocate from the cytoplasm to the nucleus, where STAT3 binds to sequence-specific DNA elements in the promoters of STAT3 target genes. STAT3 activates gene transcription of many inflammation related genes, and can also repress the transcription of others. Although STAT3 transcriptional activity is associated with cell survival in several cell types including lymphomas and solid tumor epithelial cells [25-28], STAT3 takes on a different role in the post-lactational mammary gland, where STAT3 is required to initiate cell death. In the absence of STAT3, cell death was abrogated for at least 6 days after pup withdrawal, despite milk stasis [18, 19]. Conversely, loss of suppressor of cytokine signaling (SOCS)-3, a negative regulator of STAT3, accelerated involution by increasing the rate of cell death following pup withdrawal [29, 30].

A number of genes regulated by STAT3, such as CAAT/enhancer binding protein (C/ebp) δ , oncostatin M (OSM), OSM receptor (OSMR), and insulin-like growth factor (IGF) binding protein (IGFBP)-5, are also required in the post-lactational mammary epithelium to initiate cell death [31, 32]. OSM, a cytokine normally produced by macrophages but in this case produced by the mammary epithelium, is required during post-lactational involution, since OSMR knockout mice exhibited delayed involution [33]. Loss of C/ebp δ , a transcription factor involved in the acute phase response, also delayed mammary gland involution [31]. Because STAT3, and many target genes activated by STAT3, are critical triggers of cell death during post-lactational involution, it is likely that STAT3 lies at the apex of a transcriptionally-activated signaling cascade that is required to initiate cell death in the post-lactational mammary epithelium. This role of STAT3 as an apoptosis inducer lies in contrast with observations that STAT3 is frequently activated in several cancer entities [28], correlating with heightened malignancy [34, 35]. Further, constitutive STAT3 activity promotes tumor formation in skin [36] and lung [37, 38]. The apparent discrepancy may be related to tissue specificity of STAT3 activity, or the activity of STAT3 in the tumor microenvironment (for example, in inflammatory cells) versus its role in the epithelial compartment of the tumor.

2.4. NF- κ B

Although the role of STAT3 in the induction of post-lactational apoptosis is clear, STAT3 alone is insufficient to induce involution in the absence of nuclear factor- κ B (NF- κ B) signaling [39]. NF- κ B comprises a family of five structurally and functionally related transcription factors [40]. Based on their transactivation properties, NF- κ B proteins are divided into two classes: Class I consists of RelA/p65, RelB, and c-Rel, while Class II includes NF- κ B1/p50 and NF- κ B2/p52. Each can dimerize in almost any combination but only class I proteins possess the C-terminal transactivation domains required for NF- κ B-mediated transcription of target genes. Under basal conditions, NF- κ B dimers are sequestered in the cytoplasm bound to the protein Inhibitor of κ B (I κ B). Several signaling pathways can activate the I κ B kinases (IKKs) that phosphorylate I κ B, thus liberating NF- κ B dimers and allowing their nuclear translocation, where they bind to specific DNA sequences in target genes.

Among this family of transcription factors, two NF- κ B subunits, RelA (p65) and p50 are expressed at different levels in the mammary epithelium throughout mammary gland

development. Furthermore, NF- κ B activity as measured *in vivo* using a transgenic NF- κ B reporter model demonstrated two major peaks of NF- κ B-mediated trans-activation: one that occurs during pregnancy, and another that occurs during involution [41]. These data are consistent with reports showing that NF- κ B induced transcription of pro-survival genes [42-45], and other reports showing that NF κ B activated transcription of pro-apoptotic genes [46-48]. Therefore, it is possible that NF- κ B might drive cell growth and survival in the breast epithelium in some cases, but may regulate breast epithelial cell death in others. In support of the idea that NF- κ B might promote cell death, increased NF- κ B activity is rapidly induced after weaning, with strong increases seen within one hour of milk stasis in mouse mammary glands. NF- κ B activity remains elevated through the first four days of murine post-lactational involution. Furthermore, loss of NF- κ B signaling in a genetically engineered mouse model of conditional IKK- β disruption decreased post-lactational NF- κ B signaling, resulting in decreased caspase-3 cleavage and delayed post-lactational apoptosis [39], confirming the importance of NF κ B signaling in post-lactational cell death of the secretory epithelium. Conversely, constitutively active IKK- β increased NF- κ B signaling, thus causing accelerated induction and higher rates of apoptosis during post-lactational involution [49]. Even in the absence of milk stasis, constitutively active IKK- β was capable of inducing apoptosis in the mouse secretory mammary epithelium, and therefore interfered with successful lactation by nursing dams.

2.5. Akt/PI3K

Intense interest in survival signaling pathways has revealed that phosphatidyl inositol 3-kinase (PI3K) is a potent regulator of cell survival [50, 51]. Cancer cells frequently utilize PI3K signaling to promote cell survival under conditions of hypoxia, nutrient stress, or even to escape the cytotoxic effects of therapeutic anti-cancer treatments. It is clear, however, that non-transformed cells also use the PI3K signaling pathway to promote cell survival, and that increased PI3K signaling can interfere with physiological cell death [52]. PI3K is a heterodimer comprised of p110 (the catalytic domain) and p85 (the regulatory domain) [50, 53]. Under basal conditions, p85 represses the catalytic activity of p110. However, SH2 domains in p85 interact with phosphorylated tyrosines within YxxM motifs of receptor tyrosine kinases (RTKs) such as the insulin-like growth factor (IGF)-1 receptor (IGFR) or adaptor proteins, such as the insulin receptor substrate proteins. This relieves p85-mediated inhibition of p110, allowing p110 to phosphorylate phosphatidyl inositol 2-phosphate (PIP₂), thus generating PIP₃, a powerful membrane-associated second messenger that recruits pleckstrin homology (PH)-domain containing proteins to the cell membrane. PDK1 and Akt are two PH-domain containing proteins recruited to the membrane in response to RTK activation [52, 54, 55]. PDK1 is a serine-threonine kinase that phosphorylates Akt, another serine-threonine kinase that stimulates cell survival by interacting with members of the Bcl2 family of apoptosis regulators [56-58], which are also involved in the induction of cell death during involution [59-61]. For example, mammary-specific loss of the Bcl2 family member Bax, a known cell death inducer, decreased apoptosis during early involution [60, 62-64], while overexpression of Bax within the secretory mammary epithelium increased

post-lactational apoptosis and promoted precocious STAT3 activity [7, 65]. Loss of the anti-apoptotic protein Bcl-xL in the mammary gland during post-lactational involution accelerated cell death [66, 67]. By inactivating Bax and activating Bcl-xL, Akt activity increases cell survival in the secretory mammary epithelium.

The role of PI3K/Akt signaling in suppressing post-lactational apoptosis is supported by genetically engineered mouse models that result in increased PI3K/Akt signaling. For example, a mouse model in which mammary-specific expression of myristoylated p110 α [68], a modified p110 α that is restricted to the cell membrane, resulted in aberrantly elevated PI3K activity in the mammary epithelium and delayed post-lactational involution. Similarly, mammary-specific transgenic overexpression of Akt1 or Akt2 promoted cell survival and delayed post-lactational involution in mice [69, 70]. Conversely, ablation of Akt1, but not the ablation of Akt2 or Akt3, promoted apoptosis and accelerates involution [71], demonstrating isoform-specificity in the gene-dosage effects of Akt (overexpression versus ablation), and highlights the importance of Akt1 in the post-lactational mammary gland. Other studies demonstrated that Akt signaling is sustained during lactation by prolactin signaling [72-74]. This observation was confirmed in an independent transgenic mouse model of mammary-specific STAT5 activation, in which STAT5 activity, when aberrantly sustained through post-lactational involution, upregulated Akt1 transcription and impaired apoptosis. These studies suggest that high levels of prolactin-induced STAT5 activity, as seen during lactation, maintains Akt1 expression and activity to promote cell survival, but when lactation ceases STAT5-induced Akt expression must be depleted in order for cells to undergo apoptosis [75].

Like prolactin signaling, other ligand-activated signaling cascades are capable of driving PI3K/Akt signaling during lactation, and if not turned off, can delay post-lactational apoptosis. For example, cell signaling initiated by IGF-1, which activates IGFR thus causing tyrosine phosphorylation of insulin receptor substrate proteins [76], potentially activates the PI3K/Akt signal transduction cascade in the mammary epithelium during lactation. Overexpression of IGF-1 in the mouse mammary gland delayed post-lactational involution, suggesting that suppression of IGF-mediated cell survival is required for apoptosis to occur, and supporting the idea that PI3K signaling must be interrupted to initiate post-lactational apoptosis [77]. IGF-1 bio-availability is tightly controlled by IGF binding proteins (IGFBPs), which can sequester IGFs in the extracellular microenvironment of the mammary epithelium [78]. Consistent with the ability of IGF-1 to interfere with post-lactational cell death, one of the earliest transcriptional events during post-lactational involution is the upregulation of IGFBP-2 mRNA (4-fold), IGFBP-4 (6-fold) and IGFBP-5 mRNA (50-fold) [79]. This profound increase in IGFBP-5 is also seen at the protein level, and is conserved across several species. Increased expression of IGFBPs may limit IGF1-induced signaling, thus limiting IGF1-induced PI3K/Akt signaling [77, 80, 81]. Transgenic overexpression of IGFBP-5 in the mouse mammary gland increased caspase-3 cleavage (an indicator of apoptosis) and decreased the expression of the pro-survival factors Bcl-2 and Bcl-xL [80-84], suggesting that IGFBP-5 is pro-apoptotic. An IGF-1 analogue which binds weakly to IGFBP-5 partially overcame IGFBP-5-induced cell death during post-lactational involution,

suggesting that IGFBP-5 was acting, at least in part, by inhibiting IGF action. Conversely, *Igfbp5* null mammary glands exhibit delayed post-lactational apoptosis [78].

