Reproduction and Breeding 3 (2023) 125–130

Contents lists available at ScienceDirect

Reproduction and Breeding

journal homepage: www.keaipublishing.com/en/journals/reproduction-and-breeding

Human breast organoid models for lactation research

Jenny Lee [a], Yan Liu [b], Edward Ray [b], Armando E. Giuliano [b], Xiaojiang Cui [b,*]

[a] The University of California Los Angeles, College of Letters and Science, CA, 90048, USA
[b] Department of Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, 90048, USA

ARTICLE INFO

Keywords:
Breast cancer
Lactation
Mammary gland
Milk
Organoid

ABSTRACT

The human mammary gland is the major organ involved in lactation. In the mammary gland, alveoli secrete milk and myoepithelial cells contract to propel the milk through branched structures called ducts and eventually to the nipple. It is through this process of lactation that infants receive milk, which is essential for proper infant growth and development. The lactation process is comprised of sophisticated interactive networks at the cellular level that are not well understood. Whereas the majority of published mammary gland lactation studies have relied on mouse mammary glands, recent advancements in techniques to study mammary glands enable *in vitro* reproduction of lactation using human-representative frameworks. Currently, the 3D breast organoid is the state-of-the-art model in human mammary gland research, utilizing induced pluripotent stem cells (iPSCs) or processed patient-derived breast tissues embedded in a special matrix that are then able to grow into complex structures that recapitulate aspects of native human breast tissue. Gaining comprehensive biological insight into the process of lactation through these breast tissue-mimetic 3D models is essential for further studies on lactation-associated human mammary gland diseases, human milk composition, and potential solutions to challenges in maternal milk accessibility. In this short review, the benefits and potential utility of 3D breast organoids in understanding the underlying science of lactation and advancing further human mammary gland studies are discussed.

1. Mammary gland development and lactation

The human mammary gland is a complex organ consisting of intricate structures that remodel over the course of a female's lifetime. During development, precursor luminal stem cells give rise to ductal and alveolar luminal cells of the mammary gland. In contrast, precursor basal stem cells give rise to myoepithelial cells that line the outside of the luminal cells and make contact with the extracellular matrix (ECM). At birth, the female mammary gland has a primitive ductal structure and is in a latent phase. At the onset of puberty, substantial development begins as ductal branches and bulbous structures at the tips of the ducts called the terminal end buds (TEBs) elongate through the fat pad due to a series of interactions between major signaling molecules [1]. The TEBs then degenerate and only the extended ductal tree remains [1]. In each cycle of menstruation, the fluctuating levels of ovarian hormones, including the major hormones estrogen and progesterone, drive the periodic reconstruction of mammary gland structures [2].

During pregnancy, progesterone promotes further side-branching of ducts and alveologenesis [3]. Maturation of the lobules are also aided by chorionic gonadotropin [4]. The development of these structures play an important role in lactogenesis — the process of mammary epithelial cell differentiation into the mature lactating mammary gland [5]. Lactogenesis consists of two consecutive stages known as secretory differentiation and secretory activation [4]. Secretory differentiation occurs in the second half of pregnancy and begins with the differentiation of mammary epithelial cells into milk-producing lactocytes [6]. Previous studies using cell and organ cultures have shown that the stimulation of secretory differentiation relies on a combination of prolactin, insulin, and steroids [5]. Although the secretory differentiation stage makes the alveoli capable of secreting certain milk proteins such as lactose and casein, high progesterone and estrogen levels prevent premature milk secretion [7,8]. Progesterone and estrogen decrease by the third trimester of pregnancy and after removal of the placenta post-delivery, which is a major secretor of progesterone [4,9]. Thereafter, secretory activation occurs when milk is secreted due to low progesterone levels and high prolactin, insulin, and cortisol levels [6]. Continued prolactin secretion by the lactotrophs of the anterior pituitary gland is stimulated by suckling, and prolactin subsequently stimulates lactocytes in the

* Corresponding author. Cedars-Sinai Medical Center, 8700 Beverly Blvd, Davis Building 2065, Los Angeles, CA, 90048, USA.
E-mail address: Xiaojiang.Cui@cshs.org (X. Cui).

https://doi.org/10.1016/j.repbre.2023.08.003
Received 26 May 2023; Received in revised form 17 August 2023; Accepted 21 August 2023
Available online 27 August 2023

alveoli to produce milk [8,10]. In addition, suckling stimulates the posterior pituitary gland to release oxytocin, which prompts the myoepithelial cells to contract and propel the milk from the alveoli through the ducts and eventually to the nipple [8].

After lactation, the mammary gland undergoes involution. When suckling or stimulation is halted and milk is no longer ejected from the ducts, milk buildup creates a negative feedback effect by activating apoptosis-associated signaling [11]. Matrix metalloproteases (MMPs) secreted by stromal cells have been shown to augment apoptosis by remodeling the ECM around the alveoli, causing alveoli collapse [11]. Through involution, the mammary gland is reverted to the pre-pregnancy state [11].

