

RESEARCH ARTICLE

USP25 inhibits DNA damage by stabilizing BARD1 protein in a house dust mite-induced asthmatic model in vitro and in vivo

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Abstract

House dust mites (HDM) can cause DNA double-strand breaks in the lungs of asthmatic patients. However, the molecular mechanisms driving DNA damage and repair in HDM-induced asthma are yet to be elucidated. Thus, in this study, HDM treatment was applied to BEAS-2B cells and mice to mimic the pathological process of asthma in vitro and in vivo, respectively. γ -H2AX foci and expression were measured by immunofluorescence staining and western blot, respectively. The levels of interleukin (IL)-4, IL-6, IL-13, and tumour necrosis factor α (TNF α) were measured using enzyme-linked immunoassay. The expression of USP25 and BARD1 was measured by reverse transcription quantitative PCR and western blot. Co-immunoprecipitation and ubiquitination assays were employed to detect the relationship between USP25 and BARD1. As per the results, it was found that the deubiquitylating enzyme USP25 repressed HDM-induced DNA damage and the production of proinflammatory cytokines, including TNF- α , IL-4, IL-8, and IL-13, in BEAS-2B cells; in contrast, the depletion of USP25 led to the opposite effects. USP25-mediated inhibition of DNA damage and inflammation was facilitated by the stabilizing protein BARD1, which is a tumor suppressor that principally functions by promoting DNA repair and replication in BEAS-2B cells. Furthermore, USP25 was found to robustly augment BARD1 protein abundance and prevent HDM-induced DNA damage and inflammation in vivo. Taken together, these results suggest a novel mechanism contributing to DNA damage and repair in HDM-induced asthma and that selectively modulating this pathway could lead to a novel therapeutic approach for controlling and managing asthma due to HDM exposure.

KEYWORDS

asthma, BARD1, DNA damage, house dust mites, USP25

Abbreviations: CHX, cycloheximide; DSBs, DNA double-strand breaks; DUBs, deubiquitinases; HDM, house dust mites; H&E, hematoxylin and eosin; RONS, reactive oxygen and nitrogen species; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SD, standard deviation; USP, ubiquitin-specific peptidase.

Cong Gan and Ye Wang contributed equally to this work.

1 | INTRODUCTION

Allergic asthma represents the most common type of asthma; it is known to be predominantly elicited by environmental allergens, such as house dust mites (HDM) (Chan et al., 2016a; Sleiman & Flory, Imielinski, et al., 2010; Sleiman & Hakonarson, 2010). In such patients, large amounts of genotoxic reactive oxygen and nitrogen species (RONS), including peroxides and nitric oxide, are produced mainly in immune cells in response to HDM exposure. These RONS can result in a sustained inflammatory response characterized by enhanced production of various proinflammatory cytokines including tumour necrosis factor α (TNF- α), thereby leading to bronchial obstructions and hyperresponsiveness (Cheng et al., 2013; Wang et al., 2018). Moreover, RONS may damage large biological molecules, such as proteins, lipids, and nucleic acids, which could result in further deterioration of patients' physiological conditions (Ho et al., 2012). DNA double-strand breaks (DSBs) exemplify one of the major HDM-RONS-induced harmful effects on nucleic acids in patients with allergic asthma. Being one of the most cytotoxic forms, DSBs can cause genomic rearrangements, instability, and cell death (Panier & Durocher, 2013).

Two main mechanisms have been identified to be involved in the repair of DNA DSBs, namely nonhomologous end-joining and homologous recombination (Hartlerode & Scully, 2009; Scully et al., 2019; Sung & Klein, 2006; Symington, 2002). Non-homologous end-joining, which is defined as a rapid pathway commonly associated with insertions and deletions at DSBs, can join two DNA ends in a DNA sequence-independent manner. However, homologous recombination requires intact DNA, which relies on a high sequence homology between donor and broken DNAs. Thus, enabling the template DNA is a critical step during the repair process. BRCA1 Associated RING Domain 1 (BARD1) is one of pivotal protein during homologous recombination and associated with lung cancer cell sensitivity to cisplatin (Zhang et al., 2021). Depletion of BARD1 renders DNA damage sensitivity, HR deficiency, and genome destabilization. The ablation of BARD1 in mice leads to cancer susceptibility, and probable disease-causing mutations are found in patients with breast cancer (Mccarthy et al., 2003; Shakya et al., 2008).

An increasing body of evidence supports that posttranslational modification of proteins by ubiquitin and ubiquitin-like modifiers plays a critical role in facilitating cellular responses and re-establishing genome integrity following DSBs (Schwertman et al., 2016). The importance of ubiquitin-dependent signaling during cellular biological processes is evidenced by the complexity of the ubiquitin system, which is known to comprise more than 1000 factors (Kim et al., 2011; Wagner et al., 2011). Given their versatility and reversibility, ubiquitination signals are ideal players in the regulation of dynamic and complex cellular processes like DSB repair. While the protein ubiquitination process is catalyzed by ubiquitinases, ubiquitin is usually removed by deubiquitinases (DUBs). Indeed, a recent study showed that ubiquitin-specific peptidase 15 (USP15), a member of the cysteine protease DUBs subfamily, is vital in the regulation of

homologous recombination in human breast cancer and is essential for DSB repair (Peng et al., 2019). Tarsounas and Sung (2020) have demonstrated that MDC1 assists in recruiting USP15 to DSBs, which in turn deubiquitinates BARD1, thereby resulting in persistent retention of BARD1/BRCA1 at DSBs to ensure complete repair of DNA damage.

