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MicroRNA–21 attenuates BDE-209-induced lipid accumulation in THP-1 macrophages by downregulating Toll-like receptor 4 expression

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ABSTRACT

Growing evidence demonstrates a possible response of specific microRNA (miRNA) to environmental pollutant stimuli in multiple biological processes. We previously reported that a persistent organic pollutant, decabromodiphenyl ether (BDE-209), can enhance Toll-like receptor 4 (TLR4)-dependent lipid uptake in THP-1 macrophages; whether miRNAs are involved in this process remains unclear. In the present study, we investigated the levels of several miRNAs related to TLR4 signaling, including miRs–9, –21, −27b, −125b, −132, −146a, −147, −155, and –let-7e, in THP-1 macrophages after stimulation by BDE-209 and oxidized low-density lipoprotein. The results showed that the levels of miR–21 were significantly suppressed by BDE-209 at concentrations of 6.25, 12.5 and 25 μM, in a dose-dependent manner; whereas there was no significant changes for the other miRNAs investigated. Moreover, the suppression of miR–21 was accompanied by an upregulated TLR4 expression, at both mRNA and protein levels. Further analysis showed that the up-regulated TLR4 induced by BDE-209 was inhibited in macrophages transfected with miR–21 mimic; meanwhile opposite results were exhibited when an anti-miR–21 inhibitor was transfected to the macrophages. Additionally, transfection with miR–21 mimic effectively attenuated BDE-209-induced lipid accumulation in macrophages. Together, these data illustrate that miR–21 inhibits BDE-209-triggered lipid accumulation in macrophages through down-regulating TLR4 expression.

1. Introduction

MicroRNAs (miRNAs) are small (~22 nucleotides long), single-stranded non-coding RNAs that are encoded in the genome (Ambros, 2004; Bartel, 2004). They regulate gene expression at the post-transcriptional level by base-pairing, often imperfect, with the 3′-untranslated region (UTR) of their target mRNA transcripts, leading to mRNA degradation and/or translational repression (Djuranovic et al., 2011). Currently, more than 2650 distinct mature miRNAs have been identified in the human genome (http://www.mirbase.org). Bioinformatics predicted that mammalian miRNAs can regulate as many as 60% of all protein-coding genes; thus, they are potentially involved in all physiological and pathological processes (Friedman et al., 2009). Therefore, miRNAs exert salient roles in various biological processes such as cell differentiation, organ development, immune reaction, and adaptation to stress (Ambros, 2004; Bartel, 2004). However, once the normal miRNA machinery is altered under specific conditions, it will potentially lead to a disease or pre-disease state (Bartel, 2004; Tokar, 2016). Over the last decade, growing epidemiological and laboratory evidence is substantiating a link between alterations of miRNA expression and the many human pathologies, including cancer, autoimmune diseases, and cardiovascular diseases (Xiao and Rajewsky, 2009; Fang et al., 2010; Pogribny et al., 2016; Jansen et al., 2017; Pereira-da-Silval et al., 2018; Donaldson et al., 2018). Like other genetic factors, recent studies have also pointed to a possible response of specific miRNA to environmental stimuli such as exposure to environmental agents and pollutants, through the combined effects of genes and environmental stimuli and/or the interactions between them (Sonkoly and Pivarcsi, 2011; Jardim, 2011; Li et al., 2015; Vrijens et al., 2015; Yu and Cho, 2015; Krauskopf et al., 2017; Sollome et al., 2016).

Excessive lipid accumulation in macrophages and the subsequent foam cell formation is one of the key processes responsible for the

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initiation and development of cardiovascular diseases (Moore and Tabas, 2011; Moore et al., 2013), the leading cause of morbidity and mortality in modern society (Pagidipati and Gaziano, 2013). During this process, Toll-like receptor 4 (TLR4), a signal transducing integral membrane protein, plays an important role (den Dekker et al., 2010; Roshan et al., 2016). It is well known that activation of TLR4 signaling promotes the uptake of modified low density lipoproteins (LDL), e.g., oxidized LDL (oxLDL), in macrophages and accelerates foam cell formation (den Dekker et al., 2010; Roshan et al., 2016). Emerging evidences have also demonstrated that miRNAs such as miRs–9, –21, −27b, −125b, −132, −146a, −147, −155 and –let-7e are important controllers of TLR4 signaling in macrophages (O'Neill et al., 2011; Virtue et al., 2012; Donaldson et al., 2018). These miRNAs can regulate TLR4 signaling pathways by targeting TLR4 expression, TLR4 signaling proteins, transcription factors, cytokine mRNAs, or TLR4 signaling regulators (O'Neill et al., 2011). Although regulation of TLR4 expression by miRNAs is a well-known mechanism, it is not clear yet whether similar mechanisms are involved in the chemical-mediated TLR4 signaling.

