Review

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Human Breastmilk miRNAs, Diversity and Potentials for Preventive Strategy in Nutritional Therapy

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Abstract: The endogenous miRNAs of breast milk are the products of more than 1,000 nonprotein-coding genes, giving rise to mature small regulatory molecules of 19–25 nucleotides. They are incorporated in macromolecular complexes, loaded on Argonaut proteins, sequestrated in exosomes, lipid complexes, or present in exfoliated cells of epithelial, endothelial, or immune origins. Their expression is dependent on the stage of lactation, however their detection depends on progress in RNA sequencing and the reappraisal of small RNAs definition. Some miRNAs from plants are detected in breast milk, opening the possibility of stimulation of immune cells of the allergic repertoire. Each miRNA harbors a seeding sequence, which targets mRNAs, gene promoters, or long noncoding RNAs. Their activities depend on their bioavailability. Efficient doses of miRNAs are estimated at roughly 100 molecules in the cytoplasm of target cells from in vitro and in vivo experiments. Each miRNA is included in networks of stimulation/inhibition/sequestration driving the expression of cellular phenotypes. Three types of stress applied during lactation to manipulate miRNA supply, have been explored on the rodent offspring: foster mother, cafeteria diet, early weaning. The review present the main mature miRNAs described across current mothers' cohorts, their bioavailability in experimental models, and the studies assessing the potentials of miR-26 or miR-320 miRNA families to alter offspring phenotype.

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1. Introduction

Milk is a complex food produced by the mammary gland, providing the baby with essential nutrients for growth, along with an immunity kit for short-term survival, and long-term epigenetic information influencing the health of the future adult. During lactation, the composition of milk is tuned to the baby' needs from colostrum at birth up to the end of lactation at weaning [1]. Milk from different mammals can be used to feed a human baby, suggesting that molecules like miRNAs known to retain biological activity from flies to mammals, can be easily tested on a relevant animal model for potential preventive use in nutritional therapy of infants. Many ribo and desoxiribo nuclei acids of complex food are recycled by the digestive system. However some escape digestion, and are used in cell-to-cell signaling or provided by immune or exfoliated epithelial cells directly to the offspring. A mature miRNA is defined as a single-stranded ribonucleic acid of 19–25 nucleotides in length, which is generated by the RNase-III-type enzyme Dicer from an endogenous transcript that contains a local hairpin structure [2,3]. The miRNAs are encoded either within the introns of protein-coding genes or transcribed under the control of their own promoters. They are transcribed as long primary transcripts that are trimmed into the hairpin intermediates (pre-miRNAs) in the nucleus, subsequently exported in the cytoplasm, then cleaved into mature miRNAs. The hairpins are usually coding for a 3p and a 5p mature miRNAs with differential tissular expression [4]. The miRNAs function as post-transcriptional repressors of their target genes when bound to the specific sites in the 3′ untranslated region (UTR) of the target mRNA. The miRNAs binding relies mainly on 'seed pairing' (the perfect or near-perfect complementary match of nucleotides 2–8 of the mature miRNA

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product [5]. *In silico* database exploration predict binding sites on promoter, 5'UTR, coding domain of mRNA, as well as on lnc-RNA. These molecules can be detected in cytoplasm, nucleus, and nucleolus [6]. Non-coding RiboNucleic Acids (small or long ncRNA) are involved in epigenetic regulation directly silencing or activating chromatin at specific loci or through their integral role in the machinery that drives DNA methylation. Breastmilk is a rich source of miRNAs [7] which have been proposed to act as milk epigenetic regulators [8–11].

The review will present the main mature miRNAs described across current mothers' cohorts, conditions of their bioavailability in experimental models, and the studies assessing the potentials of miR-26 or miR-320 miRNA families to alter offspring phenotype.

1. RNA content and mature miRNA diversity

The miRNA composition of breast milk is explored by RNA sequencing, performed with kits designed with an RNA extraction step [12] or without [13] like recently in a breast milk cohort [14]. The q-PCR is used for specific exploration of known miRNAs and confirmation studies, with normalization by reference endogenous genes like miRNAs, or by spiking samples with xenogenous miRNA like cel-lin4-5p.

