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ORIGINAL ARTICLE

Anti-inflammatory effects of PRIMA-1^{MET} (mutant p53 reactivator) induced by inhibition of nuclear factor-κB on rheumatoid arthritis fibroblast-like synoviocytes

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Abstract

Fibroblast-like synoviocytes (FLSs), the main pathological cells in rheumatoid arthritis (RA), display tumor-like phenotype, including hyper-proliferation, apoptosis resistance, and aggressive phenotype. Excessive proliferation and insufficient apoptosis of RA-FLSs can lead to hyperplastic synovial pannus tissue, excess production of inflammatory mediators, and destruction of joints. In this article, we investigate the effect of PRIMA-1^{MET} on the apoptosis induction and inhibition of pro-inflammatory cytokines in RA-FLSs. Synovial tissue samples were obtained from 10 patients with RA. The FLSs were treated with different concentrations of PRIMA-1^{MET}. The rate of apoptosis and cell survival was assessed by flow cytometry and MTT assay and Real-time quantitative PCR was performed to evaluate the transcription of p53, IL-6, IL-1β, TNF-α, Noxa, p21, PUMA, Bax, Survivin, and XIAP in treated RA-FLSs. The protein level of p53, IκBα, and phospho-IκBα were measured using Western blotting. The results showed that PRIMA-1^{MET} induced apoptosis in RA-FLSs and increased significantly the expression of Noxa, and decreased significantly IL-6, IL-1β, p53, and phospho-IκBα expression. PRIMA-1^{MET} can induce apoptosis in RA-FLSs through induction of Noxa expression while p53 was downregulated. Furthermore, PRIMA-1^{MET} treatment results in the suppression of pro-inflammatory cytokine production and NF-κB inhibition. Given the role of p53 and NF-κB in RA-FLSs, PRIMA-1^{MET} can be considered as a new therapeutic strategy for rheumatoid arthritis.

Keywords Fibroblast-like synoviocytes · NF-κB · Noxa · P53 · PRIMA-1^{MET} · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is an inflammatory, chronic, and invasive autoimmune disease, with a prevalence of about 1%. RA is associated with irreversible joint disabilities, systemic complications, premature death, and socioeconomic burden (Hewagama and Richardson 2009). The main pathological features of RA are joint inflammation, proliferative synovitis, and destruction of cartilage and bone. Joint involvement is characterized by the formation of hypertrophied synovium that is called "pannus". Pannus tissue is composed of aggressive fibroblast- and macrophage-like synoviocytes. The development of the pannus stimulates the production of degrading enzymes such as matrix metalloproteinases (MMPs) and pro-inflammatory cytokines that promote the destruction of cartilage and bone (Firestein 2003).

Intimal lining layer fibroblast-like synoviocytes (FLSs or type B synoviocytes) are among the most critical cells involved in the pathogenesis of RA. In the inflammatory

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milieu of RA synovium, FLSs display tumor-like phenotype, including hyper-proliferation, apoptosis resistance, and aggressive phenotype. Excessive proliferation and insufficient apoptosis of RA-FLSs can lead to hyperplastic synovial pannus tissue, excess production of inflammatory mediators, and destruction of joints. Therefore, RA-FLSs are directly responsible for inflammation, cartilage destruction, and autoimmunity (Bottini and Firestein 2013).

The p53 tumor suppressor gene plays a pivotal role in cell cycle regulation, particularly apoptosis. P53 protein has a protective role in many autoimmune and inflammatory diseases by inhibiting the production of inflammatory cytokines, extracellular matrix-degrading enzymes, and induction of apoptosis (Yamanishi et al. 2005). Several studies have shown that the function of p53 protein is impaired in malignant neoplasms and non-malignant diseases such as RA (Firestein et al. 1997; Greenblatt et al. 1994). P53 protein dysfunction is the result of somatic mutations caused by genotoxic stress (Yamanishi et al. 2005). It has been reported that more than 40% of p53 cDNAs in RA synovium have mutations, most of which have been found in FLSs (Inazuka et al. 2000). Besides, p53 is overexpressed in synovial samples of arthritis patients compared to healthy synovial samples (Firestein et al. 1996). Despite the increased expression of p53, apoptosis resistance in RA-FLSs has been reported which may be related to the dominant-negative mutations (Han et al. 1999).

P53 reactivation and induction of massive apoptosis 1 (PRIMA-1) is a small non-peptide molecule that was first introduced in the American Cancer Center as a compound with anti-cancer properties (Bykov et al. 2002b). PRIMA-1^{MET}, the methylated form of drug, targets the p53 protein and restores the mutant p53 function. This molecule can induce apoptosis by repairing the mutant p53 protein by alkylating and activating the protein thiol groups (Lambert et al. 2009).

Besides, it has been shown that PRIMA-1-induced apoptosis in NB4 cells, acute promyelocytic leukemia (APL) cell line, was accompanied by repressed nuclear factor κB (NF-κB) activity (Farhadi et al. 2017). NF-κB signaling pathway plays an essential role in the formation of pannus, chronic inflammation, and cartilage destruction by promoting proliferation, inhibiting cell apoptosis, producing inflammatory cytokines, and metalloproteinases (Makarov 2001).

