Biochemical and Biophysical Research Communications 444 (2014) 555–561

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dihydro-CDDO-trifluoroethyl amide suppresses inflammatory responses in macrophages via activation of Nrf2

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ARTICLE INFO

Article history:
Received 14 January 2014
Available online 29 January 2014

Keywords:
Nrf2
Dh404
Macrophages
Inflammation

ABSTRACT

Nuclear factor erythroid 2-related factor (Nrf2) is the major regulator of cellular defenses against various pathological stresses in a variety of organ systems, thus Nrf2 has evolved to be an attractive drug target for the treatment and/or prevention of human disease. Several synthetic oleanolic triterpenoids including dihydro-CDDO-trifluoroethyl amide (dh404) appear to be potent activators of Nrf2 and exhibit chemo-preventive promises in multiple disease models. While the pharmacological efficacy of Nrf2 activators may be dependent on the nature of Nrf2 activation in specific cell types of target organs, the precise role of Nrf2 in mediating biological effects of Nrf2 activating compounds in various cell types remains to be further explored. Herein we report a unique and Nrf2-dependent anti-inflammatory profile of dh404 in inflamed macrophages. In lipopolysaccharide (LPS)-inflamed RAW264.7 macrophages, dh404 dramatically suppressed the expression of pro-inflammatory cytokines including inducible nitric oxide synthase (iNOS), monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1 beta (MIP-1β), while minimally regulating the expression of interleulin-6 (IL-6), IL-1β, and tumor necrosis factor alpha (TNFα). Dh404 potently activated Nrf2 signaling; however, it did not affect LPS-induced NF-κB activity. Dh404 did not interrupt the interaction of Nrf2 with its endogenous inhibitor Kelch-like ECH associating protein 1 (Keap1) in macrophages. Moreover, knockout of Nrf2 blocked the dh404-induced anti-inflammatory responses in LPS-inflamed macrophages. These results demonstrated that dh404 suppresses pro-inflammatory responses in macrophages via an activation of Nrf2 independently of Keap1 and NF-κB, suggesting a unique therapeutic potential of dh404 for specific targeting a Nrf2-mediated resolution of inflammation.

1. Introduction

Nuclear factor-erythroid 2-related factor 2 (Nrf2), a member of the Cap'n' collar (CNC) family of basic-leucine zipper transcription factors, is a major negative regulator of oxidative stress and inflammatory responses [1,2]. Because of the wealth of evidence that has accumulated showing that Nrf2 is the major regulator of cellular defenses against various pathological stresses in a variety of organ systems including lung, liver, gastrointestinal tract, bladder, kidney, brain, skin, ovary, and heart, Nrf2 has evolved to be an attractive drug target for the treatment and/or prevention of human disease [1]. Several natural or synthetic compounds have been studied to target Nrf2-mediated signaling for cancer chemoprevention [1]. Of these, the synthetic triterpenoid derivatives of oleanolic acid seem to be the most promising drug candidates [3]. Indeed the efficacy for the treatment of chronic kidney disease associated with type II diabetes of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oicacid (CDDO), CDDO methyl ester (CDDO-Me) has been demonstrated [4] despite concerns regarding a potential deleterious effect of CDDO-Me in the kidney as well as other unexpected side effects such as muscle spasm, hypomagnesemia, elevation in alanine aminotransferase levels and gastrointestinal effects [5–7]. The underlying mechanisms of the adverse events associated with CDDO-Me in chronic kidney patients are presently unknown. Nevertheless, Nrf2 still holds promise as a therapeutic target in kidney and other organs [8].

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http://dx.doi.org/10.1016/j.bbrc.2014.01.101
0006-291X/

It is worthy to note that Nrf2 signaling is cell type specific [9]. Moreover, the magnitude of Nrf2 activation is functionally relevant in specific settings as follows: (1) constitutive activation of Nrf2 due to the global knockout of Kelch-like ECH associating protein 1 (Keap1, a major endogenous inhibitor of Nrf2) causes juvenile mortality [10]; (2) activation of Nrf2 by knockdown of Keap1 paradoxically leads to either anti-diabetic or pro-diabetic phenotypes [11,12]; (3) selected activation of hepatic Nrf2 by conditional knockout of Keap1 in the liver is beneficial [13]; and (4) over activation of Nrf2 is the dominant cause of the liver damage induced by hepatic specific deletion of autophagy related gene (Atg)7, whereas persistent activation of Nrf2 in Atg5 deficient liver is critical for the protection against acetaminophen-induced liver injury [14,15]. These results suggest that the pharmacological efficacy of Nrf2 activators may be dependent on the nature of Nrf2 activation in specific cell types of target organs. However, the precise role of Nrf2 in mediating biological effects of Nrf2 activating compounds in various cell types remains to be further explored.

The synthetic oleanolic triterpenoid of dihydro-CDDO-trifluoroethyl amide (dh404, a novel analog of CDDO-Me) is a potent Nrf2 activator [16]. Dh404 is well tolerated in rodents and primates [17] and has been shown to improve obesity, type II diabetes, and Huntington's disease, as well as cardiac maladaptive remodeling and dysfunction in rodents [18–21]. Furthermore, we have demonstrated an essential mediator role of Nrf2 in dh404-induced suppression of oxidative stress in cardiomyocytes [16]. However, the effect of dh404-Nrf2 signaling axis in other cell types is unclear. Herein, we report a unique anti-inflammatory profile of dh404 which is Nrf2 dependent and NF-κB independent in inflamed macrophages, suggesting a novel potential of dh404 for specific targeting Nrf2-mediated resolution of inflammation in macrophages.

