

Therapeutic effects of scavenger receptor MARCO ligand on silica-induced pulmonary fibrosis in rats

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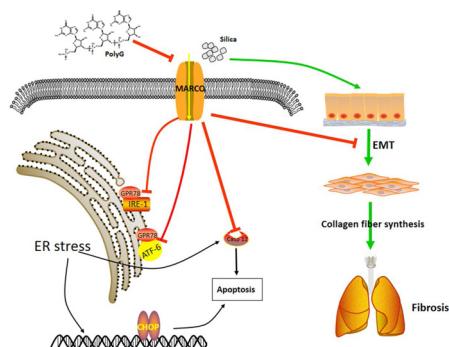
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GRAPHICAL ABSTRACT

Pharmacological targeting of MARCO with polyG (MARCO inhibitor) attenuated the silica-induced fibrosis through suppressing the ERS-associated apoptosis and inhibiting the EMT process.



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ABSTRACT

Pulmonary fibrosis induced by prolonged exposure to silica particles is a chronic and irreversible lung disease without effective treatment till now. Our previous study has shown that early intervention with MARCO antagonist PolyG could alleviate pulmonary fibrosis in silica-exposed rats. However, the therapeutic effects of PolyG on silica-induced pulmonary fibrosis have rarely been reported. In this study, we explored the effects of administration (on the 28th day after silica exposure) of PolyG (MARCO inhibitor) on an established rat silicosis model. The lungs were analyzed histopathologically in rats using HE and Masson staining. The silica-induced ERS-related apoptosis, EMT and fibrosis were evaluated using western blotting, qRT-PCR and

Abbreviations: AMs, alveolar macrophages; ERS, endoplasmic reticulum stress; MARCO, macrophage receptor with collagenous structure; EMT, epithelial-mesenchymal transition; AECs, alveolar epithelial cells; PRRs, pattern recognition receptors; UPR, unfolded protein response; ATF6, activating transcription factor 6; IRE1, inositol-requiring enzyme-1α; CHOP, C/EBP homologous protein; IPF, idiopathic pulmonary fibrosis; SR-A1, scavenger receptors type 1; SRB1, scavenger receptor class B type 1; SCARA5, scavenger receptor class A member 5; CD36, cluster of differentiation 36

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immunohistochemical analyses. The results suggested that silica exposure could increase the MARCO activity, and induce ERS and EMT in lung tissues. Pharmacological targeting of MARCO with PolyG attenuated the development of pulmonary fibrosis in silica-exposed rats. Further study indicated that PolyG could inhibit silica-induced ERS-related apoptosis and EMT process. Together, our findings reveal an essential function of ERS-related apoptosis and EMT in the processes of pulmonary fibrosis caused by silica, and identify MARCO as a potential therapeutic pharmacological target for silicosis.

1. Introduction

Crystalline silica is one of the main components of the earth's crust, and silica exposure is common in occupational and living environments. Environmental exposure to silica occurs during volcanic explosions, sandstorms and industrial contaminations. More situation, long-term exposure to respirable crystalline silica is present in many industries such as construction, metal or coal mining, glass or clay manufacturing and certain earth industries and such exposure is the direct cause of silicosis (Leung et al., 2012). Silicosis, characterized by pulmonary inflammation and progressive fibrosis, affects tens of millions of workers involved in dusty occupations in many countries (Li et al., 2016). This incurable disease has been studied extensively with related pathological mechanisms involving apoptosis and epithelial-mesenchymal transition (EMT). Unfortunately, potential mechanisms for silicosis remain unclear because multiple pathways are contained in the pathogenesis.

After exposure to silica particles, the acute inflammatory response is ignited through subsequent uptake of silica particles by alveolar macrophages (AMs). AMs play a major role in the innate host responses, and could respond to exogenous particulates and pathogens through interactions with pattern recognition receptors (PRRs) (Beamer et al., 2016). The class A scavenger receptors are a group of PRRs with a common clearance function for endogenous molecules or apoptotic cells, as well as exogenous microbial and particulates (Canton et al., 2013). Recent reports have focussed on the role of the class A scavenger receptor, especially macrophage receptor with collagenous structure (MARCO), in regulating inflammation and tissue remodeling (Maler et al., 2017). When pretreated with MARCO antibody in vitro, the cytotoxicity

induced by silica is almost completely inhibited with the reduced oxidative stress and apoptosis (Hamilton et al., 2008; Thakur et al., 2009b). Simultaneously, when compared with wild-type mice, the severity of lung fibrosis in a model of asbestosis was significantly decreased in MARCO-deficient mice. (Murthy et al., 2015). However, contradictory evidence suggests that inflammatory responses to silica particles are enhanced in MARCO-deficient macrophages and mice (Thakur et al., 2009a). Thus, the exact role played by scavenger receptor MARCO in silica-induced pulmonary fibrosis needs further investigation.

More evidence indicates some particulates could bind to the scavenger receptor MARCO and initiate apoptosis (Mukhopadhyay et al., 2011; Stichling et al., 2018). Apoptosis plays a central role in silicosis by activation of cell surface death receptor (Fas/FasL) pathway and mitochondrial apoptotic pathway (McIlwain et al., 2013; Yao et al., 2011). In addition to the mitochondria, endoplasmic reticulum (ER) is also involved in the activation of apoptosis through the unfolded protein response (UPR), which can cause endoplasmic reticulum stress (ERS). ERS-induced apoptosis may play an essential mechanistic component in the development and progression of fibrosis in various tissues including lung, liver, kidney, and heart (Tanjore et al., 2013). More recent studies suggest that silica-induced ERS is involved in the apoptosis of alveolar macrophages (Hu et al., 2017) and play a key role in the development of silicosis.