In clinical practice, breast milk is classified as a non-invasive sample, available in maternity or collected at home, on a worldwide scale. The main parameters to ponder when designing clinical plan, are the mother's delivery (term or preterm), the capacity of mammary glands to deliver the food (difference between breasts, the composition variation during suckling between fore, middle, and hind milk, the circadian rhythm of production ; Figure 1A). To control for differences between breasts, mothers are sometimes instructed to utilize the same breast for sampling at each time-point. Minimal differences in miRNA content exist between fore- and hind-milk [15]. It has been advised to utilize pre-feed samples in order to minimize confounding [16]. The impacts of freeze-thaw cycles have been found minimal on milk miRNAs [17,18], which is not surprising as milk of domestic animals retain cryoprotective properties. All these parameters have to be taken into account when designing sampling procedure [19].

In clinical trials the available volumes are reported varying between 100 µL and 100 mL (Figure 1B). Even if qPCR is a sensitive method, the design of breast milk analysis with 100 µL (down to 50 µL; [18]) is challenging but a prerequisite for exploring milk samples from the same mother during a nycthemeron, or to share samples between multiple analyses (transcriptomic, lipidomic, metabolomic; [20]). We have to consider the sampling of a single drop of breast milk as clearly of less informative value than a volume of over 5 mL. Multiplexed techniques to bulk extract from low volumes of crude milk are deeply needed in the field.

The RNA content of whole breast milk after classical phenol/chloroform extraction is wide, for instance, 90 to 1000 ng/µL on 8 parturientes [21]. Breast milk contains high amount of small or very small non-coding RNAs [22] along with transfert RNA, messenger RNA, long-non-coding RNA [23,24].

In lipid fraction of breast milk (Figure 1C), the variation of known and newly described miRNAs have been explored on mothers with normal diet [16,25,26] or comparatively with mothers consuming high fat diet [27]. Many short RNAs are undescribed and, beyond an interest as biomarkers, new regulatory processes may be discovered when exploring total content of whole milk. The classification of miRNAs is designed on biogenesis [2,28], with 4 criteria: (1) the need to confirm expression by hybridization to a size-fractionated RNA sample, (2) the small RNA sequence should be present in one arm of the hairpin precursor, which lacks large internal loops or bulges, (3) the small RNA sequences should be phylogenetically conserved. The sequence conservation should also be seen in the precursor hairpin, usually to a lesser extent than in the mature miRNA segment, (4), the evidence can be strengthened if the precursor accumulates in the presence of reduced Dicer function. More recently, the definition has been reassessed to distinguish function from biogenesis of miRNAs from other classes of RNA by dissociating the three notions of 'miRNA gene' (Mir), 'miRNA precursor' (pre-MiRNA), and 'mature miRNA product' (MiRNA). Under that assumption, miRNA genes produce mature miRNAs but some miRNAs may originate from genes transcribed into other

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types of noncoding RNAs [29]. These definitions are of importance when performing RNA sequencing with the aim of discovering new miRNAs.