2. Materials and methods

2.1. Synthesis and preparation of dh404

Triterpenoid, dihydro-CDDO-trifluoroethyl amide (dh404) of 2-cyano-3,12-dioxooleana-1-ene-28-trifluoroethylamide ($C_{33}H_{45}F_3N_2O_3$) with a molecular weight of 574.338, was synthesized, purified, and analytically characterized using methodology as previously described [1]. The preparation of dh404 for experimental use involved dissolving it in DMSO and then diluting it in the culture media to a final DMSO concentration of 0.01% (v/v).

2.2. Animals and cell cultures

All of the animal procedures were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the University of South Carolina Institutional Animal Care and Use Committee. Littermate wild-type (WT) and Nrf2 knockout ($Nrf2^{-/-}$) mice were generated by breeding heterozygous Nrf2 ($Nrf2^{+/-}$) mice as previously described [22]. Bone marrow-derived macrophages were generated from bone marrow progenitors of adult male 8–16 weeks old WT and $Nrf2^{-/-}$ littermates as previously described [23,24]. Briefly, bone marrow cells flushed from femora of the littermates were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 20% mouse L929 (American Type Culture Collection, ATCC) cell supernatant as a source of colony-stimulating factor (CSF), penicillin (100 U/ml) and streptomycin (100 mg/ml) in a 95% O_2: 5% CO_2 humidified atmosphere at 37 °C. Eight days after the culture, flow cytometer (BD Accuri™ C6, BD Biosciences) analysis confirmed that almost 100% of these cells are double positive with macrophage specific biomarkers CD11b and F4/80 using

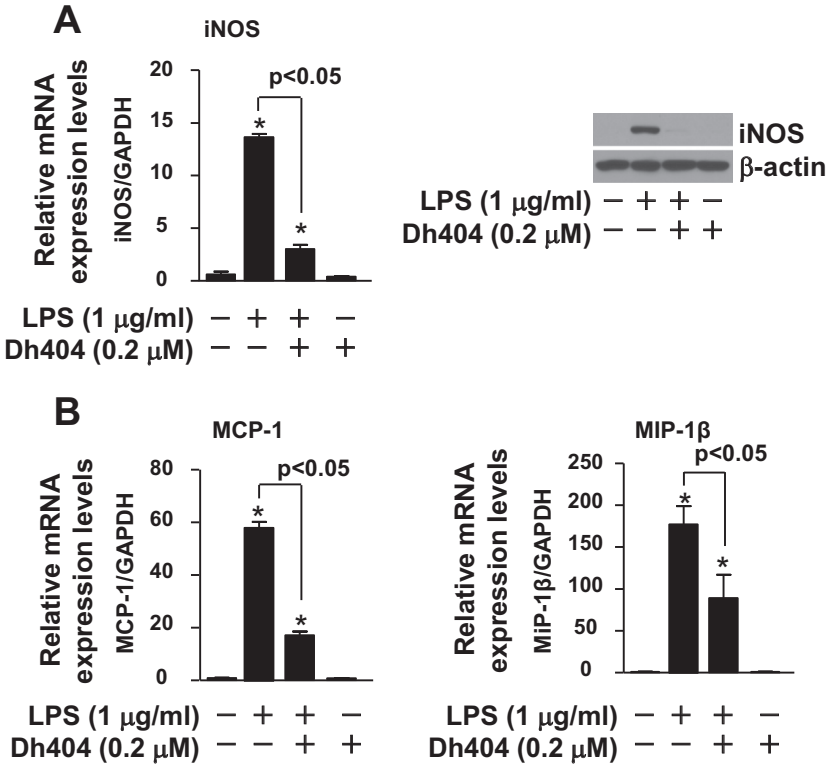

Fig. 1. Effect of dh404 on the expression of iNOS, MCP-1, and MIP-1β in inflamed RAW264.7 macrophages. (A, Left panel and B) RAW264.7 cells were treated with or without LPS (1 μg/ml) and dh404 (0.2 μM) in DMEM with 2% FBS as indicated for 4 h and subjected to Q-PCR analysis. $n = 4$, $^*p < 0.05$ vs. control (–). (A, Right panel) RAW264.7 cells were treated with or without LPS (1 μg/ml) and dh404 (0.2 μM) for 6 h and subjected to Western blot analysis. Results are representatives of three independent experiments.

anti-CD11b (cat# 14-0112, eBioscience) and anti-F4/80 (cat# 123109, Biolegend) as previously reported [23,24]. In addition, RAW 264.7 cells (ATCC), a murine macrophage cell line, were cultured in DMEM (Invitrogen) supplemented with 10% FBS as previously described [25]. Cell viability assays were determined by monitoring the release of the cytoplasmic enzyme, lactate dehydrogenase (LDH), utilizing a cytotoxicity detection kit (Clontech Laboratories, Inc.) as previously described [26].

2.3. Quantitative real-time PCR (Q-PCR)

Q-PCR was performed as previously described [25]. Expression levels of target genes were normalized by concurrent measurement of GAPDH mRNA levels. All primers used are listed in Supplementary Table 1 of Online Supporting Information.

