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Review

Mature white adipocyte plasticity during mammary gland remodelling and cancer

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ABSTRACT

Mammary gland growth and differentiation predominantly rely on stromal-epithelial cellular communication. Specifically, mammary adipocytes play a crucial role in ductal morphogenesis, as well as in the proliferation and differentiation of mammary epithelial cells. The process of lactation entails a reduction in the levels of white adipose tissue associated with the MG, allowing for the expansion of milk-producing epithelial cells. Subsequently, during involution and the regression of the milk-producing unit, adipocyte layers resurface, occupying the vacated space. This dynamic phenomenon underscores the remarkable plasticity and expansion of adipose tissue. Traditionally considered terminally differentiated, adipocytes have recently been found to exhibit plasticity in certain contexts. Unraveling the significance of this cell type within the MG could pave the way for novel approaches to reduce the risk of breast cancer and enhance lactation performance. Moreover, a comprehensive understanding of adipocyte *trans*- and de-differentiation processes holds promise for the development of innovative therapeutic interventions targeting cancer, fibrosis, obesity, type 2 diabetes, and other related diseases. Additionally, adipocytes may find utility in the realm of regenerative medicine. This review article provides a comprehensive examination of recent advancements in our understanding of MG remodelling, with a specific focus on the tissue-specific functions of adipocytes and their role in the development of cancer. By synthesizing current knowledge in this field, it aims to consolidate our understanding of adipocyte biology within the context of mammary gland biology, thereby fostering further research and discovery in this vital area.

1. Introduction

Throughout embryonic and postnatal mammary gland (MG) development, interactions between epithelial and mesenchymal cells are required for proper ductal morphogenesis (Cunha & Hom, 1996; Robinson, Karpf, & Kratochwil, 1999). Notably, the most common mesenchymal mammary cell is the adipocyte (Gouon-Evans, Rothenberg, & Pollard, 2000; Silberstein, 2001). Fibroblasts, migrating macrophages, eosinophils, nerve cells, and endothelial cells are the other types of cells present in the MG. Several studies in both foetal (Sakakura et al., 1976, 1982) and adult mice (Daniel, Berger, Strickland, & Garcia, 1984; Sakakura et al., 1982) have shown that mammary fat tissue is essential in the morphogenesis of the mammary parenchyma during MG development. It suggests that the mammary fat pad is required for epithelial cell growth and morphogenesis and the size restriction of the mammary tree (DeOme, Faulkin, Bern, & Blair, 1959).

The size restriction of the mammary tree refers to the natural limitation on the size of the mammary gland that is imposed by the surrounding mammary fat pad. The mammary fat pad acts as a physical barrier that restricts the expansion of the mammary gland, preventing it from growing beyond a certain size. The size restriction is important because unchecked growth of the mammary gland could lead to abnormal development and potentially increase the risk of breast cancer. The mammary fat pad provides a natural mechanism to limit the size of the mammary gland and ensure that it develops normally. It is important to note that the size restriction of the mammary tree is not fixed and can vary depending on various factors, including genetics, hormonal changes, and environmental factors. For example, during pregnancy, the mammary gland undergoes significant growth to accommodate milk production, and the size restriction of the mammary fat pad may be temporarily overcome to allow for this growth. Overall, the size restriction of the mammary tree is a critical aspect of mammary gland

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development and maintenance, and its regulation is tightly controlled by the interactions between the mammary epithelial and stromal cells within the mammary gland microenvironment. In addition, it was demonstrated in a transplantation study using chimaera of mammary specific mesenchymes and mammary epithelial cells that fat pad precursors are required for the formation of mammary-specific patterning (Daniel et al., 1984; DeOme et al., 1959; Sakakura et al., 1976, 1982).

During MG development and remodelling, the adipocytes and the luminal epithelial cells work in synchronisation (Fig. 1). It is the "synchronized ON and OFF" of adipo-alveolar cells at various developmental stages (Cinti, 2018). Alveoli growth during pregnancy and their removal during lactation correspond to the presence and reduction of MG's adipocytes (act in synchronisation). During pregnancy, glandular epithelial cells are recruited from ductal stem cells and then die in lactation through apoptosis, explaining how plasticism occurs in adult MG tissue (Cinti, 2018). Besides that, lipids are transferred from adipocytes to alveolar cells, which secrete fat as a milk component. As a result of the transfer, adipocytes will shrink in size ('slimming'), appearing to have vanished. Slimmed adipocytes fill their cytoplasm with lipids after lactation, restoring their characteristic white morphology (Cinti, 2018). However, further studies are required on this hot topic to solve the mysterious adipocyte puzzle and their role in MG development.

However, due to the unique plastic properties of adipocytes, cellular differentiation studies showed that white adipocytes could be converted to brown adipocytes (Barbatelli et al., 2010; Granneman et al., 2005). The adipose progenitor cells (preadipocytes) of the lineage of fatty tissue originated from mesenchymal stem cells can produce chondrocytes, osteoblasts, and myocytes. Contrariwise, de-differentiated adipocyte-and theroiderived progeny cells (DFAT cells) can differentiate back into adipocytes (Bielczyk-Maczynska, 2019). DFAT cells are an adipogenic progenitor cell line produced from mature adipocytes in ddY mice. Matsumoto et al. however, demonstrate that DFAT cells can develop into adipogenic, osteogenic, and chondrogenic lineages under the appropriate culture settings, despite their clonal expansion (Matsumoto et al., 2008). However, due to lack of strong evidence about the origin of DFAT from differentiated adipocytes using lineage-tracing system. It implies that the process by which DFAT cells are generated in vitro may be different than what occurs in vivo, or that they may be an artifact of the culture conditions due to changes in cell micromanagement, or that they may come from contaminating non-adipocyte populations.

It raises intriguing questions about the connection of breast adipocytes to epithelial cells and how they interact with one another. Do adipocytes start apoptosis during pregnancy or survive throughout the pregnancy-lactation phase, and how do they keep disappearing until the completion of lactation? The mechanism underlying adipocyte and luminal epithelial cell dynamics and plasticity remains unknown. To answer the questions, a research group ha s employed an inducible mature adipocyte-specific tracking system called the "AdipoChaser mouse" to ascertain the process (Wang, Tao, Gupta, & Scherer, 2013). Others have investigated the distinct fates of epithelial and adipocyte lineages in MG development using techniques such as in vivo proliferation, long-term genetic lineage tracing, pharmacological inhibition of adipogenesis, and teat sealing assays. In this review, we discussed important issues and highlighted new discoveries, elucidating the molecular processes underlying the flexibility of mgWAT growth throughout the pregnancy-lactation-involution cycle. Furthermore, understanding the fundamentals of glandular development is necessary in order to answer the unresolved MG adipocyte-epithelial cell crosstalk questions.

2. Mammary gland and adipose tissue: role of adipocytes in MG epithelial remodelling

The MG is a bilayered complex tissue made up of a branching epithelial structure. It produces a primitive branching ductal tree composed of myoepithelial and luminal cells during early embryonic development (Giordano, Smorlesi, Frontini, Barbatelli, & Cinti, 2014). Interestingly, the majority of its development takes place post-natally. At puberty, it expands rapidly and then undergoes cyclic growth under the influence of systemic hormones (progesterone and estrogen) in each menstrual cycle as well as by local cell-stromal interactions (Cunha & Hom, 1996). Surprisingly, approximately 90% of the resting MG (non--lactating) is composed of adipose tissue (Giordano et al., 2014). For instance, most MG in rodents comprises adipocytes and epithelial cells, whereas, in humans, the adipocytic component is significantly underrepresented, with more fibrous connective tissue instead (Cinti, 2018). In any case, adipocytes are necessary for normal MG development. Studies have predominantly examined the gland epithelial component, while only a few reports explore neighbouring adipocytes' role in both physiological and pathological states (Cinti, 2018; Cristea & Polyak, 2018). The MG-associated white adipose tissue (mgWAT) origin during reproduction is instead a contentious topic. Since many reports indicate that mammary lobular-alveolar expansion from certain types of mammary stem cells occurs (Eirew et al., 2008; Shackleton et al., 2006; Tiede & Kang, 2011; Wang et al., 2015), other controversial studies indicate that MG adipocytes can *trans*-differentiate into epithelial cells during pregnancy and lactation. They are reversed into adipocytes after lactation (Giordano et al., 2014; Morroni et al., 2004; Zwick, Guerrero-Juarez, Horsley, & Plikus, 2018). Hence, the mammary gland white adipose