2. Mammary organoids

2.1. Need for new mammary modeling techniques

Despite great advancements in understanding the dynamic mammary gland structures and chemical pathways of lactogenesis and involution, the full scope of biomolecular mechanisms remains unknown. Most importantly, current knowledge of human mammary gland development and lactation largely originates from animal model studies. The most commonly used mouse mammary gland is substantially different from humans in morphology, development, and signaling pathways. At birth, the mouse mammary gland displays a single ductal network that begins at the nipple in contrast to the human mammary gland which has several ductal networks converging at the nipple [12]. Additionally, mouse mammary glands only display lobular growth during pregnancy, whereas human mammary glands experience lobular growth at the onset of puberty [12]. Furthermore, human mammary glands have been shown to express more complex signaling pathways during early development. One such example is the absence of luminal marker cytokeratin (CK) 19 during mouse embryonic development in contrast to early mammary gland structures of human infants [12]. These comparisons account for only a small fraction of the differences between mouse and human mammary glands. Therefore, it is crucial that new biological methods and experimental systems are used to advance our understanding in the various topics of human lactation biology, lactation-associated diseases, and human milk composition.

Because animal or murine mammary gland studies cannot fully recapitulate the architecture and molecular underpinnings of human mammary gland tissue, 3D breast organoids have been monumental in reproducing the human mammary gland without reliance on animal microenvironments. In contrast to animal xenograft models, organoids allow easy control and observation of breast tissue, provide results of added or removed genes or hormones without the influence of other variables, maintain spatial morphology, preserve local signaling pathways, and allow ECM interactions. Through breast organoid studies, more accurate understandings of the human mammary gland can be obtained, and clinical applications can be developed.

2.2. Current mammary organoid studies

Human breast organoids can either be prepared by differentiation of human induced pluripotent stem cells (iPSCs) or digestion of patient-derived breast tissue samples. In a previous study by Qu et al., human iPSC-derived non-neural ectoderm cell-containing spheres successfully differentiated into mammary-like organoids in 3D culture [13]. The organoids were able to form alveolar structures, showing recapitulation of breast tissue morphology. Furthermore, the researchers found both luminal markers such as EpCAM and CK18, and basal markers such as CK14, showing that cell heterogeneity was preserved [13]. The ability to use somatic cells (e.g., from the blood or skin) to generate iPSCs is one of the major advantages of iPSC-derived breast organoids. In addition to increased opportunities for organoid research, iPSCs also improve accessibility to a large patient population for more diversity-inclusive mammary gland studies.

Many studies have also used patient-derived breast organoids for 3D culturing. In a study by Rosenbluth et al., normal human breast organoids expressed both luminal and basal cell proteins such as CK8 and CK14, respectively, and certain cells were also found to express estrogen receptors (ER) [14]. Further flow cytometry analyses confirmed that breast organoid cells could be characterized into luminal progenitor, mature luminal, or basal/stem cell subtypes, supporting that cell heterogeneity could be maintained [14]. Additionally, the organoids displayed similar ductal and alveolar structures as in native human breast tissue [14]. Sachs et al. also presented that ER, progesterone receptor, and HER2 markers were maintained for a majority of breast organoids when compared with the original tumor tissues [15]. The study further showed that breast organoids can be used for drug screening, as the model system yielded drug experiment results consistent with those of *in vivo* studies [15]. A great advantage of patient-derived organoids of 3D models in comparison to iPSC-derived breast organoids is the ability to preserve stromal components and interactions from digested breast tissue samples in organoid culture. One study using mouse breast organoids found that macrophages co-cultured with breast organoids allowed basal cells to maintain stem cell activity by activating TNF-α and PI3K, demonstrating the importance of stromal cells in organoid culture studies [16]. Importantly, studies have also shown that patient-derived breast organoids can undergo long term culture and cryopreservation, enabling establishment of organoid biobanks [14,15].

Most organoid studies utilize a matrix that allows organoids to "float", and the matrix is subsequently covered in culture medium. The matrix allows 3D growth and facilitates in reproducing ECM conditions [17,18]. Matrix stiffness and the Matrigel to Collagen I ratio in the matrix have important implications on the morphology and growth of the organoid [17,19,20]. The techniques used to culture the organoids can also vary. For example, previous studies have used the dome/droplet form culture, cultures with different ratios of Matrigel and Collagen I, the hanging drop array system, and the inverted mammary organoid model (Fig. 1) [14,15,21–24].

