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Review Article

MicroRNA as a new bioactive component in breast milk

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ABSTRACT

Breast milk is a complex and multifaceted fluid that plays a critical role in the development of infants. It is composed of water, carbohydrates, fats, proteins, vitamins, and minerals, as well as numerous bioactive compounds such as hormones, oligosaccharides, and immune proteins. Additionally, breast milk contains microRNAs, which have been found to regulate gene expression and impact various aspects of infant development. This text provides an overview of the components of human breast milk and their importance in infant development, with a focus on microRNAs.

MicroRNAs are short RNA sequences that regulate gene expression posttranscriptionally, and they play an important role in shaping the mechanisms of immunity, protecting against oxidative stress, and promoting thermogenesis. The composition of breast milk can vary in the same mother between different feedings, as it changes in response to various factors such as the infant's age, feeding frequency and duration, time of day, and maternal health status. Despite the variations in breast milk composition, it still provides complete nutrition for the infant. The unique microRNA profiles in breast milk and how they are affected by various factors can have significant implications for disease prevention and treatment. Further research is needed to better understand the functions of individual microRNA molecules and their potential therapeutic applications.

1. Introduction

For years, breastfeeding has been considered the best way to provide ideal nutrition for the health and development of infants. According to the guidelines of the World Health Organization (WHO) [1], the American Academy of Pediatrics (AAP) [2] and the European Society of Gastroenterology, Hepatology and Pediatric Nutrition (ESPGHAN) [3], exclusive breastfeeding is the recommended method of feeding infants at least until 6 months of age, and continued breastfeeding with complementary foods is recommended until age 2 or beyond. The composition of milk has been the subject of many studies, which allowed to gain extensive knowledge about the components of human milk and their importance.

Human breast milk (HMB) contains 87–88% water, 7% carbohydrates, 3.8% fats and 1% protein. It also contains macronutrients and micronutrients such as vitamins (A, E and K, C, B2, B3 and B5), minerals (sodium, potassium, chloride, calcium, iron, zinc, copper, magnesium and selenium) [4]. Hormones (insulin, estrogen, androgens, prolactin, gastrin, leptin, adiponectin, gonadotropin-releasing hormone (GnRH), progesterone, resistin and ghrelin) were also detected [4,5]. In addition, HBM contains oligosaccharides (bifidus factor) that stimulate the growth of over 200 desirable bacterial strains. Probiotic bacteria, including Bacteroides spp., which play an important role in the early stages of neonatal intestinal colonization by fermenting lactic acid bacteria, and Bifidobacterium spp., which support intestinal barrier function and modulate the immune system response are the most important bacteria for infant development [4].

More attention has been paid to the fact that, in addition to its nutritional components, HBM is particularly rich in immunological components. Representative immune proteins include α-lactalbumin, lactoferrin, lysozyme and secretory immunoglobulin A (sIgA) [6]. sIgA accounts for approximately 80–90% of all immunoglobulins in HBM. Only about 10% is absorbed through the intestines and transferred to the bloodstream, while the remaining play a leading role in the local immunity acquired from the mother through the mammary-enternal pathway. sIgA molecules additionally affect the binding of pathogens such as *Escherichia coli*, *Vibrio cholerae*, Campylobacter, Haemophilus influenzae, rotavirus, cytomegalovirus and Candida albicans. Lactoferrin found in HBM is an antimicrobial compound with high affinity for iron. It has a bacteriostatic effect in relation to pathogens requiring iron and has a bactericidal effect in relation to some pathogens. In addition, lactoferrin affects the production and expression of various cytokines

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that affect the immune system.

Lysozyme, through its synergistic action with lactoferrin, inhibits the proliferation of pathogenic bacteria (especially Gram-negative bacteria) [7]. α-Lactalbumin is essential for lactose biosynthesis and facilitates the absorption of trace elements and minerals such as calcium and zinc [6]. In 2007, the presence of microRNAs in HBM was first described [8]. Since then, the importance of microRNAs in HBM has been the subject of many studies.

