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ORIGINAL ARTICLE

Regulatory roles of miR-155 and let-7b on the expression of inflammation-related genes in THP-1 cells: effects of fatty acids

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Abstract

The main aim of this investigation was to study the regulatory roles of let-7b and miR-155-3p on the expression of inflammation-associated genes in monocytes, macrophages, and lipopolysaccharide (LPS)-activated macrophages (AcM). A second goal was to analyze the potential modulatory roles of different fatty acids, including oleic, palmitic, eicosapentaenoic (EPA), and docosahexaenoic (DHA), on the expression of these miRNAs in the three cell types. This hypothesis was tested in human acute monocytic leukemia cells (THP-1), which were differentiated into macrophages with 2-O-tetradecanoylphorbol-13-acetate (TPA) and further activated with LPS for 24 h. Monocytes, macrophages, and AcM were transfected with a negative control, or mimics for miR-155-3p and miR-let-7b-5p. The expression of both miRNAs and some proinflammatory genes was analyzed by qRT-PCR. Interestingly, let-7b mimic reduced the expression of *IL6* and *TNF* in monocytes, and *SERPINE1* expression in LPS-activated macrophages. However, *IL6*, *TNF*, and *SERPINE1* were upregulated in macrophages by let-7b mimic. *IL6* expression was higher in the three types of cells after transfecting with miR-155-3p mimic. Similarly, expression of *SERPINE1* was increased by miR-155-3p mimic in monocytes and macrophages. However, *TLR4* was downregulated by miR-155-3p in monocytes and macrophages. Regarding the effects of the different fatty acids, oleic acid increased the expression of let-7b in macrophages and AcM and also increased the expression of miR-155 in monocytes when compared with DHA but not when compared with non-treated cells. Overall, these results suggest anti- and proinflammatory roles of let-7b and miR-155-3p in THP-1 cells, respectively, although these outcomes are strongly dependent on the cell type. Noteworthy, oleic acid might exert beneficial anti-inflammatory effects in immune cells (i.e., non-activated and LPS-activated macrophages) by upregulating the expression of let-7b.

Keywords SERPINE1 · Macrophages · Monocytes · Oleic acid · DHA · EPA

Introduction

Non-coding RNAs are emerging as a critical group of modulators on gene expression. Indeed, some of these regulatory RNAs are termed microRNAs (miRNAs), which control biological and physiopathological phenomena by blocking target genes through the inhibition of protein translation or by promoting mRNA degradation [21]. Recent findings have evidenced that miRNAs play important roles in cell proliferation, differentiation, and development, as well as in the regulation of genes implicated in many inflammation-related diseases. A pioneer research linking miRNA with immunocompetence arrived from miRNA expression analyses in a monocytic cell line treated with lipopolysaccharide (LPS), a toll-like receptor (TLR)-4 ligand [35]. Currently, cells of the immune

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system are known to express hundreds of miRNAs, having the potential to broadly influence molecular pathways controlling the development and function of immune responses.

Specific miRNAs, including miR-155 and let-7b, were initially linked with inflammatory responses as they appeared upregulated by TLRs ligands, inflammatory cytokines (e.g., IL-6, TNF-α), and specific antigens in multiple immune cell lineages [24]. In this context, miR-155 has been implicated in cell differentiation and activation of cells concerning innate and adaptive immune systems, and existing proofs support miR-155 as a depressing controller of innate immune or inflammatory functions [3]. Contrariwise, some investigations have found that miR-155 may also have a proinflammatory role [41]. In the same way, let-7b, a modulator of cell proliferation and developmental timing, is able to mediate immune responses and adjust inflammation [2]. Actually, a screening of targets related to let-7b by using Targetscan® version 6.0 (www.targetscan.org) found that *TLR4*, *SERPINE1*, and *TNF* are putative target genes of this miRNA. However, the impact of let-7b on gene expression machinery in monocytes and macrophages has been scarcely investigated. In addition, the regulatory role of these miRNAs on inflammation-related gene expression profiling remains unknown.

In this context, a Mediterranean-based nutritional intervention was able to induce changes in the expression of miR-155-3p and let-7b in white blood cells from subjects with metabolic syndrome features after 8 weeks of intervention [20]. Moreover, a low intake of lipids and saturated fat was associated with overexpression of let-7b after the dietary intervention [20]. In this sense, recent studies have analyzed the role of different miRNAs on the pathogenesis of diseases associated with the intake of high-fat diets and different types of fatty acids [8]. Noteworthy, it has been demonstrated that the adult offspring of mothers prenatally fed a high-fat diet before conception presented lower levels of some miRNAs (e.g., let-7 family) during pregnancy and lactation, suggesting that epigenetic processes are likely involved in diet-induced changes affecting gene expression, which may be maintained until later life stages and adulthood [45].

To our knowledge, there are no evidences about the effects of anti- and proinflammatory fatty acids (FAs) on the expression of miRNA-155-3p and let-7b. Therefore, the current study aimed to clarify the potential modulatory roles of let-7b and miR-155 on inflammation-associated genes (*IL6*, *TNF*, *SERPINE1*, and *TLR4*). Furthermore, we investigated the influence of different FAs on the expression of these miRNAs in THP-1 monocytes, macrophages, and LPS-activated macrophages (AcM).

