Melatonin bioengineered: A New Possible Strategy for Treatment of Breast Cancer

Rubian Trindade da Silva Fernandes¹, Aron Carlos de Melo Cotrim², Eduardo Luzía França³, Adenilda Cristina Honorio-França⁴, Inês Aparecida Tozetti⁵

¹Master in Basic and Applied Immunology and Parasitology - Federal University of Mato Grosso (UFMT), Barra do Garças, MT, Brazil - Doctorate in Health and Development in the Central West Region Medical School (FAMED) Federal University of Mato Grosso do Sul UFMS), Campo Grande, MS / Brazil. Email: rubiantdsf@gmail.com
²Master in Science of Materials - Federal University of Mato Grosso (UFMT), Barra do Garças, MT, Brazil - PhD in Biosciences (InBio), Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS / Brazil. Email: aroncarlosbg@gmail.com
³Master in Tropical Diseases, UNESP, Botucatu, SP / Brazil - PhD in Immunology from the ICB of USP, São Paulo, SP / Brazil - Postdoctoral fellow by the Institute of Biosciences, UNESP Botucatu and in Pharmaceutical Sciences by UFSJ - Associate Professor of the ICBS-CUA of the Federal University of Mato Grosso (UFMT), Barra do Garças, MT / Brasil. Email: dr.eduardo.franca@gmail.com
⁴Master of Science (Immunology) from the University of São Paulo, SP / Brazil - PhD in Immunology from the University of São Paulo with a sandwich period at the Necker Hospital, INSERM Unit 25 - Paris, France - Post-doctorate from the Faculty of Medicine, State University Paulista Júlio de Mesquita Filho and the Medical School of Ribeirão Preto, University of São Paulo, SP / Brazil. Associate Professor at ICBS-CUA, Federal University of Mato Grosso (UFMT), Barra do Garças, MT / Brasil. Email: adenildachf@gmail.com
⁵Master in Immunology from the University of São Paulo, SP - PhD in Immunology from the University of São Paulo, SP / Brazil. Associate Professor at the Laboratory of Immunology, Bioassays and Molecular Biology of the Center for Biological and Health Sciences / UFMS, Campo Grande, MS / Brasil. Email: inestozetti@ufms.br

Abstract — Breast cancer is an important public health problem, with an estimated 3.2 million new cases by the year 2050. Diet plays a key role in the etiology of breast cancer and breastfeeding is associated with a lower incidence of breast cancer. On the other hand, the improvement of the therapeutic properties of bioactive compounds through their incorporation into microcarriers is an important strategy in obtaining new therapies, since cyclical changes in concentration are eliminated; there is biological availability of the compound as well as the reduction in toxicity, number dose and suppression of adverse reactions. Studies using hormones such as melatonin extracted from human milk adsorbed onto polyethylene glycol (PEG) microspheres showed that the controlled release of this compound was able to reduce viability and induce apoptosis in MCF-7 cell lines. Colostrum differs from most of the secretions because it contains viable leukocytes during the first days of lactation with a quantity and activity comparable to blood leukocytes, and has several defense components such as antibodies and hormones, such as melatonin (MLT). This review details the influence of the soluble and cellular components present in human colostrum, such as the MLT hormone, as the modified release systems influence the action of MLT and the possible mechanisms involved that contribute to the hypothesis of reduction of breast cancer in women who breastfed.

Keywords — colostrum, melatonin, polyethylene glycol, breast cancer, bioengineering.

I. INTRODUCTION

Breast cancer cases have increased worldwide, and are directly related with more life expectancy, exposure to risk factors, and habits. Breast cancer is a multifactorial disease with a higher incidence among women, leading to death [1]. Cancer and chronic inflammation are closely linked and the imbalance between reactive oxygen species and antioxidant enzymes favors the emergence of these diseases. Thus the control of the production of reactive species as well as the maintenance of oxidative balance are primordial for the control of tumor progression [2, 3].

Some factors such as healthy lifestyle and breastfeeding are related to the prevention of breast cancer [4]. Human colostrum differs from other secretions by containing large quantities of viable leukocytes comparable to those found in blood acting as anti-inflammatory mediators. It is believed that both soluble and cellular components interact with each other and may be important for antitumor immunity [5,6]. Breast milk is rich in soluble and cellular components, such as...
phagocytes, secretory IgA immunoglobulin (SIgA), and hormones, especially melatonin (MLT) [5,7,8].

Melatonin, a hormone produced by the pineal gland, is involved in several physiological processes, including the functional regulation of breast milk. In milk it is related to the anti-inflammatory effects [9] and pro-oxidant and antioxidant effects of paramount importance in the oxidative stress balance as a protection mechanism [10].

Some studies have reported that the bioavailability and biofunctional function of melatonin may be potentiated when associated in modified release systems [11,12]. Among these systems, polyethylene glycol (PEG) microspheres have been considered an important vehicle for the administration of various drugs, natural products and hormones [13,14,15,16,17].

The administration of drugs adsorbed to carrier systems such as PEG microspheres has been an alternative treatment for various diseases [18], including breast cancer. These release systems are promising for the release of the hormone melatonin [11], preventing it from the degradation promoted by the metabolic enzymes increasing their bioavailability in the organism [19]. How much combined with the MLT has been demonstrated its capacity to reduce cell viability and induce apoptosis in tumor cell lines from breast cancer [12,11].