2. Characteristics of microRNAs

MicroRNAs (also called miRNAs or miRs) are short, non-coding RNA sequences (approximately of 22 nucleotides) that act as post-transcriptional regulators of gene expression. The first stage of microRNA biogenesis is transcription initiated by the enzyme RNA polymerase II, producing precursor microRNAs (primary microRNA or pri-microRNA) [9]. These molecules are then modified by various RNAse III family proteins. In the nucleus, an enzyme complex called Drosha-DGCR8 (DiGeorge syndrome critical region gene 8 protein) processes the pri-microRNA to form a shorter molecule called pre-microRNA. Then, the pre-microRNA is transferred to the cytoplasm, where the Dicer enzyme is responsible for the formation of mature, double-stranded microRNAs. The mature microRNA, which is bound to the Argonaute protein (AGO), binds to the RNA-induced silencing complex (RISC) where a single strand is inhibited [10].

Post-transcriptional regulation of gene expression is possible due to the complementarity of base pairs with messenger RNA molecules. Gene silencing can take place through degradation of a specific mRNA or as a result of inhibition of transcript translation. MicroRNAs are attached to the 3′ untranslated region (3′UTR) of the target mRNA. If there is complete complementarity, the Ago2 protein can cleave the mRNA molecule leading to its direct degradation. In the case of incomplete complementarity, silencing is done by translation blocking. After the degradation of a specific transcript, the microRNA is not damaged and can regulate the translation of subsequent genes [11].

HBM is one of the richest sources of microRNAs in comparison to other human bodily fluids [12]. MicroRNAs are present in each milk fraction, namely in skimmed, lipid and cellular fraction. However, studies have shown that the cellular and lipid fractions contain a significantly higher amount of microRNAs compared to the skimmed fraction [10]. The MicroRNAs cargo are in small transport extracellular vesicles, called exosomes, which are involved in intercellular communication. The lipid bilayer membrane of exosomes protects microRNAs cargo against degradation by low pH, RNases and digestion and enables their transport to various tissues in the body [13].

3. Effect of physical and chemical factors on microRNAs in human milk

An important aspect in terms of the quality of further research and the use of microRNA in medicine is the sensitivity of the molecules to various chemical and physical conditions.

Leiferman et al. [14] compared the composition of HBM stored at 4 °C for 4 weeks. They observed a gradual reduction in the number of exosome-sized vesicles to 49% ± 13% compared to fresh milk samples. Wang et al. [15] performed the analysis of exosomes and microRNAs in previously frozen milk in −80 °C. The number of exosomes obtained was about two orders of magnitude lower than in fresh HBM. Both microRNA-30d-5p and microRNA-125a-5p were detectable in the exosomes of milk collected in the early stage of hospitalization and decreased to below the detection limit in mid- and late-hospitalization. microRNA-423-5p was not detectable at any stage.

The effects of Holder Pasteurization (HoP) and High Pressure Processing (HPP) methods on microRNAs in HBM were studied [16]. Milk samples were subjected to HoP, HPP or left unpasteurized as controls. HPP resulted in a statistically insignificant decrease in the number of microRNA reads compared to unprocessed material, while HoP led to an 82-fold decrease in total material ($p = 0.0288$) and a 302-fold decrease in exosomes ($p = 0.0021$). Changes in microRNA fraction composition before and after HPP indicated uneven stability of individual molecules under high pressure conditions, with microRNA-30d-5p identified as relatively stable and the microRNA-29 family as sensitive to HPP.

Milk exosomes have also been shown to protect the microRNAs within them from enzymes, chemicals or mechanical degradation. Under acidic conditions that mimic gastric and pancreatic digestion, milk exosomes prevent the degradation of sensitive microRNAs [17,18]. Indirect evidence based on numerous microRNA stability studies, both in vitro and ex vivo, confirm the uptake and functionality of microRNAs in the body of the infant. Intestinal permeability, which is increased in the postpartum period and in inflammatory bowel conditions, may promote intestinal uptake of microRNA-enriched milk exosomes [19].

