

The Presence of Stem Cells in Human Breast Milk and Research Implications

Melody (Brooke) Peterson

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Mary A. Highton, DNP, MSN, NNP-BC
Thesis Chair

Linda Gregory MSN, RN
Committee Member

Kimberly A. P. Mitchell, Ph.D.
Committee Member

Marilyn Gadowski, Ph.D.
Honors Assistant Director

Date

Abstract

Stem cell therapy research is an expanding area of study as stem cell therapy is believed to have the potential to provide treatment options for numerous disease processes. Currently, embryonic stem cell research is the method of choice to evaluate the potential for stem cell therapy. The use of human embryos for stem cell research raises moral and ethical controversies. Stem cells are found in a variety of mediums, but until the recent discovery of stem cells in human breast milk, the most versatile stem cells have been those found in the human embryo. Human breast milk stem cells could offer a new and less controversial medium of study. Both pluripotent and multipotent stem cells have been found in breast milk samples. Breast milk could provide stem cells compatible for extensive research without the moral constraints of embryonic stem cell research. The implications of such a find could mean increased stem cell availability for further investigation into the therapeutic use of stem cells in the management, treatment, and cure for a wide variety of health ailments. Human breast milk stem cells offer specific therapeutic potentials based on their particular affinities that other stem cell sources lack.

The Presence of Stem Cells in Human Breast Milk and Research Implications

Breast Milk Overview

Breast milk offers a wide host of benefits for the infant, including the proper and necessary nourishment to support and encourage growth and development. According to Ballard and Morrow (2013) human breast milk should not be described as *merely nutrition* for the infant. The cellular and bioactive components of breast milk make it much more than a way to provide calories for an infant. When studying breast milk, it becomes obvious very early on that one of the most important purposes of breast milk is to provide immunity for the baby (Piper, Berry & Cregan, 2007). Right after birth, the mother's first thick milk fills the ducts of the breast; this milk is known as colostrum. Colostrum is most significantly comprised of immune factors rather than lactose. Lactose provides most of the nutritional support that the baby requires. The abundance of immune factors in colostrum indicates that breast milk serves a greater purpose for the newborn than simple nutrition. The breast milk immune factors include T-cells, macrophages, stem cells, lymphocytes, cytokines, immunoglobulins, and oligosaccharides. Each of these factors supplements the infant's inadequate immune system until the child can properly develop his/her own immunity. Breast milk specifically includes the immunoglobulins IgG, IgM, and IgA which help the infant develop his/her immune system. Oligosaccharides found in milk are important in helping the baby develop a positive normal flora in the intestines. These oligosaccharides are known as prebiotics as they encourage beneficial organism growth in the gastrointestinal

tract. The cytokines play a role in reducing the risk of allergy development in breastfed children (Ballard & Morrow, 2013).

Breast milk contains a variety of growth factors that help the infant grow and mature properly. These growth factors include epidermal, neuronal, insulin-like, and vascular endothelial growth factors as well as erythropoietin, somatostatin, calcitonin, and adiponectin. The growth factors work to regulate the breastfed infant's growth in several different areas. The growth factors appear to play a particular role in the development of the gastrointestinal (GI) tract through maturation, repair, development, and even by improving peristalsis (Ballard & Morrow, 2013). Proteins in the form of whey and caseins are present in breast milk to give the infant the nutrition that it needs to grow properly. These proteins offer beneficial antimicrobial activity throughout the body and aid in the absorption of key nutrients (Piper, Berry, & Cregan, 2007).

There are other important macronutrients in breast milk, such as the lipid or fat portion of the breast milk. The lipids found in human milk provide nearly half of the energy in the breast milk. Lipids contain docosahexaenoic acid (DHA) which plays a significant role in neural and retinal development. Carbohydrates are also found in human breast milk, most commonly in the form of lactose. The carbohydrate components of breast milk serve as a significant energy source for the infant as they are broken down into glucose. The oligosaccharides that are frequently found in breast milk have shown the ability to protect infants against a variety of GI ailments, including multiple causes of diarrhea (Piper et al., 2007).

