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RESEARCH ARTICLE

The effect of fenofibrate, a PPARα activator on toll-like receptor-4 signal transduction in melanoma both in vitro and in vivo

N. Dana[1] · S. Haghjooy Javanmard[1] · G. Vaseghi[1,2]

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Abstract

Background The anti-cancer effect of peroxisome proliferator-activated receptor (PPAR) α ligands on growth and metastatic potential of melanoma cells has been shown previously. However, the mechanism underlying these effects remains to be elucidated. Here, we investigated the effects of fenofibrate (PPAR ligand) on Toll-like receptor-4 (TLR-4) signaling in mice melanoma.

Methods Mice melanoma cells (B16F10) were treated with fenofibrate or LPS or LPS + fenofibrate or pre-treated with CLI-095 (a TLR4 inhibitor), followed by fenofibrate. In in vivo model, C57BL/6 mice were subcutaneously injected with B16F10 cells (with/without LPS pre-treatment), and fenofibrate was administrated after development of palpable tumors. Cell proliferation, the expression level of *Tlr4*, *Myd88*, *Nf-κb1* genes, TLR-4 protein expression, TNF-α levels, and tumor volume were measured.

Result Our results indicated that fenofibrate significantly inhibited the *Tlr-4*, *Myd-88*, and *Nf-kb1* mRNA expression and TNF-α concentration in B16F10 LPS-stimulated cells. In addition, blocking TLR-4 signaling increased the anti-inflammatory potential of fenofibrate. Also fenofibrate can reduce LPS-induced tumor volume, *Tlr-4*, *Myd-88*, *Nf-kb1* mRNA, and TLR-4 protein expression in tumor tissue and also TNF-α level in tumor tissue lysate.

Conclusion Our data indicate that fenofibrate may exert its anti-melanoma effects via interaction with TLR4-dependent signaling pathway (TLR-4/MyD-88/ NF-kB).

Keywords PPAR alpha · Toll-like receptor-4 · Melanoma

Introduction

Melanoma is one of the most lethal and aggressive form of skin cancers [1], which continues to increase, worldwide [2]. Melanoma is defined as a heterogenous disease [3]. Recent studies have shown that stimulation of Toll-like receptor-4 (TLR-4), a critical transmembrane protein in pathogen recognition and activation of innate immunity, in melanoma cells with specific ligands, significantly upregulates pro-inflammatory and immunosuppressive cytokines, and also inflammatory factors [4].

TLR4 recognizes exogenous and endogenous ligands such as bacterial lipopolysaccharides (LPS) and heat shock protein, which consequently activate inflammatory and innate immune responses [5] and then initiate intracellular signaling cascades, ultimately causing the activation of nuclear factor-kB (NF-kB) and the expressions of pro-inflammatory cytokines [6]. Mice lacking TLR4 are markedly protected from carcinogenesis [7, 8].

PPARs are ligand-activated transcription factors which belong to the nuclear hormone receptor superfamily. PPAR α is one of the three members of the PPAR family and is mainly responsible for fatty acid oxidation and ketogenesis, simultaneously inhibiting glycolysis and fatty acid synthesis [9].

There are strong evidences indicating that PPARα activator, fenofibrate (a drug of the fibrate class), can be useful as a complementary adjunct treatment of cancer [10–12].

✉ G. Vaseghi
golnazvaseghi@yahoo.com

[1] Applied Physiology Research Center and Department of Physiology, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran

[2] Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran

Fenofibrate can strongly inhibit melanoma cell migration and proliferation in a PPARα-independent manner [9]. Fenofibrate anti-inflammatory activity is mediated through NF-κB downregulation and also PPARα independent mechanisms.

To date, various studies have demonstrated that PPARα agonists are potent inhibitors of TLRs activation in different diseases and they have shown cross talk between them [13–15]. PPARα stimulation can change the expression level of *TLR4* in various ways, which can lead to modulating its effects. For example, fenofibrate can modulate angiotensin II-induced inflammatory responses in vascular smooth muscle cells via the TLR4-dependent signaling pathway [6]. Although PPARα serves anti-cancer action by transrepressing inflammatory signaling pathways, much less is known about the underlying mechanisms of the inhibitory effects of fenofibrate on cancer treatment and the TLR4-dependent signaling pathway involved.

Herein, we examined whether fenofibrate is able to inhibit melanoma progression via interfering with the TLR4-dependent signaling pathway and if elucidates its anti-inflammatory and anti-cancer mechanisms via this pathway.

