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P53 supports endothelial barrier function via APE1/Ref1 suppression

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ABSTRACT

The tumor suppressor protein P53 is strongly involved in orchestrating cellular defenses in the diverse variety of human tissues. Anomalies to lung endothelium permeability are streaming severe consequences towards human health, often associated with fatal outcomes. Ongoing investigations suggest that P53 exerts a prominent strategic role in crucial signaling cascades, in charge of both the maintenance and defense of pulmonary endothelium against toxic intruders. The current study employs human and bovine lung microvascular cells, as well as pharmacologic and genetic P53 modulators to demonstrate the negative regulation of APE1/Ref1 by P53. Moreover, it includes real time measurements of endothelial permeability, to reveal the disruptive role of APE1/Ref1 towards endothelial integrity. Those findings supports our efforts to elucidate the highly sophisticated regulatory network that enact endothelial adaptations under the plethora of challenging environmental factors.

1. Introduction

The endothelial cells form a semipermeable barrier, which regulates the blood fluid and proteins migration through the vascular wall. This dynamic structure is subjected to a constant remodeling, in response to the plethora of extra- and intra- cellular stimuli (Barabutis et al., 2016). The function of this vascular wall is affected in a plethora of pathophysiological conditions, including inflammation (Breton-Romero and Lamas, 2014), oxidative (Han et al., 2016), and endoplasmic reticulum stress (Luchetti et al., 2017; Alam et al., 2017), sepsis (Barabutis et al., 2017), and cancer (Jung et al., 2017; Li et al., 2019). Indeed, a severe anomaly of the endothelial barrier may lead to serious respiratory abnormalities, such as Acute Lung Injury and Acute Respiratory Distress Syndrome. The latter cardiovascular conditions is the consequence of impaired endothelium function, which in turn results to anomalous transportation of essential respiratory elements across the endothelial and epithelial barriers of the lung (Herrero et al., 2018).

Microvascular permeability is increased by various inflammatory and carcinogenetic stimuli, such as LPS, growth factors (i.e. Growth Hormone Releasing Hormone), cytokines, and reactive oxygen and nitrogen species (Fischer and Braga, 2018; Barabutis et al., 2018a). These stimuli "trigger' signaling events that abuse the normal function of barrier integrity, by modulating the properties of junction and adhesion proteins, and inducing cytoskeleton remodeling (Zhang et al., 2018; Nakamura and Murata, 2018). Multifarious cellular messengers modulate molecular cascades that reform cytoskeletal integrity and contractility, causing fluctuations of the endothelial barrier function (Simmons et al., 2019; Yurdagul et al., 2016). Hence, the discovery of the molecular components that regulate the activities of the endothelial barrier function, may lead to new therapeutic strategies against ALI/ARDS.

Our recent efforts on the elucidation of the molecular machinery that operates towards the maintenance of the endothelial barrier, revealed that the tumor suppressor P53 (Case and Domann, 2014) is a key player on the endothelium defense against LPS (Barabutis et al., 2015). The guardian of the genome prevented the MLC2–triggered formation of actin stress fibers (Barabutis et al., 2015, 2018b), and sabotaged the actin severing activity of cofilin, via the activation of the Rac1 (Barabutis et al., 2017, 2018b). The latter Rho-GTPase protects the vasculature by preserving cortical actin, which in turn fully integrates junctional elements into the barrier (Barabutis et al., 2016, 2015). Hsp90 inhibitors, which exert strong anti-inflammatory activities, suppressed the LPS-induced P53 phosphorylation(s). The latter modification has been shown to lead to P53 degradation (Xia et al., 2009;

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Tergaonkar, 2009). Moreover, those anti-inflammatory compounds prevented the intracellular P53 loss, by increasing the abundance of the Hsp90/P53 complexes in the intracellular niche (Barabutis et al., 2015). Indeed, 17–DMAG, a potent hsp90 inhibitor, opposes the LPS-inflicted vascular leak in mouse lungs, by reducing the IL-2 and IL-10 BALF levels (Barabutis et al., 2019).

Apurinic/apyrimidinic endonuclease 1 (APE1) is a multitalented transcription factor, associated with a diverse variety of intracellular activities. APE1 is in charge with DNA repair due to oxidative base damage, and exerts a prominent role towards the redox regulation of transcription factors responsible for driving cell survival pathways. The same protein is also known as redox effector factor 1 (Ref-1) (Kelley et al., 2012). Dysregulation of apurinic/apyrimidinic endonuclease 1/redox effector factor 1 (APE1/Ref1) is linked to various human pathologies, such as inflammation, cancer, cardiovascular diseases and neurodegeneration (Thakur et al., 2015). The importance of that molecule as a potential therapeutic target, is underlined by the fact that intense efforts are oriented towards the development of different classes of APE1/Ref1 inhibitors that directly affect its nuclease, redox and nucloeoplosmin activity (Laev et al., 2017). Remarkable, APE1/Ref1 negatively affects the activation of the Rac1 pathway (Simmons et al., 2019), which has been previously shown by our group to be induced by P53(18). In light of those associations, we decided to proceed with the present study.