Production of Stem Cells

Differential Abilities

Stem cells are cells that have not yet been differentiated into one specific cell type, but retain the possibility of dividing into multiple cells of varying cell type. Stem cells have designations based on their abilities to differentiate and multiply. Totipotent cells have the ability to differentiate into all cell types including a functional organism. Pluripotent stem cells have the ability to differentiate into all cell types, excluding a functional organism. Multipotent stem cells have a limited ability to differentiate into a variety of cell types and are often referred to as progenitor cells (Hassiotu et al., 2012). Both pluripotent and multipotent stem cells have been found in breast milk (Serakinci & Keith, 2006).

Cellular Identification

Cell markers are used to identify specific cell populations present in breast milk. Stem cell markers are determined by the types of molecules that bind to the receptor sites on the cell. Stem cells can also be identified by cytokeratin (CK) markers. Flow cytometry has been employed to evaluate the cell constitution of breast milk. Flow cytometry evaluates the breast milk for the quantity and the characteristics of the milk's cellular components. Stem cells have been found in varying amounts by their specific stem cell markers (Fan et al., 2010).

The CK markers and cluster of differentiation (CD) markers were used in many research studies to identify the cellular components of breast milk and especially stem cell presence. Some of the most commonly cited cellular markers found for

hematopoietic stem cells include CD34, CD133, and CD117. Those used for mesenchymal stem cells include CD90, CD105, and CD73. Myoepithelial cells include CD9 and CD44. Immune cells that are found in significant amounts in human breast milk are CD209, CD86, CD83, CD14, CD13, and HLADR. Mammary stem cells are marked by the expression of CK5, CK14, and CK19 markers. Proper cellular identifications allows for accurate determination of the cell's differential abilities. CD or CK markers have not yet been discovered for some cells; therefore, cellular identification for these cells is based on differential ability (Indumathi et al., 2012).

Components of Breast Milk

Stem Cell Components

Breast milk provides cellular components that encourage proper growth and ideal functioning of a child. Out of the cellular components of breast milk such as leukocytes, lymphocytes, phagocytes, epithelial cells, neutrophils, monocytes, and colostrum corpuscles, a variety of stem cells and progenitor cells have been found (Indumathi, Dhanasekaran, Rajkumar, & Sudarsanam, 2012). Specific stem cells found in breast milk include hematopoietic stem cells, mammary stem cells, mesenchymal stem cells, neuro-progenitor cells, and myoepithelial progenitor cells (Fan, Chong, Choolani, Cregan, & Chan, 2010). The initial discovery of the stem cells in human breast milk occurred as breast milk was cultured and found to have stem cell markers including markers such as CK5 and nestin. Both of those markers are implicated as mammary stem cell markers. Once these markers were found, more research began to look at the cellular constituents of milk and the potential for the presence of stem cells. There is a significant presence of

stem cells in breast milk and specifically to the presence of stem cells that expressed pluripotent factors in subpopulations of human breast milk (Hassiotou, Geddes, et al., 2013).

Pluripotent stem cells

The finding of pluripotent human breast milk stem cells (hBSCs) is a significant discovery with implications for infants consuming breast milk as well as research developments and therapeutic and regenerative medicine using stem cells. The hBSCs are able to self-renew while maintaining their pluripotency genes. They are able to differentiate into cells of all three germ layers and into a variety of cell lineages (Twigger, Hodgetts, Filgueira, Hartmann, Hassiotou, 2013). Differentiation of hBSCs can occur spontaneously or through guided direction, as seen in specific cell culturing. Culturing hBSCs in a variety of ways allows them to show properties of neurons, cardiomyocytes, osteoblasts, chondrocytes, adipocytes, hepatocytes, and pancreatic beta cells. It is believed that the stem cells found in human breast milk are not solely derived from the mammary glands. They are thought to have come from a variety of tissues within the maternal body and then expressed through the breast milk. The differential and proliferative abilities of hBSCs make them very similar to human embryonic stem cells, the most frequently used stem cells for scientific research. Human breast milk stem cells may be a useful alternative in research (Hassiotou, Geddes, et al., 2013). This may offer amazing potential in stem cell research and therapeutic interventions.