2.3. Lactation studies using organoids

Recent studies have indicated the potential and utility of breast organoids to model lactation *in vitro*. In the study by Qu et al., researchers were able to demonstrate that the addition of a lactation medium containing prolactin, insulin, and hydrocortisone to iPSC-derived breast organoids were able to induce milk protein expression (Fig. 2a) [13]. Another study by Sumbal et al. embedded patient-derived breast organoids in Matrigel and plated the mixture in a dome/droplet form (Fig. 2b) [25]. When the organoids were exposed to the growth factor FGF2 and lactation medium containing prolactin and hydrocortisone, organoids showed darker lumen suggesting milk secretion, produced lipid droplets, and displayed increased expression of the milk proteins β-casein and *Csn2* [25]. The expression of these two milk proteins were also preserved in long-term lactation medium culture [25]. Staining of luminal and myoepithelial cells showed that the architecture of the organoids was intact during long-term lactation medium culture, lactation medium withdrawal, and oxytocin-stimulated myoepithelial contraction [25]. Notably, lactation medium withdrawal was able to reduce the size and branching of the organoids and caused increased expression of MMPs, suggesting the ability of organoids to model involution [25]. The protocol used in this research study has also been further standardized [26].

3. Prospects of human breast organoids

3.1. Breast organoid models for furthering lactation studies

Previous research using breast organoids to recapitulate structural and chemical characteristics of normal and cancerous breast tissue,

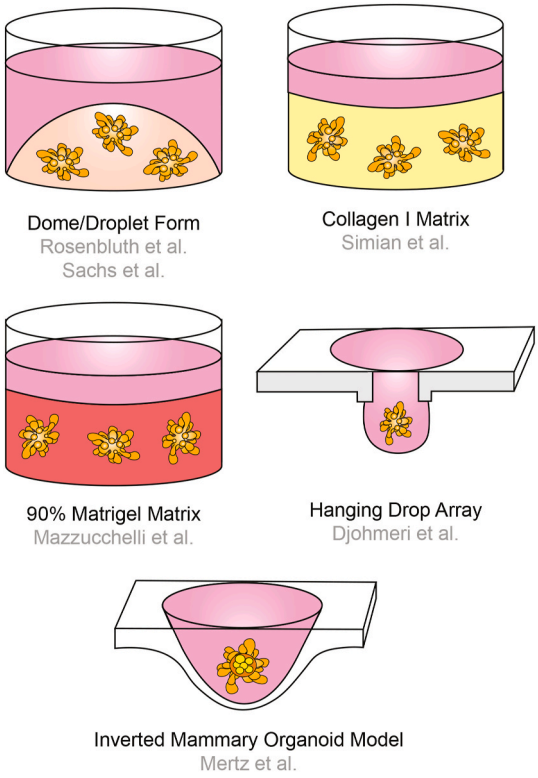

Fig. 1. Types of Breast Organoid Models Used in Reported Studies
(1) Top left, organoids are mixed in a matrix of basal membrane extract (shown in orange). A droplet of the mixture is placed on a 24-well suspension culture plate in studies by Sachs et al. and Rosenbluth et al. Medium is placed over the matrix (shown in pink). (2) Top right, organoids are embedded in matrix consisting of only collagen I (shown in yellow), and medium is placed over the matrix (shown in pink) in the Simian et al. study. (3) Middle left, organoids are embedded in matrix consisting of 90% Matrigel (shown in red), and medium is placed over the matrix (shown in pink) in the Mazzucchelli et al. study. (4) Middle right, organoids are mixed in cell suspension solution containing Matrigel (shown in pink) in the hanging drop array. The solution can be directly renewed by removing and adding small amounts when needed. (5) Bottom, the inverted hanging drop array in the Mertz et al. study uses similar cell suspension solution as the hanging drop array. However, this method is different from other organoid models in that adipose tissue self-assembles to be in the interior of the mammary epithelial cells.

human mammary gland microenvironments, and lactation demonstrate high promise of organoids in furthering lactation studies. Future organoid studies should study the signaling pathways of important molecules involved in lactation such as FGF2, insulin, and cortisol, as well as additional molecular players in involution. Because Sumbal et al. did not co-culture with adipocytes and previous studies by Watson et al. observed adipocyte differentiation during involution, co-culturing organoids with adipocytes and other stromal components should also be conducted in order to understand the full scope of involution. Solidified knowledge on the biomolecular mechanisms of the stages of lactation using breast organoids will consequently allow for a strengthened comprehension of lactation-associated breast cancer and human milk composition (Fig. 3).

3.2. *Breast organoid models for studying pregnancy- and lactation-associated breast cancer*

Although breast cancer during pregnancy is uncommon, the prevalence of breast cancer occurrence during pregnancy may be increasing in alignment with the growing number of women delaying childbearing until a later age [27]. Currently, the breast cancer incidence rate during pregnancy is approximately 1 in 3,000 pregnancies and comprises approximately 2% of all breast carcinoma cases [27]. Furthermore, these breast cancers generally present poor histologic and prognostic features. The effects of fluctuations in hormones, signaling pathways, cell proliferation, and structural reorganization during lactation on breast cancer development and progression are unclear.