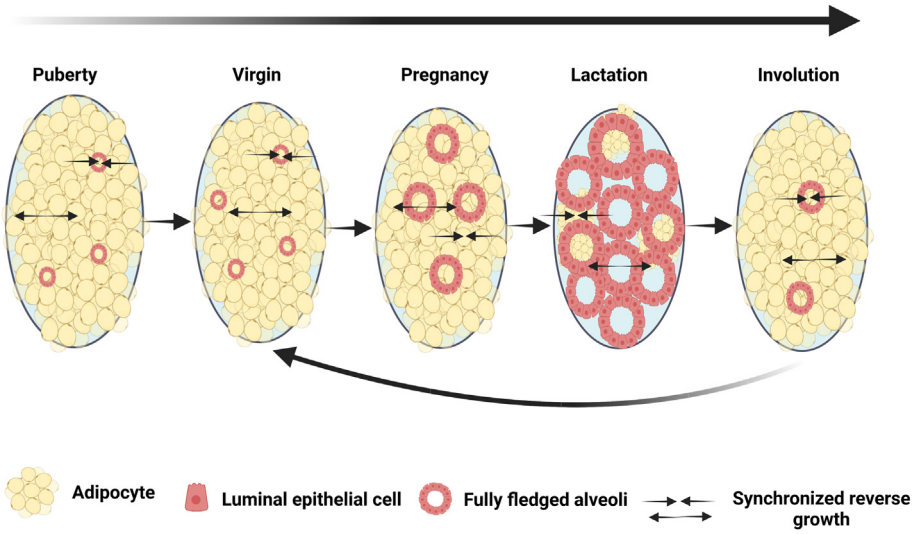

Fig. 1. During successive stages of MG development, adipocytes and luminal epithelial cells grow in a synchronized direction.

tissue (mgWAT) undergoes dynamic morphological and functional remodelling, particularly during the period from pregnancy to lactation, to facilitate milk synthesis and production. During pregnancy, the breast enlarges, displacing the fat tissue. Subsequently, lipid droplets accumulate towards the end of pregnancy, at which point they become one of the important source for milk production. These specialized cells are termed pink adipocytes. Following lactation, the mammary gland regresses, and the adipocytes return to their original positions (Ross et al., 2000; Watanabe et al., 2011).

Pink adipocytes: Pink adipocytes, also known as "beige" or "brite" adipocytes, are a specialized subset of adipose cells found within white adipose tissue (WAT) depots, exhibiting unique characteristics that allow them to switch between white and brown adipocyte-like phenotypes. Arising from distinct lineages, pink adipocytes originate from Myf5-negative, PDGFRα-expressing progenitors, in contrast to the traditional white and brown adipocyte lineages. These cells store lipid droplets and express white adipocyte markers like resistin and leptin, while also expressing key thermogenic markers associated with brown adipocytes, including UCP1, which facilitates heat production. The activation of pink adipocytes leads to "browning" or "beiging," a process characterized by increased thermogenesis and energy expenditure, contributing significantly to metabolic regulation. Factors such as cold exposure, exercise, hormones, and dietary components influence pink adipocyte activation. The study of pink adipocytes has opened up new avenues in obesity and metabolic research, holding promise as potential targets for therapeutic interventions against obesity and metabolic disorders, as they play a vital role in maintaining energy homeostasis and improving glucose metabolism (Giordano et al., 2014).

Initially, adipocyte formation, or adipogenesis, begins with the differentiation of embryonic mesenchymal stem cells (MSCs). MSCs are undifferentiated cells that can differentiate into a variety of cell types, including adipocytes and myoblasts. MSCs-derived adipocytes go through a cellular differentiation process that involves the activation and inactivation of various signalling pathways. One of the important is the WNT pathway, where WNT (Wingless-related integration site) protein binds to the frizzled receptor and coreceptor LRP (low density lipoprotein - receptor-related protein), allowing them to move to the nucleus and activate osteogenic genes while inhibiting adipogenesis (Fig. 2A). However, Serine/threonine-kinase receptor-bound BMPs cause phosphorylation and migration of SMAD proteins to the nucleus to activate adipogenic programme gene transcription (Fig. 2B). This BMP (Bone Morphogenetic Protein)/Smad signalling pathway promotes adipogenesis by activating the peroxisome proliferator-activated receptor γ (PPARγ). Adipogenic gene expression is induced by PPARγ and CCAAT/enhancer-binding proteins (C/EBPs). To induce the adipocyte phenotype, C/EBPβ and C/EBPδ expression are increased at the start of adipogenesis (Bowers et al., 2006; Farmer, 2006). The NOTCH receptor becomes proteolytically cleaved, and its internal domain is released into the nucleus, allowing its target genes to be transcriptionally stimulated (Fig. 2C). Hedgehog proteins bind to the PATCH receptors, inhibiting their suppressive SMO activity, allowing Gli proteins to activate and migrate to the kernel, inhibiting adipogenesis (Fig. 2D) (Alvarenga, Vasconcellos, & Medrado, 2019).

The hormonal milieu directs the terminal bifurcation of luminal epithelial cells during pregnancy, resulting in a dissectible alveolar structure via functional differentiation of alveolar epithelial cells (AECs). This creates a stable microenvironment for the production and secretion of milk-associated components such as carbohydrates, proteins, and secretory lipids. Furthermore, the process leads to the lactation stage, where most adipocytes lose the lipid content known as "slimming" to synthesise milk fat production (Bandyopadhyay, Lee, Guzman, & Nandi, 1995; Bartley, Emerman, & Bissell, 1981; Elias, Pitelka, & Armstrong, 1973; Hovey & Aimo, 2010; Rudolph, Neville, & Anderson, 2007). Finally, weaning activates the cellular events of involution when the suckling stimulus is lost, leading to a gradual cessation of milk production in the mammary glands. It leads to the regression of AECs of the alveoli structure and extensive expansion of adipocytes, repopulating the stroma and remodelling the MG as the final non-lactating stage (Rudolph, McManaman, Hunter, Phang, & Neville, 2003; Watson & Kreuzaler, 2011). The process of involution is completed in 10 days in mice and can be divided into two phases. The first is reversible, which lasts 2–3 days and is majorly completed by epithelial cell apoptosis (Furth, Bar-Peled, & Li, 1997; Lund et al., 1996; Monks, Geske, Lehman, & Fadok, 2002). Second, an irreversible phase during which alveoli collapse where proteases degrade alveoli in the lobular-alveolar architecture of MGs, includes degrading basement membranes, stroma, and extracellular matrix (ECM), to allow proper remodelling of the mammary epithelium in preparation for subsequent rounds of lactation (Watson & Kreuzaler,

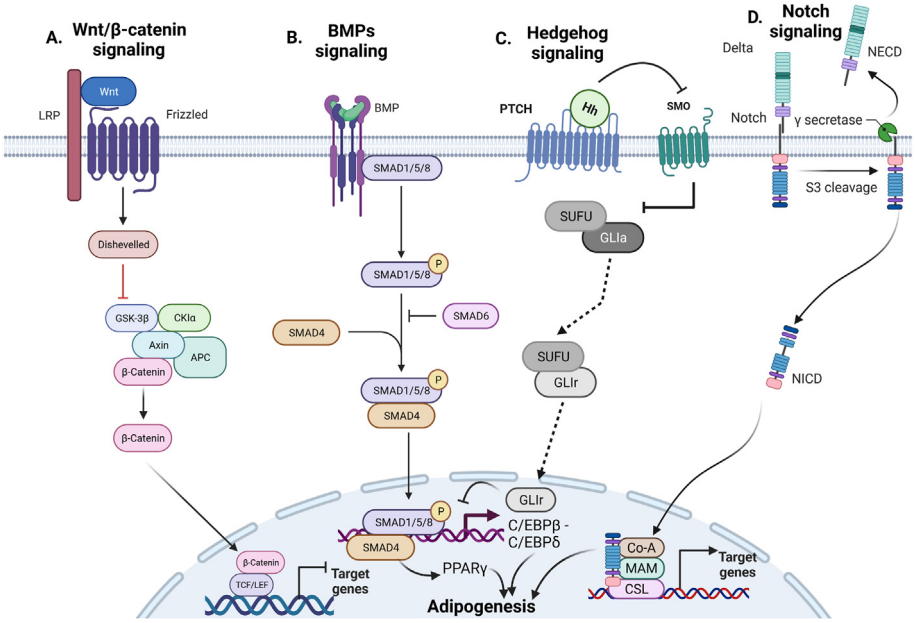

Fig. 2. The activation and inactivation of numerous signalling pathways occur during the cellular development of MSC-derived adipocytes. The BMP/Smad signalling pathway promotes adipogenesis by activating *PPAR-Ɣ*, whereas *C/EBPβ* and *C/EBPδ* expression are raised at the commencement of adipogenesis to produce the adipocyte phenotype.