Materials and methods

Cell culture and treatment

B16F10 melanoma cell line (obtained from National Cell Bank of Iran, Tehran) was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (Gibco, Newcastle, NSW, Australia) in 37 °C and 5% CO_2 atmosphere. For experiments, cells were seeded in 24-well (2×10^5 cells/well) plates and incubated for 24 h. In all of the experiments, specific stimulation of TLR-4 was done with Ultrapure LPS-EB from *E. coli* 0111: B4 (5 µg/ml) from InvivoGen. All the experiments were repeated three times and reported as three independent replicate experiments.

For drug preparation, in in vitro fenofibrate was dissolved in DMSO and for mice drug administration fenofibrate was dissolved in polyethylene glycol.

In in vitro procedure after 24 h, the seeded cells were divided into the control, fenofibrate (1, 25, 50,100 µmol/L), LPS (5 µg/ml) and LPS + fenofibrate (corresponding LPS group plus 1, 25, 50,100 µmol/L fenofibrate) groups. Initially, the cells were stimulated with different concentrations of fenofibrate for 1 h. Following stimulation, the cells were treated with LPS. Subsequently, after 24 h, cells were used for assessment of cell viability and mRNA levels of *Tlr-4*, *Myd-88*, and *Nf-kb1*, and the cell supernatant was used for the measurement of TNF-α concentration.

Animals

6-week-old male C57BL/6 mice were purchased from the Pasteur Institute of Iran (Tehran). All experiments were approved by the Ethics Committee of Isfahan University (approval ID: IR. MUI. REC.1394.3.617).The animals were placed in a 12/12 h light/dark cycle at 25 ± 2 °C and 50% humidity. The mice were randomly divided into eight groups ($n=6$), fed with a regular diet and housed and monitored in a pathogen-free environment.

B16F10 cells were cultured in complete medium. After 24 h stimulation of cells with or without LPS (5 µg/ml), animals were divided into two major groups, one group received treated cells and the other received untreated cells. Tumors were induced by injecting 1×10^6 cells in 200 µL of PBS subcutaneously on the right back flank [16]. Following the development of palpable tumors (5–7 days after injection), each of the main groups was divided again into four groups. Then mice were treated as follows. Four LPS groups were administered fenofibrate in different dose ranges (0, 50, 100, 200 mg/kg/day) for 10 days by gavage. Their control group received only vehicle. The other four groups that were injected with untreated cells received the same fenofibrate treatment and their control group was administrated vehicle for 10 days.

Finally, at the end of the study (day 20), animals were euthanized and tumor sizes were measured. Tumor samples were cut into pieces and immediately frozen in liquid nitrogen for RNA and protein extraction. The remaining tumors were fixed in 10% formalin for histological analysis.

RNA isolation

Total RNA from treated cells was extracted using a GeneJET RNA purification kit (Thermo Scientific (EU), Lithuania), according to the manufacturer's description. In our in vivo model, frozen tumor tissues were homogenized in lysis buffer (Thermo Scientific) using Micro Smash MS-100R (Tomy Digital Microbiology Co Ltd, Tokyo, Japan) and total RNA was extracted. RNA samples were treated by RNase-free DNase (Qiagen, USA) to eliminate the genomic DNA. First-strand cDNA was synthesized from RNA templates by using a RevertAid First-Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania).

Quantitative real-time PCR (qRT-PCR)

Gene expression on mRNA level was assessed using Maxima SYBR Green Rox qPCR master mix kit (Fermentas, Vilnius, Lithuania) according to the instructions. Real-time

qPCR was carried out on a Corbett machine, Rotorgene 6000 (Australia).

The PCR conditions consisted of an initial denaturation at 95 °C for 15 min; followed by 40 cycles of denaturation (95 °C for 15 s), annealing and extension for 1 min at 60 °C. A second 1-min annealing period was completed at 72 C before a final extension at 72 C for 10 min.

The primers of *Tlr-4*, *Myd-88*, *Nf-kb1*, and *beta-actin* were designed from the sequence list of GeneBank database (National Centre for Biotechnology Information, NCBI) using Beacon designer 8 software and then blasted against GeneBank database sequences (see Table 1 for the sequences).

The relative expression ratios of the target gene in the tested group versus those in the control group were calculated by the $2^{-\Delta\Delta Ct}$method using the housekeeping gene beta-actin (β-actin) as the endogenous reference gene to normalize the level of target gene expression [17].

Detection of TNF-α (ELISA) in vivo and in vitro

The level of TNF-α in the cell culture supernatant, mice serum samples, and tumor tissue lysate was measured using commercially available enzyme-linked immunosorbent (ELISA) assay kit (eBioscience). The OD values were read in a microplate reader at 450 nm. The concentration of cytokines in each sample was calculated using a standard curve generated using recombinant cytokines.