Decabromodiphenyl ether (BDE-209) is a ubiquitous environmental pollutant and is widely presented in the blood serum of the general population (Verreault et al., 2018; Fromme et al., 2016). We previously reported that BDE-209 can enhance TLR4-dependent lipid accumulation in cultured human macrophages (Zhi et al., 2018). However, the possible role of miRNAs as mediators in this process is not clear yet. In the present study, we investigated the possible changes in the levels of several miRNAs, including miRs–146a, −132, −155, −147, −125b, –let-7e, −21, −27b and −9, in THP-1 derived macrophages after BDE-209 exposure. These miRNAs were chosen because they have been implicated in TLR4 signaling in clinical and/or experimental studies (O'Neill et al., 2011; Virtue et al., 2012; Donaldson et al., 2018). Our study provides evidence that miR–21 is involved in the process of BDE-209-stimulated lipid accumulation in THP-1-derived macrophages. We also demonstrate that miR–21 inhibits BDE-209-triggered foam cell formation by down-regulating TLR4 expression.

2. Materials and methods

2.1. Chemicals and reagents

BDE-209 (95.9% purity) was obtained from Dr. Ehrenstorfer GmbH (Germany). Dimethyl sulfoxide (DSMO) was purchased from Sigma-Aldrich (USA). BDE-209 was firstly dissolved in DMSO in a 2 mM stock solution and was further diluted for testing.

2.2. Cell culture and BDE-209 treatment

THP-1 human monocyte-derived cells were from the American Type Culture Collection (ATCC, USA) and cultured as described previously (Zhi et al., 2018). Briefly, The THP-1 cells were maintained in RPMI-1640 media supplemented with 10% (v/v) fetal bovine serum (FBS), 20 IU/mL penicillin, and 20 g/mL streptomycin at 37 °C. THP-1 cells were differentiated into macrophages by administrating 200 ng/mL of phorbol 12-myristate 13-acetate (Sigma, USA) and incubated for 48 h.

To examine the possible changes in miRNA levels induced by BDE-209, macrophages were exposed to 25 μM of BDE-209, according to a previous described method (Zhi et al., 2018). In brief, macrophages were exposed to BDE-209 for 24 h at 37 °C; which did not significantly modify cell viability based on CCK-8 assay (Zhi et al., 2018). The concentration of DMSO in the culture medium is 1.25% (v/v), which had no effects on the measured miRNAs level based on preliminary experiments performed with and without vehicle. Thus, macrophages incubated with culture medium containing a final concentration of 1.25% of DMSO were also processed in parallel as a vehicle control. After the 24-h exposure, the supernatant was removed and the cells were washed twice with PBS. Then, the macrophages were administrated 50 μg/mL of oxLDL (Yeasen, China) and were further incubated for 24 h. To explore whether the influence of BDE-209 on the expression of miR–21 is dose-dependent, macrophages were exposed to 1.56, 3.12, 6.25, 12.5, and 25 μM of BDE-209 for 24 h, further exposed to 50 μg/mL of oxLDL for 24 h, and then tested for miR–21 levels. Macrophages transfected with miR–21 mimic or miR–21 inhibitor were also exposed to 25 μM of BDE-209 for 24 h and then exposed to oxLDL (50 μg/mL) for 24 h, to assess the role of miR–21 in regulating TLR4 expression. To identify whether miR–21 can inhibit lipid accumulation, macrophages were transfected with miR–21, exposed to 25 μM of BDE-209 for 24 h, further exposed to 50 μg/mL of oxLDL for 24 h and tested for lipid accumulation.

2.3. RNA isolation and quantification

Total RNA was isolated from THP-1-derived macrophages using the TRIzol Reagent (Transgene Biotech Co., China), whereas miRNAs were isolated from the cells using the mirVana™ miRNA Isolation Kit (Ambion Gmbh, Germany), according to the instructions provided by the manufacturers.

