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Зміст

Оригінальні дослідження

Антипкін Ю.Г., Лапшин В.Ф., Уманець Т.Р., Камінська Т.М., Банадига Н.В., Колоскова О.К., Личковська О.Л. , Гаращенко Т.А., Подольський Вл.В., Подольський В.В.

Аналіз поширеності COVID-19 серед дитячого населення України в перший рік пандемії 7

Абатуров О.Є., Крючко Т.О., Кривуша О.Л., Бабич В.Л., Токарєва Н.М., Ткаченко О.Я.

Вплив поєднаної терапії солями кальцію та вітаміном D на концентрацію макро- та мікроелементів у слині дітей раннього віку 12

Марушко Т.В., Онуфреїв О.Є., Марушко Ю.В., Герман О.Б.

Рівень антитіл до адалімумабу в дітей з ювенільним ідіопатичним артритом на різних етапах лікування..... 17

Гавриленко Ю.В., Кияниця М.А.

Ефективність і безпечність застосування Зіпелор льодяників у дітей при лікуванні гострого фарингіту 25

Мозирська О.В.

Вивчення впливу атопічного дерматиту в дітей на якість життя сімей..... 30

Пишник А.І., Няньковський С.Л.

Нутритивне забезпечення дітей дошкільного віку з порушеннями психомоторного розвитку 35

Крючко Т.О., Ткаченко О.Я., Щербак В.В., Климко Д.О., Олійніченко М.О., Клишта В.В.

Роль комплексного застосування назальної іригаційної та антигістамінної терапії в лікуванні алергічного риніту в дітей..... 45

Абатуров О.Є., Товарницька А.О.

Протизапальний вплив miR-148a грудного молока на стан слизових оболонок у недоношених новонароджених дітей 59

Ковальчук Т.А., Боярчук О.Р.

Показники якості життя у дітей із нетравматичними несинкопальними транзиторними втратами свідомості та їхніх родин..... 67

Contents

Original Researches

Yu.G. Antypkin, V.F. Lapshyn, T.R. Umanets, T.M. Kaminska, N.V. Banadyha, O.K. Koloskova, O.L. Lychkovska, T.A. Harashchenko, Vl.V. Podolskiy, V.V. Podolskiy

Analysis of the COVID-19 prevalence among children in Ukraine during the first year of the pandemic..... 7

O.E. Abaturov, T.O. Kryuchko, O.L. Kryvusha, V.L. Babych, N.M. Tokareva, O.Y. Tkachenko

The effect of combined therapy with calcium salts and vitamin D on the concentration of macro- and microelements in saliva in young children 12

T.V. Marushko, O.Ye. Onufreiv, Yu.V. Marushko, O.B. German

Levels of antibodies to adalimumab in children with juvenile idiopathic arthritis at different stages of treatment 17

Yu.V. Havrylenko, M.A. Kyianytsia

Effectiveness and safety of Zipelor lozenges in children in the treatment of acute pharyngitis 25

O. Mozyrska

Study of the impact of atopic dermatitis in children on the quality of life of families 30

A.I. Pushnyk, S.L. Nyankovskyy

Nutrient support for preschool children with psychomotor disorders..... 35

T.O. Kryuchko, O.Ya. Tkachenko, V.V. Shcherbak, D.O. Klymko, M.O. Oliinichenko, V.V. Klyshta

The role of a combined use of nasal irrigation and antihistamine therapy in the treatment of allergic rhinitis in children 45

O.E. Abaturov, A.O. Tovarnytska

Anti-inflammatory effect of breast milk miR-148a on the state of mucous membranes in premature newborns 59

T.A. Kovalchuk, O.R. Boyarchuk

Quality of life indicators in children with non-traumatic and non-syncopal transient loss of consciousness and their families..... 67

Здоров'я дитини

Оригінальні дослідження / Original Researches

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Anti-inflammatory effect of breast milk miR-148a on the state of mucous membranes in premature newborns