Tissue lysates were prepared after snap freezing of tumor tissue in liquid nitrogen using Ripa buffer, with a protease inhibitor cocktail (Calbiochem, San Diego, CA). Tissue lysates were cold centrifuged at 12,000 rpm for 30 min and then the supernatants were collected. Protein concentration was determined by the Bradford method using Bio-Rad Protein Assay Dye and microtiter plate reader (ELX 800-BioTek-USA) to normalize the protein concentration in all samples.

In the other experiment, we determined whether CLI-095 (TLR4-specific inhibitor) has inhibitory effects on the TNF-α production in the cell supernatant. Briefly, B16F10 cells were plated in 24-well plates and pretreated with CLI-095 (1 μM) for 1 h prior to the addition of fenofibrate (100 μmol/l) for 1 h, and subsequently stimulated with LPS (5 μg/ml) for 24 h. A 100 μl aliquot of culture medium supernatant was collected to determine TNF-α concentration by the same ELISA kit.

Table 1 Primers used for real-time PCR analysis

Gene	Primer sequence
Tlr-4	5′-AGTGGCTGGATTTATCCAGGTGTG-3′
	5′-TTGAGAGGTGGTGTAAGCCATGCC-3′
Myd-88	5′-AAGTCTAGGAAGGCCCCAAA-3′
	5′-CTGGGGAGAAAACAGCTGAG-3′
Nf-kB1	5′- ACACGAGGCTACAACTCTGC-3′
	5′- GGTACCCCCAGAGACCTCAT-3′
β-actin	5′- GCTGTATTCCCCTCCATCGTG -3′
	5′- CACGGT TGGCCT TAGGGTTCAG -3′

Determination of tumor volume

To determine tumor volume by external caliper, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined. Tumor volumes based on caliper measurements were calculated by the modified ellipsoidal formula [18].

$$\text{Tumor volume} = {}^{1}\!/_{2}\left(\text{length} \times \text{width}^2\right).$$

Immunohistochemistry

Tumor tissues were fixed in 10% formalin, embedded in paraffin, and sectioned. Paraffin sections were deparaffinized and rehydrated with serially diluted ethanol and transferred to PBS. The endogenous peroxidase activity was blocked by 3% hydrogen peroxide (dissolved in methanol for 20 min). Sections were incubated with a blocking serum (normal horse serum) for 1 h. Excess of serum was drained and the sections were incubated with the TLR4 antibody (mouse monoclonal, sc-293072 HRP) overnight at 4 °C. The peroxidase reaction was developed using 3, 3-diaminobenzidine (diaminobenzidine tetrahydrochloride) substrate and finally all the sections were counterstained with hematoxylin. Phosphate-buffered saline (pH 7.6) was used for rinsing between the different steps. Finally, images were captured using the Leica microscope equipped with a Leica camera (DFC450 C) at objective lens. The TLR-4 protein expression was evaluated by ImageJ software.

Statistical analysis

Data were expressed as means ± SEM. Differences between the two groups were determined either by unpaired Student's *t* test or by one-way ANOVA followed by post hoc Dunn's multiple comparison tests. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS 19 software.

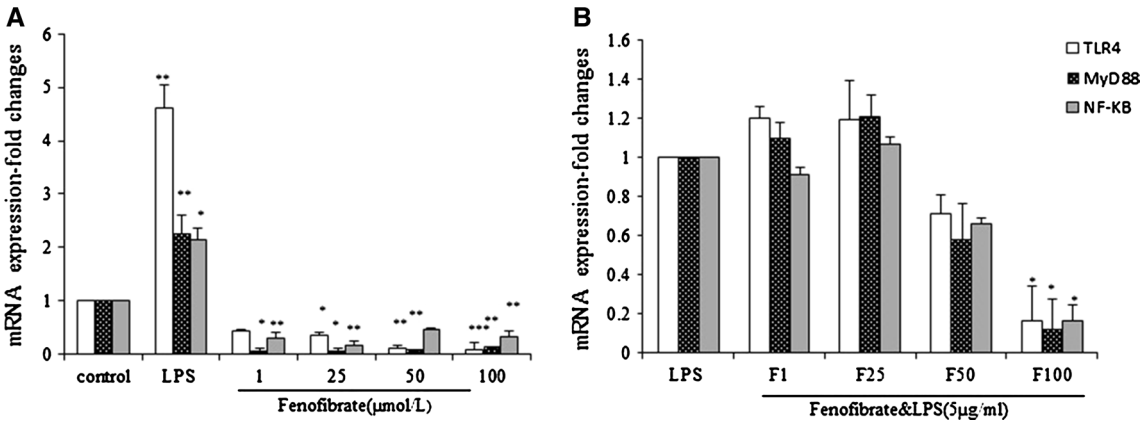

Fig. 1 Effect of fenofibrate and LPS on expression of *Tlr-4*, *Myd-88*, and *Nf-kb1* in vitro. **a** B16F10 cells were incubated with fenofibrate (1, 25, 50, 100 μM) or LPS(5 μg/ml) for 24 h. **b** B16F10 cells were pre-incubated for 1 h with or without fenofibrate (1, 25, 50, 100 μM), followed by cell incubation with LPS (5 μg/ml) for 24 h. mRNA expression was measured using quantitative real-time PCR. $^{*}P<0.05$, $^{**}P<0.01$,$^{***}P<0.001$ compared with the negative control One representative experiment of three is depicted. Each graph has been represented as mean ± SEM

