

# At the Dawn of a New Discovery: The Potential of Breast Milk Stem Cells<sup>1–4</sup>

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## ABSTRACT

Breast milk contains bioactive molecules that provide a multitude of immunologic, developmental and nutritional benefits to the infant. Less attention has been placed on the cellular nature of breast milk, which contains thousands to millions of maternal cells in every milliliter that the infant ingests. What are the properties and roles of these cells? Most studies have examined breast milk cells from an immunologic perspective, focusing specifically on the leukocytes, mainly in the early postpartum period. In the past decade, research has taken a multi-dimensional approach to investigating the cells of human milk. Technologic advances in single cell analysis and imaging have aided this work, which has resulted in the breakthrough discovery of stem cells in breast milk with multilineage potential that are transferred to the offspring during breastfeeding. This has generated numerous implications for both infant and maternal health and regenerative medicine. This review summarizes the latest knowledge on breast milk stem cells, and discusses their known in vitro and in vivo attributes as well as potential functions and applications. *Adv Nutr* 2014;5:770–778.

## Introduction

Breastfeeding is unique to mammals, which have developed specialized organs, the mammary glands, dedicated to the synthesis, secretion, and delivery of milk to the newborn offspring (1). Milk not only provides ideal nourishment, but also ensures maximal immunologic support of the otherwise susceptible young, whose immune system is still immature at birth (1,2). Increasing evidence demonstrates that, in addition to these attributes, milk contains bioactive molecules that facilitate the normal development of the offspring via targeted programming. For example, hormones in breast milk assist in the development of appetite control mechanisms that not only confer short-term regulation of feeding, but also protect from overweight and obesity in adolescent and adulthood (3–5).

The functions of milk for the offspring are highly conserved; nevertheless, milk composition differs substantially among mammalian species, demonstrating a species-specific milk evolution that reflects the environment, needs, and growth rate of the young for which it is intended (1,6). To a lesser extent, milk composition varies within a species, and many factors may be associated with this variation, including the maternal diet and environment and potentially genetic factors (2,7). Additional complexity is generated by intraindividual variations in breast milk composition, which were attributed to the stage of lactation, the degree of breast fullness, infant feeding, the health status of the breastfeeding dyad, and other factors (1,8–11).

Despite these variations in milk composition both between and within species, the main building blocks of milk are common to all species. These include molecules (e.g., proteins, lipids, carbohydrates, and vitamins) as well as viable cells, both of which have functionalities. Most of the milk compositional research has been centered on its bioactive molecules. In contrast, very little is known about milk cells, their origin and properties, and the factors influencing them. This lack of knowledge has perpetuated the thought that the leukocyte is the predominant cell type in human milk. This belief was actually based on studies of bovine milk and a few human milk studies that focused on colostrum, both of which are indeed abundant in leukocytes

<sup>1</sup> This article is a review of the symposium “It’s Alive: Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant,” held 29 April 2014 at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2014 in San Diego, CA. The symposium was sponsored by ASN and the ASN Lactation RIS, and supported by an educational grant from Medela, Inc.

<sup>2</sup> A summary of the symposium “It’s Alive: Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant” was published in the September 2014 issue of *Advances in Nutrition*.

<sup>3</sup> Supported by an unrestricted research grant from Medela AG (Switzerland). The company had no input in writing or the decision to publish the manuscript.

<sup>4</sup> Author disclosures: F. Hassiotou and P. E. Hartmann, no conflicts of interest.

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(2). However, recent analyses using state-of-the-art techniques of cell characterization are revealing that leukocytes constitute only a small minority ( $\leq 2\%$ ) of the cells in mature human milk when both the mother and the infant are healthy (10,12,13). Even so, this low percentage translates to hundreds to thousands of immune cells being ingested every day by breastfed infants, if we consider the known range in total breast milk cell content ( $10^4$  to  $13 \times 10^6$  cells/mL milk) (2) and daily infant breast milk intake (470–1350 mL) (8). Extraordinarily, the remaining nonimmune cells ingested in a day by the breastfed infant can reach billions. This situation is reversed during periods of infection of either the mother or the infant, providing strong evidence of the striking dynamic ability of breast milk to boost the immune system of the infant and readily respond to his changing immunologic needs (10). Nevertheless, the consistently low percentage of leukocytes under healthy conditions clearly now indicates that we must embrace a paradigm shift regarding the main cell type in human milk and acknowledge that for the majority of the lactation period the infant predominantly receives nonimmune cells from breast milk.

This raises a question: What exactly are these nonimmune cells and what are their functions? The latest research shows that the majority of nonimmune cells in breast milk are of epithelial origin. They include mature mammary cells, such as lactocytes (milk-secretory cells) and myoepithelial cells originating from the ducts and alveoli of the lactating mammary gland (Fig. 1) (2,14). In the last 5 y, however, there have been ground-breaking reports demonstrating the presence of epithelial progenitors (15) and cells with stem cell properties in breast milk (16–18). It is not surprising that these discoveries are now stimulating research in both the lactation and stem cell fields, with potential implications that span from the role(s) of these stem cells in the lactating mammary gland and in the breastfed infant to their use in lactation biology and regenerative medicine. This review summarizes the current knowledge on the cellular hierarchy of human milk, with emphasis on its stem cells and their origin, classification, known attributes, and future applications.