2011). As involution progresses, mammary epithelial cells undergo apoptosis, leading to their removal. Concomitantly, adipocytes reappear, and the lobular-alveolar structure is reorganized to resemble that of virgin glands. (Alexander, Selvarajan, Mudgett, & Werb, 2001). The sudden decline in the number of mature adipocytes is observed during the formation of the alveolar structures at the pregnancy and lactation stages. Upon involution, AECs undergo immense apoptosis; simultaneously, the process of adipogenesis occurs to restore the adipocyte population. Whereas the mgWAT depot generally requires mammary epithelial development (Couldrey et al., 2002; Hovey & Aimo, 2010; Landskroner-Eiger, Park, Israel, Pollard, & Scherer, 2010; Rudolph et al., 2007), the process by which adipocytes reappear and support epithelial remodelling after lactation is unknown. Nonetheless, little is known about mgWAT's fate during the remodelling of the pregnancy-lactation-involution cycle.

On the contrary, the dynamic behaviour of WAT has been studied to better understand its response to a variety of physiological stimuli, including starvation and other stresses, obesogenic diet, wound healing, irradiation, dermal infection, chemotherapy, and hair cycling (Jeffery et al., 2016; Plikus et al., 2017). However, little is known about the mechanism of WAT expansion and regression. Although it is established that mature adipocytes are post-mitotic, a recent finding is spotted light for depot-specific adipose tissue growth (Jeffery et al., 2016; Zwick, Guerrero-Juarez, et al., 2018). This information gives rise to two mechanisms for WAT expansion (Rutkowski, Stern, & Scherer, 2015; Tang & Lane, 2012). First, adipogenesis (production of new mature adipocytes) requires the proliferation of adipocyte precursors (APs), their differentiation followed by absorption of triglyceride specific lipid droplets. Second, the growth of pre-existing adipocytes through triglyceride specific lipid droplet consumption and/or formation (Zwick, Rudolph, et al., 2018). More research is required to precisely describe whether one or both of the mentioned mechanisms apply to mgWAT expansion during different stages of MG development.

There are two forms of adipose tissue: white (WAT) and brown (BAT). WAT stores energy, whereas BAT dissipates it (Brenot, Hutson, & Harris, 2019; Klaus, 1997). Brown adipocytes' thermogenic activity is mediated by a specific mitochondrial protein known as uncoupling protein (UCP). Although the exact type of adipocyte found in the MG has never been determined, WAT has always been assumed (Hovey, McFadden, & Akers, 1999). According to this study, BAT is a critical element of the mammary fatty stroma, which is temporally governed during the early postnatal development period (Gouon-Evans & Pollard, 2002). Abolishing BAT in the UCP-DTA mice model causes complex mammary ductal outgrowth/TEB (Terminal end buds) formation, resulting in alveoli and β-casein gene induction (Gouon-Evans & Pollard, 2002). The systemically-induced UCP-DTA transgenic mice showed an increase in the growth of the mammary ductal system (Gouon-Evans & Pollard, 2002). Thus, it suggests that BAT prevents the development of breast epithelial cells during the onset of ductal elongation in prepubertal mice. Lactating and pregnant mice, on the other hand, show epithelial differentiation because wild-type mice lack mammary UCP-1 transcripts. In order to investigate the early differentiation of alveoli, the authors also determined if β-casein was present in transgenic mice. β-Casein transcripts were never found in the MGs of wild-type mice as young as 5 weeks old, but were found in two of three transgenic mice (Gouon-Evans & Pollard, 2002). The findings indicate that BAT inhibits mammary epithelial cell differentiation systemically during prepubertal ductal outgrowth when ductal outgrowth should take precedence over ductal differentiation.

The MG fully develops during puberty and only achieves a mature functional state during pregnancy. MG adipocytes develop into their typical phenotype three days after birth, although the mammary epithelium remains quiescent from birth to puberty (Inman, Robertson, Mott, & Bissell, 2015). Adipocytes develop from preadipocytes with a few small lipid vacuoles to mature white adipocytes with a single large, unilocular lipid vacuole covering most of the cytoplasm, according to MG ultrastructural studies (Bani-Sacchi, Bianchi, Bani, & Bigazzi, 1987). It is also worth mentioning that estradiol promotes the hyperplasia and hypertrophy of MG adipocytes, resulting in new adipocytes clustered near ductal structures. In addition, the MG is often packed with multilocular adipocytes co-localised with blood arteries and inguinal lymph nodes in mice deficient in oestrogen (Bani-Sacchi et al., 1987).

Moreover, several lipodystrophic (LD) mice that lack adipose tissue have defects in the development of the mammary gland (Couldrey et al., 2002; Landskroner-Eiger et al., 2010; Moitra et al., 1998; Pajvani et al., 2005). Adipose tissue within the mammary gland plays a crucial role as an endocrine organ in regulating mammary gland development and function. The functionalities attributed to mammary gland adipose tissue, including adipokine secretion and differentiation, are regulated by the hypothalamic-pituitary axis. During mammary gland remodelling phases, such as puberty, pregnancy, lactation, and involution, the adipose tissue undergoes significant changes in size and composition, leading to alterations in adipokine secretion and adipocyte differentiation. Adipokines such as leptin, adiponectin, and resistin are known to regulate mammary gland development, lactation, and involution by acting on mammary epithelial cells and modulating their proliferation, differentiation, and survival. Additionally, adipose tissue-derived stem cells have been identified in the mammary gland and shown to contribute to mammary gland development and regeneration. Therefore, the endocrine role of adipose tissue in mammary gland remodelling phases is critical for proper mammary gland development and function (Couldrey et al., 2002; Landskroner-Eiger et al., 2010; Moitra et al., 1998; Pajvani et al., 2005). Hence, lose of adipose tissues associated with the following settings, including (1) adipokine loss, (2) hormonal signalling downstream from adipokine loss (meaning the absence of oestrogen because of leptin loss) and (3) cell-cell interaction among adipocyte-epithelial cells loss.

The MG epithelium network is buried inside and invades adipose tissue via TEB during pregnancy and lactation. However, it was discovered that the MG in LD mice is abnormally underdeveloped, lacking TEB, and exhibits a phenotype that is not ameliorated by either estradiol administration or by normalisation of adipokine levels. Moreover, several studies using targeted knockout or knockdown approaches to impair adipocyte function have been conducted to demonstrate adipocytes' critical role during the lactation cycle. Targeted deletion of the central regulator of endoplasmic reticulum adaptive responses XBP1 in adipocytes, for example, resulted in increased adiposity and impaired lactation (Gregor et al., 2013). Whereas in adipocytes, deletion of the peroxisome regulator Pxmp2 resulted in impaired MG development during pregnancy (Vapola et al., 2014). Evidence suggests that functional adipocytes play an essential role during the lactation cycle. To better understand the importance of adipocytes in MG development, researchers used an ob/LD-based system to dissect the contributions of leptin, oestrogen, and cell-cell interactions.

LD mice have been able to conceive for 6–11 days after being injected with leptin. Brenot et al. investigated that leptin is necessary for pregnancy but not for milk production. The MG phenotype was not caused by a defect in the epithelia of the MG. Surprisingly, when transplanted into a WT host environment, the lipodystrophic epithelium was able to grow. This suggests that estradiol alone is not enough to promote development. Despite these obstacles, the gland develops typically when the MG epithelium of LD mice is removed and transplanted into wild-type animals with normal adipose tissue. LD mice could produce less milk, but it had roughly the same protein or lipid content as wild-type mice's milk. The findings show that adipose tissue is also not necessary for milk production. Furthermore, the study discovered that, in addition to estradiol and leptin, epithelial-adipocyte interaction is required for proper MG development (Brenot et al., 2019). Thus, brown MG adipocytes and white MG adipocytes may perform distinct developmental functions in the gland, which must be meticulously evaluated. However,

study results showed that lacking adipose tissue exhibits MG growth inadequacies (Hovey et al., 1999).

3. The origins of adipocytes and their differentiating fates during pregnancy and lactation

3.1. Role of adipocyte precursors in MGs?

The presence of adipocyte precursors (APs) is a major source of adipocytes during MG remodelling of various types of WAT in MG, including subcutaneous, visceral, and dermal WAT (sWAT, vWAT, and dWAT, respectively) via the process of adipogenesis (Festa et al., 2011; Jeffery et al., 2016; Zhang et al., 2015). Recent research shows that different types of adipocyte precursors are used during embryonic MG development than in adult MG tissue depots. It was shown that Pparϒ+ levels exhibit distinct site-specific expression behaviour in fetal to adult adipose precursors (Jiang, Berry, Tang, & Graff, 2014; Tang et al., 2008). In the context of MG adipocyte development, a noteworthy observation pertains to the differential expression of perilipin and adiponectin in two distinct adipocyte populations - fetal $CD24^{+}$ adipocyte precursor cells and adult maturing adipocytes. Specifically, it has been reported that perilipin and adiponectin are expressed in the former, which are responsible for generating mature adipocytes in fetal inguinal WAT depots. However, in contrast, their expression is entirely suppressed in the latter, indicating that these two proteins play distinct roles in the metabolic and regulatory functions of these two adipocyte populations (Hong et al., 2015). This observation underscores the dynamic nature of adipocyte biology and highlights the importance of studying its intricacies in the context of mammary gland physiology and its implications for lactation biology and breast cancer. Further research in this area may lead to a better understanding of the mechanisms that regulate mammary gland adipocyte function and their potential therapeutic implications for human health. Furthermore, the presence of Akt 2 is not essential for WAT but significant for proliferating $CD24^{+}$ APCs, suggesting different ways of developing WAT for fetal/early postnatal development and in adult animals to select new adipocytes (Jeffery, Church, Holtrup, Colman, & Rodeheffer, 2015). These demonstrated the complexity of WAT growth in vivo and the importance of understanding WAT formation in various physiological situations.