Liao et al. [20] conducted a study of simulated gastro-pancreatic digestion. They confirmed that exosomes enter human intestinal crypt-like cells (HIECs). They identified 288 mature microRNAs. A large percentage of microRNAs were associated with synapse development and immunity. The most abundant was hsa-microRNA-22-3p. After digestion, the overall abundance of microRNAs in human milk exosomes was stable. They demonstrated the resistance of microRNAs contained in human milk exosomes to digestive processes in the stomach. Therefore, it is possible that milk exosomes are absorbed through the intestinal barrier into the infant's bloodstream. The possibility of vertical transmission of microRNA signaling from milk through the digestive tract of the newborn has recently been confirmed by Weil et al. [21]. Milk-derived microRNAs survived the passage through the digestive tracts of human and porcine neonates. Bovine-specific molecules were accumulated in intestinal epithelial cells of preterm infants after enteral feeding with colostrum/bovine formula. In piglets, colostrum supplementation with cel-microRNA-39-5p/-3p resulted in increased levels of cel-microRNA-39-3p and "argonaute RISC catalytic component 2 (AGO2)" in blood.

4. The importance of milk exosomal microRNAs for infant growth and development

The functions of individual microRNA molecules and their importance for the growth and development of infants are still the subject of many studies.

4.1. Immune system

The so-called immunological microRNA molecules, i.e. microRNA-148a-3p, microRNA-181a-5p, microRNA-182-5p, microRNA-16-5p, and microRNA-99b-5p, take part in the regulation of the processes of maturation and differentiation of B and T lymphocytes [12]. In the process of maturation, B lymphocytes undergo processes of positive and negative selection. Therefore, they are able to produce high-affinity antibodies, which are necessary for the removal of pathogens. MicroRNA-155 is crucial in the regulation of B lymphocyte maturation. Nakagawa et al. [22] demonstrated that microRNA-155 is co-expressed with the c-MYC proto-oncogene in positively selected B cells. Functionally, microRNA-155 protected these cells from apoptosis, allowing clonal population proliferation, providing an explanation why microRNA-155 deletion impairs affinity maturation and promotes premature germinal center lysis. MicroRNA-155 directly inhibits the Jumonji family member JARID2, which increases B cell apoptosis.

During breastfeeding, HBM microRNAs support B-cell proliferation through epigenetic upregulation of BCL6 (by inhibition of DNA methyltransferase 1 (DNMT1)) via microRNA-148a-3p and microRNA-155-5p/microRNA-29b-5p. After weaning with physiological termination of microRNA signaling, infant BCL6 expression and B-cell proliferation decline, while BLIMP1-mediated B-cell maturation increases, to ensure adequate self-antibody production. Epidemiological evidence supports

an association between the consumption of cow's milk and the risk of diffuse large B-cell lymphoma (DLBCL), which is the most common non-Hodgkin's lymphoma in the world [23]. Upregulation of PI3K-AKT-mTORC1 signaling is a common feature of DLBCL. Increased BCL6 gene expression and suppression of maturation protein induced by B1 cells (BLIMP1)/PR1 domain containing protein (PRDM1) are the key pathological abnormalities in DLBCL. Since human and bovine microRNAs have identical nucleotide sequences, consumption of pasteurized cow's milk in adults can de-differentiate B cells, which have an increased BLC6/BLIMP1 expression ratio.