2.6. TGF β 3

While prolactin, IGF-1, and several RTK-activating ligands can activate PI3K/Akt signaling to promote cell survival, other ligands are capable of inducing cell death during post-lactational involution, such as leukemia inhibitor factor (LIF) [85-87], serotonin [88], Fas ligand (FasL) [89] TRAIL [90], and transforming growth factor (TGF)- β 3. Transcripts encoding TGF- β 3, but not TGF- β 1 or TGF- β 2, substantially increase in the milk-producing cells during post-lactational involution [91, 92]. This rapid induction of TGF- β 3 transcription in the secretory mammary epithelium occurs as early as 3 hours after pup withdrawal in response to milk stasis [93], and is among the most rapid gene expression changes occurring in response to post-lactational involution, suggesting that TGF β 3 might be an initiating signal for cell death during involution. It would be interesting to determine the impact of mechanical stress on expression from the TGF β 3 promoter. Consistent with the proposed role for elevated TGF- β 3 in inducing apoptosis during involution, transgenic over-expression of TGF- β 3 in the secretory cells of the mouse mammary gland accelerated apoptosis during early post-lactational involution. Conversely, loss of TGF β 3 reduced post-lactational apoptosis by nearly 70% [93], suggesting that autocrine TGF- β 3 signaling initiates cell death following pup removal.

The importance of the TGF β 3-induced signaling pathway for post-lactational apoptosis has been further investigated in genetically engineered mouse models. For example, loss of the TGF β -regulated transcription factor Smad3 decreased post-lactational apoptosis by nearly 40% [94]. Similarly, loss of TGF- β receptor type II (T β RII) in the mammary epithelium, or transgenic expression of dominant negative (DN) T β RII decreased apoptosis during early post-lactational involution [95-97], consistent with a critical role for TGF- β 3 signaling through T β RII and Smad3 to induce apoptosis during early involution. However, there is some discrepancy regarding the role of TGF β signaling during involution, as transgenic expression of constitutively active T β RI decreased apoptotic cells in the mammary gland [98]. Perhaps elevated T β RI signaling activates signaling pathways not normally active under physiological conditions.

2.7. Stromal-epithelial interactions

The signaling pathways described above focus on those events occurring within the secretory epithelium that are responsible for initiating cell death during post-lactational involution. However, it is becoming more apparent that stromal cells contribute substantially to post-lactational apoptosis [99-103]. This was recently demonstrated in a transgenic mouse model referred to as MAFIA (macrophage Fas-induced apoptosis) [104]. Macrophages from MAFIA mice express a modified Fas receptor that, in response to a dimerization-inducing small molecule (AP20187), triggers Fas-mediated apoptosis. Depletion of macrophages immediately prior to weaning impaired apoptosis within the secretory mammary epithelium, despite milk stasis and STAT3 activation [105]. These results demonstrate that macrophages

are necessary to initiate apoptosis in the mammary epithelium. The underlying mechanism remains unclear at this point. However, it is possible that macrophages respond to signals emanating from mechanically stressed epithelia by producing factors that may activate the signaling pathways necessary for induction of apoptosis, or repress signaling pathways that may otherwise limit apoptosis. The transcriptional signatures generated from mouse mammary glands during involution were derived from whole tissue RNA, which would include not only epithelia but also the dynamic stromal components of the mammary gland. Therefore, it is possible that many expression events detected during early post-lactational involution are occurring within macrophage populations.

Mast cells are also heavily recruited to the mammary gland during post-lactational involution [106], and like macrophages, are critical for epithelial apoptosis during mammary gland involution [107]. Specifically, mast cells produce plasma kallikrein (PKaI), the primary activator of plasminogen in the mammary gland. Expression of PKaI rapidly increases during involution, and while PKaI, plasminogen, and other serine proteases undoubtedly have a major role in tissue remodeling during later stages of involution, evidence suggests that PKaI also drives epithelial apoptosis. Inhibition of mast cell-derived PKaI during post-lactational involution impaired epithelial cell death, suggesting that mast cells are vital for triggering apoptosis in the post-lactational mammary gland. Interestingly, in the absence of STAT3 within the mammary epithelium, mast cells and macrophages are not recruited to the mammary gland during post-lactational involution [108], suggesting that recruitment of stromal cells to the involuting mammary gland is initiated by early apoptotic signaling events occurring within the epithelial compartment (**Fig. 1**).

2.8. Lysosomal membrane polarization

Although most studies suggest that mammary gland involution occurs by apoptosis, it has been proposed recently that several morphological features of the involuting mammary gland may resemble necrosis rather than apoptosis [109]. These include cytoplasmic swelling, lack of membrane blebbing, and lack of nuclear fragmentation. Using mice deficient for both caspase 3 and 6, it was shown that mammary gland involution could proceed in the absence of these two classical activators of apoptosis, suggesting that perhaps alternative mechanisms of programmed cell death may exist in the post-lactational mammary gland. The authors proposed that STAT3 activity could upregulate expression of lysosomal cathepsins, which may leak from lysosomes to activate cell death pathways [110, 111]. In support of this idea, cathepsin L is upregulated strongly with the onset of mammary involution [112]. Mice treated with a specific cathepsin L inhibitor during the first three days of involution demonstrated reduced cell death as compared to untreated mice [112]. Cathepsin-induced cell death can be simulated by ectopic addition of reactive oxygen species (ROS) to cultures of mammary epithelial cells. Interestingly, the ROS nitric oxide (NO) can trigger mammary gland involution after weaning [113] in mice. While the role of apoptotic cell death in the mammary gland is widely accepted, investigators should be aware of alternative cell death pathways that contribute to programmed cell death during involution.

Figure 1. Apoptosis in the post-lactational mammary epithelial cell (MEC) is initiated by three molecular signals that are each required: TGFβ3, NFκB, and STAT3. Their loss impairs post-lactational apoptosis, despite continued milk stasis. Apoptotic MECs are cleared from the mammary gland by efferocytosis, or the phagocytic engulfment of dying cells. In the post-lactational mammary gland, MECs and macrophages engulf neighboring apoptotic cells. The phagocyte uses cell surface receptors to recognize, bind, and engulf apoptotic cells. These receptors include MerTK, Axl, α_vβ₃ integrin and others. Intracellular signaling pathways that regulate cytoskeletal rearrangements (such as Rac signaling) are necessary for efferocytosis. Once engulfed into vesicles, apoptotic cells are degraded by the lysosomal pathway. Efferocytosis activates NF-κB and STAT3, upregulating cytokines that are critical for post-lactational mammary remodeling.

2.9. Pathologies of the breast due to aberrant regulation of apoptosis

In the clinical setting, post-lactational involution of the secretory epithelium begins with milk stasis, at which point the secretory cells undergo apoptosis. Clearance of dying cells and residual milk is accomplished by phagocytes within the breast [114]. Regrowth of stromal adipose tissue and continued tissue remodeling returns the breast to a relatively quiescent state comprised of morphological structures similar to those found in nulliparous women. Rarely, the process of involution may be delayed. Failure to remove unnecessary lactational cells may result in symptomatic inflammatory tissue damage. Delayed involution in the human breast is characterized by the maintenance of secretory structures, loss of post-

lactational apoptosis, and infiltration of the breast by inflammatory cells. Focal calcification may also be present [5]. Ductal distention of accumulated milk can be painful. Stagnant milk can be a source for infection and mastitis, to which the gland would respond with secretion of acute inflammatory cytokines and recruitment of leukocytes [115]. Similar to this clinical scenario, mouse models of delayed post-lactational cell death commonly develop mastitis [49, 108, 116].