The control of the process of carcinogenesis is closely related to the control of apoptosis, since the tumor cell is able to alter this system, favoring its proliferation and promotion [20]. Thus, cancer treatments are directed at inducing increased apoptosis of tumor cells [21,22], which may be favored by the use of immunomodulatory agents such as MLT, for acting on immunocompetent cells and assisting in tumor eradication.

This review details the influence of the soluble and cellular components present in human colostrum, such as the MLT hormone, and how they may be responsible for mechanisms that reinforce the hypothesis that breastfeeding reduces the risk of breast cancer.

II. BREAST CANCER

Breast cancer is a public health problem, with around 59,700 new cases in Brazil in the year 2018 [23]. The worldwide incidence and mortality of this disease are highly related and it is estimated that by the year 2050 will appear about 3.2 million new cases of breast cancer in the world. Despite technological advances, there are still several mechanisms that must be elucidated in the eradication of this disease that affects the world population [24].

Breast cancer is considered a heterogeneous disease, both morphologically and clinically, and is due to a disordered proliferation of breast tissue cells. About 80% of the types of breast tumors originate in the ductal epithelium, known as invasive ductal carcinoma [25]. Invasive carcinomas are so called because they have high metastatic potential, since carcinomas in situ have low metastatic potential and may arise in both lobes and mammary ducts [26]. During carcinogenesis, genetic mutations are accumulating and the cell phenotype is changing through malignant lesions, evolving into invasive cancer [27].

The structure of the breast is composed of glandular tissues composed of the milk producing glands and the ducts through which milk produced and stromal tissues pass, which are fibrous and fibrous connective tissues. In addition to these tissues the breast is also composed of the tissue of the immune system and lymphatic system [28]. For normal development of breast tissue to occur, there is a need to balance cell proliferation and apoptosis. In tumor growth, there is a reduction in apoptosis and an increase in cell proliferation [27].

The balance between a protective cytotoxic response and a non-protective response can be regulated by the individual’s overall immune status [29]. A major challenge for tumor research has been the identification of molecular and immunological changes associated with the different stages of tumor progression, and advances in these studies have been hampered by technical limitations to the pre-invasive stages of tumors [30].

The study with in vitro breast cancer cells began in 1973 from isolated cells from pleural effusion of a 69-year-old woman with metastatic disease [31]. MCF-7 cells are useful for in vitro breast cancer studies by having several particular ideal characteristics of the mammary epithelium, such as the ability to process estrogen in the form of estradiol via estrogen receptors in the cell cytoplasm. This cell line is positive for the estrogen receptor (ER) and for the progestosterone receptor and negative for HER2. These cells are very well studied with the immense number of protocols defined which allows researchers to use this cell line for study pathogenesis and in the search for treatment of breast cancer through reliable means in vitro assays [32]. During the last decade, several work on the mechanisms related to the interaction between the cells of the immune system and tumor progression. The results indicate that an immune response to a tumor is determined by the different cell types, such as lymphocytes, NK cells, neutrophils and others, as well as by the interactions between hormones, proteins and receptors present on the cell surface [33].

On the other hand, tumor cells arise from a mutation in DNA (deoxyribonucleic acid) that can be caused by radiation, bacteria, fungi, viruses, chemicals, etc. Although the components of the immune system are present and active, cancer cells can progressively grow.

www.jaers.com
and spread, thus, the body weakened by poor diet, genetic predisposition, advanced age and exposure is the perfect environment for the development of cancer. In addition, the cancer cells are very similar to the own cells of the organism, which hampers even more the response of the immune system [28]. Among the mutations, the most important alterations that occur are self-sufficiency in signs of proliferation, insensitivity to growth inhibitory signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis and tissue invasion and metastasis [20]. The carcinogenesis process is directly involved with the generation of reactive oxygen species. Oxidative stress participates in all stages of cancer development. At initiation, oxidative species damage DNA by introducing genetic mutations and structural alterations; in the promotion, there is an increase of the initiated cell population, which is proliferation with consequent decrease of apoptosis; already in progression participate in the development of irreversible cancer growth [34].

III. BREASTFEEDING AND THE IMPORTANCE OF BREAST CANCER PREVENTION

Breast cancer is the neoplasm most feared by women, since its occurrence causes great psychological, functional and social impact, acting negatively on issues related to self-image, social interaction and the perception of sexuality. It is considered of great importance in the health care of women, due to the high prevalence, morbidity and mortality [35].

The role of the immune system in cancer prevention is complex and partially understood. It is widely known that diet plays a fundamental role in the etiology of cancer [33] and that breastfeeding is associated with lower incidence of breast cancer. In this sense, studies have tried to elucidate the effects of lactation on breast cancer [36, 37]. There is evidence that human milk may confer long-term benefits and an increasing number of studies have indicated that breastfeeding provides protection against ovarian and breast cancer [38,39].

However, the effects of breastfeeding on the risk of breast cancer have been difficult to study because of the high correlation with parity [40, 41]. Reproductive factors may induce permanent changes in the epithelium of the mammary gland or in the surrounding stromal tissue [42,43]. Although the mechanisms have not been fully elucidated, the hypothesis of reducing the risk of breast cancer through breastfeeding seems to occur because of breast tissue differentiation or reduction in the number of ovulatory cycles [44].

Studies have revealed that the relative risk of having breast cancer reduces about 7.0% for each child born and about 4.3% for every 12 months of breastfeeding. This suggests that breastfeeding duration mothers is crucial to ensure the immunity components acts against the breast cancer [37,45]. There is still evidence that breastfeeding protects women who have had their children under 50 years of age [46]. Another study reported that women who breastfed several children had the lowest risk of developing breast cancer, and mothers who breastfed four or more children had a 60 percent reduction in breast cancer risk. The magnitude of the protective effect is directly related to the time of breastfeeding [47].