2.4. Western blot analysis

Western blot analysis was performed as previously described [27]. The primary antibodies included: anti-iNOS (BD610431, BD Biosciences); anti-IκBα (sc-371, Santa Cruz Biotechnology, Inc.); anti-Nrf2 (sc-722, Santa Cruz Biotechnology, Inc.); anti-NQO-1 (ab34173, Abcam Inc.); anti-HO-1 (SPA-896, Stressgen Biotechnologies); and anti-β-actin (Sigma A1978, Sigma–Aldrich).

2.5. Transfection and reporter gene luciferase assay

Transcriptional activity of NF-κB and Neh2 activity [28] were determined by measurement of luciferase (Luc) activities in RAW 264.7 cells that were transfected with their reporter plasmids (pNF-κB-TA-Luc or pNeh2-Luc with pRL-TK-renilla) as previous described [25].

2.6. Immunofluorescence and confocal microscopic analysis

Raw 264.7 cells cultured on Lab-Tek® Chamber Slides (Thermo Scientific) were treated by dh404 (200 nM) for 1 h and then fixed with 4% paraformaldehyde at room temperature for 15 min. Immunofluorescence staining was performed with rabbit anti-Nrf2 (sc-13032, Santa Cruz Biotechnology, Inc.) antibodies as previously described [22]. Nuclei were labeled using blue dye of 4′, 6-diamidino-2-phenylindole (DAPI, Sigma–Aldrich). F-actin was labeled using green dye of Alexa Fluor® 488 phalloidin (Invitrogen). Images were acquired using a confocal microscope (LSM510META, Carl Zeiss Inc.).

2.7. Statistics

Values are expressed as mean ± SD in the text and figures. The data were analyzed using ANOVA with the Newman–Keuls' test

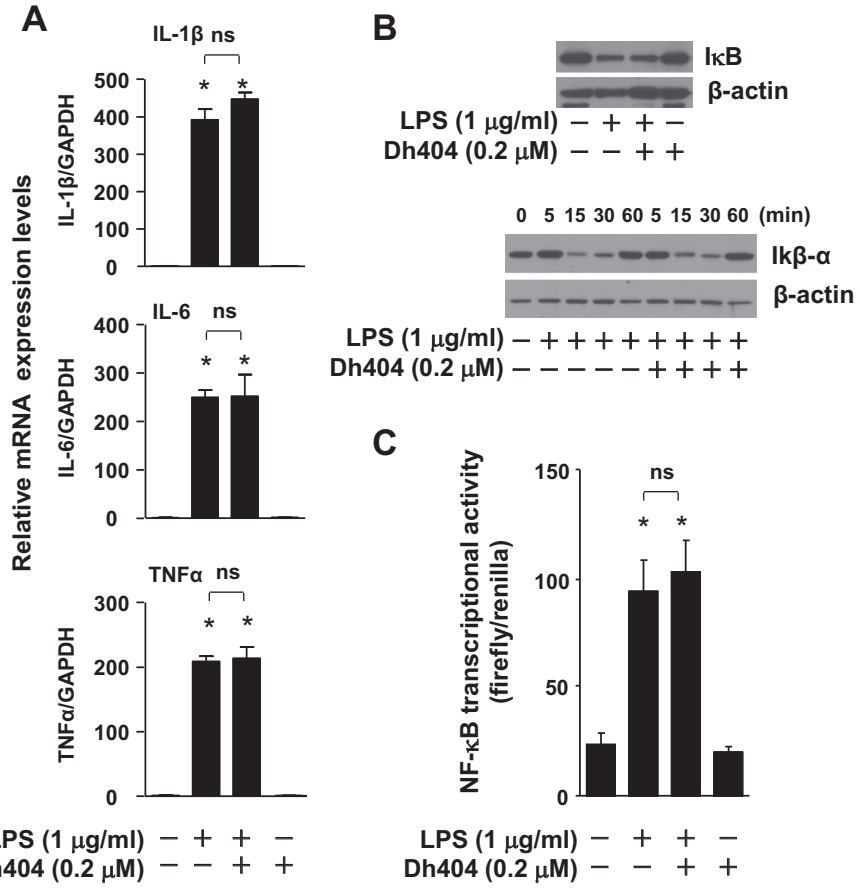

Fig. 2. Effect of dh404 on the expression of IL-1β, IL-6, and TNFα as well as the activation of NF-kB pathway in inflamed RAW264.7 cells. (A) RAW264.7 cells were treated and analyzed as in Fig. 1B. $n = 4$, $^{*}p < 0.05$ vs. control (−). (B) RAW264.7 cells were treated with or without LPS (1 μg/ml) and dh404 (0.2 μM) in DMEM with 2% FBS as indicated for 30 min (Upper panel) or different time periods (Lower panel) and the subjected to Western blot analysis. Results are representatives of three independent experiments. (C) RAW264.7 cells transfected with pNF-κB-TA-Luc and pRL-TK-renilla were treated with or without LPS (1 μg/ml) and dh404 (0.2 μM) in DMEM with 2% FBS as indicated for 6 h and then subjected to measurement of NF-κB transcriptional activity. $n = 4$, $^{*}p < 0.05$ vs. control (−).