Abstract. ***Background.*** *Breast milk (BM) is an optimal nutritional product for newborns and a source of exogenous microRNAs (miR). MiR-148a is one of the most highly expressed miR of BM. Currently, there is a lack of data on the miR-148a effect on the development of necrotizing enterocolitis (NEC) in premature newborns. The purpose of the study was to determine the influence of miR-148a of the mother's BM on the risk of NEC development in preterm newborns.* ***Materials and methods.*** *We examined 74 newborns, who were treated in neonatal departments. We determined the level of miR-148a in the BM of 44 mothers of exclusively breastfed children. In parallel, we evaluated gene expression of the transcription factor T-bet in scrapings of the buccal mucosa of all the newborns. Three groups for comparison were selected: group 1 consisted of 32 newborns up to 37 weeks of gestation on breastfeeding (BF); group 2 — of 30 preterm newborns on artificial feeding; the control group — 12 full-term newborns on BF.* ***Results.*** *The gestational age median of group 1 children was 33 (31; 34) weeks; group 2 — 32.5 (32; 35) weeks; and it was comparatively higher in the control group ($p < 0.001$) — 40 (39; 41) weeks. Neonatal encephalopathy as the main diagnosis occurred more often among full-term newborns ($p < 0.001$). Children of groups 1 and 2 did not differ significantly in the frequency of cases of respiratory distress syndrome and neonatal encephalopathy ($p > 0.05$). In group 2 compared to the first one, manifestations of NEC occurred significantly more often ($p < 0.05$): 9/30.0 ± 8.4 % vs 3/9.4 ± 5.2 %. We determined that the level of miR-148a expression in the BM of the mothers of premature children on BF was significantly lower ($p < 0.001$) than in the group of full-term children: 0.089 (0.048; 0.142) c.u. vs 1.0 (1.0; 1.0) c.u. Furthermore, the level of the transcription factor T-bet expression in the cells of the buccal mucosa scrapings was higher in premature children with clinical NEC ($p = 0.022$): 2.36 (1.94; 3.17) c.u. vs 1.49 (1.0; 3.27) c.u. in children without signs of NEC. We proved the presence of direct positive correlation between the T-bet level and NEC manifestations ($r = 0.271$; $p = 0.021$) and determined the inverse correlation between the level of miR-148a expression in the mother's BM and the level of T-bet expression ($r = -0.371$; $p = 0.043$).* ***Conclusions.*** *The miR-148a expression level is relatively lower in the BM of the mothers whose children were born prematurely and have problems with adaptation than in the mothers who gave birth at term. However, in case of NEC development, there is an increase of miR-148a level in the mother's BM, which contributes to a decrease in the T-bet expression in the mucous membranes of the child and has a protective impact on intestinal walls.*

Keywords: *miR-148a; T-bet; preterm infants; premature newborns; necrotizing enterocolitis*

Introduction

Human breast milk (BM) has been considered the best food for newborn nutrition since ancient times. The WHO experts stress that exclusive breastfeeding (BF) for the first six months of life is the most optimal nutrition for babies to ensure the development and formation of all organs and systems of the child's body. Breastfeeding helps to reduce the risk of a wide range of diseases [1, 2].

In recent years, special attention has been paid to the effect of the microRNA (miR) of BM on the health of newborn babies. MicroRNAs are small non-coding RNA molecules containing 18–25 nucleotides [3, 4]. Most BM miRs are contained in extracellular vesicles, which contributes to their absorption in the child's digestive tract [5]. Exogenous miRs that entered the body with milk were identified in brain, kidneys, and liver of experimental animals [6].

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Each miR regulates the expression of several genes. Thereby, miRs influence metabolism, differentiation, and development processes in cells of various tissues; and immune system maturation [3, 4, 7, 8]. MiR-148a is a representative of the highly concentrated miR pool of BM. According to some authors, the share of miR-148a varies from 12 to 35.79 % of the miR transcriptome of BM [5, 9–11]. It has been demonstrated that miR-148a is involved in the regulation of inflammatory responses [8, 12]. However, currently, there is a lack of data on the miR-148a effect on necrotizing enterocolitis (NEC) development in premature newborns.

The aim of the present study was to investigate the effect of miR-148a in maternal BM on the probability of NEC development in preterm newborns.

Material and methods

The study was conducted as part of the research work "Genotype-associated diagnostic and treatment process personalization in children with diseases of the respiratory, endocrine and digestive systems" (state registration number 0118U006629) of the Department of Pediatrics 1 and Medical Genetics of the Dnipro State Medical University. The study was conducted by following modern scientific standards. Measures are provided to ensure the health of the patient; respect for his rights; human dignity; moral and ethical norms per under the Declaration of Helsinki; the European Convention on Human Rights and Biomedicine; the Universal Declaration on Bioethics and Human Rights (UNESCO); relevant legislative acts of Ukraine (Constitution of Ukraine (Articles 3, 21, 24, 28, 32); Fundamentals of Ukrainian legislation on health care (Articles 43.1, 44.1); Law of Ukraine "On Medicinal Products" (Article 7, 8).

The study was conducted on the basis of the Department for Post-Intensive Care and Nursing of Newborns of the Communal Enterprise "Dnipropetrovsk Regional Perinatal Center with Hospital" of DRC" and the Department for Premature Newborns of the Communal Non-Commercial Enterprise "City Multidisciplinary Clinical Hospital for Mother and Child named after Prof. M.F. Rudnev" of the Dnipro City Council" for 2021–2022.