Mesenchymal stem cells

The research conducted by Patki, Kadam, Chandra and Bhonde (2010) specifically evaluated the presence of mesenchymal stem cells in human breast milk. Mesenchymal stem cells are non-mammary stem cells. The stem cells were evaluated after being supplemented with human umbilical cord blood serum which helps the cells show increased proliferation rates. The mesenchymal stem cells found in the cultured samples were true mesenchymal stem cells by their expression of CD44, CD29, and SCA1 as surface markers. These mesenchymal stem cells were then evaluated for their lineage-specific abilities to differentiate into adipogenic, chondrogenic, and osteogenic cells when exposed to the proper induction medium. The human breast milk mesenchymal stem cells (MSCs) all demonstrated morphologic changes in cell shape in response to the exposure to the appropriate medium. As the MSCs began the process of differentiation, their cellular shape began to change to mirror the shape of the desired cell line of the medium (Patki et al., 2010).

The changes that occurred as the MSCs moved through the process of induced differentiation, typically after about three weeks, which allowed for full differentiation, were also seen in the cellular expression of phenotype. The differentiated MSCs began to express the phenotype seen in the desired cell line after the exposure to that cell line's induction medium. The adipogenic differentiated cells developed lipid droplets in the cell. The chondrogenic differentiated cells developed sulfated proteoglycans which are specific to the chondrocytes. The osteogenic differentiated cells showed mineralization and calcium deposits. Together, the differential abilities observed in these cells are

suggestive of the potential that hBSCs can serve. These cells are believed to have been derived from the myoepithelial cell line in the lactating breast. However, the mesenchymal nature of the stem cells, based on the potential for multilineage differentiation, shows the true power that these stem cells hold, in that they can be reprogrammed through different induction mediums to become a variety of human tissues (Patki et al., 2010).

Variety among Breast Milk Samples

The presence of the stem cells in the breast milk varies widely amongst obtained samples. There are several factors that could determine the concentration of stem cells in the breast milk at a given time. The cellular constitution of breast milk is subject to change frequently based on the mother's stage of lactation, her health status, the permeability of her basement membranes, and her breast fullness. The maternal body is made to respond to the needs of her infant as well as those of the lactating mother. One of the most significant ways the maternal body responds to her infant when breast feeding is through the composition of her breast milk. This child specific response is part of the reason every mother's breast milk is different, and why one mother's breast milk can vary significantly in composition when compared to that of another mother. Changes in breast milk composition occur as the infant grows and his/her maturity levels change. The breast milk composition will adapt to meet the child's needs. Breast milk differences have also been noted relative to the method implemented for breast milk removal including breast feeding, pumping, or hand expression (Hassiotou, Geddes, et al., 2013).

It has been documented that the immunity cellular components found in breast milk show dramatic rises if either the mother or the child is sick, but return to more conservative levels when both are healthy (Hassiotou, Geddes, et al., 2013). The beginning stages of milk formation, when the thickest colostrum is secreted, have been found to express the most significant number of stem cells. The composition of mature milk still reflects a child's specific needs, but in comparison to early milk, is less focused on the rich cellular components and more focused on nutrition. The concentration of cellular components may decrease based on the child's stage of lactation due to dilutional factors. As growth occurs, a greater volume of milk is required which decreases the amount of cellular components in each sample. The milks composition adapts to the child's needs to provide optimal nutrition and functional support (Hassiotou, Hepworth, et al., 2013).

Purpose of Breast Milk Stem Cells

A large portion of the stem cells found in breast milk is derived from the mammary stem cells which the breast creates as it undergoes remodeling to become a secretory organ. The cells that the breast tissue creates to remodel itself are then passed through the breast into the milk and then through the milk to the baby. These mammary stem cells have been found to be able to self-renew and differentiate into specific cell types (Fan et al., 2010). Once ingested, the stem cells travel through the infant and are able to engraft into various tissues and organs. The functions of stem cells are to repair and regenerate, which allows the infant to grow, develop, and maintain homeostasis. Stem cells have a very similar role in adults allowing tissue to repair and regenerate as

necessary. It is the ability to repair and regenerate that makes stem cells appealing for therapeutic use (Hassiotou, Geddes, et al., 2013).