On the other hand, the deposition of silica particles in the lung also leads to epithelial cell injury through prolonged interaction with immune cell populations, which could stimulate the process of EMT. EMT plays an essential role in wound healing, organ fibrosis, tumor metastasis and other developmental processes. (Bartis et al., 2014). Early

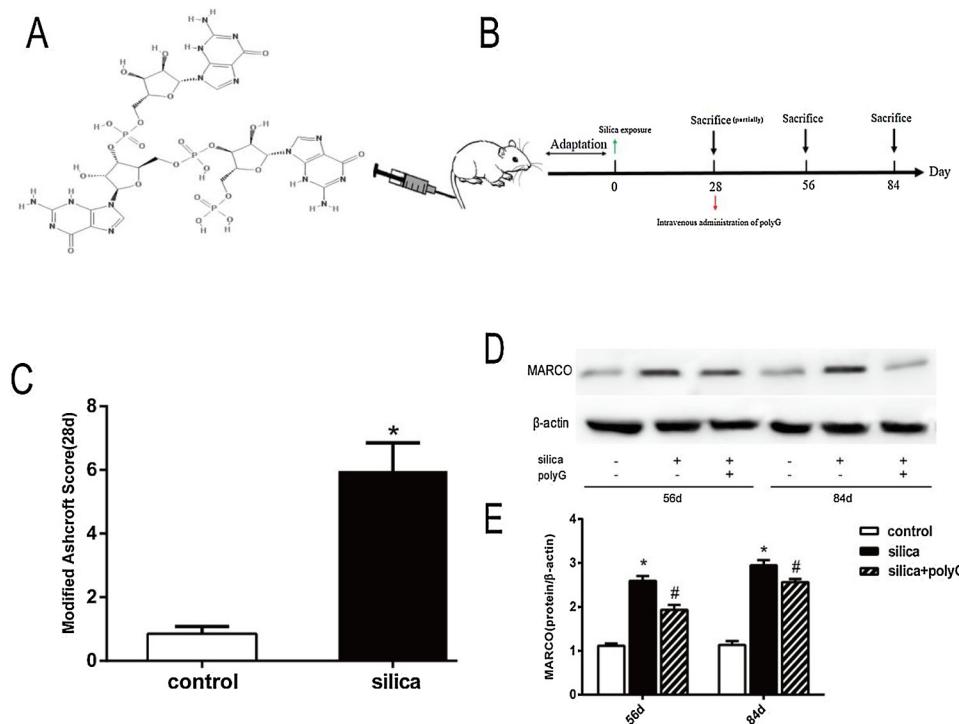


Fig. 1. Scavenger receptor MARCO expression was decreased by PolyG. (A)Structural formula of PolyG. (B)Experimental design for intratracheal instillation of silica and administration of PolyG. (C) Severity of pulmonary fibrosis measured using modified Ashcroft score (after silica exposure for 28 days). (D) The expression level of MARCO protein in lung tissues was measured by Western blot analysis. (E) The relative protein expression of MARCO was quantified by Image J. Data are presented as mean \pm S.D. * P < 0.05 compared with the control group; # P < 0.05 compared with the silica group.

researches have indicated that EMT is a worthy physical phenomenon occurred in alveolar epithelial cells (AECs) and involved in the development of silicosis (Heise et al., 2011; Yan et al., 2016).

Intriguingly, recent study has reported that MARCO, which is also expressed on epithelial cells in the lung, could be involved in the engulfment of amorphous silica nanoparticles (Lara et al., 2018). In addition, some scavenger receptors like scavenger receptor class B type 1(SRB1), scavenger receptor class A member 5(SCARA5) and cluster of differentiation 36(CD36) have been thought to take part in the regulation of EMT process in hepatocellular carcinoma and diabetic nephropathy (Hou et al., 2015; Liu et al., 2013; Yu et al., 2015). Moreover, recent reports have suggested the involvement of ERS and EMT in the progression of silicosis and a possible link between ERS pathway and EMT process has been introduced in AECs (Liu et al., 2018; Zhong et al., 2011). Our previously published results indicate that inhibiting MARCO through preventive intervention using PolyG could decrease the levels of mitochondrial apoptosis and alleviate silica-induced fibrosis (Yang et al., 2018). However, whether scavenger receptor MARCO mediates the downstream ERS pathway and the therapeutic effects of PolyG on EMT and silica-induce pulmonary fibrosis is still unknown.

Here, we attempted to investigate whether ERS-related apoptosis and EMT were involved in the pathway by which scavenger receptor MARCO regulate silica-induced fibrosis. To address this issue, a rat model of silica-induced pulmonary fibrosis was developed and PolyG was treated as an inhibitor to block the activity of MARCO. The role of PolyG on the progression of pulmonary fibrosis and potential influence on ERS-associated apoptosis and the EMT process was further evaluated.

2. Material and methods

2.1. Animals

Male Sprague-Dawley (SD) rats at 6 to 8 weeks of age were obtained from Beijing Weitong Lihua Experimental Animal Technology Co. Ltd. (Beijing, China). All animals were housed under a specific pathogen-free environment (room temperature $22 \pm 2^\circ\text{C}$; 12/12 h light/dark cycles; relative humidity 50–60%), with food and water ad libitum. All animal experiments were approved by the Animal Care and Use Committee at Xinxiang medical university and conducted complying with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Experimental groups and silicosis model

Rats were randomly divided into three groups ($n = 16$ per group): (a) control group, (b) silica group, (c) silica + PolyG group. PolyG (Sigma Aldrich, St. Louis, USA), a scavenger receptor ligand, was intravenously administered as a single dose at 2.5 mg/Kg after 28-days of silica exposure. PolyG was dissolved in sterile saline to a final concentration of 1 mg/mL. PolyG (Fig. 1A) is a polynucleotide comprised of guanosine units connected via 3'-5' phosphodiester linkages. It contains a GMP 3'-end residue, a GMP 5'-end residue and a guanosine 5'-monophosphate residue. Eight rats from each group were sacrificed respectively at 56th day and 84th day after silica exposure. The experimental schedule was shown in Fig. 1B. Silica particulates preparation was conducted according to previously published methods (Yang et al., 2018). Briefly, we ground the silica particulates (approximately 97% between 1 and 5 μm diameter; Frederick, MD, USA) for 3 h using an agate mortar, and then weighed and suspended them in sterile saline (50 g/L). Finally the suspensions were sonicated for 10 min prior to instillation. The silicosis model was induced as follows: Rats were anesthetized with pentobarbital sodium (40 mg/kg) by intraperitoneal injection and then were administered 50.0 g/L silica suspensions 1 mL by non-surgical intratracheal instillation. Control rats were treated with

1 mL sterile saline.