Studies on the relationship between lactation and breast cancer also have divided results. A previous study analyzed data from the 1997 Nurses' Health Study II and presented inconsistencies in the relationship between premenopausal breast cancer and breastfeeding [28]. For women without a family history of breast cancer, there was no significant association between having breastfed at least once in their lifetime or never having breastfed and premenopausal breast cancer [28]. However, women who never breastfed and took lactation supplements had lower breast cancer incidence rates than those who never breastfed and did not take lactation suppressants [28]. Additionally, for women with a first-degree relative with a history of breast cancer, those who breastfed at least once in their life showed significantly lower premenopausal breast cancer incidence rates than those who had never breastfed [28]. Another study postulated that breastfeeding may reduce the risk of breast cancer by reducing the total number of menstrual cycles throughout a woman's life [29]. These reports are among those that demonstrate the lack of understanding on the impact of lactation on breast cancer development. Thus, whether lactation lowers breast cancer incidence rates remains an unresolved question and warrants further investigation. More studies on the relationship between dramatic apoptosis and remodeling of the ECM during involution and breast cancer risk should also be conducted.

Because 3D breast organoids are able to pinpoint the time-dependent molecular and cellular changes induced by specific hormones, physiological perturbations, and genomic mutations, organoids could be used to identify potential risk factors of breast cancer initiation and progression during the pregnancy and lactation stages.

3.3. *Breast organoid models and human milk*

Human milk contains distinctive components that are essential for proper organ development, microbiome development, and overall growth [30]. One study demonstrating the importance of maternal milk presented that breastfeeding for six months decreased the risk of childhood obesity by 42% [31]. In human milk, antibodies, macronutrients such as proteins and fatty acids, vitamins, and growth factors are known as important components [30].

According to the Centers for Disease Control and Prevention, 83.2% of infants born in 2019 received some breast milk after birth but only 62.6% exclusively received breast milk [32]. By 6 months, the percentage of infants born in 2019 who received some breast milk decreased to 55.8% and only 24.9% exclusively received breast milk [32]. This suggests that infants may have relied on infant formula for alternatives of breast milk. However, infant formula is derived from cow milk and has been shown in previous studies to contain different qualities and amounts of ingredients than those in human milk [33]. For example, cow milk has greater levels of fat, protein, and minerals [34]. Cow milk also contains inadequate amounts of certain essential factors such as vitamin E and iron while containing disproportionately high levels of sodium and potassium [34]. Moreover, human milk contains important substances such as human milk oligosaccharides that support critical functions including immunity and neurocognitive development, and greater levels of lactoferrin which increases the bioavailability of iron in human milk [35,36].

However, even the benefits of maternal milk may differ between women based on various factors. 3D breast organoids may be helpful in elucidating how gene expression, external exposures, diet, and lifestyle can affect human milk production and composition. For example, organoid studies could clarify inconsistencies between the association of human milk and infant health conditions, such as the relationship