The enzymatically digested single-cell suspension of MG tissue from the involution stage generates a stromal vascular fraction (SVF) containing immune, endothelial, and mesenchymal cells that show APs in it (Zuk et al., 2001). The flow cytometry analysis of AP specific lineage markers include CD45– and $CD31^{-}$, $CD34^{+}$, $CD29^{+}$, Sca-1+, CD24± (Muzumdar, Tasic, Miyamichi, Li, & Luo, 2007a; Rios, Fu, Lindeman, & Visvader, 2014; Rivera-Gonzalez et al., 2016; Schmidt & Horsley, 2013). On day 1 of involution, in vitro culture of the non-epithelial ($Epcam^{-}$) cell population in the SVF fraction with adipogenic stimuli resulted in the differentiation of lipid-filled APs cells. Furthermore, in human omental subcutaneous WAT obtained from mammoplasty tissue, researchers found the presence of resident APs exclusively expressing specific markers, namely $CD45^{-}$, $CD34^{+}$, and $CD90^{+}$. (Zuk et al., 2001). Thus, it is established that multiple WAT depots are generated using adipogenic precursors through adipogenesis, depending on the physiological condition. An interesting question is the involvement of the APs in remodelling of the mgWAT after the lactation stage. The process of adipogenesis takes place in multiple stages, initially by the vigorous proliferation of APs and converting them into terminally differentiated mature adipocytes, and also by filling them through hypertrophy with lipid either using a de novo synthesis pathway or by uptake of the lipids retained during the lactation process (Festa et al., 2011; Jeffery et al., 2015, 2016; Rivera-Gonzalez et al., 2016; Rutkowski et al., 2015; Tang & Lane, 2012). However, EdU pulse study revealed that during the involution stage of the mammary gland, there was a significant increase in adipocyte progenitors (APs) that underwent proliferation and differentiation into adipocytes. Specifically, the proportion of EdU-positive APs increased from 0.9% in virgins to 5% on the first day of involution. However, the most striking observation was that on the third day of involution, a substantial proportion of APs (30.2%) were EdU-positive. This finding suggests that the involution stage is characterized by a significant acute burst of APs undergoing vigorous proliferation, leading to adipogenesis. The observation that the highest proportion of EdU-positive APs occurred on the third day of involution is particularly noteworthy. This is likely due to the fact that the initial stage of involution involves the clearance of milk-producing cells, leading to a reduction in the mammary gland epithelium. The acute burst of APs during this stage is thought to be triggered by factors released during the clearance process, which promotes adipogenesis to replace the lost milk-producing cells. The results provide important insights into the mechanisms that regulate adipogenesis during the involution stage, highlighting the importance of APs in the process (Zwick, Guerrero-Juarez, et al., 2018). The use of both EdU and BrdU in this study served as important tools for investigating the proliferative capacity of mammary gland cells, thereby contributing to a greater understanding of the mechanisms that regulate mammary gland development and function.

3.2. The fate of mature adipocytes in the MG during pregnancy and lactation

3.2.1. Do adipocytes transdifferentiate into mammary epithelial cells (adipoepithelial transdifferentiation)?

During pregnancy, a milk-secreting gland replaces the adipose tissue in the breast; when lactation is interrupted, the process reverses. As a result, much research has been conducted to explore the in vivo dynamics of adipogenesis. Using transgenic mouse models, promoters specific for whey acidic protein (WAP) were used for secretory epithelium cells while fatty acid-binding protein 4 (FABP4) was used for adipocytes. The study established that breast adipocytes can transform into AECs and vice versa (Giordano et al., 2014; Morroni et al., 2004). It is essential to report that de-differentiation is the process when cells with a more specialized differentiation state revert to a more progenitor or stem cell-like identity. In contrast, transdifferentiation is when differentiated cells transfer into another differentiated cell type (Merrell & Stanger, 2016). Likewise, a recent finding showed transdifferentiation of AECs into brown adipocytes (Giordano et al., 2014). These results are a bit enigmatic since it is believed that adipocytes are terminally differentiated cells. Previously, morphological and bromodeoxyuridine studies demonstrated that mouse mammary adipocytes convert into secretory epithelial cells during pregnancy and then revert to adipocytes (transdifferentiation) after lactation. Prior to pregnancy, aP2-Cre/R26R mice exhibit labelled adipocytes in their mammary gland. During pregnancy, these mice develop labelled secretory epithelial cells expressing the lacZ gene. However, after pregnancy, during the involution phase, the mammary gland of WAP-Cre/R26R mice contains labelled adipocytes and secretory epithelial cells. Finally, it indicates that reversible adipocyte-to-epithelium and epithelium-to-adipocyte transdifferentiation occurs in adult mouse MGs during pregnancy and lactation (Morroni et al., 2004).

Cell-specific promoters used the Cre-loxP system to control β-gal expression in the secretive epithelial cells to obtain additional evidence of glandular cells transdifferentiating into brown adipocytes (Birling, Gofflot, & Warot, 2009; Soriano, 1999). Previous studies with ultrastructural explants and lineage tracing have shown that plastic changes found in the subcutaneous deposition of pregnant mice are due, in particular, to the reversible transdifferentiation of white adipocytes into milk-secreting epithelial cells (De Matteis et al., 2009; Morroni et al., 2004; Prokesch et al., 2014). Notably, milk-secreting epithelial cells are densely packed with cytoplasmic lipid vacuoles, which fits the typical adipocyte definition. Thus, “pink adipocytes” refers to epithelial milk-secreting cells formed due to adipocyte transdifferentiation (Giordano et al., 2014). Therefore, some researchers used an ultrastructural and lineage tracing study to determine whether alveolar epithelial cells

and white adipocytes can transdifferentiate into brown adipocytes during MG involution. Using the WAP promoter, which is specific for milk-producing cells, was used to induce β-gal expression in double transgenic mice (WAP-Cre/R26R). The lactating gland's alveoli were stained positive for X-Gal, the substrate of β-gal, while brown adipocytes were found negative. However, numerous X-Gal-positive multilocular cells were observed in the mixed areas between the BAT and the involuting glands after lactation.

Furthermore, immunohistochemistry was used to confirm that these multilocular adipocytes were brown adipocytes by looking at UCP1 expression in sections that had previously reacted to X-Gal. The X-Gal-positive multilocular cells were also UCP1-positive, verifying their brown phenotype (Giordano et al., 2014). Thus, it is concluded that in WAP-Cre/R26R double transgenic mice, a subpopulation of brown adipocytes is actively engaged in the *trans*-differentiation process. For decades, Cinti and his colleagues demonstrated that MG adipocytes could transdifferentiate into epithelial milk-secreting cells at the time of pregnancy and involution (both white and brown) (Colleluori, Perugini, Barbatelli, & Cinti, 2021) (Fig. 3A). Specifically, during late pregnancy (mice at 17-18th day), an intermediate phenotype between adipocytes and alveolar structures were coloured positively both for alveologenesis and mature adipocyte markers. Then the concept of 'pink adipocytes' becomes apparent. Alveolar cells exhibit morphology similar to an adipocyte on days 17–18 of pregnancy, called "pink adipocytes", because they contain a high concentration of cytoplasmic lipids and are parenchymal cells that appear pink during pregnancy (Cinti, 2018).

Numerous lineage tracing systems have been conducted to investigate whether white-pink *trans*-differentiation is reversible. Using the $Ap2^{Cre}R26^{LacZ}$ lineage-tracing model system, it has been found that pink adipocytes were unable to express aP2 on days 17–18 of pregnancy, but 70% expressed the reporter gene lacZ, indicating white to pink *trans*-differentiation. LacZ has been expressed in brown or white adipocytes, but not in virgin epithelial ductal cells (Morroni et al., 2004). After serving their functional purpose, most alveolar structures deteriorate, and the remaining space is quickly occupied by adipose tissue. The most commonly accepted interpretation is that these alveolar cells die due to apoptosis. The hidden slimmed adipocytes take over by repopulating because the Swap gene is exclusively expressed in milk-producing epithelial MGs and not in adipocytes and other types of cells in virgin mice. However, during pregnancy and lactation, reporter gene expression was seen in MG ducts and alveoli epithelial cells and developing and mature adipocytes, mostly during the early post-lactation period in WAP–Cre/R26R virgin mice (Robinson et al., 1995).