With regards to the PRIMA-1^{MET} effects on apoptosis induction and downregulation of NF-κB activity and given the role of apoptosis and inflammation in RA pathogenesis, in the present study, we evaluated the effect of PRIMA-1^{MET} on RA-FLSs. Our results showed that PRIMA-1^{MET} could induce apoptosis and has an anti-inflammatory effect in RA-FLSs.

Materials and methods

Chemicals and reagents

PRIMA-1^{MET} was purchased from Sigma-Aldrich (Sml1789, USA). Antibodies against CD90 (Thy-1) (ab225), CD13 (ac227663), CD44 (ab6124), CD68 (ab31630), fibroblast surface protein (ab11333), p53 (ab131442), IκBα (ab97783), and β-actin (ab8226) purchased from abcam (USA), and the antibody against phospho-IκBα (#2859) was obtained from cell signaling technology (USA). Anti-rabbit HRP conjugated (PZ5610) was from Kalazist (IRAN).

Study subject

Synovial tissue specimens from 10 inactive patients with rheumatoid arthritis who were candidates for knee replacement surgery were taken from an inflamed joint replacement tissue by an orthopedic surgeon at Shariati Hospital. The mean of patients' ages and disease durations were 59.12 ± 11.81 and 18.6 ± 2.3 years, respectively. All patients fulfilled the American College of Rheumatology/European league against rheumatism 2010 criteria (ACR/EULAR 2010 criteria) for RA (Aletaha et al. 2010) and provided their written consent to participate in the current study. Prednisolone, Hydroxychloroquine, and Sulfasalazine were the major administrated drugs in patients. This study was approved by the Ethics Committees of Tehran University of Medical Sciences (Approval No: IR.TUMS.VCR.REC.1397.968).

FLS isolation and cell culture

The synovial tissue was first rinsed with 70% ethanol and phosphate-buffered saline (PBS) solution (Gibco) containing 2% penicillin/streptomycin (Biosera) and 2% amphotericin B (Sigma-Aldrich), and then was cut into 5-mm slices. After that, the fragmented tissue was digested and minced with 1 mg/ml collagenase type VIII (Sigma–Aldrich, USA) in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) at 37 °C for 80 min.

After centrifugation (1000 g, 10 min) and washing, the cells were resuspended in DMEM supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) (Biosera) and incubated in T25 culture flasks at humidified 37 °C with 5% CO2. The medium was replaced every 48 h and FLSs were sub-cultured when reached 70–80% confluency. FLSs at passage 3 were confirmed and stored in a nitrogen tank. For future experiments, FLSs from passages 4–6 were used.

FLS confirmation: immunofluorescence assay and flow cytometry

Flow cytometry and immunofluorescence assay were used to identify the purity of the isolated cells from synovial tissues. After the third passage, the cells were characterized using immunofluorescence assay for fibroblast surface protein and by four antibodies, CD44, CD13, CD68, and CD90, using flow cytometry. A fluorescence-activated cell sorting (FACS) calibur flow cytometer (BD Biosciences, USA) was used for the analysis.

Cytotoxicity analysis with MTT assay

FLSs were seeded at a density of 7.5×10^3 cells/well into a 48-well culture plate in triplicate and incubated overnight at 37 °C in a CO_2 incubator, then treated with various concentrations (25, 30, 35, 40 and 55 µM) of PRIMA-1^{MET} for 12 and 24 h. After that, cells were incubated with 50 µl of MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] solution (Sigma-Aldrich, USA) for 4 h at 37 °C with 5% CO_2. After 4 h incubation, the medium was discarded and 500 µl of dimethyl sulfoxide (DMSO) (Sigma–Aldrich, USA) was added to each well to solve the formazan and the absorbance was measured at 570 nm wavelength by an enzyme-linked immunosorbent assay (ELISA) reader (BioTek-ELx800, USA).

Detection of apoptosis by flow cytometry

FLSs were plated overnight in a 6-well culture plate at a density of 75×10^3 cells/well. The next day, the cells were exposed to different concentrations (25, 30, 35, 40 and 55 µM) of PRIMA-1^{MET} for 24 h. After incubation, cells were harvested and annexin V-FITC and PI solution were added based on the manufacturer's instruction. The resulting fluorescence was detected by BD FACSCalibur flow cytometer (BD biosciences, USA) and data analysis was performed with the FlowJo software (Tree Star, Ashland, USA) (version 7.6.1).

Quantitative real-time PCR (qRT-PCR)

After 24 h treatment of FLSs with 35 µM of PRIMA-1^{MET}, total RNA was extracted using SinaPure-RNA kit (SinaClonBioScience, Iran). Complementary DNA (cDNA) synthesis was performed with a PrimeScript™ RT reagent kit (Takara BIO INC. Japan), according to the manufacturer's instruction. Quantitative real-time PCR was performed for gene expression analysis using the RealQ Plus Master Mix Green-high ROX (Ampliqon, Denmark) on a StepOne Real-Time PCR System (Applied Biosystems, USA). Each sample was analyzed in triplicate and data were analyzed based on the comparative Ct ($2^{-\Delta\Delta Ct}$) method. The expression of target genes was normalized to GAPDH expression.

