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Remodeling of Murine Mammary Adipose Tissue during Pregnancy, Lactation, and Involution

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Abstract

White adipocytes in the mammary gland stroma comprise the majority of the mammary gland mass. White adipocytes regulate numerous hormonal and metabolic processes and exhibit compositional and phenotypic plasticity. This plasticity is exemplified by the ability of mammary adipocytes to regress during lactation, when mammary epithelial cells expand to establish sufficient milk-producing alveoli. Upon weaning, the process reverses through mammary involution, during which adipocytes extensively regenerate, and alveolar epithelial cells disappear through cell death, returning the mammary gland to the non-lactating state. Despite intensive studies on the development and involution of the mammary alveolar epithelium, the fate of mammary adipocytes during pregnancy and lactation, and the origins of mammary adipocytes regenerated during mammary involution, is poorly understood. Here, we discuss the recent discoveries of the fate of mammary adipocytes during pregnancy and lactation in a number of different mouse models, and the lineage origin of mammary adipocytes regenerated during involution.

Keywords Adipocyte · Mammary gland · Lactation · Involution · Remodeling · Dedifferentiation · Obesity · Breast Cancer

Remodeling of Mammary Adipose Tissue during Lactation and Involution

Human breast tissue is predominantly comprised of mammary epithelium and its surrounding stroma; in the non-lactating gland, white adipose tissue in the stroma comprises the majority of the mammary gland mass [1]. White adipocytes regulate numerous hormonal and metabolic processes and exhibit compositional and phenotypic plasticity [2–5]. The mammary epithelium and its surrounding mammary adipocytes remodel dramatically during reproduction [6–9]. During pregnancy and lactation, the mammary epithelium proliferates extensively, as the mammary ducts branches and the mammary epithelium expands to fill in the stroma between the ducts to form the alveolar structures for milk production. At the same time, the mammary adipocytes in the stroma disappear, ceding space to the expanding mammary glands. After weaning, during mammary involution, the mammary alveolar structures collapse as the secretory epithelial cells undergo cell death and the basement membrane and extracellular matrix dissolve. Meanwhile, the mammary adipocytes regenerate rapidly and fill up the space that they originally occupied prior to pregnancy. The remodeling potential of mammary adipocytes is a remarkable example of the capacity of adipocytes to adapt to the altered spaciotemporal requirements of the mammary gland over the course of pregnancy, lactation, and involution. Interestingly, this remodeling predominantly happens in mammary adipose tissue, and to a much smaller extent in other subcutaneous or visceral adipose depots.

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Mammary Adipocytes Do Not Contribute to the Mammary Alveolar Cells

During gestation, the mammary gland undergoes dramatic restructuring to establish the alveolar structures to allow the onset of milk production and secretion. The mammary alveolar epithelium is consisted of two layers of epithelial cells: the inner layer of secretory luminal epithelial cells (the alveolar cells that secrete milk) and the outer myoepithelial cell with

contractile properties. It is generally accepted that the mammary alveolar epithelium develops from adult mammary stem cells (MaSCs) [10–12]. With genetic lineage-tracing models, quantitative 3D imaging, and single-cell sequencing, intensive efforts have been dedicated to determining the identity, location, and differentiation potential of MaSCs. However, the exact lineages of alveolar cells and myoepithelial cells are somewhat controversial and have been subject to debate in the literature. Many studies suggested that alveologenesis is accomplished through a cooperative outgrowth of cells from luminal- and myoepithelial-specific lineages [13–18]. A recent report suggested that the PDGFRα^+ mesenchymal progenitors, usually considered to be adipocyte progenitors, also contribute to the mammary epithelial niche, primarily giving rise to hormone receptor (HR)- negative luminal epithelial cells [19]. However, other studies suggested that a group of lineage-restricted, bipotent MaSCs actively and stochastically develop into both cell types in the mammary alveolar epithelium [20–25]. Interestingly, based on the altered morphology observed through elegant electron microscopy images, and aP2-Cre (for adipocyte) and WAP-Cre (milk protein secreted by mammary epithelial cells) based β-gal histochemistry, Cinti and colleagues suggested that mammary adipocytes could trans-differentiate into mammary alveolar cells during pregnancy and lactation [26, 27].