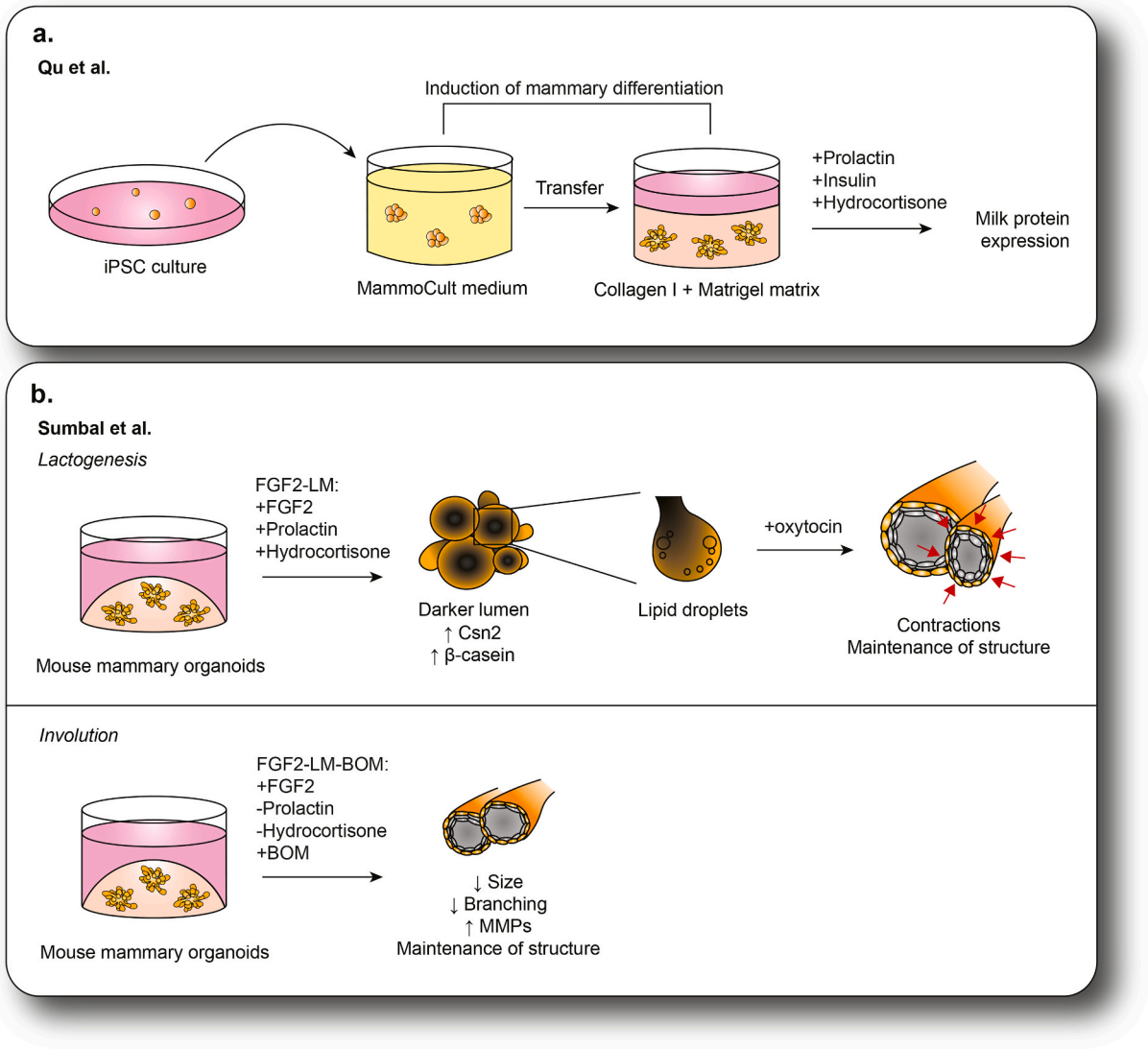

Fig. 2. Capability of Mammary Organoids to Model Lactogenesis and Involution
(a) Qu et al. model. iPSC-derived non-neural ectoderm cell-containing spheres are enriched in Mammocult medium (shown in yellow). The spheres are placed in 3D culture containing matrix composed of Collagen I and Matrigel (shown in orange) to further mammary differentiation and organoid growth. Medium is placed over the matrix (shown in pink). Addition of lactation medium consisting of prolactin, insulin, and hydrocortisone results in milk protein expression. (b) Sumbal et al. model. Mouse mammary organoids are embedded in Matrigel in the dome/droplet form (shown in orange) in a 24-well culture plate. Medium is placed over the matrix (shown in pink). Milk secretion and lipid droplet production are detected after the addition of lactation medium, and myoepithelial contraction occurs after the addition of oxytocin. Removing lactation medium reproduces activity indicative of involution.

between breastfeeding and increased risk of asthma or respiratory illness in infants [37]. Additionally, certain circumstances or situations may affect a woman's ability to breastfeed. One study found that women who experience delayed secretory activation of lactogenesis could have decreased abilities to exclusively breastfeed or breastfeed for long durations [38]. Another study was able to conclude that women who had remaining placental fragments post-delivery had difficulty in entering the secretory activation stage [39].

Further 3D breast organoid studies may advance our understanding of human milk composition and shed light on ways to improve human milk production and accessibility. It is envisioned that breast organoid systems may be developed, refined, and scaled up to manufacture "human milk formula" for infants who have special nutritional requirements, or be used as enhanced maternal milk alternatives when maternal milk is not produced or available in order to ensure healthy infant growth.

4. Conclusion

Current abilities of 3D breast organoids show the potential of breast organoid models in improving our general knowledge of the cellular and molecular biology of lactogenesis and involution. In addition, breast organoids may be used to investigate the development of breast cancer during pregnancy and lactation stages. Furthermore, breast organoids may be exploited as potential sources of human milk to aid in the development of healthy infants.

However, current abilities of 3D breast organoids also have its limitations in that reproduction of the breast tissue microenvironment and biological processes that occur in the native human mammary gland must be further improved. Additionally, long term culture of breast organoids — although possible — is still a challenge and culturing procedures must be standardized. These and other limitations must be addressed before the applicable benefits of 3D breast organoid systems can be reaped. Nonetheless, well-developed human breast organoid