USP25 is another DUB implicated in cell cycle progression and DNA repair (Valero et al., 1999; Valero et al., 2001). USP25 has been shown to lead to neuroinflammation and induce synaptic and cognitive dysfunction (Zhu et al., 2021) and to negatively regulate signaling triggered by IL-17, which is a mediator of inflammation that plays critical roles in autoimmunity and infection (Zhong et al., 2012). Moreover, disruption of the proteasome system via alterations of USP25 activity has been associated with the processes of invasion and migration in various cancers (Zhu et al., 2021). Interestingly, a recent study found that USP25 promoted the stability of proteins involved in DNA repair in lung epithelial cells of patients with increased susceptibility to microbial infection due to frequent cigarette smoking (Long et al., 2020). Other studies have reported that USP25 inhibits lipopolysaccharide-induced proinflammatory cytokines such as interleukin (IL)-6 and TNF- α (Ding et al., 2017), IL-17 (Zhong et al., 2012), and the Toll-like receptor-nuclear factor kappa B signaling pathway (Zhong et al., 2013). In this present study, we aimed to delineate the role of USP25 in repairing DNA damage in HDM-induced asthma and determine whether such repair is facilitated by USP25-induced deubiquitination, which in turn enhances the stability of BARD1 protein.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Human bronchial epithelial BEAS-2B cells were obtained from American Type Culture Collection, and maintained in RPMI media with 10% fetal bovine serum (Sigma Aldrich), and antibiotics penicillin/streptomycin (Invitrogen). Cells were treated with HDM (200 μ g/ml) for 0, 6, 12, and 24 h at 37°C, 95% humidity, and 5% CO₂.

2.2 | Cell transfection

Small interfering RNAs (siRNAs) targeting human USP25 (siUSP25#1, 5'-GGAGACAACUACUACCAATT-3'; siUSP25#2, 5'-GCAGAUG GAUGAAGUACAATT-3'; siUSP25#3, 5'-GCAGGGUUCUUUAAGG CAATT-3') were constructed by Shanghai GenePharma Co., Ltd. and transfected into BEAS-2B cells using DharmaFECT 1 siRNA transfection reagent (Thermo Fisher Scientific). Nonspecific siRNA (siNC) was used as a negative control. pLKO.1 lentiviral vectors containing small hairpin RNA (shRNA) targeting human BARD1 (shBARD1#1, 5'-GCTAGCCACTGCTCAGTAA-3'; shBARD1#2, 5'-G CTGTTGCCAAAGCTGTTT-3'; shBARD1#3, 5'-CCTTCAAACACCA

TCCAAA-3') were constructed by Sangon Biotech. The human USP25 ectopic expression vector was constructed by placing the open reading frame of USP25 into pLVX-Puro. 293T cells were then transfected with pLKO.1-BARD1-shRNA (shBARD1) or pLVX-Puro-USP25 (USP25) for 4–6 h using Lipofectamine 2000 reagent (Invitrogen). Vectors were harvested and transduced into BEAS-2B cells. The pLKO.1-nonspecific shRNA (shNC) or empty pLVX-Puro vector served as the control.

2.3 | Immunofluorescence staining

BEAS-2B cells were seeded and treated with HDM (200 µg/ml) for 0, 6, 12, and 24 h at 37°C. Plates were then incubated with antibodies against γ-H2AX (ab81299; Abcam), USP25 (sc-398414; Santa Cruz Biotechnology, Inc.), or BARD1 (ab226854; Abcam), followed by DAPI staining. Images were captured with a Zeiss fluorescence microscope (Carl Zeiss) and quantified with Bitplane Imaris 5.7 software (Bitplane AG) as previously described (Chan et al., 2016b).

2.4 | Comet assay

BEAS-2B cells transfected with USP25 siRNA or transduced with the USP25 expression vector and BARD1 shRNA vector were exposed to HDM (200 µg/ml) for 6 h at 37°C. Subsequently, the comet assay was performed as previously described (Chan et al., 2016a).

2.5 | Enzyme-linked immunoassay (ELISA) assays

BEAS-2B cell culture supernatants were harvested. Cytokine levels were determined using commercially available ELISA kits for TNF-α, IL-4, IL-8 (Jiancheng Bioengineering Institute, China), and IL-13 (CUSABIO) according to the manufacturers' instructions. All samples were then assayed in duplicate.

2.6 | Reverse transcription quantitative PCR (RT-qPCR)

Total RNA was extracted using TRIzol reagent (Invitrogen). Reverse transcription was performed using a PrimeScript™ RT reagent kit (Takara). RT-qPCR was performed using SYBR® Premix Ex Taq™ GC (Takara). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the normalization control. The primers used for PCR are as follows: USP25-F: 5'-GCCAAGCAGATACAAATG-3', USP25-R: 5'-T AGGTGTCCTCTTCAAAC-3'; BARD1-F: 5'-TGTTGTTGTTCTCGG TGATG-3', BARD1-R: 5'-TGTCCTTGGATGGTGTGTTG-3'; GAPD H-F: 5'-AATCCCATCACCATTCTC-3', and GAPDH-R: 5'-AGGCTG TTGTCATACTTC-3'. The fold changes were determined using the $2^{-\Delta\Delta CT}$ method.

2.7 | Western blot

Briefly, whole cell lysates were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After transfer to polyvinylidene difluoride (BioRad), we blocked the membranes with 5% fat-free milk for 2 h. This was followed by incubation with the appropriate primary antibody, including anti-USP25 (ab187156; Abcam), anti-BARD1 (ab226854; Abcam), γ-H2AX (ab81299; Abcam), or anti-GAPDH (#5174; CST), at room temperature for 1 h. After washing three times in phosphate-buffered saline, we incubated the membranes with horseradish peroxidase-conjugated second anti-mouse or rabbit antibodies as appropriate (Beyotime). The blots were developed using an enhanced chemiluminescence detection kit (Pierce Biotechnology).

2.8 | Co-immunoprecipitation and ubiquitination assays

For the co-immunoprecipitation assay, cell lysates were suspended in RIPA buffer and incubated with anti-USP25 (sc-398414; Santa Cruz Biotechnology) or anti-BARD1 (ab50984; Abcam), with the normal immunoglobulin G antibody (Cell Signaling) used as a control. Subsequently, the lysates were incubated with Protein A/G PLUS-Agarose beads (sc-2027; Santa Cruz Biotechnology) for 2 h at 4°C. The immunoprecipitate was analyzed by western blot analysis using anti-USP25 (ab187156; Abcam) or anti-BARD1 (ab226854; Abcam) antibody. For the ubiquitination assay, cells with or without USP25 overexpression were subjected to lysis using RIPA lysis buffer. Ubiquitination of BARD1 was detected by immunoprecipitation with the anti-BARD1 (ab50984; Abcam) antibody and then by western blot with an anti-ubiquitin (ab7780; Abcam).