To achieve the goal, we enrolled 74 children and collected breast milk samples from 44 mothers, whose children were in the observation group and were breastfeeding. Inclusion criteria were as follows: exclusive breastfeeding or exclusively artificial feeding (AF) from birth; age at the examination time up to 28 days. Exclusion criteria: severe and clinically unstable condition of newborns; mixed diet or change in diet in the anamnesis; the age of the baby older than 28 days; the presence of inflammatory diseases of the mother that required medical intervention; the presence of inflammatory signs of inflammation of the mucous membrane of oral cavity, nasopharynx, and oropharynx of the infants; mixed nutrition of the neonates.

We selected 3 groups of children:

— the first group — 32 children who were born prematurely and were exclusively breastfed and, accordingly, their 32 mothers;

— the second group — 30 children who were born prematurely and were fed exclusively with artificial formulas;

— the control group — 12 full-term exclusively breastfed newborns and, respectively, 12 of their mothers.

In all cases of AF, the start of formula intake was connected with the mother's hypogalactia.

Molecular and genetic research methods included determination of T-bet transcription factor gene expression in newborn buccal mucosa scraping cells, determination of extracellular miR-148a in mothers' BM by polymerase chain reaction with reverse transcription in real time. A certified kit of Applied Biosystems™ TaqMan™ Small RNA Assays was used to determine miRNAs. For statistical analysis, the normalized expression levels of miR-148a in the BM of the mothers of the first group and the expression of T-bet in the mucous membranes of the babies of the first and second groups of the study were calculated in relation to the control group, where the expression level of the specified factors was taken as 1.

Molecular and genetic methods of research were carried out in the certified laboratory "PCR lab Interdepartmental Training and Research Laboratory" of Ternopil National Medical University.

Statistical processing of the results was carried out using the Statistica 6.1 software product (StatSoft Inc., serial number AGAR909E415822FA). The analysis of the obtained data with the assessment of the statistical probability of differences was analyzed using parametric and non-parametric methods of statistics. In case of normal distribution of quantitative data (Shapiro-Wilk test), we used the arithmetic mean (M), its standard error (± m), ANOVA analysis of variance with a posteriori comparison according to the Tukey test. In case of abnormal distribution, we applied the median (Me) with an interquartile range (25%; 75%), non-parametric Kruskal-Wallis analysis with pairwise comparison by Dunn and Mann-Whitney tests. To characterize and compare relative values, we used the frequency index with error (F ± m %), Pearson's agreement criterion (χ^2), and Fisher's two-tailed exact test (FET). The relationship between various factors was assessed using the Spearman rank correlation coefficient (r). The critical level of statistical significance when testing all null hypotheses is taken as equal to 0.05 (5 %).

Results

Newborn children of the main and control groups differed significantly in terms of gestational age and anthropometric indexes ($p < 0.001$). The median gestational age of premature babies on BF was 33 (31; 34) weeks, on AF — 32.5 (32; 35) weeks, meanwhile, in the group of full-term newborns it was 40 (39; 41) weeks. The material was collected significantly later in the group of premature newborns on AF ($p < 0.05$): at 9.0 (6.5; 13.5) days of life versus 6.5 (5.5; 7.0) days of life in the group of premature babies on BF. The start of enteral feeding was later in the group of preterm infants on AF compared to the group of preterm infants on BF ($p < 0.05$).

The main reasons for newborn monitoring in neonatal units were: respiratory distress syndrome (RDS), NEC, and neonatal encephalopathy (NE). NEC was observed only in the groups of premature newborns (on BF and AF). Features of clinical and anamnestic data are shown in Table 1.

NE significantly prevailed in the group of full-term children (91.7 ± 8.0 %). There was no difference ($p > 0.05$) in the frequency of RDS cases (65.6 ± 8.4 % vs 50.0 ± 9.1 %), NE cases (31.3 ± 8.2 % vs 30.0 ± 8.4 %) between the groups of premature newborns on BF and AF. However, NEC developed significantly more often-in premature children on AF than on BF ($p < 0.05$): 30.0 ± 8.4 % versus 9.4 ± 5.2 %.

The miR-148a expression level in BM of the mothers of premature children on BF was significantly lower ($p < 0.001$) than in the group of full-term children: 0.089 (0.048; 0.142) c.u. vs 1.0 (1.0; 1.0) c.u. (Fig. 1). Premature newborns of the second comparison group were on exclusive AF. We did not determine miR-148a expression level in formula because of the potential deficiency of miR there.