unless otherwise specified. Values of $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Dh404 treatment suppresses a selected set of pro-inflammatory factors in RAW 264.7 macrophages

Considering the potent anti-inflammatory effects of triterpenoids [3,29], we postulated that dh404 treatment could suppress the inflammatory response in macrophages. We used the expression of iNOS to reflect the pro-inflammatory status in LPS-inflamed RAW 264.7 macrophages as previously described [25]. To optimize our experimental system, we first examined the impact of dh404 on cell viability in RAW 264.7 macrophages. In a dose response study, we observed that dh404 did not affect the cell viability below the concentration of 500 nM in serum free condition for 24 h (Supplementary Fig. 1). Then, we treated cells with a dh404 non-cytotoxic dose of 200 nM. As expected, we observed dh404 to potently suppress LPS-induced iNOS expression at both mRNA and protein levels (Fig. 1A), suggesting an anti-inflammatory effect of dh404 in macrophages. In addition, we determined whether dh404 is capable of suppressing other pro-inflammatory cytokines including MCP-1, MIP-1β, IL-1β, IL-6, and TNFα in LPS-inflamed RAW264.7 macrophages. Interestingly, dh404 suppressed the expression of MCP-1 and MIP-1β while hardly affecting the expression of IL-1β, IL-6, and TNFα (Figs. 1B and 2A). These results reveal a unique anti-inflammatory profile of dh404 in macrophages.

3.2. Dh404 treatment minimally regulates NF-κB pathway in RAW 264.7 macrophages

Synthetic oleanane triterpenoids, such as CDDO-Me, has an inhibitory effect on NF-κB signaling [3] and NF-κB is known to be a key transcriptional regulator for orchestrating the expression of many inflammatory genes including those aforementioned [30]. Therefore, we sought to determine whether dh404 is capable of suppressing the expression of these inflammatory cytokines via interfering with the NF-κB pathway in macrophages. While LPS robustly activated NF-κB pathway as evidenced by the enhancement of IκB degradation and the subsequent activation of NF-κB transcriptional activity in RAW264.7 macrophages, dh404 treatment was unable to prevent the activation of the NF-κB pathway in the inflamed macrophages (Fig. 2B). These results indicate that dh404 inhibits pro-inflammatory responses in macrophages via a mechanism that is independent of the NF-κB signaling pathway.

3.3. Dh404 activates Nrf2 in RAW 264.7 macrophages

Since Nrf2 appears to be a critical suppressor of inflammatory responses [2] and we have demonstrated that dh404 is a strong Nrf2 activator in cardiomyocytes [16], we next examined whether dh404 activates Nrf2 in RAW264.7 macrophages. As shown in Fig. 3A, dh404 treatment rapidly increased Nrf2 protein expression and the increased expression of Nrf2 was sustained for at least 6 h. The subsequent induction of Nrf2 downstream genes, such as NAD(P)H:quinone oxidoreductase (NQO)1 and heme oxygenase 1 (HO-1) [1], at the mRNA and protein levels was also apparent