### The Cellular Hierarchy of the Mammary Gland

In accordance with its principle function to synthesize and deliver milk to the infant during lactation, the mammary gland is largely immature during most of the life of a woman, developing into a mature functional organ only during pregnancy and lactation (1,19). Outside this period, the adult female mammary gland is in a “resting state,” consisting of a network of bilayered epithelial ducts embedded within supporting stromal and adipose tissue (Fig. 2A) (1). During pregnancy, a gradual remodeling of the gland occurs, which is facilitated by the orchestrated secretion of a lactogenic hormone complex, mainly including estrogen, progesterone, prolactin, placental lactogen, and insulin, that acts on mammary stem and progenitor cells to induce ductal branching, alveolar morphogenesis, and secretory differentiation (20–22) (Fig. 2B). Mammary stem cells

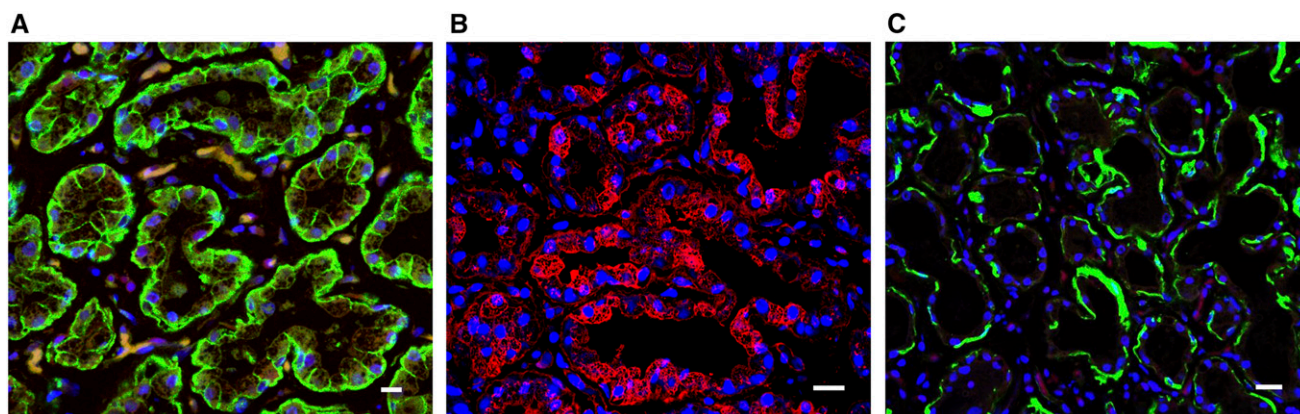
[with the known marker profile cluster determinant (CD)<sup>5</sup> 49F<sup>+</sup>/CD29<sup>+</sup>/CD24<sup>low</sup>/cytokeratin (CK)5<sup>+</sup>] are multipotent cells thought to exist in the basal ductal layer in the resting breast, with the ability to self-renew, but also to differentiate through a line of progenitor cells into the 2 main types of mammary epithelial cells: the luminal cells (known to express CK18) and the basal myoepithelial cells [known to express CK14 and smooth muscle actin (SMA)] (2,23,24). Mammary stem cells are scarce in the resting breast, but proliferate during pregnancy to facilitate the remodeling of the mammary gland toward a milk-secretory organ (2). After parturition, a decrease in circulating progesterone occurring via withdrawal of the placenta and with the presence of high prolactin concentrations results in secretory activation of the differentiated luminal cells (lactocytes) at the alveolar sites, which are then capable of copious milk synthesis and secretion (20,25). The delivery of milk from the lactocytes to the infant during breastfeeding occurs via oxytocin-induced contraction of the myoepithelial cells surrounding the lactocytes, which results in ejection of milk toward the nipple (milk ejection reflex) (2).

Although it is well understood that the breast cellular hierarchy includes mammary stem cells, mature luminal and myoepithelial cells, and progenitor cells as various intermediate steps in this pathway, the marker profiles and properties for each cell type of this hierarchy are still not well defined. This may be partly due to the use of resting breast tissue by most previous studies, in which stem and progenitor cells are scarce, and which does not contain the complete cellular hierarchy seen in the lactating breast. On the other hand, the latter is very difficult to access, because few women die during lactation and it is unethical to solicit breast biopsies of lactating women when it is not medically warranted. A solution to this problem is provided by the use of breast milk as a noninvasive, ethical, and plentiful source of cells from the lactating breast epithelium.