Material and methods

Cell culture

Human acute monocytic leukemia cells (THP-1) were purchased from the American Type Culture Collection (ATCC, TIB-202, VA, USA) and cultured in RPMI-1640 medium (ATCC) supplemented with 4500 mg/L glucose, 2 mM L glutamine, 1 mM sodium pyruvate, 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), 1500 mg/L sodium bicarbonate, 100 U/ml penicillin-streptomycin, and 10% fetal bovine serum (BSA) in a 5% CO_2 humidified atmosphere at 37 °C.

Cells were grown at a density of 4×10^5 cells/mL, as recommended by the ATCC. For the differentiation process into macrophages, THP-1 monocytic cells were grown with 25 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, MO, USA) for 48 h at 37 °C in a 5% CO_2 incubator. Thereafter, macrophages were activated with 100 ng of LPS during 24 h. Finally, cells were photographed with a laser-scanning confocal imaging system (Olympus; FV1200).

mirVana® miRNA mimic transfection

Cells in the three differentiation states were transfected with either 15 nM mirVana® miR-Let-7b-5p mimic, mirVana® miR-155-3p mimic, or mirVana® miRNA mimic negative control (Applied Biosystems, CA, USA) using Lipofectamine® RNAiMAX Transfection Reagent (Applied Biosystems) according to manufacturer's protocol. In all the transfection experiments, the group transfected with the mirVana® miRNA mimic negative control was considered the negative control for those transfected with let-7b or miR-155 mimics. The BLOCK-iT Alexa Fluor Red Fluorescent Oligo control (Invitrogen, CA, USA) was also transfected into THP-1 cells by using Lipofectamine® RNAiMAX Transfection Reagent to assess transfection efficiency. The number of experiments (*N*) indicated in the figure or the figure legend means independent experiments.

Treatment with fatty acids

THP-1 monocytes, macrophages, and LPS-activated macrophages were incubated with oleic, palmitic, eicosapentaenoic (EPA), or docosahexaenoic (DHA) acids at a concentration of 100 μM. Palmitic acid, EPA, and DHA were dissolved in ethanol whereas oleic acid was dissolved in endotoxin-free water. All of them were then mixed with bovine serum albumin (BSA) in a 1:2 M ratio (BSA: free fatty acid). Then, the fatty acids were added to RPMI-1640 medium supplemented with BSA 10% and treated for 30 h at 37 °C in a 5% CO_2 humidified atmosphere. After incubation, the cell pellets were collected for further analyses.

RNA isolation and quantitative real-time PCR

Total RNA was isolated from THP-1 cells by using Trizol reagent according to the manufacturer's instructions (Invitrogen). The purity and concentration of RNA were determined at 260/280 nm with a NanoDrop spectrophotometer (Thermo Fisher Scientific, DE, USA). cDNA was synthesized from 1 μg of total RNA using random primers and MultiScribeTM MMLV reverse transcriptase (Applied Biosystems). For mature miRNAs, 20 ng of RNA were reverse transcribed with the Taqman MicroRNA RT kit (Applied Biosystems) and miRNA-specific primer sets provided by the manufacturer. The references of the specific PCR primers and probes used for the measurement of the expression of the miRNAs (TaqMan® MicroRNA Assays) were the following: Let-7b—002619; miR-155-3p—002287; U6—001973; U48—001006. The references of the specific TaqMan® Assays used for mRNA quantification were the following: *IL6*—Hs00985639_m1; *TNF*—Hs01113624_g1; *SERPINE1*—Hs01126606_m1; *TLR4*—Hs00152939_m1; *GAPDH*—Hs02758991_g1; *ACTB*—Hs01060665_g1.

Quantitative real-time PCR was performed with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The screened genes were selected on the basis of earlier studies supporting their possible involvement in inflammatory pathways related to miR-155 and let-7b. β-actin (*ACTB*) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as internal references for mRNA analyses, whereas U6 and U48 were used as control miRNAs. All assays were performed in triplicate. Data analysis was performed using the 2-ΔΔCt method to determine the relative quantitative level and was expressed as a fold-difference to the relevant control. Thus, the amount of target, normalized to endogenous gene and relative to the control, is expressed as $2^{-\Delta\Delta Ct}$ [ΔCt = Ct (target gene) − Ct (endogenous gene); ΔΔCt = ΔCt for any sample − ΔCt for the control]. This normalization was performed in all figures except in Fig. 1, in which there was not a control group and the results were expressed as ΔΔCt and directly compared between the three cell types.

Statistical analysis

Results were expressed as mean values ± SEM or SD and analyzed by Student's *t* test when comparing two groups or repeated measures ANOVA for more than two groups. Analyses were carried out using REST 2009 (Relative Expression Software Tooland) SPSS 15.1 software for Windows (SPSS Inc., IL, USA). The GraphPad Prism® version 5.0 (GraphPad Software Inc., CA, USA) was used to show graphically the results. Differences were considered as statistically significant when the *p* value was lower than 0.05.

Results

LPS-activated macrophages (AcM) showed higher basal expression of *IL6*, *TNF*, and *SERPINE1* than the other cell types ($p < 0.05$ for all) after normalization by the respective negative control (Fig. 1). In the same way, THP-1-derived macrophages expressed more *TLR4* than monocytes and AcM ($p < 0.05$ for all). Finally, THP-1-derived macrophages showed higher expression of *SERPINE1* but lower mRNA levels of *TNF* than monocytes (Fig. 1; $p < 0.05$ for all).

