

DNA methyltransferase 1-targeting miRNA-148a of dairy milk: a potential bioactive modifier of the human epigenome

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ABSTRACT:

Background: The perception of milk has changed from a “simple food” to a more sophisticated bioactive functional signaling system that promotes mTORC1-driven postnatal anabolism, growth, and development of the newborn infant. Accumulating evidence supports the view that milk’s miRNAs significantly contribute to these processes. The most abundant miRNA of milk found in milk fat and milk exosomes is miRNA-148a, which targets DNA methyltransferase 1 (DNMT1), a pivotal epigenetic regulator that suppresses transcription. Furthermore, milk-derived miRNA-125b, miRNA-30d, and miRNA-25 target TP53, the guardian of the genome that interacts with DNMT1 and regulates metabolism, cell kinetics, and apoptosis. Thus, the question arose whether cow’s milk-derived miRNAs may modify epigenetic regulation of the human milk consumer.

Methods: To understand the potential impact of dairy milk consumption on human epigenetics, we have analyzed all relevant research-based bioinformatics data related to milk, milk miRNAs, epigenetic regulation, and lactation performance with special attention to bovine miRNAs that modify gene expression of DNA methyltransferase 1 (*DNMT1*) and p53 (*TP53*), the two guardians of the mammalian genome. By means of translational research and comparative functional genomics, we investigated the potential impact of cow’s milk miRNAs on epigenetic regulation of human *DNMT1*, *TP53*, *FOXP3*, and *FTO*, which are critically involved in immunologic and metabolic programming respectively. miRNA sequences have been obtained from mirbase.org. miRNA-target site prediction has been performed using TargetScan release 7.0.

Results: The most abundant miRNA of cow's milk is miRNA-148a, which represents more than 10% of all miRNAs of cow's milk, survives pasteurization and refrigerated storage. The seed sequence of human and bovine miRNA-148a-3p is identical. Furthermore, human and bovine DNMT1 mRNA share 88% identity. The miRNA-148a 7mer seed is conserved in human and bovine DNMT1 mRNA respectively, which may allow for the strong binding of bovine miRNA-148a to human DNMT1 mRNA. Consequently, we hypothesize that bovine milk miRNA-148a - protected by highly resistant milk exosome membranes - may reach the systemic circulation of the milk consumer targeting and suppressing human *DNMT1* mRNA. Attenuated DNMT1 expression associated with reduced CpG promoter methylation upregulates gene expression of developmental genes such as *FOXP3* and *FTO*. Milk-derived miRNA-125b, miRNA-30d, and miRNA-25 via targeting *TP53* may downregulate p53, which physically interacts with and stabilizes DNMT1. Enhancement of dairy lactation performance is associated with increased expression of bovine milk miRNA-148a, a modification that may further increase the miRNA-148a load of dairy milk.

Conclusion: Translational evidence and comparative functional genomics support our hypothesis that bovine milk miRNA signaling may suppress human DNMT1-mediated epigenetic regulation and p53 signaling, which closely interacts with the epigenetic and transcriptional regulation of growth, metabolism, cell cycle progression, and apoptosis. Human and bovine milk miRNAs are able to target *DNMT1* and *TP53* mRNAs, share identical seed sequences, and resist pasteurization. Pasteurization and refrigeration of dairy milk conserves the gene regulatory software of milk and allows its unrestricted entry into the human food chain. The continued exposure of modern humans to milk's epigenetic machinery since the widespread distribution of refrigerators is a novel change of human nutrition which may promote diseases of Western civilization.