### The Cellular Composition of Human Milk

Breast milk has been known to contain cells since the time of microscopist Anthony van Leeuwenhoek (26). His observations were later confirmed by Donn  (1838) (27) and Henle (1841) (28). Investigators in subsequent years speculated as to the nature of these cells, some classifying them as epithelial, others as immune/mesenchymal or a mix of both (29–34). However, it is only recently, with advancements in single cell analysis and characterization, that we are beginning to appreciate the nature and properties of the heterogeneous cellular composition of human milk. Similar to cell content, breast milk cell composition is dynamic, with the proportion of the different cell types being influenced by many factors, such as infant feeding, the health status of the dyad, and potentially the stage of lactation (2,11). However, the main

<sup>5</sup> Abbreviations used: BSC, breast milk stem cell; CD, cluster determinant; CK, cytokeratin; EMT, epithelial to mesenchymal transition; hBSC, human breast milk stem cell; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell



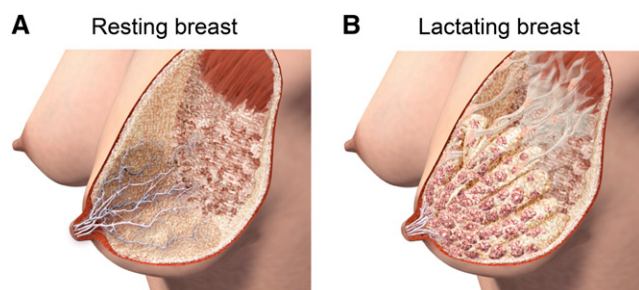
**FIGURE 1** Cross-sectional view of the human lactating breast, showing mature alveoli (A). The epithelial cell adhesion molecule is shown in green. Each alveolus comprises a luminal layer of epithelial cells that contains the lactocytes, expressing milk proteins such as  $\alpha$ -lactalbumin (shown in red) (B). Myoepithelial cells lining alveoli in the lactating breast (C). Smooth muscle actin is shown in green; nuclei are shown in blue (DAPI stain). Scale bars: 10  $\mu$ m.

cell types present in breast milk are thought to be common both among and within women with normal lactation.

As discussed earlier in this review, although leukocytes are abundant in colostrum, they constitute the minority of cells ( $\leq 2\%$ ) in mature human milk when both the mother and infant are healthy. They originate from the maternal bloodstream and are actively recruited in the breast in response to infections of the dyad, returning to low baseline concentrations upon recovery (10). This raises the question of the nature of the remaining  $<98\%$  of breast milk cells. Because breast milk originates from the mammary gland, it is logical to assume that it contains cells from the mammary epithelium. The cells that are in direct contact with the ductal and alveolar lumen, and thus with the milk in these compartments, are the luminal cells, which include the lactocytes. Indeed, breast milk contains a large number of luminal epithelial cells (CK18<sup>+</sup>) and lactocytes synthesizing milk proteins, with the latter amounting to 32–86% of total cells based on expression of the lactocyte marker  $\beta$ -casein examined by flow cytometry (2). In addition to lactocytes,

CK14/SMA-positive cells are found in breast milk at varying levels (1,2,14,16), suggesting the presence of myoepithelial cells. It is noteworthy that CK18/CK14 double-positive cells can also be found in breast milk (F. Hassiotou, unpublished data), potentially suggesting the presence of progenitors (35) and highlighting the fact that care must be taken in interpreting tissue immunostaining and milk flow cytometry data, because the markers currently known to distinguish different mammary cell populations are not definitive. For example, Indumathi et al. also reported the presence of myoepithelial cells in colostrum, but the markers used to identify these and other cell types were not myoepithelial cell-specific (36).

Based on current evidence, luminal and the myoepithelial cells together are thought to constitute up to  $<98\%$  of the cells in human milk under healthy conditions (2). Interestingly, this epithelial compartment of breast milk has been suggested to include not only mature epithelial cells, but also their precursors (2). Exciting advances are revealing that breast milk harbors populations of stem and progenitor cells, and much attention is now intensified on delineating their origin, properties, and functions.



**FIGURE 2** Anatomy of the human adult breast in its resting state (in nonpregnant, nonlactating women) (A) and its lactating state (B). During pregnancy and lactation, elongation of existing ducts and synthesis of new ducts occur, along with formation of alveolar structures at the duct termini, where milk is synthesized during lactation. Reproduced with permission from Medela AG.

### Breast Milk Stem Cells

Stem cells have 2 unique capabilities: they can self-renew, i.e., create copies of themselves, and they can differentiate through progenitor steps toward different mature functional cell types under specific conditions (18). There are different categories of stem cells, depending on their differentiation potential. Pluripotent stem cells, typically found in the embryo (embryonic stem cells), have multilineage properties, because they can differentiate into cells from all 3 germ layers, being 1 of the most plastic cell types known to date (37). Stem cells that can only differentiate into cells of 1 organ or tissue are called multipotent because of their restricted differentiation capabilities. These are typically found in adult organs, facilitating tissue homeostasis during cell turnover or injury (38). More recently, however, it was shown that some adult tissues harbor rare populations of

stem cells that have broader differentiation potential outside their dermal origin when they are placed in corresponding microenvironments both *in vitro* and *in vivo* (39–44). Bone marrow, for example, is known to contain rare populations of stem cells with multilineage potential (43,44).