Western blot analysis

After FLSs treatment (35 µM, 24 h), treated and untreated FLSs were lysed in radioimmunoprecipitation assay (RIPA) buffer (Cytomatingene, Iran) containing protease inhibitor cocktail (P8340, Sigma–Aldrich, USA). Equal amounts of cell lysates were used to separate proteins by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membrane (Thermo Scientific, USA). The PVDF was blocked with 5% skim milk (Sigma-Aldrich, USA), followed by incubation with primary antibodies including anti-IκBα, anti-phospho-IκBα, anti-P53 (1:1000), and anti-β-actin as the loading control. After washing three times with Tris Buffered Saline with Tween 20 (TBST), the blots were incubated with goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies. Finally, the protein expression levels were visualized on X-ray film using an enhanced chemiluminescence (ECL) kit (GE Healthcare, USA).

Statistical analysis

IBM SPSS software version 24 (SPSS Inc., Chicago, IL, USA) was used to perform the analysis. The normal distribution of variants was analyzed using the Kolmogorov–Smirnov test. Mean comparison analysis between two groups (treated and untreated) was performed using Student's paired *t* or Wilcoxon signed-rank tests. *P* values less than 0.05 were considered statistically significant and all data were represented as the mean ± standard error of the mean (SEM). GraphPad Prism software version 8.0.2 (GraphPad Software, La Jolla California USA) was used for drawing the plots.

Results

Identification of FLSs

Flow cytometry and immunofluorescence assay were used to identify isolated cells from synovial tissues. After 3 passages, a typical bipolar configuration was seen under inverted microscopy which confirmed a morphologically homogeneous population of FLSs. Most cells (> 98%) expressed the fibroblast surface protein (Fig. 1a), and were positive for CD90 (99.44% ± 3.7%), CD44 (99.33 ± 2.21%), and CD13 (98.63 ± 2.06%) markers, and were negative for the expression of CD68 (0.16 ± 4.01%) (Fig. 1b). Synoviocytes from passages 4–6 were used for further experiments.