Keywords: adipogenesis, dairy, DNA methyltransferase 1, epigenetics, exosome, miRNA-148a, miRNA-125b, milk, obesity, p53, Parkinson disease, prostate cancer

BACKGROUND

Milk has been identified as a postnatal signaling system that promotes postnatal metabolic and immunological programming [1-8]. According to the functional hypothesis [9,10], milk-derived miRNAs may play a key role in milk signaling. Milk is the human body fluid that contains the highest amounts of RNAs and miRNAs [10]. These miRNAs are predominantly secreted by mammary epithelial cells (MECs) and are transported via extracellular vesicles (exosomes) to fulfill their regulatory tasks in the complex setting of mammalian reproduction [5-8,12]. There is increasing evidence that this specific encapsulation of milk miRNAs in exosomes (30-100 nm in diameter) and exosome-like vesicles (> 100 nm) confers protection against miRNA degradation and creates a long-distance signaling pathway for intestinal and vascular endothelial transport by endocytosis, in addition to miRNA delivery to peripheral tissues [13-20]. It has recently been demonstrated that human milk exosomes and their miRNAs survive digestion *in vitro* and are taken up by human intestinal cells [21]. Moreover, trans-epithelial transport of bovine milk exosomal miRNAs across intestinal Caco-2 cell monolayers indicated their potential to cross the human intestinal barrier [13,22]. Recent labeling studies support the view that bovine milk exosomes

including their RNAs reach the systemic circulation and are bioavailable after oral administration to mice [23-26].

Cow's milk exosomes protect miRNAs against harsh digestive processes and enable their crossing of the intestinal barrier to reach the blood circulation for distant cellular effects [22]. Notably, there is no significant difference in the levels of miRNA-148a, miRNA-21, and miRNA-25 between *in vitro* digested exosomes and their respective undigested controls [22]. Importantly, miRNA-148a has been identified as the most prevalent miRNA of human and bovine skim milk and milk fat comprising more than 10% of all milk-derived miRNAs [27-29]. It has recently been confirmed that the great majority of human and bovine milk miRNAs including miRNA-148a and miRNA-200c survive pasteurization [27, 29]. Consumption of commercial milk resulted in a dose-dependent increase of miRNA-200c and miRNA-29b in peripheral blood mononuclear cells of healthy adult human volunteers and corresponding changes in gene expression [30]. Thus, accumulating evidence points to the functional capability of human and bovine milk miRNAs to survive gastrointestinal degradation, pasteurization, homogenization, and refrigerated storage [15, 21, 27, 29, 31, 32]. It has recently been demonstrated that the expression of miRNA-148a of normal human colon cells (CRL1831) and K562 human chronic myeloid leukemia cells increased after incubation with either milk exosomes or the fat layer isolated from human milk [29]. In fact, the increased cellular expression of miRNA-148a was associated with a significant decrease in the expression of DNMT1 [14]. Taken together, accumulating indirect evidence supports our hypothesis that bovine milk miRNA-148a enters the human food chain and may modify human DNMT1 expression and systemic gene regulation of the milk recipient [7].

METHODS

By means of translational research and comparative functional genomics, we investigated the potential impact of bovine milk miRNAs on epigenetic regulation of human *DNMT1* and *TP53*, which are critically involved in gene regulation and transcription. miRNA sequences have been obtained from mirbase.org. Target site prediction has been performed using Target Scan (Release 7.0) [33].

RESULTS

Human DNMT1: A potential target of bovine milk miRNA-148a

DNA methylation is one of the best characterized epigenetic modifications. It is generally accepted that DNA methylation promotes gene silencing, while DNA demethylation enhances transcription [34]. DNA methylation serves as a cellular memory system and is dynamically regulated through the action of DNA methyltransferases (DNMTs) [34]. Remarkably, DNA methylation and histone methylation are coupled processes. Notably, DNA methyltransferase 1 (DNMT1), its accessory protein UHRF1 and their associated proteins help maintain DNA methylation and histone methylation through mitotic cell division in a coordinated way [35]. The function of DNMT1 is tightly related to growth control [36]. DNA methylation represses genes partly by the recruitment of the methyl-CpG-binding protein MeCP2, which in turn leads to recruitment of histone deacetylases [37]. DNMT1 is associated with histone deacetylase activity *in vivo* [37]. One of the known histone deacetylases, HDAC1, directly binds to DNMT1 [37]. Thereby, DNMT1-mediated