The relative levels of let-7b, when compared with the respective negative control groups, were overexpressed with let-7b mimic: 23-fold in monocytes, 121-fold in macrophages, and 421-fold in AcM (Fig. 2(A)). In the same way, the transfection with miRNA-155 mimic increased the expression of this miRNA: 30-fold in monocytes, 193-fold in macrophages, and 509-fold in AcM (Fig. 2(B)).

After transfection with let-7b, mRNA levels of *TNF* and *IL6* were downregulated in monocytes (in comparison to negative control group) by a mean factor of 0.50 (S.E. range = 0.26–0.72; $p = 0.014$) and 0.33 (S.E. range is 0.21–0.50; $p < 0.001$), respectively (Fig. 3). However, the transfection with the same miRNA increased the expression of *IL6*, *TNF*, and *SERPINE1* in THP-1-derived macrophages ($p < 0.05$). These results were not found in AcM, with only *SERPINE1* being downregulated in AcM by a mean factor of 0.67 (S.E. range is 0.46–0.95; $p = 0.046$) (Fig. 3).

The transfection of miR-155 mimic led to the upregulation (in comparison to control group) of *IL6* in monocytes (mean factor = 1.44; S.E. range = 1.20–1.99; $p = 0.024$), macrophages (mean factor = 2.12; S.E. range = 1.24–4.10; $p = 0.010$), and AcM (mean factor = 2.17; S.E. range = 1.08–4.37; $p = 0.030$), as shown in Fig. 4. Also, *SERPINE1* was upregulated in monocytes and macrophages by a mean factor of 1.34 (S.E. range = 1.10–1.99; $p = 0.041$) and 1.28 (S.E. range is 1.00–1.76; $p = 0.016$), respectively. However, *TLR4* was downregulated in transfected monocytes and macrophages by a mean factor of 0.71 (S.E. range = 0.57–0.92; $p = 0.005$) and 0.71 (S.E. range = 0.51–0.99; $p = 0.002$) as plotted (Fig. 4).

The relative expression of let-7b was inhibited in monocytes as a result of the incubation with DHA (mean factor = 0.67 ± 0.11; $p = 0.030$) when compared with the negative control (Fig. 5). However, let-7b was upregulated when monocytes were incubated with EPA when compared to DHA ($p = 0.030$). In THP-1-derived macrophages and AcM, the expression of let-7b was higher as a result of the treatment with oleic acid (7.61 ± 0.63 and 5.27 ± 1.28, respectively; $p < 0.05$ for both cell types). Moreover, palmitic acid increased the expression of let-7b in AcM when compared with the non-treated and PUFA (DHA or EPA)-treated cells ($p < 0.05$). Concerning miR-155, oleic acid was able to increase the expression of this

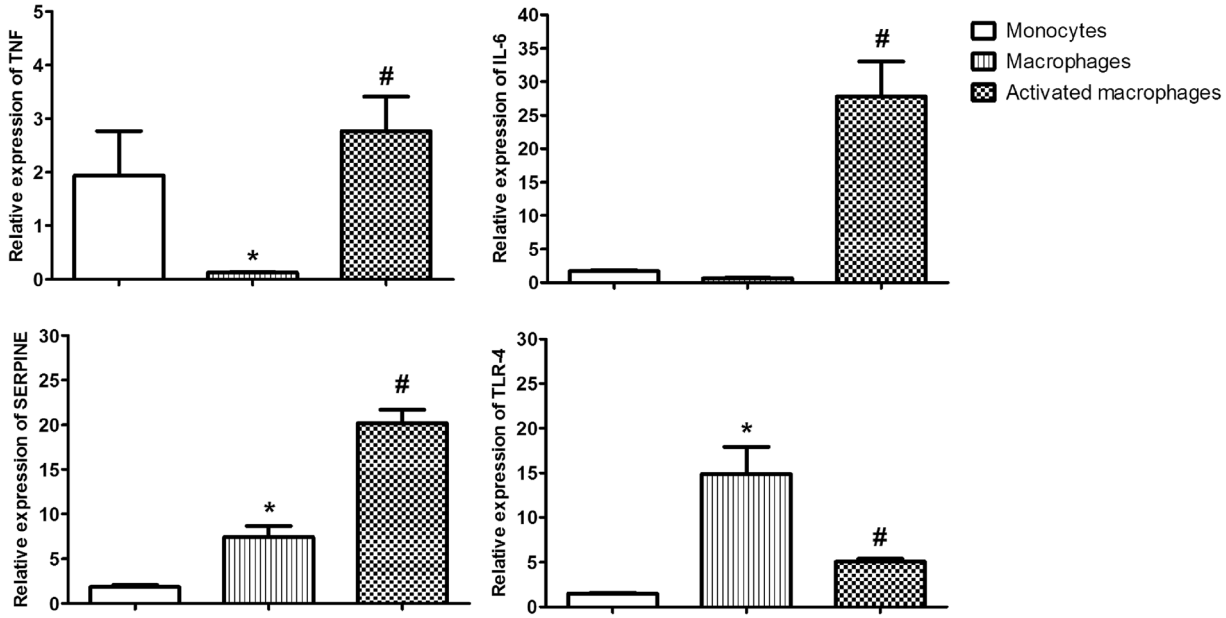

Fig. 1 Basal expression of TNF, IL6, SERPINE, and TLR4 in THP-1 monocytes, macrophages, and LPS-activated macrophages ($n = 14$ in each group). Data are expressed as ΔΔCt and shown as the means ± standard deviation. As there was not a control group, the results were directly compared between the three cell types. *$P < 0.05$ when compared with monocytes. #$P < 0.05$ when compared with the other cell groups

miRNA in THP-1 monocytes when compared with DHA ($p < 0.05$) but not when compared with non-treated cells (Fig. 6). Moreover, the incubation with oleic acid overexpressed miR-155 in THP-1-derived macrophages (3.38 ± 0.43; $p < 0.001$).