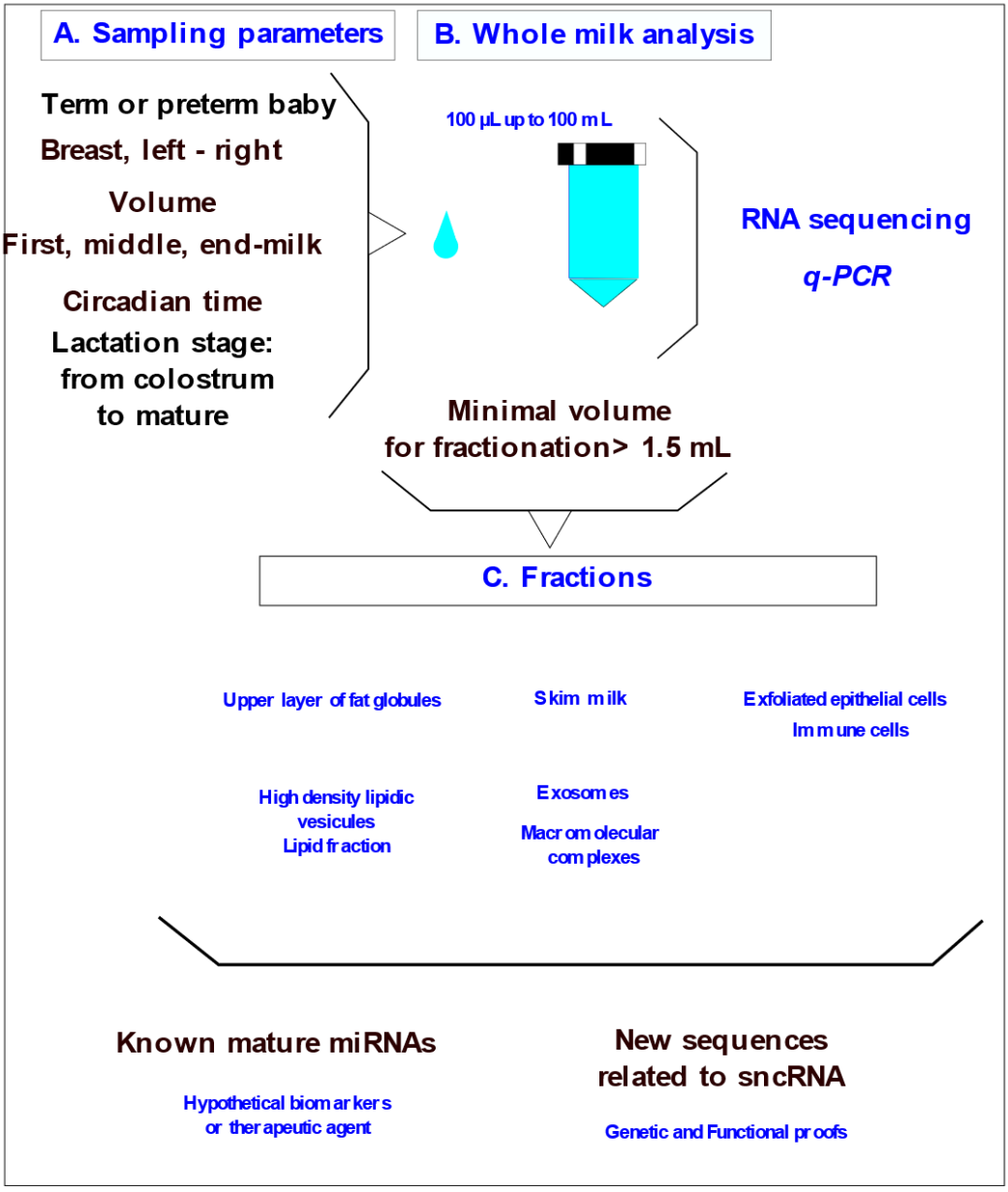

Figure 1. Human breast milk composition: sampling parameters, and analyses of whole milk, and fractions. Sampling parameters (A) have been explored on whole milk by RNA-sequencing or q-PCR. (B). Clinical samples are posing a major problem of legal pressure to reduce the sample volume. Milk fractions have been explored on down to 1.5 mL of whole milk (C). Recommendations have been made to centrifuge breast milk immediately after sampling to remove exfoliated cells, then to store at -80°C or better under liquid nitrogen. A step at -20°C is frequently applied when mothers are sampled at home.

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The recovery of exosomes is influenced by the procedure of sampling (Figure 1C), and a systematic centrifugation step of the milk has been proposed before storage in order to remove cells and debris [30]. A minimal volume of 1.5 mL whole milk has been used to purify exosomes [17], which is a technical lock, up to higher volume like 20 mL [31].

The diversity of miRNAs has been related to the stage of lactation. A strikingly different composition in miRNAs between colostrum and mid-lactation on 18 parturientes has been found (seven miRNAs confirmed by q-PCR: miR-511-3p, 429, 29c-3p, 885-5p, 30b-5p, 183-5p, 623; [32]). Previously, a different composition in miRNAs of one colostrum against five breast milk: miR-518c-3p was uniquely detected in breast milk [7] and is also expressed in the placenta with a putative use as preeclampsia biomarker [33]. Seven miRNAs (miR-148a-3p, 22-3p, 26a-5p, 21-5p, 7b-5p, 7g-5p, 24-3p) have been found common to nipple aspirate, serum, plasma, breast tissue, and breast milk [34]. Surprisingly, the lists have no common miRNAs, notably in the let-7 family wich is higly expressed in tissues, and a source of reference genes. As the let-7g-5p is highly expressed [34] and used as reference gene [18], it should be underlined that in exosomes from mothers suffering of type-1 diabetes, aberrant levels of microRNAs of the let-7 family have been reported [41].