mRNA and miRNAs were quantified by a real-time qPCR. For analysis of gene expression of TLR4, cDNA was prepared with a reverse transcription kit (Transgen Biotech Co., China) from 2 μg of total RNA, following the manufacture's instruction. Real-time PCR of the cDNA products was performed with a TransStart ® Top Green qPCR SuperMix (Transgene Biotech Co., China). The miRNA levels were quantified using specific Taqman assays for miRNA (Applied Biosystems, USA) and Taqman Universal Master Mix (Applied Biosystems, USA). The thermocycler programs for all target genes were performed in accordance with the recommendation by the manufacture. GAPDH and U6 were used as the endogenous control for gene-expression analysis and miRNA analysis, respectively. Primers used for TLR4, GAPDH and U6 were as follows: TLR4, forward: 5′-GGT GAT TGT TGT GGT GTC CCA-3′, and reverse: 5′-AGT GTT CCT GCT GAG AAG GCG-3'; GAPDH, forward: 5′-AGA AGG CTG GGG CTC ATT TG-3′, and reverse: 5′-AGG GGC CAT CCA CAG TCT TC-3'; U6, forward: 5′-GCG CGT CGT GAA GCG TTC-3′, and reverse: 5′-GTG CAG GGT CCG AGG T-3'. Specific primers for miRNAs were obtained from Genepharma Company (China).

2.4. Western blot analyses

Western blots were carried out as previously described (Zhi et al., 2018). In brief, protein samples were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA), and probed with commercially available primary antibody against TLR4 (1:1000 dilution; R&D, USA). After incubation with the primary antibody, the membrane was incubated with a secondary HRP-conjugated antibody (Santa Cruz Biotechnology, USA). Densitometric analysis of the gels was performed as described previously (Zhi et al., 2018).

2.5. miR–21 inhibition and overexpression

For inhibition of miR–21, macrophages were transfected with anti-miR–21 inhibitor or anti-miR-negative control (0.4 nM; Genepharma, China) with 2% Lipofectamine 2000 (Invitrogen; USA), following manufacturer standard protocols. For overexpression of miR–21, macrophages were transfected with miR–21 mimic (0.4 nM; Genepharma, China) or miR-negative control (0.4 nM; Genepharma, China). Transfection of miRNA mimic, inhibitor and negative control were performed as described previously (Zhi et al., 2019). Cells were allowed to recover for 48 h before BDE-209 treatment.

2.6. Oil Red O staining

Lipid deposition in the foam cells derived from macrophages were

identified by Oil Red O staining, according to previously reported methods (Zhi et al., 2015, 2018). Briefly, macrophages incubated with oxLDL in the presence or absence of BDE-209 were fixed in 10% paraformaldehyde-PBS. Then, the cells were rinsed quickly with 60% isopropyl alcohol and stained with 0.3% Oil Red O in 60% isopropanol. The cells were then counter-stained with hematoxylin and mounted in 30% glycerol. Foam cells were imaged under a light microscope. For quantification of intracellular lipid droplets stained with Oil Red O, Oil Red O was extracted from the macrophages by isopropanol. The optical density (OD510) of the extracted Oil Red O was then detected as described previously (Zhi et al., 2015, 2018).

2.7. Statistical analysis

All values of the measured parameters are presented as the means ± standard error. Comparisons between groups were assessed with *t*-test unpaired for normal distributions of at least three independent experiments. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. BDE-209 inhibits miR–21 expression in macrophages

We recently revealed that BDE-209 exposure can significantly upregulate TLR4 expression in THP-1-derived macrophages incubated with oxLDL (Zhi et al., 2018). Given that some miRNAs, including miRs-146a, −132, −155, −147, −125b, -let-7e, −21, −27b, and −9, can act as mediators and have the propensity to fine-tune gene expression of TLR4 in macrophages (O'Neill et al., 2011; Virtue et al., 2012; Donaldson et al., 2018), we sought to explore whether the levels of these miRNAs have been altered in THP-1 macrophages exposed to 25.0 μM of BDE-209, an exposure concentration which can significantly increase TLR4 expression in macrophages (Zhi et al., 2018). The results showed that the miR–21 level in BDE-209-exposed group was significantly decreased compared with the control group (Fig. 1). In contrast, the levels of miRs–146a, −132, −155, −147, −125b, –let-7e, −27b, or −9 were not significantly different between the exposed group and the control (Fig. 1).

