

# Making milk

## A new link between STAT5 and Akt1

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**T**he transcription factor STAT5A is essential for two processes during mammary gland development. First, it controls the development of luminal progenitor cells from stem cells<sup>1</sup> and second, it has a role during pregnancy where it is required for alveologenesis<sup>2,3</sup> the production of clusters of luminal cells that synthesize and secrete milk during lactation. Thus, deletion of STAT5A in late pregnancy results in lactation failure. Alveologenesis requires the proliferation of a different lineage of luminal epithelial cells in response to the pregnancy hormones progesterone and prolactin, the latter of which activates STAT5. Prolactin is required additionally during lactation to ensure adequate milk production and the transcription of several milk protein genes has been shown to be regulated by STAT5.<sup>4,5</sup> On the other hand, the PI3K/Akt pathway is essential for the synthesis of other milk components such as lipids and lactose.<sup>6</sup> In recent elegant work from Lewis Chodosh's laboratory, published in *Genes and Development*, these two pathways are now shown to be directly linked.<sup>7</sup> More specifically, it is shown that the PI3K/Akt pathway induces autocrine prolactin production and that this is required for the initiation of lactation.

The mammary gland is comprised of a branched network of ducts that are embedded within a fatty stroma. The ducts are composed of a bi-layered epithelium with a network of basal myoepithelial cells surrounding an inner layer of luminal epithelial cells.<sup>8</sup> Stem cells are predominantly found dispersed

throughout the basal layer although unipotent stem cells are found in both luminal and basal locations.<sup>9</sup> The mammary gland undergoes a cycle of further development with every pregnancy. In response to the major hormones of pregnancy, progesterone and prolactin, tertiary branching and the formation of lobuloalveolar structures is initiated. The progenitors for this lineage have not been clearly defined but it is thought that the alveolar lineage arises from progenitors that are localized in the basal layer of epithelium. Several studies using genetically altered mice have demonstrated the importance of the prolactin/JAK2/STAT5 pathway for mammary gland development and mice deficient in pathway components have similar phenotypes characterized by the absence of alveolar structures and failed lactation.<sup>2,10-12</sup>

While the prolactin/JAK2/STAT5 pathway is essential for alveologenesis and milk protein gene expression, the PI3K/Akt pathway is important for the synthesis of lactose and lipids in addition to glucose transport. The three principal components of the PI3K/Akt pathway are phosphatidylinositol 3-kinase (PI3K), its antagonist phosphatase and tensin homolog deleted on chromosome ten (Pten) and the serine/threonine kinase Akt/protein kinase B. There are three different Akt isoforms, Akt1, Akt2 and Akt3, which are encoded by different genes but share structural similarities.<sup>13</sup> The specific functions of these isoforms was revealed by the use of knockout mice which demonstrated that Akt1 and Akt2 have opposing functions in mammary gland since ablation of Akt1 results in delayed development and differentiation during pregnancy and lactation

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whereas loss of Akt2 promotes precocious differentiation. The consequences of Akt1 loss include a failure to upregulate a number of processes including glucose uptake, Glut1 surface localization, and lipid synthesis concomitantly with a failure to downregulate lipid catabolic enzymes.<sup>14</sup> Furthermore, Akt1-deficient glands involute more rapidly following lactation while this process is delayed in Akt2-deficient mammary glands. Akt3 seems to have a minor role.<sup>15</sup> Interestingly, only the Akt1 knockout phenotype is cell autonomous and furthermore, affects phosphorylation of STAT5A during late pregnancy and lactation. Thus Akt appears to regulate STAT5 signaling during pregnancy.