2.3. Histological analysis

Lung samples were harvested at 56 and 84 days post silica administration. Lung was removed and was fixed in 10% formalin for 48 h and then embedded in paraffin. Paraffin sections (4- μm thick) were used for hematoxylin and eosin (HE) and Masson staining to evaluate the inflammatory infiltrates and the extent of fibrosis in the lung. A modified Ashcroft histopathology scoring scale was used to quantify fibrotic alterations by three reviewers unaware of the treatment groups. For immunohistochemistry staining, the primary antibodies including α -SMA(1:100) and vimentin(1:100) (Cell Signaling Technology Inc, Beverly, MA, USA), E-cadherin(1:50) (Santa Cruz Biotechnology, Santa Cruz, CA) and a biotinylated secondary antibody(1:100) were used. Rabbit IgG was used as an isotype control. For quantitative analysis of immunohistochemical staining, we generated a graphic with the mean intensity in each section of the stack and defined ten areas from each section and measured sections from three or four eyes per treatment, and we then batch-processed them with customized macros and algorithms generated for Image-Pro Plus 6.1.

2.4. Quantitative real-time RT-PCR

Total RNA was isolated using the Trizol Reagent (Thermo Fisher Scientific, Waltham, MA) and reverse transcribed to cDNA with the TransScript First-Strand cDNA Synthesis SuperMix (Invitrogen). The sequences of specific primer pairs are described below: E-cadherin, 5'-GATTACAAGTTGCCGCCATC-3' and 5'-CTTGACCACCGTTCTCCTC-3'; α -SMA, 5' CACCATGGGAATGAACACTTC-3' and 5'-CTGTCAGCAATG CCTGGGTA-3'; vimentin, 5'-AGGAACAGGATGTCAAATCG-3' and 5'-AAGGGCATCCACATCACTCGGT-3'; Col1a1, 5'CAATCGGCTAAAGAA GTCTGTC-3' and 5'-AGGTGGGTACAGTGTAGCCT-3'; Col3a1, 5'-AGGA GGGTACAGTGTAGCCT-3' and 5'-GATCGCATAGGTAACAGGTGTT-3'; GAPDH, 5'-GTGATTGATGCCGACGGCAG-3' and 5'-CAGGAGCATGGAT TGGAGT-3'. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was set as an endogenous control for normalization and $\Delta\Delta\text{CT}$ methods were used to evaluate the relative mRNA expression.

2.5. Western blot analysis

Total proteins extracted from tissues were estimated using the Pierce BCA Protein Assay Kit (Thermo Scientific, USA). Equal amounts of total protein (60 μg) from each sample were separated by application of a constant voltage of 110 V for 2 h and then transferred onto NC membranes at constant electric current of 300 mA for 1.5 h. After blocking the nonspecific binding with 5% non-fat milk for 1 h, membranes were washed and then incubated at 4°Covernight with primary antibodies: α -SMA(1:1000), vimentin(1:1000), and β -actin(1:2000) (Cell Signaling Technology Inc, Beverly, MA, USA), Collagen I(1:1500), Collagen III(1:1500), GPR78(1:1000), activating transcription factor 6(ATF6)(1:1500), inositol-requiring enzyme-1 α (IRE1)(1:1500), C/EBP homologous protein(CHOP)(1:1000), Caspase-12(1:1000) (Affinity Biosciences. OH. USA), MARCO(1:200) and E-cadherin(1:200) (Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were then incubated with HRP-conjugated goat anti-rabbit(1:8000) (Cell Signaling Technology, Beverly, MA, USA) for 1 h. Blots were developed with Amersham Imager600 (GE Healthcare) and β -actin was used as a loading control.

2.6. Statistical analysis

All analyses were performed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA). All data from independent experiments were presented as mean \pm S.D. One-way ANOVA analysis with a post hoc test was performed and the differences between any two groups were determined

using Tukey's multiple-comparisons test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. PolyG antagonize expression of scavenger receptor MARCO in silica-exposed rats

To determine whether the silicosis model was established on the 28th day (PolyG administration time) after silica exposure, we performed HE and Masson trichrome staining in the control group and silica group (see Supplemental Fig. 1). Seriously damaged alveolar structure with extensive inflammatory cells infiltration and collagen fiber deposition were observed in the rat lungs of silica group. Semi-quantitative analysis of lung histopathology indicated that the

pulmonary fibrosis in silica group was significantly more severe than that in the control group (Fig. 1C). Furthermore, in comparison with the control group, the expressions of MARCO were enhanced not only on the 28th day (Yang et al., 2018), but also on the 56th day and 84th day after silica exposure. Exogenous administration of PolyG significantly inhibited the expression of MARCO in all time points when compared with the silica groups (Fig. 1D and E). The results indicated that therapeutic administration of PolyG could also antagonize the expression of MARCO.

3.2. Histopathological analysis of lungs from silica-exposed rats treated with PolyG

The rats were sacrificed on the 56th day and 84th day (28 days and 56 days after administration of PolyG), and the lung tissues of each

