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Review

Multipotent stem cells of mother's milk

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Stem cells: present and future
Guest Editors: Gavino Faa, Vassilios Fanos, Antonio Giordano

Abstract

In recent years the presence of stem cells (hBSCs: human breastmilk-derived stem cells) and epithelial progenitors has been demonstrated in mother's milk (MM). Stem cells present in samples of fresh MM exhibit a high degree of vitality and this makes possible the performance of cell cultures and to evaluate the differentiation capacity of the hBSCs. The most important datum that expresses the enormous potential of the use of MM stem cells is the presence of a cell population capable of differentiating into the three mesoderm, endoderm and ectoderm lines. The small number of studies and MM samples analyzed and the different sampling methods applied suggest standardization in the collection, analysis and culture of MM in future studies, in consideration of the well-known extreme variability of MM composition, also from the standpoint of cells.

The analysis of literature data confirms the uniqueness of MM and its enormous potential.

Keywords

Human breastmilk-derived stem cells (hBSCs), epithelial progenitors, mesenchymal stem cells, embryonic stem cells.

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Introduction

Mother's milk (MM) is an active biological liquid the composition of which is still not completely known. It shows a variability that is not only interindividual, but even intraindividual. We know that MM in the same woman changes both qualitatively and quantitatively in the different stages of lactation, during the day and even during a single breastfeed, adapting qualitatively also to the mother's and child's state of health. Functionally, it is possible to distinguish a nutritional and a bioactive component in MM [1]. The latter is composed of growth and immunological factors, as well as a conspicuous cell component.

Knowledge on the cell component of MM is still scanty. We know that the cell component is 10^4-$13x10^6$/ml of MM: essentially, it was thought that leukocytes predominated, but this observation derived mostly from studies performed on bovines and cholesterol [2]. Recent data demonstrate that in mature mother's milk (MMM) leukocytes are present in a percentage of ≤ 2%, different from colostrum in which from 13% to 70% of the cell component is represented by leukocytes. Thus, most cells present in MMM are those of an epithelial nature: lactocytes and myoepithelial cells deriving from the ducts and alveoli of the lactating mammary gland.

In recent years the presence of stem cells and epithelial progenitors has also been demonstrated [3].

Stem cells have been identified in numerous tissues of our organism: thanks to their capacity to regenerate and differentiate, they play a key role in controlling homeostasis and in tissue regeneration processes. Different kinds of stem cells have been identified on the basis of their degree of differentiation: totipotent stem cells are capable of differentiating in the three germ layers and extraembryonic tissues; pluripotent ones differentiate only in the three germ layers; multipotent ones (progenitors) are oriented cells that produce specialized cells with specific functions; unipotent ones are capable of differentiating into a single cell line.

It is possible to distinguish the different cell populations by means of flow cytometry and immunofluorescence which allow identification of specific surface markers or transcription factors (TFs). Stem cells present in samples of fresh MM exhibit a high degree of vitality and this makes possible the performance of cell cultures and to evaluate the differentiation capacity of the human breastmilk-derived stem cells (hBSCs).

We shall see that on the basis of data now available MM is a quite promising source of stem cells with the therapeutic implications deriving from them.

Stem cells of mother's milk (human breastmilk-derived stem cells): a heterogeneous population

Research into and studies of stem cells in mother's milk are of recent interest. In 2007, Cregan demonstrated the presence of multipotent stem cells in MM for the first time [3].

Based on knowledge at hand, we know that MM contains a heterogeneous population of stem cells with an evolutionary hierarchy: thus it is possible to find in it immature pluripotent cell forms as well as the more mature committed or unipotent forms.

Epithelial stem cells

In MM, the presence of stem cells belonging to the mammary line CK5, CK14 and CK18 has been demonstrated. Such antigens are in fact the expression of the degree of differentiation of mammary epithelial cells. CK5 is the marker of mammary progenitors, while CK14 and CK18 are specific antigens present on the cells of the two mature mammary epithelial lines (myoepithelial and luminal/ductal cells) [3-5].