Results

Effects of fenofibrate on mRNA expressions of Tlr-4, Myd-88, and Nf-kb1 in B16F10 cells

As shown in Fig. 1a, LPS upregulated mRNA expressions of *Tlr-4* ($P<0.01$), *Myd-88* ($P<0.05$), and *Nf-κb1* ($P<0.05$). On the other hand, treatment of cells with fenofibrate has shown that this PPAR alpha agonist can significantly decrease *Tlr-4* in all doses except at dose 1 μM. We found a significant reduction in *Myd-88* and *Nf-κb1* (except 50 μM) mRNA expression levels after fenofibrate treatment.

To investigate whether fenofibrate depresses LPS-induced mRNA expressions of the genes, B16F10 cells were pretreated with LPS (5 μg/ml) for 1 h prior to the addition of fenofibrate (100, 50, 25,1 μM) for 24 h. As shown in Fig. 1b, compared with the control (LPS treated cells), stimulating the cells with fenofibrate resulted in significantly decreased mRNA expression of *Tlr-4* ($P<0.01$) *Myd-88* ($P<0.05$), and *Nf-κb1* ($P<0.05$). On the other hand, treatment of the cells with the combination of LPS and fenofibrate significantly reversed the effect elicited by LPS alone.

Fenofibrate reduces TNF-α in LPS-stimulated B16F10 cells supernatant

As shown in Fig. 2a, the results indicated that the levels of TNF-α induced by LPS in B16F10 cell culture supernatant increased significantly with LPS dose, as compared with untreated control ($P<0.001$). Treatment of cells with fenofibrate alone decreases the levels of TNF-α only with high dose (100 μM) in comparison with the control group (untreated cells) ($P>0.05$). Moreover, TNF-α levels after treatment with fenofibrate in the combination of LPS were significantly lower than treatment with LPS alone for all doses of fenofibrate ($P<0.001$).

Blocking TLR-4 with CLI-095 increases the anti-inflammatory potential of fenofibrate in LPS-stimulated B16f10 cells

As shown in Fig. 2b, compared with the control, stimulating the cells with LPS led to TNF-α elevation, whereas CLI-095 and fenofibrate each reversed the LPS-induced effect on TNF-α in B16F10 cells (data not shown). Moreover, treatment of the cells with a combination of CLI-095 and fenofibrate synergistically reversed the effects induced by LPS in comparison with the treatment of the CLI-095 or fenofibrate alone. Considering that the TLR4 inhibitor antagonizes the effects of LPS on TNF-α, and fenofibrate also downregulates TLR4 expression in B16F10 cells, the modulatory effects of fenofibrate on TNF-α production in these cells is related to TLR4.

Fenofibrate inhibits melanoma tumor development in mice

At first, to determine the antineoplastic effect of fenofibrate in vivo, male C57bl6 mice were used. As soon as the tumor became palpable, about 5 days after cell injection, the treatment schedule was performed using fenofibrate (50, 100, 200 mg/kg/day) or vehicle by gastric gavage and continued on diets for 10 more days. Tumor size was measured throughout the study. Untreated control mice