Discussion

In our study, overexpression of miR-155 was able to significantly inhibit the expression of *TLR4* in both monocytes and macrophages. Apparently, this is the first research that

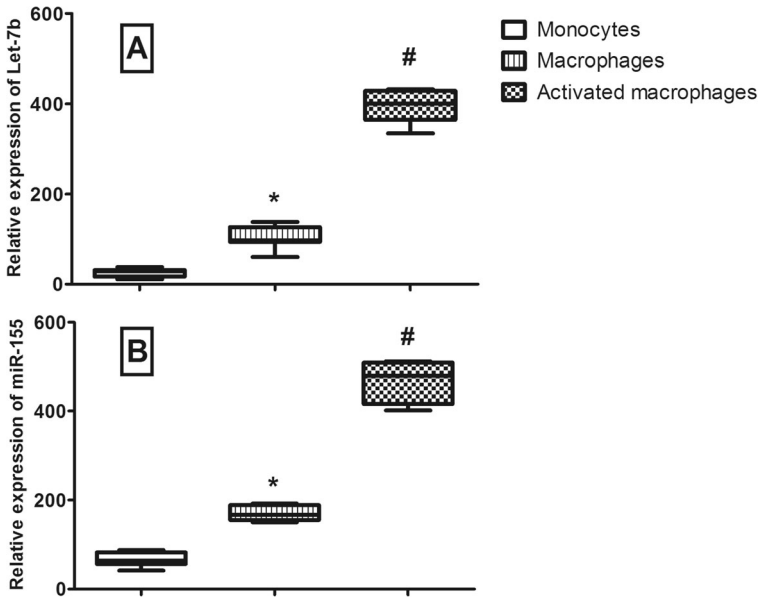

Fig. 2 Expression of let-7b (A) and miR-155-3p (B) in THP-1 monocytes, macrophages, and LPS-activated macrophages transfected with the respective miRNA ($n = 10$) when compared with the respective negative control. Data are expressed as $2^{-\Delta\Delta Ct}$ and shown as the means ± standard error range. All the results are in relation to the expression of the miRNAs in the groups of monocytes (left), macrophages (middle), and LPS-activated macrophages (right) transfected with the mirVana® miRNA mimic negative control. According to the $2^{-\Delta\Delta Ct}$ method, miRNA expression in the groups transfected with the negative control was 1. *$P < 0.05$ when compared with monocytes. #$P < 0.05$ when compared with the other groups

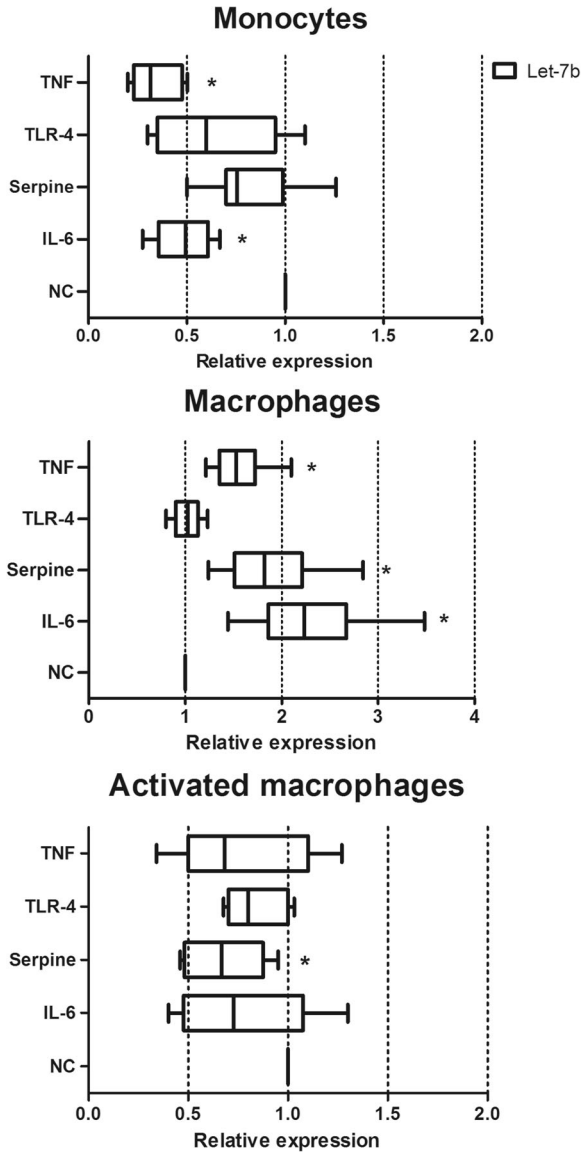

Fig. 3 Relative expression of inflammation-related genes after transfection with let-7b mimic in THP-1 monocytes, macrophages, and LPS-activated macrophages ($n = 8$) compared with the respective negative control group (NC). Data are expressed as $2^{-\Delta\Delta Ct}$ and shown as the means ± standard error range. The NC was the group of cells that was transfected with the mirVana® miRNA mimic negative control. *$P < 0.05$ when compared with the respective NC