Maternal cells, including maternal stem cells, are found in a child's tissues and body systems directly after birth and in the first few years of life. Recently, maternal cells have been discovered in some children past those first few years. Maternal cells can be present in her offspring well into early adult life, if not longer. It is believed that this cellular exchange occurs through a process known as microchimerism. This maternal-child microchimerism can occur during fetal development in utero, or this can occur after birth through oral exposure to maternal cells found in breast milk (Sani et al., 2015). It appears that in order for the maternal cell lines and stem cells to last into adulthood both in-utero and oral exposure need to occur. Microchimerism can occur without the presence of both, but the cell lines are not found to last as long in the offspring. Cellular transfer can occur through oral exposure alone, and does not require fetal exposure. This was demonstrated as maternal cells were able to colonize in the liver of mice who were only exposed to those maternal cells through breast milk. This is indicative of the power of breast milk stem cells to be able to transfer across the gastrointestinal lining of an infant to be fully incorporated into the infant's developing body with the potential to fully engraft into the newborn's tissues and organs (Dutta & Burlingham, 2010).

Stem Cell Research

Adult and Embryonic Stem Cells

Adult stem cells are different from embryonic stem cells. Embryonic stem cells have more pluripotent abilities. Adult stem cells have significant multipotent potential,

with a much smaller fraction of pluripotent cells (Serakinci & Keith, 2006). The cellular differences give embryonic research an advantage over adult stem cell research.

Embryonic stem cells show greater proliferation rates than adult stem cells. This makes embryonic stem cells preferable for research because fewer cells are required. The embryonic stem cells collected can be allowed to proliferate to reach the desired amount of stem cells (Cogle, Guthrie, Sanders, & Allen, 2003). The stem cells found in human breast milk include cells that have both multipotent and pluripotent potential, allowing them to be a feasible alternative to embryonic stem cell research. Breast milk is a non-invasive source of stem cells that are easy to obtain (Fan et al., 2010).

Embryonic Stem Cell Research

Embryonic stem cell research is the current popular method of stem cell research. The moral implications of using an embryo for research have caused a heated debate amongst researchers, the scientific community, religious organizations, and the public at large. Embryonic potential for human life has led many to discourage the use of embryos in research. Stem cell research of breast milk stem cells holds a solution to the moral/ethical dilemma. Breast milk is a readily available source of stem cells that hold both multipotent and pluripotent abilities without the constraints and restrictions of embryonic research (Murdoch et al., 2012).

Gaining access to human embryos has been difficult logistically, ethically and morally. The advances in infertility treatments throughout the past several decades through in-vitro fertilization (IVF) have given rise to multitudes of embryos that have the potential to be used for research. Most couples who decide to use IVF elect to have

multiple embryos created and then only have a few actually implanted at a time. The embryos that are not implanted can undergo cryopreservation to be used by the couple at a later time, donated to another couple, destroyed, or donated to scientific research (Murdoch et al., 2012). Many choose to donate their unused or unwanted embryos to science. The process of the donation involves the informed consent of the parents choosing to donate (National Institutes of Health, 2009).

Ethical Considerations of Stem Cell Research

A significant question involving embryos and hESC research is the definition of an embryo. Scientifically, there is no universally accepted definition of an embryo; the definition is subject to change amongst agencies, communities, and especially countries. The various definitions typically all include the idea that an embryo is a fertilized egg in its very early stages of development, or at least an organism with the potential for life. It is that definition which in turn helps to define what human embryonic stem cells are. In the United States a new definition of human embryonic stem cells was created by the National Institutes of Health in 2009. This definition states that hESCs are: “Cells that are derived from the inner cell mass of blastocyst stage human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers” (National Institutes of Health, para. 12). This definition directly addresses the two main points of stem cells which are their differential and proliferative abilities (Murdoch et al., 2012).

Aside from the strictly scientific definition of what makes an embryo, there are the more controversial views which much of the population in the United States hold.