Many studies on whole milk are attempting to derive a specific set of miRNAs common to all mothers at a specific stage of lactation, or related to the mother's pathological status. The list of the most highly expressed breastmilk families of miRNAs has been proposed on the meticulous analysis of 19 studies, as follows: let-7-5p family, miR-30-5p family, miR-148a-3p, miR-200a-3p + miR-141-3p, miR-22-3p, miR-181-5p family, miR-146b-5p, miR-378a-3p, miR-29-3p family and lastly miR-200b/c-3p [35].

The content of known miRNAs can be related to immune function, epigenetics, insulin regulation and growth. According to the analysis of current milk formulations, miR-148a-3p and miR-125b-5p are highly expressed [36] and considered as targeting the gastrointestinal tract [8].

Studies agree on the high expression of miR-146a-5p, which has been linked to food allergy as the human milk oligosaccharide 2'-fucosyllactose attenuates β-lactoglobulin-induced food allergy through the miR-146a-mediated toll-like receptor 4/nuclear factor-κB signaling pathway [37].

Analysis on whole milk revealed that expression of miR-146b-5p, which is not abundant in plasma, was abundantly expressed in breast milk [26,38,39]. The miR-146b-5p has been found to be crucial in the homeostasis of alveolar mammary gland [40].

Exploration of epigenetic properties of breast milk miRNAs has been done on miR-148a-3p and on the families of miR-26 and miR-320, which are frequently reported in analysis, but in lower amount. The expression of DNA methyltransferase 1 (dntm1), a target gene of miR-148a, was found to be down-regulated in cultured cells with up-regulation of miR-148a after incubation with human milk-derived miRNA from the skim and fat layers [42], as well as on comparative functional genomics [9]. Importance of these microRNAs for lactation, particularly for miR-148a-3p, which has been consistently reported as the most abundant microRNA in the different milk fractions (fat, whey, and extracellular vesicles) [43]. However, in milk of mothers suffering from Gestational Diabetes Mellitus, the expression of miR-148a-3p is lower than in normal milk [44]. The miR-148a-3p expression in the fat and skim layers of human milk is found similar as well as for bovine and goat fractions. The expression of miR-148a-3p is significantly lower in the infant formula than in human milk [45].

Evidence suggests that levels of specific miRNAs in human milk lipid fractions are modulated by maternal diet [27]; notably on miR-148a-5p and miR-146b-5p, and maternal weight [46,47]. The miR-26a-5p has also been described on another cohort in lipid milk fraction [26]. However, the impact of the maternal environment on specific changes in milk miRNA content and their potential involvement in metabolic programming in the offspring are not fully understood. Human breastmilk supply of specific miRNAs is affected by maternal overweight/obesity and may influence infant body mass index [47].

The hsa-miR-26a-5p MIMAT0000082, « UUCAAGUAAUCCAGGAUAGGCU » is highly similar to 26b-5p ; just like the hsa-miR-26a-1-3p MIMAT0004499, "CCUAUUCUUGGUUACUUGCACG" is highly similar to 26a-2-3p and 26b-3p. The family is widely expressed in mammals [48].

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Among the newly detected miRNA in whole milk, the isoform e of the miR-320 family [14], strongly in favor of a wide expression of miR-320 family (a to e). The miR-320 family contains 5 members in human (a, b, c, d, e) all from 3p. The hsa-miR-320-3p is used here because of sequence identity in rat (MIMAT0000903) and mouse (MIMAT0000666). In human it is mainly known as 320a (or 320-3p) (5 -AAAAGCUGGGUUGAGAGGGCGA-3 ; MIMAT0000510). The 18 nucleosides in 5 are identical to all family and constitute e form; the 3 end of b is "GCAA," of c "GU" and of d "A." This miRNA does not harbor hexanucleotide element putatively facilitating addressing to nucleus (43). However, on exosome purified from breast milk of a population of the Faroe island, the miR-320e-3p is described in one of the 4 Clusters [49], suggesting that more studies are needed to delineate the importance of ethnicity. On milk samples collected from 38 healthy mothers from preterm (n = 15) and full-term infants (n-23), the MiRNA-320 was more highly expressed in the colostrum of fullterm than in the milk of mothers with preterm infants [50]. The expression of MiRNA-148 was higher in preterm mother's milk than of full-term colostrum. MiRNA-320 and MIRNA-148a expression were up-regulated in cells incubated with milk exosomes [50], which lead to a decrease in their target genes Fatty acid synthase 1 and DNA methyltransferase 1, respectively.