2.9 | Protein stability assay

BEAS-2B cells transfected with the indicated vectors were treated with 100 µg/ml cycloheximide (CHX; Millipore) for the indicated times, and then harvested. We then determined the protein expression of BARD1 by western blot analysis.

2.10 | Adenovirus packaging

Mouse USP25 ectopic expression vectors were constructed by placing ORF sequences of USP25 into the pShuttle-H1 adenovirus vector. Vectors encoding the target genes and packaging plasmids were cotransfected into HEK 293T cells with FuGENE HD transfection reagent (Promega).

2.11 | Animals

Sensitization and HDM treatment of female (approximately 6–8 weeks) BALB/c mice were performed as previously described

(Chan et al., 2016a). We injected the pShuttle-H1-USP25 adenovirus vector (1×10^9 titer) intratracheally at day 13/14 and at day 1/2 post-HDM treatment. Mouse bronchoalveolar lavage fluids were collected and the cytokines levels in the bronchoalveolar lavage fluid were assessed using ELISA. On day 3 after HDM treatment, mouse lung tissues were isolated and hematoxylin and eosin and immunofluorescence staining were performed. Antibody used was targeted at γ -H2AX (ab81299; Abcam). We quantified inflammatory cell infiltration in the lungs as previously described (Bao et al., 2009). All protocols involving animals were approved by

the Institutional Animal Care and Use Committee of the Children's Hospital of Nanjing Medical University.

2.12 | Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0.2 (GraphPad Software). Data were presented as the mean $\pm$ standard deviation. The two-tailed Student's *t* test and analysis of variance were used to analyze the differences between the two groups or

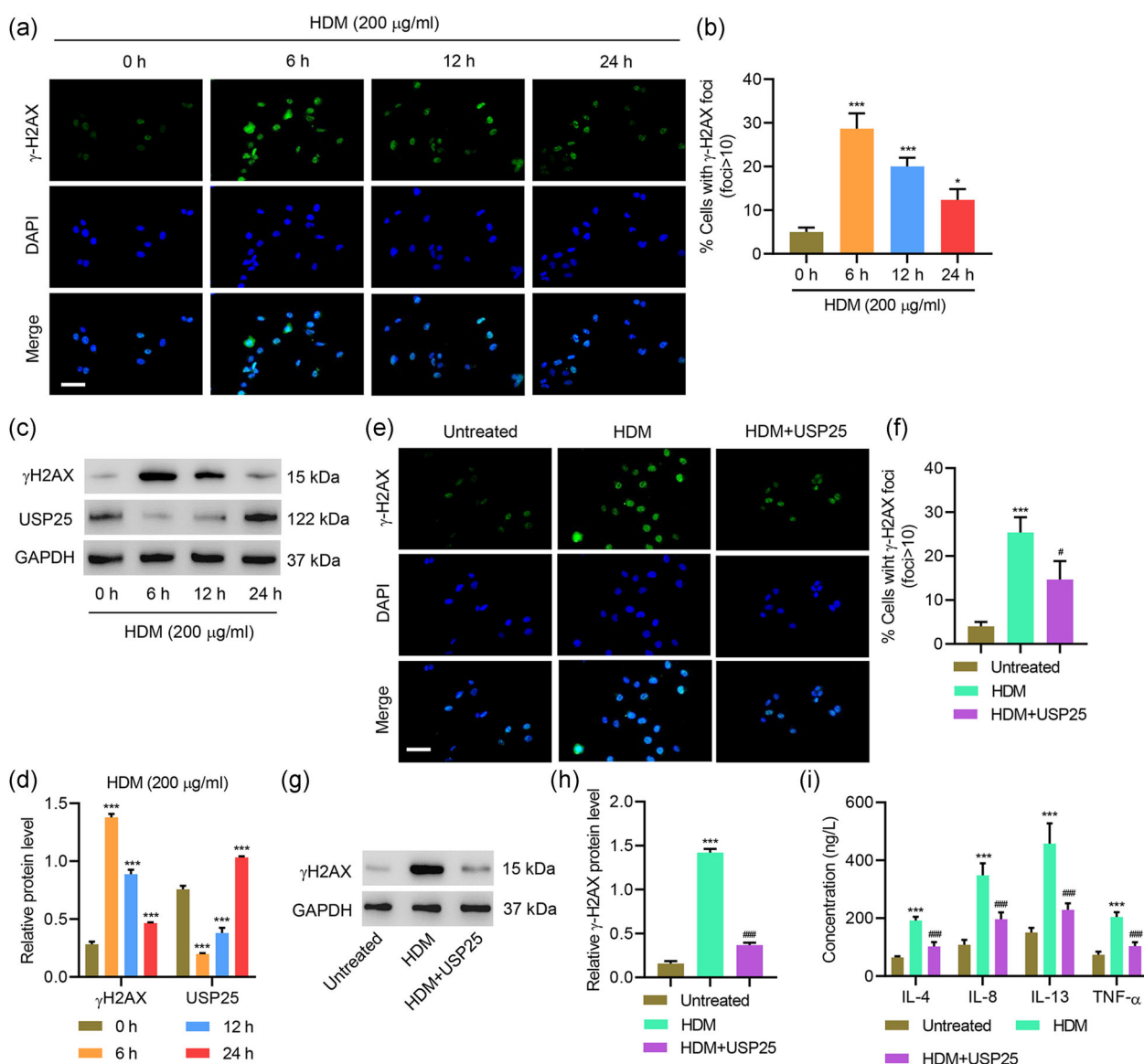


FIGURE 1 USP25 overexpression inhibits HDM-induced DNA damage in BEAS-2B cells. BEAS-2B cells were treated with 200 μ g/ml HDM for the indicated times. (a) Cells were stained for γ -H2AX (green) and the nuclei were stained with DAPI (blue). (b) Quantification of γ -H2AX-positive cells (foci > 10). (c,d) Expression of γ -H2AX and USP25 measured by western blot. BEAS-2B cells transduced with the indicated vectors were treated with 200 μ g/ml HDM for 6 h. (e) Cells were stained for γ -H2AX (green), and the nuclei were stained with DAPI (blue). (f) Quantification of γ -H2AX-positive cells (foci > 10). (g,h) Expression of γ -H2AX was measured by western blot. (i) IL-4, IL-8, IL-13, and TNF- α levels were measured by ELISA. Scale bar: 50 μ m. Data are expressed as the mean $\pm$ SD ($n = 3$). * $p < .05$, *** $p < .001$ compared with the 0 h or untreated group. # $p < .05$, ### $p < .001$ compared with the HDM group. ELISA, enzyme-linked immunoassay; HDM, house dust mites; IL, interleukin; TNF, tumour necrosis factor