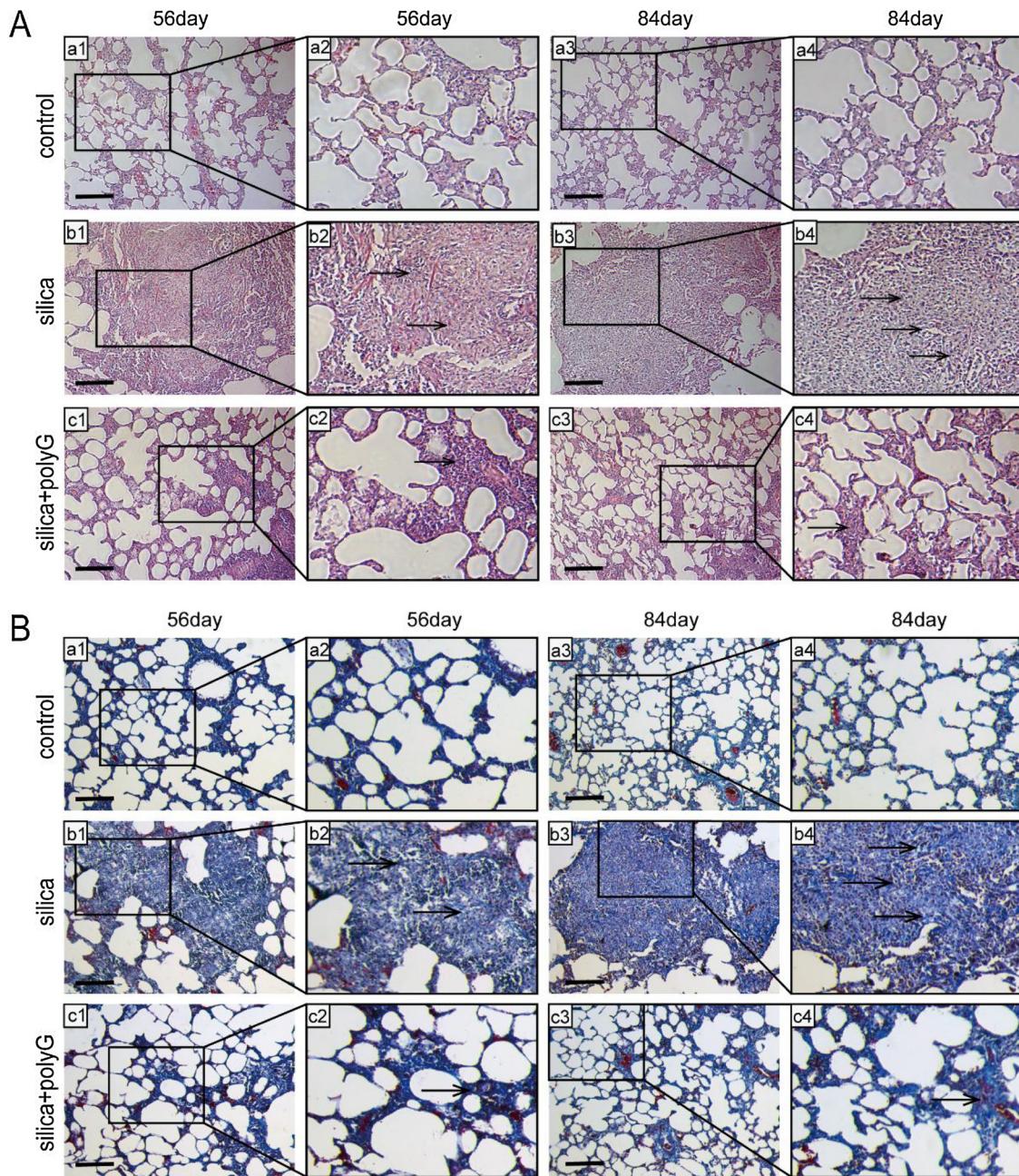


Fig. 2. HE staining and Masson staining of lung tissues in each group on the 56th day and 84th day after silica exposure. (a1-c1 & a3-c3), original magnification $\times 100$. Scale bar 100 μm . (a2-c2 & a4-c4), higher magnification view from the boxed areas in left panels. Arrows show pathological changes in lung tissues.

group were histologically examined. HE staining results showed more infiltrating inflammatory cells and silicotic nodules in silica-exposed rats compared with the control group on the 56th day and 84th day after silica exposure (Fig. 2). Pharmacological blockade of MARCO decreased the number of infiltrating inflammatory cells and alleviated the dispersion of silicotic nodules when compared with the silica groups. Masson staining showed that excessive collagen deposition (Figs. 2 and 3A) were found in pulmonary mesenchyme in silica-exposed rats compared with the control groups. Nonetheless, after therapeutic administration of PolyG, the collagen deposition area in rats was also diminished when compared with those in the silica groups.

3.3. Scavenger receptor MARCO targeting protect against pulmonary fibrosis in silica-exposed rats

After silica exposure, we assessed the expression of collagen at mRNA and protein levels in lung tissues on the 56th day and 84th day respectively (Fig. 3B–F). Results showed that levels of these fibrotic indicators were significantly higher in silica-exposed rats when compared with those in the control groups. In the PolyG treatment groups, a markedly decrease on the levels of Col1a1, Col3a1, Colland Col III was observed when compared with the silica groups. Thus, in vivo MARCO targeting down modulated silica-induced the expression of profibrotic genes and collagen deposition.

3.4. Antagonism of scavenger receptor MARCO suppressed ERS-related apoptosis in silica-exposed rats

To ascertain whether ERS was involved in the process of silica-induced apoptosis and the effects of PolyG on ERS-related apoptosis, GRP78 (the ERS responsive marker), ATF6 and IRE1 were measured in rat lung tissues. As shown in Fig. 4, the protein expression levels of GRP78, ATF6 and IRE1 were significantly higher in the silica groups compared with the control groups. After administration of the PolyG on the 28th day, a remarkable reduction in the expression levels of GRP78, ATF6 and IRE1 in rat lung tissues was found on the 56th day and 84th

day when compared with the silica groups (Fig. 4A–E). Moreover, pharmacological intervention with PolyG markedly suppressed the silica-induced upregulation of the pro-apoptotic proteins CHOP and Caspase-12 when compared with the silica groups on the 56th day and 84th day after silica exposure (Fig. 4A and F–H). Taken together, these results suggested that ERS played an important role in the silica-induced lung injury and mediated cell-mediated apoptosis, and MARCO inhibition could attenuate apoptosis.

3.5. Therapeutic effects of PolyG on the expression of EMT markers in silica-exposed rats

Moreover, we examined whether antagonism of MARCO could modulate the EMT process in silica-exposed rats. E-Cadhein-positive cells were expressed on the surface of the alveolar wall cavity and α -SMA and vimentin-positive cells were not found in lung tissues of rats in the control groups. After silica exposure, increased number of vimentin and α -SMA-positive cells (mainly expressed in interstitial fibrotic areas and silicotic nodules) and decreased number of E-cadherin-positive cells (Figs. 5 and 6) were observed in lung tissue when compared with the control group. The silica-exposed groups that were administrated with PolyG showed a significant elevated number of E-cadherin-positive cells and a significant reduced number of vimentin and α -SMA-positive cells compared with the silica groups. Our results suggested that EMT occurred in silica-exposed rats and therapeutic administration of PolyG could suppress the progression of this process.