EMS receptor	Bridging Molecule (BM)	EMS	Mammary Tissue and Cell Line Expression
PSR	-	PS [117]	Primary mouse mammary epithelia [117]
TIMs	-	PS [118-124]	unknown
BAI1	-	PS [125]	unknown
Stabilin-2	-	PS [126, 127].	unknown
ABCA1	unknown	PS [128, 129]	Bovine [130], mouse, human mammary [129]
$\alpha_v\beta_3$ integrin	Vitronectin [131] Thrombospondin [132] MFG-E8 [133]	PS [131, 132]	Human MCF10A, MCF-7, and MDA-MB-231 cells [134] bovine [135], mouse mammary gland [136, 137]
Tyro3	Gas6 [138] Protein S [139, 140]	PS [138]	unknown
Axl	Gas6 [138, 141]	PS [138, 141]	Human breast [142]
MerTK	Gas6 [138] Protein S[140]	PS [138, 140]	Mouse mammary [116]
CR3/CR4	unknown	C3bi [143]	unknown
CD14	unknown	ICAM [144]	Bovine [145], canine [146], mouse [4], and human mammary [144, 147]
CD68	unknown	unknown	Human, mouse mammary macrophages [148]
CD163	unknown	unknown	Human breast [148]
CD36	Thrombospondin [132]	unknown	MDA-MB-435, MDA-MB-231 human cells in mouse mammary [149]
LRP	β_2 GP1 [150] C1q [151]	PS [150] Calreticulin [151]	Rat mammary gland [152] Normal, transformed mammary epithelia [153]
Marco [154]	unknown	unknown	unknown

Table 1. Key Factors Involved in Efferocytosis

Recent data garnered from mouse models of delayed post-lactational involution suggest that deregulation of post-lactational apoptosis may facilitate mammary tumor formation [34, 70, 73, 155-157]. Observations made in human populations also suggest that altered post-lactational involution may associate with tumor formation in the breast [100-102, 158]. This may reflect micro-environmental influences, or may be a function cell death-dependent removal of unnecessary breast epithelial cells in a regulated fashion. In many cancers, intrinsic cell deaths mechanisms become suppressed, contributing to the net growth of the transformed cell population. For example, activation of STAT5 in post-lactational mouse mammary glands delays apoptosis, and results in formation of mammary tumors that express estrogen and progesterone receptors (ER+PR+), as well as activated STAT3 and STAT5 [159]. Moreover, post-lactational transcriptional programs initiated by NF- κ B and STAT3 not only support cell death, but also enhance tumor formation and progression by inducing expression of pro-tumorigenic cytokines [23, 24, 28, 34, 160-162]. Indeed, the transiently increased risk of developing breast cancer in the five years following a pregnancy may be greatly influenced by a deregulated tumor microenvironment developed in the post-lactational breast [101].

While post-lactational involution and age-related lobular involution are distinct processes, recent studies indicate that both are related to breast cancer development. Clinical studies show that completion of lobular involution may reduce future breast cancer incidence [163-166]. With aging, there is a gradual loss of breast epithelial tissue that typically begins in peri-menopause, which then accelerates during menopause. Lobular involution is characterized by the apoptosis-mediated decrease in the size and complexity of the ductal tree and of the TDLU. This is distinct from post-lactational involution, which occurs very rapidly by comparison. However, similar mechanisms controlling apoptosis of the breast epithelium may occur in these two distinct models of involution.

Lobular involution, like post-lactational involution, may inversely correlate with breast cancer risk, since premenopausal women who underwent partial or complete lobular involution had a substantially decreased incidence of breast cancer, while postmenopausal women who showed delayed lobular involution were found to have a correspondingly elevated breast cancer incidence [164]. While much remains to be learned about how lobular involution is regulated, some clinical studies and animal models suggest that IGF-1 may inhibit involution of lobules in the breast [163]. Clinically, a cross-sectional study among 472 women demonstrated that higher IGF-1 levels associated with incomplete lobular involution, supporting the idea that IGF-1/PI3K/Akt-induced survival pathways prevent physiologic cell death, leading to pathological consequences.

2.10. Introduction to efferocytosis in the breast

Following apoptosis, one final event is needed to truly complete the life of the cell. This final step is phagocytic engulfment of apoptotic cells or 'efferocytosis'. The term 'efferocytosis' was recently coined by Hensen et al. to distinguish phagocytic apoptotic cell removal from phagocytic pathogen removal [167]. While both processes are executed by phagocytes, they

result in distinctly different biological responses, one characterized by a dampened acute inflammatory response and upregulation of tolerogenic and wound healing effectors (efferocytosis), while the other is characterized by a pro-inflammatory response (pathogen removal).

Efferocytosis is a carefully regulated process involving recruitment of phagocytes to the apoptotic cell, recognition of the apoptotic cell by the phagocytes, engulfment of the apoptotic cell by the phagocyte, and final breakdown of apoptotic cell components. If disrupted, apoptotic cells will undergo necrotic lysis, leading to acute inflammation, tissue damage and autoimmunity. Therefore, efferocytosis is critical for tissue homeostasis. However, recent discoveries indicate that the normal process of efferocytosis may be undesirable under certain pathological conditions, such as in the tumor microenvironment. We will discuss apoptotic cell clearance in the normal post-lactational breast and in the breast tumor microenvironment.

2.11. The process of efferocytosis

In general, clearance of apoptotic cells is often executed by macrophages and dendritic cells (DCs), but can also be performed by fibroblasts, endothelial and epithelial cells. A cell that engulfs an apoptotic cell through phagocytic mechanisms is called an efferocyte, regardless of its origin. Studies performed in cell culture and *in vivo* demonstrate that MECs and macrophages are both capable of engulfing apoptotic MECs during post-lactational involution of the secretory mammary epithelium [114, 116, 168].

Macrophages, the ‘professional phagocytes’ of the immune system, must infiltrate the mammary gland in response to the physiological presence of apoptotic cells during involution. Large quantities of bone marrow-derived and spleen-derived macrophages infiltrate the post-lactational mouse mammary gland in response to STAT3 activation and apoptosis of the mammary secretory epithelium. It is thought that apoptotic cells may release soluble chemo-attractants, or ‘find me’ signals, which recruit macrophages to the post-lactational mammary gland. For example, Monocyte Chemo-attractant Protein-1 (MCP-1/CCL2) is released from apoptotic cells in an NF- κ B-dependent manner [169]. Interestingly, MCP-1 expression is strongly induced in the mammary gland at day 2 of involution, a time point that follows NF- κ B-induced cell death, and that precedes macrophage influx in the post-lactational mammary gland. These observations are consistent with NF- κ B-induced apoptosis followed by NF- κ B-induced expression of an efferocyte chemo-attractant, although this has not yet been demonstrated. Additional chemokines including CX3CL1, CCL6, CCL7, CCL8, and CXCL14, are induced during post-lactational involution and may be signals that recruit macrophages to the involuting mammary gland to clear the accumulating apoptotic cell burden [170, 171] [4, 16].

Histological evidence of apoptotic cells within cytoplasmic vacuoles of mammary macrophages confirms that mammary macrophages engulf apoptotic cells during involution. Once present within the mammary gland, the macrophage identifies apoptotic

cells, scanning for signals that are present on the apoptotic cell but not a healthy cell, often referred to as an 'eat me' signal (EMS, **Table 1**). The earliest and most recognized EMS is surface exposure of phosphatidylserine (PS). Healthy cells actively maintain PS on the inner plasma membrane leaflet. At the onset of apoptosis, PS is presented to the outer leaflet thus acting as a marker for a dying cell that requires engulfment [172-174]. EMSs are recognized by macrophages that express EMS receptors on their cell surface. EMS receptors may bind directly to the EMS on the dying cell. For example, the PS receptor (PSR), a transmembrane protein expressed by macrophages [128] and MECs [129], directly binds PS. Brain angiogenesis inhibitor 1 (BAI1) also binds PS directly, and is important for macrophage-mediated efferocytosis [125], but has not yet been studied in the mammary gland. Stabilin-2 and members of the T cell immunoglobulin and mucin (TIM) family of receptors also directly bind PS [118-124, 126, 127], demonstrating mechanistic redundancy in the efferocytic pathways. It should be noted, however, that PS is an insufficient EMS, as macrophages fail to recognize live cells in which PS is forced to the outer leaflet [173, 175].

While some EMS receptors bind apoptotic cell EMSs directly, other EMS receptors bind an extracellular bridging molecule that simultaneously binds the EMS and the EMS receptor (**Table 1**). For example, the bridging ligand milkfat globule epidermal growth factor-like 8 (MFG-E8) binds to PS on apoptotic cells [133, 176, 177], while binding $\alpha\text{v}\beta_3$ and $\alpha\text{v}\beta_5$ integrins expressed by macrophages. Growth arrest specific gene 6 (Gas6) and Protein S [178, 179] are bridging molecules that bind to the EMS receptors MerTK, Axl, and Tyro3 [180] expressed by macrophages, while simultaneously binding PS on the apoptotic cell.

Once the apoptotic cell is bound to the macrophage, intracellular signaling pathways must remodel the actin cytoskeleton to drive phagocytic ingestion of the apoptotic cell. Most of these events have been mapped out in *Caenorhabditis elegans* (*C. elegans*), which are discussed in detail within comprehensive reviews by Reddien et al. and Ravichandran et al. [181, 182]. In mammalian macrophages, intracellular signaling networks that regulate actin cytoskeletal dynamics are required for apoptotic cell engulfment. For example, a protein complex comprised of CrkII, DOCK180, and ELMO causes activation of the Rac GTPase, a master regulator of actin cytoskeletal dynamics. Actin-dependent membrane extensions physically engulf the apoptotic cell [183-187]. Rac-mediated actin rearrangements are countered by RhoA [188], which prevents the formation of cell extensions needed for efferocytosis [189]. The engulfed apoptotic cell is then consumed by the efferocyte, primarily through lysosomal degradation. Many signaling factors that drive apoptotic cell engulfment also enhance lysosomal degradation of apoptotic cells. For example, Rac is essential for maturation of phago-lysosomes in macrophages [190-193].