Although prolactin is secreted by the pituitary gland, it is also known to be produced elsewhere in the body where it may have autocrine effects although its regulation is not clearly defined. During early pregnancy in rodents, the levels of prolactin are low but rise toward the end of pregnancy. One of the genes regulated by prolactin is Elf5, a master regulator of the alveolar lineage and forced expression of Elf5 in virgin mice induced alveologenesis and milk production.<sup>16</sup>

An exciting new insight into crosstalk between STAT5, prolactin and PI3K/Akt signaling was recently revealed in the study by Chen and coworkers.<sup>7</sup> Using a number of mouse models, they show that Akt signaling induces the production of prolactin by the mammary epithelium and that this induces, in an autocrine manner, STAT5 activation and terminal differentiation of mammary epithelium. First, the authors conditionally activated the PI3K pathway in virgin mice in two ways: (1) by expressing a doxycycline-inducible activated (myristoylated) allele of Akt in mammary epithelium under the control of the MMTV promoter and (2) by conditional deletion of the negative regulator of this pathway, Pten. In both situations, ducts became distended with lipid and protein and resembled alveoli in lactating tissue. Remarkably, milk proteins were expressed almost at lactation levels. Thus, activation of the PI3K pathway induces secretory differentiation in vivo in the absence of other signals.

It was then shown that activation of Akt, or deletion of Pten, results in

phosphorylation of STAT5. This was confirmed to be due to Akt expression in the mammary epithelium by fat pad transplantation experiments of Akt1-expressing cells into wild-type fat pads. Thus, STAT5 is activated in a cell intrinsic manner.