3.6. Scavenger receptor MARCO inhibition attenuated the process of EMT in silica-exposed rats

To assure the therapeutic effect of MARCO antagonism on EMT markers expression, the epithelial marker E-Cadherin and mesenchymal markers vimentin and α -SMA were measured in rat lung tissues. Compared with the control groups, reduced E-Cadherin expression and increased vimentin and α -SMA expression were observed both at mRNA and protein levels (Fig. 7) in silica groups. However, PolyG treatment

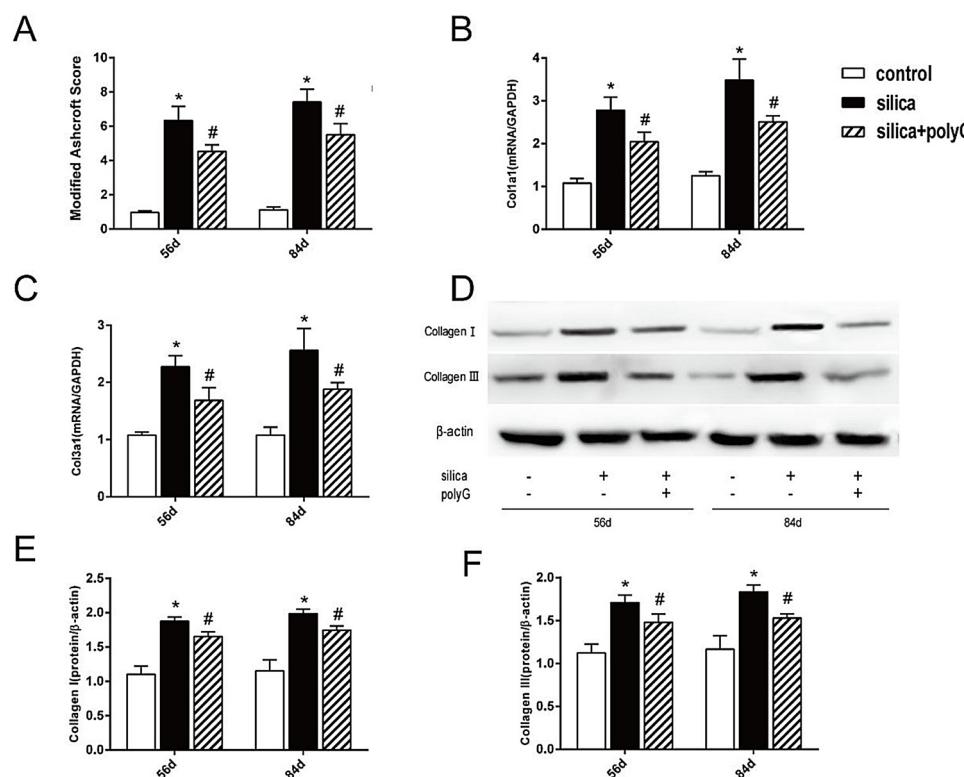


Fig. 3. Silica-induced lung fibrosis was prevented by pharmacological scavenger receptor MARCO targeting in rats. (A) The mean intensity of deposited collagen with Masson staining was scored using Image-Pro Plus software. (B–C) The relative mRNA expression of Col1a1 and Col3a1 in lung tissues was measured by quantitative RT-PCR. (D) The expression levels of Collagen I and III in lung tissues were measured by Western blot analysis. (E–F) The relative protein expression of Collagen I and III were quantified by Image J. Data are presented as mean \pm S.D. * P < 0.05 compared with the control group; # P < 0.05 compared with the silica group.

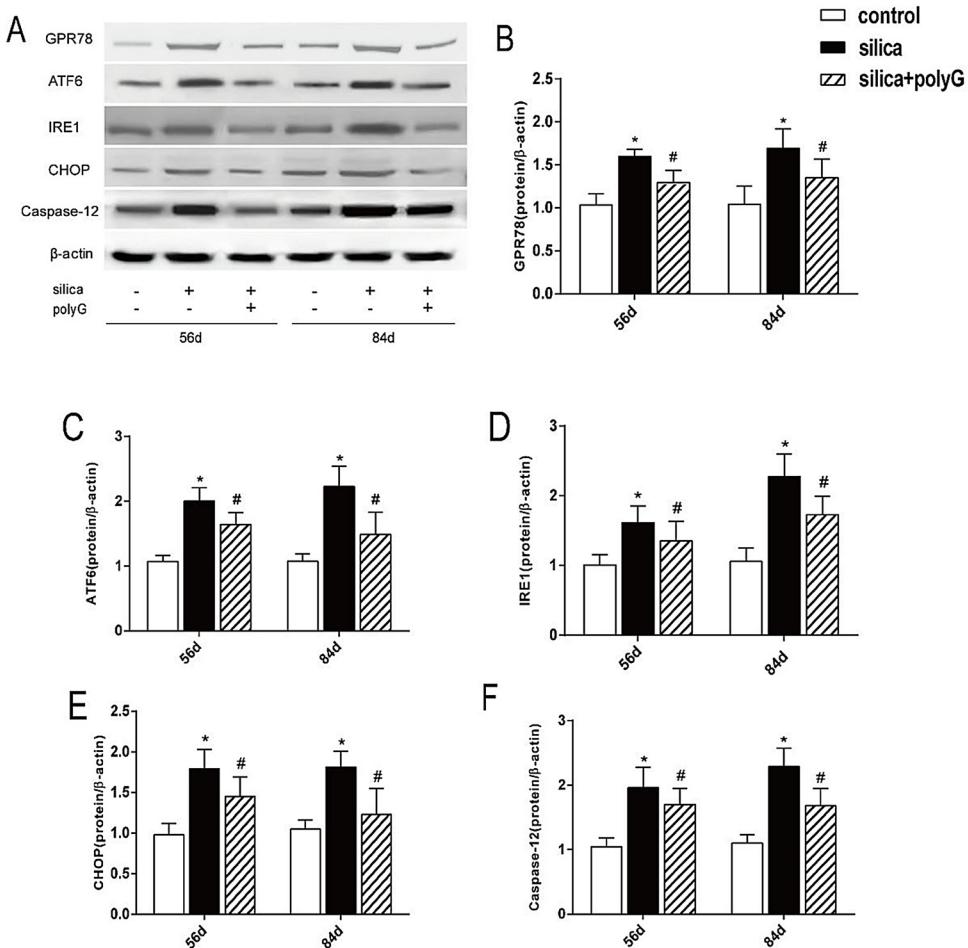


Fig. 4. MARCO antagonism suppressed the UPR pathways and ERS-related apoptosis. (A) The expression levels of and ERS and UPR pathway related proteins in lung tissues were measured by Western blot analysis. (B) The relative protein expression of GPR78, ATF6, IRE1, CHOP and Caspase-12 to β-actin was expressed in the bar graphs. Data are presented as mean ± S.D. * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the silica group.

induced a significant increase in the expression level of E-Cadhein on the 56th day and 84th day when compared with silica exposed rats. Likewise, the expression levels of vimentin and α-SMA in PolyG treatment groups were down-regulated at both time points in rat lung tissues when compared with the silica groups.