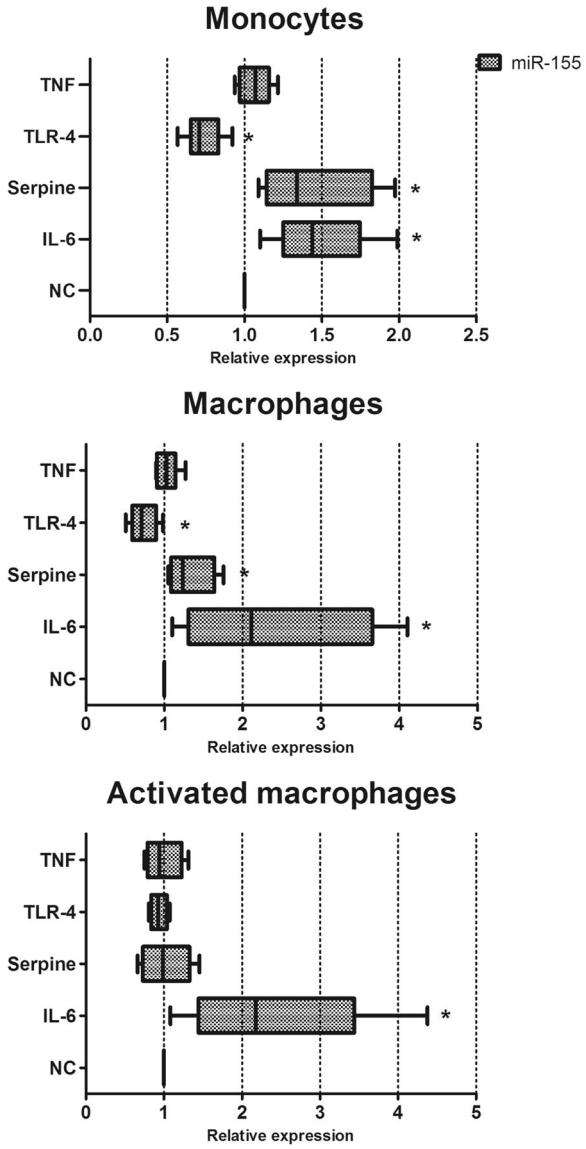

Fig. 4 Relative expression of inflammation-related genes after transfection with miR-155 mimic in THP-1 monocytes, macrophages, and LPS-activated macrophages ($n = 8$) compared with the respective negative control group (NC). Data are expressed as $2^{-\Delta\Delta Ct}$ and shown as the means ± standard error range. The NC was the group of cells that was transfected with the mirVana® miRNA mimic negative control. *$P < 0.05$ when compared with the respective NC

demonstrates this inverse relationship. In mammalian immune cells, TLRs recognize pathogen-associated molecules or pathogen-associated molecular patterns (PAMPs), playing a crucial role in innate immune response signaling. TLR4 is one of the most widely studied TLRs. It is expressed in a number of cell types including blood cells, especially in professional antigen-presenting cells such as monocytes, macrophages, and dendritic cells [44].

On the other hand, it has been previously reported that let-7b mediates TLR4 expression via post-transcriptional regulation and inhibits NF-κB activity through a MyD88-dependent pathway [36]. However, we did not find differences in *TLR4* expression in let-7b-transfected cells. A similar result was reported by Teng et al. [36] in gastric epithelial cell lines, when the treatment with let-7b mimics or let-7b inhibitors provoked changes in TLR4 protein expression with no significant modifications in *TLR4* mRNA expression. The inconsistence of these results suggests that, in different cell types, the let-7 family can modulate TLRs in a distinct manner despite their high degree of homology. Moreover, in contrast to their traditional roles as repressors of gene activation, new evidences allow to hypothesize that, in some circumstances,