Lactating mammary glands are an integral part of the mucosal immune system, and the antibodies and cells present in the milk reflect the antigenic stimulation of the Mucosa-Associated Lymphoid Tissue (MALT) in both the intestine and the respiratory tract. The literature reports that antibodies and human milk cells have specificity for a variety of antigens from intestinal and respiratory pathogens [48].

Colostrum differs from most of the secretions by containing viable leukocytes (10⁹ cells / ml) during the first days of lactation [49], with amount and activity comparable to blood leukocytes [5]. On the other hand, other defense components present in the secretion that may be associated with protective activity, such as lactoferrin, analogous complexes (receptors), fatty acids (lipids), mucins [50], cytokines and chemokines [51,52,53,54], antibodies such as IgA [55,56], lysozymes [52], probiotics [57], antioxidant factors [58], among other components produced by the maternal immune system, as well as several hormones such as melatonin [59, 60, 15].

The concentrations of melatonin in human colostrum and mature milk are similar the concentration of this hormone in the bloodstream. Also, immunocompetent colostrum cells can start to produce melatonin after stimuli from injuries, such as those caused by bacteria or fungal metabolites. Studies have shown that lymphocytes and peritoneal macrophages from rats and human colostrum phagocytes produce melatonin in response to activation, and this production of melatonin in the site activates lymphocytes and macrophages to produce IL-12, IL-6, IFN-γ which increases the production of T lymphocytes, the presentation of antigens and the phagocytic activity of macrophages, thus increasing the local inflammatory aspect [61,62,63] and this synthesis of melatonin occurs by the same enzymatic pathway that occurs in human pinealocytes [64].

Immunocompetent breast cells remain highly permeable after childbirth, which makes this type of cell suitable for signaling pathways when collected in a non-invasive manner, suggesting that these cells play an
important role in the protection of the newborn in pathological conditions these cells will be the defense of the newborn, thus consolidating the importance of breastfeeding [62].

IV. MELATONIN

Melatonin is synthesized by the pineal gland [65]. It plays an important role in circadian rhythm control, reproduction, sleep-wake, is directly linked to the regulation of several neuroendocrine axes, protection against cancer and action against free radicals, acting on cell protection [66, 67]. Studies have shown that melatonin may increase the action of innate and acquired immunity and stimulate mainly leukocytes, an immunomodulatory property, which represents an important mechanism of protection for several diseases [9, 68, 8], as well as showing remarkable functional versatility oncotic properties, antioxidants and antiaging [69].

The action of direct melatonin against free radicals has been increasingly studied and its indirect role as an antioxidant has been tested and the effect has been highly effective in reducing oxidative stress in the body when compared to the antioxidants better known as vitamins C and E. Melatonin and its metabolites have positive aspects that make them effective in fighting free radicals. They easily cross the blood-brain and placental barrier, in addition to all maternal organs which leads to greater protection of the placenta and the fetus. Another positive aspect of melatonin is that it can be produced in other compartments, external to the pineal gland [66], and it has been speculated that all cells can synthesize melatonin, mainly in their mitochondria and this local production has the function of protection against radicals free [70].

The production of melatonin by other kinds of cells and organs has been reported, such as the retina, thymus, brain, intestine, bone marrow, ovary, testis, placenta, skin and lymphocytes [71]. High concentrations of melatonin have been found in skin keratinocytes, suggesting that the production of melatonin outside the pineal gland is not only related to the light / dark circadian rhythm, but rather as an antioxidant and anti-inflammatory agent and as a mechanism of stress protection oxidative. This production of melatonin in response to oxidative stress occurs in all living beings, such as plants, unicellular beings, animals and man, and must have been the main function of melatonin in the primitive beings, since they did not have resources in the fight against free radicals.

The production of melatonin by cells of the immune system occurs by activation of pro-inflammatory agents such as cytokines, increases the phagocytic capacity of macrophages and lymphocytes and induces the synthesis of interleukin-2 (IL-2), which has autocrine action and paracrine [69].

Melatonin acts on inflammatory processes and allergic diseases by attenuating the activation of NF-κB, reducing the production of TNF-α and IL-6 and promoting the survival of mast cells via a series of enzyme kinase activation and inhibition processes [72].

Melatonin exerts antioxidant action, which decreases the formation of free radicals, reducing the number of lesions that may affect cellular DNA [73]. It exerts an antiproliferative effect on physiological dose dependent breast cancer MCF-7 (human breast adenocarcinoma cell line) cells, in addition to reducing the rates of invasive and metastatic properties of this cell type [74]. Studies have shown that melatonin decreased cell proliferation and increased expression of p53 and p21 proteins in MCF-7 cells, inhibiting proliferation and inducing apoptosis. The p53 protein is an important tumor suppressor gene and is involved in the regulation of the cell cycle [75]. Melatonin, via activation of the melatonin 1 receptor (MT1) [76], is associated with suppression of growth and development of breast cancer through regulation of growth factors, regulation of gene expression, inhibition of tumor cell invasion and metastasis and by regulation of mammary gland development [77].