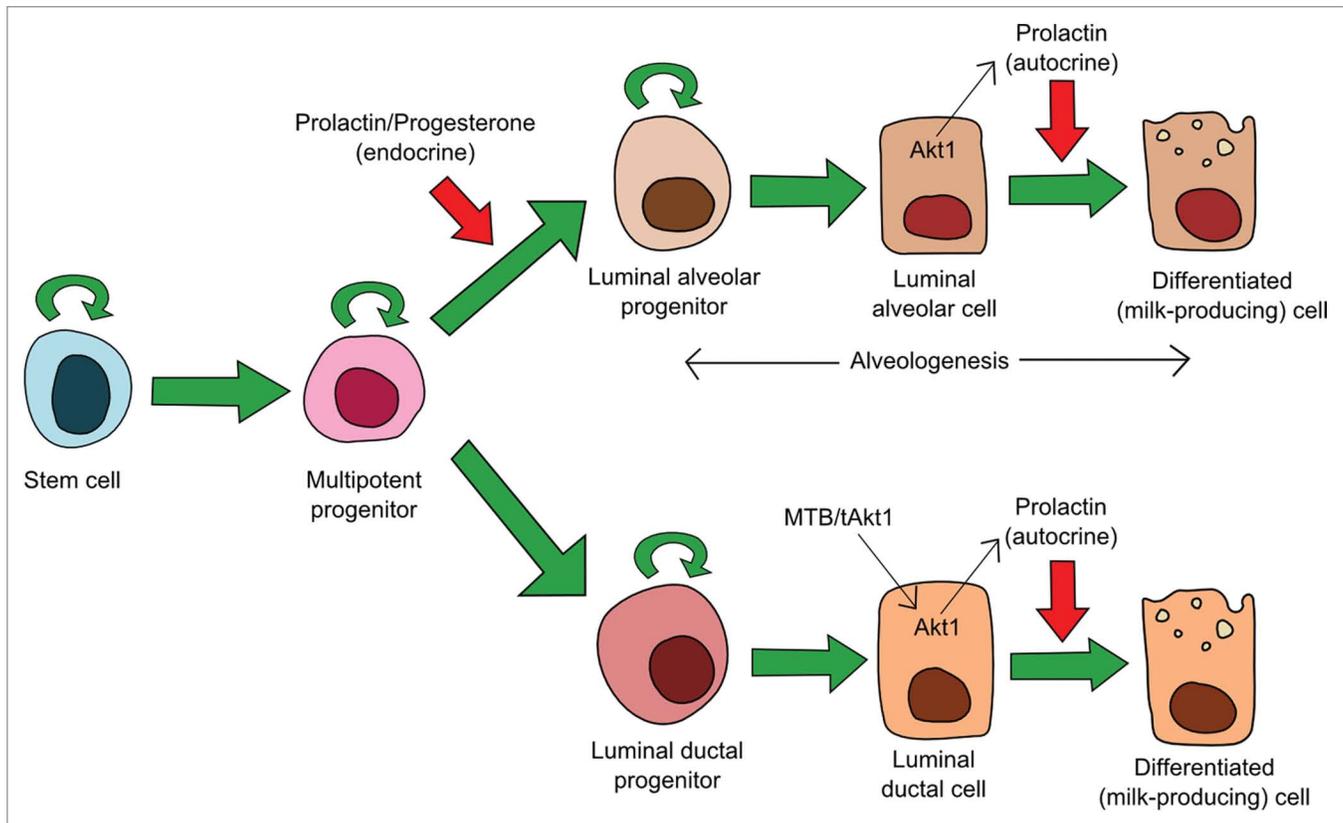
This raises the obvious question—is Akt-induced differentiation dependent on STAT5? To address this, mice harboring hypomorphic alleles of STAT5A/B were crossed to *MTB/tAkt1* transgenic animals. Although ducts were still somewhat distended in *MTB/tAkt1;Stat5a/b<sup>-/-</sup>* mice compared with *MTB;Stat5a/b<sup>-/-</sup>*, expression of milk proteins was almost completely abrogated. This was associated with much reduced levels of pSTAT5 (a truncated protein is expressed in these hypomorphic mutants). Similar results were obtained with deletion of the prolactin receptor and in in vitro studies. The authors then hypothesized that Akt could induce the expression of a locally-acting factor and demonstrated first that conditioned medium from explanted *MTB-tAkt1* mammary tissue could induce STAT5 phosphorylation and then showed that prolactin expression is induced in virgin glands of Akt1-expressing or Pten deleted mice at the protein level but not at the mRNA level. Thus Akt can regulate autocrine prolactin levels post-transcriptionally. The failure to induce pSTAT5 in explanted mammary glands from *MTB/tAkt1;Pr<sup>l</sup><sup>-/-</sup>* mice, and the absence of autocrine prolactin in *Akt1<sup>-/-</sup>;Akt2<sup>+/-</sup>* mammary glands, provides convincing evidence that Akt1 induces autocrine prolactin. Interestingly, Elf5 is induced by Akt1 in virgin *MTB/tAkt1;Stat5a/b<sup>-/-</sup>* and *MTB/tAkt1;Pr<sup>l</sup><sup>-/-</sup>* mice even though pSTAT5 is not, suggesting that Akt1 is a transcriptional regulator of Elf5 independently of STAT5. The authors conclude that activation of the PI3K-Akt pathway in mammary gland is necessary and sufficient for the induction of autocrine prolactin, activation of STAT5 and terminal differentiation (i.e., milk production) even in the absence of pregnancy hormones.

One interesting outcome of this work is that the activation of Akt1 in virgin animals causes only the ductal cells to differentiate into milk-producing cells and alveologenesis is not induced. According to previous knockout studies, both

STAT5 and PrlR are required for alveolar development, which leaves us with a conundrum: why has the activation of the Prl/JAK2/STAT5 pathway not resulted in lobuloalveolar development as well as differentiation?

Is it possible the mammary gland can distinguish between autocrine and endocrine sources of prolactin? This seems unlikely as the hormone is presumably identical regardless of point of origin. The authors propose that in late pregnancy STAT5 is activated by this autocrine source of prolactin as endocrine levels are very low at this stage. This could be tested by using implanted prolactin pellets in *Akt1<sup>-/-</sup>/Akt2<sup>+/-</sup>* mice during pregnancy. If milk production is rescued in these mice then any source of prolactin can have the same effect and autocrine prolactin is needed simply because there is insufficient circulating endocrine prolactin. However, regardless of whether the source of prolactin that stimulates differentiation and milk production is autocrine or endocrine, this does not explain why it sometimes induces alveologenesis and sometimes differentiation and milk production.