DNA methylation alters the chromatin state via HDAC activity [37]. The DNMT1 complex with HDAC1, Rb, and E2F1 represses transcription from E2F-responsive promoters [37], suggesting their involvement in transcriptional repression of chromatin by means of histone deacetylation [38]. DNMT1 activity via maintenance of the appropriate histone H3 modifications contributes to the preservation of the correct organization of large heterochromatic regions [39]. Furthermore, DNMT1 has been shown to physically interact and bind to the guardian of the genome p53 and co-localizes with p53 in the nucleus [38]. Upon p53 induction, a reporter construct containing the promoter of the anti-apoptotic gene survivin (*BIRC5*), which contains a natural p53 binding site, was methylated in wild type HCT116 cells but not in DNMT1 null or p53 null cells. Endogenous survivin gene repression involves cooperation between DNMT1 and p53, which is relieved by introduction of DNMT1- or p53-specific small inhibitory RNA (siRNA) [38]. p53 stabilizes the p53-DNMT1-HDAC1 complex [38]. DNMT1 potentially provides a key interaction via DNA methylation recruiting HDAC1 and acting as a bridge between nuclear p53 and chromatin, reinforcing a repressed chromatin state [40].

It is conceivable that milk, which is donated during the postnatal growth period of newborn mammals, enhances gene transcription and growth by suppressing DNMT1 gene expression. Do et al. [41] recently confirmed that miRNA-148a belongs to the most abundantly expressed miRNAs of bovine milk since it accounts for more than 10% of the read counts in each stage of dairy cow lactation. miRNA-148a has also been identified as the most abundant miRNA in human milk [21, 28, 29]. miRNA-148a, miRNA-148b, and miRNA-152 are three members of the miRNA-148/152 family which share substantial homology in their seed sequences [42]. Notably, the seed sequence of human (*hsa*) and bovine (*bta*) miRNA-148a-3p are identical (Table 1). It is of functional importance that miRNA-148a directly targets DNMT1 [43]. It has been demonstrated that the expression of miRNA-148a of normal human colon cells (CRL1831) and K562 human leukemia cells increased after incubation with milk exosomes and the fat layer isolated from human milk, respectively [29]. This was associated with a significant decrease in the expression of DNMT1 in both cell types [29]. This experiment is a proof-of-concept demonstrating that the addition of milk exosomes, a source of MEC-derived miRNA-148a, increases intracellular miRNA-148a levels of recipient cells resulting in corresponding downregulation of DNMT1 expression. For instance, gene expression of the key transcription factor of regulatory T-cells, *FOXP3*, critically depends on epigenetic regulation and is upregulated by *FOXP3* promoter demethylation [44]. Admyre *et al.* [45] showed that the addition of human milk exosomes to peripheral blood mononuclear cells significantly enhanced the expression of FoxP3. In infants with cow's milk allergy, a linear correlation between the extent of *FOXP3* promoter demethylation and FoxP3 expression could be detected [46]. These observations are in line with our view that milk exosome-derived miRNA-148a via targeting DNMT1 enhances FoxP3 expression [4,27]. There are further genes such as *INS*, *IGF1*, and *FTO* which promote anabolism and growth, that are also epigenetically upregulated via promoter demethylation recently reviewed elsewhere [7]. Apparently, epigenetic upregulation of developmental and lactation-related genes by miRNA-148a-mediated suppression of DNMT1 appears to represent a key epigenetic signature of lactation and milk signaling.

miRNA-21 is another abundant miRNA of human and cow's milk [29, 47], which indirectly inhibits DNMT1 expression by targeting Ras guanyl nucleotide-releasing protein-1 (RASGRP1)

[46]. The expression of DNMT1 has been inversely related to the expression miRNA-148a and its homolog miRNA-152 [29,48,49].

Table 1. Sequence of DNMT1-targeting human and bovine miRNA-148a (seed in bold; mirbase.org)

hsa-miRNA-148a-3p 5' UCAGUG CACUACAGA ACUUUGU	bta-miRNA-148a-3p 5' UCAGUG CACUACAGA ACUUUGU
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The recent study of Golan-Gerstl et al. [29] demonstrates that miRNA-148a-3p sequences are highly conserved among various species including humans (*Homo sapiens*) and cows (*Bos taurus*). This means that bovine milk miRNA-148a may be able to target human DNMT1 mRNA.