V. POLYETHYLENE GLYCOL (PEG) AND THERAPEUTIC APPLICATION IN BIOENGINEERING

Studies aimed at reducing adverse drug effects have been developed as novel therapeutic systems, known as modified release systems [78, 79, 80, 81, 82, 83]. The improvement of the therapeutic properties of bioactive compounds through their incorporation into microcarriers is an important strategy in obtaining new therapies, since cyclical changes in concentration are eliminated; there is biological availability of the compound as well as the reduction in toxicity, number of administered doses and suppression of adverse reactions [84].

PEG-drug conjugates and microemulsions-drug, are being studied as possible modified release systems for a variety of molecules and drugs [85, 86, 87, 88, 89, 90, 91, 92, 83]. This combination has many advantages such as prolonged residence in the organism, decreased degradation by metabolic enzymes and reduction or elimination of the immunogenicity of proteins [87].

Several studies have shown that the association of PEG microspheres with molecules, hormones or proteins increases the immunomodulatory capacity of both blood and colostrum phagocytes and suggests that the adsorption of these compounds to PEG microspheres has
immunostimulatory effects and can be considered an important material/vehicle, with potential for future therapeutic applications in infectious diseases or tumors [13, 15, 14, 17, 16, 93].

Studies using hormones such as melatonin and secretory IgA antibodies extracted from human milk adsorbed onto PEG microspheres showed that the controlled release of this compound was able to reduce viability and induce apoptosis in MCF-7 cell lines [11,12]. Other herbicidal and barium chloride studies, adsorbed to PEG microspheres on human blood mononuclear cells co-cultured with breast cancer cell lines (MCF-7), showed a pro-apoptotic effect in breast cancer cells MCF-7 human [94, 95]. Immunotherapy for tumor treatments based on cytotoxic properties of immunocompetent cells has also been the focus of many studies. Both T cells and phagocytic cells are considered effectors with antitumor activity [96]. The melatonin adsorbed to the PEG microsphere was able to increase the functional activity of colostrum phagocytes and that this modified hormone release system may represent an alternative in the treatment of diseases [17].

Here we hypothesize that melatonin adsorbed on PEG microsphere may be effective in the treatment of breast cancer. The possible interactions between components present in human milk and therapy of bioengineered melatonin as a strategy for the prevention and treatment of breast cancer are shown in figure 1.

VI. CONCLUSION

Major advances in cancer therapy have been occurring, and the study of the use of melatonin in cell culture or in vivo oncology has shown promise. The mechanisms of action of melatonin in reducing oxidative stress and the activation of apoptosis in cancer cells has put this hormone as a highlight in the adjuvant use of cancer treatment.

And considering that the breast tissue is in constant and direct contact with the soluble and cellular immune components in the milk, and the numerous immunological constituents of the breast milk, among these high concentrations of melatonin, macrophages, it is possible that interactions of these components, directly or modified release systems with factors present in tumor cells may be an alternative for tumor immunotherapy.

There is still much to study and develop to further increase the cure rates of cancer patients, as well as eradicate the occurrence of adverse reactions that both discomfort and incapacitate the patient, often leading to withdrawal of treatment. There is a need to improve the studies around melatonin as an immunomodulatory agent of colostrum phagocytes in the action against breast cancer cells, since these cells are present in large quantities, mainly in women who have breastfed, which can increase even more the chances of prevention against breast cancer.

REFERENCES

[1] Ghoncheh, M., Pournamdar, Z., & Salehiniya, H. (2016). Incidence and mortality and epidemiology of
breast cancer in the world. *Asian Pac J Cancer Prev*, 17(S3), 43-6.

[2] Tas, F., Hansel, H., Belce, A., Iván, S., Argón, A., Camlica, H., & Topuz, E. (2005). Oxidative stress in breast cancer. *Medical Oncology*, 22(1), 11.

[3] Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: how are they linked?. *Free Radical Biology and Medicine*, 49(11), 1603-1616.

[4] Gradim, C. V. C., Magalhães, M. C., Faria, M. D. C. F., & Arantes, C. I. S. (2011). Aleitamento materno como fator de proteção para o câncer de mama. Revista da Rede de Enfermagem do Nordeste, 12(2).

[5] Honorio-França, A. C., Carvalho, M. P. S. M., Isaac, L., Trabulsi, L. R., & Cameiro-Sampaio, M. M. S. (1997). Colostral mononuclear phagocytes are able to kill enteropathogenic *Escherichia coli* opsonized withcolostral IgA. *Scandinavian Journal of Immunology*, 46(1), 59-66.

[6] Honorio-França, A. C., Launay, P., Cameiro-Sampaio, M. M. S., Monteiro, R. C. (2001) Colostral neutrophils express IgA Fc receptors (CD89) lacking y chain association that mediate non-inflammatory properties of secretary IgA. *Journal of Leukocyte Biology*, 69(2), 289-296.

[7] França, E. L., Feliciano, N. D., Silva, K. A., Ferrari, C. K., & Honorio-França, A. C. (2009). Modulatory role of melatonin on superoxide release by spleen macrophages isolated from alloxan-induced diabetic rats. *Bratisl Lek Listy*, 110(9), 517-22.

[8] Morceli, G., Honorio-França, A. C., Fagundes, D. L., Calderon, I. M., & França, E. L. (2013). Antioxidant effect of melatonin on the functional activity of colostral phagocytes in diabetic women. *PLoS One*, 8(2), e56915.

[9] França-Botelho, A. C., França, J. L., Oliveira, F. M., França, E. L., Honório-França, A. C., Caliari, M. V., & Gomes, M. A. (2011). Melatonin reduces the severity of experimental amoebiasis. *Parasites & vectors*, 4(1), 62.