In addition to efferocytosis in the mammary gland, macrophages are also critical for cytokine modulation and extracellular matrix remodeling during the second phase of involution, underscoring the important role of macrophages in the post-lactational breast. Given their known role as professional phagocytes and their massive influx to the post-lactational mammary gland, it is perhaps not surprising that efferocytosis by macrophages occurs in the post-lactational breast. What is more surprising is that apoptotic cell clearance occurs on a profound scale prior to the influx of macrophages to the involuting mammary

gland. Recent evidence demonstrated that MECs are the primary efferocytes of the breast during the earliest stages of post-lactational involution, the first three days prior to the influx of macrophages. The ability of MECs to act as efferocytes ensures a rapid response to the massive level of apoptosis that occurs during post-lactational involution. Loss of MEC-mediated efferocytosis impairs post-lactational homeostasis, resulting in chronic mammary inflammation, scarring and inhibition of future lactation.

Interestingly, MECs utilize many of the same EMS receptors used by macrophages to recognize apoptotic MECs. For example, MerTK is critical for MEC-mediated efferocytosis during post-lactational involution. MerTK loss from the mouse mammary epithelium causes apoptotic cell accumulation and milk stasis [116], despite the presence of wild-type macrophages. Interestingly, mRNA and protein expression of MerTK is dramatically upregulated by post-lactational day 1 within the luminal mammary epithelium. Similarly, the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are expressed in the early post-lactational mammary epithelium. The $\alpha_v\beta_5$ bridging ligand, MFG-E8, is simultaneously induced [194], increasing the physical interaction between MFG-E8 and PS [136] and driving the clearance of membrane-coated milk components from the involuting mammary gland [195].

2.12. Physiological and pathological consequences of efferocytosis

After the efferocyte removes the apoptotic cell, transcriptional events in the efferocyte result in cytokine, chemokine and growth factor production. The combined profile of the factors produced by the efferocyte promotes wound healing through enhanced tissue remodeling, angiogenesis, proliferation and resolution of acute inflammation. The efferocytosis-induced wound healing cytokine profile contrasts sharply to the cytokine profile produced in response to phagocytosis of pathogens, which is characterized by acute inflammatory cytokines [196]. In fact, efferocytosis is thought to be a key step in resolving or dampening acute inflammatory cytokine expression following tissue injury or pathogen exposure, resulting in repair and homeostasis [197]. Microarray analyses of mammary glands harvested at early post-lactational involution time points displayed a pronounced wound healing expression signature [3, 4, 158, 168, 198, 199], consistent with transcriptional changes that result from efferocytosis. The prominent role of efferocytosis in re-establishing mammary homeostasis following widespread apoptosis of the secretory epithelium was shown by experiments in which loss of efferocytosis resulted in apoptotic cell accumulation, sustained milk stasis within ductal lumens, inflammation and scarring [116]. These pathological changes impaired lactation in future pregnancies.

Although key to re-establishing homeostasis in the post-lactational mammary gland [116], recent evidence indicates that efferocytosis may support a more malignant tumor microenvironment [101, 102, 105, 106, 158, 198, 200-202]. Researchers are beginning to address the overlapping roles of efferocytic and metastasis-promoting cytokines. In agreement with this idea, mouse studies show that breast tumors grew more rapidly, invaded more readily, and formed distant metastases more efficiently when implanted in the post-lactational mammary gland as compared to implantation into a nulliparous

mammary gland [101]. One explanation for this observation is that post-lactational efferocytosis promotes breast tumor malignancy through production of wound healing cytokines, which are known to drive breast cancer growth and invasion. In support of this idea, MFG-E8 [203] and its receptor $\alpha_v\beta_3/5$, as well as Gas6 and its ligand MerTK [116, 204] are frequently overexpressed in breast cancers. One recent study demonstrated that the pro-tumorigenic cytokine IL-6 induces expression of MerTK, enhancing the ability of macrophages to engulf apoptotic cells and increasing production of wound healing cytokines such as IL-4 and IL-10 [205]. Recently published data implicates MerTK in breast cancer metastasis [206].

This observation has clinical relevance to pregnancy associated breast cancers (PABCs), defined as breast cancers that arise during the 5 years following a pregnancy. PABCs are among the most malignantly aggressive breast cancers, and are thus associated with poor prognosis. A better understanding of the processes outline above will undoubtedly expand the therapeutic options for these patients.

3. Conclusion

Altogether, these data support the hypothesis that targeting mediators of efferocytosis may limit pro-tumorigenic cytokine production. Moreover, it is becoming increasingly apparent that many factors within the mammary gland cooperate to ensure apoptosis and apoptotic cell clearance, highlighting the complexity of these processes and the need for more detailed investigations. Due to the dominant role of apoptosis and efferocytosis in maintaining tissue homeostasis, especially during post-lactational involution, the mammary gland provides an ideal platform for future study.

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4. References

- [1] Humphreys, R.C., et al., Apoptosis in the terminal endbud of the murine mammary gland: a mechanism of ductal morphogenesis. *Development*, 1996. 122(12): p. 4013-22.
- [2] Andres, A.C. and R. Strange, Apoptosis in the estrous and menstrual cycles. *J Mammary Gland Biol Neoplasia*, 1999. 4(2): p. 221-8.

- [3] Atabai, K., D. Sheppard, and Z. Werb, Roles of the innate immune system in mammary gland remodeling during involution. *J Mammary Gland Biol Neoplasia*, 2007. 12(1): p. 37-45.
- [4] Stein, T., et al., 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*, 2004. 6(2): p. R75-91.
- [5] de Almeida, C.J. and R. Linden, Phagocytosis of apoptotic cells: a matter of balance. *Cell Mol Life Sci*, 2005. 62(14): p. 1532-46.
- [6] Hinck, L. and G.B. Silberstein, Key stages in mammary gland development: the mammary end bud as a motile organ. *Breast Cancer Res*, 2005. 7(6): p. 245-51.
- [7] Mailleux, A.A., et al., BIM regulates apoptosis during mammary ductal morphogenesis, and its absence reveals alternative cell death mechanisms. *Dev Cell*, 2007. 12(2): p. 221-34.
- [8] Li, M., et al., Mammary-derived signals activate programmed cell death during the first stage of mammary gland involution. *Proc Natl Acad Sci U S A*, 1997. 94(7): p. 3425-30.
- [9] Marti, A., et al., Milk accumulation triggers apoptosis of mammary epithelial cells. *Eur J Cell Biol*, 1997. 73(2): p. 158-65.
- [10] Quaglini, A., et al., Mechanical strain induces involution-associated events in mammary epithelial cells. *BMC Cell Biol*, 2009. 10: p. 55.
- [11] VanHouten, J.N., Calcium sensing by the mammary gland. *J Mammary Gland Biol Neoplasia*, 2005. 10(2): p. 129-39.
- [12] VanHouten, J.N., M.C. Neville, and J.J. Wysolmerski, The calcium-sensing receptor regulates plasma membrane calcium adenosine triphosphatase isoform 2 activity in mammary epithelial cells: a mechanism for calcium-regulated calcium transport into milk. *Endocrinology*, 2007. 148(12): p. 5943-54.
- [13] VanHouten, J., et al., PMCA2 regulates apoptosis during mammary gland involution and predicts outcome in breast cancer. *Proc Natl Acad Sci U S A*, 2010. 107(25): p. 11405-10.
- [14] Tremblay, G., et al., Local control of mammary involution: is stanniocalcin-1 involved? *J Dairy Sci*, 2009. 92(5): p. 1998-2006.
- [15] Hasilo, C.P., et al., Nuclear targeting of stanniocalcin to mammary gland alveolar cells during pregnancy and lactation. *Am J Physiol Endocrinol Metab*, 2005. 289(4): p. E634-42.
- [16] Clarkson, R.W., et al., Gene expression profiling of mammary gland development reveals putative roles for death receptors and immune mediators in post-lactational regression. *Breast Cancer Res*, 2004. 6(2): p. R92-109.
- [17] Chapman, R.S., et al., The role of Stat3 in apoptosis and mammary gland involution. Conditional deletion of Stat3. *Adv Exp Med Biol*, 2000. 480: p. 129-38.
- [18] Chapman, R.S., et al., Suppression of epithelial apoptosis and delayed mammary gland involution in mice with a conditional knockout of Stat3. *Genes Dev*, 1999. 13(19): p. 2604-16.