The presence of stem cells in the mammary gland has been the subject of many decades of research, culminating in recent years. The existence of stem and progenitor cells in this organ was postulated in the 1970s based on the ability of the mammary gland to substantially expand during pregnancy and to differentiate into a milk-secreting organ during lactation, suggesting that these were the primary cellular targets responsible for these changes (45). This was supported by the generation of cell cultures that could be passaged from mammary epithelial cells; importantly, the fact that these cultures have often been generated from mammary secretions (46–48) was an indication that such cells may also be present in milk.

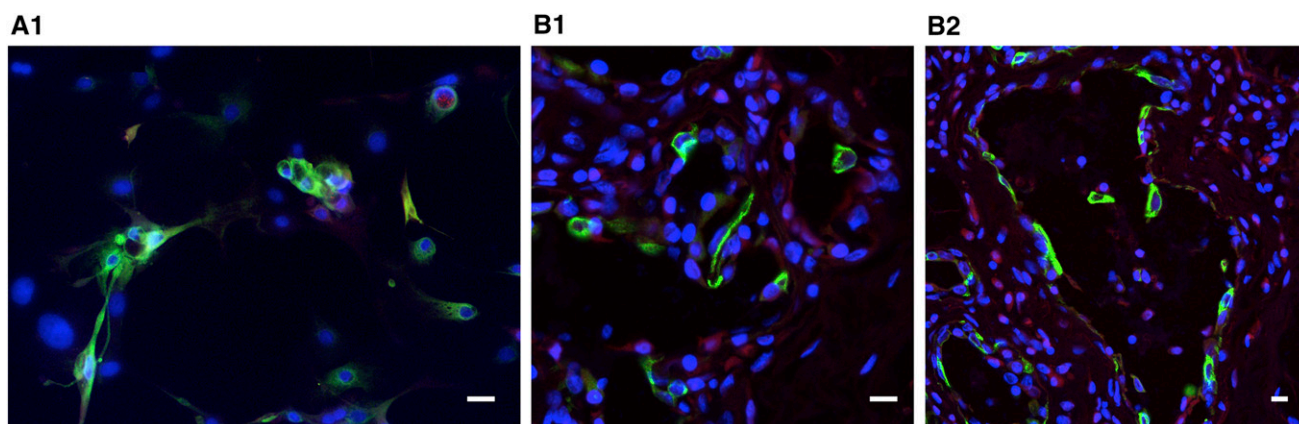
In 2007 Cregan et al. (16) provided the first evidence that breast milk contains cells with stem cell/progenitor properties. They showed that cell colonies established in culture from breast milk contained cells positive for the mammary stem cell marker CK5 and the general stem cell marker nestin (Fig. 3). The *ex vivo* presence of CK5<sup>+</sup> and nestin<sup>+</sup> cells in breast milk was later confirmed by Fan et al. (14). Subsequently, expression of the mammary stem cell marker CD49f and the epithelial progenitor marker p63 by breast milk-derived cells was demonstrated by Thomas et al. (15,49,50). These cells were shown to be multipotent *in vitro*, being able to both self-renew and differentiate into the 2 main mammary epithelial lineages, CK18<sup>+</sup> luminal cells synthesizing milk proteins, and CK14<sup>+</sup> myoepithelial cells (15,49).

These studies included colony-forming assays and essentially established the presence of cells in breast milk with mammary stem/progenitor properties. The origin of these cells, although not examined by these studies, was assumed to be the lactating mammary epithelium. Indeed, there is a