[10] Korkmaz, A., Topal, T., Tan, D. X., & Reiter, R. J. (2009). Role of melatonin in metabolic regulation. *Reviews in Endocrine and Metabolic Disorders*, 10(4), 261-270.

[11] França, E. L., Honorio-França, A. C., da Silva Fernandes, R. T., Marins, C. M. F., de Souza Pereira, C. C., & de Pilla Varotti, F. (2016). The effect of melatonin adsorbed to polyethylene glycol microspheres on the survival of MCF-7 cells. *Neurommunomodulation*, 23(4), 27-32.

[12] Honorio-França, A. C., Nunes, G. T., Fagundes, D. L. G., de Marchi, P. G. F., da Silva Fernandes, R. T., França, J. L., ... & França, E. L. (2016). Intracellular calcium is a target of modulation of apoptosis in MCF-7 cells in the presence of IgA adsorbed to polyethylene glycol. *OncoTargets and therapy*, 9, 617.

[13] Scherer, E. F., Honorio-França, A. C, Hara, C. P., Reinaque, A P.B., Côrtes, M. A., França, E.L. (2011). *Immunomodulatory effects of poly (ethylene glycol) microspheres adsorbed with nanofractions of Momordica charantia L on diabetic human blood phagocytes*. *Science of Advanced Materials*, 3(5), 687-694.

[14] Reinaque, A. P. B., França, E. L., Scherer, E. F., Côrtes, M. A., Souto, F. J. D., & Honorio-França, A. C. (2012). Natural material adsorbed onto a polymer to enhance immune function. Drug design, development and therapy, 6, 209.

[15] Fagundes, D.G., França, E.L., Hara, C.C.P., Honorio-França, A.C. (2012). *Immunomodulatory effects of poly (Etilene Glicol) Microspheres adsorbed with cortisol on activity of colostrum phagocytes*. *International Journal of Pharmacology*. 1(6) 510-518.

[16] Guimarães, P. C. L., Honorio-França, A. C., Hara, C. D. C. P., Fagundes, D. L. G., Ratto, S. H. B., & França, E. L. (2013). *Modulation of human colostrum phagocytic activity by the glycine-adsorbed polyethylene glycol microspheres*. *Journal of Chemistry*, 2013.

[17] Hara, C. D. C. P., Honorio-França, A. C., Fagundes, D. L. G., Guimarães, P. C. L., & França, E. L. (2013). *Melatonin nanoparticles adsorbed to polyethylene glycol microspheres as activators of human colostrum macrophages*. *Journal of Nanomaterials*, 2013.

[18] Jevščar, S., Kunstelj, M., & Porek, V. G. (2010). *PEGylation of therapeutic proteins*. *Biotechnology Journal: Healthcare Nutrition Technology*, 5(1), 113-128.

[19] Yu, D., Peng, P., Dharap, S. S., Wang, Y., Mehlig, M., Chandna, P., ... & Borchard, G. (2005). *Antitumor activity of poly (ethylene glycol)–camptothecin conjugate: The inhibition of tumor growth in vivo*. *Journal of Controlled Release*, 110(1), 90-102.

[20] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57-70.

[21] Nicholson, D. W. (2000). From bench to clinic with apoptosis-based therapeutic agents. *Nature*, 407(6805), 810.

[22] Wong, R. S. (2011). *Apoptosis in cancer: from pathogenesis to treatment*. *Journal of Experimental & Clinical Cancer Research*, 30(1), 87.

[23] INCA, 2018. Estimativa 2018: incidência de câncer no Brasil / Instituto Nacional de Cáncer José Alencar Gomes da Silva. Coordeniação de Prevenção e Vigilância. – Rio de Janeiro: INCA, 2018.
[24] Tao, Z., Shi, A., Lu, C., Song, T., Zhang, Z., & Zhao, J. (2015). Breast cancer: epidemiology and etiology. Cell biochemistry and biophysics, 72(2), 333-338.

[25] INCA, 2016. Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA). Ministério da Saúde. Estimativa 2016: incidência de câncer no Brasil.

[26] Richie, R. C., & Swanson, J. O. (2003). Breast cancer: a review of the literature. Journal of Insurance Medicine-New York Then Denver, 35(2), 85-101.

[27] Parton, M., Dowsett, M., & Smith, I. (2001). Studies of apoptosis in breast cancer. BMJ: British Medical Journal, 322(7301), 1528.

[28] Sharma, G. N., Dave, R., Sanadaya, J., Sharma, P., & Sharma, K. K. (2010). Various types and management of breast cancer: an overview. Journal of advanced pharmaceutical technology & research, 1(2), 109.

[29] DeNardo, D. G., & Coussens, L. M. (2007). Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Research, 9(4), 212.

[30] Macchetti, A. H., Marana, H. R. C., Silva, J. S., Andrade, J. M. D., Ribeiro-Silva, A., & Bighetti, S. (2006). Tumor-infiltrating CD4+ T lymphocytes in early breast cancer reflect lymph node involvement. Clinics, 61(3), 203-208.

[31] Soule, H. D., Vazquez, J., Long, A., Albert, S., & Brennan, M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. Journal of the National Cancer Institute, 51(5), 1409-1416.

[32] Comşa, Ş., Cimpean, A. M., & Raica, M. (2015). The story of MCF-7 breast cancer cell line: 40 years of experience in research. Anticancer research, 35(6), 3147-3154.