There are four general views of embryos. The first view is that life clearly begins at conception, and as such embryos have the moral rights of a person. The second view is that embryos hold intrinsic value based on their potential to become a person and experience life. The third view is that in order for morality to be granted to human beings, they must have certain cognitive abilities that an embryo does not yet possess. Finally, some hold that an embryo is sacred and holds symbolic significance (Master & Crozier, 2012). The knowledge of these common perspectives, especially in light of the accepted scientific definition of an embryo in the United States, helps to bring understanding and ultimately can help all of those involved come to an agreement of compromise as this area of research moves forward.

The ethical concerns of using embryonic stem cells in scientific research and medical therapies raise important considerations not only for those conducting, participating in, and funding the research, but for humanity as a whole. As in-vitro fertilization grew in popularity, questions began to arise around the issue of what to do with the embryos that were not implanted. One of the proposed solutions was to donate them to scientific research. The basis of the ethical argument surrounds the consideration of the embryo's potential for life against the embryo's potential to play a significant role in the development of new and advanced medical therapies. These arguments often debate the origin of life and the implication of interrupting this process. There are both moral and ethical considerations involved in denying the scientific use of unused embryos because of their vast potential in the scientific and medical arena. Those who promote the use of human embryos to study hESCs typically hold a paternalistic

viewpoint. They believe that using embryos for research, even if those embryos should receive respect due to their potential to hold human life, could provide a wealth of knowledge which could help to manage, treat, or cure many significant diseases. These potential benefits offer more to society as a whole than one embryo could in this perspective (Ramos-Zúñiga, González-Pérez, Macías-Ornelas, Capilla-González & Quiñones-Hinojosa, 2012).

Outlining the ethical and moral concerns can easily become quite complex if each individual or even every group's beliefs are considered. It is much easier to contemplate the moral and ethical concerns that surround stem cell research when generalizing the most common viewpoints held by those who live in the United States. The overwhelming number of citizens fall into one of the following categories: either they support stem cell research because of the potential benefits it offers for a variety of disease processes, or they do not support stem cell research due to a variety of moral and ethical considerations. The first group finds it morally unacceptable not to continue with stem cell research because of its potential. The second group typically has two concerns revolving around the ultimate destruction of the embryos during the research process or the risks that the women who choose to donate their ova face (Master & Crozier, 2012). As the ethical and moral considerations of embryonic research are presented and analyzed, the need for other, less controversial, sources of stem cells can be clearly seen.

Alternatives to embryonic stem cell research

One proposed solution is a moral compromise where both sides are willing to relinquish certain aspects so that the greater good can be accomplished while maintaining

their moral integrity. The problem with the moral compromise is that it may attempt to allow both sides to maintain their moral integrity, but in such a moral dilemma, even if both are willing to acquiesce some of their more salient points, both will have a breach in their integrity. Other resolutions are offered, but the alternative solutions mentioned are morally controversial, scientifically impossible at the given time, or dangerous and unpredictable. One resolution was to create a pluripotent line of cells from the patient's own cells which would not use embryonic stem cells; but this is not yet scientifically possible. A second option was to find a way to remove some of the embryo's cells but still leave the embryo the potential for life. The consequence of removing any cells from an embryo makes that embryo undesirable for implantation because of the risk for life-long repercussions. The concerns for the safety and risks involved for female ova donors are significant because at this time it does require an invasive procedure, in addition to the social risks of exploitation by researchers and doctors. Part of these concerns includes the controversy over whether to compensate or offer an outright payment to women for their ova donations. Conversely, halting scientific advancement, which could offer treatments, therapies, and cures, as well as a deeper knowledge of the body, also seems to be morally void (Master & Crozier, 2012).