Up to now, the number of studies on the composition of non-coding RNA is relatively modest according to the population of women of reproductive age, the ethnic diversity, and the diet in use on a world scale. The consequence is that the discrepancies between studies cannot be properly explained according to the huge diversity of environmental factors like food or mother's ethnicity. Due to the high connectivity of miRNAs, new integrative strategies between omics and clinical parameters of the mother have to be designed to evidence existing logical links. The studies on miR-26 and miR-320 families illustrate that more work under the redundancy hypothesis of action of near-identical molecules or paralogs are needed for proper therapeutic use. The next section is presenting the data available on the conditions of miRNA bioavailability in the digestive tract.

2. Bioavailability of mature miRNAs in the digestive system

Systemic RNA interference–deficient transporter (sidt1) is the main receptor of dietary and orally administered miRNAs in the digestive system [51], indicating the possibility of the natural transit of miRNAs present in breastmilk from mother to offspring. However, miR-375-3p is unable to cross the digestive tract of mice [52], and even by using the breast milk of a mouse engineered to produce high amount of miR-30b, the demonstration of plasma loading reveals impossible [53].

As a consequence, the bioavailability of miRNA depends on sheltering these molecules from the molecular environment. It determines the capacity of miRNA molecules to reach target cells with a concentration high enough to trigger a biological response, a crucial step in digestive fluids highly loaded with RNases. *In vitro* data on cell cultures have shown that a ratio of 100 miRNAs molecules delivered in the target cell cytoplasm triggers a mesurable physiological response [54]. *In vivo*, after a rough estimation of the total cells of the gastric mucosa to adapt the concentration of miR-320-3p or miR-375-3p, we confirm that this ratio can be used in oral gavage [55,56].

Milk is rich in exosomes, which can serve as natural cargo for miRNAs [57]. However, these molecules are frequently widely present in all milk fractions. So fluorescently labeled miRNAs like miR-375-3p must be transfected in exosomes in order to show accumulation in liver, spleen, and brain after suckling or oral gavage [58]. Another approach is to take advantage of the milk composition related to some pathology of the mother. Human milk exosomes from Gestational Diabetes Mellitus (GDM) and healthy parturients exhibit distinct regulatory bioactivities in both cultured HepG2 cells and Balb/c mice liver which have been related to natural loading by miR-101-3p [31]. Previous studies verified as well that the target gene of miR-101-3p is mTOR, and miR-101-3p suppressed mTOR by binding the 3′-UTR regions of mRNAs [59].

Works on milk exosomes are a very active field of investigation because (1) a food source of a single miRNA species to supplement diet with crude product does not exist, (2) exosomes can protect RNA from digestive enzymes, (3) they can be tailored by genetic engineering of cell lines to load miRNAs with molecular addressing to relevant cell or tissue [60]. Some natural miRNAs can be

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addressed to exosomes [61], and with a step of loading the miRNA on Argonaut proteins before loading into exosome [62].

However, miRNA obtained by chemical synthesis can also be loaded in artificial vector like lipoaminoglycoside Dioleyl-Succinyl Paromomycin [63]. The loading of miR-375-3p has been measured in a transgenic rat model on the enteroendocrine cell lineage [56]. Taking this specific miRNA as an example, the miR-375-3p is proposed as a key regulator in malignant breast cancer [64]. In cohorts of breast-fed infants, the consumption of miR-375-3p has been associated with protection from atopy [65], opening for consideration on the prevention of allergic diseases through breastfeeding and described as unmodified in the breast milk of mothers treated with probiotics [66]. These divergent properties illustrate the problem paused by supplying miRNA in animal model, most of the time, the site of delivery will impact differently cellular phenotypes. Another level of complexicity is added if one considers that immune cells involved in allergy may be stimulated also by miRNAs from plant present in breast milk [21]. As the chemistry of plant miRNAs are different from eucaryotes, complicating the situation of breast milk analysis, the presence of these xenogenous molecules have to be taken more into account in futures allergy studies.