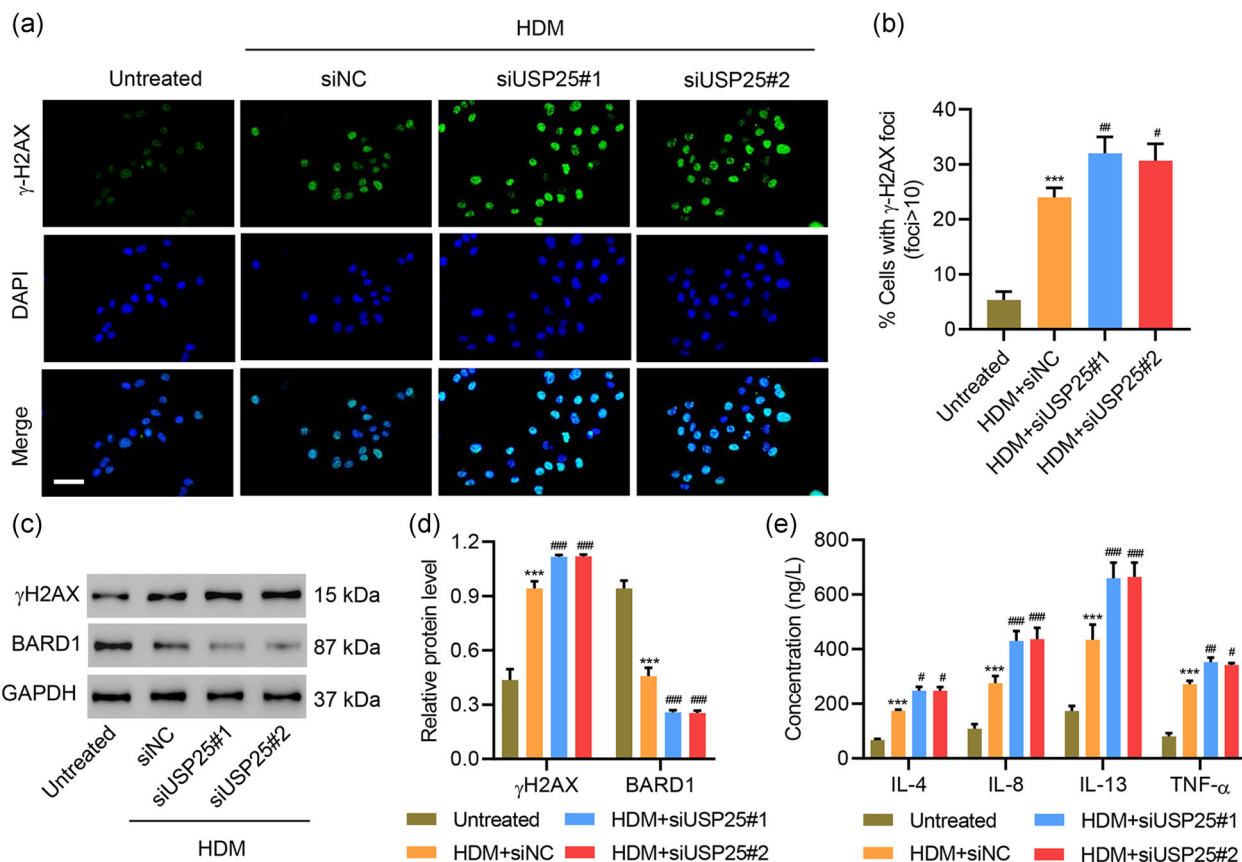


FIGURE 2 USP25 silencing promotes HDM-induced DNA damage in BEAS-2B cells. BEAS-2B cells transfected with the indicated siRNAs were treated with 200 µg/ml HDM for 6 h. (a) Cells were stained for γ-H2AX (green); the nuclei were stained blue. (b) Quantification of γ-H2AX-positive cells (foci > 10). (c,d) γ-H2AX and BARD1 were analyzed via western blot. (e) IL-4, IL-8, IL-13, and TNF-α levels were measured by ELISA. Scale bar: 50 µm. Data are expressed as the mean ± SD (n = 3). ****p* < .001 compared with the siNC group. #*p* < .05, ##*p* < .01, ###*p* < .001 compared with the HDM + siNC group. HDM, house dust mites; IL, interleukin; TNF, tumour necrosis factor

more than two groups, respectively. All *p* values < .05 were considered statistically significant.

3 | RESULTS

3.1 | USP25 represses HDM-induced DNA damage

To determine the inhibitory effect of USP25 on HDM-induced DNA damage, we first treated BEAS-2B cells with HDM (200 µg/ml). As shown in Figure 1a–d, 6 h exposure to HDM can lead to the peak abundance of γ-H2AX foci, which is a highly specific molecular indicator of DNA damage in eukaryotes (Mah et al., 2010). γ-H2AX foci (Figure 1a,b) and expression (Figure 1c,d) gradually abated with increasing HDM treatment duration. The western blot results indicated changes in USP25 protein levels in response to HDM exposure (Figure 1c,d). HDM treatment led to the lowest USP25 level at 6 h, which actually increased with increasing incubation duration (Figure 1c,d). Taken together, these data suggest that HDM-induced DNA damage in BEAS-2B cells can be reversed by corresponding upregulation of USP25 expression.