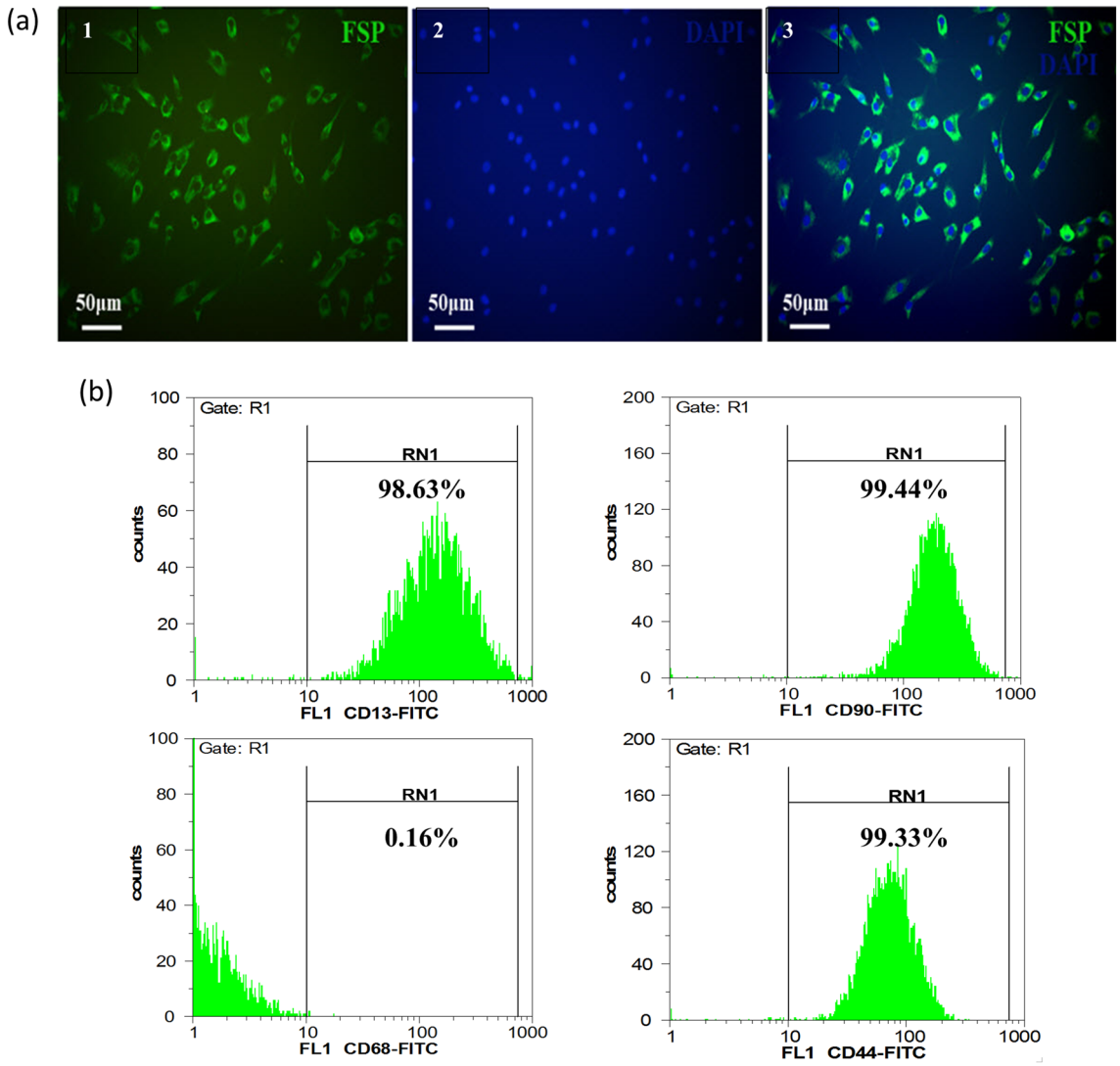

Fig. 1 **a** Confirmation of purity of cultured FLSs by immunofluorescence assay. 1) Specific staining of FLSs using anti-Fibroblast Surface Protein (green). 2) Staining the cell nucleus using DAPI (blue). 3) Merge two pictures indicating the purity of the cultured FLS. (Inverted phase contrast microscope with 200×magnification). **b** Characterization of FLSs. After 3 passages, isolated cells were analyzed by flow cytometry for CD90, CD44 and CD13, and CD68. FLSs are positive for CD90, CD44, and CD13 and negative for CD68. FLS, fibroblast-like synoviocytes; FITC, fluorescein isothiocyanate; DAPI, 4′,6-diamidino-2-phenylindole (Color figure online)

PRIMA-1^{MET} reduces RA-FLSs viability

MTT assay was used to determine the effect of different doses of PRIMA-1^{MET} on cell viability. The results showed that PRIMA-1^{MET} treatment leads to decreased cell survival in a dose-dependent manner in RA-FLSs. Using various concentrations (25, 30, 35, 40 and 55 µM) of PRIMA-1^{MET} for 12 and 24 h, we found a significant decrease in cell viability with approximately 65% and 99% at concentrations of 40 and 55 µM, respectively (Fig. 2).

PRIMA-1^{MET} induces apoptosis in RA-FLSs

To determine the cell death induced by PRIMA-1^{MET} through apoptosis, annexin V/PI staining by flow cytometry was used. As shown in Fig. 3, the rate of apoptosis in PRIMA-1^{MET}-treated-RA-FLSs was increased in a dose-dependent manner. The percentage of annexin V positive cells after treatment of RA-FLSs with 25, 30, 35, 40 and 55 µM of PRIMA-1^{MET} was increased by 7.5, 16.11, 35.73, 61.75 and 95.38%, respectively.

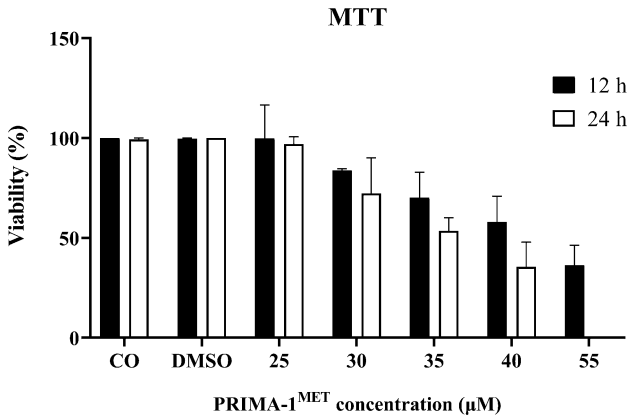

Fig. 2 Decreases cell viability and metabolic activity of RA-FLSs. FLSs were cultured in complete medium with different concentrations of PRIMA-1^{MET} in 48-well plates for 12 and 24 h and cell viability was measured using the MTT assay. The metabolic activity of RA-FLSs upon PRIMA-1^{MET} treatment decreased in a dose-dependent manner ($n=2$). *FLS* fibroblast-like synoviocytes, *RA* rheumatoid arthritis, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *CO* control, *DMSO* dimethyl sulfoxide

PRIMA-1^{MET} treatment downregulates p53 gene and protein expression in RA-FLSs

Previous studies have shown that PRIMA-1 treatment in cancer cells has no effect on p53 expression and only affects p53 protein function (Farhadi et al. 2017; Izetti et al. 2014). As described in Fig. 4, using quantitative RT-PCR and Western blotting, there is a significant decrease in p53 expression at the gene (P value = 0.002) and protein levels in the treated group compared to the control group (Supplementary Fig. 1).

PRIMA-1^{MET} upregulates Noxa as a pro-apoptotic target gene of p53 in RA-FLSs

Previous studies have shown that PRIMA-1^{MET} induces p53 pro-apoptotic target genes such as phorbol-12-myristate-13-acetate-induced protein 1 (Noxa), (cyclin-dependent kinase inhibitor 1A (CDKN1A) or P21, and Bcl-2-associated X protein (BAX) through the restoration of mutant p53 sequence-specific DNA-binding and results in apoptosis in the cancer cells (Liang et al. 2009; Messina et al. 2012; Wang et al. 2007). As indicated in Fig. 5A, unlike a significant decrease in p53 gene and protein expression, mRNA expression of Noxa was significantly increased upon PRIMA-1^{MET} treatment (P value = 0.039). Other pro-apoptotic p53 target genes like P53 upregulated mediator of apoptosis (PUMA), P21, and Bax mRNA expression levels were not altered significantly after PRIMA-1^{MET} treatment (P value = 0.633, 0.109, 0.25 respectively).