To elucidate whether the BDE-209-stimulated changes in miR–21 expression are dose-dependent, the macrophages were further exposed to various concentrations of BDE-209 (1.56, 3.12, 6.25, 12.5, and 25.0 μM). As illustrated in Fig. 2, concentrations of 6.25, 12.5 and 25.0 μM of BDE-209 exposures significantly decreased the levels of miR–21, with approximately 1.4-, 2.5- and 4.3-fold decreases compared

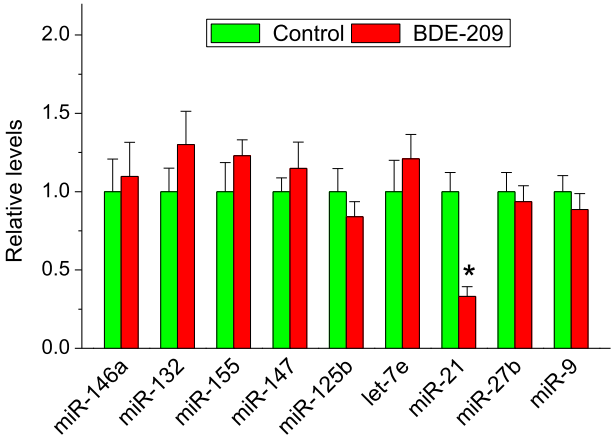

Fig. 1. Relative levels of miRNAs related to TLR4 signaling in macrophages exposed to 25 μM of BDE-209 and 50 μg/mL of oxLDL. Error bar represents 1SE. An asterisk (*) means significant ($p < 0.05$) difference when compared with the control.

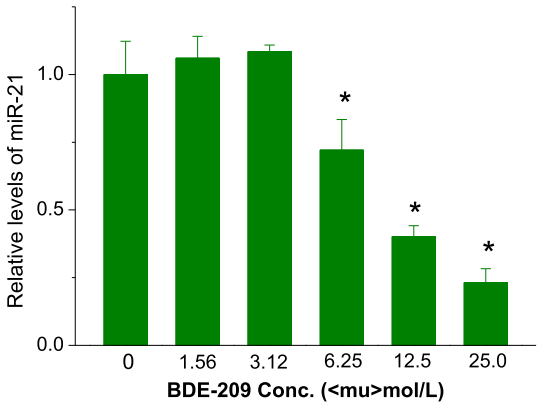

Fig. 2. Relative levels of miR–21 in macrophages exposed to various concentrations of BDE-209 and 50 μg/mL of oxLDL. Error bar means 1SE. An asterisk (*) represents significant ($p < 0.05$) difference when compared with the control.

with those in the control group, respectively. The lower doses (1.56 and 3.12 μM), however, did not significantly change miR–21 levels in the macrophages (Fig. 2). These observations suggested that BDE-209 exposure inhibited miR–21 expression in THP-derived microphages incubated with oxLDL, in a dose-dependent manner.

3.2. miR–21 down-regulates TLR4 expression in macrophages exposed to BDE-209

To investigate the influence of miR–21 on TLR4 expression, we determined the mRNA and protein levels of TLR4 in the macrophages exposed to 1.56, 3.12, 6.25, 12.5, and 25.0 μM of BDE-209 (Fig. 3A, B, and C). A regression analysis between the changes in miR–21 levels and TLR4 expression levels in the macrophages was further performed. The results showed that the changes of miR–21 levels were significantly correlated with those of TLR4 expression, both at mRNA and protein levels (Fig. 3D and E). This observation suggested that miR–21 may be involved in the TLR4 expression in macrophages exposed to BDE-209.

In order to further identify the role of miR–21 in TLR4 expression, we overexpressed and silenced the expression levels of miR–21 in macrophages exposed to 25.0 μM of BDE-209. After transfection with miR–21 mimics, a significant decrease in the mRNA expression of TLR4 was observed in the macrophages compared with the control group (Fig. 4A). In contrast, transfection with anti-miR–21 inhibitor notably reduced the mRNA expression of TLR4 (Fig. 4A). The western blot analysis showed that transfection with miR-21 mimic also significantly suppressed the protein expression of TLR4 in the macrophages compared with the control, whereas transfection with anti-miR–21 inhibitor significantly upregulated the protein expression of TLR4 (Fig. 4B and C). These results indicated that miR–21 negatively regulated TLR4 expression in the BDE-209 exposed macrophages, at both the mRNA and protein levels.