Considering the moral implications surrounding the use of human embryos for stem cell research, there is great value in attempting to find other, less controversial, resources for stem cell research. Adult stem cells offer significant potential in the field of stem cell research, but retrieval of most adult stem cells, especially the most viable options for stem cell research, requires invasive procurement measures. These adult

sources include bone marrow and adipose tissue (Master & Crozier, 2012). A novel source to consider is definitely breast milk. The recent finding of stem cells in human breast milk offers a new, morally acceptable, virtually unlimited, and easily accessible source of stem cells for research. Based on the differential and proliferative abilities, it appears that stem cells found in human breast milk may indeed be compatible for use in stem cell research. There are many similarities found between hBSCs and hESCs which suggest such a conclusion. The similarities between the hBSCs and hESCs will be discussed in depth, but the ability of hBSCs to be attained without harm makes them an appealing resource for further evaluation and consideration (Twigger et al., 2013).

Compatibility of Breast Milk Stem Cells for Stem Cell Research

Pluripotency of Human Breast Milk Stem Cells

The presence of pluripotent stem cells in human breast milk suggests potential use of these cells in stem cell research. Human breast milk stem cells are similar to embryonic stem cells which provide a predominant avenue for stem cell research. Human breast milk stem cells have shown similar phenotype, morphology, and functionality as embryonic stem cells. These similarities are promising for the use of breast milk stem cells in a research manner comparable to that of embryos (Twigger et al., 2013).

The pluripotential of hBSCs permits them to be fit for stem cell research. Cellular pluripotency, found in human embryonic stem cells, is what makes hESCs more compatible for stem cell research in comparison to adult stem cells. Specifically, it is the ability to maintain self-renewal, proliferative functioning, and pluripotent genetic

expression over time that makes embryonic stem cells so desirable. The pluripotency of these cells is important because it allows for further investigations into the development of cell differentiation. Understanding normal development and abnormal development then allows for new therapies to be developed based on the idiosyncrasies found (Cogle et al., 2003).

Teratoma Assay Findings

Of particular interest is the fact, that when studied, the stem cells found in human breast milk demonstrate a negative teratoma assay finding. This test is typically performed to determine if cells demonstrate in-vivo pluripotency. The teratoma assay involves injecting the cells being studied, in this case hBSCs, subcutaneously into immunodeficient mice. The assay is positive if the mice grow tumors, but negative if the mice remain tumor free. The positive result indicates that the stem cells are pluripotent. The teratoma formed will be a variety of differentiated stem cells if the stem cells are pluripotent. Having this test result return negative could appear as problematic for the use of hBSCs in in-vivo therapies and treatment options (Müller, Goldmann, Löser, & Loring, 2010). However, the negative teratoma assay is not necessarily an unfavorable finding. More research will need to be conducted to fully determine the implications of the negative teratoma assay Hassiotou et al. (2012).

It is possible that the negative teratoma assay could be beneficial and help to decrease secondary risks associated with stem cell therapies such as tumor formation. The true question is whether the stem cells found in human breast milk can or cannot fully integrate into damaged tissue. If the cells cannot integrate, as is possible with the

negative assay, further research will need to be done to determine the extent in which these cells can be used in therapies and treatments. The hBSCs did show the ability to integrate into the damaged tissue of the immunodeficient mice, even though these stem cells failed the teratoma assay typically used to determine cellular ability to integrate into tissue. If the cells can fully integrate into damaged human tissues, this could show significant potential in the use of hBSCs in both research and medical therapies and treatment options (Hassiotou et al., 2012).

Healthcare Implications

The healthcare field hopes to be able to harness stem cell research in a therapeutic manner. The desired outcome is that stem cell therapies will eventually be able to treat, cure, or manage disease such as hematopoietic, neurological, cardiovascular, and bone disorders. Breast milk stem cell presence allows for continued and possibly preferred methods of stem cell research to develop these therapies (Trounson, 2009). Human breast milk stem cells have already shown an ability to differentiate well into cells that function in a manner similar to pancreatic beta islet cells and hepatocytes. These stem cells were able to produce insulin and albumin respectively, suggesting the potential for these specific stem cells to be used in diabetes and liver disease (Hassiotou, Geddes, et al., 2013). The use of autologous stem cell therapy would be plausible for mothers, allowing them to use their own stem cells, reducing the immune reactions that are often seen during stem cell therapies (Cedar, 2006).