We have found a tendency to the increase of the miR-148a expression level in the BM of mothers of premature children on BF who had signs of NEC to compare with the BM of mothers of children of the same group who did not have gastrointestinal disorder. Nonetheless, the difference was unreliable: 0.25 (0.05; 1.75) against 0.08 (0.05; 0.13) with $p = 0.156$ according to the Mann-Whitney test. The lack of significant differences between the median values of miR-148a expression in our study can be explained by the relatively small number of cases of NEC in the group of preterm infants on BF, its significant variability ($p < 0.001$ according to Levene's test), and the dependence of the NEC development on a number of other factors.

Table 1. Clinical and anamnestic characteristics of the comparison groups

Index	Comparison groups			Significance of difference between the groups
	Group 1 (n = 32)	Group 2 (n = 30)	Controls (n = 12)	
Gestational age, weeks, Me (25%; 75%)	33 (31; 34)	32.5 (32; 35)	40 (39; 41)	$p_{1-2} = 1.00$ $p_{1-c} < 0.001$ $p_{2-c} < 0.001$
Birth weight, g, Me (25%; 75%)	1925 (1490; 2200)	1890 (1450; 2250)	3600 (3050; 4100)	$p_{1-2} = 1.00$ $p_{1-c} < 0.001$ $p_{2-c} < 0.001$
Apgar score at the 1st min, points, Me (25%; 75%)	6 (4; 7)	6 (5; 7)	6 (5; 7)	$p_{1-2} = 0.818$ $p_{1-c} = 1.00$ $p_{2-c} = 1.00$
Apgar score at the 2nd min, points, Me (25%; 75%)	6 (5.5; 7)	7 (6; 7)	7 (6; 7)	$p_{1-2} = 0.132$ $p_{1-c} = 0.201$ $p_{2-c} = 1.00$
Age at the time of material collection, days, Me (25%; 75%)	9.0 (6.5; 13.5)	11.5 (7.0; 16.0)	6.5 (5.5; 7.0)	$p_{1-2} = 1.00$ $p_{1-c} = 0.043$ $p_{2-c} = 0.005$
Start of enteral feeding, days, Me (25%; 75%)	1 (1; 1)	1 (1; 3)	1 (1; 1)	$p_{1-2} = 0.008$ $p_{1-c} = 1.00$ $p_{2-c} = 0.081$
Operative delivery (caesarean section), n F ± m %	12 37.5 ± 8.6	17 56.7 ± 9.0	4 33.3 ± 13.6	$p_{1-2} = 0.131$* $p_{1-c} = 0.798$ $p_{2-c} = 0.172$
RDS, n F ± m %	21 65.6 ± 8.4	15 50.0 ± 9.1	–	$p_{1-2} = 0.303$** $p_{1-c} < 0.001$ $p_{2-c} = 0.003$
Total duration of respiratory support, days, Me (25%; 75%)	3 (1; 11)	2 (1; 6)	2 (1; 6)	$p_{1-2} = 1.00$ $p_{1-c} = 0.673$ $p_{2-c} = 1.00$
NEC, n F ± m %	3 9.4 ± 5.2	9 30.0 ± 8.4	–	$p_{1-2} = 0.041$** $p_{1-c} = 0.551$ $p_{2-c} = 0.041$
NE, n F ± m %	10 31.3 ± 8.2	9 30.0 ± 8.4	11 91.7 ± 8.0	$p_{1-2} = 1.00$** $p_{1-c} < 0.001$ $p_{2-c} < 0.001$
Duration of inpatient stay, days, Me (25%; 75%)	19.5 (13; 32)	31.5 (13; 38)	11.0 (9.5; 13.5)	$p_{1-2} = 0.359$ $p_{1-c} = 0.004$ $p_{2-c} < 0.001$

Notes: * — according to Pearson's χ^2 test; ** — according to the Fisher's two-tailed exact test, in other cases — according to Dunn's test; p_{1-2}, p_{1-c}, p_{2-c} — the level of statistical significance of the differences in indexes between the corresponding groups.

The analysis of the T-bet transcription factor expression in the cells of buccal mucosa scrapings of all the newborns showed that the T-bet expression was higher in premature children with the clinic of NEC (p = 0.022): 2.36 (1.94; 3.17) c.u. against 1.49 (1.0; 3.27) c.u. in children without signs of NEC. Moreover, we have traced the correlation between the T-bet expression level and NEC development in children (r = 0.271; p = 0.021). The distribution of T-bet expression levels among newborns with and without signs of NEC is shown in the Table 2.

We have proved the presence of the direct correlation between the level of T-bet and manifestations of NEC (r = 0.271; p = 0.021) and determined the inverse correlation between the level of miR-148a expression in the maternal BM and the level of T-bet expression: r = –0.371; p = 0.043 (Fig. 2).