Do mammary adipocytes really become alveolar cells during pregnancy and lactation? We have previously developed the AdipoChaser-LacZ mouse model, a doxycycline-based, tet-responsive labeling system for the inducible, permanent labeling of adiponectin expressing cells as LacZ positive cells for pulse-chase experiments [28]. This model has allowed us to uniformly label all existing white adipocytes ("pulse"), and then identify newly generated adipocytes in vivo (during a "chase" period) [28]. To determine if mammary alveolar cells could be developed from mammary adipocytes, we utilized the same mouse model to pre-label all the white adipocytes and tracked the fate of these cells during pregnancy and lactation [29]. Instead of trans-differentiating into mammary gland alveolar cells, we found that mammary adipocytes not only lose lipid droplets, but dedifferentiate into Pdgfrα^+, bona fide adipocyte precursor cells during lactation [29] (Fig. 1). These dedifferentiated mammary adipocytes occupy the space between the milk-secreting alveolar structures. Importantly, although the Cinti group observed aP2+ cells in the mammary epithelial cells, our lineage tracing results did not reveal any labeled cells among the alveolar epithelial cells. A recent report by the Horsley group also did not detect the adipocyte-epithelial transdifferentiation during lactation [30]. In this study, they performed lineage tracing for adiponectin$^+$ and K14$^+$ (labels mammary myoepithelial cells), individually, and in both experiments adipocytes were clearly distinct from the mammary epithelium structure. Moreover, we demonstrated that these dedifferentiated mammary adipocytes can be isolated from the stromal vascular fraction of the lactating mammary gland, proliferated in a dish, and differentiated into adipocyte in vitro. This challenges the widely held believe that the mature adipocyte has only two options – it can undergo a change in cell size or undergo cell deat. These labeling experiments in the mammary gland demonstrate that mature adipocytes have the additional option to undergo a full-blown de-differentiation program.

The Lineage Origin of Mammary Adipocytes Generated during Involution

Upon cessation of suckling of the offspring, the postpartum involution of the mammary gland is initiated. This period is characterized by a cessation of milk production, followed by massive cell death of the epithelial cells within the alveolar structures, leaving the ductal tree behind [31]. As the mammary epithelium undergoes involution, the mammary adipocytes rapidly regenerate, and massive clusters of adipocytes become visible within as little as two days after weaning. Thus, postpartum involution utilizes coordinated programs of epithelial cell death and stromal remodeling to return the mammary gland back to the pre-pregnancy state. Previous studies have identified multiple signaling pathways that modulate this dramatic remodeling process of the mammary epithelium [32, 33]. However, the lineage of the rapidly regenerating mammary adipocytes remains unclear. As the Cinti group observed WAP$^+$ cells in regenerated adipocytes, they subsequently suggested that these mammary alveolar cells undergo a reverse trans-differentiation and reconvert back to adipocytes. In our recent study, we not only established that mammary adipocytes dedifferentiate into precursor-like cells during gestation, but we also observed that these dedifferentiated adipocytes proliferate and re-differentiate back into mature adipocytes post-weaning [29]. In the first few days after mammary involution is initiated, these dedifferentiated adipocytes start to proliferate as judged by the fact that these cells actively incorporate BrdU. We further demonstrated that even as far out as two months after weaning, the regenerated adipocytes within the mammary gland are exclusively derived from pre-existing mammary adipocytes. Similaryly, with tamoxifen-dependent lineage tracing, the Horsley group observed very low levels of de novo adipogenesis during mammary involution, and they suggest that adipogenesis from adipocyte precusors is not likely to be the major contributor of adipocyte regeneration [30]. Thus, unlike other types of adipogenesis, the mammary adipocytes regenerated during involution are not derived from de novo differentiated "virgin" adipocyte precursors. Rather, these mature adipocytes derive from pre-existing mature mammary adipocytes. Importantly, this cycle of mammary adipocyte