Similarly, numerous other electron microscopy findings show that pink adipocytes pile up cytoplasmic lipids after lactation, are extruded from the alveoli further into interstitial space, or even develop into unilocular white adipocytes with no evidence of apoptosis. These cells have cytoplasmic structures not found in adipocytes but in alveolar epithelial cells that produce and secrete milk proteins and vacuoles containing milk protein granules. Furthermore, a member of the S-100 family of proteins, white adipocyte marker S-100 B (Cinti, 2018; Cinti et al., 1989), was expressed by 10–15 percent of early post-lactation adipocytes, whereas slimmed (or slimmed refilling) adipocytes were not detected at this time point (Cinti, 2018). These findings altogether support the transdifferentiation of pink to white adipocytes when taken together. However, some cell transdifferentiation from pink-to-brown supported by multilocular adipocytes expressed the reporter gene in WAP–Cre/R26R mice and UCP1, a metabolically active brown adipocyte marker in Postactivation (Cinti, 2018). Lineage tracing experiments to permanently mark a specific cell type to track its fate throughout its lifetime, such as the $WAP^{Cre}R26^{LacZ}$ model and $Ap2^{Cre}R26^{LacZ}$ model, revealed a consistent finding supporting adipo-epithelial transdifferentiation during pregnancy (Colleluori et al., 2021).

3.2.2. *Perspectives on the de- and re-differentiation of adipocytes*

Wang et al. used the AdipoChaser system to track adipogenesis processes in the MG to better understand the fate of adiponectin-expressing adipocytes during pregnancy, lactation and involution cycles (Wang et al., 2018). These triple transgenic mice can permanently label all the mature adipocytes expressing adiponectin cells with LacZ upon doxycycline stimulation. Determining the fate of adipocytes at developmental stages of MG showed low count at lactation period without lipid content.

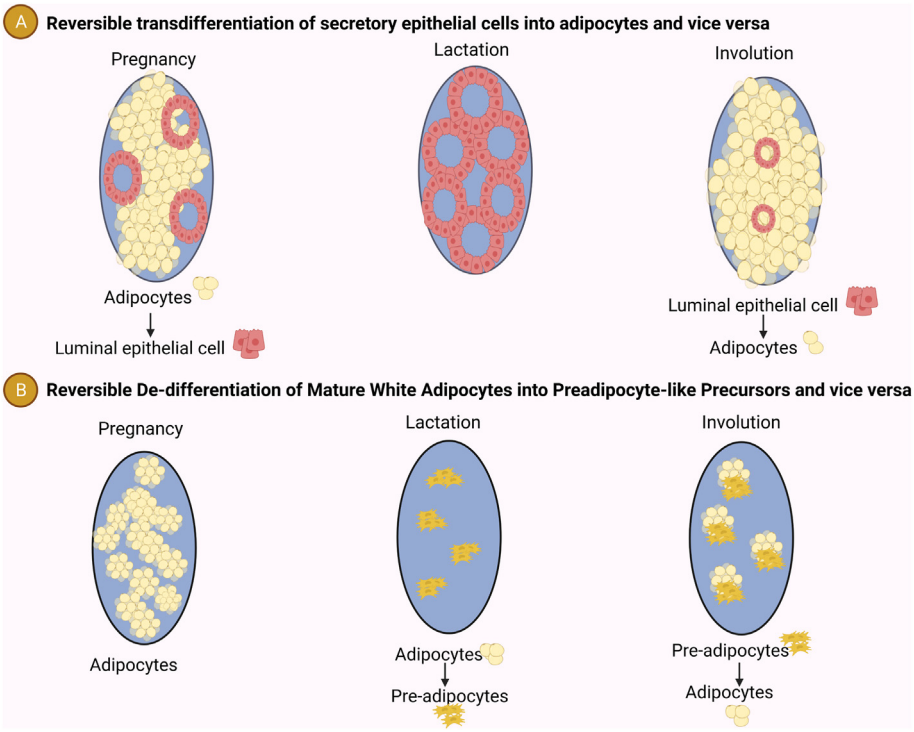

Fig. 3. There are two theories to consider about the existence of adipocytes, A. transdifferentiation and B. de-differentiation.

The counterpart to the involution was most of the cells identified as LacZ+, signifying their presence as pre-existing mature adipocytes. Concurrently, no LacZ + cells were seen in the AECs, strongly disproving that adipocytes can transdifferentiate into epithelial cells and validating the finding that alveolar epithelial cells are derived from their stem cell lineage (Vapola et al., 2014). Furthermore, Wang and colleagues discovered that adipocytes do not lose their lipids during pregnancy; instead, they de-differentiate into PDGFR + preadipocytes and fibroblast-like cells and maintain this phenotype throughout lactation. The discrepancy in results is mainly attributable to using two different promoter systems, FABP4 by Marroni et al. or adiponectin by Wang et al. (Morroni et al., 2004; Wang et al., 2013).

Besides, even after second parity leading to involution, it showed almost 100% LacZ + activity, proving that these adipocytes were rigorously undertaking multiple rounds of de-and re-differentiation cycles during reproduction instead of de novo adipogenesis from other precursor cells. These results imply that mammary adipocytes have a remarkable level of plasticity for cyclical de-differentiation and re-differentiation during reproductive cycles (Fig. 3B). In physiological conditions, mature adipocytes, commonly thought of as post-mitotic cells, potentiate to undergo de-differentiation, proliferation, and further differentiation into their mature phenotype numerous times. Also, another elegantly performed study found that PDGFRα+mesenchymal preadipocytes produce epithelial cells at the time of pubertal mammary expansion, as well as lobuloalveolar structures during pregnancy (Joshi et al., 2019). In mice, during mammary parenchymal growth, they showed the plasticity of mesenchymal cells inside the stroma by converting adipocyte progenitor cells into epithelial progenitor (PDGFRα+) cells. In addition to their role as adipocyte progenitors, PDGFRα+ cells contribute de novo to the luminal and basal epithelia throughout mammary morphogenesis. From early embryonic development to postnatal morphogenesis, PDGFRα+ progeny can be found in mammary epithelial lineages (Joshi et al., 2019). These stromal progenitors are recruited into the developing mammary epithelium and the adult gland specifically in response to steroid sex hormone exposure during pregnancy. Steroid sex hormones activate PDGF signals in the epithelium, causing PDGFRα+ mesenchymal niche cells to migrate via chemotactic migration. As a result, PREF-1 positive mesenchymal adipocyte precursors differentiate into adult mammary epithelial cells, whereas mature adipocytes do not. Additionally, they describe the contribution of PDGFRα+ mesenchymal cells to the mammary epithelial network during early development and pregnancy (Joshi et al., 2019).

Indeed, these studies conclude that these adipocytes switch into a cell type that shows similarity to neither adipocytes nor secretory mammary epithelial cells, but can re-differentiate into adipocytes during the weaning process of the continuous reproductive cycle. Given the findings of the previous two studies, it is reasonable to speculate that mature adipocytes can de-differentiate into PDGRFα+ progenitors and then differentiate into lobuloalveolar cells during pregnancy (Joshi et al., 2019). Other cells multiply and develop back into mature adipocytes during gland involution, although this process happens across several pregnancies (Wang et al., 2018) (Fig. 4). Based on this contradictory evidence, it is clear that the specific biological events defining MG adipo-epithelial remodelling during the lactation cycle remain unknown. Given the controversial empirical results of lineage tracing experiments (WAPCre vs Hypoadiponectinemia), it could be beneficial to investigate such phenomenal processes at the single-cell level to distinguish between various cellular populations and differentiation status at the time of the lactation cycle.

4. The proliferation and expansion of mature adipocytes during involution

4.1. Does involution lead to the proliferation and enlargement of mature adipocytes?

The mature adipocytes in the adult MG are considered post-mitotic, which are strongly regulated during the developmental process. Despite continuous remodelling during reproduction, its homeostasis is maintained by balancing the adipogenesis and adipocyte death rate. Consequently, it is essential to understand the mechanistic continual turnover rate of mgWAT to explore its development and disease conditions. Multiple research groups generated and utilised the dual fluorescent membrane-localised tdTomato/eGFP (mT/mG) reporter Cre-mediated excision system to delineate the mature adipocytes in the MG stroma. To visualize mature adipocytes, the transgenic mouse line's heritable expression system can switch from tomato to mGFP expression by expressing Cre recombinase under the control of the Adipoq promoter (Berry & Rodeheffer, 2013).