As shown in Table 2 human DNMT1 mRNA contains a potent 7mer target site for human and bovine miRNA-148a.

Table 2. Predicted base pairing of human and bovine miRNA-148a with position 48-55 of human DNMT1 3'UTR (seed in bold; TargetScan)

hsa-miRNA-148a-3p	3'	UGUUUCAAGACAUC ACGUGACU
		II II III
DNMT1 3'UTR (position 48-55)	5' ...	CAGGAAUCCCCAACA UGCACUGA ...
		II II III
bta-miRNA-148a-3p	3'	UGUUUCAAGACAUC ACGUGACU

Bovine DNMT1 mRNAs displays a strong sequence homology to human DNMT1 mRNA [50, 51]. Remarkably, all of the DNMTs with an important role in DNA methylation including DNMT1 show a greater degree of structural similarity between human and bovine than between human and mouse [51]. Human DNMT1 contains 1616 amino acids and bovine DNMT1 1611 amino acids, respectively. Amino acid sequence identity of 88% has been reported [51].

Bovine miRNA-148a promotes lactation performance

Genetic and epigenetic selection of dairy cows intended to increase lactation performance and milk yield further enhanced the expression of lactation-promoting miRNAs, especially of miRNA-148a [41, 52]. Co-expression network and pathway analyses correlated abundantly expressed bovine miRNA-148a with milk yield [53]. Accordingly, Wang *et al.* [54] reported that the expression of the miRNA-148a family member miRNA-152 significantly increased during lactation in MECs of dairy cows producing high quality milk compared to the lower miRNA-152 levels in cows producing low quality milk. The forced expression of miRNA-152 in dairy cow MECs resulted in a marked reduction of DNMT1 at both the mRNA and protein levels [54]. Additionally, miRNA-148a has been shown to induce milk triacylglycerol synthesis in goat MECs [55]. Intriguingly, the expression of miRNA-148a was significantly enhanced by hormone (dexamethasone, insulin, and

prolactin) treatment. Significantly, increased extracellular levels of miRNA-148a have been detected in MEC culture medium pointing to an extracellular transfer of miRNA-148a [56]. Altogether, increased dairy cow lactation performance is associated with the enhanced expression of bovine miRNA-148a/152, which may increase in dairy milk obtained from high performance dairy cows. Thus, experimental evidence supports the fact that bovine miRNAs of the miRNA-148a/152 family suppress DNMT1 increasing lactation and milk yield.

Interaction between DNMT1 and p53

Endogenous survivin gene repression involves cooperation between DNMT1 and p53 and is relieved by introduction of DNMT1- or p53-specific small inhibitory RNA [38]. It is thereby conceivable that milk-derived miRNAs may target TP53 mRNA and further augment the epigenetic activity of miRNA-148a-mediated DNMT1 silencing. In fact, miRNAs are important regulators of p53 expression and its signaling pathway [57]. As discussed for the survivin promoter, p53 stabilizes the p53-DNMT1-HDAC1 complex (Fig. 1) [38]. The expression of the p53 gene (*TP53*) is tightly regulated via transcriptional and post-translational modulations. Le *et al.* [58] demonstrated that miRNA-125b is a *bona fide* negative regulator of p53 in both zebrafish and humans. miRNA-125b-mediated down-regulation of p53 is strictly dependent on the binding of miRNA-125b to a miRNA response element in the 3'-UTR of *TP53* mRNA. Notably, miRNA-125b regulation of p53 is conserved at the network level in all vertebrates [59]. Milk contains abundant miRNA-125b, which has been demonstrated in human [60], bovine [15, 32], and porcine milk exosomes [61] respectively. Further known p53- targeting miRNAs are miRNA-30d, miRNA-25, and miRNA-504 [62]. miRNA-25 and miRNA-30d directly target the 3'-UTR of *TP53* downregulating p53 protein levels, thereby reducing the expression of p53-regulated genes [62]. miRNA-30d has been detected as a signature miRNA of mature raw and commercial milk of dairy cows [47]. miRNA-30d has also been found in porcine milk exosomes and in human milk [32, 63, 64]. Additionally, miRNA-25-3p has been observed in human and porcine milk exosomes [60, 63]. Remarkably, the mature seed sequences of human and bovine miRNA-125b, miRNA-25, in addition to miRNA-30d, are identical (Table 3).