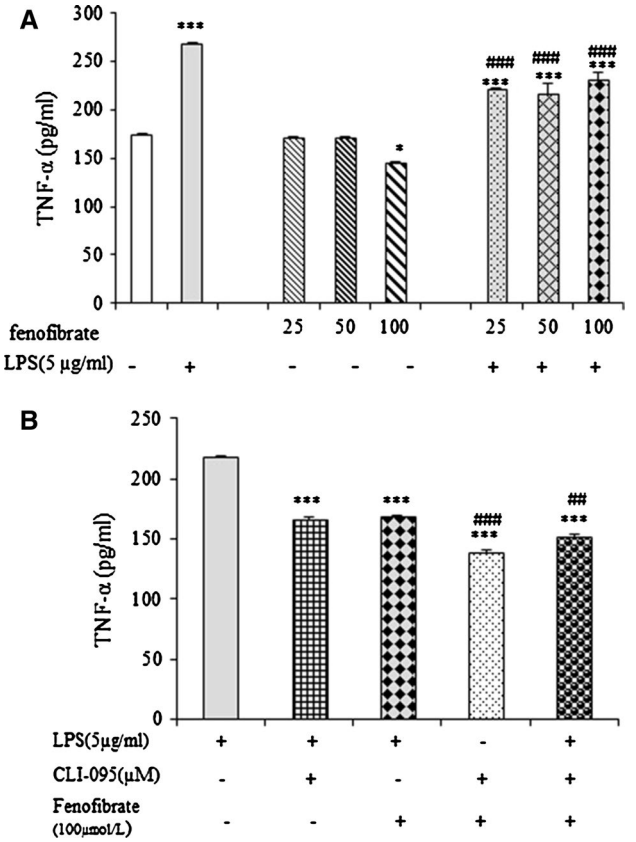

Fig. 2 Effect of fenofibrate and LPS on TNF-α production in B16F10 cell supernatant with or without TLR4 inhibitor. **a** The cells were pretreated with fenofibrate alone or in combination with LPS. $^{*}P<0.05$, in comparison with control group and $^{\#}P<0.01$ compared to the LPS group. **b** The cells were pretreated with fenofibrate alone or in combination with CLI-095 before LPS treatment. Following 24 h treatment, the amounts of TNF-α production was measured in the supernatants. $^{*}P<0.05$, $^{***}P<0.001$ compared to the LPS-treated cells; $^{\#\#\#}P<0.001$ compared to the LPS plus fenofibrate-treated cells. Data are means ± SEM of three independent experiments

rapidly developed visible tumors and dramatic growth was observed throughout the course of the study. In contrast, treatment of mice with fenofibrate markedly attenuated the ability of cells to develop tumors. Tumors in groups of mice that received fenofibrate 50 ($P<0.01$) and 100 mg/kg/day ($P<0.05$) were significantly smaller than tumors from untreated control mice (Fig. 3a). These data clearly demonstrate that fenofibrate has anti-melanoma activity in vivo.

In the LPS groups, LPS treatment of B16F10 cells before injection caused a significant increase in tumor volume compared to the other group that was injected with untreated cells ($P<0.05$).

As demonstrated in Fig. 3a, administration of fenofibrate led to a significant reduction in tumor volume only in 50 mg/kg/day dose compared to their control group ($P<0.05$).

Effects of fenofibrate and LPS separately on expressions of Tlr-4, Myd-88, and Nf-kb1 in mice tumor

Pretreatment of cells with LPS before injection into mice significantly elevated the levels of *Tlr-4* ($P<0.001$), *Myd-88* ($P<0.01$), and *Nf-kb1* ($P<0.001$) mRNAs in tumor tissue.

Fenofibrate concentration dependently downregulated mRNA expressions of *Tlr-4*,*Myd-88*, and *Nf-kb1*. As shown in Fig. 3c, *Tlr-4* expression was significantly decreased only by 50 mg/kg/day fenofibrate ($P<0.05$) and the other fenofibrate doses had no significant effect on *Tlr-4* expression. Fenofibrate treatment for 10 days significantly decreased *Myd-88* expression at doses of 50 ($P<0.001$), 100 ($P<0.01$), and 200 mg/kg/day ($P<0.01$).Also, the expression of *Nf-kb1* was reduced significantly at doses of 50 ($P<0.001$), 100 ($P<0.05$), and 200 mg/kg/day ($P<0.05$).

The inhibitory effect of fenofibrate on the LPS-induced expression of Tlr-4, Myd-88, and Nf-kb1 in mice

Fenofibrate in all concentrations significantly suppressed LPS-stimulated increase of *Tlr-4* expression in comparison to LPS alone treated group ($P<0.001$). Fenofibrate significantly decreased the *Myd-88* expression at the concentration of 50 ($P<0.01$), 100 ($P<0.01$) and 200 ($P<0.05$). As demonstrated in Fig. 3d, administration of fenofibrate at doses of 50 ($P<0.01$) and 100 ($P<0.01$) mg/kg/day significantly decreased the expression of *Nf-kb1* as compared to the control group, but the dose of 200 mg/kg/day had no significant effect.

Immunohistochemical evaluation of TLR-4 protein expression

All the samples, including control, LPS, fenofibrate 50 (mg/kg/day), fenofibrate 100 (mg/kg/day) and LPS + fenofibrate 50 (mg/kg/day) group, were positive for TLR-4 protein expression. As shown in Fig. 4 compared with the control group, the TLR-4 expression was significantly increased in the LPS group ($P<0.001$). We found reduced TLR-4 protein expression in fenofibrate group, but it was not significant expression when compared with the control group ($P<0.05$). Interestingly, we found reduced TLR-4 protein expression in the group that received LPS and fenofibrate 50 (mg/kg/day) compared with the LPS group ($P<0.001$).