- [19] Humphreys, R.C., et al., Deletion of Stat3 blocks mammary gland involution and extends functional competence of the secretory epithelium in the absence of lactogenic stimuli. *Endocrinology*, 2002. 143(9): p. 3641-50.
- [20] Song, L., et al., Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells. *Oncogene*, 2003. 22(27): p. 4150-65.
- [21] Zhong, Z., Z. Wen, and J.E. Darnell, Jr., Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science*, 1994. 264(5155): p. 95-8.
- [22] Pensa, S., C.J. Watson, and V. Poli, Stat3 and the inflammation/acute phase response in involution and breast cancer. *J Mammary Gland Biol Neoplasia*, 2009. 14(2): p. 121-9.
- [23] Dauer, D.J., et al., Stat3 regulates genes common to both wound healing and cancer. *Oncogene*, 2005. 24(21): p. 3397-408.
- [24] Aggarwal, B.B., et al., Signal transducer and activator of transcription-3, inflammation, and cancer: how intimate is the relationship? *Ann N Y Acad Sci*, 2009. 1171: p. 59-76.
- [25] Aoki, Y., G.M. Feldman, and G. Tosato, Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma. *Blood*, 2003. 101(4): p. 1535-42.
- [26] Kanda, N., et al., STAT3 is constitutively activated and supports cell survival in association with survivin expression in gastric cancer cells. *Oncogene*, 2004. 23(28): p. 4921-9.
- [27] Catlett-Falcone, R., et al., Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. *Immunity*, 1999. 10(1): p. 105-15.
- [28] Bromberg, J.F., et al., Stat3 as an oncogene. *Cell*, 1999. 98(3): p. 295-303.
- [29] Sutherland, K.D., G.J. Lindeman, and J.E. Visvader, Knocking off SOCS genes in the mammary gland. *Cell Cycle*, 2007. 6(7): p. 799-803.
- [30] Sutherland, K.D., et al., c-myc as a mediator of accelerated apoptosis and involution in mammary glands lacking Socs3. *EMBO J*, 2006. 25(24): p. 5805-15.
- [31] Thangaraju, M., et al., C/EBPdelta is a crucial regulator of pro-apoptotic gene expression during mammary gland involution. *Development*, 2005. 132(21): p. 4675-85.
- [32] Thangaraju, M., S. Sharan, and E. Sterneck, Comparison of mammary gland involution between 129S1 and C57BL/6 inbred mouse strains: differential regulation of Bcl2a1, Trp53, Cebpb, and Cebpδ expression. *Oncogene*, 2004. 23(14): p. 2548-53.
- [33] Tiffen, P.G., et al., A dual role for oncostatin M signaling in the differentiation and death of mammary epithelial cells in vivo. *Mol Endocrinol*, 2008. 22(12): p. 2677-88.
- [34] Abdulghani, J., et al., Stat3 promotes metastatic progression of prostate cancer. *Am J Pathol*, 2008. 172(6): p. 1717-28.
- [35] Ranger, J.J., et al., Identification of a Stat3-dependent transcription regulatory network involved in metastatic progression. *Cancer Res*, 2009. 69(17): p. 6823-30.
- [36] Kataoka, K., et al., Stage-specific disruption of Stat3 demonstrates a direct requirement during both the initiation and promotion stages of mouse skin tumorigenesis. *Carcinogenesis*, 2008. 29(6): p. 1108-14.

- [37] Yeh, H.H., et al., Ha-ras oncogene-induced Stat3 phosphorylation enhances oncogenicity of the cell. *DNA Cell Biol*, 2009. 28(3): p. 131-9.
- [38] Yeh, H.H., et al., Autocrine IL-6-induced Stat3 activation contributes to the pathogenesis of lung adenocarcinoma and malignant pleural effusion. *Oncogene*, 2006. 25(31): p. 4300-9.
- [39] Baxter, F.O., et al., IKKbeta/2 induces TWEAK and apoptosis in mammary epithelial cells. *Development*, 2006. 133(17): p. 3485-94.
- [40] Karin, M. and Y. Ben-Neriah, Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol*, 2000. 18: p. 621-63.
- [41] Brantley, D.M., et al., Dynamic expression and activity of NF-kappaB during post-natal mammary gland morphogenesis. *Mech Dev*, 2000. 97(1-2): p. 149-55.
- [42] Li, Z.W., et al., The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. *J Exp Med*, 1999. 189(11): p. 1839-45.
- [43] Maeda, S., et al., IKKbeta is required for prevention of apoptosis mediated by cell-bound but not by circulating TNFalpha. *Immunity*, 2003. 19(5): p. 725-37.
- [44] Senftleben, U., et al., IKKbeta is essential for protecting T cells from TNFalpha-induced apoptosis. *Immunity*, 2001. 14(3): p. 217-30.
- [45] Romashkova, J.A. and S.S. Makarov, NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature*, 1999. 401(6748): p. 86-90.
- [46] Fujioka, S., et al., Stabilization of p53 is a novel mechanism for proapoptotic function of NF-kappaB. *J Biol Chem*, 2004. 279(26): p. 27549-59.
- [47] Ashkenazi, A. and V.M. Dixit, Death receptors: signaling and modulation. *Science*, 1998. 281(5381): p. 1305-8.
- [48] Baetu, T.M., et al., Disruption of NF-kappaB signaling reveals a novel role for NF-kappaB in the regulation of TNF-related apoptosis-inducing ligand expression. *J Immunol*, 2001. 167(6): p. 3164-73.
- [49] Connelly, L., et al., Activation of nuclear factor kappa B in mammary epithelium promotes milk loss during mammary development and infection. *J Cell Physiol*, 2010. 222(1): p. 73-81.
- [50] Engelman, J.A., J. Luo, and L.C. Cantley, The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet*, 2006. 7(8): p. 606-19.
- [51] Wong, K.K., J.A. Engelman, and L.C. Cantley, Targeting the PI3K signaling pathway in cancer. *Curr Opin Genet Dev*, 2010. 20(1): p. 87-90.
- [52] Wickenden, J.A. and C.J. Watson, Key signalling nodes in mammary gland development and cancer. Signalling downstream of PI3 kinase in mammary epithelium: a play in 3 Akts. *Breast Cancer Res*, 2010. 12(2): p. 202.
- [53] Cantley, L.C., The phosphoinositide 3-kinase pathway. *Science*, 2002. 296(5573): p. 1655-7.
- [54] Manning, B.D. and L.C. Cantley, AKT/PKB signaling: navigating downstream. *Cell*, 2007. 129(7): p. 1261-74.

- [55] Vivanco, I. and C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*, 2002. 2(7): p. 489-501.
- [56] Chipuk, J.E., et al., Mechanism of apoptosis induction by inhibition of the anti-apoptotic BCL-2 proteins. *Proc Natl Acad Sci U S A*, 2008. 105(51): p. 20327-32.
- [57] Kuwana, T. and D.D. Newmeyer, Bcl-2-family proteins and the role of mitochondria in apoptosis. *Curr Opin Cell Biol*, 2003. 15(6): p. 691-9.
- [58] Datta, S.R., et al., Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*, 1997. 91(2): p. 231-41.
- [59] Heermeier, K., et al., Bax and Bcl-xs are induced at the onset of apoptosis in involuting mammary epithelial cells. *Mech Dev*, 1996. 56(1-2): p. 197-207.
- [60] Schorr, K., et al., Bcl-2 gene family and related proteins in mammary gland involution and breast cancer. *J Mammary Gland Biol Neoplasia*, 1999. 4(2): p. 153-64.
- [61] Metcalfe, A.D., et al., Developmental regulation of Bcl-2 family protein expression in the involuting mammary gland. *J Cell Sci*, 1999. 112 (Pt 11): p. 1771-83.
- [62] Zeng, X., H. Xu, and R.I. Glazer, Transformation of mammary epithelial cells by 3-phosphoinositide-dependent protein kinase-1 (PDK1) is associated with the induction of protein kinase Calpha. *Cancer Res*, 2002. 62(12): p. 3538-43.
- [63] Pinner, S. and E. Sahai, PDK1 regulates cancer cell motility by antagonising inhibition of ROCK1 by RhoE. *Nat Cell Biol*, 2008. 10(2): p. 127-37.
- [64] Alessi, D.R., et al., Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol*, 1997. 7(4): p. 261-9.
- [65] Rucker, E.B., 3rd, et al., Forced involution of the functionally differentiated mammary gland by overexpression of the pro-apoptotic protein bax. *Genesis*, 2011. 49(1): p. 24-35.
- [66] Walton, K.D., et al., 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*, 2001. 109(2): p. 281-93.
- [67] Schorr, K., et al., Gain of Bcl-2 is more potent than bax loss in regulating mammary epithelial cell survival in vivo. *Cancer Res*, 1999. 59(11): p. 2541-5.
- [68] Renner, O., et al., Activation of phosphatidylinositol 3-kinase by membrane localization of p110alpha predisposes mammary glands to neoplastic transformation. *Cancer Res*, 2008. 68(23): p. 9643-53.
- [69] Dillon, R.L., et al., Akt1 and akt2 play distinct roles in the initiation and metastatic phases of mammary tumor progression. *Cancer Res*, 2009. 69(12): p. 5057-64.
- [70] Schwertfeger, K.L., M.M. Richert, and S.M. Anderson, Mammary gland involution is delayed by activated Akt in transgenic mice. *Mol Endocrinol*, 2001. 15(6): p. 867-81.
- [71] Maroulakou, I.G., et al., Distinct roles of the three Akt isoforms in lactogenic differentiation and involution. *J Cell Physiol*, 2008. 217(2): p. 468-77.
- [72] Creamer, B.A., et al., Stat5 promotes survival of mammary epithelial cells through transcriptional activation of a distinct promoter in Akt1. *Mol Cell Biol*, 2010. 30(12): p. 2957-70.