Table 3. Sequences of p53-targeting human and bovine microRNAs (seed in bold; mirbase.org)

hsa-miRNA-125b 5' UCCCUGACACCCUAACUUGUGA	bta-miRNA-125b 5' UCCCUGACACCCUAACUUGUG
hsa-miRNA-30d 5' UGUAAACAUCCCCGACUGGAAG	bta-miRNA-30d 5' UGUAAACAUCCCCGACUGGAAGCU
hsa-miRNA-25-3p 5' AGUCUGGCUCUGUUCACGUUAC	bta-miRNA-25-3p 5' AGUCUGGCUCUGUUCACGUUAC

miRNA-125b exhibits the strongest binding affinities of the three TP53-targeting miRNAs detected in milk. It is significant to mention that these milk-derived miRNAs target different sites of the *TP53* 3'UTR (Table 4).

Table 4. Predicted base pairing of miRNA-125, miRNA-30d, and miRNA-25 with human *TP53* 3'UTR mRNA (seed in bold; TargetScan).

hsa-miRNA-125-5p	3'	AGUGUCAAUCC CAGAGUCCCU	
			I I I I I I
<i>TP53</i> 3'UTR (position 1022-1026)	5'...	AAGACUUGUUUUAUG CUCAGGGU ...	
			I I I I I
bta-miRNA-125p	3'	GUGUCAAUCC CACAGUCCCU	
<hr/>			
hsa-miRNA-30d-5p	3'	GAAGGUCAGCCCCUA- - CAAUGU	
			I I I I I
<i>TP53</i> 3'UTR (position 576-582)	5'	UGCAGUUAAGGGUUAG UUUACAA ...	
			I I I I I
bta-miRNA-30d-5p	3'	UCGAAGGUCAGCCCCU ACAAUGU	
<hr/>			
hsa-miRNA-25-3p	3'	AGUCUGGCUCUGUUC ACGUUAC	
			I I I I I
<i>TP53</i> 3'UTR (position 381-387)	5'...	CUUUGAACCCUUGCU UGCAAUAG ...	
			I I I I I
bta-miRNA-25-3p	3'	AGUCUGGCUCUGUUC ACGUUAC	

Altogether, these data imply that milk-derived miRNAs, especially milk miRNA-125b, may impair the expression of p53 and consequently p53-mediated gene regulation in milk consuming humans. Importantly, p53, the guardian of the genome [65], controls a vast regulatory transcriptional network including most critical checkpoints of cell cycle progression, apoptosis, and metabolism [66, 67], in addition to interacting with and stabilizing DNMT1 [38].

DISCUSSION

The abundance of miRNA-148a in milk and its high interspecies homology implies that milk miRNA-mediated regulation is a highly conserved archaic signaling pathway of mammals. Recent data support the view that dairy milk miRNAs including miRNA-148a survive pasteurization, processing, and refrigerated storage and thereby enter the human food chain. Efforts of the dairy industry to increase milk yield are associated with exaggerated expression of milk miRNA-148a/miRNA-152 and suppression of bovine MEC *DNMT1* [41, 53-55]. Significantly, attenuated bovine p53 expression of domestic cows with extended lactation persistence compared to the wild type *Bovidae*, which has recently been explained by a loss of Bov-A2, a short interspersed nuclear element in the promoter region of *TP53* [68]. The identical seed sequence of human and bovine miRNA-148a and its transport via milk exosomes and milk fat globules allows the prediction that dairy milk miRNA-148a reaches the systemic circulation of the milk consumer where it may attenuate human *DNMT1* mRNA expression. DNMT1 is a key regulator of DNA methylation,

histone methylation, histone deacetylation, and p53 interaction. Thus, it is conceivable that bovine milk-derived miRNA-148a via targeting human *DNMT1* modifies human gene expression. Physiologically, milk-induced epigenetic regulation may serve to enhance gene expression only during the period of postnatal development and infant growth. Significantly, promoter CpG demethylation of *FTO* and *FOXP3* and other important developmental genes such as *INS*, *IGF1*, *NRF2*, *CAVI*, *SREBP1*, *GLUT1*, *NR4A3*, and *LCT* have been associated with increased gene expression involved in metabolic programming and immune homeostasis [69, 70] (Figure 1).

