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Review

Breast milk stem cells: four questions looking for an answer

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Stem cells: present and future
Guest Editors: Gavino Faa (Cagliari, Italy), Vassilios Fanos (Cagliari, Italy), Antonio Giordano (Philadelphia, USA)

Abstract

The finding of stem/progenitor cells in the maternal milk and the discovery of their multilineage potential, associated with some evidence regarding the ability of maternal cells to cross the gastrointestinal barrier and integrate into the organs of the breastfed neonate, has opened an intriguing debate, regarding the strict relationship between mother and son in the postnatal period. In particular, thanks to the discovery of the presence in high quantities of mammary stem cells, a new vision of maternal milk is emerging, in which breastfeeding appears as an unique occasion for reinforcing the physiological development of the newborn, putting all the formulas at a different level of relevance for the neonate. In this contribution the authors try to give an answer to the following 4 questions:

1. is there heterogeneity and a hierarchy among breast milk stem cells?
2. can stem cells present in breast milk enter into the newborn organism?
3. can breast milk stem cells integrate in the neonatal organs and differentiate toward different tissues, including neurons and neuroglia?
4. could metabolomics be useful for the study of stem cells in the human milk?

Keywords

Milk, stem cells, neurons, neuroglia.

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Introduction

In recent years, considerable progress has been made towards a better understanding of the composition of the human breast milk. Previously, it was generally considered as a peculiar supply of nutrients and bioactive factors, but it is now widely assumed that human breast milk represents the source of a heterogeneous population of cells and, in particular, of stem/progenitor cells with multilineage differentiation potential [1], including mesenchymal and embryonic stem cells, admixed with exfoliated luminal epithelial cells [2, 3]. The significance of the presence of one or multiple stem/progenitor cell pools in the breast milk is still debated. According to some authors, through stem cells human breast milk might provide long-term benefits not previously hypothesized for the lactating newborns [4]. Thanks to the ability of maternal breast-derived stem cells to integrate into the newborn tissues and differentiate into functional cells, maternal milk might play a relevant role in the postnatal development of multiple organs, including brain, of every neonate undergoing breastfeeding [5]. This hypothesis has been recently demonstrated in a mouse model: breast milk-derived maternal stem cells were identified, 3 weeks after birth, in the stomach wall, in the thymus and in the liver of lactating pups, clearly indicating the ability of maternal stem/progenitor cells to migrate and integrate into multiple organs of the lactating newborn [6].

The finding of stem/progenitor cells in the maternal milk and the discovery of their multilineage potential, associated with some evidence regarding the ability of maternal cells to cross the gastrointestinal barrier and integrate into the organs of the breastfed neonate, has opened an intriguing debate, regarding the strict relationship between mother and son in the postnatal period. In particular, thanks to the discovery of the presence in high quantities of mammary stem cells, a new vision of maternal milk is emerging, in which breastfeeding appears as an unique occasion for reinforcing the physiological development of the newborn, putting all the formulas at a different level of relevance for the neonate. Given so many implications for both infants and mothers generated by the knowledge that breast milk stem cells might be transferred to the infant during breastfeeding, many questions are emerging on the ethical and physiological implications of this discovery. Here we will try to give an answer to the most important questions regarding this fascinating field of neonatology of the next future. In the final part of this article, some questions for which, at the best of our knowledge, actually there is no answer will be introduced, with the aim that further studies based on large numbers of observations will clarify this intriguing field of postnatal physiology.

First question: is there heterogeneity and a hierarchy among breast milk stem cells?

To try to give an answer to this question, we have to analyze the current status of our knowledge regarding the stem cell pools existing in the site of origin of breast milk stem cells: the mammary gland. According to recent studies mainly based on lineage tracing, the mammary stem cell compartment is heterogeneous, comprising at least two subsets of stem/progenitor cells, namely $Lgr5^+$ [7] and $Lgr5^-$ [8] mammary stem cells. The identification of mammary stem/progenitor cells has proven difficult at histology: mammary progenitors are thought to be intermingled among the basal cells, approximately one in 18 cells within the basal layer being able to generate the entire mammary gland [9]. Recently, a hierarchy among stem/progenitor cells has been shown in the mouse mammary gland. According to this study, bipotent mammary stem cells might originate both luminal and basal progenitors, from which ductal, alveolar and myoepithelial cells might take their origin, eventually driving morphogenesis of the mammary ductal tree [10]. Moreover, accumulating evidence continues to surface that implicates the existence in the mammary gland of a heterogeneous compartment of stem/progenitor cells, including the following pools: fetal primitive

mammary epithelial stem cells [11], slow-cycling or quiescent asymmetrically dividing stem cells [12], and activated stem cells driving morphogenesis in puberty and in early pregnancy [13].