3.3. miR–21 attenuates BDE-209-triggered lipid accumulation in macrophages

BDE-209 can augment lipid accumulation in cultured human macrophages through enhancing TLR4-dependent lipid uptake in the cells (Zhi et al., 2018). In this process, miR–21 can negatively regulate TLR4 expression in the macrophages. Thus, we hypothesized that miR–21 may inhibit BDE-209-induced lipid accumulation in the macrophages. To verify this hypothesis, we transfected miR–21 mimic into the macrophages, exposed them to 25.0 μM of BDE-209, and examined the lipid content in the cells. The results of Oil red O staining showed that the lipid content significantly decreased in the macrophages transfected with miR–21 mimic (Fig. 5A and B). Taken together, our results

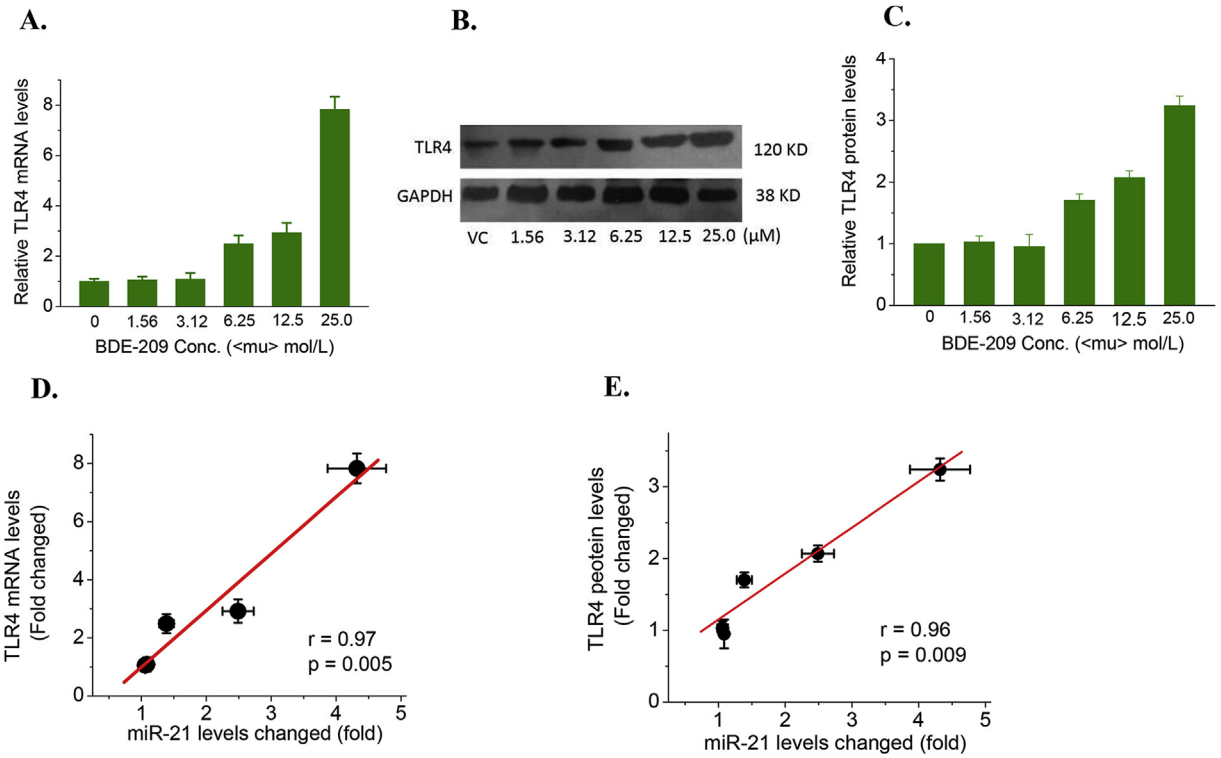

Fig. 3. Correlations of the changes in miR–21 levels and TLR4 expression levels in macrophages exposed to various concentrations of BDE-209 and 50 μg/mL of oxLDL. (A) The mRNA expression levels of TLR4 in macrophages exposed to various concentrations of BDE-209. Error bar represents 1SE. (B) Immunoblots of TLR4 in macrophages exposed to various concentrations of BDE-209. VC = vehicle control. (C) The protein expression levels of TLR4 in macrophages exposed to various concentrations of BDE-209. (D) Correlation of changes in miR–21 levels and TLR4 mRNA expression levels. (E) Correlation of changes in miR–21 levels and TLR4 protein expression levels.

suggested that miR–21 can attenuate BDE-209-triggered lipid accumulation in cultured human macrophages by downregulating TLR4 expression.