Next, we tested the effects of USP25 overexpression on DNA damage. BEAS-2B cells were transduced with the USP25 expression vector and USP25 expression was measured by RT-qPCR and western blots (Figure S1a–c), followed by HDM treatment (200 µg/ml) for 6 h. As expected, ectopic expression of USP25 led to the inhibition of DNA damage in BEAS-2B cells treated with HDM, as revealed by the significant decreases in γ-H2AX foci (Figure 1e,f) and expression (Figure 1g,h) as well as the percentage of DNA in the comet tail (Figure S2a,b). These findings further support the inhibitory role of USP25 on DNA damage induced by HDM in BEAS-2 cells. The proinflammatory cytokines IL-4, IL-8, IL-13, and TNF-α are closely associated with chronic airway inflammation, such as asthma (Wang et al., 2018). As shown in Figure 1i, overexpression of USP25 has abolished the HDM-induced increases in IL-4, IL-8, IL-13, and TNF-α levels in BEAS-2B cells. However, USP25 overexpression did not induce DNA damage and inflammation in human lung epithelial cells without HDM treatment (data not shown). Taken together, these results suggest that USP25 can sufficiently suppress HDM-induced DNA damage and inflammation in human lung epithelial cells.

3.2 | Depletion of USP25 triggers DNA damage in human lung epithelial cells

siRNA-mediated gene silencing was used to assess whether USP25 is essential for the inhibition of DNA damage in human lung epithelial cells. BEAS-2B cells were transfected with siRNAs targeting USP25 silencing, USP25 expression was measured by RT-qPCR and western blots (Figure S3a–c), and HDM treatment (200 μ g/ml) was applied for 6 h. We found that depletion of USP25 resulted in significant increases of γ -H2AX foci (Figure 2a,b) and expression (Figure 2c,d) as well as the percentage of DNA in the comet tail (Figure S2a,b) in HDM-treated BEAS-2B cells.

USP15 has been shown to deubiquitinate BARD1 to promote its stability and the repair of DNA lesions in breast cancer cells (Peng et al., 2019). Here we examined whether USP25 possesses similar effects. Indeed, silencing of USP25 has markedly reduced the abundance of BARD1 protein (Figure 2c,d), but it significantly enhanced IL-4, IL-8, IL-13, and TNF- α levels in HDM-treated BEAS-2B cells (Figure 2e). However, USP25 knockdown did not induce DNA

damage or inflammation in human lung epithelial cells without HDM treatment (data not shown). These data support the essential role of USP25 in controlling and repairing DNA damage induced by HDM, suggesting that such effects are facilitated by stabilized BARD1 protein levels in human lung epithelial cells.

3.3 | USP25 deubiquitinates BARD1 to promote stability

First, co-immunoprecipitation and immunofluorescence staining were performed to confirm a direct interaction between USP25 and BARD1 in BEAS-2B cells (Figure 3a,b). As shown in Figure 3c,d, knockdown of USP25 did not alter the messenger RNA (mRNA) levels of BARD1, but it did reduce its protein abundance. We further showed that such inhibitory effects on BARD1 protein levels were reversed by the addition of MG132, which is a potent proteasomal inhibitor (Guo & Peng, 2013). These data suggest that USP25 regulates BARD1 levels in a proteasome-dependent