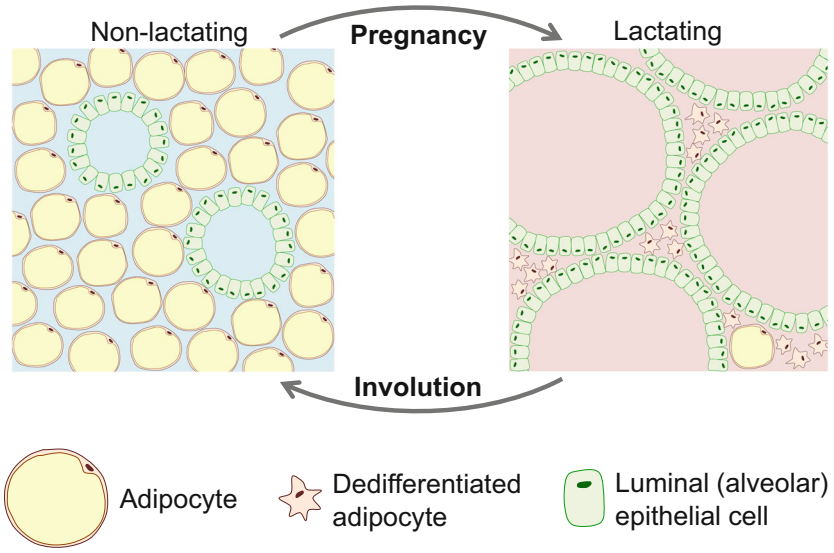

Fig. 1 A schematic summary of adipocyte remodeling in the mammary gland during pregnancy, lactation, and involution

de-differentiation and re-differentiation repeats during multiple rounds of pregnancies [29].

Mammary Adipocyte Dedifferentiation and Self-Renewal Bring New Insights to Adipocyte Plasticity and Obesity

Although many terminally differentiated cells have been discovered to be able to re-enter the cell cycle and self-renew in vivo [34, 35], this had not been reported for adipocytes previously. Our discovery of adipocyte plasticity contributes significantly to understanding adipose tissue remodeling. As the de-differentiation and re-entry into the cell cycle happens under physiological conditions in vivo, it is highly likely that these processes are not restricted to adipocyte in the mammary gland.

To date, the molecular mechanisms and source of critical signaling components for mammary adipocyte remodeling are completely unknown. Mammary alveologenesis and lactogenesis are regulated by pregnancy hormones as well as endocrine hormones driving lactation, such as estrogen, progesterone, prolactin, and somatotropin [36–39]. Are the mechanisms of mammary adipocyte dedifferentiation primarily governed by these hormones? Mammary adipocytes are in close contact with the mammary epithelium, and the remodeling of these cells is perfectly coordinated with the remodeling of the mammary epithelium. Is it possible that the mammary epithelium secretes critical paracrine signaling molecule(s) that lead to mammary adipocyte dedifferentiation and maintenance of the dedifferentiated status? It has been suggested that mammary epithelial cells induce lipolysis in adjacent mammary adipocytes [40]. This raises the question as to whether lipolysis the trigger or result of adipocyte dedifferentiation? Future studies by us and others will hopefully reveal the underlying cellular and molecular mechanism(s) for mature adipocyte dedifferentiation and will bring new insights into both adipose and mammary biology.