4. Discussion

BDE-209 is a ubiquitous environmental pollutant which has been recently listed in the Stockholm Convention as a persistent organic pollutant (POP) substance and was hence restricted in the commercial use globally (Stockholm Convention, 2017). Despite of the restriction, humans are still exposed to BDE-209 due to its environmental persistence and bioaccumulation nature, raising a worldwide concern on its potential adverse effects (Huang et al., 2014; Fromme et al., 2016). BDE-209 is structurally similar to some of the legacy POPs such as polychlorinated biphenyls (PCBs), which have been demonstrated to be potentially associated with incidence of cardiovascular diseases (Perkins et al., 2016; Henríquez-Hernández et al., 2017; Singh and Chan, 2018), the leading cause of death and disability worldwide (Pagidipati and Gaziano, 2013). To date, however, little research has focused on the possible cardiovascular toxicity of this new POP and the underlying mechanisms involved. We recently reported that BDE-209 can promote the adhesion of monocyte onto cultured human aortic endothelial cells and can augment foam cell formation in cultured human macrophages, suggesting potential cardiovascular toxicity of this POP substance (Zhi et al., 2018, 2019). We previously revealed that the augmented lipid accumulation in macrophages is via TLR4-dependent lipid uptake in the cells (Zhi et al., 2018), but the molecular mechanisms are not well understood. In the present study, we illustrate that BDE-209 inhibits miR–21 expression, which negatively regulates the expression of TLR4 at both mRNA and protein levels, resulting in enhanced lipid accumulation in the macrophages and the formation of foam cells.

While evidence is now emerging indicating that the human miRNA machinery could be altered in response to environmental toxicants (Sonkoly and Pivarcsi, 2011; Jardim, 2011; Li et al., 2015; Vrijens et al., 2015; Yu and Cho, 2015; Krauskopf et al., 2017), information on the effects of BDE-209 are still limited. Our study is one of the few to investigate the potential effects of BDE-209 on miRNA expression in human cells. Our results clearly demonstrate such an effect that BDE-209 inhibits miR–21 expression in cultured human macrophages. Previous epidemiological and model studies also illustrated that BDE-209 was associated with changes in the expressions of several miRNAs. A birth cohort study assessed the possible linkage of various known environmental pollutants and placental expression of miRNAs and observed a positive association between placental BDE-209 concentrations and expression levels of miR–188-5p (Li et al., 2015). A recent report assessing the effects of BDE-209 on the early human embryonic development showed that miRs–145 and –335 were upregulated in human embryonic stem cell lines exposed to BDE-209 (Du et al., 2016). More recently, we demonstrated that BDE-209 could suppress miR–141 expression in cultured human aortic endothelial cells (Zhi et al., 2019). The BDE-209-induced changes in miRNA expression may relate to the toxicities of this compound. It was demonstrated that BDE-209 inhibited the expression of pluripotency genes such as *OCT4* and induced apoptosis in human embryonic stem cells via increasing the expressions of miR–145 and miR–335 (Du et al., 2016). Additionally, BDE-209 potentiates monocyte–endothelial cell interaction by suppress the expression of miR–141 in cultured human aortic endothelial cells (Zhi et al., 2019). Given miRNAs are able to regulate diverse gene networks including cell proliferation, apoptosis and differentiation (Ambros, 2004; Bartel, 2004), the current findings together with the previous results may suggest that interaction with miRNAs is possibly one of the important mechanisms behind the toxicity of BDE-209; which may provide a new insight into the molecular mechanisms of the toxicity of