Discussion

Necrotizing enterocolitis is the most common acute gastrointestinal pathology affecting mostly premature newborns. NEC incidence decreases with the increase of gestational age and weight of a newborn. Despite the fact that early diagnosis and active treatment of this disease have improved clinical outcomes, NEC remains the cause of serious long-term pathological conditions [13–15]. The preterm infant`s intestine is relatively more immature in structure and immune function; and is characterized by the development of an exaggerated inflammatory response toward the striking factors [16]. Genetic predisposition stands apart from the factors contributing to the development of NEC. Several studies indicate possible genetic variants associated with overexpression of Toll-like receptors 4 (TLR4), the stimulation of which promotes the release of IL-1 and IL-6 [17, 18].

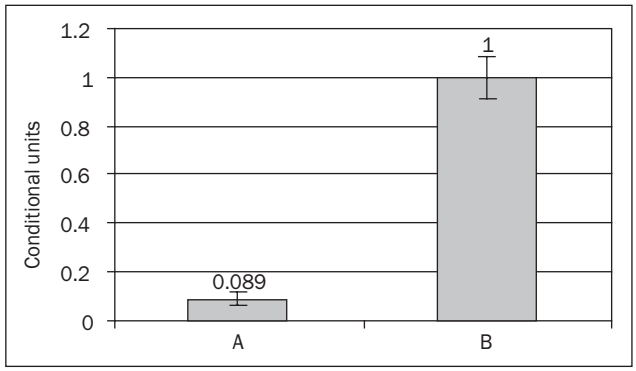

Figure 1. MiR-148a level comparison in the breast milk of the mothers of breastfed preterm neonates (A) and breastfed full-term neonates (B)

MicroRNA 148a is one of the most highly concentrated miRs in BM and is a representative of the miR-148/miR-152 family, which consists of three highly conserved and mature miRs (miR-148a, miR-148b, and miR-152), whose molecules are characterized by similar sequences and the presence of the UCAGUGCA region. The miR-148/miR-152 precursor with a stem-loop structure is cleaved in the nucleus and cytoplasm of the cell to form miR-148a, miR-148b, and miR-152. The human miR-148a coding sequence is located on the chromosome 7p15.2. The sequences encoding miR-148/miR-152 are similar to the human homeobox (HOX) genes [19].

The maximum concentration level of miR-148a in BM is noted in the early postpartum period. The concentration level of miR-148a in mature BM is significantly lower than in transitional BM. Milk formulas have significantly lower quantities of miR-148a compared to BM [20]. The results of our study demonstrate a probable decrease in the miR-148a concentration in the BM of mothers of preterm neonates, compared to the BM of mothers of full-term babies. At the same time, Yaffa Elbaum Shiff et al. [21] showed that the miR-148a concentration is higher in the BM of mothers of preterm infants than in the BM of mothers of full-term infants. Probably, the contrariety of the obtained data of our study and the study of Yaffa Elbaum Shiff et al. [21] is due to the peculiarities of the cohort of mothers of preterm newborns, as the concentration of miR-148a in BM depends on

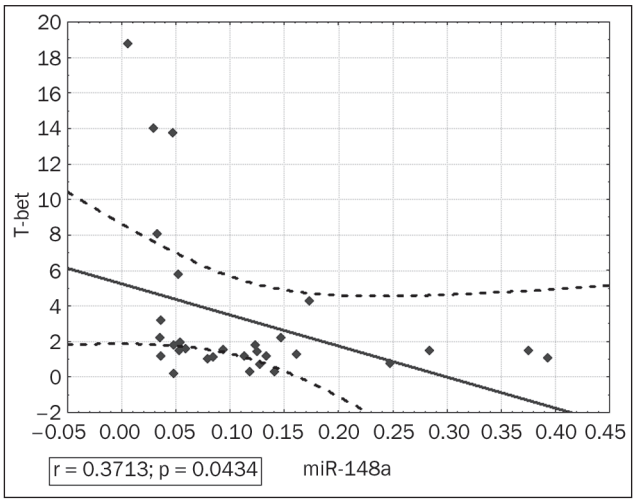

Figure 2. Correlation between the relative miR-148a expression levels in maternal breast milk and the T-bet gene expression in the buccal mucosa scraping cells of premature newborns who were breastfed

Table 2. MiR-148a levels in breast milk of the mothers, whose infants were born prematurely, and T-bet levels in buccal mucosal scraping cells of premature newborns with and without signs of NEC

Index	Expression level, c.u.		Significance of difference between the groups
	Premature newborns		
	with signs of NEC	without signs of NEC	
miR-148a	0.25 (0.05; 1.75)	0.08 (0.05; 0.13)	P_N-p_{wN} = 0.156
T-bet	2.36 (1.94; 3.17)	1.49 (1.0; 3.27)	P_N-p_{wN} = 0.022

Note: P_N-p_{wN} is the level of statistical significance of differences in indexes between the groups with signs of NEC (P_N) and without signs of NEC (p_{wN}) according to the Mann-Whitney test.

the state of health of the mothers and the medical treatment they receive. In particular, stress, negative emotions, and excess body weight of the mother contribute to a decrease of the miR-148a concentration in BM [22, 23], whereas oxytocin, melatonin, prolactin, dexamethasone intake has a positive effect on the level of miR-148a in BM [8].