[33] Parodi, P. W. (2007). A role for milk proteins and their peptides in cancer prevention. Current pharmaceutical design, 13(8), 813-828.

[34] Klaunig, J. E., Xu, Y., Isenberg, J. S., Bachowski, S., Kolaja, K. L., Jiang, J., ... & Walborg Jr, E. F. (1998). The role of oxidative stress in chemical carcinogenesis. Environmental health perspectives, 106(Suppl 1), 289.

[35] Pinheiro, A. B., Lauter, D. S., Medeiros, G. C., Cardozo, I. R., Menezes, L. M., Souza, R. M. B. D., Abrahão, K.; Casado, L.; Bergman, A.; Thuler, L. C. (2013). Cancer de mama em mulheres jovens: análise de 12.689 casos. Rev. Bras. Cancerol. (Online), 59(3), 351-359.

[36] França-Botelho, A. D. C., Ferreira, M. C., Franca, J. L., Franca, E. L., & Honorio-Franca, A. C. (2012). Breastfeeding and its relationship with reduction of breast cancer: a review. Asian Pacific Journal of Cancer Prevention, 13(11), 5327-5332.

[37] França, E.L., França-Botelho, A.C., França, J.L., Ferrari, C.K., Honorio-Franca, A.C.(2013). Repercussions of Breastfeeding for Diabetes and Breast Cancer. Asian Pacific Journal of Cancer Prevention (14) 6233-6239.

[38] Davis, M. K. (2001). Breastfeeding and chronic disease in childhood and adolescence. Pediatric Clinics of North America, 48(1), 125-141.

[39] Kent, J. C. (2007). How breastfeeding works. The Journal of Midwifery & Women’s Health, 52(6), 564-570.

[40] Barnett, G. C., Shah, M., Redman, K., Easton, D. F., Ponder, B. A., & Pharoah, P. D. (2008). Risk factors for the incidence of breast cancer: do they affect survival from the disease? Journal of Clinical Oncology, 26(20), 3310-3316.

[41] Alsaker, M. D., Opdahl, S., Åsvold, B. O., Romundstad, P. R., & Vatten, L. J. (2011). The association of reproductive factors and breastfeeding with long term survival from breast cancer. Breast cancer research and treatment, 130(1), 175-182.

[42] Russo, J., Moral, R., Balogh, G. A., Mailo, D., & Russo, I. H. (2005). The protective role of pregnancy in breast cancer. Breast Cancer Research, 7(3), 131.

[43] Russo, J., Balogh, G. A., & Russo, I. H. (2008). Full-term pregnancy induces a specific genomic signature in the human breast. Cancer Epidemiology and Prevention Biomarkers, 17(1), 51-66.

[44] Yang, L., & Jacobsen, K. H. (2008). A systematic review of the association between breastfeeding and breast cancer. Journal of women’s health, 17(10), 1635-1645.

[45] Ip, S., Chung, M., Raman, G., Chew, P., Magula, N., Trikalinos, T., & Lau, J. (2007). Breastfeeding and maternal and infant health outcomes in developed countries. Evid Technol Asses (Full Rep), 153(153), 1-186.

[46] Lipworth, L., Bailey, L. R., & Trichopoulos, D. (2000). History of breast-feeding in relation to breast cancer risk: a review of the epidemiologic literature. Journal of the National Cancer Institute, 92(4), 302-312.

[47] Romieu, I., Hernandez-Avila, M., Lazcano, E., Lopez, L., & Romero-Jaime, R. (1996). Breast cancer and lactation history in Mexican women. American journal of epidemiology, 143(6), 543-552.

[48] Goldman, A. S. (2002). Evolution of the mammary gland defense system and the ontology of the immune system. Journal of mammary gland biology and neoplasia, 7(3), 277-289.