Oral administration of miRNAs is a current problem in targeted nutritional therapy and requires further studies both to design new prokaryotic or eukaryotic vectors [67,68] and to test miRNA cocktails. The biological relevance of low-abundance miRNAs is contrary to the conventional logic that stipulated that higher abundance should correlate with biological relevance [69]. Continued uptake of milk-derived exosomes that carry dnmt targeting miRNAs may promote diabesity, allergy [35], neurodegenerative diseases, and cancer later in life [11].

A hypothesis driven by an *in silico* approach has related miR-148a-5p to dnmt1 regulation opening the possibility that breast milk could manipulate the homeostasis of tissue like the pancreas [70]. Targeted nutritional therapy needs not only screening miRNAs with potential epigenetic activity, but also designing natural or biomimetic vectors addressing the load of molecules to a specific cell lineage. In the last section, studies addressing the long-term consequences of manipulating breast milk miRNAs will be presented.

3. PotentialsofmiRNAsforinvivotreatmentofoffspring

Gestation and lactation constitute a unique window of opportunity to supplement babies for minimizing the negative effects of early-nutritional programming [72] among which obesity [73]. Early postnatal life is a critical period where stressful experiences may potentially lead to long-term programming with consequences on the adult health. The application of preventive and therapeutic approaches during early-life-sensitive periods is likely promising. For example, if the epigenetic patterns disrupted by exposure to stress can be modified through specific epigenome-targeted therapeutic interventions, it would be possible to correct the impaired gene expression patterns to prevent stress-induced chronic pathologies and improve human health and longevity. Three types of stress applied during lactation to manipulated miRNA supply, have been explored on the rodent offspring. The effect of a foster mother in a model using a/a and Avy /a mice on C57Bl6J background and the consequences on first and second generations on survival parameter as end-point and ovocyte metabolism (Figure 2A, [74]). The other studies discussed here are limited to the first generation. A cafeteria diet consumed by lactating mothers is decreasing the level of miR-26a-3p with consequences on the F1 generation (Figure 2B, [75]). Likewise, the effect of an oral supply of miR-320-3p on polr3d on brain cells and the digestive epithelia of rat pups submitted or not to early-weaning (Figure 2C, [56]).

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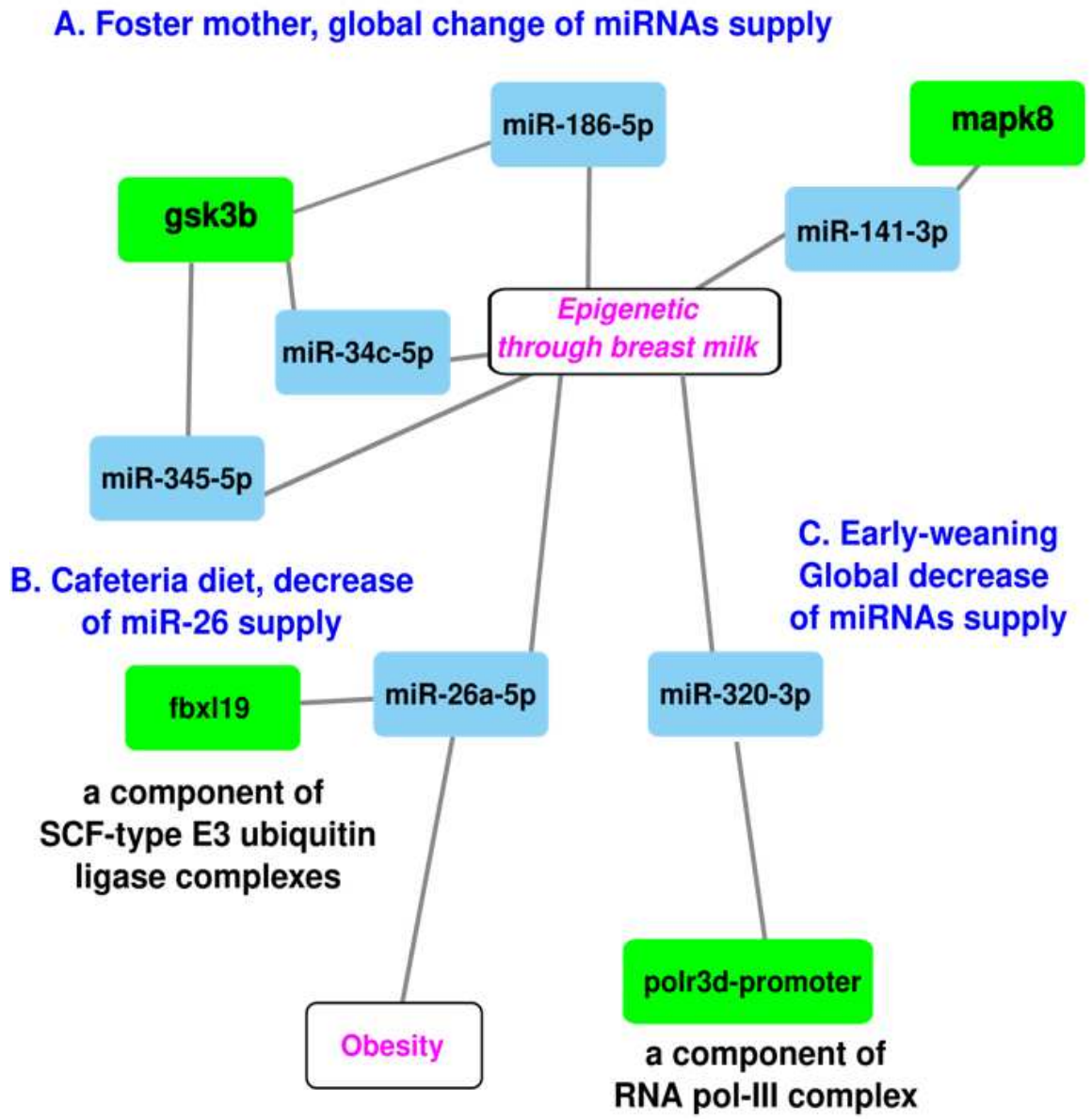