The molecular targets of miR-148a are mRNAs of a wide range of proteins with various biological functions (Table 3) [24].

MicroRNAs of BM play a key role in the intestinal cells maturation, the establishment of intestine's barrier function and prevent NEC development [8]. MicroRNA 148a of BM reduces the expression of DNA methyltransferase 1 (DNMT1), which is its direct target, and promotes an increase in the expression of survivin, which inhibits the expression of apoptosis proteins. Therefore, miR-148a of BM promotes the survival and proliferation of epithelial cells of the digestive tract in newborn children [20]. Suppression of the miR-148a expression in BM can cause a decrease in ZO-1 level in the intestine due to an increase in DNMT1 activity. That will contribute to enhance of paracellular permeability [22]. MicroRNA 148a along with miR-143, miR-145,

Table 3. Molecular targets of miR-148a [25]

Gene	Functional group or protein action	miR-148a effects
1	2	3
BACH2	Transcription factor	Plasmatic cells development
BCL2l11	Apoptosis facilitator	Regulation of immune tolerance
CaMKIIα	Kinase	Regulation of innate response and antigen-presenting capacity of dendritic cells
CAND1	Regulator of ubiquitin ligases	Promotion of cell proliferation
CCKBR	G-protein coupled receptor	Cell proliferation
CDC2B	Kinase	Inhibition of cell growth and survival
CDKN1B	Cyclin-dependent kinase inhibitor 1B	Cell cycle inhibitor
c-myc	Transcription factor	Involvement in cell apoptosis
CTNNB1	β-catenin	Involvement in cell apoptosis
DNMT1	DNA methyltransferase	Inhibition of cell proliferation and migration, induction of apoptosis
DNMT3b	DNA methyltransferase	
ERBB3	Kinase	Cell proliferation
Gadd45a	Growth arrest and DNA damage inducible	Increased neuroblast differentiation and apoptosis
HLA-G	Human leukocyte antigen	Inhibition of immune evasion
HOTAIR	lncRNA	Inhibition of cell migration, invasion, cell proliferation, cell cycle progression
HPIP	Hematopoietic transcription factor pre-B cell leukemia	Inhibition of cell proliferation, migration, invasion
IGF-IR	Insulin-like growth factor receptor	Cell proliferation, colony formation, tumor angiogenesis
IRS1	Insulin receptor substrate	Cell proliferation, colony formation, tumor angiogenesis
ITGA11	Integrin	Inhibition of migration
ITGB8	Integrin	Inhibition of migration
MET	Kinase	Inhibition of mesenchymal-epithelial transition and metastasis
MIG6	Mitogen-inducible gene	Inhibition of cell growth and survival
MITF	Transcription factor	Development of plasmatic cells
MMP7	Peptidase 7	Tumor invasiveness
MSK1	Kinase	Attenuation of drug-resistance
NRP1	Neuropilin 1	Suppression of metastasis
PTEN	Phosphatase, tumor suppressor	Regulation of immune tolerance
QKI	RNA binding protein	Inhibition of cell migration, angiogenesis

The end of Table 3

1	2	3
ROCK1	Kinase 1	Inhibition of cell proliferation, mesenchymal-epithelial transition, migration, invasion
RUNX3	Transcription factor	Tumor suppression
S1P1	Chemoattractant receptor	Egress of lymphocytes from primary to secondary lymphatic organs
SKP1	Ubiquitin ligase complex	Inhibition of cell migration, angiogenesis
SMAD2	Intracellular signal transducer and transcriptional modulator	Inhibition of cell migration and invasion
TGIF2	Transcription factor	Cell proliferation and invasion
USP4	Ubiquitin-specific protease	Inhibition of cell growth, migration
VAV2	Guanine nucleotide exchange factor	Inhibition of migration
WASL	Wiskott-Aldrich syndrome protein	Inhibition of migration
WNT1	Signaling protein	Inhibition of cell metastasis
WNT10B	Signaling protein	Inhibition of migration

miR-452 and miR-26a is involved in the regulation of the inflammatory response. Thus, the miR-148 family representatives, by controlling the expression of IKKα, IKKβ, IL1R1, GP130 and TNFR2, reduce the NF-κB-associated pathway activation [26]. MiR-148a overexpression lowers the expression of TLR4 and significantly downregulates the expression level of MyD88, IRAK1 and TRAF6, which play a key role in the TLR/IL-1R signaling pathway activation. Consequently, the level of pro-inflammatory cytokines IL-1β and TNF-α decrease [27].