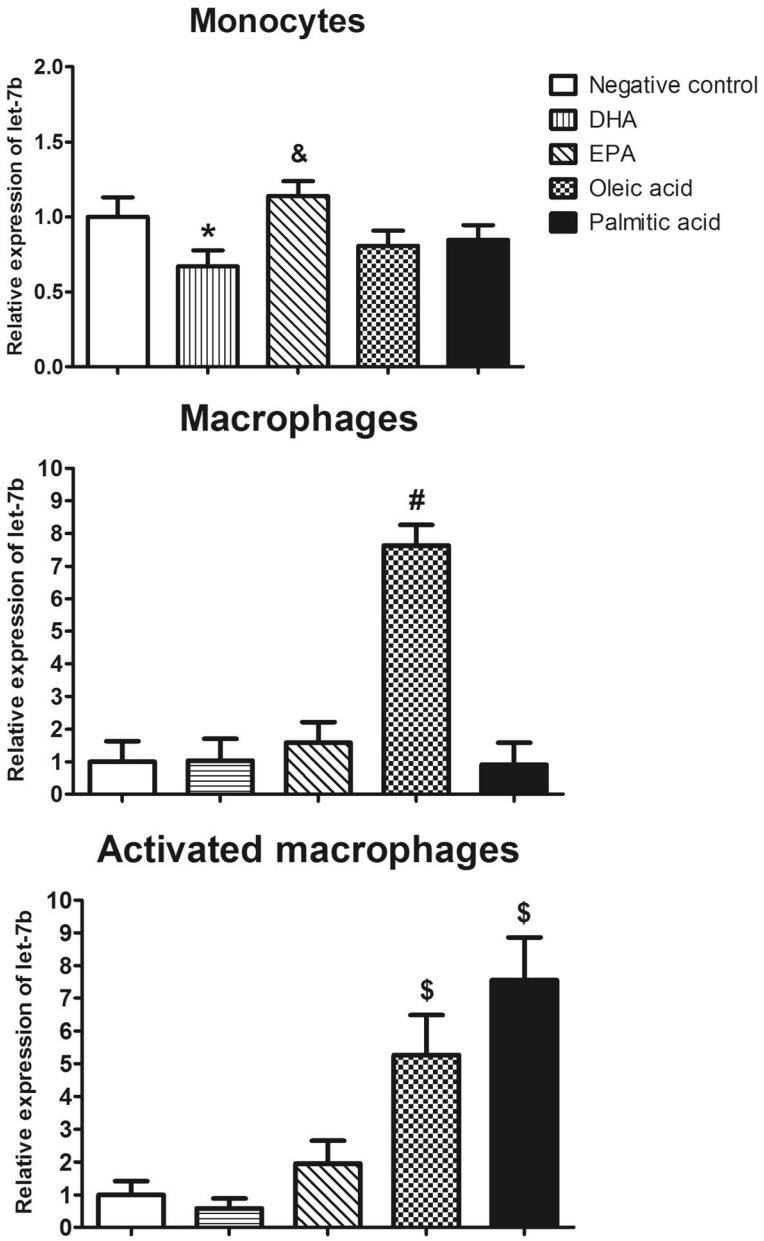

Fig. 5 Relative expression of let-7b in THP-1 monocytes, macrophages, and LPS-activated macrophages after treatment with different fatty acids. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. Data are expressed as $2^{-\Delta\Delta Ct}$ and shown as the means ± standard deviation. Cells of the control group were not incubated with fatty acids. $^{*}P < 0.05$ when compared with the NC. $^{\&}P < 0.05$ when compared with the DHA-treated group. $^{\#}P < 0.05$ when compared with the other treated groups. $^{\$}P < 0.05$ when compared with NC, DHA, and EPA groups

specific miRNAs could switch from activation to repression of protein translation during the cell cycle [40]. These findings emphasize the complexity of the miRNA-mediated regulatory mechanisms involved in gene expression control.

miR-155, which is conserved across vertebrate species, is implicated in the inflammatory response [41]. For example, it has been reported that miR-155 enhanced TNF-α and IL-6 production in peripheral blood-derived macrophages in humans [16]. On the other hand, the selective inhibition of TNF-α resulted in a significant decline of miR-155 levels [25].

Other reports have also shown a direct relationship between miR-155 and TNF-α in diverse pathological conditions. However, our research was not able to find an association between miR-155 and *TNF* expression in THP-1 cells. Divergences in the kinetics of expression between miR-155 and some of its target genes have been anteriorly reported, presenting delays of 8–12 h [37]. These variations could be attributed to some mechanisms, such as the rate of miR loading into the RISC complex, the rate of mRNA decay, and the rate of transcription [11].

In this context, several studies have reported that miR-155 is stimulated by LPS, IFN-β, and various TLR ligands in murine macrophages [24, 39]. Once induced, miR-155 participates in the activation of TNF-α and IL-6 by eliciting the Fas-associated death domain protein (FADD), Ik B kinase ε (IKKε), and receptor (TNF receptor superfamily)-interacting serine-threonine kinase 1 or RIPK1 [39]. Macrophages and