Neuronal Therapy

There is another specific opportunity that hBSCs offer in the field of stem cell therapies and this is in the area of neural damage and disorders. The prevalence of neurodegenerative disorders is increasing and the stem cells found in human breast milk offer a potential source of stem cells to be used in research and therapy. Neurogenesis, the process of generating new neural cells from adult stem cells, offers a new scope of potential when looking at using hBSCs rather than adult stem cells for their therapies. Some of the stem cells found in human breast milk have been found to express the cell marker nestin which is seen in neural stem cells. As mentioned before, hBSCs tend to behave in a similar manner to hESCs. This nature, combined with the expression of nestin, make it a logical choice to evaluate the hBSCs' abilities to be used in the emerging area of neural regeneration and neural cell therapies (Hosseini, Talaei-Khozani, Sani & Owringi, 2014).

It is believed that the neural potential for hBSCs is found in the development of both the mammary gland and the nervous system from a common origin and with similar regulators. Those regulators are the same regulators that control the self-renewal and differentiation of hESCs. This combination, allows hBSCs to hold great potential when considering their ability to be used in the neurological field. Hosseini et al. (2014) evaluated this potential by looking at the ability of hBSCs to differentiate into all three levels of neural cell lineages including neurons, astrocytes, and oligodendrocytes. The results found that small subpopulations of the hBSCs expressed nestin and CD133 which are markers for neural stem cells. Other cells found were: neuron-like cells that

expressed β tubulin III, oligodendrocyte-like cells with anti-O4 antibodies, and astrocyte-like cells with anti-GFAP antibodies. Together these findings indicate the need for further research into the incorporation of hBSCs in new neural stem cell therapies (Hosseini et al., 2014).

Even further implications for the use of neural stem cells include the potential for transplantation of neural stem cells directly into recipient brain tissue. The brain shows remarkable affinity for stem cell transplantation which is derived significantly from the brain's immune response, according to Ramos-Zúñiga et al. (2012). The stem cells, once transplanted, are believed to offer significant improvements through several of their properties including the ability to regenerate/repair damaged areas, the excretion of neuroprotective factors, and their immunomodulation abilities. Together, these cells can help to repair and regenerate any damaged areas in the brain and help to protect the brain in cases of inflammation and neural degeneration. These conclusions are true for both adult neural stem cells and embryonic neural stem cells (Ramos-Zúñiga et al., 2012). It is reasonable to include hBSCs as a potential resource to use in these specific neural stem cell transplantations given their specific affinity for differentiation into neural stem cells. The negative teratoma assay response could show a benefit in this situation because both adult and embryonic stem cells have shown the risk for tumor formation. Further research would be necessary to ensure the hBSCs could be safely transferred and useful for in-vivo purposes (Hosseini et al., 2014).

Microchimerism and Transplantation Implications

Microchimerism was briefly discussed previously in regard to the purpose of hBSCs in the infant. The mechanism of action that occurs in maternal-child microchimerism may be beneficial for transplantation purposes. The microchimerism discussed happens both from fetus to mother and from mother to fetus in utero through the placenta. The mother to fetus transfer is then increased significantly and substantiated through the ingestion of breast milk. Without being breastfed, the children do not continue to have maternal cells into adulthood as those who were breastfed do. It is reasonable to hypothesize that many of the cellular components absorbed through the breast milk and adopted into the child's body are indeed the stem cells found in breast milk. The hBSCs have shown great differential capacity as well as the ability to be absorbed into circulation and distributed to tissue in the infant's body (Aoyama, Matsuoka & Teshima, 2010).

Breast milk contains a specific regulatory T cell type (Foxp3+) which is able to help the breastfed-infant's body to suppress the immunity against those maternal cells. Exposure to any cells, other than a person's own cells, carries with it the possibility of triggering an immune reaction. This immune reaction does not happen between the mother and the infant when cells are transferred through breast feeding. It is then possible to imagine that if these non-inherited maternal antigens (NIMAs) did not spur an immune reaction while in utero and breast feeding, that it is possible that NIMA-mismatched donor tissue could allow for a permissible mismatch. The allowance of permissible mismatches then opens up a significant increase in the number of potential

donors that would otherwise be considered either risky or even incompatible. Typically, these mismatches can be seen most frequently amongst siblings and used for transplantation purposes. Two of the most significant transplantation options that are increased through the use of NIMA-mismatched donors include hematopoietic stem cell transplantation and bone marrow transplants. Those receiving permissible mismatches who were breastfed and had NIMA exposure to those same mismatches showed less graft-versus-host disease (Aoyama et al., 2010).