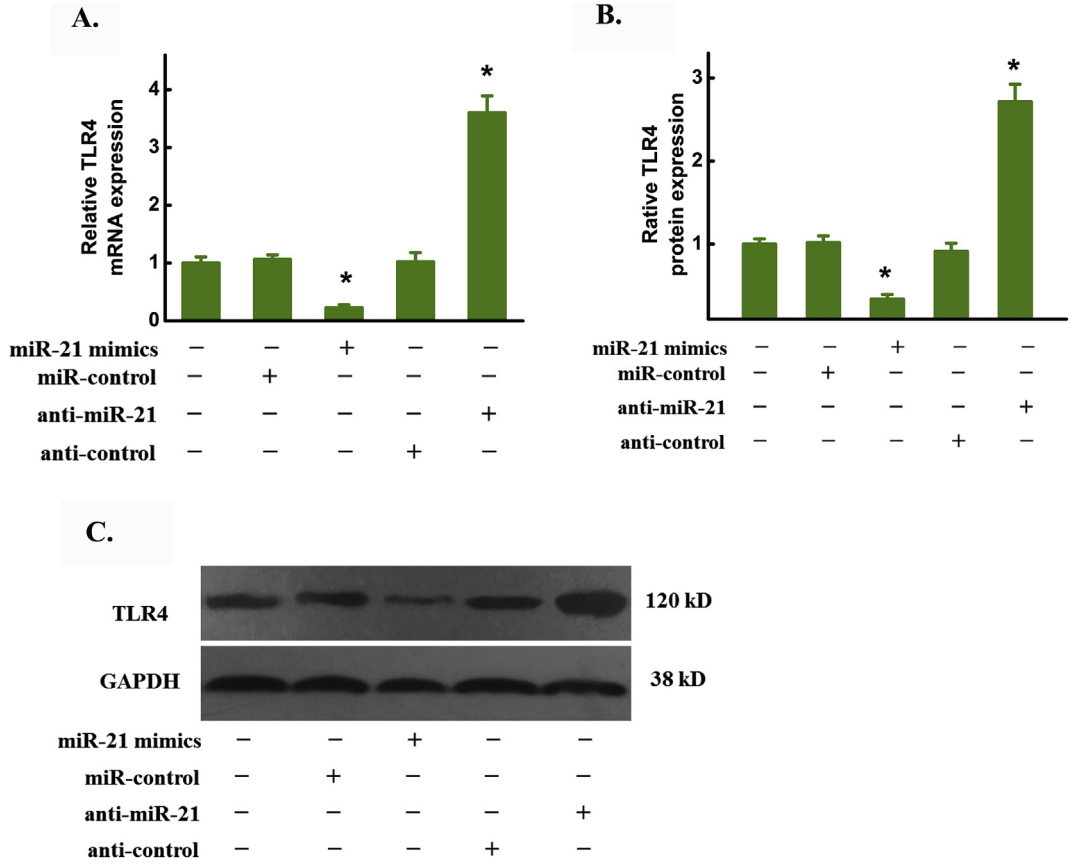

Fig. 4. miR–21 inhibits TLR4 expression in macrophages exposed to 25 μM of BDE-209 and 50 μg/mL of oxLDL. (A) The mRNA expression levels in macrophages transfected with miR–21 mimic, anti-miR–21 inhibitor or their controls. Error bar means 1SE. An asterisk (*) represents significant ($p < 0.05$) difference when compared with the control. (B) The protein expression levels in macrophages transfected with miR–21 mimic, anti-miR–21 inhibitor or their controls. (C) Immunoblots of TLR4 in macrophages transfected with miR–21 mimic, anti-miR–21 inhibitor, or their controls.

this new POP. From this perspective, specific miRNA signatures may have a great potential as novel biomarkers of exposure and effects of BDE-209 in target cells.

Although specific miRNAs are known to be altered in response to environmental toxicants, the underlying mechanisms remain unclear. It has been reported that BDE-209 exposure can generate intracellular reactive oxygen species (ROS), which may be associated with the changes in levels of specific miRNAs in human embryonic stem cells (Du et al., 2016). We also examined the ROS levels in our macrophages exposed to diverse levels of BDE-209. However, we have not found a significant change in ROS levels in the BDE-209 exposed macrophages (data not shown). Recently, Sollome and co-workers identified several transcription factors as transcriptional regulators of miRNAs in response to environmental toxicants, using an *in silico* bioinformatic approach (Sollome et al., 2016). The candidate transcription factors included SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, and member 3 (SMARCA3) (Sollome et al., 2016). Whether BDE-209 exposure suppressed the expression of miR–21 in macrophages is through such a mechanism warrants further study.

Information on the effects of miR–21 stimulated by exogenous chemicals to TLR4 signaling in human cells is scarce. In the present study we revealed that the BDE-209-induced miR–21 inhibition negatively regulated TLR4 expression in cultured human macrophages. A previous study also demonstrated that an intravenous anesthetics, propofol (2,6-diisopropylphenol), could regulate the expression of TLR4 through miR–21 in human umbilical vein endothelial cells (Ma et al., 2017). In spite of insufficient data on the effects of miR–21 stimulated by exogenous chemicals to TLR4 signaling, there exists some toxicological characterizations of miR–21 on human TLR4 expression. Bioinformatics analysis revealed that the sequence of miR–21 is partially complementary to a region within the 3′-UTR of the transcript for human TLR4 (Ma et al., 2017; Xue et al., 2017). This is further confirmed by a luciferase assay showing that miR–21 is able to directly bind to the 3′-UTR of TLR4 and inhibit TLR4 expression (Ma et al., 2017; Xue et al., 2017).