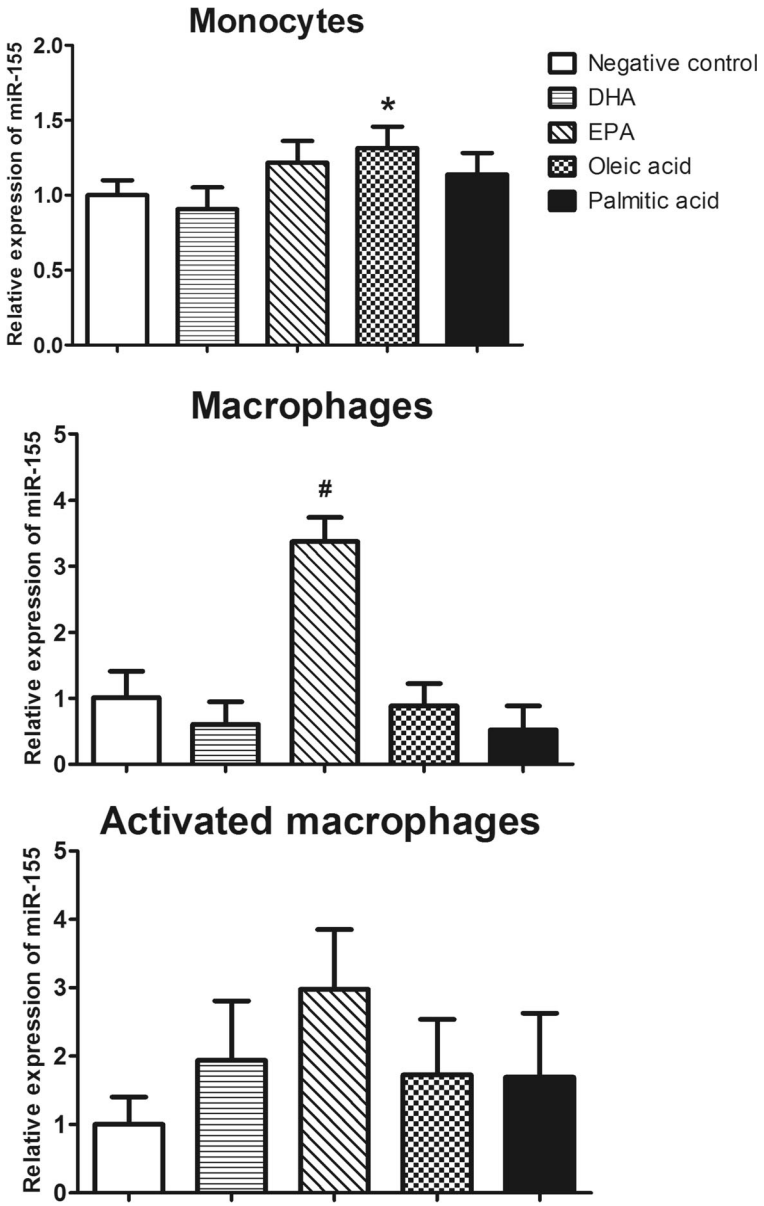

Fig. 6 Relative expression of miR-155 in monocytes, macrophages, and LPS-activated macrophages after treatment with different fatty acids. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. Data are expressed as $2^{-\Delta\Delta Ct}$ and shown as the means ± standard deviation. Cells of the control group were not incubated with fatty acids. $^{\#}P < 0.05$ when compared with the DHA group. $^{\&}P < 0.05$ when compared with the other treated groups

monocytes overexpressing miR-155 reduced inositol polyphosphate-5-phosphatase D (*SHIP1*) expression, which may lead to increased production of proinflammatory cytokines [26]. Also, miR-155 performs a role in the innate immune response by regulating suppressor of cytokine signaling (SOCS1), a negative modulator of dendritic cell antigen-presenting capacity [39]. All these findings are corroborated in the present work, either by miR-155 upregulation in AcM or by the upregulation of proinflammatory genes in all transfected cell types. For example, miR-155 mimic upregulated *IL6* in monocytes, macrophages, and AcM. In a recent study, a significant reduction in *IL6* expression was found after LPS stimulation by miR-155 knockout (− 15,820-fold reduction) or silencing (− 15,968-fold reduction) when compared with control microglial cells [41], revealing that miR-155 is crucial for the expression of *IL6* in response to inflammatory challenges. Indeed, the way miR-155 increases the expression of IL6 is still unknown as there are no predicted miR-155-binding sites in the 3-UTR of the *IL6* mRNA transcript [15]. Interestingly, SOCS1, which normally inhibits IL-6/IL6ST (gp130) JAK/STAT3 signaling, is a recognized murine and human miR-155 target. In human microglia and macrophages, miR-155 also elicits IL-6 induction, putatively via SOCS1 repression [23]. SOCS1 repression enhances the IL-6/IL6ST (gp130) JAK/STAT3 axis, while STAT3 stimulation in turn upregulates *IL6* and progliogenic genes including *GFAP*, skewing the differentiation of neural stem cells into glial cells [27].

Plasminogen activator inhibitor-1 (PAI-1), encoded by the *SERPINE1* gene, is recognized as a proinflammatory protein

involved in cardiovascular risk. In our models, *SERPINE1* expression was increased in monocytes and macrophages transfected with miR-155 mimic. These findings might be probably explained by a parallel effect of this miRNA on factors controlling *SERPINE1* expression. For example, *SMAD2*, known to regulate *SERPINE1*, is directly targeted by miR-155 [19]. On the other hand, let-7b was able to reduce the expression of *SERPINE1* in LPS-activated macrophages but, controversially, it upregulated *SERPINE1* in non-activated macrophages. According to available bioinformatics tools, such as microSniper and Targetscan, *SERPINE1* gene has predicted target binding sites for a number of miRNAs of the let-7 family. For example, let-7g improved several endothelial functions by inhibiting in vivo and in vitro *SERPINE1* expression [18]. In the same study, stroke patients with higher PAI-1 levels were associated with lower serum levels of let-7g. The authors proposed a regulation of PAI-1 by SMAD2, which is also modulated by let-7g, reinforcing the importance of other miRNAs like let-7b on this process.

It is generally assumed that miRNAs predominantly exert an inhibitory effect on gene expression. However, when an internal environmental factor changes, they eventually may switch to positively regulate gene functions. Thus, the different expression of let-7b suggests that other mechanisms and phenomena may be involved aside from microRNA/target gene. One possible explanation is that the treatment of phorbol 12-myristate 13-acetate/ionomycin (PMA) promotes the phagocytic properties of the cells and induces a constitutive production of proinflammatory cytokines [22]. The actions of PMA may have been influenced more than the inhibition by let-7b, resulting in higher expressions of other proinflammatory genes (*TNF* and *IL6*). In this context, it is important to highlight that these properties also increased the efficiency of miRNA transfection. Moreover, proinflammatory miRNAs and genes are dramatically upregulated (approximately 80-fold) by LPS [1], in agreement with other reports [24, 39]. These events led to higher expression of let-7b in LPS-activated macrophages than in non-activated macrophages. Therefore, our results suggest that the increased expression of let-7b was strong enough to downregulate *SERPINE1* in AcM when compared with the respective reference group.