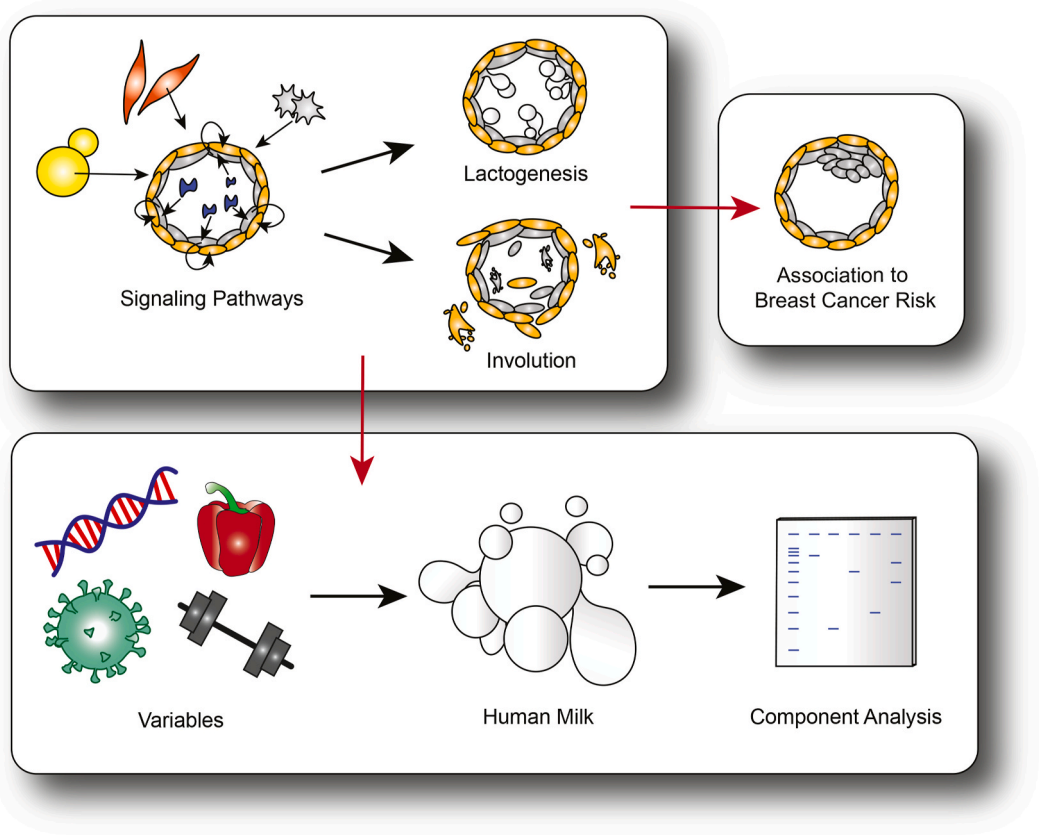

Fig. 3. Potential Applications of Breast Organoid Models in Lactation Research
Breast organoids may serve as useful models to delineate epithelial and stromal interactions in human breast tissue and can contribute to the elucidation of the cellular and molecular signaling pathways governing lactogenesis and involution. A strong knowledge base in these lactation stages can further enhance our understanding of the associations between lactation stages and breast cancer, as well as the effects of various internal and external variables on milk composition and production.

models coupled with multiplex staining, single-cell RNA sequencing, and spatial gene expression profiling technologies would open up an avenue for the elucidation of lactation biology.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Xiaojiang Cui is supported by National Institutes of Health (2R01CA151610 and R21CA280458), Department of Defense (W81XWH-18-1-0067), and Samuel Oschin Cancer Institute Research Development Fund. Armando E. Giuliano is supported by the Fashion Footwear Charitable Foundation of New York, Inc, the Margie and Robert E. Petersen Foundation, and Linda and Jim Lippman Fund.