- [73] Neilson, L.M., et al., Coactivation of janus tyrosine kinase (Jak)1 positively modulates prolactin-Jak2 signaling in breast cancer: recruitment of ERK and signal transducer and activator of transcription (Stat)3 and enhancement of Akt and Stat5a/b pathways. *Mol Endocrinol*, 2007. 21(9): p. 2218-32.
- [74] Flint, D.J., et al., Prolactin inhibits cell loss and decreases matrix metalloproteinase expression in the involuting mouse mammary gland but fails to prevent cell loss in the mammary glands of mice expressing IGFBP-5 as a mammary transgene. *J Mol Endocrinol*, 2006. 36(3): p. 435-48.
- [75] Furth, P.A., et al., Signal transducer and activator of transcription 5 as a key signaling pathway in normal mammary gland developmental biology and breast cancer. *Breast Cancer Res*, 2011. 13(5): p. 220.
- [76] Heidegger, I., et al., Targeting the insulin-like growth factor network in cancer therapy. *Cancer Biol Ther*, 2011. 11(8): p. 701-7.
- [77] Flint, D.J., et al., Role of insulin-like growth factor binding proteins in mammary gland development. *J Mammary Gland Biol Neoplasia*, 2008. 13(4): p. 443-53.
- [78] Sureshbabu, A., E. Tonner, and D.J. Flint, Insulin-like growth factor binding proteins and mammary gland development. *Int J Dev Biol*, 2011. 55(7-9): p. 781-9.
- [79] Flint, D.J., et al., Insulin-like growth factor binding proteins initiate cell death and extracellular matrix remodeling in the mammary gland. *Domest Anim Endocrinol*, 2005. 29(2): p. 274-82.
- [80] Lochrie, J.D., et al., Insulin-like growth factor binding protein (IGFBP)-5 is upregulated during both differentiation and apoptosis in primary cultures of mouse mammary epithelial cells. *J Cell Physiol*, 2006. 207(2): p. 471-9.
- [81] Marshman, E., et al., Insulin-like growth factor binding protein 5 and apoptosis in mammary epithelial cells. *J Cell Sci*, 2003. 116(Pt 4): p. 675-82.
- [82] Allar, M.A. and T.L. Wood, Expression of the insulin-like growth factor binding proteins during postnatal development of the murine mammary gland. *Endocrinology*, 2004. 145(5): p. 2467-77.
- [83] Tonner, E., et al., Insulin-like growth factor binding protein-5 (IGFBP-5) potentially regulates programmed cell death and plasminogen activation in the mammary gland. *Adv Exp Med Biol*, 2000. 480: p. 45-53.
- [84] Tonner, E., et al., Insulin-like growth factor binding protein-5 (IGFBP-5) induces premature cell death in the mammary glands of transgenic mice. *Development*, 2002. 129(19): p. 4547-57.
- [85] Quaglino, A., et al., Mouse mammary tumors display Stat3 activation dependent on leukemia inhibitory factor signaling. *Breast Cancer Res*, 2007. 9(5): p. R69.
- [86] Schere-Levy, C., et al., Leukemia inhibitory factor induces apoptosis of the mammary epithelial cells and participates in mouse mammary gland involution. *Exp Cell Res*, 2003. 282(1): p. 35-47.

- [87] Kritikou, E.A., et al., A dual, non-redundant, role for LIF as a regulator of development and STAT3-mediated cell death in mammary gland. *Development*, 2003. 130(15): p. 3459-68.
- [88] Matsuda, M., et al., Serotonin regulates mammary gland development via an autocrine-paracrine loop. *Dev Cell*, 2004. 6(2): p. 193-203.
- [89] Song, J., et al., Roles of Fas and Fas ligand during mammary gland remodeling. *J Clin Invest*, 2000. 106(10): p. 1209-20.
- [90] Sohn, B.H., et al., 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*, 2001. 360(Pt 1): p. 31-8.
- [91] Faure, E., et al., Differential expression of TGF-beta isoforms during postlactational mammary gland involution. *Cell Tissue Res*, 2000. 300(1): p. 89-95.
- [92] Robinson, S.D., et al., Regulated expression and growth inhibitory effects of transforming growth factor-beta isoforms in mouse mammary gland development. *Development*, 1991. 113(3): p. 867-78.
- [93] Nguyen, A.V. and J.W. Pollard, Transforming growth factor beta3 induces cell death during the first stage of mammary gland involution. *Development*, 2000. 127(14): p. 3107-18.
- [94] Yang, Y.A., et al., Smad3 in the mammary epithelium has a nonredundant role in the induction of apoptosis, but not in the regulation of proliferation or differentiation by transforming growth factor-beta. *Cell Growth Differ*, 2002. 13(3): p. 123-30.
- [95] Gorska, A.E., et al., Transgenic mice expressing a dominant-negative mutant type II transforming growth factor-beta receptor exhibit impaired mammary development and enhanced mammary tumor formation. *Am J Pathol*, 2003. 163(4): p. 1539-49.
- [96] Joseph, H., et al., Overexpression of a kinase-deficient transforming growth factor-beta type II receptor in mouse mammary stroma results in increased epithelial branching. *Mol Biol Cell*, 1999. 10(4): p. 1221-34.
- [97] Bierie, B., et al., TGF-beta promotes cell death and suppresses lactation during the second stage of mammary involution. *J Cell Physiol*, 2009. 219(1): p. 57-68.
- [98] Muraoka-Cook, R.S., et al., Activated type I TGFbeta receptor kinase enhances the survival of mammary epithelial cells and accelerates tumor progression. *Oncogene*, 2006. 25(24): p. 3408-23.
- [99] Cunha, G.R. and Y.K. Hom, Role of mesenchymal-epithelial interactions in mammary gland development. *J Mammary Gland Biol Neoplasia*, 1996. 1(1): p. 21-35.
- [100] Howlett, A.R. and M.J. Bissell, The influence of tissue microenvironment (stroma and extracellular matrix) on the development and function of mammary epithelium. *Epithelial Cell Biol*, 1993. 2(2): p. 79-89.
- [101] Lyons, T.R., et al., Postpartum mammary gland involution drives progression of ductal carcinoma in situ through collagen and COX-2. *Nat Med*, 2011. 17(9): p. 1109-15.

- [102] O'Brien, J., et al., Alternatively activated macrophages and collagen remodeling characterize the postpartum involuting mammary gland across species. *Am J Pathol*, 2010. 176(3): p. 1241-55.
- [103] Reed, J.R. and K.L. Schwertfeger, Immune cell location and function during post-natal mammary gland development. *J Mammary Gland Biol Neoplasia*, 2010. 15(3): p. 329-39.
- [104] Burnett, S.H., et al., Conditional macrophage ablation in transgenic mice expressing a Fas-based suicide gene. *J Leukoc Biol*, 2004. 75(4): p. 612-23.
- [105] O'Brien, J., et al., Macrophages are crucial for epithelial cell death and adipocyte repopulation during mammary gland involution. *Development*, 2012. 139(2): p. 269-75.
- [106] Ramirez, R.A., et al., Alterations in mast cell frequency and relationship to angiogenesis in the rat mammary gland during windows of physiologic tissue remodeling. *Dev Dyn*, 2012. 241(5): p. 890-900.
- [107] Lilla, J.N., et al., Active plasma kallikrein localizes to mast cells and regulates epithelial cell apoptosis, adipocyte differentiation, and stromal remodeling during mammary gland involution. *J Biol Chem*, 2009. 284(20): p. 13792-803.
- [108] Hughes, K., et al., Conditional deletion of Stat3 in mammary epithelium impairs the acute phase response and modulates immune cell numbers during post-lactational regression. *J Pathol*, 2012. 227(1): p. 106-17.
- [109] Kreuzaler, P.A., et al., Stat3 controls lysosomal-mediated cell death in vivo. *Nat Cell Biol*, 2011. 13(3): p. 303-9.
- [110] Margaryan, N.V., et al., New insights into cathepsin D in mammary tissue development and remodeling. *Cancer Biol Ther*, 2010. 10(5): p. 457-66.
- [111] Lockshin, R.A. and Z. Zakeri, Caspase-independent cell death? *Oncogene*, 2004. 23(16): p. 2766-73.
- [112] Burke, M.A., et al., Cathepsin L plays an active role in involution of the mouse mammary gland. *Dev Dyn*, 2003. 227(3): p. 315-22.
- [113] Zaragoza, R., et al., Nitric oxide triggers mammary gland involution after weaning: remodelling is delayed but not impaired in mice lacking inducible nitric oxide synthase. *Biochem J*, 2010. 428(3): p. 451-62.
- [114] Monks, J., et al., Epithelial cells remove apoptotic epithelial cells during post-lactation involution of the mouse mammary gland. *Biol Reprod*, 2008. 78(4): p. 586-94.
- [115] Kumar, Y., et al., Delayed involution of lactation presenting as a non-resolving breast mass: a case report. *J Med Case Reports*, 2008. 2: p. 327.
- [116] Sandahl, M., et al., Epithelial cell-directed efferocytosis in the post-partum mammary gland is necessary for tissue homeostasis and future lactation. *BMC Dev Biol*, 2010. 10: p. 122.
- [117] Fadok, V.A., et al., A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature*, 2000. 405(6782): p. 85-90.
- [118] DeKruyff, R.H., et al., T cell/transmembrane, Ig, and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells. *J Immunol*, 2010. 184(4): p. 1918-30.