The inhibitory role of let-7b on *TNF* and *IL6* expression in monocytes has been found in previous reports. For example, Guo et al. [10] noted that let-7b had different expression patterns in inflamed tissues in comparison with healthy controls. The let-7b/TLR4 pathway may contribute to cell polarization and inflammatory ablation. Further investigations of the mechanisms that regulate let-7b expression have demonstrated that a TLR4/NF-κB/STAT3/AKT regulatory signaling pathway plays a key function in the control of inflammatory system plasticity [38]. Moreover, let-7b may trigger inflammation and immune responses by activating NF-κB and mediating IL-6 downregulation [36].

The ω-3 long-chain polyunsaturated FAs (PUFA) EPA (20:5n-3) and DHA (22:6n-3) are the FAs most frequently associated with health-promoting effects. In addition to their anti-hypertriglyceridemic properties, they are usually considered as anti-inflammatory mediators. Indeed, it has been demonstrated that ω-3 PUFAs, including DHA, can modify the expression of miRNAs [6] in cancer and other diseases. In the present study, DHA treatment was able to decrease the expression of let-7b in monocytes whereas EPA increased the expression of miR-155-3p in macrophages. In this context, a previous report showed let-7 downregulation in rats treated with dietary fish oil [6]. However, it is unclear whether the effects of ω-3 FAs on miRNA expression are the consequence of direct interactions or are mediated by other metabolites synthesized from essential FAs, such as maresins, protectins, lipoxins, and resolvins, which are endogenously produced from essential FA precursors during inflammation. Some of these proresolving lipid mediators may regulate the expression of genes involved in the resolution of inflammation by modulating specific miRNAs [31]. On the other hand, DHA (from fish oil) has been reported to enhance mitochondrial Ca^{2+} accumulation, thereby inducing apoptosis [14]. Thus, elevated DHA levels may affect some processes related to cellular death and influence indirectly the expression of let-7b and miR-155-3p.

Similarly to PUFAs, monounsaturated FAs or MUFAs (especially oleic acid) have shown anti-proliferative effects in cancer and have been associated with decreased cardiovascular risk. For example, a high MUFA intake or saturated FA replacement (SFAs) by MUFAs improved visceral fat distribution, insulin signaling [7], and postprandial cellular oxidative stress in patients with metabolic syndrome [29]. One of the mechanisms involved in oleate action could be the prevention of JNK-1/2 or NF-κB activation in response to palmitate or TNF-α [28]. In endothelial cells, oleate reduced intercellular adhesion molecule 1 (ICAM-1) and monocyte chemotactic protein 1 (MCP-1) and increased endothelial nitric oxide synthase (eNOS) expression induced by proinflammatory cytokines. Moreover, oleate impaired the proliferation induced by palmitate, angiotensin II, or TNF-α, and the apoptosis induced by TNF-α [28]. However, the molecular mechanisms by which oleate exerts its protective role are not fully understood. For instance, let-7f was induced by oleic acid in Caco-2 cells [9]. This result was similar to that found for let-7b in the present work: Let-7b levels were higher in AcM and macrophages incubated with oleic acid. To our knowledge, this is the first time that these results are observed in monocytes and macrophages, providing

evidence to better understanding the actions of oleic acid in the regulation of the expression of miRNAs.

On the other hand, some SFAs including palmitic acid have been associated with adverse cardiovascular effects [33]. There are several mechanisms that attempt to explain these deleterious effects of SFAs. For example, SFAs can act as ligands for TLR and activate diverse signaling pathways implicated in the inflammatory response [32]. For example, it has been described that palmitate may induce insulin resistance in myotubes through TLR2, activating JNK, NF-κB, and p38 [34] and decreasing the levels of IκB-α [30]. Palmitate also induces insulin resistance in muscle cells and other non-neuronal cell types [42]. Nevertheless, oleate has seemingly a greater protective effect against palmitate damage to neuronal cells, even compared to other PUFAs such as DHA (ω-3) and linoleate (ω-6) that have received higher attention [17]. However, other authors have described that both palmitate and oleate induced insulin resistance in primary hepatocytes, in association with an accumulation of diacylglycerols and/or ceramide [4]. In our study, palmitic acid treatment increased the expression of let-7b only in AcM.

This study presents some limitations that should be considered. Firstly, it remains unclear whether transfected miRNAs behave similarly to endogenous miRNAs; hence, it cannot be discarded that some of the observed changes in gene expression are indirect, for example, by affecting the expression of transcription factors [5]. Secondly, the supraphysiological levels of mature miRNAs and these artefactual RNA species may lead to non-specific changes in gene expression [12]. However, to minimize the potential bias, a negative control with the same concentration of the miRNA mimic was used in all transfection experiments. Thirdly, we have not measured the secreted protein to the medium and it is possible that changes in mRNA expression might not be accompanied by shifts in protein levels. However, a screening of putative targets of the selected microRNAs was carefully performed by using specific Targetscan® and miRWalk®, and other studies have reported the influence of these specific microRNAs on the expression of the cited proteins [13, 43].

In summary, deepening into the regulatory mechanisms that certain miRNAs play on the inflammatory processes [4, 21], the current findings suggest some pro- and anti-inflammatory properties of let-7b and miR-155-3p on THP-1 cells, respectively. However, depending on the differentiation state of the cells, these miRNAs may act in an unexpected way. Moreover, our data suggest that the modulation of the expression of miRNAs through a nutrition approach (i.e., ω-3 PUFAs) might be a future alternative or adjunct to current pharmacologic therapy by targeting endogenous miRNAs. Finally, this study contributes to understand the miRNA-mediated mechanisms of action for different fatty acids in inflammation-related cells.

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