4.2. Prevention of atopic and autoimmune diseases

The influence of microRNAs in HBM on the prevalence of atopy in infants is still under investigation. Regulatory T cells (Tregs) play an important role in the control of autoimmunity and immune tolerance. Milk microRNAs may promote a two-step selection process converting self-reactive thymocytes into stable Treg regulatory cells, thereby lowering the level of atopy. MicroRNA-155 molecules control key genes involved in their regulation, including the expression of FoxP3, a main regulator of Treg cell development and function. MicroRNA-155 mediates the suppression of SOCS1, thereby upregulating the expression of FoxP3 [24]. Hypermethylation of the FoxP3 gene is associated with reduced Treg function and the development of allergy. Conversely, consumption of farm milk is associated with higher FoxP3 demethylation and higher Treg counts [25]. The microRNA-155 molecule controls key genes involved in regulating FoxP3 expression, IL-4 signaling, immunoglobulin class switching to IgE and FcεRI expression. MicroRNA-148a demethylates FoxP3 by directly lowering the expression of DNA methyltransferases (DNMT1 and DNMT3b), while microRNA-21 indirectly inhibits DNMT1 expression by targeting microRNAs. MicroRNA-29 targets DNMT3a and DNMT3b. Interestingly, the nucleotide sequences of microRNA-148a-3p, microRNA-29b and microRNA-21 of *Homo sapiens* and *Bos taurus* are identical [26] Hicks et al. [27] have recently shown in their work that infants who did not develop atopy, consumed higher concentrations of microRNA-375-3p and microRNA-148b-3p. Recent evidence also indicates that the concentrations of microRNA-148a-3p and let-7d-3p in colostrum correlated with the frequencies of activated Treg cells at 24 months, similarly to microRNA-181a-3p and microRNA-181c-3p in mature milk [28].

It is suggested that microRNAs found in milk exosomes have a protective effect on the occurrence of autoimmune diseases. Stremmel et al. [29] studied the effect of cow's milk exosomes on the occurrence of ulcerative colitis in a mouse genetic model. Milk exosomes prevented the appearance of the severe phenotype of ulcerative colitis. The macroscopic colitis score decreased from a mean of 3.33 in untreated mice to 0.75 index points ($p < 0.01$) in exosome-treated mice, which included a significant improvement in sub-scores of fecal improvement and colon weight and length.

4.3. Gastrointestinal maturation and prevention of necrotizing enterocolitis

The intestinal epithelium is an important connection between commensal microorganisms and the body. Epithelial damage and impaired barrier function resulting from the inflammatory response are common pathological features of gastrointestinal diseases, including necrotizing enterocolitis (NEC). Endothelial cells not only absorb nutrients, but also absorb exosomes containing immunomodulatory molecules, including microRNAs [30]. MicroRNA-transporting exosomes in HBM have a beneficial effect in preventing NEC by reducing inflammation and restoring tightness of the intestinal epithelium. Guo et al. [31] conducted a study on a mouse model of NEC and showed that treatment with HBM exosomes had a significant protective effect in NEC mice, both suppressing inflammation and improving intercellular tight junctions. The microRNA-148a-3p/p53/SIRT1 axis has a significant protective effect on NEC. MicroRNA-148a-3p directly targets TP53 in its 3′ UTR. MicroRNA-148a-3p mimic treatment significantly reduces p53 expression and increases sirtuin 1 (SIRT1) levels in intestinal epithelial cells. In addition, reduced nuclear translocation of nuclear factor-κB (NF-κB) and cell apoptosis were observed. In vivo delivery of agomir microRNA-148a-3p plays a similar protective role on NEC, which is accompanied by changes in p53 and SIRT1. Abrogation of agomir microRNA-148a-3p protection against NEC was observed in Sirt1-deficient (Sirt1+/−) mice.

MicroRNA-182-5p also promoted T cell-mediated immune responses. Another microRNA identified is microRNA-22-3p, which has been confirmed to inhibit the activity of NF-κB, a key inflammatory signaling molecule that can cause NEC [32].

Martin et al. [33] conducted a study, in which purified exosomes from HBM were added to intestinal epithelial cells. The epithelial cells were then treated with different concentrations of H_2O_2. Oxidative stress with H_2O_2 resulted in a 50% decrease in cell viability, and HBM-derived exosomes had a statistically significant protective effect.

A similar study was also conducted by Dong et al. [34], in which intestinal stem cells (ISCs) would be protected by HBM-derived exosomes from oxidative stress-induced damage in vitro, possibly mediated by the Wnt/β-catenin signaling pathway. This indicates that oral administration of HBM-derived exosomes may be a promising agent for the treatment or prevention of NEC.