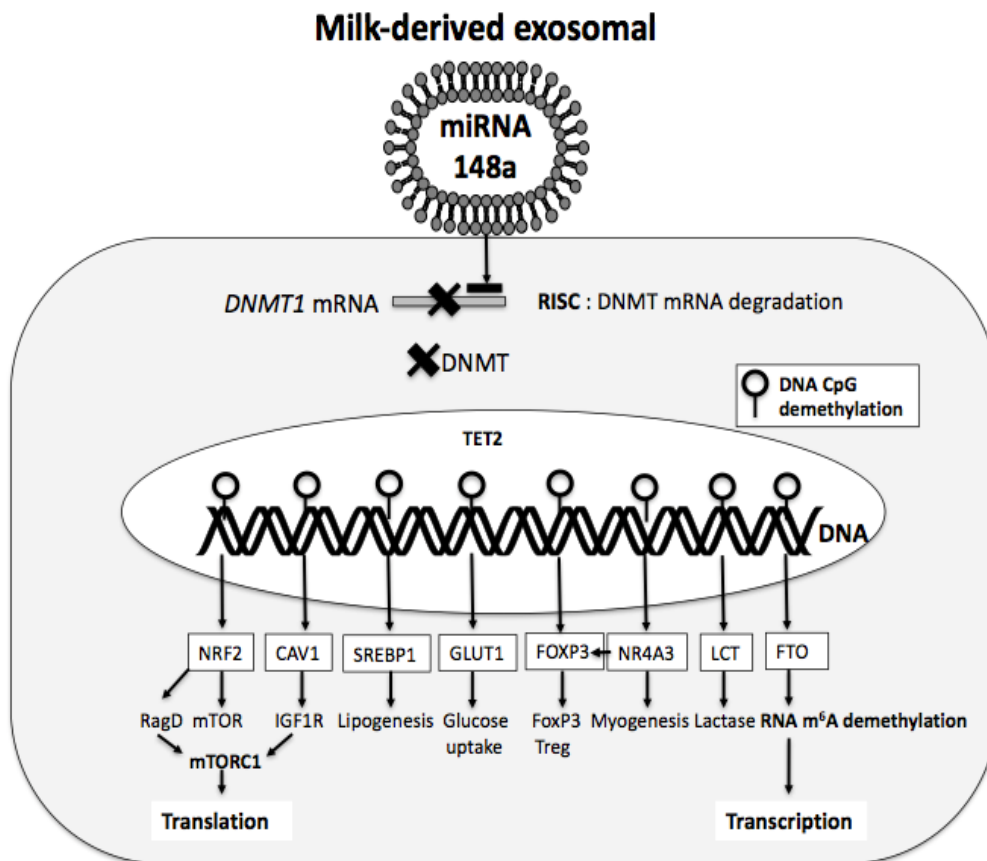


Figure 1. Working model of milk-miRNA-148a-mediated DNMT1 silencing associated with increased gene expression of important developmental genes. Modified according to [7].

Obesity. Recently, miRNA-148a has been associated with obesity in humans and adipocyte differentiation of mesenchymal stem cells through Wnt signaling [71]. miRNA-148a is highly abundant in adipose tissue [72, 73]. miRNA-148a-mediated silencing of DNMT1 accelerates adipocyte differentiation of 3T3-L1 cells [74]. Additionally, miRNA-148a has been identified as a downstream effector of X-box-binding protein 1 that silences Wnt10b during adipogenesis of 3T3-L1 cells [75]. Furthermore, Wnt10b, a strong negative regulator of adipogenesis, has been identified as a direct target miRNA-148a [76]. Persistent intake of bovine miRNA-148a by continued consumption of pasteurized whole and skim milk may thereby be an unrecognized pathogenic factor promoting adipogenesis and obesity in civilized milk-consuming societies.