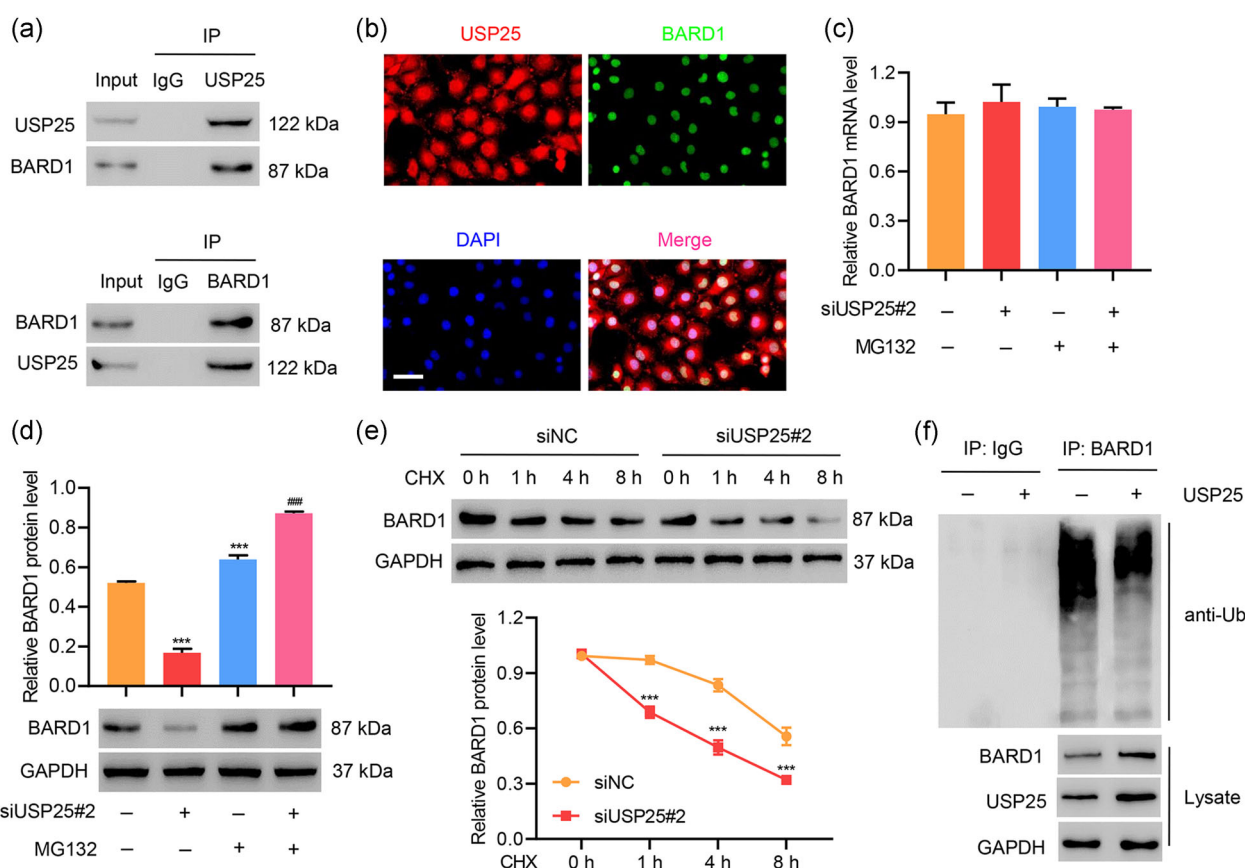


FIGURE 3 USP25 interacts with and deubiquitinates BARD1. (a) Immunoprecipitation was performed with an IgG control, anti-USP25, or anti-BARD1 antibody, followed by incubation with the indicated antibodies. (b) The subcellular localization of USP25 (red) and BARD1 (green) was measured by immunofluorescence staining. Nuclei were stained with DAPI (blue). Scale bar: 50 μ m. (c,d) Expression of BARD1 in BEAS-2B cells transfected with the indicated siRNA in the presence of 10 μ M MG132, measured by RT-qPCR and western blot. (e) BEAS-2B cells transfected with the indicated siRNA were treated with CHX (100 μ g/ml); BARD1 expression was determined via western blot analysis. (f) USP25-overexpressed BEAS-2B cells immunoprecipitated with BARD1 or IgG antibody for evaluating ubiquitination. Data are expressed as the mean $\pm$ SD ($n = 3$). *** $p < .001$ compared with the siNC group or neither siUSP25#2 or MG132 treated group. #### $p < .001$ compared with the siUSP25#2 group. IgG, immunoglobulin G; RT-qPCR, reverse transcription quantitative polymerase chain reaction; siRNA, small interfering RNA

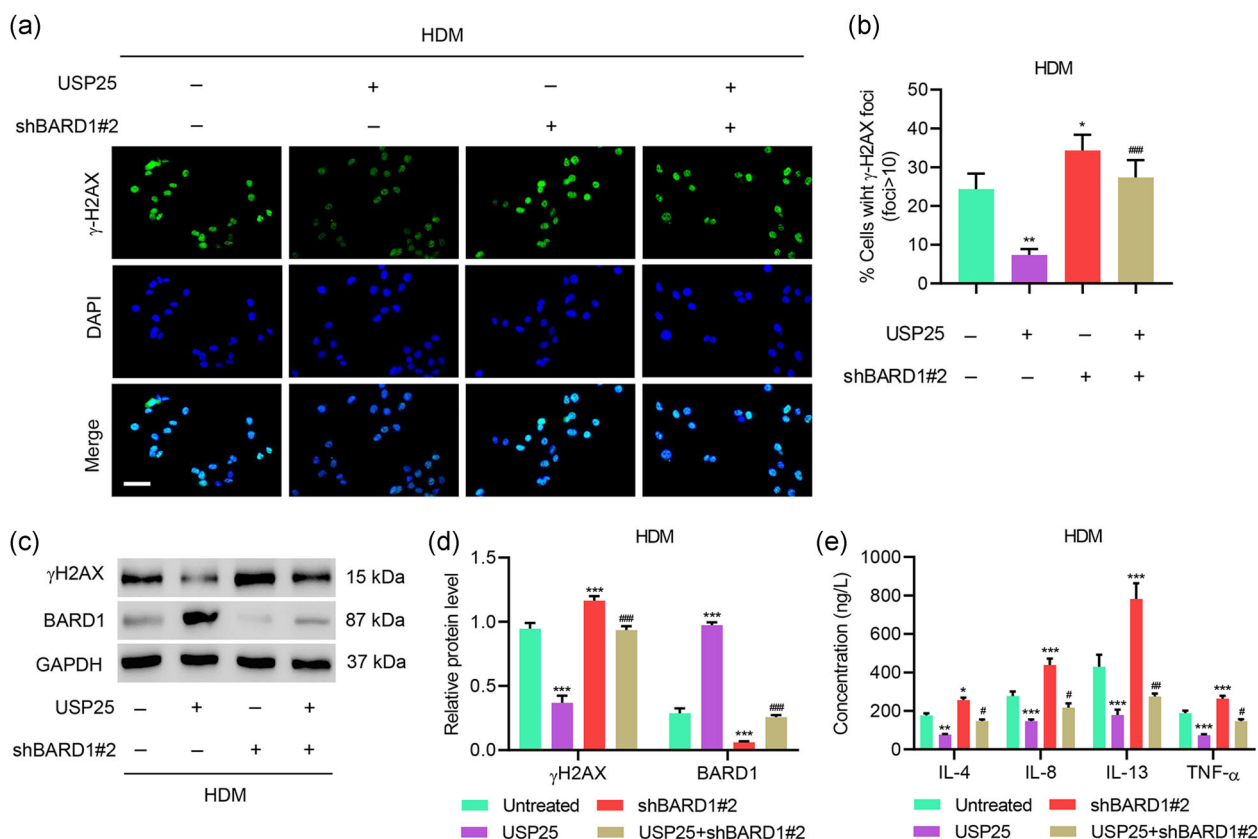


FIGURE 4 USP25 overexpression inhibits HDM-induced DNA damage in BEAS-2B cells by stabilizing BARD1 protein. BEAS-2B cells transduced with the indicated vectors were treated with 200 μg/ml HDM for 6 h. (a) Cells were stained for γ-H2AX (green) and the nuclei were stained with DAPI (blue). (b) Quantification of γ-H2AX-positive cells (foci >10). (c,d) Expression of γ-H2AX and BARD1 was measured by western blot. (e) IL-4, IL-8, IL-13, and TNF-α levels were measured by ELISA. Scale bar: 50 μm. Data are expressed as the mean ± SD (n = 3). **p* < .05, ***p* < .01, ****p* < .001 compared with the untreated group. #*p* < .05, ##*p* < .01, ###*p* < .001 compared with the USP25 group. ELISA, enzyme-linked immunoassay; HDM, house dust mites; IL, interleukin; TNF, tumour necrosis factor

manner. To test whether BARD1 was unstable, we treated BEAS-2B cells with CHX to inhibit protein synthesis (Schneider-Poetsch et al., 2010) and determined the half-life of BARD1. Immunoblotting results showed that BARD1 is a labile protein with a half-life ~8 h in BEAS-2B cells (Figure 3e). Moreover, BARD1 stability was noted to have dramatically decreased in USP25-depleted BEAS-2B cells, further supporting that USP25 stabilizes BARD1 (Figure 3e). Finally, HDM treatment promoted the ubiquitin levels of BARD1 in BEAS-2B cells (Figure S4), whereas overexpression of USP25 notably reduced ubiquitination levels of BARD1 but increased its protein abundance (Figure 3f). Collectively, these data suggest that USP25 enhances the stability of BARD1 by deubiquitinating it in human lung epithelial cells.