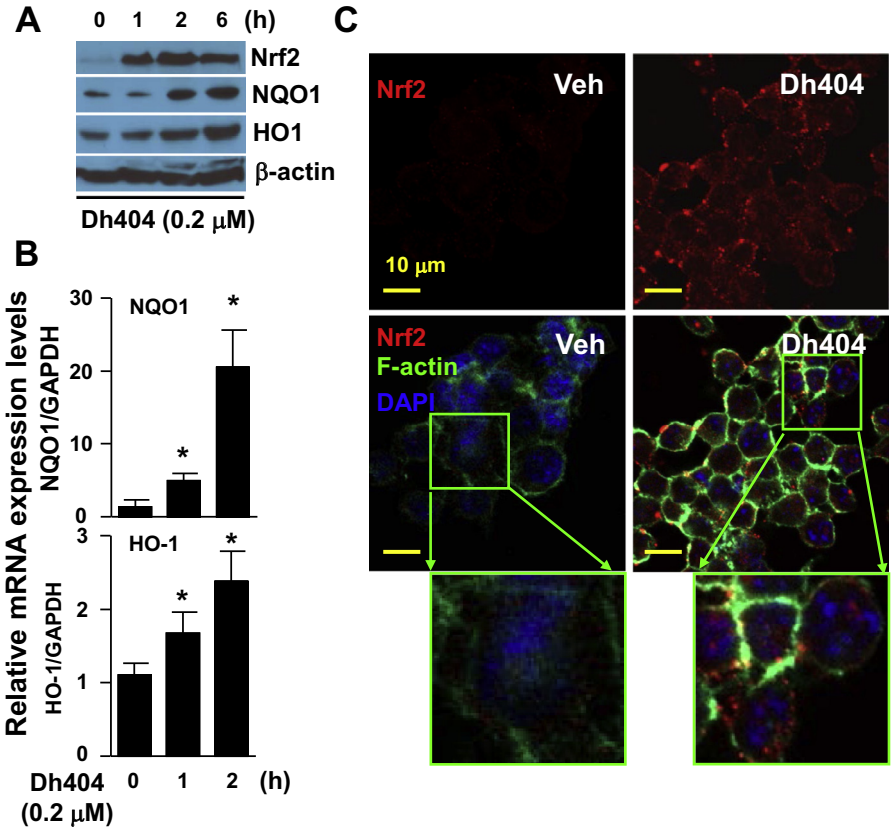

Fig. 3. Effect of dh404 on the activation of Nrf2 signaling in RAW264.7 macrophages. (A) Western blot analysis of the expression of Nrf2 and its downstream genes. The cells were treated with dh404 (0.2 μM) as indicated. Results are representatives of three independent experiments. (B). qPCR analysis of the mRNA expression of Nrf2 downstream gene NQO1 and HO-1. The cells were treated with dh404 (0.2 μM) as indicated. $n = 4$, $^*p < 0.05$ vs. control (0). (C). Immunofluorescence staining of Nrf2. The cells were treated with or with dh404 (0.2 μM) for 1 h. Nrf2 (red); F-action (Green); Nuclei (Blue).

(Fig. 3A and B). In addition, confocal microscopic analysis revealed that the dh404-upregulated Nrf2 proteins translocated into nuclei (Fig. 3C). These results clearly demonstrate that dh404 activates Nrf2 signaling in macrophages. Mechanically, we have observed that dh404 does not interfere with Keap1 binding with Nrf2. Instead it inhibits the Keap1-dependent degradation of Nrf2. As a result, the newly synthesized Nrf2 proteins saturate the capacity of Keap1 to bind with Nrf2, accumulate in the cytoplasm and subsequently translocate into the nucleus thereby activating Nrf2 signaling [16]. Because the protein turnover of Nrf2 is rapid with the half-life of Nrf2 in cells estimated to be less than 20 min [1], it is conceivable that dh404 activates Nrf2 via the same mechanism in macrophages. However, utilizing a Neh2-luciferase reporter system reflecting the amount of Nrf2 which is degraded via directly binding with Keap1 [28], we found that dh404 did not affect the binding of Keap1 and Nrf2 (Neh2) and the Keap1-mediated Nrf2 (Neh2) degradation whereas the positive control (American ginseng crude extract) interrupted Keap1 and Nrf2 binding thereby blocking the Keap1-mediated Nrf2 (Neh2) degradation in RAW246.7 cells (Supplementary Fig. 2). These results reveal that dh404 upregulates Nrf2 expression via a mechanism independent of Keap1 in macrophages.

3.4. Knockout of Nrf2 blocks dh404-suppressed expression of iNOS, MCP-1, and MIP-1β in primary culture of bone marrow-derived macrophages

Finally, we examined whether Nrf2 is a critical mediator for dh404-induced anti-inflammation effects in macrophages using the primary cultures of bone marrow-derived macrophages from WT and Nrf2$^{-/-}$ mice. As shown in Fig. 4, dh404 dramatically inhibited LPS-induced expression of iNOS, MCP-1, and MIP-1β in bone marrow-derived macrophages from WT; however, the anti-inflammatory effect of dh404 was blocked in LPS-inflamed bone marrow-derived macrophages from Nrf2$^{-/-}$ mice. The exaggerated expression of these pro-inflammatory cytokines in LPS-inflamed bone marrow-derived macrophages from Nrf2$^{-/-}$ mice reveals that Nrf2 is a suppressor of inflammatory responses in macrophages (Fig. 4). Taken together, these results clearly demonstrate that Nrf2 is an essential mediator for dh404-induced resolution of inflammatory responses in macrophages.

4. Discussion

In the present study, we demonstrate first the time that dh404 could specifically activate Nrf2-mediated resolution of inflammatory responses in macrophages which is independent of Keap1 and NF-κB, highlighting a unique potential of dh404 for the treatment of diseases that are associated with chronic inflammatory responses in macrophages.

The finding of dh404-induced Keap1 independent activation of Nrf2 is particularly intriguing although the precise mechanism by which dh404 activates Nrf2 in macrophages remains unknown. Previous studies have shown that amplification of Nrf2 by pharmacological inducers or genetic deletion of hepatic Keap1 attenuates inflammatory damage of liver [13], whereas the activation of Nrf2 by genetic knockout or knockdown of Keap1 is not always beneficial [10,12,14]. While it remains unclear as to whether Keap1 is required for realizing Nrf2-mediated beneficial effects such as suppression of inflammation; it is likely that the activation of Nrf2 due to the Keap1 loss-of-function is detrimental in certain specific pathological settings. Thus, it is plausible that the maximal beneficial effects of Nrf2 might be achieved by targeting Nrf2 while keeping Keap1 function intact.