4.4. Effect on carcinogenesis

MicroRNAs reach the systemic circulation and can influence the epigenetic programming of various organs. They control the expression of DNMT1 involved in the regulation of insulin secretion, insulin-like growth factor-1, α-synuclein and forkhead box P3. MicroRNA-148a and microRNA-30b molecules can stimulate the expression of uncoupling protein 1, a key thermogenesis inducer that transforms brown adipose tissue (BAT) [35].

Research is being conducted on the effect of milk exosomal microRNA on the inhibition of the proliferation of various cancer cells in vitro and in vivo. Suppression of p53 enhances proliferation in the first stages of life, epigenetic doping is restricted to the postnatal breastfeeding period. Continued intake of bovine milk exosomes is a matter of concern because sustained proliferation enhances the risk of cancer [36]. MicroRNAs found in milk, especially microRNA-125b, directly target TP53. TP53 regulates the expression of key genes involved in cell homeostasis such as FOXO1, PTEN, SESN1, SESN2, AR, IGF1R, BAK1, BIRC5 and TNFSF10 [37]. The nuclear interaction of p53 with DNMT1 controls gene silencing. The key molecule microRNA-148a directly targets DNMT1. Reduced DNMT1 expression further impairs the activity of histone deacetylase 1 (HDAC1) involved in regulating chromatin structure and transcription access. The presented microRNA-p53-DNMT1 pathway provides a new explanation for the epidemiological link between milk consumption and acne vulgaris and prostate cancer. Constant consumption of pasteurized cow's milk, due to the similarity or even identity of bovine microRNAs and human microRNAs in cow's milk and HBM, attenuates p53 and DNMT1 signaling and may be a risk factor for acne vulgaris, prostate cancer and other p53/DNMT1-related diseases [38]. Therefore, bovine milk bioactive microRNAs should be eliminated from the human food chain.

Milk exosomes can be used as carriers for oral administration of chemotherapeutic agents. By modifying exosomes with a tumor-targeting ligand, it is possible to deliver drugs to tumor sites [39]. In their study Manca et al. [13] evaluated the bioavailability and distribution of exosomes and their microRNA cargoes from cow, porcine and mouse milk within species and across species. Different types of microRNAs were shown to have unique distribution profiles and accumulated in the intestinal mucosa, spleen, liver, heart or brain. Nevertheless, it does raise the hypothesis that milk exosomes loaded with special anticancer agents or antisense microRNAs may be helpful in

reaching tumor tissues such as the brain, as milk exosomes are able to cross the blood-brain barrier and accumulate in brain tissues.

4.5. Milk exosomes and the gut microbiome

Human milk contains its own unique microbiome, including beneficial, commensal and potentially probiotic bacteria. The HBM microbiome contributes to the colonization of the infant's gut. Studies have shown that certain strains of species such as Lactobacillus and Bifidobacterium are present in maternal HBM and infant feaces, confirming that HBM contributes to the vertical transmission of commensal bacteria [40]. There is evidence that milk exosomes are a food source for gut bacteria. The addition of milk-derived exosomes promotes the growth of *Escherichia coli* K-12 MG1655 and Lactobacillus plantarum WCFS1. These are strains of probiotic bacteria beneficial to human health [41]. The study of Zhou et al. [42] showed that the gut microbiome facilitates cell-to-cell communication via milk exosomes, crossing species boundaries. Milk exosomes facilitate communication between the animal and bacterial kingdoms. The effect of cow's milk exosomes on the growth and probiotic properties of Bifidobacterium animalis F1-3-2 was assessed. The results showed that cow's milk exosomes promoted the growth of B. Animalis F1-3-2, improved the gastrointestinal tolerance of B. Animalis F1-3-2, such as resistance to acids and bile salts. Besides, the adhesion of B. Animalis F1-3-2 was also promoted. In addition, cow's milk exosomes induced better anti-inflammatory properties of B. Animalis F1-3-2, the level of inflammatory cytokines such as TNF-α, IL-1β [43]. There is growing awareness that the gut plays an important role in many inflammatory diseases. Increased leaky gut, gut microbiome dysbiosis and intestinal inflammation are not only associated with gut diseases such as colitis and Crohn's disease, but also characteristic of systemic inflammatory diseases such as lupus, multiple sclerosis and rheumatoid arthritis (RA). Exosomes found in milk have been shown to help improve intestinal barrier function and disrupt the gut-articular axis in RA. Gut microbiota dysbiosis induces an inflammatory response and is associated with the progression of RA disease [44].