Recently, to understand the dynamics of adipocytes in the MG during the pregnancy-lactation-involution cycle, Zwick et al. used the Adipoq-Cre; mT/mG system to trace adipocyte proliferation status (Zwick, Guerrero-Juarez, et al., 2018). The transgenic mice at the virgin stage displayed intense mGFP+ expression than in the pregnant mammary stroma and few in the lactation-stage MG stroma. Furthermore, the mTomato+ expression was discovered to be more expensive in the pregnancy and lactation stage than in the virgin stage, an indication of the high presence of alveolar epithelial cells. As expected, the involution

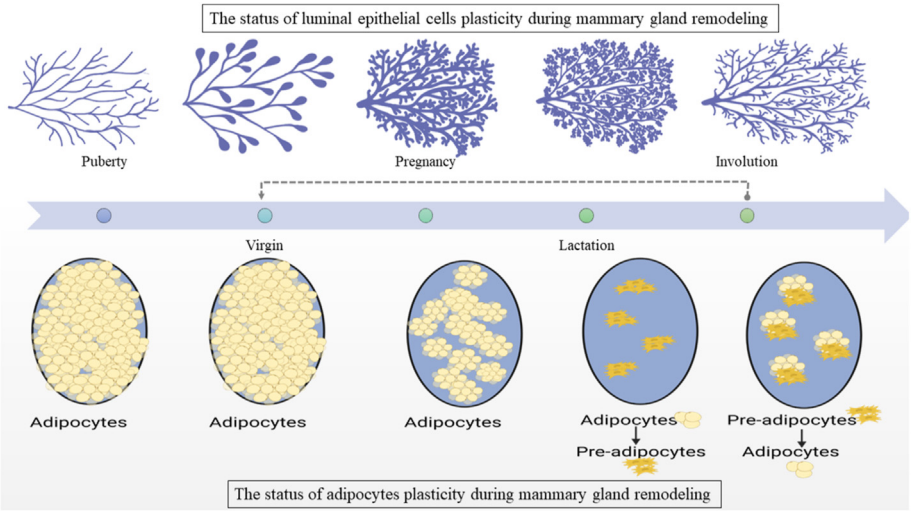

Fig. 4. During different stages of gland development, the status of both cells in the MG.

leads to the regression of mTomato+ alveolar ducts, but surprising leads to the expansion of mgWAT, leading to a high number of mGFP+ cells. The PAT proteins (perilipin, adipophilin, and TIP47) are considered crucial lipid accumulation supervisors. Notably, it was identified that the 99.7% expression of perilipin (Berry & Rodeheffer, 2013; Brenot et al., 2019; Russell et al., 2007) protein was explicitly associated with mature adipocytes on the 7th day of involution along with consistent expression of adiponectin in mGFP+ cells. Nevertheless, an interesting finding is the disclosure of a small mass of mGFP+ cells found positive for perilipin during lactation and showing the expression of mature adipocyte marker proteins including Cep/α, Pparγ2, Leptin Adipoq, and Perilipin (Berry & Rodeheffer, 2013). Thus, it confirms the presence of a small mass of mature adipocytes in the lactation stage of the MG (Zwick, Guerrero-Juarez, et al., 2018).

Further, to understand the hypertrophy of pre-existing lactating adipocytes during involution, the morphometric based cross-sectional area (CSA) analysis showed an increment of the 4.1-fold size of adipocytes during involution from 3 to 7 days compared to day 1. This is consistent with the perilipin+ lipid droplet size increasing during MG involution's reversible and irreversible phases (Li, Matheny, & Scarpace, 1997). Other mature adipocyte cell numbers were also significantly increased from lactation to the 7th day of involution, but the gross area of isolated MGs decreased by 4.7-fold during the equivalent period. As shown in the overview Fig. 4, the increase in adipocyte counts during involution vis-a-vis resulted in an increment in adipocyte density compared to the overall size of the MG shrinks (Zwick, Guerrero-Juarez, et al., 2018).

4.2. Mature adipocytes expand mgWAT during involution

The intriguing question was whether pregnant and lactating adipocytes completely de-differentiate or simply lose their lipid droplets. To answer that, the purified SVF and preadipocyte marker PDGFRα protein-based fluorescence-activated cell sorting (FACS) sorted cells exposed to adipogenic stimuli in vitro differentiation assay showed 87% LacZ positive, implying their origin from pre-existing adipocytes. These results validate the finding that PDGFRα containing LacZ+ cells transform into preadipocytes during lactation instead of just conceiving lipid-avoided adipocytes.

To determine and typify these preadipocytes, the scientists utilised another dual colour co-labelling AdipoChaser-mT/mG system (Ye et al., 2015) (Vishvanath et al., 2016). All the mature adipocytes were pre-labelled with GFP and analysed during pregnancy and lactation using bromodeoxyuridine (BrdU) for proliferation. The results showed no colour convergence observed in the pregnancy and the lactating stage, but at three days of weaning, the co-localisation of the GFP and BrdU, proving the active division of these precursor cells. The further characterisation of GFP+ cells found in the SVF fraction in the lactating stage showed a high population of PDGFRα+ cells, strongly suggesting the precursor state instead of transdifferentiation. The comparison of the SVF fraction of GFP+/PDGFRa+ lactating, PDGFR + virgin and murine-derived preadipocyte cell lines revealed a common preadipocyte and fibroblast marker in the virgin and lactating stages. However, a dissimilar marker in mature adipocytes strongly implies that mature adipocytes lost their characteristics during de-differentiation and acquired preadipocyte characteristics.

5. Mechanistic pathway for the adaptation of mgWAT

The deep understanding of mechanistic pathways for the plasticity of mgWAT in diverse pathophysiological conditions to support tissue homeostasis and function is an active area of research (Zwick, Guerrero-Juarez, et al., 2018). We compiled the data provided in recent articles to support the findings that the proliferative APs inhabited the involution stage during MG remodelling. At the same time, mgWAT growth in involution is prominent because of hypertrophy of pre-existing adipocytes by taking up the retained lipid droplets from the lactating epithelial lumen and cells, as modelled in Fig. 3. These significant findings of hypertrophy-driven mgWAT expansion at the time of involution are in contrast with the decade-old findings showing that adipose tissue formation at different adipose depots takes place by hypertrophy and adipogenesis (Festa et al., 2011; Jeffery et al., 2015, 2016). However, it mimics the metabolic WAT depots of the pre-existing adipocyte expansion process after starvation followed by 1–4 days of refeeding (O'Brien, Martinson, Durand-Rougely, & Schedin, 2011). These findings enforce understanding of the mechanisms of neighbouring cells, including macrophages (Lilla, Joshi, Craik, & Werb, 2009) and ECM remodelling (Alexander et al., 2001; Joe et al., 2010; Selvarajan, Lund, Takeuchi, Craik, & Werb, 2001) that involves the growth of mgWAT. Therefore, determining the micro environment-specific growth of adipocytes during mgWAT might give clues about proliferation, hypertrophy, and lipid trafficking during the involution stage.

Multiple lines of experimental evidence suggest adipogenesis at different time points in the MG developmental cycle, such as in the last week of lactation and the initial 7 days of involution. For example, the subtle adipogenesis of approximately 10% of mgWAT was observed during the lineage-tracing experiment in pregnant animals. Likewise, on the 3rd day of involution, AP proliferation was seen and considered to contribute to mesenchymal lineage cells such as fibroblasts or the production of new adipocytes on the 7th-day of involution (Jeffery et al., 2014). The latter possibility of proliferative adipocytes resonates with the timing of adipogenesis under the influence of a high-fat diet, as also, after the first week of high-fat feeding, the results showed AP proliferation. Nonetheless, the newly formed adipocytes are continuously observed for 7 weeks following proliferation (Jeffery et al., 2015).

On the contrary, the above studies result in controversy about the findings of transdifferentiation of adipocyte to epithelial and vice versa during the lactation stage (Morroni et al., 2004; Prokesch et al., 2014). The difference in the results may be the use of different promoters in the lineage tracing model for experiments. For instance, to trace the mature adipocytes reversible transdifferentiation (Morroni et al., 2004), the use of the aP2-Cre system was utilised, which also labels cells external to the adipocyte lineage (Lee et al., 2013; Rivera-Gonzalez et al., 2016). In comparison, the other studies reported using an unambiguous adipocyte Adipoq promoter system (Vishvanath et al., 2016; Wagner et al., 1997). Similarly, the use of β-gal, a cytoplasmic reporter protein, is not appropriate for adipocyte lineage tracing investigations (Morroni et al., 2004) because the tiny expression of this reporter protein can not be interpreted accurately. In contrast, precision can be attained by using the membrane-specific localised reporters per se mT/mG protein system (Berry et al., 2014; Berry & Rodeheffer, 2013; Sanchez-Gurmaches & Guertin, 2014; Sargeant et al., 2014).