Furthermore, milk-derived miRNAs such as miRNA-125b via targeting *TP53* may disrupt p53-DNMT1-HDAC1 interaction promoting gene expression and unfolding of chromatin structure. The suppression of DNMT1 and p53 apparently represents milk's epigenetic mode of action promoting transcription, postnatal growth, and adipogenesis (Figure 2).

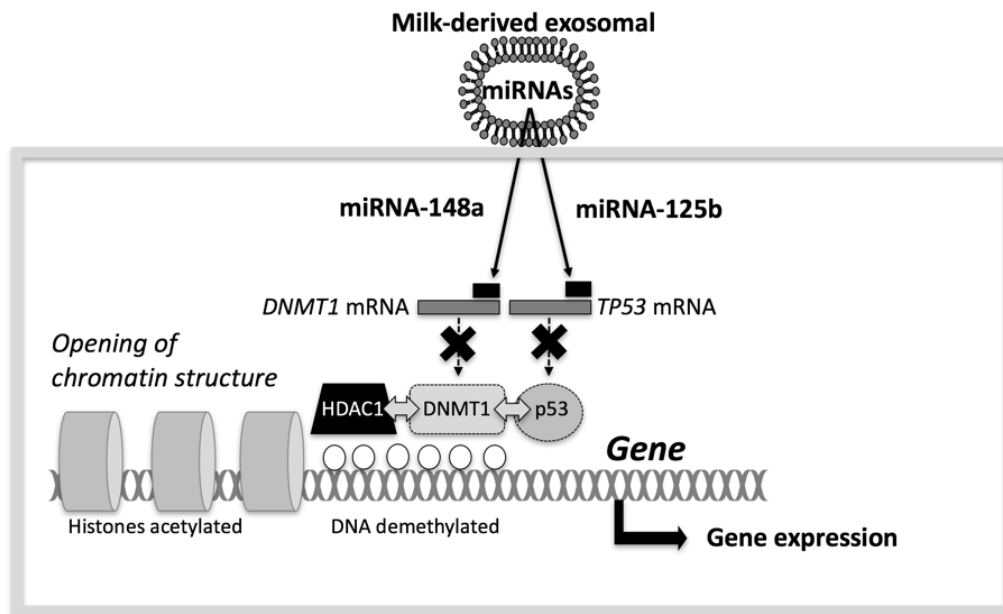


Figure 2. Working model illustrating the potential impact of milk-derived miRNAs targeting DNMT1 and p53 expression, the key epigenetic and transcriptional regulators of the milk recipient promoting gene expression and transcription.

Interestingly, p53 knockout enhanced adipogenesis and the expression of adipogenic marker genes, whereas its overexpression markedly reduced adipogenesis and marker gene expression in 3T3-L1 preadipocytes [77]. Newly weaned mice who had access to whole cow's milk for 17 weeks consumed more calories and increased body and fat mass [78]. Remarkably, a recent meta-analysis identified *MIR148A* as a new locus associated with increased body mass in individuals of African ancestry [79], which is associated with obesity and type 2 diabetes mellitus [80]. The EPIC-Interact study (n=340, 234) [81] demonstrated an increased association of type 2 diabetes mellitus with the consumption of unfermented cow's milk in contrast to fermented milk products such as yoghurt, which are depleted in exosomal miRNAs [19].

Prostate cancer. Lee *et al.* [82] demonstrated that reduced expression of DNMT1 plays an important role in the induction of epithelial-mesenchymal transition (EMT) and cancer stem cells (CSC) phenotype by prostate cancer (PCa) cells, with enhanced tumorigenesis and metastasis. The authors confirmed that silencing DNMT1 is correlated with enhancement of the induction of EMT and the CSC phenotype in PCa cells [82]. Thus, milk-miRNA-148a-mediated suppression of DNMT1 may promote EMT and CSC phenotype of PCa cells. A population-based Swedish study provided evidence that lactose intolerance, which is associated with a reduced intake of milk-derived miRNAs, reduces the risk of lung, breast, and ovarian cancers [83]. The incidence of lactose intolerance in PCa patients has been reported to be less than that in the general population

[84]. A significantly greater number of PCa patients in the lactose-tolerant group had a milk intake >500 ml/day than those in the intolerant group [84].