Research results of Li G. et al. [28] prove that an increase in the miR-148a level downregulates the level of p-p38 protein. This leads to a decrease in the level of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. It is believed that the miR-148/152 family representatives suppress the activity of TNF-α expression also by reducing the expression of matrix metallopeptidase (MMP) 10 and MMP13 [29]. Therefore, a low level of miR-148 concentration in the BM of mothers of preterm neonates may be one of the factors associated with a high risk of inflammation development in the intestinal mucosa in preterm newborns. On the other hand, we have proved that there is a significant increase in the miR-148a concentration in the BM of mothers, whose preterm children had clinical signs of NEC. In our opinion, the increase in the miR-148a concentration in the BM of mothers of preterm neonates with NEC is a protective reaction of the mother's body that prevents the development of the inflammatory process in the intestines of the child. In the experimental study, Miao-Miao Guo et al. [30] demonstrated that miR-148a-3p significantly reduces the expression of p53 and

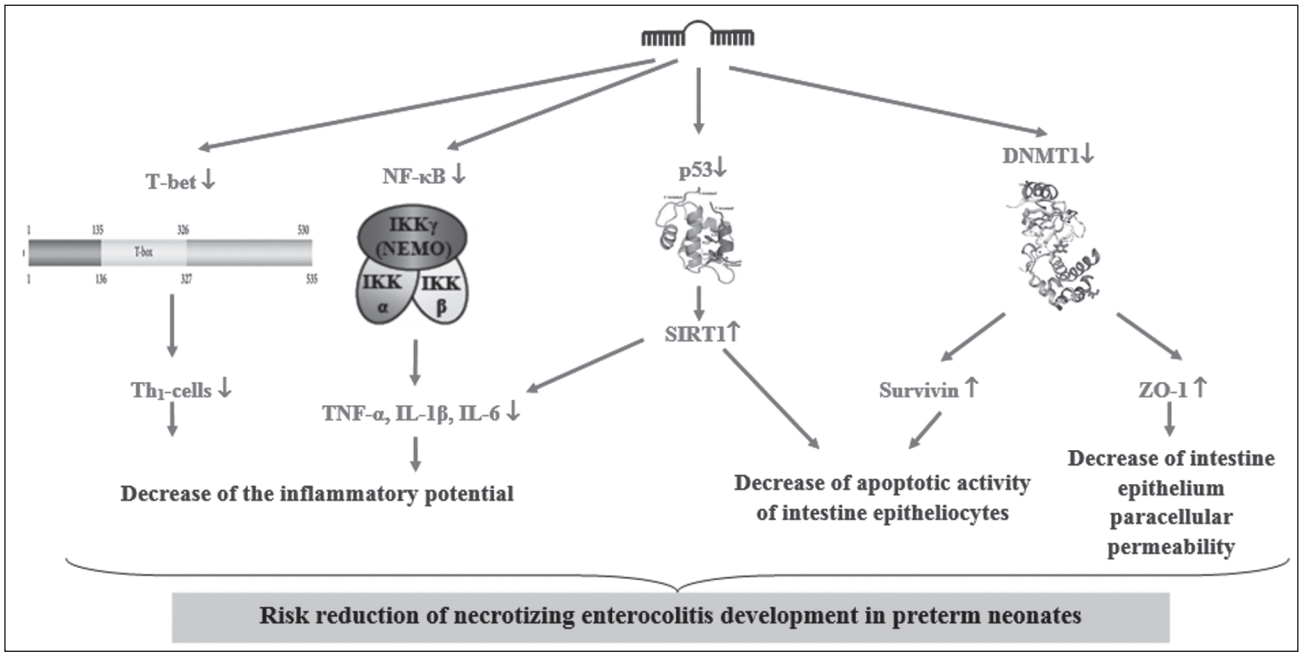

Figure 3. Scheme of maternal breast milk miR-148a impact on the necrotizing enterocolitis development in preterm newborns

increases the level of NAD-dependent deacetylase sirtuin 1 (SIRT1) in IEC6 intestinal epithelial cells treated with lipopolysaccharide. The authors believe that the miR-148a-3p/p53/SIRT1 axis has a protective effect, preventing the development of NEC, and agomir therapy may become a new direction for the NEC treatment. In our study, a tendency towards a decrease in the concentration of miR-148a in the BM of mothers of preterm newborns with NEC was revealed. In our opinion, the decrease in the concentration of miR-148a in BM may be a factor contributing to the NEC development in an infant.