Effect of PRIMA-1^{MET} on anti-apoptotic genes

It has been shown that PRIMA-1^{MET} can decrease anti-apoptotic genes like Bcl-2 and XIAP expression and has no effect on survivin expression in cancer cells (Farhadi et al. 2017). Paradoxically, it has been reported that a derivative of PRIMA-1 increases XIAP expression (Soans et al. 2014). To investigate the effect of PRIMA-1^{MET} on anti-apoptotic genes, we evaluated XIAP and survivin mRNA expression in PRIMA-1^{MET} –treated RA-FLSs. As described in Fig. 5B, the transcription of XIAP and survivin mRNA were significantly upregulated after 24 h exposure to PRIMA-1^{MET} (P value = 0.012, 0.002, respectively).

PRIMA-1^{MET} treatment inhibits NF-κB through IκBα dephosphorylation

PRIMA-1 treatment in APL cell line (NB4) showed that PRIMA-1 can inhibit NF-κB activity. In the present study, the effect of PRIMA-1^{MET} on NF-κB was evaluated by Western blotting of p-IκBα and IκBα proteins in the treated and control (untreated) groups (Fig. 5C and Supplementary Fig. 1). The results of Western blotting showed that PRIMA-1^{MET} treatment increases IκBα dephosphorylation.

PRIMA-1^{MET} downregulates pro-inflammatory cytokines in RA-FLSs

The effect of PRIMA-1 on inflammation and pro-inflammatory cytokines has not been investigated but it has been reported that a derivative of PRIMA-1 can upregulate tumor necrosis factor-alpha (TNF-α) (Soans et al. 2014). To evaluate whether PRIMA-1^{MET} can reduce inflammatory cytokines in RA-FLSs, PRIMA-1^{MET}-treated FLSs were assessed for pro-inflammatory cytokine genes. As indicated in Fig. 5D, comparison and statistical real-time PCR analysis showed that the exposure to PRIMA-1^{MET} led to a significant decrease in mRNA expression of IL-6 and IL1-β as the most important pro-inflammatory cytokines in RA (P value = 0.016, 0.031 respectively). TNF-α in the treated-FLSs group were not significantly changed compared to the untreated group (P value = 0.055).

Discussion

Transcription factor p53 has multiple functions in the cells, the most important of which is induction of apoptosis (Colman et al. 2000). However, it has been reported that p53 can activate pro-survival pathways, too (Vousden 2006). Reactivation of mutant p53 is a promising and effective therapeutic strategy in cancer cells (Khoo et al. 2014). Therefore, according to the presence of mutant p53 in RA-FLSs,