This offers a significant testimony, although somewhat indirectly, to the power of the cellular, and most definitely stem cell, components found in breast milk. The NIMAs exist because of exposure to maternal cells both in-vitro and through breast milk. It is the breast milk that contains the necessary factors to prevent an immune response to the foreign maternal cells. The stem cells passed through the milk and absorbed into a variety of the infant's body systems offer development support at the time of ingestion. As time passes, those engrafted stem cells help to widen the pool of potential donors if transplantation is ever needed as well as reduce the risk for graft-versus-host disease. Breast milk offers a multitude of life-long benefits for the breastfed infant (Aoyama et al., 2010).

Conclusion

Breast milk provides the optimal nutrition for an infant. The incredible complexity of breast milk, which allows it to provide perfectly for an infant, also gives it promise in the areas of scientific research and medical therapies. Breast milk is composed of a variety of components which combine to offer an infant exactly what he

or she needs to thrive. One of the most remarkable components of human breast milk is stem cells. These stem cells offer a non-invasive source of pluripotent and multipotent stem cells with the ability to self-renew and differentiate into a wide variety of cells.

These characteristics make hBSCs favorable for stem cell research. The stem cells found in human breast milk have shown specific affinity for stem cell therapies. Two of the most significant medical therapies for these stem cells include neuronal therapies and transplantation.

The constitution of breast milk is ever changing. Changes in composition occur within each woman, within each feeding, and are subject to change based even on the time of day. The composition will change based on maternal health, infant health, and developmental stage of the baby. Breast milk can vary significantly between individuals and cultures. These fluctuations are especially reflected in the presence of stem cells found in breast milk samples. The stem cells are thought to be derived considerably from the remodeling of the breast as it becomes a secretory organ. The purposes of hBSCs in the breast feeding child include growth, regeneration, and repair of tissues. The stem cells, once ingested, can integrate into the systemic circulation through the intestines. The integration of these cells is an example of maternal-child microchimerism. The microchimerism begins in utero and is solidified through breast feeding.

Stem cells are identified in breast milk by their cell markers, typically through evaluation with flow cytometry. Both adult and embryonic stem cells have been used in research, but the pluripotency of human embryonic stem cells allows them to be more valuable for research. Adult stem cells are typically difficult to obtain and require

invasive retrieval. Embryonic stem cells are most frequently obtained through donation of extra embryos created during IVF. Similarly, breast milk donations provide researchers with access to hBSCs. The creation of embryos through IVF, and the subsequent donation of unused embryos, requires an invasive procedure to retrieve the maternal oocytes. The donation or collection of breast milk is non-invasive and does not pose any significant threats to the mother or her child. Human embryonic stem cells and human breast milk stem cells show many similarities that make hBSCs a promising new source for stem cell research over hESCs. The hBSCs offer an inexpensive and easily accessible source of stem cells whereas hESCs are a complicated resource because of ethical and moral considerations. These ethical and moral considerations significantly limit the ability of hESCs to be used for the time being, but hBSCs do not have such reservations.

The healthcare implications are substantial if hBSCs are able to be used not only for stem cell research, but for stem cell therapies. These therapies include help for disease such as hematopoietic, neurologic, cardiovascular, and bone disorders. Of special significance are neuronal therapies, because of the compatibility of hBSCs as neural stem cells, and transplantation considerations, due to the microchimerism that occurs during breast feeding. Breast milk is often viewed solely as nutritional support for children, but the true power of breast milk is yet to be unearthed. With more research, one of the most significant roles of breast milk could include the use of its stem cells for both stem cell research and medical therapies/treatments.

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