The inhibitory effect of fenofibrate and LPS on TNF-α production in mice serum and tumor lysate

To examine the effect of LPS on TNF-α concentration in serum and tumor lysate, its level was determined by Elisa

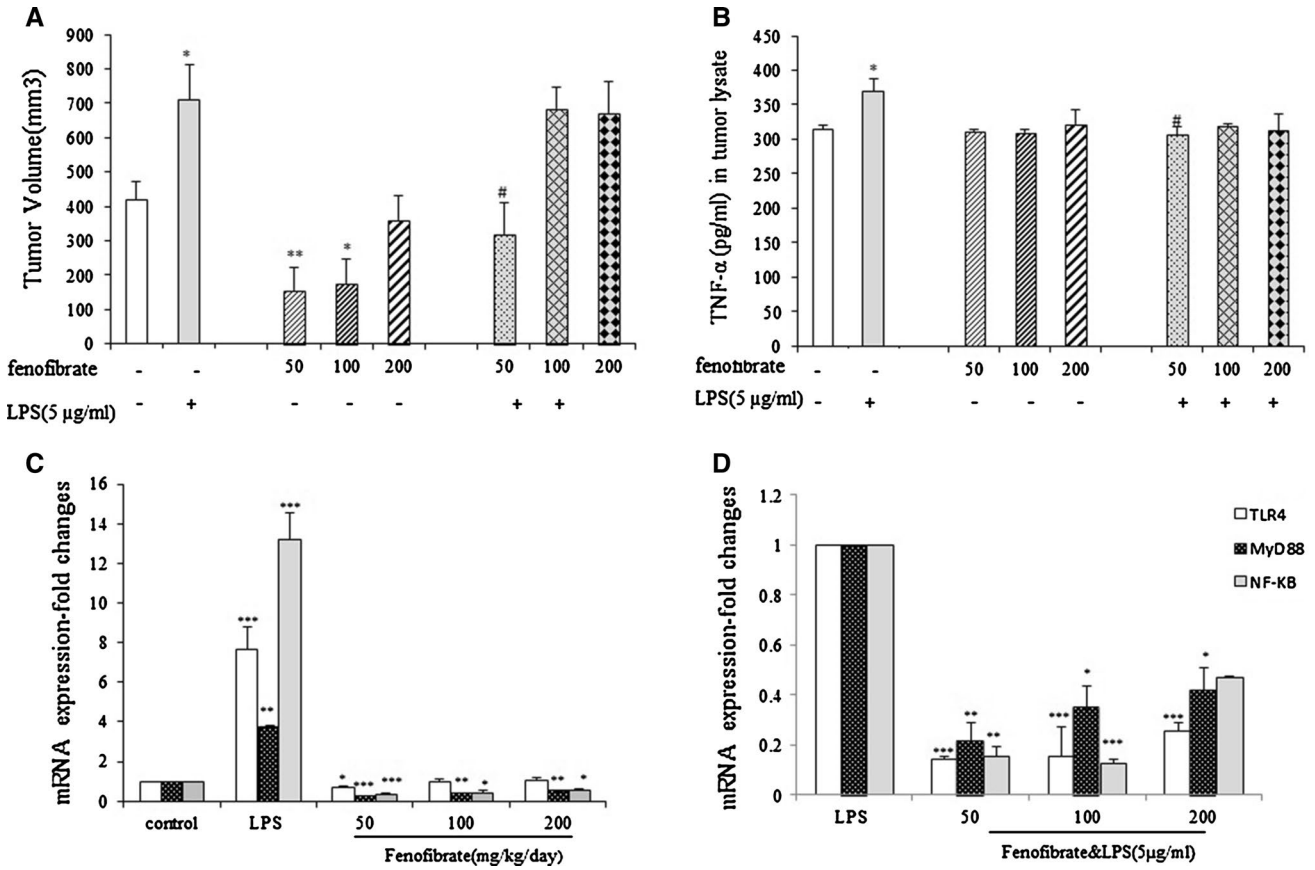

Fig. 3 Effect of fenofibrate with or without LPS on tumor volume, TNF-α production in tumor lysate, and *Tlr4, Myd-88*, and *Nf-kb1* gene expression in different groups of mice. **a** To determine tumor volume by external caliper, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were determined. $^{*}P<0.05$, $^{**}P<0.01$, in comparison with the control group and $^{\#}P<0.01$ compared to the LPS group. **b** The level of TNF-α was measured by Elisa kits in tumor lysate.$^{*}P<0.05$, in comparison with the control group and $^{\#}P<0.01$ compared to the LPS group. **c** Effect of fenofibrate on *Tlr4, Myd-88*, and *Nf-kb1* expression in tumor tissue of groups that received untreated cells. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ compared with the control group. **d** Effect of fenofibrate on *Tlr4, Myd-88*, and *Nf-kb1* expression in tumor tissue of experimental groups that were injected with LPS-treated cells and administered fenofibrate in different dose ranges. The mRNA expression data were normalized to the β-actin signal. Fold changes relative to control are presented as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ compared with the control group