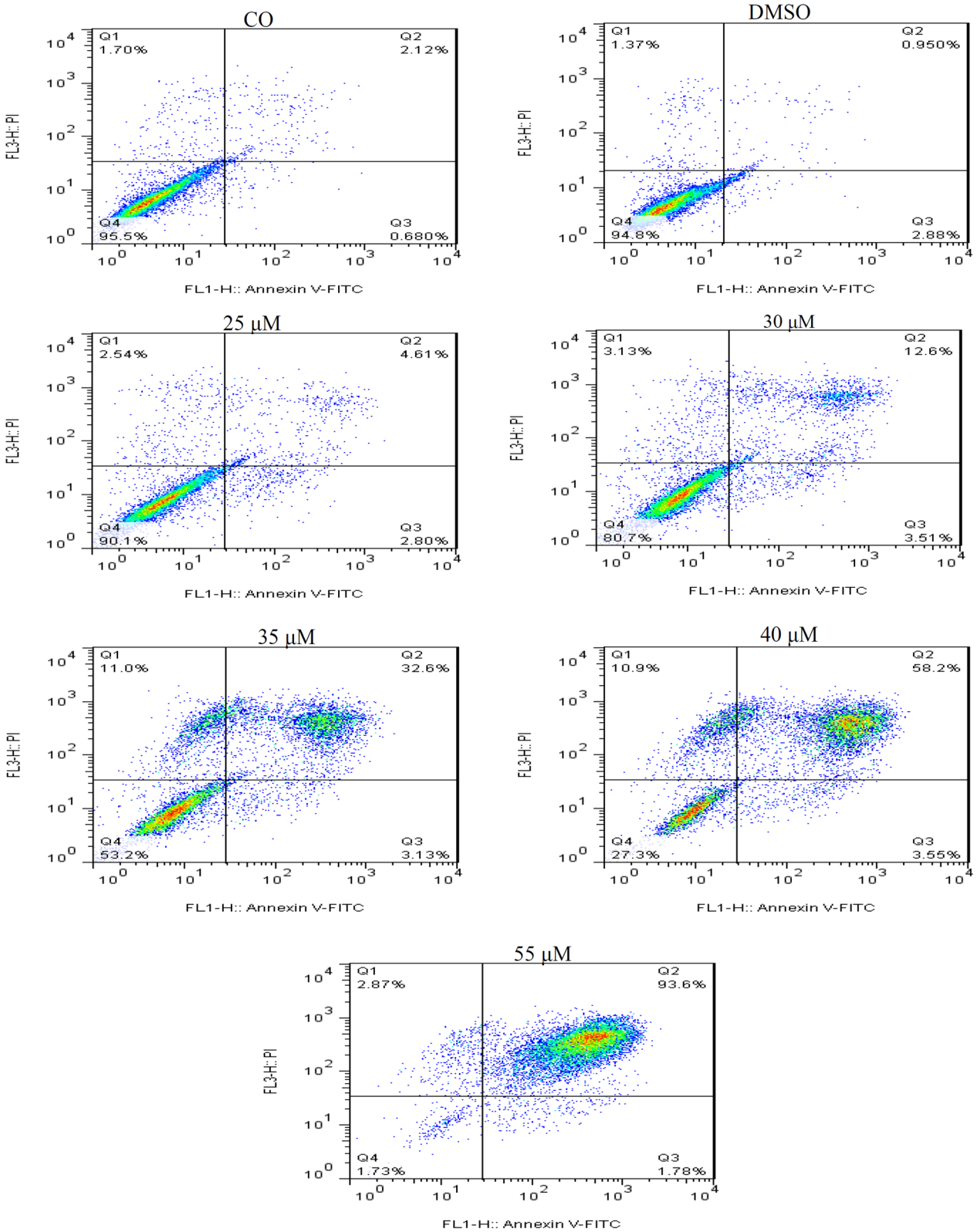

Fig. 3 Apoptotic effect of PRIMA-1^{MET} on RA-FLSs. Cells were treated with different concentrations (25, 30, 35, 40, and 55 µM) of PRIMA-1^{MET} for 24 h. Then cells were analyzed for annexin-V, PI by flow cytometry ($n=2$). *FLS* fibroblast-like synoviocytes, *RA* rheumatoid arthritis, *PI* propidium iodide, *CO* control, *DMSO* dimethyl sulfoxide, *FITC* fluorescein isothiocyanate

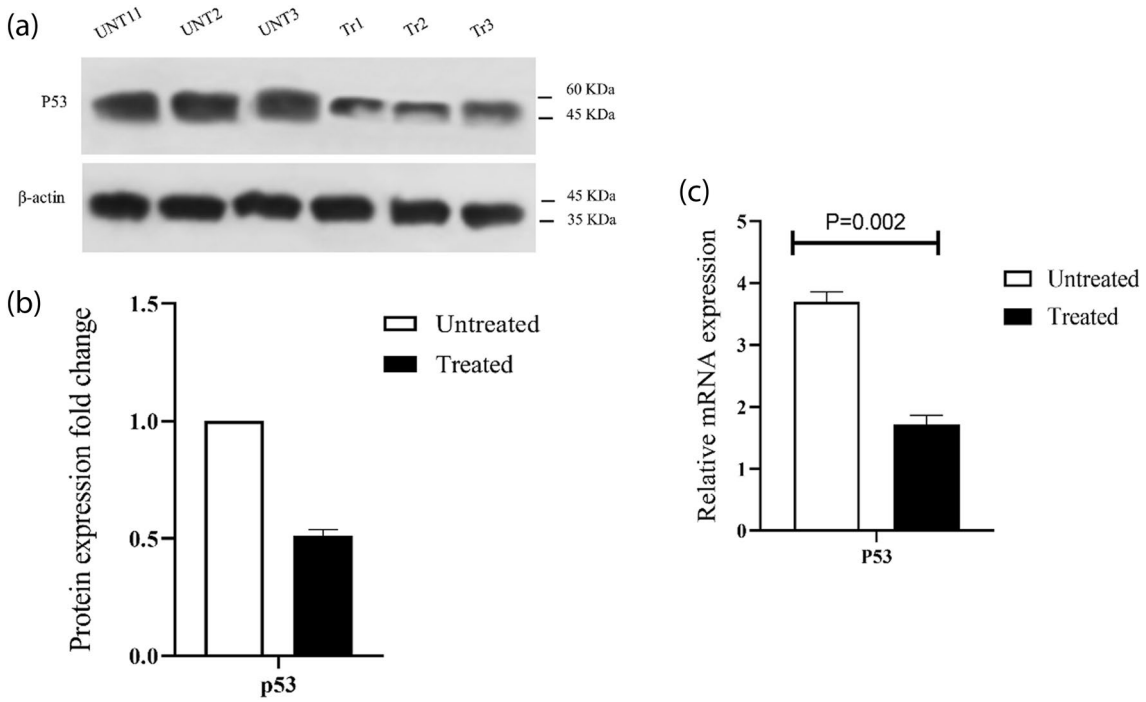

Fig. 4 Downregulation of p53 in RA-FLSs by PRIMA-1^{MET}. **a** The effect of PRIMA-1^{MET} on p53 protein expression. After exposure of cells with 35 µM of PRIMA-1^{MET} for 24 h, total cell lysates were prepared and Western blotting was performed ($n=3$). Actin serves as a loading control. **b** The densitometric analysis of Western blots by ImageJ software. **c** The effect of PRIMA-1^{MET} on p53 mRNA expression. RA-FLSs were treated with 35 µM PRIMA-1^{MET} for 24 h, after which RNA was extracted, and the expression of the p53 gene was measured by quantitative RT-PCR and normalized to the expression of GAPDH ($n=10$). *FLS* fibroblast-like synoviocyte, *RA* rheumatoid arthritis, *mRNA* microRNA, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *RT-PCR* reverse transcription-polymerase chain reaction, *UNT* untreated

restoration of mutant p53 by recently developed small molecules such as PRIMA-1^{MET} may induce apoptosis in RA-FLSs. PRIMA-1 and its methylated version restore p53 function through modification of thiol group in mutant p53 (Lambert et al. 2009) without any changes in p53 expression (Farhadi et al. 2017; Izetti et al. 2014). However, the results of the present study indicate a significant decrease in p53 expression at both mRNA and protein levels. Since PRIMA-1^{MET} affects multiple targets and pathways, it is possible that downregulation of p53 is not a direct effect of PRIMA-1^{MET} and is related to the activation of other regulatory proteins (Bykov et al. 2018; Rangel et al. 2019).