Parkinson's disease. DNMT1 is abundantly expressed in the adult brain [50] and is mainly located in the nuclear compartment, where it has access to chromatin. Hypomethylation of CpG islands at intron 1 of the SNCA gene has been reported to result in overexpression of α -synuclein in Parkinson's disease (PD) [85]. In fact, reduced nuclear DNMT1 levels have been detected in human postmortem brain samples from PD patients and dementia with Lewy bodies (DLB) [85]. Quantitative analysis by clonal assay showed that the CpG 2 of SNCA was hypomethylated in PD patients compared with the normal control [86]. Furthermore, the methylation rate at CpG 4 in intron 1 of SNCA and the overall mean methylation rate at these sites were significantly lower in DLB patients than in healthy controls [87]. Milk-miRNA-148a-mediated suppression of DNMT1 may thereby explain the overexpression of α -synuclein, a key player involved in the pathogenesis of PD and DLB. Epidemiological evidence showed significant positive associations for PD risk in men with lactose intake [88], which again may be indicative of persistent milk miRNA uptake.

CONCLUSION

In all mammals except humans, milk's epigenetic signaling is restricted to the postnatal period. Only modern humans may be exposed during their entire life period to milk-derived miRNA signaling and milk-dependent epigenetic regulation. In this regard, pasteurized dairy milk represents a most critical functional food that transfers archaic, highly conserved miRNAs into the human food chain. It should be kept in mind that already minor amounts of miRNAs exert significant biological effects [89], especially if they are provided consistently. Before the era of the refrigerator, humans mostly consumed fermented milk and fermented milk products. Recent evidence underlines that fermentation deteriorates milk-derived exosomal miRNAs [19], while pasteurization and refrigerated storage of milk exposes the human milk consumer to bioactive miRNAs of milk [21, 22,27-32].

In terms of evolutionary biology, persistent consumption of the milk of another species is a relatively novel behavioral pattern in human nutrition [90]. In the 1950s, this pattern was modified by the introduction of pasteurization and refrigeration allowing milk's miRNAs to enter the human food chain [7]. Milk is apparently the most active functional food with greatest impact on human development, growth, health and disease. Continued milk-miRNA-mediated downregulation of DNMT1 and p53 may not only explain the increase in linear growth and body mass of milk consuming children [91-95], but also the long-term increased risk of PCa and PD [96-101]. A multitude of biological parameters explains why shorter or smaller people have lower risks of cardiovascular disease and greater longevity [102]. In contrast, higher milk intake increases linear growth and mortality [92, 98].

Many factors such as the precise kinetics and uptake of dairy milk miRNAs by human milk consumers are still uncertain, which thereby warrants further investigations. Future miRNA research should thus focus on milk miRNA-148a and miRNA-125b uptake and its predicted impact on DNMT1 and p53 gene expression in human milk consumers and its long-term consequences on human health. We recommend to eliminate dairy milk miRNAs from the human food chain.

List of Abbreviations: CSC, cancer stem cell; DLB, dementia with Lewy bodies; DNMT1, DNA methyltransferase 1; FDX3, forkhead box P3; FTO, fat mass- and obesity- associated gene; HDAC1, histone deacetylase 1; miRNA, micro-ribonucleic acid, MEC, mammary epithelial cell, mTORC1, mechanistic target of rapamycin complex 1; PCa, prostate cancer; PD, Parkinson disease; SNCA, synuclein alpha, TP53, tumor protein p53; UHRF1, ubiquitin-like protein containing PHD and ring finger domains 1; UTR, untranslated region;

Authors Contributions: B.C.M. designed the research, formulated the hypothesis and wrote major parts of the manuscript. G.S. checked the miRNA data bases and controlled computed miRNA base pairing research. Both authors approved the final manuscript.

Competing Interests: There are no conflicts of interest to declare.

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