[49] Islam, S. N., Ahmed, L., Khan, M. N. I., Huque, S., Begum, A., & Yunus, A. B. M. (2006). Immune components (IgA, IgM, IgG, immune cells) of...
colostrum of Bangladeshi mothers. Pediatrics international, 48(6), 543-548.
[50] Brandtzæg, P. (2010). The mucosal immune system and its integration with the mammary glands. The Journal of pediatrics, 156(2), S8-S15.
[51] Meki, A. R. M., Saleem, T. H., Al-Ghazali, M. H., & Sayed, A. A. (2003). Interleukins-6,-8 and-10 and tumor necrosis factor-alpha and its soluble receptorI in human milk at different periods of lactation. Nutrition research, 23(7), 845-855.
[52] Lönnerdal, B. (2003). Nutritional and physiologic significance of human milk proteins. The American journal of clinical nutrition, 77(6), 1537S-1543S.
[53] Kverkova, I., Cinova, J., Tuckova, L., & Tkaskalova-Hogenova, H. (2007). Cytokine profiling in human colostrum and milk by protein array. Clinical chemistry, 53(5), 955-962.
[54] Gurofalo, R. (2010). Cytokines in human milk. The Journal of pediatrics, 156(2), S36-S40.
[55] Honorio-França, A.C., Launay, P., Carneiro-Sampaio, M.M., Monteiro, R.C. (2001). Colostral neutrophils express Fc alpha receptors (CD89) lacking gamma chain association and mediate noninflammatory properties of secretory IgA. Journal of Leukocyte Biology, 69(2):289-96.
[56] Monteiro, R. C., & Van De Winkel, J. G. (2003). IgA Fc receptors. Annual review of immunology, 21(1), 177-204.
[57] Newburg, D. S. (2005). Innate immunity and human milk. The Journal of nutrition, 135(5), 1308-1312.
[58] Friel, J. K., Tsopmo, A., Diehl-Jones, B., & Aluko, R. (2008). Antioxidant Properties of Human Milk Fractions. The journal of the Federation of American societies for Experimental biology, 22(1), 446, 2008.
[59] Lönnerdal, B. (2000). Breast milk: a truly functional food. Nutrition, 16(7/8), 509-511.
[60] Miralles, O., Sánchez, J., Palou, A., & Picó, C. (2006). A physiological role of breast milk leptin in body weight control in developing infants. Obesity, 14(8), 1371-1377.
[61] García-Mauriño, S., Pozo, D., Carrillo-Vito, A., Calvo, J.R., Guerrero, J. M.(1999) Melatonin activates Th1 lymphocytes by increasing IL-12 production. Life sciences, 65(20), 2143-2150.
[62] Pontes, G. N., Cardoso, E. C., Carneiro-Sampaio, M. M., & Markus, R. P. (2006). Injury switches melatonin production source from endocrine (pinea) to paracrine (phagocytes)–melatonin in human colostrum and colostrum phagocytes. Journal of pineal research, 41(2), 136-141.
[63] Pontes, G. N., Cardoso, E. C., Carneiro-Sampaio, M. M., & Markus, R. P. (2007). Pineal melatonin and the innate immune response: the TNF-α increase after cesarean section suppresses nocturnal melatonin production. Journal of pineal research, 43(4), 365-371.
[64] Pires-Lapa, M. A., Tamara, E. K., Salustiano, E. M., & Markus, R. P. (2013). Melatonin synthesis in human colostrum mononuclear cells enhances dectin-1-mediated phagocytosis by mononuclear cells. Journal of pineal research, 55(3), 240-246.
[65] Claustrat, B., Brun, J., & Chazot, G. (2005). The basic physiology and pathophysiology of melatonin. Sleep medicine reviews, 9(1), 11-24.
[66] Reiter, R. J., Tan, D. X., Korkmaz, A., & Rosales-Corral, S. A. (2013). Melatonin and stable circadian rhythms optimize maternal, placental and fetal physiology. Human reproduction update, 20(2), 293-307.
[67] Reiter, R. J., Tan, D. X., & Galano, A. (2014). Melatonin: exceeding expectations. Physiology, 29(5), 325-333.
[68] Honorio-Franca, A. C., Hara, C. C. P., Ormonde, J. V. S., Nunes, G. T., & Franca, E. L. (2013). Human colostrum melatonin exhibits a day-night variation and modulates the activity of colostral phagocytes. Journal of Applied Biomedicine, 11(3), 153-162.
[69] Carrillo-Vico, A., Calvo, J. R., Abreu, P., Lardone, P. J., García-Mauriño, S., Reiter, R. J., & Guerrero, J. M. (2004). Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. The FASEB Journal, 18(3), 537-539.
[70] Reiter, R. J., Tan, D. X., & Galano, A. (2014). Melatonin: exceeding expectations. Physiology, 29(5), 325-333.
[71] Tan, D. X., Manchester, L. C., Terron, M. P., Flores, L. J., & Reiter, R. J. (2007). One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species?. Journal of pineal research, 42(1), 28-42.
[72] Maldonado, M. D., García-Moreno, H., González-Yanes, C., & Calvo, J. R. (2016). Possible involvement of the inhibition of NF-kB factor in anti-inflammatory actions that melatonin exerts on mast cells. Journal of cellular biochemistry, 117(8), 1926-1933.
[73] Vijayalaxmi, Reiter, R. J., Tan, D. X., Heeman, T. S., & Thomas Jr, C. R. (2004). Melatonin as a radioprotective agent: a review. International Journal of Radiation Oncology* Biology* Physics, 59(3), 639-653.
[74] Cos, S., Fernández, R., Guézmes, A., & Sánchez-Barceló, E. J. (1998). Influence of melatonin on invasive and metastatic properties of MCF-7 human
breast cancer cells. Cancer research, 58(19), 4383-4390.

[75] Cos, S., Mediavilla, M. D., Fernández, R., González-Lamuño, D., & Sánchez-Barceló, E. J. (2002). Does melatonin induce apoptosis in MF-7 human breast cancer cells in vitro? Journal of pineal research, 32(2), 90-96.

[76] Rögelsperger, O., Ekmeckioğlu, C., Jäger, W., Klimpfinger, M., Königsberg, R., Krenbek, D., ... & Thallhammer, T. (2009). Coexpression of the melatonin receptor 1 and nestin in human breast cancer specimens. Journal of pineal research, 46(4), 422-432.

[77] Hill, S. M., Frasch, T., Xiang, S., Yuan, L., Duplessis, T., & Mao, L. (2009). Molecular mechanisms of melatonin anticancer effects. Integrative cancer therapies, 8(4), 337-346.

[78] VERMA, R. K., GARG, S. (2001). Current status of drug delivery technologies and future directions. Pharmaceutical Technology, 25(2),14-16.

[79] Gil, E. C., Colarte, A. I., Bataille, B., Pedraz, J. L., Rodríguez, F., & Heinämäki, J. (2006). Development and optimization of a novel sustained-release dextran tablet formulation for propanolol hydrochloride. International journal of pharmaceutics, 317(1), 32-39.