Except for regulating TLR4 expression, miR–21, identified as one of the first mammalian miRNAs, has been established to play other critical roles in human macrophages (Jazbutyte and Thum, 2010; Sen and Roy, 2012; Sheedy, 2015). In particular, miR–21 has emerged as a key mediator in limiting inflammation induced by an injury, infection, or chemical stimuli (Sheedy, 2015). More recently, it was demonstrated that macrophage deficiency of miR–21 could promote apoptosis, plaque necrosis, and vascular inflammation during atherogenesis (Canfrán-Duque et al., 2017). These findings suggest that miR–21 inhibition in macrophages may potentially promote inflammation, which contributes to pathogenesis of inflammatory diseases including infection, atherosclerosis and cancer. Therefore, other likely effects of the downregulation of miR–21 induced by BDE-209 in macrophages deserve further investigations.

Excessive lipid accumulation in macrophages and the subsequent lipid-laden foam cell accumulation is a critical trigger for the initiation of cardiovascular diseases (Moore and Tabas, 2011; Moore et al., 2013). Therefore, blocking lipid deposition in macrophage-derived foam cells is one of the effective therapeutic strategies in dampening incidence of cardiovascular diseases (Li and Glass, 2002). In the present study, we demonstrated that overexpression of miR–21 dramatically attenuated BDE-209-induced lipid accumulation in cultured human macrophages.

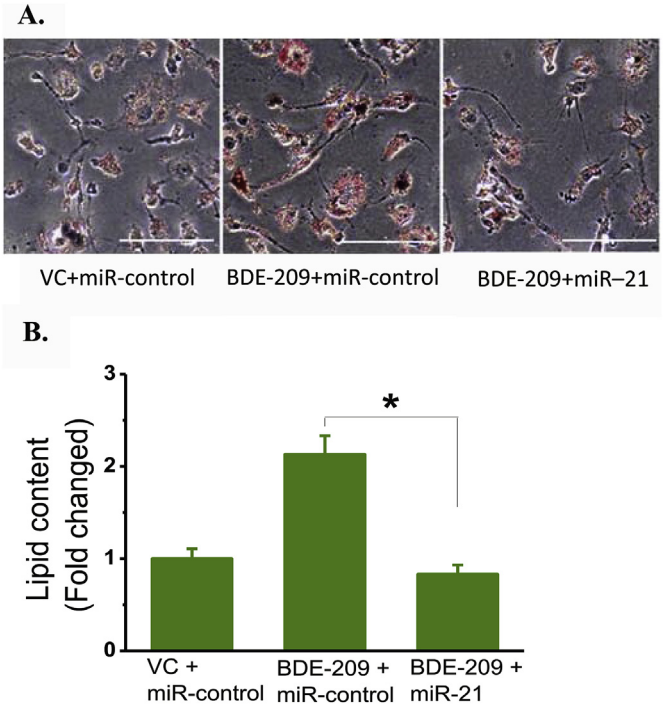

Fig. 5. miR–21 attenuates lipid accumulation in human macrophages exposed to 25 μM of BDE-209. (A) Representative photomicrographs of lipid-laden macrophages by Oil Red O staining. Scale bar = 100 μm, VC = vehicle control. (B) Transfection of miR–21 mimic into the macrophages significantly abrogated the BDE-209-induced lipid accumulation. An asterisk (*) means significant ($p < 0.05$) difference when compared with VC.

Thus, our results may provide a potential molecular target in the therapeutic aspect for prevention and treatment of the possible cardiovascular toxicity of BDE-209.

While suggestive, the positive results obtained from the present study should be interpreted with caution due to the inherent limitations in the study design. The current research is only a mechanism study. Our results showing BDE-209 inhibits miR–21 in cultured human macrophages do not mean that it will occur in the general population, because the exposure concentrations of BDE-209 in our macrophages are several orders of magnitude higher than those detected in the general population ($\sim 10^{-4}$ μM) (Zhi et al., 2018). Additionally, our results are based on an in vitro model rather than in vivo, where the real interaction of BDE-209 and miR–21 may be more complex than that occurs in vitro. More refined analyses are warranted to thoroughly capture the effects of BDE-209 on miR–21 expression in humans.

5. Conclusions

In the present study, we highlighted some mechanisms concerning the regulation of miRNAs to lipid accumulation induced by BDE-209 in cultured human macrophages. We showed that BDE-209 inhibited miR–21 expression in macrophages, which negatively regulated TLR4 expression, leading to excessive lipid uptake in the cells. We also illustrated that overexpression of miR–21 can effectively attenuate BDE-209-induced lipid accumulation in macrophages. Our data suggest the important role of miR–21 in the process of macrophage-derived foam cell formation trigged by BDE-209, and may possibly indicate miR–21 as a potential prognostic marker and future therapeutic target for BDE-209 exposure.

Acknowledgments

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