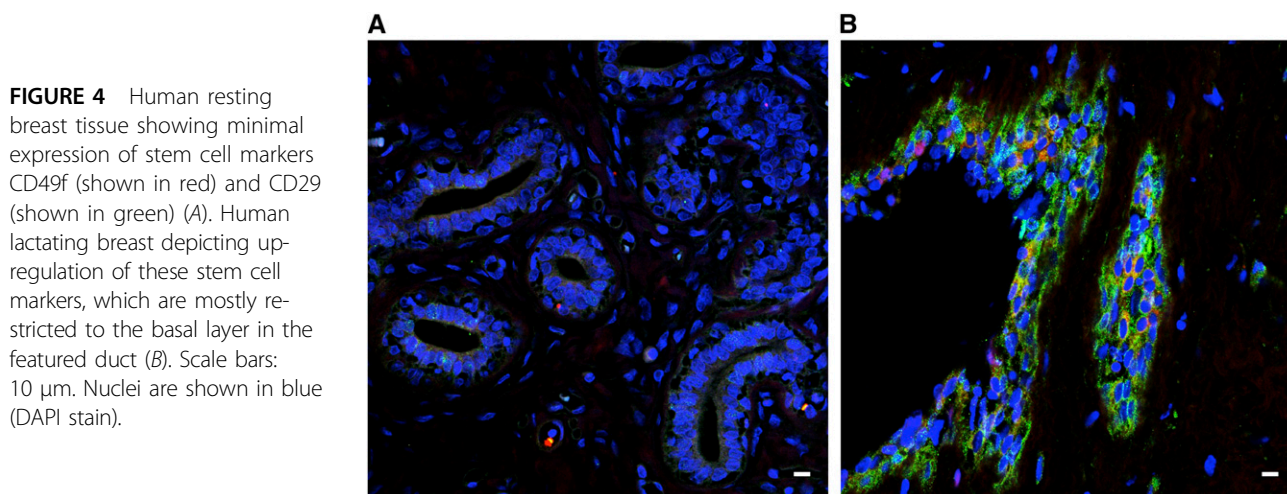
marked increase in the number of cells expressing these mammary stem/progenitor markers from the resting (in nonpregnant, nonlactating women) to the lactating breast epithelium, which supports this notion (Fig. 4) (1). Soon thereafter, the finding of stem cells in breast milk displaying multilineage potential similar to human embryonic stem cells (hESCs) and investigations of their origin once again transformed the field.

Hassiotou et al. published the first report in 2012 demonstrating expression of pluripotency markers by cell subpopulations in breast milk, and providing new data on the properties and origin of these cells, which were named human breast milk stem cells (hBSCs) (18). A whole array of pluripotency genes was shown to be expressed by freshly isolated breast milk cells (Fig. 5), including POU class 5 homeobox 1 (OCT4), sex determining region Y-box 2 (SOX2), and nanog homeobox, the core gene circuitry controlling pluripotency in embryonic stem cells (51). The levels of gene expression differed among lactating women, and subsequent evidence suggested that this may be related to a number of factors, such as the stage of lactation (52) and the degree of breast fullness (11), which are under further investigation. Interestingly, the highest expression of these genes at the mRNA level was noted in a woman who was pregnant and concurrently breastfeeding (18), which is in accordance with the expansion of the mammary gland during pregnancy and supports a role for these genes in this process.

Comparisons were also made between hBSCs and hESCs, with remarkable similarities in both gene expression and morphology during expansion in culture (18). In conditions of differentiation, hBSCs, similar to hESCs, differentiated both spontaneously and in a directed fashion into cells from the 3 germ layers. This included not only mammary gland-specific cells such as lactocytes and myoepithelial cells, but also other ectodermally-derived cells, such as neuron- and glia-like cells; mesodermal cells, such as osteoblasts, chondrocytes, adipocytes, and cardiomyocytes; and endodermal cells, such as pancreatic  $\beta$ -like cells synthesizing



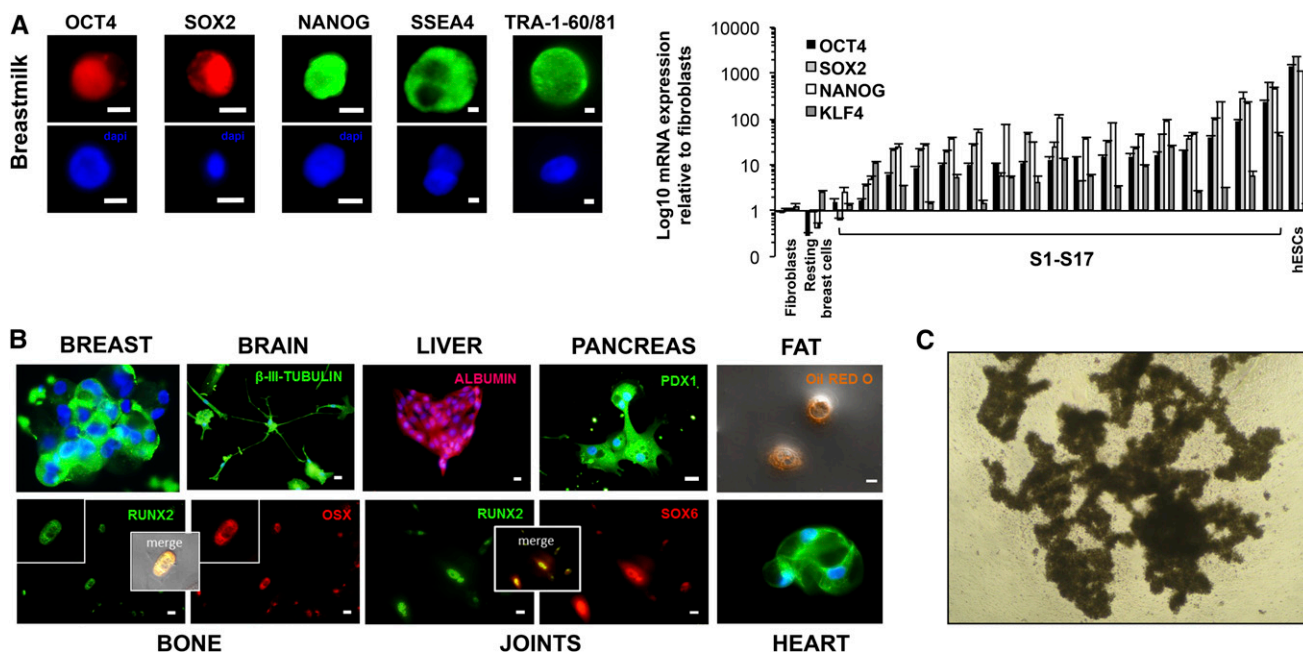
**FIGURE 3** CK5<sup>+</sup> cells (shown in green) in culture derived from breast milk (A). Scale bar: 50  $\mu$ m. CK5<sup>+</sup> cells (shown in green) in the alveoli (B1) and ducts (B2) of the lactating mammary epithelium. Scale bars: 10  $\mu$ m. In B2, CK5<sup>+</sup> cells are also evident in the ductal lumen, supporting their presence in breast milk. Nuclei are shown in blue (DAPI stain). CK5<sup>+</sup>, cytokeratin 5+.