Figure 2. Exploration of three types of stress related to miRNAs with epigenetic properties, delivered during lactation, on the first or second offspring generations. Experiments on a murine model of foster mothers have shown that miRNAs like miR-186-5p can be impacted at the ovocyte level in offspring, opening the possibility to impact first and second offspring generations (A). Independent experiments have shown that a cafeteria diet is altering the miR-26 family with consequences on the adipogenesis of the first generation (B). An oral supplementation of pups with miR-320-3p during lactation has an immediate effect on digestive cells and long-term impact on the brain compartments (C). Note that family miR-26 and 320 have several isoforms which can all be redundant molecules with similar bioactivity. Nomenclature : glycogen synthase kinase 3 beta, gsk3b, polr3d promoter, RNA polymerase III subunit 3d promoter, F-box and leucine rich repeat protein 19 (fbxl19). Illus-tration drawn with Cytoscape [71].

Alternative breastfeeding practices allowing human milk sharing, such as cross−fostering or donor-milk banking are used in hospital [76] or for convenience. The mouse model is allowing to follow the expression patterns of miR-186-5p, miR-141-3p, miR-345-5p, and miR-34c-5p and their candidate target genes Mapk8, Gsk3b, and Ppargc1a in ovarian and liver tissue [74]. The main result is to relate miR-186-5p with the glycogen synthase kinase 3 beta (gsk3b) gene in F2-ovaries, a

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negative regulator of glucose homeostasis, involved in energy metabolism, inflammation, ER-stress, mitochondrial dysfunction, and apoptotic pathways. Defects in this gene have been associated with Parkinson disease and Alzheimer disease. The miR-186-5p expression decreases in F2 milk-siblings, whereas Gsk3b increases compared to control counterparts. These data on breast milk are well in line with those of a pilot study conducted to analyze the miRNA profiling in gastric content from both breast-fed and formula-fed infants [77,78]. The gastric content of infants at autopsy were analysed and demonstrate the presence of miRNAs in gastric content. What is more interesting in these findings is the fact that the samples were isolated between 1 month and 2 years after their death. Additionally, they found differences in the miRNA content regarding the type of feeding (breastfed or formula fed) and, in this framework, they propose miR-151a and miR-186-5p as potential breast milk biomarkers [77]. These 2 studies [74,77] are paving the way for exploring long-term effect of miR-186-5p supply in transgenerational rodent models.