References

[1] I.S. Paine, M.T. Lewis, The terminal end bud: the little engine that could, J. Mammary Gland Biol. Neoplasia 22 (2) (2017) 93–108, https://doi.org/10.1007/s10911-017-9372-0.
[2] Rathi Ramakrishnan, et al., Morphological changes in breast tissue with menstrual cycle, 1 Dec. Nature News (2002) www.nature.com/articles/3880699.
[3] H. Macias, L. Hinck, Mammary gland development, Wiley Interdiscip. Rev. Dev. Biol. 1 (4) (2012) 533–557, https://doi.org/10.1002/wdev.35.
[4] Pillay, J., & Davis, T. J. (n.d.). Physiology, Lactation. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK499981/.
[5] M.C. Neville, T.B. McFadden, I. Forsyth, Hormonal regulation of mammary differentiation and milk secretion, n.d., SpringerLink, https://link.springer.com/article/10.1023/A:1015770423167.
[6] W.W. Pang, P.E. Hartmann, Initiation of Human Lactation: Secretory Differentiation and Secretory Activation, 2007, November 20. SpringerLink, https://link.springer.com/article/10.1007/s10911-007-9054-4.
[7] M.C. Neville, J. Morton, S. Umemura, Lactogenesis: the Transition from Pregnancy to Lactation, Elsevier, 2005, July 15. https://www.sciencedirect.com/science/article/pii/S0031395505702844?via%3Dihub.
[8] Infant and Young Child Feeding: Model Chapter for Textbooks for Medical Students and Allied Health Professionals, World Health Organization, 2009.
[9] A. Alex, E. Bhandary, K.P. McGuire, Anatomy and physiology of the breast during pregnancy and lactation, Adv. Exp. Med. Biol. 1252 (2020) 3–7, https://doi.org/10.1007/978-3-030-41596-9_1.
[10] M. Witkowska-Zimny, E. Kaminska-El-Hassan, Cells of human breast milk, Cell. Mol. Biol. Lett. 22 (2017) 11, https://doi.org/10.1186/s11658-017-0042-4.
[11] C.J. Watson, *Key Stages in Mammary Gland Development - Involution: Apoptosis and Tissue Remodelling that Convert the Mammary Gland from Milk Factory to a Quiescent Organ* - . BioMed Central, 2006, April 10. https://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr1401.
[12] S. McNally, T. Stein, Overview of Mammary Gland Development: A Comparison of Mouse and Human, 2016, October 29. SpringerLink. Retrieved April 20, 2023, from, https://link.springer.com/protocol/10.1007/978-1-4939-6475-8_1.
[13] Y. Qu, B. Han, B. Gao, S. Bose, Y. Gong, K. Wawrowsky, A.E. Giuliano, D. Sareen, X. Cui, Differentiation of human induced pluripotent stem cells to mammary-like organoids, Stem Cell Rep. 8 (2) (2017) 205–215, https://doi.org/10.1016/j.stemcr.2016.12.023.
[14] J.M. Rosenbluth, R.C.J. Schackmann, G.K. Gray, L.M. Selfors, C.M.-C. Li, M. Boedicker, H.J. Kuiken, A. Richardson, J. Brock, J. Garber, D. Dillon, N. Sachs, H. Clevers, J.S. Brugge, April 6). *Organoid cultures from normal and cancer-prone human breast tissues preserve complex epithelial lineages*, Nature News (2020). https://www.nature.com/articles/s41467-020-15548-7.
[15] N. Sachs, J. de Ligt, O. Kopper, E. Gogola, G. Bounova, F. Weeber, H. Clevers, A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity, Cell, 2018, December 7. https://www.sciencedirect.com/science/article/pii/S0092867417313193?via%3Dihub#sec4.
[16] Y. Zhou, Z. Ye, W. Wei, M. Zhang, F. Huang, J. Li, C. Cai, Macrophages maintain mammary stem cell activity and mammary homeostasis via TNF-α-PI3K-cdk1/