4.5.1. The influence of factors on the microRNA profile in breast milk

4.5.1.1. Lactation period. Alsaweed et al. [45] tested milk samples obtained from 10 lactating women at 2, 4 and 6 months after delivery, identifying 1195 known mature microRNAs. Similarity of microRNA profile and concentration was demonstrated between the three lactation stages examined. However, about a third were differentially expressed during the first 6 months of lactation, with more pronounced microRNA upregulation seen at the fourth month. These findings indicate that although total microRNA concentrations do not change in the first 6 months of lactation, microRNA composition is altered, particularly at 4 months compared to 2 months of breastfeeding, suggesting adaptation to the needs of the infant.

4.5.1.2. Pre-term birth. In the milk of mothers who gave birth prematurely, an altered hormonal profile and microRNA were observed. Decreased levels of prolactin, estrogen and progesterone have been detected, which may permanently affect microRNAs in the milk of women giving birth prematurely, and thus increase the potential evolutionary benefits for the premature infant, such as effects on glucose homeostasis, regulation of adipogenesis and B-cell proliferation [4].

Carney et al. [46] examined milk samples from mothers of premature infants (pMBM) obtained 3–4 weeks after delivery and from mothers of newborns obtained at birth and 3–4 weeks after delivery at term (tMBM). The expression profiles of nine microRNAs, related to elemental metabolism and lipid biosynthesis, in lipid and skimmed pMBM differed from those in tMBM. The colostrum microRNA profile was closely related to tMBM. This suggests that milk from mothers giving birth prematurely may play an adaptive role in the growth and development of premature infants. The molecule microRNA-148a-3p, which plays a key role in the milk of mothers of premature infants, is associated with reducing the inflammatory response of heart tissue and also plays a role in the differentiation of embryonic cells. It also inhibits cytokine production, including IL-12, IL-6, TNF-α and IFN-β, upregulation of MHC class II expression and DC-initiated Ag-specific T cell proliferation by targeting CaMKIIα. In addition, microRNA-148a may increase food and energy intake, which is critical for the survival of premature infants. The direct target of microRNA-148a is the hypothalamic cholecystokinin receptor 2 (CCK2R, or CCKBR), which interacts with the opioid, dopaminergic and melanocortin systems to regulate food intake. These lines of evidence support that milk-derived exosomal microRNA-148a can promote hyperphagia and energy intake by silencing negative feedback signaling mediated by CCK-CCK2R [47].

4.5.1.3. Caesarean section. The milk of mothers who gave birth by caesarean section shows altered expression of microRNA molecules. Increased levels of exogenous oxytocin during vaginal delivery have been shown to be responsible for the increased levels of microRNA-148a and microRNA-30 in human colostrum and the decreased levels of microRNA-320 [48]. Notably, microRNA-320 was highly expressed compared to microRNA-148a in the colostrum of mothers who did not receive exogenous oxytocin. In breast cancer cells and pancreatic tissue from diabetic mice, microRNA-320 was shown to attenuate PI3K/AKT/ELF3 signaling. E74-like factor 3 (ELF3) is a direct target of microRNA-320 and it has been shown that silencing of ELF3 promotes β-cell apoptosis. Thus, it is conceivable that cesarean delivery disrupts the microRNA-148a/microRNA-320 signaling balance, which may increase the risk of T2DM later in life [49].

Chiba et al. [50] have recently shown that the levels of microRNA-148a and microRNA-125b are significantly reduced in the transitional period and in mature HBM of mothers delivering by caesarean section compared to exosome microRNA levels observed in normal vaginal delivery. In addition, caesarean section reduces the frequency of early breastfeeding and thus may negatively affect postnatal epigenetic programming.

