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Review

Breast milk microRNAs harsh journey towards potential effects in infant development and maturation. Lipid encapsulation can help

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ABSTRACT

The possibility that diet-derived miRNAs survive the gastrointestinal tract and exert biological effects in target cells is triggering considerable research in the potential abilities of alimentary preventive and therapeutic approaches. Many validation attempts have been carried out and investigators disagree on several issues. The barriers exogenous RNAs must surpass are harsh and adequate copies must reach target cells for biological actions to be carried out. This prospect opened a window for previously unlikely scenarios concerning exogenous non-coding RNAs, such as a potential role for breast milk microRNAs in infants' development and maturation. This review is focused on the thorny path breast milk miRNAs face towards confirmation as relevant role players in infants' development and maturation, taking into consideration the research carried out so far on the uptake, gastrointestinal barriers and potential biological effects of diet-derived miRNAs. We also discuss the future pharmacological and pharma-nutritional consequences of appropriate miRNAs research.

1. Introduction

Breast milk is a very important source of nutrients for mammals' newborns, including humans. In addition to its nutritional functions, it consists of non-nutritional bioactive factors that makes it vital in immunological protection and other aspects of infant development. Examples include macrophages, stem cells, immunoglobulins, growth factors, hormones, anti-microbial components, oligosaccharides/glycans and mucins, among others [1,2]. In spite of the manifold breast milk beneficial effects, the way it acts on newborns and the precise role of its components are still unclear. Therefore, milk elements are still being actively studied.

Among the molecules identified in breast milk, microRNAs are particularly abundant [2–4]. These biomolecules (hereafter referred to as miRNAs or miRs) are short non-coding RNAs (between 19 and 24 nucleotides) of eukaryotes, which are involved in the regulation of various biological processes [5–7]. Although initially they were known only at the intracellular level, these biomolecules have also been found in several biological fluids such as blood, urine, sweat, saliva, tears, and milk [8,9]. Milk is one of the richest sources of miRNAs (over 1400 mature miRNAs have been described) [3]. This abundance has raised the question of whether these molecules have a role in the various benefits of milk over the infant. In this context, the presence of exogenous miRNAs (or xenomiRs), mainly coming from the diet, has been reported and these molecules were suggested to be relevant players in the regulation of gastrointestinal (GI) tract conditions and gene expression in the consumer organism [10]. Thus, the possibility that exogenous RNA can be absorbed and have specific functions in the recipient cells stimulates the hypothesis that breast milk miRNAs can enter circulation, reach specific organs of the infant, and exert specific effects on its development [3,9,11]. In this sense, proof regarding the beneficial functions of milk miRNAs during lactation and long-term effects would be of great interest, namely in technological terms, since their inclusion in maternal-like milk would have important socio-economic consequences. Several characteristics of extracellular miRNAs, such as their origin, the mechanism by which they pass into circulation, the reason for their stability, or how they are absorbed by cells, among others [9,12] are still unclear. This review collates the current

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knowledge on the potential role of breast milk-derived miRNAs on the infant's development and maturation. We also propose breast milk miRNAs as paradigmatic examples of pharmaceutical and pharmacological hurdles to overcome if we want to exploit their great potential as preventive and therapeutic agents.

2. Methods: search strategy

We used PubMed (US National Library of Medicine National Institutes of Health), Scopus, and Science Direct to search original and review articles referring to the research on breast milk miRNAs and their potential involvement in infants' development and maturation, published up to December 2017. Various combinations of the following keywords were used: microRNA; circulating microRNA; secreted microRNA; delivery; extracellular vesicle; lipid carriers; microvesicle; exosome; uptake; endocitosis; function; exogenous RNA; xenomiR; plant; milk; human milk; bovine milk; breastmilk; composition; skim milk; mammary gland; maternal; infant formula; diet; off-spring mouse; immune-related; gut; intestinal absorption; intestinal barrier; epithelium; enterocyte; isolation. We searched the bibliographies of the retrieved articles in order to identify additional articles. From the initial search, retrieved articles were evaluated for their titles and abstracts and after detailed study of the full-text reports, 145 documents were finally selected for inclusion in this review.

3. Characteristics of miRNAs

3.1. Biogenesis and functions of miRNAs

Mammalian miRNAs participate in biological processes, functioning as regulators of gene expression. In a canonical pathway, miRNAs are first transcribed as primary miRNA (pri-miRNA), then processed by the Microprocessor complex (comprising Drosha) into pre-miRNA molecules, which are exported from the nucleus, and further processed by Dicer to yield mature miRNAs. Mature miRNAs associate with the RNA-Induced Silencing Complex (RISC) and bind to complementary sequences of their target messenger RNA (usually at the 3′ untranslated region, UTR), causing its degradation or repressing translation depending on the level of complementarity [7,13,14]. Unlike what occurs in plants, where miRNAs must have complete complementarity with their target mRNAs, miRNAs complementarity is partial in metazoans [15,16]. A single miRNA is usually able to recognize hundreds of different mRNAs and numerous miRNAs can target the same mRNA. miRNA transcription is controlled by promoters and transcription factors, similarly to protein-encoding genes. As regulators of gene expression, miRNAs are involved in the regulation of many biological processes in both plants and animals. Among others, they modulate processes of differentiation, apoptosis, proliferation, lipid metabolism, intercellular communication or immune response [5,6,17–19]. In fact, they have been shown to regulate more than 50% of mammalian protein synthesis, and they have been proposed to account for more than a third of human genes.