Furthermore, our results prove the inhibitory effect of miR-148a of BM on the level of T-bet expression that probably leads to a decrease in the level of active Th1 cells in the intestinal mucosa and helps to reduce the intensity of inflammation in NEC. Previous studies have suggested a possible stimulatory effect of T-bet on the local production of miR-148a by the type of feedback [31].

In general, miR-148a of BM can be defined as a factor that significantly prevents the development of NEK in newborn children (Fig. 3).

Conclusions

1. In the breast milk of the mothers whose children were born prematurely and have a pathological course of the early adaptation period, the level of miR-148a expression is lower than in the BM of mothers whose children were born on time and have minimal signs of maladaptation.

2. An increase in the miR-148a expression is observed in the breast milk of mothers whose children were born prematurely and have signs of NEC.

3. The development of NEC is accompanied by an increase in the T-bet expression in the immunocytes of the buccal mucosa scraping, which can be used for early non-invasive diagnosis of intestinal inflammatory changes in preterm neonates.

4. MicroRNA-148a of BM downregulates the T-bet expression level in the immunocytes of the intestinal mucosa of a neonate. That helps to reduce the activity of the inflammatory process.

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Протизапальний вплив miR-148a грудного молока на стан слизових оболонок у недоношених новонароджених дітей

Резюме. Актуальність. Грудне молоко (ГМ) — оптимальний продукт харчування для новонароджених, джерело екзогенних мікроРНК (miR). Одним з представників висококонцентрованого пулу miR ГМ є miR-148a. Сьогодні бракує даних про вплив miR-148a на розвиток некротизуючого ентероколіту (НЕК) у недоношених новонароджених дітей. **Мета дослідження**: вивчити вплив miR-148a ГМ матері на ймовірність розвитку НЕК у недоношених новонароджених дітей. **Матеріали та методи**. Обстежено 74 новонароджених, які проходили лікування в неонатальних відділеннях. Визначено рівень miR-148a в ГМ 44 матерів новонароджених дітей, які перебували на виключно грудному вигодовуванні (ГВ). Паралельно проводилося визначення експресії генів фактора транскрипції T-bet у клітинах зіскрібка букальної слизової оболонки всіх новонароджених. Було виділено три групи порівняння: першу становили 32 новонароджені до 37 тижнів гестації на ГВ, другу — 30 недоношених новонароджених на штучному вигодовуванні, контрольну — 12 доношених новонароджених на ГВ. **Результати.** Медіана гестаційного віку дітей 1-ї групи становила 33 (31; 34) тижні, 2-ї — 32,5 (32; 35) тижня і порівняно вищою була в групі контролю ($p < 0,001$) — 40 (39; 41) тижнів. Неонатальна енцефалопатія як основний діагноз зустрічалася частіше серед доношених новонароджених ($p < 0,001$). Діти 1-ї та 2-ї груп суттєво не відрізнялися за частотою випадків респіраторного дистрес-синдрому, неонатальної енцефалопатії ($p > 0,05$). У 2-й групі порівняно з 1-ю вірогідно частіше ($p < 0,05$) зустрічалися прояви НЕК: 9/30,0 ± 8,4 % проти 3/9,4 ± 5,2 %. Виявлено, що рівень експресії miR-148a в ГМ матерів недоношених дітей на ГВ був вірогідно нижчий ($p < 0,001$), ніж у групі доношених: 0,089 (0,048; 0,142) ум.од. проти 1,0 (1,0; 1,0) ум.од. Рівень експресії фактора транскрипції T-bet у клітинах зіскрібка букальної слизової оболонки був вищий у недоношених дітей із клінікою НЕК ($p = 0,022$): 2,36 (1,94; 3,17) ум.од. проти 1,49 (1,0; 3,27) ум.од. у дітей без ознак НЕК. Доведено наявність прямого зв'язку між рівнем T-bet і проявами НЕК ($r = 0,271$; $p = 0,021$) та визначено зворотний кореляційний зв'язок між рівнем експресії miR-148a в ГМ матері та рівнем експресії T-bet ($r = –0,371$; $p = 0,043$). **Висновки.** У ГМ матерів, діти яких народилися передчасно і мають проблеми з адаптацією, рівень експресії miR-148a відносно нижчий, ніж у матерів, які народили в строк. При розвитку НЕК спостерігається підвищення рівня miR-148a в ГМ матері, що сприяє зниженню експресії T-bet слизових оболонок дитини і чинить протекторний вплив на стінки кишечника.

Ключові слова: miR-148a; T-bet; недоношені; новонароджені; некротизуючий ентероколіт