4. Discussion

In this study, we observed that elevated lung MARCO expression, activated ERS-associated apoptosis and EMT by in vivo exposure to silica particles. We then verified that PolyG could suppress ERS-associated apoptosis and EMT on the 56th day and 84th day after silica exposure. At the same time, pulmonary fibrosis caused by silica exposure was also attenuated by PolyG.

Firstly, we successfully established the silica-induced pulmonary fibrosis model after silica exposure for 28 days, and then administrated the MARCO inhibitor PolyG. As one of the most important class A scavenger receptors, MARCO is the major scavenger receptor for AMs to recognize and bind to silica particles (Thakur et al., 2009b). The class A scavenger receptors have turned out to be mainly expressed on macrophages with the ability to bind some polyanions (including PolyG). Previous study has showed that silica particles could induce a highly increased expression of MARCO when compared with the phosphate buffer groups, and PolyG competitively inhibited the uptake of silica particles (Gilberti et al., 2008). In addition, alveolar macrophages isolated from asbestosis patients could express higher levels of MARCO compared with normal subjects (Murthy et al., 2015). Similarly, results

presented herein suggested that therapeutic administration of PolyG could also suppress the upregulated expression of MARCO on the 56th day and 84th day after silica exposure. Generally, inhaled silica particles could be cleared out partly by the AMs through the mucociliary escalator and/or lymphatic systems (Hamilton et al., 2008). The balance between clearance and retention of silica particles in the lung by AM plays a major role in regulating the inflammatory response and fibrosis (Thakur et al., 2009b). Our findings strengthened the important role of MARCO played in the development of silicosis, however, whether antagonizing the MARCO could reduce the engulfment of silica particles by AMs and then facilitate finally silica clearance need more in-depth research.

After silica exposure, the diffusion of inflammatory cells and the accumulation of lung collagen were observed to evaluate the changes in the progression of pulmonary fibrosis on the 56th day and 84th day. Simultaneously, the severity of pulmonary fibrosis was further confirmed by modified Ashcroft score, Col1a1 and Col3a1 mRNA levels and Collagen I and III protein levels. We noticed that abundant inflammatory infiltrate and aggravated collagen deposition with alveolar structure distortion and silicotic nodules in silica-exposed rats. Furthermore, our results indicated that administration of PolyG could ameliorate the silica-induced pulmonary fibrosis. Though prolonged observation time to the 56th day or 84th day, significant lung fibrosis was strengthened. We noticed the severity of lung fibrosis in PolyG treatment groups was milder than the silica groups at all time points.

Recently, the role of ERS in pulmonary fibrosis caught much attention because it can impact profibrotic effector pathways including