[80] Batista, C. M., de Carvalho, C. M. B., & Magalhães, N. S. S. (2007). Lipossomas e suas aplicações terapêuticas: Estado da arte. Revista Brasileira de Ciências Farmacêuticas, 43(2), 167-179.

[81] Kreuter, J. (2007). Nanoparticles- a historical perspective. International Journal of Pharmaceutics, 331(1), 1-10.

[82] Grabovac, V., Föger, F., & Bernkop-Schnürch, A. (2008). Design and in vivo evaluation of a patch delivery system for insulin based on thiolated polymers. International journal of pharmaceutics, 348(1-2), 169-174.

[83] Hemandes, M. R. G., Moraes, L. C. A., Ribeiro, E. B., Fagundes, D. L. G., Honorio-França, A. C., & França, E. L. (2017). In vitro immunomodulatory effects of microemulsions with levamisole delivery systems on blood phagocytes interacting with Giardia lamblia. Parasitology international, 66(3), 299-304.

[84] Alagusundaram, K.; chetty, A.M.S.; Umushankari, A.V.; Lavanya, C.; Ramkanth, S. (2009) Microspheres as a novel drug delivery system – A review. International Journal of ChemTech Research, 1(3) 526-534.

[85] Greenwald, R. B., Choe, Y. H., McGuire, J., & Conover, C. D. (2003). Effective drug delivery by PEGylated drug conjugates. Advanced drug delivery reviews, 55(2), 217-250.

[86] Park, J., Ye, M., & Park, K. (2005). Biodegradable polymers for microencapsulation of drugs. Molecules, 10(1), 146-161.

[87] Yu, D., Peng, P., Dharap, S. S., Wang, Y., Mehlig, M., Chandra, P., ... & Borchard, G. (2005). Antitumor activity of poly (ethylene glycol)–camptothecin conjugate: The inhibition of tumor growth in vivo. Journal of Controlled Release, 110(1), 90-102.

[88] Veronese, F. M., & Pasut, G. (2005). PEGylation, successful approach to drug delivery. Drug discovery today, 10(21), 1451-1458.

[89] Salnaso, S., Semenzato, A., Bersanía, S., Chinol, M., Paganelli, G., & Caliceti, P. (2005). Preparation and characterization of active site protected poly (ethylene glycol) - avidin bioconjugates. Biochimica et Biophysica Acta (BBA)-General Subjects, 1726(1), 57-66.

[90] Heyes, J., Hall, K., Tailor, V., Lenz, R., & MacLachlan, I. (2006). Synthesis and characterization of novel poly (ethylene glycol)-lipid conjugates suitable for use in drug delivery. Journal of Controlled Release, 112(2), 280-290.

[91] Rodrigues, P. C., Roth, T., Fiebig, H. H., Unger, C., Mülhaupt, R., & Kratz, F. (2006). Correlation of the acid-sensitivity of polyethylene glycol daunorubicin conjugates with their in vitro antiproliferative activity. Bioorganic & medicinal chemistry, 14(12), 4110-4117.

[92] Scott, E. A., Nichols, M. D., Kuntz-Willits, R., & Elbert, D. L. (2010). Modular scaffolds assembled around living cells using poly (ethylene glycol) microspheres with macroporation via a non-cytotoxic porogen. Acta biomaterialia, 6(1), 29-38.

[93] França, E. L., Ribeiro, E. B., Scherer, E. F., Cantarini, D. G., Pessôa, R. S., França, F. L., & Honorio-França, A. C. (2014). Effects of Momordica charantia L. on the blood rheological properties in diabetic patients. BioMed Research International, 2014.

[94] Ribeiro, A. A., Deluque, A. L., Fagundes, D. L. G., Franca, E. L., & Honorio-Franca, A. C. (2018). Herbal Mixture Adsorbed to Polyethylene Glycol Microspheres Induces Apoptotic Effects on Breast Cancer Cells. Current drug delivery, 15(2), 227-234.

[95] Silva, F. H., Ribeiro, A. A. L., Deluque, A. L., Cotrim, A. C. D. M., de Marchi, P. G. F., França, E. L., & Honorio-França, A. C. (2018). Effects of barium chloride adsorbed to polyethylene glycol (PEG) microspheres on co-culture of human blood mononuclear cell and breast cancer cell lines (MCF-7). Immunopharmacology and immunotoxicology, 40(1), 18-24.
[96] Van Egmond, M., van Spriel, A. B., Vermeulen, H., Huls, G., van Garderen, E., & van de Winkel, J. G. (2001). Enhancement of polymorphonuclear cell-mediated tumor cell killing on simultaneous engagement of FcγRI (CD64) and FcαRI (CD89). Cancer research, 61(10), 4055-4060.

[97] Truong, K. K., Lam, M. T., Grandner, M. A., Sassoon, C. S., & Malhotra, A. (2016). Timing matters: circadian rhythm in sepsis, obstructive lung disease, obstructive sleep apnea, and cancer. Annals of the American Thoracic Society, 13(7), 1144-1154.

[98] Tammi, I., Schriever, F., & Dörken, B. (2001). Apoptosis: implications of basic research for clinical oncology. The lancet oncology, 2(1), 33-42.

[99] Parton, M., Dowsett, M., & Smith, I. (2001). Studies of apoptosis in breast cancer. BMJ: British Medical Journal, 322(7301), 1528.

[100] Bizzarri, M., Proietti, S., Cucina, A., & Reiter, R. J. (2013). Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: a review. Expert opinion on therapeutic targets, 17(12), 1483-1496.