In the same way, cells of the neuroepithelial kind have also been found with different degrees of differentiation, as demonstrated by cell positivity to nestin, Msi-1 and NFM, respectively markers of progenitors and early and late neural differentiation [3, 6].

Mesenchymal stem cells

Also recognized within the cell component of MM, although in a small percentage, is the presence of specific markers of mesenchymal stem cells (MSCs) [5-8]. The MSCs represent about 10% to 15% of the entire cell population of MM [7].

In a study conducted in 2013 on 26 milk samples, besides the progenitors of CD29 and CD44 of the myoepithelial cells, MSCs that express the typical markers CD90, CD105 and CD73 were isolated [9].

Human breastmilk-derived stem cells and embryonic stem cells

In 2012, Hassiotou demonstrated the presence of hBSCs that express the TFs OCT4, SOX2 and NANOG typical of embryonic stem cells (hESC) in fresh MM [10]. Compared to the hESC cells, the hBSCs of MM express the ESC genes with lower values and the three TFs. NANOG is expressed at higher levels in tested samples of fresh MM and is, among the TFs, the one that plays a leading role in controlling the state of cell pluripotency.

When such cells reproduce, they conserve these markers, but they are also capable of differentiating into cells of the mammary line, lactocytes and myoepithelial cells, capable of producing and secreting milk, but also capable of differentiating into cells of different lines belonging to the three germ layers. They are thus pluripotent cells [10]. In MM, the cells that express ESC genes are not all at the same stage of development, nor do they have the same potential to self-replicate or differentiate. In fact, in culture they can differentiate as follows:

a. ESC-like cells that form colonies similar to ESCs;
b. non-ESC-like cells that form colonies with variable morphology, either mesenchymal-like or epithelial-like or mixed, and express reduced levels of TFs (TRA-1-60/TRA-1-81). These colonies most likely originate from expansion of clones of more oriented progenitors in which the ESC genes are not completely under-regulated [10].

The most important datum that expresses the enormous potential of the use of MM stem cells is the presence of a cell population capable of differentiating into the three mesoderm, endoderm and ectoderm lines. In fact, after 2 to 3 weeks of culture in specific growth media, the hBSCs are capable of forming colonies having different morphologies and belonging to different cell lines [6-10].

It has been demonstrated that *in vitro* they can proceed in the differentiation process up to the formation of specific cells of the several tissues (hepatic, pancreatic, nervous and glial, adipose cells, chondrocytes, osteoblasts, cardiomyocytes) and can synthesize the specific proteins of the single cell line (insulin, albumin) [6-10]. A particularly interesting fact having to do with the therapeutic applications that may derive from this is the capacity that the hBSCs have in culture: that of spontaneously forming neurons, astrocytes and oligodendrocytes [6].

During the differentiation process it can be observed that genes typical of hESC are first upregulated and then downregulated with the appearance of upregulation of genes specific to the cell line at the same time.

However, contrary to hESC, the pluripotent cells deriving from MM are unable to cause tumors if injected into experimental animals, but may integrate and regenerate damaged tissues when inoculated into severely immunodeficient mice [10]. We can thus conclude that MM expresses a population of hBSCs similar, but not identical, to hESCs.

Variability in the composition of human breastmilk-derived stem cells of mother's milk

The individual and interindividual variability in the quali/quantitative composition of MM is well known. From a review of the literature it emerges that this variability is confirmed also for hBSCs [2, 10, 11]. In general what is observed is a progressive reduction of the number of cells in MM as lactation progresses. The expression of TFs and other genes involved in cell differentiation presents an interindividual and intraindividual variability, but it also changes in relation to the state of breast repletion. A major expression of genes OCT4, NANOG and SOX2 in women who breastfed and were pregnant at the same time has also been noted.

Origin of human breastmilk-derived stem cells

The cell heterogeneity of MM reflects that of the lactating mammary gland. The origin of hBSCs was debated for a long time. Once the hypothesis of their hematic origin was rejected, that of their fetal origin was advanced. The process of bidirectional migration of fetal and maternal cells through the placenta is in fact known. These cells

remain in various fetal tissues for a long period of time and may proliferate and differentiate into other kinds of cells [12]. It was thought that a subpopulation of cells from fetal tissues may reside in the breast after delivery and pass into the milk. However, it has recently been demonstrated that there is no fetal microchimerism in MM cells [8, 13].