The importance of lipid catabolism is a promising area of research in MG biology. Here, we highlight the unappreciated function of mature adipocytes in regulating lipid trafficking from the mammary epithelium. One recent study found the initiation of apoptosis through milk lipid uptake in alveolar epithelial cells by Stat-3 dependent leakage of lysosomes, which evokes the process of involution and establishes that lipid trafficking is a critically important player in the process (Prater, Shehata, Watson, & Stingl, 2013; Wang et al., 2012). A separate study identified the apoptosis of alveoli epithelial cells even in adipocyte-depleted mice without lipid but not completely (Bowers et al., 2006). Thus, these results suggest that Stat3 signalling is independent of lipid uptake and can be activated by other associated proteins in epithelial cells in MGs lacking adipocytes.

Nonetheless, it indicates that the complete remodelling of the MG is possible in the existence of lipid uptake and/or the presence of mature adipocytes in AECs. Furthermore, the importance of retaining lipid in adipocyte-depleted glands in AECs resulted in the improper deterioration of the epithelial by an unknown mechanism (Zwick, Guerrero-Juarez, et al., 2018). Similarly, it was also reported that adipocytes are also responsible for the re-organisation of the mammary epithelium tree through extracellular matrix proteins or inflammatory responses

(Martinson, Jindal, Durand-Rougely, Borges, & Schedin, 2014; Schedin, O'Brien, Rudolph, Stein, & Borges, 2007; Stein et al., 2004; Wang et al., 2013). Thus, we strongly suggest exploring the link between the MG adipocytes and the surrounding environment of stroma, lipid trafficking, and diverse epithelial cells for proper gland remodelling.

Apart from the milk stasis in combination with stromal factors discussed in this article, the evidence also implies that adipocyte lipid dynamics is started by epithelial cells. It is proven that MEC induces adipocyte-specific lipolysis to start the milk production process (Bandyopadhyay et al., 1995; Bartley et al., 1981; Hovey & Aimo, 2010; Rudolph et al., 2007), and hair follicles stimulate adipocyte lipid filling in the skin (Donati et al., 2014; Zwick, Guerrero-Juarez, et al., 2018). Furthermore, the lipids of the epithelial cell intake derived from adipocytes are also exclusively found in different cancer growth types, such as breast, melanoma, oral (Pascual et al., 2017), and ovarian (Nieman et al., 2011). Thus, the intense crosstalk between WAT and epithelial cells and the resultant findings also indicate the role of the involution process in pregnancy-associated breast cancer. This information is stressed to better understand adipocyte-derived lipid dynamics and organ remodelling for breast cancer progression. Furthermore, the existence of adipose tissue is not limited to just the MG. However, it is very diverse in different organ systems (Muzumdar, Tasic, Miyamichi, Li, & Luo, 2007b; Plikus et al., 2017), which implies that these lipid dynamics have profound implications for tissue homeostasis maintenance, renewal, and pathophysiological conditions.

The primary focus of this review is to highlight the very recent findings of adipocyte plasticity in connection with MG remodelling during pregnancy, lactation, and the involution cycle. In addition, we discussed the experimental evidence for the reversible re and de-differentiation of adipocyte transitions. Nonetheless, the signalling pathways of this temporary reversion during tissue remodelling remain undefined. Importantly, what kind of stimulus induces these changes and converts them back into adipocytes is unknown. Indeed, some evidence is predicted that the surrounding stroma, in conjunction with parenchymal cells and in conjunction with associated pregnancy hormones, may regulate the temporary transition cycle. Further, the deep knowledge of the plasticity of adipocytes remains to be explored, which may generate novel, useful information.

6. Breast cancer, adipocyte dysfunction, and lactation defects

Breast cancer (BC) affects one in eight women during their lifetime, and one in thirty women dies of this disease. More than 300,000 cases of invasive and in situ breast cancer were diagnosed in 2020 (Siegel, Miller, & Jemal, 2020). BC is the most frequently diagnosed female cancer and the leading cause of female cancer-related death worldwide (Bray et al., 2018). This is because BC has a high rate of invasion, metastasis, and recurrence, particularly in the brain and lungs (Ponnusamy, Natarajan, Thangaraj, & Manoharan, 2020). Numerous growing evidence suggests that adipocytes adjacent to invasive cancer cells, known as cancer-associated adipocytes (CAAs), play a role in BC progression. Adipocytes are excellent candidates for modifying tumour behaviour by releasing adipokines such as growth factors, hormones, cytokines, and other molecules through heterotypic signalling processes. The following can be introduced according to established scientific evidence in vivo, in vitro, and clinical. The characterisation of adipocyte-derived factors varies with tumour progression and is confirmed in human breast cancer samples. CAAs alter the characteristics/phenotypes of BC cells, resulting in increased aggression. In addition, BC cells significantly influence the adipocytes surrounding them (Zhao, Wu, Rong, Zheng, & Guo, 2020) (Fig. 5). Adipocytes are also one of the primary stromal cells in many tissues and play an important role in the tumour microenvironment (TME). CAAs are a type of adipocyte that lives near cancer cells and communicates with them by releasing a variety of factors that have both local and systemic effects. Intermodulation between adipocytes and cancer cells alters both cell types' phenotypes and functions, accelerating tumour progression (Wu et al., 2019).

Moreover, the basement membrane normally separates mature adipocytes from epithelial cells, which limits the interaction possible between both cells. When BC cells break through the basement membrane, they are exposed to the TME, which contains adipocytes (Pallegar et al., 2020). CAAs and BC cells orchestrate a highly complex interaction, on

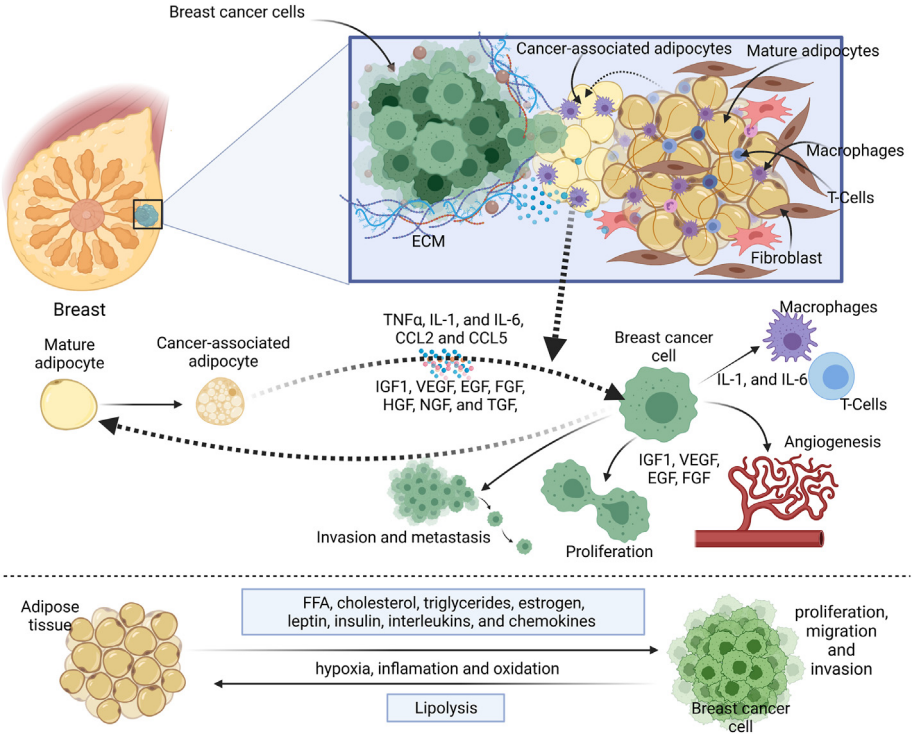

Fig. 5. The adipocyte's molecular function in the development of breast cancer.

the other hand, which has not been fully elucidated. The interaction of neighbouring CAAs and BC eventually shapes the TME favouring proliferation, tumour dissemination, angiogenesis, invasion, and metastasis, resulting in an oncogenic driven state (Zhao et al., 2020). CAAs are not only found near BC cells, but they also signal them by releasing a variety of factors that have both local and systemic effects. Adipose tissue dysfunction has been associated with accelerated growth and survival of BC cells. Several research findings have shown that CAA-BC cell crosstalk promotes cancer progression and metastasis via various secreted adipokines and their role in tumour remodelling. CAAs are mostly found adjacent to BC cells at the invasive front of breast tumours. CAAs have smaller cell sizes, irregular shapes, and small/distributed lipid droplets than mature adipocytes (Dirat et al., 2011; Suárez–Nájera et al., 2018). CAAs have a distinct phenotype, with lower lipid content, lower levels of delayed adipocyte differentiation markers, and higher levels of inflammatory cytokines and proteases (Dirat et al., 2011). CAAs secrete more adipokines than mature adipocytes, including adiponectin, leptin, chemokine ligand 2 (CCL2), chemokine ligand 5 (CCL5), interleukin (IL)-6, and other cytokines (Crake, Phillips, Kleffmann, & Currie, 2019; Wu et al., 2019). The secretion of inflammatory cytokines such as IL-1, TNFα, and IL-6, as well as chemokines (including CCL2 and CCL5), attracts M1 proinflammatory macrophages; abnormal ECM components that also contribute to the microenvironment anomalies seen in breast tumours; and fatty acids, the tumour's energetic substrate (Colleluori et al., 2021).