4.5.1.4. Maternal stress. Maternal stress negatively affects the growth and development of the child. Bozack et al. [51] evaluated the relationship between maternal stress and extracellular vesicle-derived microRNAs (hMEV-microRNAs). Among 205 hMEV-microRNAs, increased expression of six microRNAs, including microRNA-99b-3p, microRNA-96-5p, microRNA-550a-5p, microRNA-616-5p, microRNA-155-5p and microRNA-604, was significantly associated with maternal stress measurements. These hMEV-microRNAs differing in expression may be involved in the epigenetic regulation of fatty acid metabolism, steroid biosynthesis and the Hippo signaling pathway that regulates organ growth. Dysregulation of the Hippo pathway is associated with metabolic diseases, such as obesity, diabetes, hepatic and cardiovascular steatosis, and atopic diseases.

4.5.1.5. Maternal overweight and obesity. Zamanillo et al. [52] tested milk samples from 59 healthy breastfeeding mothers (38 normal weight and 21 overweight/obese (BMI $\geq$25)) and analyzed infant growth up to 2 years of age. Leptin, adiponectin and microRNA showed a decrease during lactation in mothers with a normal body weight, while their levels changed in the case of overweight/obesity In addition, in mothers with a normal body weight, negative correlations were observed between microRNA expression in milk and the concentration of leptin or adiponectin and no correlation in overweight/obesity. Moreover, microRNAs negatively correlated with infant BMI only in normal weight mothers (microRNA-103, microRNA-17, microRNA-181a, microRNA-222, microRNA-let7c and microRNA-146b). The target genes for the microRNAs listed above may be related to neurodevelopmental processes.

Shah et al. [53] tested milk samples from mothers (30 BMI normal vs 30 with overweight/obesity) and showed lower levels of microRNA-148a and microRNA-30b at 1 month of lactation in patients with increased BMI. After adjusting for gestational age, sex, and birth weight, both microRNA-148a and microRNA-30b levels were significantly associated with anthropometric measurements of infants in the first 3 months of lactation. MicroRNA-148a is the precursor of microRNA-148a-3p, the most abundant microRNA in HBM exosomes that has known neuroprotective and neuroproliferative effects. Therefore, reducing microRNA-148a may increase the risk of childhood obesity and adversely affect the neurological development of children of obese mothers.

4.5.1.6. Diabetes

4.5.1.6.1. Gestational diabetes mellitus. Shah et al. [54] tested milk from 32 women with gestational diabetes mellitus (GDM) and 62 women without GDM for metabolism-related microRNA levels (e.g., microRNA-148a, microRNA-30b, let-7a, and let-7d), which levels correlated with maternal obesity [53]. Compared it with infant height and body composition in the first six months of life. MicroRNA-148a, microRNA-30b, let-7a and let-7d levels were lower in HBM with GDM. MicroRNA-148a was negatively correlated, while microRNA-30b levels were positively correlated with body weight and body fat mass of a 1-month-old infant. It follows that mothers with GDM produced abnormal levels of microRNA that were associated with abnormal metabolic outcomes in their nursing offspring.

4.5.1.6.2. Type 1 diabetes mellitus. Type 1 diabetes mellitus (T1DM) is an autoimmune disease that usually begins in childhood. Mirza et al. [55] showed that milk exosomes from mothers with T1DM were enriched with immunomodulatory microRNAs compared to healthy mothers ($n = 26$ in each group). Of the 631 microRNAs identified, 9 hMEV-microRNAs were significantly altered in mothers with T1DM. These microRNAs are involved in cell cycle regulation and immune response processes and in the production of pro-inflammatory cytokines via PI3K/AKT. Two microRNAs microRNA-4497 and microRNA-3178, which are significantly elevated in hMEV from mothers with T1DM, increase the release of the pro-inflammatory cytokine TNF-α in vitro from THP1 transfected monocytes. However, hMEV microRNAs from mothers with T1DM did not increase the risk of T1DM or other inflammatory diseases in their offspring.