3.4 | USP25 suppresses HDM-induced DNA damage by stabilizing BARD1

We first utilized shRNAs to knockdown BARD1 to validate the silencing efficiency in BEAS-2B cells (Figure S3d-f). Subsequently, BEAS-2B cells transduced with the USP25 overexpression vector or

shRNA targeting BARD1 mRNA or a combination of them were treated with 200 μg/ml HDM for 6 h. The results showed that overexpression of USP25 inhibited DNA damage, as indicated by the decreased levels of γ-H2AX foci (Figure 4a,b), expression (Figure 4c,d), and percentage of DNA in the comet tail (Figure S2a,b), coupled with the increased BARD1 protein levels (Figure 4c,d), which were then reversed by repression of BARD1. Furthermore, the inhibitory effects of overexpression of USP25 on IL-4, IL-8, IL-13, and TNF-α were abolished with BARD1 knockdown (Figure 4e). These data suggest that USP25 stabilizes BARD1 protein abundance and, in turn, inhibits HDM-induced DNA damage and inflammatory responses in BEAS-2B cells.

3.5 | USP25 inhibits HDM-induced DNA damage in vivo

Finally, we sought to validate the inhibitory effects of USP25 on DNA damage in an HDM-induced asthmatic animal model. The timeline of HDM-induced airway inflammation in BALB/c mice is illustrated in Figure 5a. The data revealed that HDM treatment

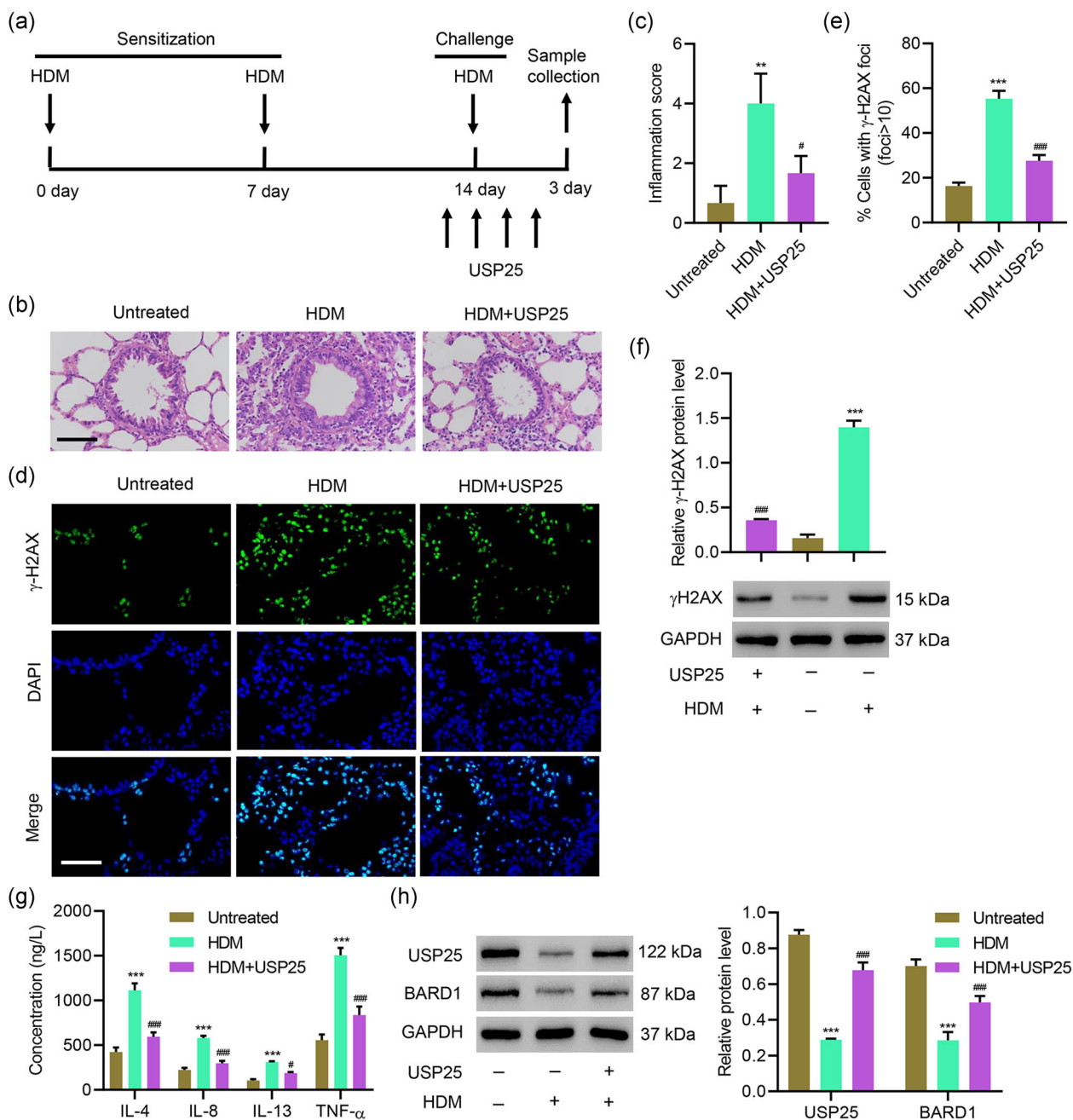


FIGURE 5 USP25 overexpression inhibits DNA damage in the lungs of HDM-induced asthmatic mice. (a) HDM-induced airway inflammation model. (b,c) Images of H&E-stained lungs. (d) Analysis of γ -H2AX (green) and DAPI (blue) in lung sections. (e) Quantification of γ -H2AX-positive cells (foci >10). (f) Expression of γ -H2AX in lung sections was measured by western blot. (g) ELISA was used to quantify IL-4, IL-8, IL-13, and TNF- α levels in bronchoalveolar lavage fluid. (h) Lung tissues were analyzed by immunoblotting to determine the levels of USP25 and BARD1. Scale bar: 50 μ m. Data are expressed as the mean $\pm$ SD ($n = 6$). ** $p < .01$, *** $p < .001$ compared with the untreated group. # $p < .05$, ### $p < .001$ compared with the HDM group. ELISA, enzyme-linked immunoassay; HDM, house dust mites; H&E, hematoxylin and eosin; IL, interleukin; TNF, tumour necrosis factor