A number of experimental studies have shown that PRIMA-1 can induce apoptosis and inhibit the growth of tumor cells by induction of pro-apoptotic target genes of p53 (Bykov et al. 2002a, 2003). In this study, the cytotoxic effect of PRIMA-1^{MET} in RA-FLSs was evaluated and we found that PRIMA-1^{MET} induces apoptosis in RA-FLSs in a dose-dependent manner.

It has been reported that PRIMA-1 enhances apoptosis through activation of transcription of pro-apoptotic p53 target genes, including P21, Bax, and Noxa in cancer cells (Farhadi et al. 2017). To determine whether PRIMA-1^{MET} induces apoptosis through induction of pro-apoptotic genes in RA-FLSs, we evaluated the expression of Noxa, Bax, PUMA, and p21. Our results showed that PRIMA-1^{MET} can just induce Noxa expression significantly in RA-FLSs. Noxa is the most important pro-apoptotic target of the p53 gene and can induce apoptosis in FLSs by inactivating Mcl-1, an anti-apoptotic protein of the Bcl-2 family (Cottier et al. 2014). Our results showed that PRIMA-1^{MET} had no effect on the expression of PUMA, Bax, and p21 which is consistent with other studies (Li et al. 2015; Mlakar et al. 2019; Synnott et al. 2017). Concerning these results and due to the pro-survival role of p53(Vousden 2006), it seems that downregulation of p53, which is not reported before, is involved in apoptosis induction in RA-FLSs.

Survivin and XIAP are members of the apoptosis inhibitory protein (IAP) family. Survivin is regulated by p53 and p53 has an inhibitory effect on this protein (Zafari et al. 2019). P53 activation also induces XIAP downregulation (Güllülü et al. 2021). Previous studies have revealed PRIMA-1 has no effect on p53 and survivin expression (Farhadi et al. 2017; Izetti et al. 2014) but in our study PRIMA-1^{MET} treatment led to a significant overexpression of survivin that may be related to decreased expression of p53.