In addition, mothers with T1DM show an altered microRNA profile in human milk exosomes, of which some differentially expressed microRNAs, including hsa-microRNA-4497 and hsa-microRNA-3178, can induce the expression of pro-inflammatory cytokines [32].

4.5.1.6.3. Type 2 diabetes mellitus. While breastfeeding protects against the development of type 2 diabetes mellitus (T2DM) later in life, accumulating epidemiological data highlight the role of cow's milk consumption in T2DM. Recent studies in rodent models have shown that pancreatic β cells are metabolically immature during breastfeeding and preferentially proliferate by activating the mechanistic target of rapamycin complex 1 (mTORC1) and suppressing AMP-activated protein kinase (AMPK). Weaning defines the β-cell metabolic transition from a proliferating, immature phenotype with low insulin secretion to a differentiated mature phenotype with glucose-stimulated insulin secretion, less proliferation, reduced mTORC1 activity but increased AMPK activity. Translational evidence presented in this perspective for the first time suggests that termination of milk microRNA transfer is the driver of this metabolic switch. MicroRNA-148a is a key inhibitor of AMPK and a phosphatase and tensin homolog, key mTORC1 suppressors. The β cells of diabetic patients revert to a postnatal phenotype with high mTORC1 activity and low AMPK activity, which is explained by the continuous transfer of cow's milk microRNAs to the human milk consumer. Cow's milk microRNA-148a apparently promotes β-cell de-differentiation to an immature mTORC1-high/AMPK-low phenotype with functional impaired insulin secretion, increased mTORC1-driven endoplasmic reticulum stress, decreased autophagy, and early β-cell apoptosis. Unlike pasteurized cow's milk, milk microRNAs are inactivated by bacterial fermentation, boiling and ultra heat treatment (UHT) and are not present in current infant formulas. Persistent milk microRNA signaling adds a new perspective to the pathogenesis of T2DM and explains the protective role of breastfeeding, but also the diabetogenic effect of continuous milk microRNA signaling by continuous consumption of pasteurized cow's milk [56].

4.5.1.7. Breastfeeding and infant formulas. In the early 20th century, the pediatric misconception that "milk is only food" was widespread [57]. Formula feeding completely bypasses epigenetic programming with exosome microRNAs. A gene associated with fat mass and obesity (FTO) has been shown to be overexpressed in the blood monocytes of formula-fed infants compared to breastfed infants. FTO is suppressed by microRNA-30b, a component of HBM also involved in brown adipose tissue-based thermogenesis [35].

Chiba et at [58]. compared the expression levels of microRNA-148a and microRNA-125b. In all analyzed infant formulas, they were lower than 1/500 and 1/100, respectively, of the level in mature human milk. In contrast, multiple studies have shown that microRNA was not detectable in infant formulas and their exosome-sized vesicles, which appeared to be casein micelle [14].

Cheshmeh et al. [59] showed that the current weight, height and head circumference in the groups of infants fed with formula and formula were significantly higher than in the exclusively breastfed group. The level of FTO and CPT1A gene expression in formula and formula fed infants was significantly higher ($p < 0.001$) than in breastfed infants, while the level of PPAR-α gene expression was significantly lower ($p < 0.05$). Breastfeeding has shown a modulating effect on the expression levels of obesity-predisposing genes and may protect against obesity and subsequent non-communicable diseases.

5. Conclusion

MicroRNAs are new bioactive components of HBM that control epigenetic programming [38]. According to the current scientific findings, microRNA can modulate the functions of genes involved in many physiological processes, including energy metabolism, immune functions and cognitive development, even if their mechanisms are not yet fully understood.

Numerous studies are being conducted to understand its function and importance for the development of breastfed infants. Tests can be conducted because milk can be stored at low temperature and be processed under high pressure. The microRNA profile varies from person to person and can be influenced by various factors, such as stress, diabetes, preterm birth. Learning the exact profiles of microRNAs and their target sites may contribute to using these molecules in the prevention and treatment of many diseases, such as NEC, atopic diseases, diabetes, obesity or cancer.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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