In all likelihood, the hBSCs arrive from the mammary gland where they were present in the embryonic period in a state of quiescence by means of a mechanism that is not completely known (by migration or as the consequence of mechanical forces). The mammary origin of hBSCs has recently been confirmed by the finding of stem cells that express TFs typical of hESC within the lumen of the glandular ducts [10].

Already in 1959 the existence of mammary stem cells (MaSCs) was hypothesized on the basis of the mammary gland's ability to expand during pregnancy and differentiate into a secretory organ during lactation [14].

The mammary gland is an organ that in a woman's lifetime goes through different stages depending mostly on stimuli of a hormonal nature. In pregnancy it undergoes a remodeling process with a ten-fold increase in the lobules and alveoli [15]. MaSCs are present in reduced numbers in the quiescent mammary gland and are arranged in the basal layer of the ducts: they are multipotent and express surface antigens CD5/CD49f+/CD29+/CD24low/CK5+. During pregnancy they proliferate and determine the transformation of the mammary gland into a secretory organ. As stem cells, they are capable of self-renewal, but also of differentiating into the two kinds of mammary epithelial cells: the luminal cells CK18, the ductal cells CK19, and into the myoepithelial cells CK14/SMA.

Recently, cells that express the embryonic TFs OCT4, SOX2, NANOG, TRA-1-60/TRA-1-81 in the lactating mammary gland have been found. They are located in the epithelium and lumen of the ducts and alveoli. The latter discovery has led to the hypothesis of the existence of hESC-like cells in MM [10].

Although the hierarchy of MaSCs is known, all the markers and properties of these cells are not yet known since studies have mostly been performed on quiescent mammary glands or those affected by pathologies. Few are the studies performed on lactating mammary glands owing to a lack of autopsy or biopsy material.

The role of the human breastmilk-derived stem cells

The stem cells of MM are capable of integration and differentiation into the several tissues. Similar to what takes place in the leukocytes, the hBSCs assumed in the milk can pass through the neonate's intestinal barrier and, through the hematic circle, can reach the different organs. This is a phenomenon of microchimerism similar to what takes place through the placenta in the fetus. This was recently discovered by Hassiotou [1, 11]. In the mouse, the TdT+ cells contained in MM and administered to wild-type offspring were found first in the stomach (attached to the wall), then in the blood and thymus, liver, pancreas, brain and spleen. Some of these cells expressed OCT4, NANOG and CD49f. In the mouse tissues a double population of TdT+ was found: one expressed undifferentiated markers (OCT4, NANOG and CD49f), the other specific markers of the tissue, thus indicating a differentiation in the host. Even after weaning, it was possible to find in the different organs of the adult mice TdT+ cells from MM [1, 11].

Considerations and conclusions

The recent discovery of hBSCs in MM and especially their pluripotency, makes it imperative to continue studies on its qualitative cell composition and the effects and functions that these have in breastfed children.

The small number of studies and MM samples analyzed and the different sampling methods applied suggest standardization in the collection, analysis and culture of MM in future studies, in consideration of the well-known extreme variability of MM composition, also from the standpoint of cells.

The analysis of data thus far collected in the literature confirms the uniqueness of MM and its enormous potential: the noninvasiveness of sample collection, the high number and heterogeneity of the stem cell component it contains make it the principal means now available for the study of human stem cells.

We can thus conclude that on the basis of present-day knowledge of MM there is a heterogeneity/hierarchy of cells and stages of development that make it inimitable both as a nutrient and a biological liquid.

Some issues remain open [16, 17]:

- what epigenetic factors act on ESC TFs and on hBSCs of the subsequent stages of differentiation?
- what conditions of the mother/child dyad interfere in the qualitative and quantitative composition of hBSCs, analogous to what takes place in the leukocytes?
- is it possible to arrive at an understanding of what metabolic processes are at the base of and regulate the development/differentiation of hBSCs through metabolomic studies?

Declaration of interest

The Authors declare that there is no conflict of interest.

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