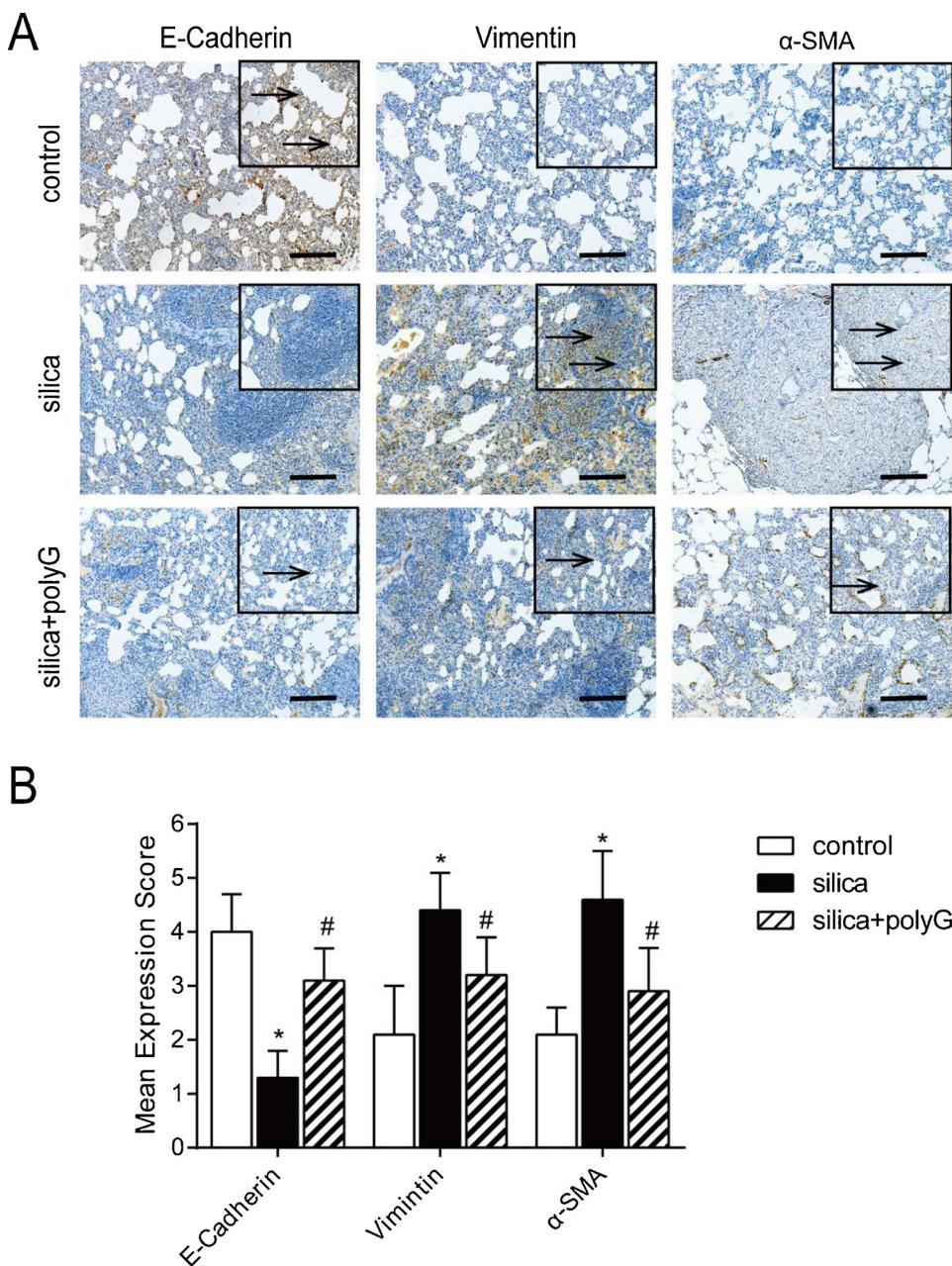


Fig. 5. Expression of E-cadherin, vimentin and α -SMA by immunohistochemical staining on the 56th day after silica exposure. (A) Representative images of positive expression of E-cadherin, α -SMA and vimentin in the lung tissues. Inset images show at a higher magnification area with positive cells (indicated by arrows). (B) The mean staining intensity of the positively stained nuclei was scored by Image-Pro Plus software. Original magnification $\times 200$. Scale bar 100 μm . * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the silica group.

apoptosis, differentiation, and inflammatory signaling (Burman et al., 2018). Our results also found that silica exposure elevated expression levels of ERS markers GPR78, ATF6 and IRE1, as well as the downstream apoptosis proteins including CHOP and Caspase-12. Similar to our results, published immunohistochemistry studies indicated that ERS markers are common in the lungs of patients with both familial and sporadic idiopathic pulmonary fibrosis (IPF) (Tanjore et al., 2012). As an ERS responsive marker, GPR78 is released from ER transmembrane signal transducers and could activate the UPR signaling pathways (Kim et al., 2016). Switching the UPR signaling from pro-survival to pro-apoptosis could be regulated by the transcriptional induction of CHOP and Caspase-12-dependent pathways (Iurlaro and Munoz-Pinedo, 2016; Kyathanahalli et al., 2015; Moorwood and Barton, 2014). Moreover, scavenger receptor signaling could regulate the ERS through unfolded protein response (UPR), and the absence of scavenger receptors type

1(SR-A1) expression prevents ERS activation after alternative stimulation (Oh et al., 2012). The UPR is initiated by an accumulation of unfolded or misfolded proteins in the lumen of the ER (Hetz and Papa, 2018). Prolonged activity of UPR indicates that ERS cannot be alleviated and homeostasis cannot be reconstituted, which leads to the activation of cell death through apoptosis (Walter and Ron, 2011). In addition, epithelial cell apoptosis is a key component of fibrotic diseases in various organs including the lungs and recent investigations have revealed that ERS associated apoptosis is prominent in AECs in pulmonary fibrosis (Tanjore et al., 2012). Thus, it is suggested that ERS may play an evidential role in silica-induced pulmonary fibrosis. Interestingly, therapeutic administration of PolyG suppressed this process in vivo model of silicosis.

Previous studies have demonstrated that ERS and EMT contribute to the pathogenesis of pulmonary fibrosis. These pathways and their

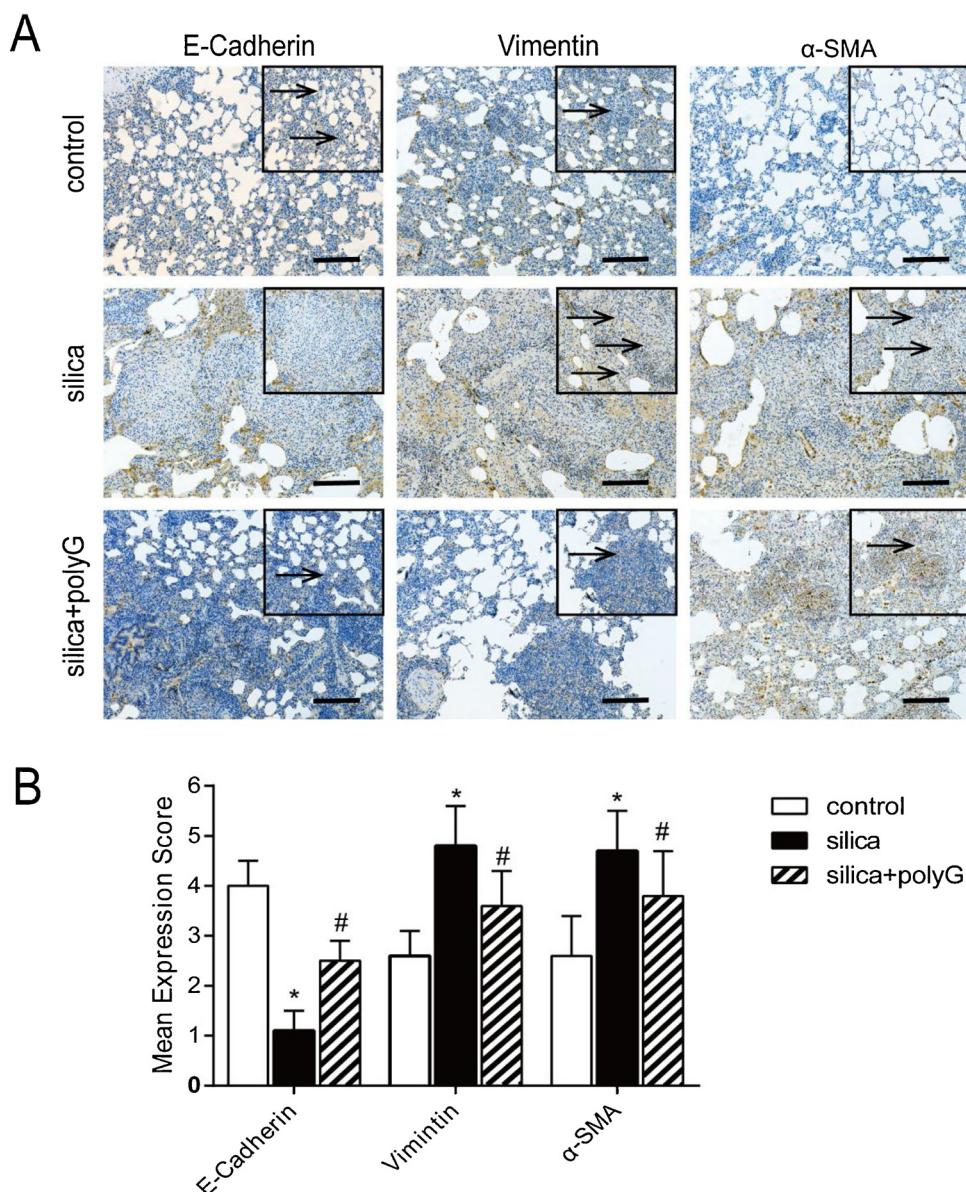


Fig. 6. Expression of E-cadherin, vimentin and α -SMA by immunohistochemical staining on the 84th day after silica exposure. (A) Representative images of positive expression of E-cadherin, α -SMA and vimentin in the lung tissues. Inset images show at a higher magnification area with positive cells (indicated by arrows). IgG was used as a control. (B) The mean staining intensity of the positively stained nuclei was scored by Image-Pro Plus software. Original magnification $\times 200$. Scale bar 100 μ m. Data are presented as mean \pm S.D. * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the silica group.