caused a significant increase in inflammation score (Figure 5b,c) and DNA damage (Figure 5d-f), as evidenced by increased γ -H2AX foci (Figure 5d,e) and expression in lung tissues (Figure 5f); these effects were markedly reversed with overexpression of USP25 in BALB/c mice. Furthermore, we found that overexpression of USP25 significantly attenuated HDM-elicited production of

proinflammatory cytokines, including IL-4, IL-8, IL-13, and TNF- α , in bronchoalveolar lavage fluid (Figure 5g), but increased USP25 and BARD1 protein abundance in lung tissues (Figure 5h). These data indicate that USP25 can robustly augment BARD1 protein abundance and, in turn, prevent HDM-induced DNA damage and inflammation in vivo.

4 | DISCUSSION

Epidemiological studies have estimated that more than 3,000,000 people globally suffer from asthma, resulting in an enormous socio-economic burden (Asher & Pearce, 2014). As one of the most common aeroallergens, HDM is implicated in nearly 85% of asthmatic cases (Gregory & Lloyd, 2011). To this end, this present study focused on understanding the molecular mechanisms underlying a type of HDM-induced asthma. Given recent research demonstrating a robust relationship between HDM exposure and DNA damage in human lung tissues (Chan et al., 2016a), our study has provided substantial *in vitro* and *in vivo* evidence supporting a novel mechanism regulating DNA damage and repair in HDM-induced asthma.

DUBs are regarded as important contributors in maintaining genome stability in human diseases, with many DUBs known to actively associate with regulation of homologous recombination (Agathangelou et al., 2017; Peng et al., 2019; Uckelmann et al., 2018). Interestingly, a recent study showed that catalytically inactive USP15 was compromised in its capacity to bind to BARD1, resulting in failed DNA repair function for the homologous recombination process (Peng et al., 2019). Our study supports the critical role of USP25 in preventing interruption in the processes of DNA damage repair and replication *in vitro* and *in vivo* (Figures 1, 2, and 5). Previous studies have suggested that BARD1 promotes the G2/M cell cycle checkpoint in response to DNA damage (Dai et al., 2019; Zhang et al., 2020). Thus, further studies are needed to explore the cell cycle checkpoint potentially involved in HDM-induced DNA damage.

The profound inhibition of proinflammatory cytokine expression with overexpressed USP25 in human lung epithelial cells highlights the importance of cross-talk between DNA damage and inflammation in the pathogenesis of HDM-induced asthma (Figures 1, 2, and 4). Inflammation can cause severe DNA damage. RONS are generated when inflammation sufficiently passes through the extracellular matrix and enters the nucleus (Ho et al., 2012). Inflammatory cells, such as macrophages and neutrophils, may use cytokines (e.g., TNF- α) to stimulate oxide formation in neighboring epithelial cells. These findings suggest that the recruitment of immune cells when USP25 is overexpressed in HDM-induced asthmatic mice should be further considered. Notably, long-lived nitric oxide and ROS actively repress the activity of various enzymes involved in DNA repair. Combined, these mechanisms could severely compromise repair of DNA damage and enhance mutation, thus leading to genomic instability (Kawanishi et al., 2017). Conversely, the response to DNA damage may cause an inflammatory microenvironment induced by high mobility group box 1, which is typically characterized by hypoxia (Kang et al., 2013). As the first defensive barrier, lung epithelial cells may contribute to increased inflammation caused by pulmonary diseases, which can often result in decreased expression of USP25, suggesting negative regulation between USP25 and lung epithelial cell inflammation (Long et al., 2020). Although it is possible that there are additional mechanism(s), this study supports that USP25 overexpression-mediated

quenching of inflammation responses is dependent on its capacity to promote repair of DNA damage in HDM-induced asthma.

Our study has shown that USP25-mediated inhibition of DNA damage resulting from HDM exposure is principally dependent on its ability of deubiquitinating BARD1, which subsequently protects it from proteasomal degradation in human lung epithelial cells (Figure 3). However, whether these interactions are stable or transient at the molecular level and stoichiometry of the interaction warrant further research. BARD1 may play an important role in repairing DNA damage in the lung tissues of asthmatic patients due to HDM exposure (Figure 4). *BRCA1* and *BARD1* are tumor suppressor genes and key players in DNA repair and replication in breast and ovarian cancers (Ghimenti et al., 2002). At the molecular level, the *BRCA1/BARD1* partners constitute part of a homologous recombination mediator-protein complex along with two other tumor genes, namely *BRCA2* and *PALB2* (the partner and localizer of *BRCA2*). Such a protein complex efficiently facilitates DNA end resection and repair of DSBs and DNA replication, essentially due to the recruitment and retention of *BRCA1/BARD1* to DNA replication forks (Tarsounas & Sung, 2020). Since *BRCA1* has been largely associated with breast and ovarian cancers, future studies should characterize novel partners to *BARD1* in lung tissues from patients with HDM-triggered asthma.

In summary, this study has described a mechanism accounting for the regulation of HDM-induced DNA damage and inflammation in asthmatic patients. Our results suggest that selectively modulating this pathway, which leads to DNA lesions and repair in human lung epithelial cells, could be a novel therapeutic approach for controlling and managing HDM-induced asthma. Future studies should test and validate the therapeutic effects of this approach *in vitro* and *in vivo*.

ACKNOWLEDGMENT

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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