Moreover, proteomic approaches discovered more than 350 proteins in mammary adipocytes. Adipokines include hormones such as leptin, adiponectin, and resistin, as well as growth factors (insulin-like growth factor 1 (IGF1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), nerve growth factor (NGF), transforming growth factor (TGF), enzymes (autotaxin), cytokines and interleukin (IL-6, IL-1, CCL5, IL-8, TNF-α) (Frühbeck, 2008; Wang, Mariman, Renes, & Keijer, 2008). These proteins are critical for adipocyte and breast epithelial function and development (Singh & Ali, 2023; Singh et al., 2023; D'Esposito et al., 2020). CAAs, in comparison to distant, mature adipocytes, may develop a more aggressive phenotype and promote BC growth and metastasis, due to their lower physical barrier and a more assertive secretome of adipokines (Wu et al., 2012). The expression and secretory spectrum of inflammatory mediators secreted by adipocytes are altered in the TME of BC, promoting tumour cell proliferation and metastasis and the formation of new blood vessels (neovascularisation). The inflammatory microenvironment of BC is a major driver of the development of the disease and is a potential target for new treatments. Potential BC treatment targets are inflammatory factors (IL-6, CCL5) and transport factors (CD36, MCT1).

BC can be regulated by adipocytes' metabolic substrates. Obesity is strongly linked to faulty adipocyte metabolism, which leads to a number of chronic disorders. Serum contains large amounts of free fatty acids, cholesterol, glycerol, and triglycerides, all of which influence the initiation, growth, and migration of breast tumours (Cunha & Hom, 1996). In vitro co-culture of mature adipocytes with breast cancer cells increased cancer cell proliferation, demonstrating that adipocytes had a direct influence on cancer cells via secreted substances (Robinson et al., 1999). Adipocytes secrete not only cytokines and growth factors, but also nonesterified fatty acids. Fatty acids are required for all of BC cells' biological functions. Lipids have two roles in breast cancer: they modify the cancer cells' metabolic pathways by rewiring them for de novo lipid biosynthesis. They can also be taken up exogenously from the cancer cells' adipocyte microenvironment or the systemic circulation (Bernard & Wellberg, 2021). Fatty acids (FFAs) released by adipocytes when stimulated by tumour secretions are stored as triglycerides in lipid droplets in tumour cells. Lipolysis is achieved in tumour cell lines through an adipose triglyceride lipase–dependent (ATGL-dependent) lipolytic pathway. ATGL is commonly found in tumours and is amplified when it comes into contact with adipocytes. FFAs are involved in fatty acid β-oxidation (FAO), an active process in the tumour, but not in healthy breast epithelial cells. However, FAO is uncoupled from ATP production in cultivated cells, which keeps AMPK/acetyl-CoA carboxylase activation looped, sustaining metabolic remodelling.

Tumour cells' increased invasive capacities are entirely negated by ATGL-dependent lipolysis or FAO pathway inhibition (Wang et al., 2017). FFAs affect BC, according to preclinical and clinical studies. Triglycerides are depleted, and cancer cell proliferation increases when BC cell lines are cultured with 3T3-L1 adipocytes (Balaban et al., 2017). BC cell proliferation is further boosted by 3T3-L1 obese adipocytes, which have increased lipid droplets and triacylglycerols (Feigelson et al., 2020). Additionally, this cycle of lipid release and uptake is further increased by BC cells that secrete lipolytic enzymes, thus amplifying the paracrine effects of lipids (Feigelson et al., 2020).

However, Adipocytes' released hormones can also regulate BC (Gouon-Evans et al., 2000). Adipose tissue also contributes to the development of BC by increasing the secretion of adipokines, commonly known as "released hormones," such as oestrogen, adiponectin, leptin, and insulin (Sakakura et al., 1976, 1982; Silberstein, 2001). In obese postmenopausal women with a high BMI, oestrogen and oestrogen derivatives are substantially induced. Obese adults had a 35% higher oestrogen level and a 130 percent higher estradiol level than those with a low BMI/athletic physique, according to a random study of postmenopausal women (Daniel et al., 1984). Usually Oestrogen is mostly produced in the ovary in premenopausal women, but it is also produced in the adipose tissues of postmenopausal women (DeOme et al., 1959). Cytochrome P450 aromatase is abundant in adipose tissue and catalyses the conversion of androgens to estrogens (Cinti, 2018). As a result, oestrogen levels in breast tumours associated with adipose tissue are tenfold higher than in the blood (Granneman, Li, Zhu, & Lu, 2005). Furthermore, cytokines produced in adipose tissues can promote the synthesis of cytochrome P450 aromatase. Obesity-induced breast cancer is associated with positive ERα, particularly in postmenopausal women (Barbatelli et al., 2010). The oestrogen-ERs complex interacts with the oestrogen response element site on DNA, controlling gene expression linked with growth, differentiation, and other disorders (Bielczyk-Maczynska, 2019). Finally, estrogens promote BC invasion and migration. Oestrogen treatment changes the cytoskeleton and activates GPCR-like proteins and the oestrogen receptor signalling pathway in vitro, resulting in breast cell metastasis (Gouon-Evans et al., 2000) (Matsumoto et al., 2008; Wang et al., 2013) (Fig. 5).

Obesity reversal through surgery, diet, exercise, and/or pharmacologic interventions, on the other hand, has been shown to target the operators as mentioned above in obesity-related BC, such as adipocyte hypertrophy, inflammation, and insulin resistance, as well as biological factors such as estrogens and insulin in some cases. Weight loss has been linked to a lower risk of breast cancer (Feigelson et al., 2020; Goodwin et al., 2020), implying that reversing obesity could lower the risk of BC and lengthen disease-free survival (Birsoy et al., 2008).

7. Outstanding questions

- ➢ What are additional molecular factors responsible for the de-differentiation of adipocytes?
- ➢ What is the difference between de-differentiated cells and pre-adipocytes in various depots?
- ➢ Do they have the ability to show pluripotent stemness behaviour and convert into other cell lineages?
- ➢ What are the molecular signals for the re-differentiation of de-differentiated preadipocytes?
- ➢ Importantly, do preadipocytes occur in other physiological processes, too, or are preadipocytes only specific to the involution process of the MG?
- ➢ Do these types of transformations occur in adipose tissue in bone marrow during lactation and involution?

➢ Does comprehensive research on these topics generate a novel therapy for obesity, diabetes, lipodystrophy, and cancer? The current review provides updates on adipocyte plasticity for recent research and future questions to work on.

8. Concluding remarks and future perspectives

Recent results from multiple lines of experiments indicate that adipocytes located in the MG and other physiological settings exhibit remarkable plasticity. The "AdipoChaser mouse" method demonstrated exclusive detection of resident APs in the MG stroma and was responsible for the repopulation of mature adipocytes following the first round of lactation. Additionally, it resolves the issue surrounding adipocyte transdifferentiation to epithelial cells and shows that alveolar epithelial cells do not form from pre-existing adipocytes. Instead, mature adipocytes are dedifferentiated into adipogenic precursors during pregnancy and lactation by expressing preadipocyte markers, including platelet-derived growth factor receptor a (PDGFRa) and depleting lipid droplets. Further, the regrowth of adipocytes in involution occurs by hypertrophy of milk-derived lipids to support epithelial regression. These results lead to conclude that adipocytes have exceptional plasticity in terms of re- and de-differentiation during MG remodelling. These findings have opened up new research avenues in mammary biology because they show that physiologic stimuli can cause genome reprogramming in mature cells, resulting in the acquisition of a new phenotype with distinct physiologic properties. There is still much more work to be done to understand the exact epigenetic regulation of adult cell physiological reversible transdifferentiation.

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Consent for publication

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The authors' contributions

PS and SAA have contributed equally and written the manuscript and arranged together. In addition, both the authors contributed to the article and approved the submitted version.

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