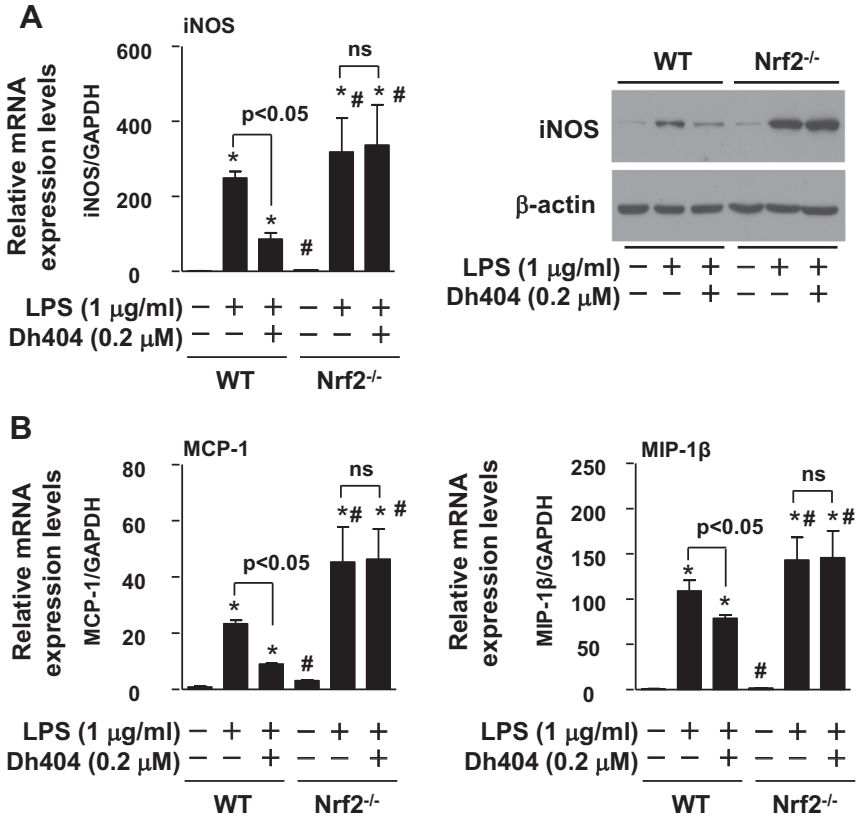

Fig. 4. Effect of dh404 on iNOS, MCP-1, and MIP-1β expression in inflamed bone marrow-derived macrophages isolated from WT and Nrf2$^{-/-}$ mice. (A, Left panel and B) The cells were treated in DMEM with 2% FBS as indicated for 4 h and then subjected to Q-PCR analysis. $n = 4$, $^{*}p < 0.05$ vs. control (−), $^{\#}p < 0.05$ vs. WT. (A, Right panel) The cells were treated in DMEM with 2% FBS as indicated for 6 h and then subjected to Western blot analysis. Results are representatives of three independent experiments.

Of note, the NF-κB pathway also plays a critical role in the suppression of the pro-inflammatory response in macrophage and the resolution of inflammation *in vivo* [31,32]. Inappropriate suppression of NF-κB signaling results in adverse consequences. For example, macrophage specific knockout of NF-κB kinase (IKK)β, which is essential for NF-κB activation and NF-κB-driven expression of inflammatory cytokines, increases atherosclerosis in LDL receptor-deficient mice [33]. Also, the loss of IKKβ or the NF-κB essential modulator IKKγ/NEMO in murine cardiomyoyctes exaggerates or results in dysregulated myocardial inflammatory responses and dilated cardiomyopathy and cardiac dysfunction [34,35]. Of interest, increased oxidative stress is a common feature in cardiomyocyte specific IKKβ and IKKγ knockout mice and is also the causative mechanism for the dilated cardiomyopathy and heart failure in these mice. In this context and unlike the other synthetic oleanane triterpenoids which directly bind IKK to suppress NF-κB [3], we observed that dh404 minimally regulates the IKK/NF-κB pathway and specifically activates Nrf2 signaling which presumably mimics the IKK-mediated antioxidant defense and resolution of inflammation. Accordingly, dh404 is positioned to be a promising drug for the maximal activation of Nrf2-driven cytoprotective signaling.

Indeed, while we have recently demonstrated that Nrf2 cardiomyocyte-specific transgenic mice are resistant to pressure overload-induced cardiac maladaptive remodeling and dysfunction (manuscript submitted), we have also found that oral administration of dh404 exerts similar effects in mice [18]. Since macrophage associated inflammation plays a critical role in cardiac dysfunction [36], the observed dh404-induced anti-inflammatory effects in macrophages appears to be an important mechanism by which dh404 protects against cardiac dysfunction. In addition, given the key role of Nrf2 in cellular defense in diverse organ systems [1], the specific targeting of Nrf2 by dh404 may have a unique pharmacological potential for the treatment of many diseases. However, whether the unique feature of dh404-induced Nrf2 activation is critical for its pharmacological efficacy *in vivo* over the other Nrf2 activators regarding inflammation resolution and side effects needs to be determined in future.

Conflict of interest

None.

Acknowledgments

We thank Dr. Irina G. Gazaryan (Weill Medical College of Cornell University, USA) for providing Neh2-Luciferase reporter. This work was supported by the Shandong University National Qianren Scholar Fund (W.-X.L.) and Taishan Scholar Fund (C.-T. and T.-D.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.101.