- [119] Ichimura, T., et al., Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. *J Clin Invest*, 2008. 118(5): p. 1657-68.
- [120] Kobayashi, N., et al., TIM-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. *Immunity*, 2007. 27(6): p. 927-40.
- [121] Miyanishi, M., et al., Identification of Tim4 as a phosphatidylserine receptor. *Nature*, 2007. 450(7168): p. 435-9.
- [122] Rodriguez-Manzanet, R., et al., T and B cell hyperactivity and autoimmunity associated with niche-specific defects in apoptotic body clearance in TIM-4-deficient mice. *Proc Natl Acad Sci U S A*, 2010. 107(19): p. 8706-11.
- [123] Santiago, C., et al., Structures of T Cell immunoglobulin mucin receptors 1 and 2 reveal mechanisms for regulation of immune responses by the TIM receptor family. *Immunity*, 2007. 26(3): p. 299-310.
- [124] Wong, K., et al., Phosphatidylserine receptor Tim-4 is essential for the maintenance of the homeostatic state of resident peritoneal macrophages. *Proc Natl Acad Sci U S A*, 2010. 107(19): p. 8712-7.
- [125] Park, D., et al., BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature*, 2007. 450(7168): p. 430-4.
- [126] Park, S.Y., et al., Epidermal growth factor-like domain repeat of stabilin-2 recognizes phosphatidylserine during cell corpse clearance. *Mol Cell Biol*, 2008. 28(17): p. 5288-98.
- [127] Park, S.Y., et al., Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. *Cell Death Differ*, 2008. 15(1): p. 192-201.
- [128] Luciani, M.F. and G. Chimini, The ATP binding cassette transporter ABC1, is required for the engulfment of corpses generated by apoptotic cell death. *EMBO J*, 1996. 15(2): p. 226-35.
- [129] Mani, O., et al., Expression, localization, and functional model of cholesterol transporters in lactating and nonlactating mammary tissues of murine, bovine, and human origin. *Am J Physiol Regul Integr Comp Physiol*, 2010. 299(2): p. R642-54.
- [130] Mani, O., et al., Identification of ABCA1 and ABCG1 in milk fat globules and mammary cells--implications for milk cholesterol secretion. *J Dairy Sci*, 2011. 94(3): p. 1265-76.
- [131] Yebra, M., et al., Requirement of receptor-bound urokinase-type plasminogen activator for integrin α v β 5-directed cell migration. *J Biol Chem*, 1996. 271(46): p. 29393-9.
- [132] Savill, J., et al., Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J Clin Invest*, 1992. 90(4): p. 1513-22.
- [133] Akakura, S., et al., The opsonin MFG-E8 is a ligand for the α v β 5 integrin and triggers DOCK180-dependent Rac1 activation for the phagocytosis of apoptotic cells. *Exp Cell Res*, 2004. 292(2): p. 403-16.
- [134] D'Mello, V., et al., The urokinase plasminogen activator receptor promotes efferocytosis of apoptotic cells. *J Biol Chem*, 2009. 284(25): p. 17030-8.

- [135] Hvarregaard, J., et al., Characterization of glycoprotein PAS-6/7 from membranes of bovine milk fat globules. *Eur J Biochem*, 1996. 240(3): p. 628-36.
- [136] Nakatani, H., et al., Weaning-induced expression of a milk-fat globule protein, MFG-E8, in mouse mammary glands, as demonstrated by the analyses of its mRNA, protein and phosphatidylserine-binding activity. *Biochem J*, 2006. 395(1): p. 21-30.
- [137] Oshima, K., et al., Lactation-dependent expression of an mRNA splice variant with an exon for a multiply O-glycosylated domain of mouse milk fat globule glycoprotein MFG-E8. *Biochem Biophys Res Commun*, 1999. 254(3): p. 522-8.
- [138] Nagata, K., et al., Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. *J Biol Chem*, 1996. 271(47): p. 30022-7.
- [139] Stitt, T.N., et al., The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. *Cell*, 1995. 80(4): p. 661-70.
- [140] Prasad, D., et al., TAM receptor function in the retinal pigment epithelium. *Mol Cell Neurosci*, 2006. 33(1): p. 96-108.
- [141] Varnum, B.C., et al., Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. *Nature*, 1995. 373(6515): p. 623-6.
- [142] Berclaz, G., et al., Estrogen dependent expression of the receptor tyrosine kinase axl in normal and malignant human breast. *Ann Oncol*, 2001. 12(6): p. 819-24.
- [143] Mevorach, D., et al., Complement-dependent clearance of apoptotic cells by human macrophages. *J Exp Med*, 1998. 188(12): p. 2313-20.
- [144] Moffatt, O.D., et al., Macrophage recognition of ICAM-3 on apoptotic leukocytes. *J Immunol*, 1999. 162(11): p. 6800-10.
- [145] Baravalle, C., et al., Proinflammatory cytokines and CD14 expression in mammary tissue of cows following intramammary inoculation of *Panax ginseng* at drying off. *Vet Immunol Immunopathol*, 2011. 144(1-2): p. 52-60.
- [146] Krol, M., et al., Density of tumor-associated macrophages (TAMs) and expression of their growth factor receptor MCSF-R and CD14 in canine mammary adenocarcinomas of various grade of malignancy and metastasis. *Pol J Vet Sci*, 2011. 14(1): p. 3-10.
- [147] Feng, A.L., et al., CD16⁺ monocytes in breast cancer patients: expanded by monocyte chemoattractant protein-1 and may be useful for early diagnosis. *Clin Exp Immunol*, 2011. 164(1): p. 57-65.
- [148] Shabo, I. and J. Svanvik, Expression of macrophage antigens by tumor cells. *Adv Exp Med Biol*, 2011. 714: p. 141-50.
- [149] Koch, M., et al., CD36-mediated activation of endothelial cell apoptosis by an N-terminal recombinant fragment of thrombospondin-2 inhibits breast cancer growth and metastasis in vivo. *Breast Cancer Res Treat*, 2011. 128(2): p. 337-46.
- [150] Maiti, S.N., et al., Beta-2-glycoprotein 1-dependent macrophage uptake of apoptotic cells. Binding to lipoprotein receptor-related protein receptor family members. *J Biol Chem*, 2008. 283(7): p. 3761-6.

- [151] Vandivier, R.W., et al., Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. *J Immunol*, 2002. 169(7): p. 3978-86.
- [152] Ghosal, D., N.W. Shappell, and T.W. Keenan, Endoplasmic reticulum luminal proteins of rat mammary gland. Potential involvement in lipid droplet assembly during lactation. *Biochim Biophys Acta*, 1994. 1200(2): p. 175-81.
- [153] Li, Y., et al., In vitro invasiveness of human breast cancer cells is promoted by low density lipoprotein receptor-related protein. *Invasion Metastasis*, 1998. 18(5-6): p. 240-51.
- [154] Rogers, N.J., et al., A defect in Marco expression contributes to systemic lupus erythematosus development via failure to clear apoptotic cells. *J Immunol*, 2009. 182(4): p. 1982-90.
- [155] Lofgren, K.A., et al., Mammary gland specific expression of Brk/PTK6 promotes delayed involution and tumor formation associated with activation of p38 MAPK. *Breast Cancer Res*, 2011. 13(5): p. R89.
- [156] Cheng, X., et al., Activation of murine double minute 2 by Akt in mammary epithelium delays mammary involution and accelerates mammary tumorigenesis. *Cancer Res*, 2010. 70(19): p. 7684-9.
- [157] Dillon, R.L. and W.J. Muller, Distinct biological roles for the akt family in mammary tumor progression. *Cancer Res*, 2010. 70(11): p. 4260-4.
- [158] O'Brien, J. and P. Schedin, Macrophages in breast cancer: do involution macrophages account for the poor prognosis of pregnancy-associated breast cancer? *J Mammary Gland Biol Neoplasia*, 2009. 14(2): p. 145-57.
- [159] Vafaizadeh, V., P.A. Klemmt, and B. Groner, Stat5 assumes distinct functions in mammary gland development and mammary tumor formation. *Front Biosci*, 2012. 17: p. 1232-50.
- [160] Barbieri, I., et al., Stat3 is required for anchorage-independent growth and metastasis but not for mammary tumor development downstream of the ErbB-2 oncogene. *Mol Carcinog*, 2010. 49(2): p. 114-20.
- [161] Connelly, L., et al., Inhibition of NF-kappa B activity in mammary epithelium increases tumor latency and decreases tumor burden. *Oncogene*, 2011. 30(12): p. 1402-12.
- [162] Desrivieres, S., et al., The biological functions of the versatile transcription factors STAT3 and STAT5 and new strategies for their targeted inhibition. *J Mammary Gland Biol Neoplasia*, 2006. 11(1): p. 75-87.
- [163] Rice, M.S., et al., Insulin-like growth factor-1, insulin-like growth factor binding protein-3 and lobule type in the Nurses' Health Study II. *Breast Cancer Res*, 2012. 14(2): p. R44.
- [164] Ghosh, K., et al., Independent association of lobular involution and mammographic breast density with breast cancer risk. *J Natl Cancer Inst*, 2010. 102(22): p. 1716-23.