Could it be that the autocrine prolactin must impact on alveolar progenitors? The *MTB/tAkt1* model used by Chen and colleagues appears to limit expression of the transgene to the luminal cell layer, as shown by co-expression with luminal marker cytokeratin (CK)8. A co-stain for a basal marker such as CK14 would confirm that this is indeed the case. Since the stem and progenitor cell populations are thought to reside predominantly in the basal layer then the effect of Akt1 activation on these cell types will not have been addressed by this investigation. Furthermore, not all cell populations are present in the virgin gland since alveolar cells arise during pregnancy and work from our laboratory has shown that the balance of alveolar cell populations during pregnancy is necessary for normal alveolar development.<sup>17</sup> As such, the development of a model with inducible Akt1 expression in the basal layer could prove illuminating as one could postulate that the activation of STAT5 in say a multipotent progenitor may stimulate alveolar development whereas activation in a ductal/alveolar luminal cell results in differentiation. It



**Figure 1.** Model of dual role of prolactin/STAT5 in the mammary gland. Multipotent progenitors arise from the stem cell pool. These can generate a number of cell populations including ductal and alveolar progenitors. Alveolar progenitors appear during pregnancy and initiate alveogenesis. Evidence would suggest that progesterone and prolactin are required for the development of this population. Later in pregnancy, Akt1 stimulates the production of autocrine prolactin which activates STAT5 to promote the production of milk components and the terminal differentiation of luminal cells. The MTB/tAkt1 mice used in the study by Chen et al. 2012 drives the activation of Akt1 in luminal ductal cells resulting in their terminal differentiation but cannot give rise to alveogenesis as this would require prolactin to act instead on the multipotent progenitors.

would be interesting to explore how Akt1 and STAT5 fit into the control of the balance of alveolar cell populations.

Alternatively, the absence of alveogenesis could be due to the absence of other signaling pathways or transcription factors normally present in the pregnant gland. The most notable absence is that of progesterone which has been shown to be required for alveogenesis and is present at high levels throughout pregnancy. Ideally one could add progesterone to the MTB/tAkt1 virgin animals and observe if alveogenesis would now take place. However, since the addition of progesterone alone does stimulate some alveogenesis<sup>18</sup> an enhanced effect may not be observed with the additional activation of Akt1.

The data present here and in previous studies suggests that prolactin-induced STAT5 activation is necessary but not sufficient for alveolar formation but is necessary and sufficient for milk production.

One potential model of prolactin/STAT5 action on the mammary gland, based on the work from Chen and coworkers, is outlined in **Figure 1**. In summary, at the onset of pregnancy multipotent progenitors arise from the stem cell pool and prolactin and progesterone drive the formation of the alveolar progenitors that have activated STAT5 and thereby drive alveogenesis. Following this early pregnancy period, levels of endocrine prolactin diminish until shortly before birth. The presence of autocrine prolactin, as stimulated by activated Akt1, acts on alveolar and ductal luminal cells in later pregnancy to promote differentiation and milk protein production.

Not only has this work elegantly and conclusively shown that the PI3K/Akt pathway induces autocrine prolactin production which is required for the initiation of lactation, but it has the additional merit of the use of genetically altered

mouse models to study the activation of STAT5 by prolactin *in vivo* in the correct cellular environment so we can be confident that the data are biologically relevant. It also highlights the difficulties in interpreting and comparing data from models that use specific promoters to drive the tetracycline-dependent transactivator or Cre recombinase. How do we explain that inducible overexpression of Elf5 using MMTV-driven rtTA (MTB) mice results in precocious alveogenesis<sup>16</sup> while MTB driven overexpression of activated Akt1 (which induces expression of Elf5) results in differentiation of ducts but does not induce alveogenesis?

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