kit. Our results have shown that the mice group that was injected with LPS-treated cells had TNF-α in tumor lysate significantly higher than the control group ($P<0.05$) (Fig. 3b). In LPS groups only administration of fenofibrate 50(mg/kg/day) decreased TNF-α level in tumor lysate. Our results illustrated that LPS and fenofibrate had no significant effect on TNF-α concentration in mice serum compared to 'the control group (data not shown).

Discussion

In the present study, we showed that PPAR-α agonist, fenofibrate, could decrease *Tlr4*, *Myd-88*, and *Nf-κb1* gene expression and TNF-α production in both in vivo and in vitro. We found that in ultrapure LPS-stimulated melanoma cells in vivo and in vitro, fenofibrate exhibited a significant and exact anti-inflammatory effect through decreasing *Tlr4*, *Myd-88*, and *Nf-κb1* gene expression and TNF-α production. In LPS groups, our immunohistochemistry results were confirmed by mRNA expression data. As in the group that received LPS and fenofibrate 50 (mg/kg/day), TLR-4 protein expressions were significantly reduced compared with the LPS group. Moreover, co-administration of fenofibrate and LPS showed more significant effects than fenofibrate alone.

We observed that fenofibrate at the highest dose did not decrease tumor size significantly compared with lower doses, which can be explained by the ketogenic effect of fenofibrate in high doses. It also can reduce weight in such doses which may disrupt the result [19, 20].

Our findings confirm the possible interaction between fenofibrate and the TLR-4 pathway in the inhibition of LPS-mediated inflammatory responses in B16F10 cells via interfering with the TLR4/MyD-88/NF-kB signaling pathway.