- [165] Gierach, G.L., L.A. Brinton, and M.E. Sherman, Lobular involution, mammographic density, and breast cancer risk: visualizing the future? *J Natl Cancer Inst*, 2010. 102(22): p. 1685-7.
- [166] Ginsburg, O.M., L.J. Martin, and N.F. Boyd, Mammographic density, lobular involution, and risk of breast cancer. *Br J Cancer*, 2008. 99(9): p. 1369-74.
- [167] Henson, P.M., Dampening inflammation. *Nat Immunol*, 2005. 6(12): p. 1179-81.
- [168] Monks, J., et al., Epithelial cells as phagocytes: apoptotic epithelial cells are engulfed by mammary alveolar epithelial cells and repress inflammatory mediator release. *Cell Death Differ*, 2005. 12(2): p. 107-14.
- [169] Nagaosa, K., A. Shiratsuchi, and Y. Nakanishi, Concomitant induction of apoptosis and expression of monocyte chemoattractant protein-1 in cultured rat luteal cells by nuclear factor-kappaB and oxidative stress. *Dev Growth Differ*, 2003. 45(4): p. 351-9.
- [170] Graham, K., et al., Gene expression profiles of estrogen receptor-positive and estrogen receptor-negative breast cancers are detectable in histologically normal breast epithelium. *Clin Cancer Res*, 2011. 17(2): p. 236-46.
- [171] Truman, L.A., et al., CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. *Blood*, 2008. 112(13): p. 5026-36.
- [172] Fadok, V.A., et al., Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol*, 1992. 148(7): p. 2207-16.
- [173] Borisenko, G.G., et al., Macrophage recognition of externalized phosphatidylserine and phagocytosis of apoptotic Jurkat cells--existence of a threshold. *Arch Biochem Biophys*, 2003. 413(1): p. 41-52.
- [174] Martin, S.J., et al., Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J Exp Med*, 1995. 182(5): p. 1545-56.
- [175] Suzuki, J., et al., Calcium-dependent phospholipid scrambling by TMEM16F. *Nature*, 2010. 468(7325): p. 834-8.
- [176] Hanayama, R., et al., Identification of a factor that links apoptotic cells to phagocytes. *Nature*, 2002. 417(6885): p. 182-7.
- [177] Hanayama, R., et al., Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science*, 2004. 304(5674): p. 1147-50.
- [178] Nakano, T., et al., Cell adhesion to phosphatidylserine mediated by a product of growth arrest-specific gene 6. *J Biol Chem*, 1997. 272(47): p. 29411-4.
- [179] Ishimoto, Y., et al., Promotion of the uptake of PS liposomes and apoptotic cells by a product of growth arrest-specific gene, gas6. *J Biochem*, 2000. 127(3): p. 411-7.
- [180] Hall, M.O., et al., Both protein S and Gas6 stimulate outer segment phagocytosis by cultured rat retinal pigment epithelial cells. *Exp Eye Res*, 2005. 81(5): p. 581-91.
- [181] Reddien, P.W. and H.R. Horvitz, The engulfment process of programmed cell death in *Caenorhabditis elegans*. *Annu Rev Cell Dev Biol*, 2004. 20: p. 193-221.

- [182] Ravichandran, K.S. and U. Lorenz, Engulfment of apoptotic cells: signals for a good meal. *Nat Rev Immunol*, 2007. 7(12): p. 964-74.
- [183] Reddien, P.W. and H.R. Horvitz, CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in *Caenorhabditis elegans*. *Nat Cell Biol*, 2000. 2(3): p. 131-6.
- [184] Gumieny, T.L., et al., CED-12/ELMO, a novel member of the CrkII/Dock180/Rac pathway, is required for phagocytosis and cell migration. *Cell*, 2001. 107(1): p. 27-41.
- [185] Hasegawa, H., et al., DOCK180, a major CRK-binding protein, alters cell morphology upon translocation to the cell membrane. *Mol Cell Biol*, 1996. 16(4): p. 1770-6.
- [186] Kiyokawa, E., et al., Activation of Rac1 by a Crk SH3-binding protein, DOCK180. *Genes Dev*, 1998. 12(21): p. 3331-6.
- [187] Van Aelst, L. and C. D'Souza-Schorey, Rho GTPases and signaling networks. *Genes Dev*, 1997. 11(18): p. 2295-322.
- [188] Nakaya, M., et al., Opposite effects of rho family GTPases on engulfment of apoptotic cells by macrophages. *J Biol Chem*, 2006. 281(13): p. 8836-42.
- [189] Riento, K. and A.J. Ridley, Rocks: multifunctional kinases in cell behaviour. *Nat Rev Mol Cell Biol*, 2003. 4(6): p. 446-56.
- [190] Wang, Q.Q., et al., Integrin beta 1 regulates phagosome maturation in macrophages through Rac expression. *J Immunol*, 2008. 180(4): p. 2419-28.
- [191] Stephens, L., C. Ellson, and P. Hawkins, Roles of PI3Ks in leukocyte chemotaxis and phagocytosis. *Curr Opin Cell Biol*, 2002. 14(2): p. 203-13.
- [192] Lerm, M., et al., Inactivation of Cdc42 is necessary for depolymerization of phagosomal F-actin and subsequent phagosomal maturation. *J Immunol*, 2007. 178(11): p. 7357-65.
- [193] Erwig, L.P., et al., Differential regulation of phagosome maturation in macrophages and dendritic cells mediated by Rho GTPases and ezrin-radixin-moesin (ERM) proteins. *Proc Natl Acad Sci U S A*, 2006. 103(34): p. 12825-30.
- [194] Oshima, K., et al., Secretion of a peripheral membrane protein, MFG-E8, as a complex with membrane vesicles. *Eur J Biochem*, 2002. 269(4): p. 1209-18.
- [195] Hanayama, R. and S. Nagata, Impaired involution of mammary glands in the absence of milk fat globule EGF factor 8. *Proc Natl Acad Sci U S A*, 2005. 102(46): p. 16886-91.
- [196] Sanchez-Mejorada, G. and C. Rosales, Signal transduction by immunoglobulin Fc receptors. *J Leukoc Biol*, 1998. 63(5): p. 521-33.
- [197] Silva, M.T., Secondary necrosis: the natural outcome of the complete apoptotic program. *FEBS Lett*, 2010. 584(22): p. 4491-9.
- [198] Stein, T., et al., A mouse mammary gland involution mRNA signature identifies biological pathways potentially associated with breast cancer metastasis. *J Mammary Gland Biol Neoplasia*, 2009. 14(2): p. 99-116.
- [199] Deonaraine, K., et al., Gene expression profiling of cutaneous wound healing. *J Transl Med*, 2007. 5: p. 11.
- [200] McDaniel, S.M., et al., Remodeling of the mammary microenvironment after lactation promotes breast tumor cell metastasis. *Am J Pathol*, 2006. 168(2): p. 608-20.

- [201] Schedin, P., Pregnancy-associated breast cancer and metastasis. *Nat Rev Cancer*, 2006. 6(4): p. 281-91.
- [202] Schedin, P., et al., Microenvironment of the involuting mammary gland mediates mammary cancer progression. *J Mammary Gland Biol Neoplasia*, 2007. 12(1): p. 71-82.
- [203] Aziz, M., et al., Review: milk fat globule-EGF factor 8 expression, function and plausible signal transduction in resolving inflammation. *Apoptosis*, 2011. 16(11): p. 1077-86.
- [204] Linger, R.M., et al., TAM receptor tyrosine kinases: biologic functions, signaling, and potential therapeutic targeting in human cancer. *Adv Cancer Res*, 2008. 100: p. 35-83.
- [205] Frisdal, E., et al., Interleukin-6 protects human macrophages from cellular cholesterol accumulation and attenuates the proinflammatory response. *J Biol Chem*, 2011. 286(35): p. 30926-36.
- [206] Png, K.J., et al., A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. *Nature*, 2012. 481(7380): p. 190-4.