cyclin B1 Axis. Nature News. https://www.nature.com/articles/s41536-023-00296-1, 2023, May 2.
[17] S.C. Mohan, T.-Y. Lee, A.E. Giuliano, X. Cui, Current status of breast organoid models, Frontiers (2021, October 22). https://www.frontiersin.org/articles/10.3389/fbioe.2021.745943/full#B9.
[18] Hynes, R. O., & Naba, A. (n.d.). Overview of the matrisome–an inventory of extracellular matrix constituents and functions. U.S. National Library of Medicine. https://pubmed.ncbi.nlm.nih.gov/21937732/.
[19] M.J. Paszek, N. Zahir, K.R. Johnson, J.N. Lakins, G.I. Rozenberg, A. Gefen, C. A. Reinhart-King, S.S. Margulies, M. Dembo, D. Boettiger, D.A. Hammer, V. M. Weaver, Tensional homeostasis and the malignant phenotype, Cancer Cell 8 (3) (2005) 241–254, https://doi.org/10.1016/j.ccr.2005.08.010.
[20] Nguyen-Ngoc, K., & Ewald, A. (n.d.). Mammary ductal elongation and myoepithelial migration are regulated by the composition of the extracellular matrix. Wiley Online Library. https://onlinelibrary.wiley.com/doi/full/10.1111/jmi.12017.
[21] M. Simian, Y. Hirai, M. Navre, Z. Werb, A. Lochter, M.J. Bissell, The Interplay of Matrix Metalloproteinases, Morphogens and Growth Factors Is Necessary for Branching of Mammary Epithelial Cells, The Company of Biologists, 2001, August 15. https://journals.biologists.com/dev/article/128/16/3117/41319/The-interplay-of-matrix-metalloproteinases.
[22] S. Mazzucchelli, F. Piccotti, R. Allevi, M. Truffi, L. Sorrentino, L. Russo, M. Agozzino, L. Signati, A. Bonizzi, L. Villani, F. Corsi, *Establishment and Morphological Characterization of Patient-Derived Organoids from Breast Cancer - Biological Procedures Online*. BioMed Central, 2019, June 15, in: https://biologicalproceduresonline.biomedcentral.com/articles/10.1186/s12575-019-0099-8.
[23] S.I. Djomehri, B. Burman, M.E. Gonzalez, S. Takayama, C.G. Kleer, A reproducible scaffold-free 3D organoid model to study neoplastic progression in breast cancer - journal of cell communication and Signaling, SpringerLink, https://link.springer.com/article/10.1007/s12079-018-0498-7, 2018, December 4.
[24] D.R. Mertz, E. Parigoris, J. Sentosa, J.-H. Lee, S. Lee, C.G. Kleer, G. Luker, S. Takayama, Triple-negative Breast Cancer Cells Invade Adipocyte/preadipocyte-Encapsulating Geometrically Inverted Mammary Organoids, OUP Academic, 2023, April 4. https://academic.oup.com/ib/article/doi/10.1093/intbio/zyad004/7103404.
[25] J. Sumbal, A. Chiche, E. Charifou, Z. Koledova, H. Li, Primary Mammary Organoid Model of Lactation and Involution, Frontiers, 2020, January 24. https://www.frontiersin.org/articles/10.3389/fcell.2020.00068/full.
[26] Charifou, E., Sumbal, J., Koledova, Z., & Chiche, A. (n.d.). A robust mammary organoid system to model lactation and involution-like processes. U.S. National Library of Medicine. https://pubmed.ncbi.nlm.nih.gov/34124297/.
[27] L.P. Middleton, M. Amin, K. Gwyn, R. Theriault, A. Sahin, Breast carcinoma in pregnant women: assessment of clinicopathologic and immunohistochemical features, Cancer 98 (5) (2003) 1055–1060, https://doi.org/10.1002/cncr.11614.
[28] A.M. Stuebe, W.C. Willett, F. Xue, K.B. Michels, Lactation and incidence of premenopausal breast cancer: a longitudinal study, Arch. Intern. Med. 169 (15) (2009) 1364–1371, https://doi.org/10.1001/archinternmed.2009.231.
[29] Y. Feng, M. Spezia, S. Huang, C. Yuan, Z. Zeng, L. Zhang, X. Ji, W. Liu, B. Huang, W. Luo, B. Liu, Y. Lei, S. Du, A. Vuppalapati, H.H. Luu, R.C. Haydon, T.C. He, G. Ren, Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis, Gene. Dis. 5 (2) (2018) 77–106, https://doi.org/10.1016/j.gendis.2018.05.001.
[30] Ballard, O., & Morrow, A. L. (n.d.). Human Milk Composition: Nutrients and Bioactive Factors. Elsevier. https://linkinghub.elsevier.com/retrieve/pii/S0031395512001678.
[31] Wang, L., Collins, C., Ratliff, M., Xie, B., & Wang, Y. (n.d.). Breastfeeding Reduces Childhood Obesity Risks. National Library of Medicine. https://www.liebertpub.com/doi/10.1089/chi.2016.0210.
[32] Centers for Disease Control and Prevention, *Breastfeeding Report Card*. Centers for Disease Control and Prevention, 2022, August 31. https://www.cdc.gov/breastfeeding/data/reportcard.htm.
[33] Z. Liu, M. Neuringer, J.W. Erdman Jr., M.J. Kuchan, L. Renner, E.E. Johnson, X. Wang, C.D. Kroenke, The effects of breastfeeding versus formula-feeding on cerebral cortex maturation in infant rhesus macaques, Neuroimage 184 (2019) 372–385, https://doi.org/10.1016/j.neuroimage.2018.09.015.
[34] C.R. Martin, P.R. Ling, G.L. Blackburn, Review of infant feeding: key features of breast milk and infant formula, Nutrients 8 (5) (2016) 279, https://doi.org/10.3390/nu8050279.
[35] D.R. Hill, J.M. Chow, R.H. Buck, Multifunctional benefits of prevalent HMOs: implications for infant health, Nutrients 13 (10) (2021) 3364, https://doi.org/10.3390/nu13103364.
[36] Institute of Medicine (US) Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, Infant Formula: Evaluating the Safety of New Ingredients, National Academies Press (US), 2004.
[37] K. Miliku, M.B. Azad, Breastfeeding and the developmental origins of asthma: current evidence, possible mechanisms, and future research priorities, Nutrients 10 (8) (2018) 995, https://doi.org/10.3390/nu10080995.
[38] E. Brownell, C.R. Howard, R.A. Lawrence, A.M. Dozier, Delayed onset lactogenesis II predicts the cessation of any or exclusive breastfeeding, J. Pediatr. 161 (4) (2012) 608–614, https://doi.org/10.1016/j.jpeds.2012.03.035.
[39] M.R. Neifert, S.L. McDonough, M.C. Neville, Failure of lactogenesis associated with placental retention, Am. J. Obstet. Gynecol. 140 (4) (1981) 477–478, https://doi.org/10.1016/0002-9378(81)90056-9.