Coming back to our question, stem cells are not restricted to the developing mammary gland, subsets of pluripotent mammary stem cells being physiologically present in the adult human breast [14]. Pluripotent stem cells with multilineage differentiation potential have been reported in the adult mammary gland and the possible persistence of fetal mammary stem/progenitor cells into adulthood has been reported as well [1]. Moreover, multiple evidences suggest that, during pregnancy, the stem/progenitor cell pools of the mammary gland might undergo a dramatic transient remodulation, characterized by the expansion of some peculiar pregnancy-specific progenitors lying at the interface between the basal and the luminal subsets [15]. These relevant changes in the stem cell burden of the mammary gland during pregnancy have been shown to be induced by hormonal cues, including steroid hormone signaling [16], and coordinated by Ezh2 [17]. A recent spate of insightful papers have clarified the mammary cell hierarchy and the composition of the mammary stem cell niche [18]. According to these studies, the organization of the mammary stem cell niche might change significantly in the different developmental stages of the gland. The niche should include stem cells, stem/progenitor daughter cells, multipotent progenitors, committed progenitors and mature cells of the myoepithelial and luminal lineage. Moreover, in the mammary niche a major role should be played by the extracellular matrix, that might influence cell fate of the mammary precursors in association with the steroid hormone signaling [16].

All these data taken together, it is tempting to speculate that the mammary gland during pregnancy undergoes a complex process of remodeling of its stem/progenitor cell burden, with the aim of introducing the proper progenitors in the breast milk, useful for the postnatal development of the newborn. According to the complexity of stem/progenitor cells observed in the maternal mammary gland, in particular during gestation, the finding of multiple subtypes of progenitors in the breast milk appears the most probable occurrence. Immunohistochemical studies are needed, in order to verify the precise number and the subtype of progenitor cells detectable in the human breast milk, number that actually is yet to be determined. Moreover, given the hierarchy detected among the mammary stem cells in the maternal mammary gland, we may hypothesize that a hierarchy should be present even among the maternal stem/progenitor cells received by the neonate during breastfeeding. The discovery of the peculiar role of each subset of mammary stem/progenitor in the neonatal development represents a fascinating field regarding the intimate interplay between mother and son after birth, introducing the new hypothesis of maternal breast programming of the neonate with consequences of his/her health and disease status for the whole life.

Second question: can stem cells present in breast milk enter into the newborn organism?

The human breast milk, other than containing a multitude of nutritional and immunologic factors that are fundamental for the proper development of the newborn, contains huge amounts of maternal cells, thousands to millions in every milliliter, that enter the gastrointestinal tract of the breasfed infant [19]. In recent years, the breakthrough discovery of the ability of a part of these cells, in particular of mammary stem/progenitor cells, to be transferred into the tissues of the infant during breastfeeding has opened a new field of research in neonatology focused on the strict relationship between mother and the breastfeed neonate [20]. Recently, an elegant experiment carried out in a mouse model [6, 19] allowed a considerable progress towards a better understanding of breastmilk stem cell transfer from mother to the breastfed neonate. In this insightful experimental work, maternal milk-derived stem cells were detected in the stomach, thymus and liver of newborn mice, providing clear evidence of their migration and integration into multiple organs of the neonate. The discovery that maternal stem/progenitor cells with multilineage potential may be transferred to the offspring during breastfeeding is shaking up, in our opinion, the neonatal health care world. Breast milk should not be simply considered as a mix of nutrients, but also as a tool utilized by the mother to transfer stem cells to her neonate, helping her/him to reach an optimal postnatal development, reinforcing the newborn organs and protecting her/him from multiple diseases later in life. In short, this new vision of the postnatal relationships between mother and son could be at the basis of a new entity in the field of the fetal programming, that might be defined as the “maternal physiological regenerative medicine”.

Third question: can breast milk stem cells integrate in the neonatal organs and differentiate toward different tissues, including neurons and neuroglia?