References

[1] J. Li, T. Ichikawa, J.S. Janicki, T. Cui, Targeting the Nrf2 pathway against cardiovascular disease, Expert Opin. Ther. Targets 13 (2009) 785–794.
[2] J. Kim, Y.N. Cha, Y.J. Surh, A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders, Mutat. Res. 690 (2010) 12–23.
[3] K.T. Liby, M.M. Yore, M.B. Sporn, Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer, Nat. Rev. Cancer 7 (2007) 357–369.
[4] P.E. Pergola, P. Raskin, R.D. Toto, C.J. Meyer, J.W. Huff, E.B. Grossman, M. Krauth, S. Ruiz, P. Audhya, H. Christ-Schmidt, J. Wittes, D.G. Warnock, Bardoxolone methyl and kidney function in CKD with type 2 diabetes, N. Engl. J. Med. 365 (2011) 327–336.
[5] G.M. McMahon, J.P. Forman, Bardoxolone methyl, chronic kidney disease, and type 2 diabetes, N. Engl. J. Med. 365 (2011) 1746 (author reply 1746–1747).
[6] A. Upadhyay, M.J. Sarnak, A.S. Levey, Bardoxolone methyl, chronic kidney disease, and type 2 diabetes, N. Engl. J. Med. 365 (2011) 1746 (author reply 1746–1747).
[7] K.S. Rogacev, J.T. Bittenbring, D. Fliser, Bardoxolone methyl, chronic kidney disease, and type 2 diabetes, N. Engl. J. Med. 365 (2011) 1745–1746 (author reply 1746-1747).
[8] L.M. Shelton, B.K. Park, I.M. Copple, Role of Nrf2 in protection against acute kidney injury, Kidney Int. (2013).
[9] M. Kobayashi, L. Li, N. Iwamoto, Y. Nakajima-Takagi, H. Kaneko, Y. Nakayama, M. Eguchi, Y. Wada, Y. Kumagai, M. Yamamoto, The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds, Mol. Cell. Biol. 29 (2009) 493–502.
[10] N. Wakabayashi, K. Itoh, J. Wakabayashi, H. Motohashi, S. Noda, S. Takahashi, S. Imakado, T. Kotsuji, F. Otsuka, D.R. Roop, T. Harada, J.D. Engel, M. Yamamoto, Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation, Nat. Genet. 35 (2003) 238–245.
[11] A. Uruno, Y. Furusawa, Y. Yagishita, T. Fukutomi, H. Muramatsu, T. Negishi, A. Sugawara, T.W. Kensler, M. Yamamoto, The Keap1-Nrf2 system prevents onset of diabetes mellitus, Mol. Cell. Biol. 33 (2013) 2996–3010.
[12] J. Xu, S.R. Kulkarni, A.C. Donepudi, V.R. More, A.L. Slitt, Enhanced Nrf2 activity worsens insulin resistance, impairs lipid accumulation in adipose tissue, and increases hepatic steatosis in leptin-deficient mice, Diabetes 61 (2012) 3208–3218.
[13] W.O. Osburn, M.S. Yates, P.D. Dolan, S. Chen, K.T. Liby, M.B. Sporn, K. Taguchi, M. Yamamoto, T.W. Kensler, Genetic or pharmacologic amplification of nrf2 signaling inhibits acute inflammatory liver injury in mice, Toxicol. Sci. 104 (2008) 218–227.
[14] M. Komatsu, H. Kurokawa, S. Waguri, K. Taguchi, A. Kobayashi, Y. Ichimura, Y.S. Sou, I. Ueno, A. Sakamoto, K.I. Tong, M. Kim, Y. Nishito, S. Iemura, T. Natsume, T. Ueno, E. Kominami, H. Motohashi, K. Tanaka, M. Yamamoto, The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1, Nat. Cell Biol. 12 (2010) 213–223.
[15] H.M. Ni, N. Boggess, M.R. McGill, M. Lebofsky, P. Borude, U. Apte, H. Jaeschke, W.X. Ding, Liver-specific loss of Atg5 causes persistent activation of Nrf2 and protects against acetaminophen-induced liver injury, Toxicol. Sci. 127 (2012) 438–450.
[16] T. Ichikawa, J. Li, C.J. Meyer, J.S. Janicki, M. Hannink, T. Cui, Dihydro-CDDO-trifluoroethyl amide (dh404), a novel Nrf2 activator, suppresses oxidative stress in cardiomyocytes, PLoS One 4 (2009) e8391.
[17] M. Chin, C.Y. Lee, J.C. Chuang, R. Bumeister, W.C. Wigley, S.T. Sonis, K.W. Ward, C. Meyer, Bardoxolone methyl analogs RTA 405 and dh404 are well tolerated and exhibit efficacy in rodent models of Type 2 diabetes and obesity, Am. J. Physiol. Renal Physiol. 304 (2013) F1438–F1446.
[18] Y. Xing, T. Niu, W. Wang, J. Li, S. Li, J.S. Janicki, S. Ruiz, C.J. Meyer, X.L. Wang, D. Tang, Y. Zhao, T. Cui, Triterpenoid dihydro-CDDO-trifluoroethyl amide protects against maladaptive cardiac remodeling and dysfunction in mice: a critical role of Nrf2, PLoS One 7 (2012) e44899.
[19] C. Stack, D. Ho, E. Wille, N.Y. Calingasan, C. Williams, K. Liby, M. Sporn, M. Dumont, M.F. Beal, Triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improve the behavioral phenotype and brain pathology in a transgenic mouse model of Huntington's disease, Free Radic. Biol. Med. 49 (2010) 147–158.
[20] C. Escartin, E. Brouillet, The Nrf2 pathway as a potential therapeutic target for Huntington disease A commentary on "Triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improve the behavioral phenotype and brain pathology in a transgenic mouse model of Huntington disease", Free Radic. Biol. Med. 49 (2010) 144–146.
[21] Y. Tan, T. Ichikawa, J. Li, Q. Si, H. Yang, X. Chen, C.S. Goldblatt, C.J. Meyer, X. Li, L. Cai, T. Cui, Diabetic downregulation of Nrf2 activity via ERK contributes to oxidative stress-induced insulin resistance in cardiac cells in vitro and in vivo, Diabetes 60 (2011) 625–633.
[22] J. Li, T. Ichikawa, L. Villacorta, J.S. Janicki, G.L. Brower, M. Yamamoto, T. Cui, Nrf2 protects against maladaptive cardiac responses to hemodynamic stress, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1843–1850.
[23] G. Boltz-Nitulescu, C. Wiltschke, C. Holzinger, A. Fellinger, O. Scheiner, A. Gessl, O. Forster, Differentiation of rat bone marrow cells into macrophages under the influence of mouse L929 cell supernatant, J. Leukoc. Biol. 41 (1987) 83–91.
[24] J. Weischenfeldt, B. Porse, Bone marrow-derived macrophages (BMM): isolation and applications, CSH Protoc. 2008 (2008) (pdb prot5080).
[25] T. Ichikawa, J. Zhang, K. Chen, Y. Liu, F.J. Schopfer, P.R. Baker, B.A. Freeman, Y.E. Chen, T. Cui, Nitroalkenes suppress lipopolysaccharide-induced signal transducer and activator of transcription signaling in macrophages: a critical role of mitogen-activated protein kinase phosphatase 1, Endocrinology 149 (2008) 4086–4094.
[26] J. Li, T. Ichikawa, Y. Jin, L.J. Hofseth, P. Nagarkatti, M. Nagarkatti, A. Windust, T. Cui, An essential role of Nrf2 in American ginseng-mediated anti-oxidative actions in cardiomyocytes, J. Ethnopharmacol. 130 (2010) 222–230.
[27] L. Villacorta, J. Zhang, M.T. Garcia-Barrio, X.L. Chen, B.A. Freeman, Y.E. Chen, T. Cui, Nitro-linoleic acid inhibits vascular smooth muscle cell proliferation via the Keap1/Nrf2 signaling pathway, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H770–H776.
[28] N.A. Smirnova, R.E. Haskew-Layton, M. Basso, D.M. Hushpulian, J.B. Payappilly, R.E. Speer, Y.H. Ahn, I. Rakhman, P.A. Cole, J.T. Pinto, R.R. Ratan, I.G. Gazaryan, Development of Neh2-luciferase reporter and its application for high