Both in physiological and pathological conditions, miRNAs transfer between cells is essential as a form of intercellular communication, where miRNA-secreting cells may influence gene expression in receptor cells [5]. miRNAs actions in the immune system are among the most studied, being known to participate in the regulation of B and T cell development, release of inflammatory mediators, proliferation of neutrophils and monocytes, and differentiation of dendritic cells and macrophages [3,17]. Furthermore, circulating miRNAs can be significantly altered in many pathological conditions (cancer, autoimmune diseases, neurodegenerative diseases, GI diseases, reproductive system dysfunctions, metabolic syndrome) in contrast to their normal physiological levels [3,6,9,18,20,21]. In this context, it has been proposed that miRNAs could be reliable biomarkers of disease development and the use of miRNAs as potential therapeutic targets is also being studied [6,9,18,22–24]. For example, synthetic miRNAs may be designed to target specific disease-associated genes or to restore the normal physiological levels of altered miRNAs [24]. The effect of miRNAs antagonists (anti-miRNAs), which target miRNAs that are overexpressed in a pathological state, has also been demonstrated [9,18,23]. Another potential use of these biomolecules has to do with regenerative medicine, since it has been seen that miRNAs are important regulators of pluripotency-related genes and can also play a role on the reprogramming of somatic cells to induced pluripotent stem cells [25].

3.2. Secreted miRNAs: origin, stability, transport and uptake

The secretion of miRNAs is an active, specific, and controlled process [5,26]. Despite the high presence of ribonucleases in human plasma, circulating miRNAs are surprisingly resistant to degradation by these enzymes even in hostile environmental conditions (e.g. acid pH or exposure to high temperatures) [27,28]. Although initially these miRNAs were thought to have some inherent property protecting them from degradation by nucleases [26], isolating and purifying miRNAs from plasma and then incubating them in whole plasma resulted in quick degradation [29]. For this reason, although it is accepted that at least one part comes from deteriorated cells (tissue damage, inflammation, apoptosis or necrosis) or cells with very low half-life such as platelets [9], the most accepted proposals on the origin of the extracellular miRNAs are based on their secretion and transport in some protected form. On the one hand, several types of extracellular vesicles (membranous compartments, differing according to their mechanism of biogenesis and secretion, released by almost all cell types) contain miRNAs, which are selectively packaged and released into recipient cells, where they regulate the expression of target genes [5,30]. On the other hand, miRNAs found in vesicles represent between 1 and 5% of extracellular miRNAs and the ones not associated with vesicles are protected by complexing with lipoproteins or RNA-binding proteins [29–31]. In addition to the protection against extracellular nucleases, the interaction of miRNAs with these protein complexes could enhance their functions once inside the receptor cell [26]. These types of clusters with membranous transporters or proteins have been found in various biological fluids, such as blood or cerebrospinal fluid, among others, including milk [6,32,33]. However, most studies have focused on showing the utility of these miRNAs as disease biomarkers and not on investigating their biological function [22].

The mechanism of uptake of secreted miRNAs by receptor cells and its specificity, which will influence their functional role, is not yet fully known. It has been proposed that vesicle-transported miRNAs enter cells by endocytosis, phagocytosis or direct fusion of the membranes, or even by a passive transfer of their contents; and that the specificity is due to cell-surface recognition molecules on these membranous transporters [5,26]. It is likely that the exact mechanism of entry into the recipient cell depends on the phagocytic capacity of the latter and on the types of lipids and proteins present on the vesicle surface [26]. In addition, it is possible that several miRNAs are simultaneously absorbed, regulating several target genes simultaneously [5]. In the case of vesicle non-associated miRNAs, transference to receptor cells may involve the recognition the proteins carrying the bound miRNA by a specific receptor [26].

4. Current knowledge on exogenous miRNAs uptake and bioavailability

4.1. Overcoming biological barriers: thorny path towards biological effects

Traditionally, miRNAs have been considered endogenous genetic regulators. However, in recent years, exogenous circulating RNAs have been discovered from external sources, which suggest that they can also be absorbed by receptor cells and exert specific functions in the recipient organism [4,9]. The main source of these exogenous miRNAs is