The miR-26a-5p, an abundant miRNAs in breast milk has been linked to adipogenesis in the offspring. Thus, milk miR-26a may act as an epigenetic regulator influencing early metabolic program in the progeny, which emerges as a relevant component of an optimal milk composition for correct development. The miR-26 suppresses adipocyte progenitor cell differentiation and functions within the adipocyte progenitor cell lineage to inhibit mobilization and subsequent adipocyte production by down-regulating FBXL19, a novel driver of adipogenesis [79]. After weaning, descendants of cafeteria-fed dams breastfed with lower levels of miR-26a displayed greater expression of Hmag1, Rb1, and Adam17 in retroperitoneal white adipose tissue in comparison with controls. Hence, alterations in the amount of miR-26a supplied through milk during lactation is able to alter the expression of target genes in the descendants and may affect adipose tissue development [75].

In breast milk, the miR-320-3p is less expressed in milk from mothers with a preterm infant than a term delivery [50], suggesting that a supplementation would be beneficial to the health of the preterm baby. The miR-320, an endogenous shRNA is highly expressed in mammals [80]. The *in vivo* delivery of miR-320-3p is targeting binding sites located both on the promoter of RNA polymerase III and on the 3′ -UTR of the transcript [55,56,81]. Polr3d is the subunit-17 of RNA polymerase-III involved in tumorigenesis. RNA polymerase III is considered to be linked to aging and longevity through TORC and insulin genes, as well as through genes related to telomerase activity [82,83]. Additional work is needed to explore the putative long-term effects on the polr3d complex, which includes 17 subunits, as well as any effect on telomerase activity [82,83]. Up to now, oral supplementation by miRNA-320-3p or miR-375-3p during lactation has long-term miRNA-specific consequences on the endogenous levels of corresponding miRNAs with a strong tissue-dependent memory. Combining miR-320-3p treatment with the weaning schedule (early or regular weaning) has shown a strong interaction between factors in all tissue extracts except the brain stem. In the hippocampus, the miR-504 was downregulated in both sexes, but in the brain stem, it was upregulated only in females, along with miR-320-3p and miR-16-5p levels. In the hypothalamus, clock was upregulated in both sexes.

Future explorations need to relate the behavior of rodents submitted to miRNA treatment during lactation in order to widen the long-term perspectives of such preventive strategy [56]. The aminoglycoside vector is efficient for delivering miRNAs in the cytoplasm of all digestive cells without loading gastric exosomes [55,56]. Such vectors allow for the administration of a cocktail of several RNA molecules, which constitutes a promising field of future exploration.

Clearly, for therapeutic applications, an effect on the offspring gonads [74] is not desired. Moreover, the influence of a single miRNA on multiple tissues [56] is also a difficult problem to address in studies in the prevention of metabolic diseases.

Conclusions and Perspectives

The concept of Developmental Origins of Health and Disease (DOHaD), highlights a critical period for the development during the 1000 first days of life, including the period from conception to the end of the second year of life. That period is suggested as the most active in terms of epigenetic

regulation, especially in terms of DNA imprinting. [84]. A growing amount of evidence supports that epigenetic programming affected by early nutrition may result in adult disease in the long run [72].

RNAs are able to interact with DNAs, RNAs, and proteins, bringing up biochemical networks within a single cell or connecting multiple cell types. Extensive validation studies are needed to consider the side effects of RNA-based drugs [85] and the reappraisal of selective pressure on the machinery at the level of genome regulation [86], as detailed descriptions of the Competing Endogenous RNA network (ce-RNA) underpinning immune cell regulation. However, the Ce-RNA hypothesis has been seriously shaken by a work on miR-122 [87] demonstrating that solutions to eradicate severe pathologies under this hypothesis are unlikely. Nevertheless, there is an exciting possibility of preventing ophthalmic diseases in the next generation by reprogramming heritability in mice, thus hypoxic stress of the retina would prepare the offspring for a beneficial response to such treatment. [88].

Designing a new milk formula taking into account the epigenetics of the child [89] means to be able to unify the molecular and global points of view of epigenetics [90]. Small transcoded RNAs (microRNA, pi-RNA, etc.) play an important role in transgenerational epigenetic inheritance. Both maternal and paternal lineages influence epigenetic alteration in offspring. Sperm not only transfers DNA to the ovum, but also transfers different kinds of RNAs including miRNAs and pi-RNAs. These transferred RNAs play an important role in embryo development by influencing various mRNA expressions [91]. Both maternal and paternal lineages influence epigenetic alteration in offspring [92].

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