throughput screening and real-time monitoring of Nrf2 activators, Chem. Biol. 18 (2011) 752–765.

[29] P. Dzubak, M. Hajduch, D. Vydra, A. Hustova, M. Kvasnica, D. Biedermann, L. Markova, M. Urban, J. Sarek, Pharmacological activities of natural triterpenoids and their therapeutic implications, Nat. Prod. Rep. 23 (2006) 394–411.

[30] D. Tugal, X. Liao, M.K. Jain, Transcriptional control of macrophage polarization, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 1135–1144.

[31] C.H. Fong, M. Bebien, A. Didierlaurent, R. Nebauer, T. Hussell, D. Broide, M. Karin, T. Lawrence, An antiinflammatory role for IKKbeta through the inhibition of "classical" macrophage activation, J. Exp. Med. 205 (2008) 1269–1276.

[32] F.R. Greten, M.C. Arkan, J. Bollrath, L.C. Hsu, J. Goode, C. Miething, S.I. Goktuna, M. Neuenhahn, J. Fierer, S. Paxian, N. Van Rooijen, Y. Xu, T. O'Cain, B.B. Jaffee, D.H. Busch, J. Duyster, R.M. Schmid, L. Eckmann, M. Karin, NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta, Cell 130 (2007) 918–931.

[33] E. Kanters, M. Pasparakis, M.J. Gijbels, M.N. Vergouwe, I. Partouns-Hendriks, R.J. Fijneman, B.E. Clausen, I. Forster, M.M. Kockx, K. Rajewsky, G. Kraal, M.H. Hofker, M.P. de Winther, Inhibition of NF-kappaB activation in macrophages increases atherosclerosis in LDL receptor-deficient mice, J. Clin. Invest. 112 (2003) 1176–1185.

[34] S. Hikoso, O. Yamaguchi, Y. Nakano, T. Takeda, S. Omiya, I. Mizote, M. Taneike, T. Oka, T. Tamai, J. Oyabu, Y. Uno, Y. Matsumura, K. Nishida, K. Suzuki, M. Kogo, M. Hori, K. Otsu, The I{kappa}B kinase {beta}/nuclear factor {kappa}B signaling pathway protects the heart from hemodynamic stress mediated by the regulation of manganese superoxide dismutase expression, Circ. Res. 105 (2009) 70–79.

[35] P. Kratsios, M. Huth, L. Temmerman, E. Salimova, M. Al Banchaabouchi, A. Sgoifo, M. Manghi, K. Suzuki, N. Rosenthal, F. Mourkioti, Antioxidant amelioration of dilated cardiomyopathy caused by conditional deletion of NEMO/IKKgamma in cardiomyocytes, Circ. Res. 106 (2010) 133–144.

[36] M. Nahrendorf, F.K. Swirski, Monocyte and macrophage heterogeneity in the heart, Circ. Res. 112 (2013) 1624–1633.