**FIGURE 4** Human resting breast tissue showing minimal expression of stem cell markers CD49f (shown in red) and CD29 (shown in green) (A). Human lactating breast depicting up-regulation of these stem cell markers, which are mostly restricted to the basal layer in the featured duct (B). Scale bars: 10  $\mu$ m. Nuclei are shown in blue (DAPI stain).

insulin and hepatocyte-like cells producing albumin (Fig. 5) (18,53). This provided the first evidence that stem cell subpopulations present in human milk are pluripotent. The varying levels of expression of these pluripotency genes by different cell subpopulations within a breast milk sample suggested that breast milk contains a cellular hierarchy, from early-stage multi-lineage stem cells, to more differentiated progenitor cells, to fully differentiated mammary epithelial cells. Interestingly, some of the progenitor cells still express these markers, but at lower levels (18,52). This

hierarchy reflects that of the lactating mammary epithelium. The relation between these multilineage stem cells and the previously described cells expressing mammary stem cell markers and displaying mammary stem cell properties in the breast and in breast milk remains to be elucidated. However, it can be inferred based on current data that the previously described population of mammary stem cells ( $CD49f^+/CD29^+/CD24^{-/low}CK5^+$ ) is part of the cellular hierarchy that starts at the multilineage stem cell state.



**FIGURE 5** Expression of pluripotency genes by freshly isolated breast milk cells at the protein level [immunofluorescence images; nuclei are shown in blue (DAPI stain)] and at the mRNA level (bar blot for 17 breastfeeding women—S1–S17—comparing expression with fibroblasts, resting breast cells, and hESCs) (A). In vitro differentiation of hBSCs into cells from all 3 germ layers (B). Under mammary differentiation conditions, hBSCs form mini-mammary glands (with primary, secondary, and tertiary structures) in the culture dish secreting milk proteins (C). A and B are adapted with permission from (18). hBSC, human breast milk stem cell; hESC, human embryonic stem cell; KLF4, Kruppel-like factor 4; NANOG, nanog homeobox; OCT4, POU class 5 homeobox 1; OSX, Sp7 transcription factor; PDX1, pancreatic and duodenal homeobox 1; RUNX2, runt related transcription factor 2; SOX2, sex determining region Y-box 2; SOX6, sex determining region Y-box 6; SSEA4, stage-specific embryonic antigen 4.

The origin of hBSCs displaying multilineage potential is still unclear. Initial investigations indicated that cells positive for pluripotency genes are present in the mammary epithelium, both its ductal and alveolar compartments, and in substantially larger numbers during lactation than in the resting breast (18,54). This further supports a role for these cells and the pluripotency genes they express in the remodeling of the mammary gland toward a milk-secretory organ. Moreover, they are found in both the basal and luminal epithelial cell layers, which is in agreement with recent reports showing that multipotent mammary stem cells are not restricted to the basal layer (55). Nevertheless, one cannot exclude the possibility that subpopulations of breast milk stem cells (BSCs) may originate from the maternal bloodstream, particularly when it is considered that 1) other parts of the body, such as the bone marrow, harbor stem cells expressing pluripotency genes (43), and 2) other blood-derived cells exist in breast milk.

Indeed, breast milk contains blood-derived leukocytes (10). In addition, preliminary investigations indicate the presence of CD34<sup>+</sup> hematopoietic stem/progenitor cells in colostrum and breast milk (14,36), which almost certainly originate from the maternal bloodstream. The properties of these cells, the mechanisms of their transfer into breast milk, and their roles remain to be established. Also, it is not yet known how maternal- and/or infant-associated factors, such as the stage of lactation or the presence of an infection, may influence the numbers of these breast milk cells. This is clearly an area deserving future attention. **Figure 6** summarizes the current knowledge of the different cell types of human milk, depicting its cellular hierarchy.