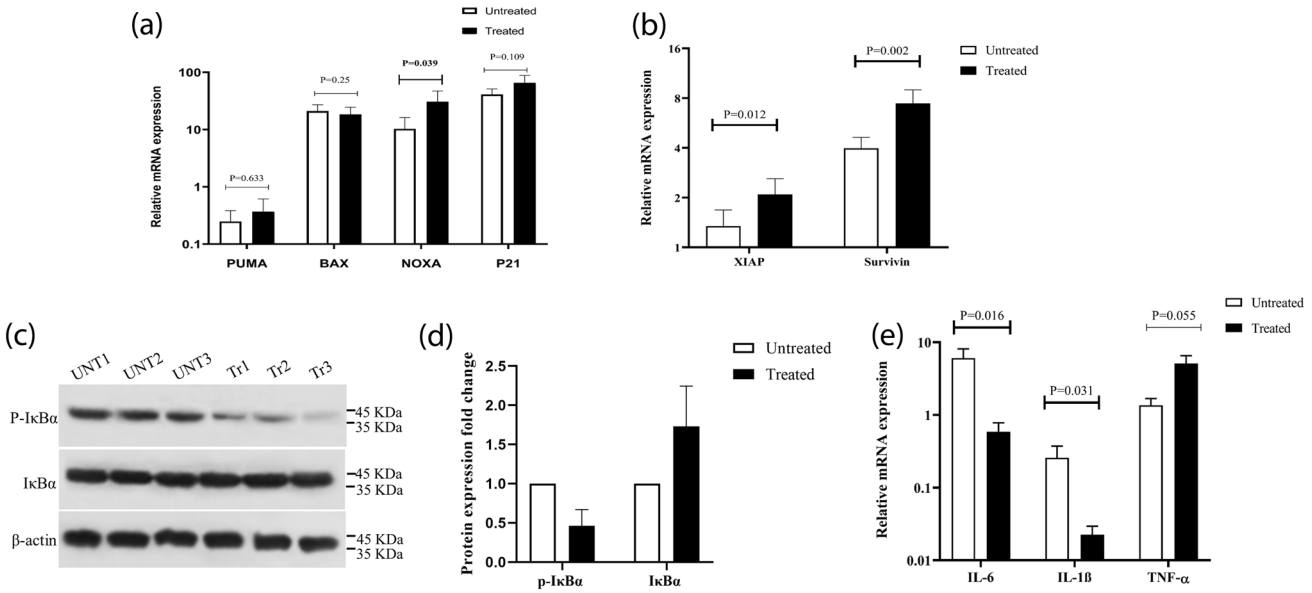

Fig. 5 RA-FLSs were treated with 35 μM PRIMA-1 for 24 h, then total RNA was extracted, and gene expression was performed using quantitative RT-PCR and normalized to the expression of GAPDH ($n=10$). **a** Modulation of p53 target genes by PRIMA-1^{MET}. **b** The effect of PRIMA-1^{MET} on the expression of XIAP and Survivin. **c** PRIMA-1^{MET} decreases NF-κB activity. RA-FLSs were treated with 35 μM PRIMA-1^{MET} for 24 h. Total cell lysates were prepared and Western blot analysis was performed ($n=3$). β-actin has been used as a loading control. **d** The densitometric analysis of P-IκBα and IκBα Western blots by ImageJ software. **e** Anti-inflammatory effect of PRIM A-1^{MET} in RA-FLSs. *FLS* fibroblast-like synoviocytes, *RA* rheumatoid arthritis, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *RT-PCR* reverse transcription-polymerase chain reaction, *PUMA* P53 upregulated mediator of apoptosis, *BAX* Bcl-2-associated X protein, *NOXA* Phorbol-12-myristate-13-acetate-induced protein 1, *XIAP* X-linked Inhibitor of Apoptosis, *NF-κB* nuclear factor-kappaB, *P-IκBα* phosphorylated IkBα, *UNT* untreated, *Tr* treated, *IL-6* Interleukin 6, *IL-1β* interleukin 1 beta, *TNF-α* tumor necrosis factor-alpha

Furthermore, PRIMA-1 can downregulate XIAP expression in cancer cells (Farhadi et al. 2017) while our results showed that PRIMA-1^{MET} increases XIAP expression significantly. Our result is consistent with Soans et al. that reported some PRIMA-1 derivatives induced XIAP expression in lung cancer cells (Soans et al. 2014). Besides, given the regulatory role of p53 on XIAP expression, it is not surprising that p53 downregulation upon PRIMA-1^{MET} treatment results in XIAP overexpression.

P53 is required for NF-κB inhibition (Murphy et al. 2011) and both of them inhibit each other's (Webster and Perkins 1999). Furthermore, some studies have reported p53 activates NF-κB through different factors (Bohuslav et al. 2004; Jänicke et al. 2008). NF-κB activation plays a critical role in RA inflammation (Makarov 2001). Besides, it has been revealed that NF-κB is involved in both the initiation and perpetuation of chronic inflammation in RA. NF-κB contributes to inflammation through the induction of pro-inflammatory cytokines expression like IL-1, IL-6, TNF-α, and IL-17 (Makarov 2001). Given the NF-κB role in RA-FLSs, the inhibition of NF-κB would be a helpful strategy to inhibit pro-inflammatory cytokine production in RA-FLSs. The results of the present study showed that PRIMA-1^{MET} has an inhibitory effect on the NF-κB pathway through decreasing phosphorylation of IκBα protein in RA-FLSs. Our results are consistent with Farhadi et al.'s study which reports that PRIMA-1 can inhibit NF-κB in acute promyelocytic leukemia cell lines (Farhadi et al. 2017). This effect of PRIMA-1^{MET} may be a direct effect or related to the decreased expression of p53 (as an NF-κB activator).

IL-1 is an important player in RA pathogenesis and induces other pro-inflammatory cytokines like IL-6 and TNF-α (Kinne et al. 2007). IL-1 plays a critical role in joint damage through the enhancement of proteoglycan degradation (Ruscitti et al. 2018). Besides, TNF-α and IL-1 act synergistically in the induction of more inflammation and joint destruction (Schett and Gravallese 2012). IL-6 disrupts the balance between Th-17 and regulatory T cells (Tregs) and leads to the differentiation of naive T cells toward Th-17 (Yoshida and Tanaka 2014). Our results showed that PRIMA-1^{MET} can significantly decrease IL-1 and IL-6 and had no effect on TNF-α expression. Given the inhibition of NF-κB upon PRIMA-1^{MET} treatment, it is not surprising to report the downregulation of these key pro-inflammatory cytokines.

Conclusion

In conclusion, our results indicate that PRIMA-1^{MET} can induce apoptosis in RA-FLSs through induction of Noxa expression while p53 was downregulated. Furthermore,

PRIMA-1^{MET} treatment results in the suppression of pro-inflammatory cytokine production and NF-κB inhibition. Given the role of p53 and NF-κB in RA-FLSs, PRIMA-1^{MET} can be considered as a new therapeutic strategy for rheumatoid arthritis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10787-022-01094-9.

Author contributions MA: acquisition of data, drafting the article, analysis and interpretation of data, final approval of the article. MNT and AS: acquisition of clinical data and patient's diagnosis and treatment, interpretation of data, drafting the article, and final approval of the article. MT, AJ, MM and EF: the conception and design of the study, revising the article critically, interpretation of data, final approval of the article.

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Data availability The data generated and/or analyzed during the current study are available from the corresponding author on reasonable request. Applicable on requested.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study was performed based on the Declaration of Helsinki guidelines and was approved by the Ethics Committee of Tehran University of Medical Sciences (Approval No: IR.TUMS.VCR.REC.1397.968).

Consent to participate The written informed consent was signed by all participants before enrolling in the study.

Consent to publication Not applicable.

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