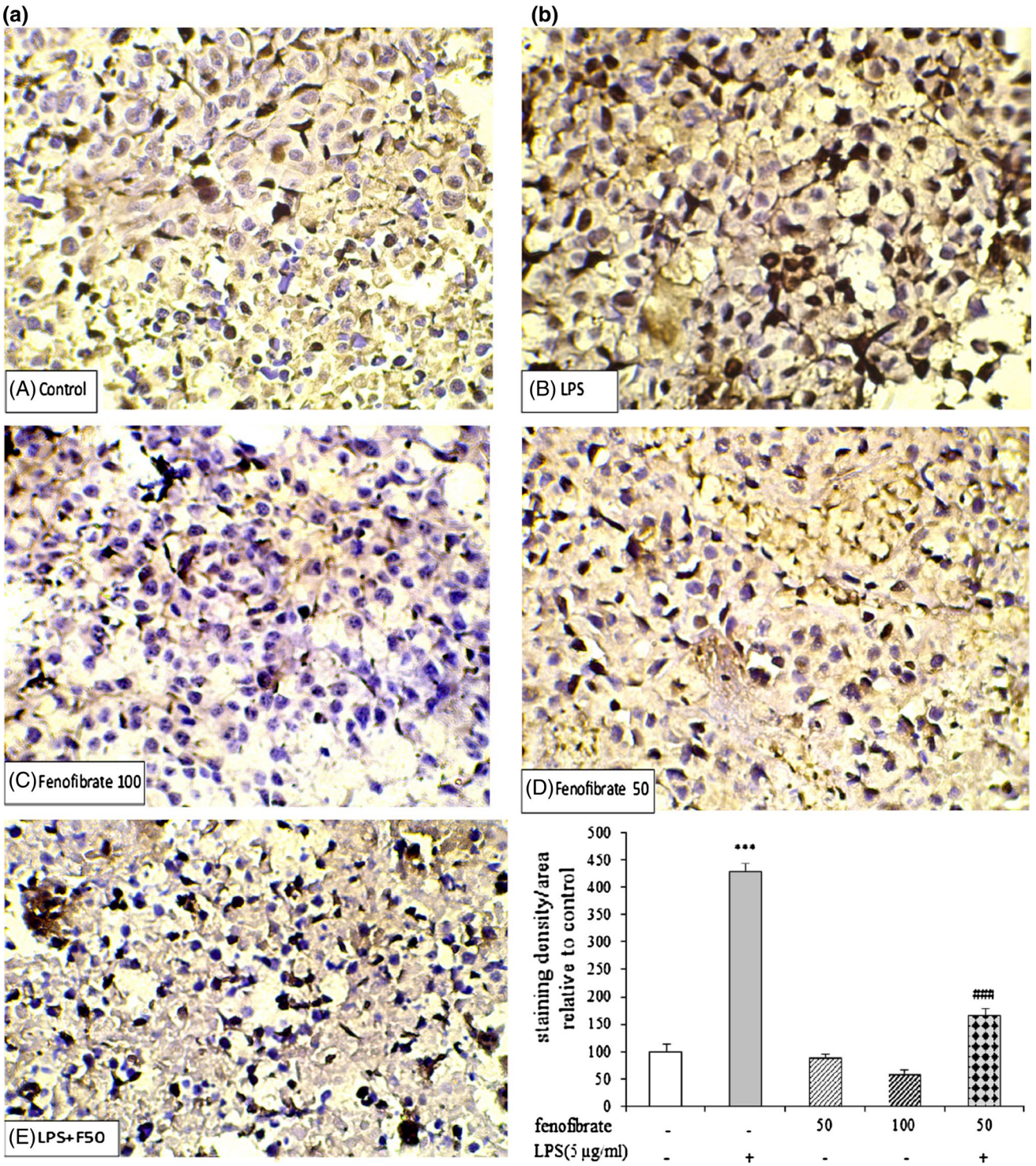

Fig. 4 Immunohistochemical evaluation of TLR4. **a** TLR-4, IHC quantifications relative to the respective nontreatment control in melanoma tumors. **b** Pictures are representative fields of tumor staining for each tumor. $^{***}P < 0.001$, in comparison with the control group, $^{###}P < 0.001$, compared to the LPS group

Since activation of NF-kB is characteristic of TLR-4, we speculate that inhibition of TLR-4 expression by PPARα agonist proceeds through this signaling pathway.

In vivo treatment of mice or in vitro treatment of B16F10 cells with fenofibrate reduced LPS-induced production of TNF-α in tumor lysate and cell supernatant, further

supporting a potential role of PPARα activation in the prevention of inflammation in melanoma cancer.

These results seem to be consistent with the fact that treatment with fenofibrate can repress the generation of TNF-α in melanoma cells [11].

TNF-α is one of the key cytokines mediating the inflammatory processes during melanoma development [21, 22]. It is known that melanoma cells produce TNF-α, but the mechanism of activation is unknown [23]. The chronic production of endogenous TNF-α in the tumor microenvironment can enhance tumor progression by inducing other cytokines/chemokines involved in cancer progression, such as IL-6 and CCL2 [21, 24].

Importantly, the present study provides the first evidence that PPARα activator fenofibrate suppresses LPS-induced *TLR4* expression in cancer cells. In other diseases such as acute pancreatitis, it has been shown that activation of PPAR-α played a protective role, partially mediated by modulation of the TLR4 pathway [25]. Also, the anti-inflammatory role of fenofibrate via interfering with the TLR4-dependent signaling pathway (TLR4/IP-10/PKC/NF-kB) has been shown to protect against atherosclerosis [6]. So far, the relationship between the effect of fenofibrate on LPS-induced inflammatory responses in melanoma cells and TLR4 remains elusive.

Therefore, we hypothesized that fenofibrate interfered with LPS via competitive interaction to B16F10 cell surface receptors, such as TLR4. To examine this possibility, we selected CLI-095 which was reported to completely suppress the production of cytokine induced by the TLR4 ligand, as a TLR4 inhibitor, and investigated its effect on TNF-α by B16F10 cells.

Pretreatment with CLI-095 potently attenuated LPS-induced TNF-α production. Therefore, we evaluated the effects of CLI-095 alone and/or in combination with fenofibrate on the production of TNF-α in B16F10 cells treated with LPS or nothing. We found that fenofibrate and CLI-095 co-treatment synergistically inhibited LPS-induced production of TNF-α.

Therefore, we presume that inhibiting TLR4 binding with LPS by CLI-095 potentially provided partial restoration of the inflammatory response in this model, indicating the antagonistic effect of fenofibrate against TLR4.

Conclusion

In conclusion, these findings provide the evidence for the beneficial effects of PPARα activator fenofibrate to cross talk with the TLR-4 signaling pathway in cancer. More importantly, the anti-inflammatory action of fenofibrate via interfering with the TLR4—dependent signaling pathway (TLR4/MyD-88/NF-kB) works in concert to protect against melanoma in vivo.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no conflict of interest.

Ethics approval The animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and the use of animals was reviewed and approved by the appropriate animal care review committee at the Ethics Committee of Isfahan University (approval ID: IR. MUI. REC.1394.3.617).

Informed consent The research did not involve human participants.

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