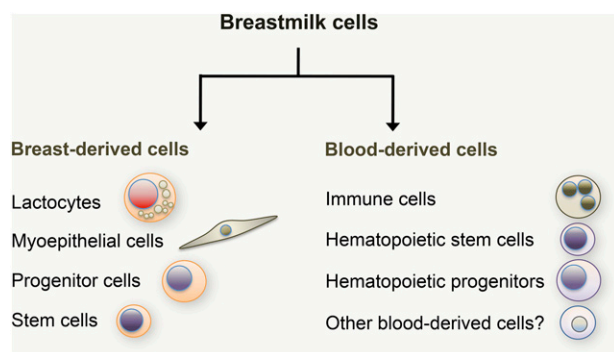
A few studies suggested that human milk contains mesenchymal stem cells (MSCs) based on markers known to be expressed by MSCs, such as STRO-1, CD90, CD105, and CD73 (14,36), and the potential of breast milk-derived cells to differentiate in culture into mesodermal cells, such as osteoblasts, chondrocytes, and adipocytes, a known feature of MSCs (17). However, caution should be exercised when interpreting these data. First, some of the mesenchymal markers tested are not restricted to mesenchymal cells; for example, STRO-1 is also expressed by the mammary

epithelium in the lactating breast (F. Hassiotou, unpublished data). Second, expression of mesenchymal markers may actually be indicative of epithelial to mesenchymal transition (EMT) in the lactating breast, and not of the presence of MSCs (2). EMT is a process that naturally occurs during embryonic development, and that is also aberrantly activated in certain types of breast cancer, equipping EMT cells with migratory properties (56). It was shown that this process is naturally activated in the normal murine lactating mammary gland (56), and recently we provided evidence toward this for the human lactating breast, where it may be involved in its normal function during lactation and/or in the active migration of cells from the epithelium into breast milk (2). Finally, the potential of multilineage hBSCs to differentiate into mesodermal cells (18) further suggests that MSCs may not actually be present in human milk. Further studies are needed to illuminate these questions.

### Functions of Breast Milk Stem Cells

Breast milk stem cells with multilineage properties were only recently discovered, and although rapid progress was made in the field in the past few years, we are still far from fully understanding their properties and function in the breast during lactation and for the breastfed infant. In both cases, indirect evidence supports the notion that they play critical roles. In the mammary gland, they appear to be important during its remodeling toward a milk-secretory organ, as is suggested by the higher expression of pluripotency genes during pregnancy, when epithelial expansion occurs, and the rarity of these cells in the resting breast (18,54). In the infant, the substantial number of these stem cells ingested daily during breastfeeding (thousands to millions) implies a function (2). In animals, including a primate model, it was shown previously that immune cell populations from milk remain unharmed in the digestive tract of the young and through diapedesis they cross the intestinal mucosa and enter the bloodstream through which they migrate to different organs, where they provide active immunity (57–60). We proposed that breast milk stem cells may have a similar fate in breastfed offspring (2,18). Dutta and Burlingham (2010) demonstrated a correlation between nursing and the number of maternal cells in the liver of mouse pups, suggesting seeding of maternal cells into the liver via breast milk; however, the nature of the transmitted cells was not determined (61). Earlier this year, we presented the first evidence showing breast milk stem cell survival in the gastrointestinal tract of the offspring, transfer to the bloodstream, and in vivo integration into different tissues (62). This now supports the notion that breast milk imparts the mother's stem cells to the infant, where they potentially function to boost infant development early in life.

This phenomenon is called microchimerism, whereby maternal cells, with all their genetic material and other constituents, are present in the offspring and may remain there alive long-term. Maternal microchimerism is known to occur in utero when stem cell exchange between the mother and the embryo is facilitated through the placenta, with



**FIGURE 6** The current knowledge of the cellular hierarchy and different cell types of human milk.

the exchanged cells surviving in the chimeras for several years (63). This cellular transfer that starts in utero appears to continue after birth during the breastfeeding period, and may facilitate further development of tolerance between mother and infant (2). Indeed, the higher acceptance of maternal transplants by individuals who were breastfed as infants (64,65) supports the establishment of a special communication and tolerance between the mother and her infant during breastfeeding, which is above and beyond what has already been established in fetal life. This tolerance may contribute to the utilization of other breast milk components by the infant and appears to have long-term status and benefits. Breast milk stem cell research is unraveling unknown attributes of breast milk far beyond its role as a food source that merit further inquiry.