interactions were thought to be targeted for therapeutic interventions in fibrotic lung diseases (Liu et al., 2018; Tanjore et al., 2015). ERS stimulated by both chemical induction and overexpression of mutant surfactant protein C could lead to EMT in lung epithelial cells (Zhong et al., 2011). Then, EMT is a process in which epithelial cells acquire migration and invasion properties to become mesenchymal cells with loss of their cell polarity and cell-cell adhesion (Thiery et al., 2009). Moreover, EMT has been considered as an essential biological process in development of silica-induced pulmonary fibrosis (Liang et al., 2016). Therefore, immunohistochemical profiles and molecular levels of epithelial marker (E-Cadherin) and mesenchymal markers (vimentin and α -SMA) were evaluated in present study and in order to determine the phenomenon of EMT after antagonism of MARCO in silica-exposed rats. In agreement with previous studies, our results showed upregulation of vimentin and α -SMA expressions and downregulation expression of E-cadherin in lung tissues of silica groups. Furthermore, in vivo therapeutic administration of PolyG suppressed the process of EMT on the 56th day and 84th day after silica exposure. Although a number

of studies indicate that the ERS could drive EMT, our results didn't identify the up/downstream relationship between two processes. Further investigations will be necessary to clarify the mechanism and crosstalk between ERS and EMT.

Taken together, we demonstrated an implication of MARCO signaling in the silica-induced ERS associated apoptosis, EMT and fibrosis in vivo. It is possible to lessen silica particles induced pulmonary toxicity and fibrosis by preventing MARCO activation with PolyG. To explore the characterization of additional MARCO ligands could expand the range of potential therapeutic strategies for silicosis.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgment

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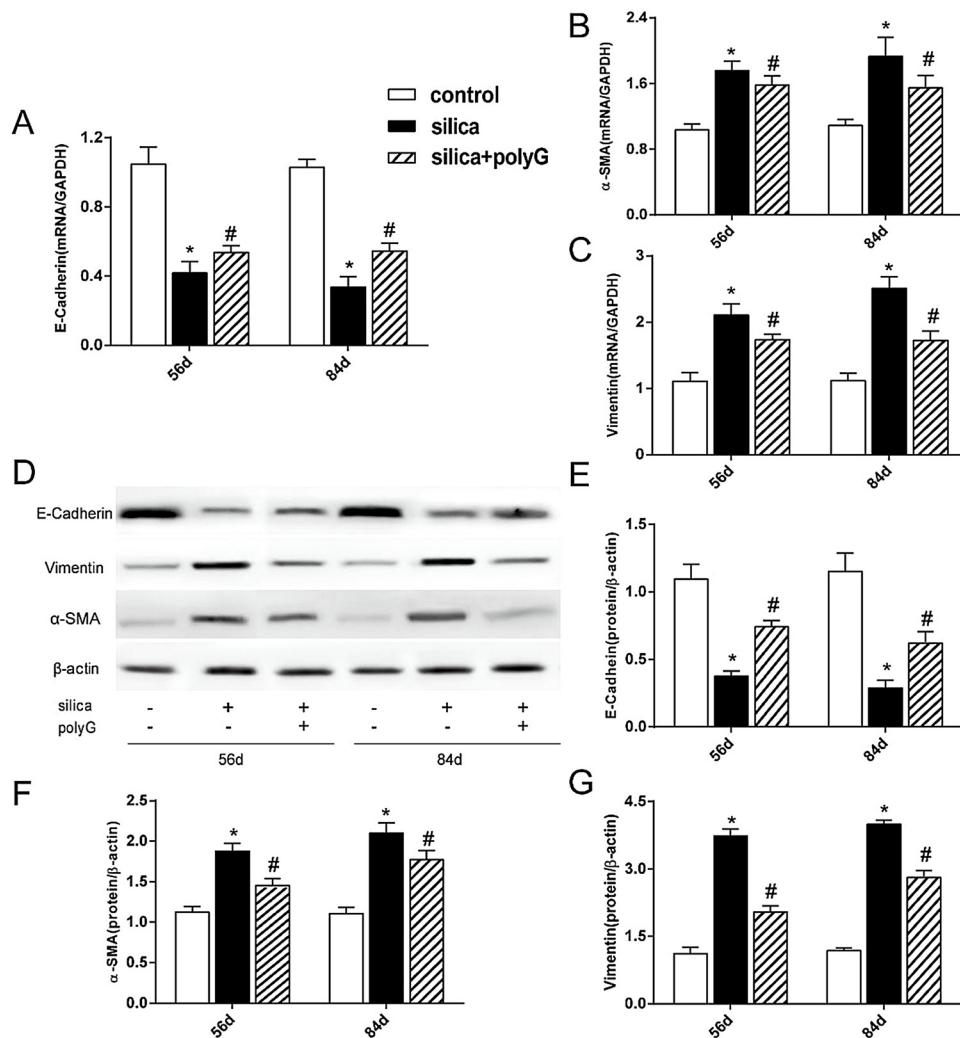


Fig. 7. Pharmacological inhibition of scavenger receptor MARCO attenuated the process of EMT in silica-exposed rats. (A–C) The mRNA levels of EMT markers in lung tissues were measured by quantitative RT-PCR analysis. (D) The expression levels of EMT markers protein in rat lungs were measured by Western blot analysis. (E–G) The relative protein expression of E-cadherin, vimentin and α -SMA were quantified by Image J. Data are presented as mean \pm S.D. * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the silica group.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxlet.2019.04.026>.

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