Stem/progenitor cells secreted by the mammary gland during lactation are characterized by their potential of a multilineage differentiation toward multiple cell types [1]. Among the human breast milk stem cells, a subset has been shown to express mesenchymal stem cell markers, whereas another subset is characterized by the expression of embryonic stem cell-associated genes, including *OCT4*, *NANOG* and *SOX2* [1]. Moreover, the presence of a subset of stem/progenitor cells expressing nestin, a typical marker of neuroectoderm, has been reported in breast milk [21]. The discovery of nestin-positive cells among breast milk stem cells, that are associated with neuroepithelial differentiation [22], together with their ability to cross the blood-brain barrier, might introduce in neonatal physiology a new linkage between maternal milk and the developing brain of the neonate [5]. The hypothesis that breast-derived stem/progenitor cells might represent a good candidate for differentiation into multiple cell types of the neural cell lineage has been recently reinforced by the fact that both the mammary gland and the neuroepithelium have the same embryological origin. The breast/milk neural differentiation hypothesis has been recently demonstrated in an *in vitro* model: exposing human breast milk-derived stem cells to culture medium leads to their differentiation into neurons, astrocytes and oligodendrocytes [23].

Fourth question: could metabolomics be useful for the study of stem cells in the human milk?

Metabolomics is an innovative technology that aims to understand the metabolic processes within the organism, capturing simultaneously a certain number of metabolites that can be intended as a pack of unique descriptors for a metabolic status. The two most used analytical approaches are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). NMR spectroscopy is fast and not destructive; it requires little sample preparation and allows to obtain consistent and reproducible results. In fact, peaks that are plotted in the NMR spectra can be assigned to specific metabolites (almost 150 metabolites detectable), which can be also quantified using specific software. MS is still considered the gold standard in metabolite detection; generally, MS methods involve their separation by gas chromatography (GC) or liquid chromatography (LC) to unravel the metabolite complexity. The huge dataset produced by metabolomics investigations are analysed by multivariate statistical methods that help the extraction of hidden information among several thousands of variables [24-26].

In the context of regenerative medicine, metabolomics seems to present a considerable advantage with respect to proteomics, the other major global functional-level analysis tool for biomaterials analysis, in that a very large number of metabolites can be detected with very small quantities of sample material [27]. The future of research will be on metabolomics, milk-oriented microbiota and stem cells. Probably from the integration of knowledge in these 3 fields we will be able to take further significant steps in understanding the miracles of mother's milk, namely stem cells [28].

Questions to be addressed in the next future

In spite of the considerable progress made toward understanding of breast-derived stem cells contained in the human milk, many open questions regarding their role and function in neonatal development are still present. First question: does preterm birth influence breast milk stem cell burden? Second question: does breast milk stem cell burden change in the postnatal period during lactation? Third question: does caesarian delivery influence breast milk stem cell burden? Fourth question: does milk-oriented microbiota interfere with breast milk stem cell burden? Fifth question: does maternal health status during gestation correlate with the breast milk stem cell burden? Future studies focusing on the interaction between maternal stem/progenitor cells and the lactating neonate would be of great interest to give an answer to these open questions.

Conclusions

"Unravelling the mistery of stem/progenitor cells in human breast milk" is the title of an article published in PlosOne by Fan and coworkers in 2010 [29], that underlines the complexity of this problem and the difficulties of the scientific community to give good answers to the multiple questions raised by this fascinating and intriguing discovery [29]. On the basis of data here reported, breast milk is emerging as a new fascinating field of research in neonatology. New methods have increased our ability for the identification and characterization

of basal and luminal breast-derived progenitors in the human milk, updating prior phenotyping that relied on CD44 [2] or CD133 [29] expression of milk stem cells. Multiple sub-populations of mammary stem/progenitor cells will be identified, in the next future, in the human milk, allowing a better understanding of the role of breast-derived progenitors in the postnatal development of every breast-fed neonate.

Finally, in our opinion researchers involved in the field of breast-milk stem cells are going to address a rather audacious goal: shaking up the neonate care world, making inroads into one of the most complex field, that of developmental perinatology. Thanks to the new discoveries on the role played by milk stem cells in neonatal development, the scientists might shed light on the postnatal interactions between mother and newborn after birth, focusing on the role of breastfeeding on the prevention of the health and disease status of the neonate later in life.

Declaration of interest

The Authors declare that there is no conflict of interest.

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