### Applications of Breast Milk Stem Cells

The cells in breast milk reflect the cellular status of the lactating mammary epithelium. Although the latter is extremely difficult to access, even in cases of breast/lactation pathologies, breast milk is plentiful, and can be accessed ethically, easily, and without the need to employ invasive procedures. Therefore, it provides a valuable source of cells from the lactating breast that can be used to examine the normal biology of this organ and deviations from it, such as lactation pathologies (e.g., insufficient milk or oversupply of milk) or breast cancer. For example, by comparing hBSCs with breast cancer stem cells, we recently observed an imbalanced expression of stem cell genes in aggressive breast cancers compared with the balanced gene expression of hBSCs, which may explain the origin of these cancers (52). Moreover, the inability of many mothers of preterm infants to both initiate copious milk production and reach a full milk supply suggests that differences exist in the developmental status of their breasts compared with mothers of term infants with normal supply. hBSCs offer a unique opportunity to illuminate these differences and noninvasively identify those mothers with underdeveloped breasts, allowing for more targeted management and monitoring, thus increasing their chances of successful lactation. Similar concepts were discussed in the dairy cow, in which the known decline in milk production with lactation was attributed to a steady rate of epithelial cell death (66,67). Developing treatments to maintain and/or boost mammary stem cell proliferation was proposed as a strategy to maintain and/or increase milk production (68,69).

The applications of breast milk stem cells, however, extend far beyond the fields of lactation and cancer. Regenerative medicine and stem cell biology may also benefit from utilizing these cells (2,53). Cell replacement therapies are being investigated intensively, with the hope of providing novel treatment options for numerous diseases as well as impairments caused by injuries. These therapies involve transplantation of stem cells or their progeny into the malfunctioning tissue to allow its normal regeneration and function (70). For example, in individuals suffering from neurodegenerative diseases such as Parkinson or Alzheimer, transplantation

of cells that can replace the nonfunctioning neurons may provide therapeutic benefits (53). Preliminary results appear encouraging, with 2 out of 3 patients who were part of a recent clinical trial evaluating the effect of injecting neural stem cells having gained considerable sensory function (71). hBSCs may be excellent candidates for the treatment of neurodegenerative diseases as well as in other areas of regenerative medicine, not only because they are highly plastic and are accessed ethically, but also because research so far indicates that they are nontumorigenic (18).

Regenerative medicine was revolutionized recently by the discovery of induced pluripotent stem cells (iPSCs), which are manufactured in the laboratory from the dedifferentiation of mature cells. However, many problems exist with this new technology, because iPSCs are highly unstable (72) and have a tendency to form immature teratomas when transplanted subcutaneously in immunodeficient mice (73). In contrast to iPSCs, hBSCs are incapable of forming any tumors in the same assay, which supports their safety (18). Although the teratoma assay has been considered the gold standard for pluripotency (74), the tumorigenicity that tested cells need to demonstrate to fulfill the assay criteria by definition excludes nontumorigenic cells from potential pluripotent cell candidates based on this assay (2,18). Indeed, pluripotent adult stem cells that cannot form teratomas were described previously in the bone marrow (43,44,75). These cells have unique DNA methylation patterns in some genes and have been proposed to lack teratoma formation based on these epigenetic characteristics (75). Future studies on the epigenetic profile of hBSCs will be crucial steps toward explaining their plasticity compared with teratoma-forming hESC and iPSCs, as well as other types of highly plastic adult stem cells.

The first evidence of *in vivo* survival and integration of BSCs was provided (62), whereas transplantation assays in animal models are on their way to further support the *in vivo* multilineage properties of BSCs. This will expand the possibilities for their use to treat diseases of different organs in both infants and adults. For example, the ability of hBSCs to differentiate *in vitro* into  $\beta$ -like cells synthesizing insulin offers a unique opportunity for the development of breast milk stem cell-based therapies for diabetic patients. We recently developed a method to expand hBSCs in culture (18), which facilitates further exploration of any therapeutic benefits, along with appropriate storage and preservation of these cells. Future studies will explore the potential of hBSCs to be used in regenerative medicine, which may prove revolutionary for the field as the most natural form of stem cell therapy, because these cells have the normal function of transferring from 1 person (the mother) to another (the infant).

### Outlook

The discovery of breast milk stem cells is an important advance in breast milk research in the last decade. Their normal function is still somewhat of a mystery; however, they open up a whole new world of possibilities for not only

the field of lactation, but also stem cell and cancer research and regenerative medicine. The greatest beneficiaries currently are breastfed infants, ingesting thousands to millions of these cells every day from mother's milk, even late in lactation. As we learn more about their multipotent nature, origin, and regulation, we will be able to use them to answer questions that have long preoccupied lactation biologists and health professionals, such as why some women do not produce sufficient volumes of milk, whereas others have an oversupply. Importantly, their discovery now reveals a component of breast milk that will never exist in artificial formulas, generating numerous implications for public policy on early infant nutrition.

### Acknowledgments

The authors thank Donna Geddes for critically reviewing the manuscript, and the late Demetrius Hassiotis for his continued support, and inspiration. Both authors read and approved the final manuscript.

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