As a global epidemic, the prevalence of obesity has increased rapidly over the past decades. The percentage of US adults with obesity has reached 39.8% in 2015–2016 [41]. Obesity is a condition of excessive white adipose tissue accumulation. As an endocrine organ, white adipose tissue is the key regulator for energy homeostasis and insulin sensitivity; its dysfunction is a major cause of whole-body insulin resistance and type 2 diabetes [42–44]. White adipose tissue has high plasticity to adapt and expand in response to excessive energy intake through both adipocyte hypertrophy or the formation of new adipocytes derived from precursors that undergo differentiation in a process referred to as adipogenesis (hyperplasia) [5]. The remodeling of mammary adipocytes serves as an excellent physiological model to study adipose tissue plasticity. Understanding the underlying mechanism(s) that regulate mammary adipocyte plasticity is of great interest for a better understanding of the physiology of adipose tissue in general.

Adipocyte Dysfunction, Lactation Defects, and Breast Cancer

Women face unique metabolic risks, especially during gestation. Unfortunately, according to the latest statistics from the CDC, adult females have a higher prevalence of obesity than adult males (41.5% vs. 38%) [41]. Women who are obese at the time of conception are more prone to excessive gestational weight gain and gestational diabetes. Lactation is often impaired or fails altogether [45–52]. This is a vicious cycle, since

lactation has been shown to reduce a mother's risk of type 2 diabetes [53–55] and protects the child from obesity, future type 2 diabetes, and other metabolic disorders [56–65]. As successful lactation period and subsequent involution of the mammary gland are accomplished through a series of complicated cellular remodeling steps not limited to the mammary epithelium only [66–71]. The lack of a mechanistic understanding of mammary gland remodeling at the whole-tissue level prevents our ability to better address impaired mammary alveologenesis and poor lactation performance. Mammary adipocytes have been shown to be essential for mammary alveologenesis and lactogenesis [66]; in obese rodent models, mammary adipocytes do not fully regress in the lactating mammary gland [72]. Thus, mammary adipocyte dysfunction caused by obesity is anticipated to contribute to these reproductive complications in obese women. Future studies will need to provide a better understanding of how mammary adipocytes cross-talk with the mammary epithelium to secure successful lactation and involution, how mammary adipocyte remodeling is impaired in the obese state and the implications for mammary gland morphogenesis and function.

The risk for breast cancer is transiently increased with each pregnancy [73], and women diagnosed with breast cancer within 5 years postpartum have a high incidence of breast cancer–related deaths [74, 75]. The mammary stromal environment plays an important role in breast cancer development. Upon weaning, an appropriate mammary involution is critical for the mammary gland to stop lactation and regain the pre-pregnancy morphology. Thus, the pathogenesis of reproduction-related breast cancer is highly likely linked to abnormal mammary gland remodeling. The regeneration of mammary adipocytes during involution has long been considered a process of a relatively "passive" refilling of the mammary gland mass, as the alveolar structures disappear through cell death and the regenerating adipocytes simply fill in. In the past twenty years, the understanding of the cellular physiology of the adipocyte has significantly progressed, and the adipocyte is now considered as an endocrine cell that actively cross-talks with many other cell types, thereby regulating many metabolic processes and cellular events in both health and diseases [42, 44]. A recent report by the Horsley group elegantly demonstrated that when adipocytes are eliminated through diphtheria toxin during early mammary involution, the mammary epithelial ducts are distended, indicating that adipocytes are critical for proper epithelial remodeling during involution [30].

Moreover, dysfunctional adipocytes are thought to actively communicate with tumor cells and promote breast cancer [76, 77]. Furthermore, since obesity is associated with an increased risk of breast cancer [78], dysfunctional mammary adipose tissue and impaired mammary adipocyte remodeling may be a critical contributors to reproduction-related increased breast cancer risk.

Concluding Remarks

The avent of improved labeling techniques that effectively allow us to "pulse" mature adipocyte with a defined color and "chase" them under different physiological conditions, combined with powerful single cell-sequencing approaches has allowed us to paint a much more refined picture of the complex cellular changes that the adipocyte undergoes and highlights a much larger plasticity of this presumably terminally differentiated cell.

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