The Hsp90 inhibitors 17-AAG, 17-DMAG, AUY-922, were employed to induce P53. They represent three different generations on the development of those compounds. 17-AAG belongs to the earlier generation of those therapeutic agents, and AUY-922 is the most advanced representative. Bovine Pulmonary Aortic Endothelial Cells (BPAEC) and Human Lung Microvascular Cells (HULEC-5a) were exposed to those compounds. Our results indicate that all those inhibitors induced P53 and suppressed APE1/Ref1 in both cell lines. Furthermore, the induction of P53 by both Nutlin and Tunicamycin (P53 inducers) resulted to similar effects. On the other hand, reduction of P53 by siRNA or Pifithrin, exerted the opposite effects, namely the increase of APE1/Ref1 expression. To directly associate APE1/Ref1 function with endothelial permeability, we silenced APE1/Ref1 expression in the cells with siRNA, and we measured their barrier function under AUY-922 exposure via transendothelial resistance means. Our results indicated that APE1/Ref1 weakens the endothelial barrier function, and in its absence due to siRNA transfection, the effects of Hsp90 inhibition/P53 induction towards the vascular barrier enhancement were much stronger.

The purpose of the current project is to investigate the hypothesis that P53 exerts its beneficial effects in the vasculature by suppressing APE1/Ref1. Our findings, in support of our hypothesis, enrich our understanding on the complex regulatory network that dictates endothelial function, and expands our knowledge on the molecular mechanisms that support the role of the “Endothelial Defender” (Uddin and Barabutis, 2019) in the vasculature. Indeed, they enhance our armamentarium against severe respiratory disorders such as ALI/ARDS, by providing novel insights on the development of advanced and targeted therapeutic approaches against endothelial dysfunction.

2. Materials and methods

2.1. Reagents

The Hsp90 inhibitors 17-AAG (cat. no. AAJ66960-EX3), 17-DMAG (cat. no. 102513-662), AUY-922 (cat. no. 101756-820), Nutlin (cat. no. 101761-034), Pifithrin (cat. no. 10187-524), Tunicamycin (cat. no. 89156-900), RIPA buffer (cat. no. AAJ63306-AP), anti-mouse (cat. no. 95017-554) and anti-rabbit IgG HRP linked antibodies (cat. no. 95017-556), as well as the nitrocellulose membranes (cat. no. 10063-173) were obtained from VWR (Radnor, PA). The P53 (cat. no. 9282S) and APE1/Ref1 (cat. no. 4128S) antibodies were obtained from Cell Signaling Technology (Danvers, MA). The β-actin antibody (cat. no. A5441) was purchased from Sigma-Aldrich (St Louis, MO).

2.2. Cell culture

In-house harvested bovine pulmonary arterial endothelial cells (BPAEC) were subcultured from primary cultures and used at an early passage. Those cells were maintained in DMEM medium supplemented with 10% fetal bovine serum. Human Lung Microvascular Endothelium cells HULEC-5a (CRL-3244) were purchased from ATCC (Manassas, VA) and maintained in PromoCell Endothelial Cell Growth Medium MV. All cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 - 95% air and medium supplemented with 1X penicillin/streptomycin. All reagents were purchased from VWR (Radnor, PA).

2.3. Protein isolation, Western Blot Analysis and transfections

Proteins were isolated from cells or tissues using RIPA buffer. Protein-matched samples were separated by electrophoresis through 12% sodium dodecyl sulfate (SDS–PAGE) Tris-HCl gels. Wet transfer was used to transfer the proteins onto nitrocellulose membranes. The membranes were incubated for 1 h at room temperature in 5% non-fat dry milk in Tris-buffered saline (TBS) – 0.1% (v/v) Tween 20. The blots were then incubated at 4 °C overnight with the appropriate primary antibody (1:1000). The signal for the immunoreactive proteins was developed by using the corresponding secondary antibody (1:2000) and was visualized in a ChemiDoc™Touch Imaging System from Bio-Rad (Hercules, CA). β-Actin antibody (1:5000) was used as a loading control. All reagents were purchased from VWR (Radnor, PA). The transfections were conducted as previously described (Barabutis et al., 2015; Barabutis and Schally, 2008a)

2.4. Measurement of endothelial barrier function

The barrier function of endothelial cell monolayers was estimated by electric cell-substrate impedance sensing (ECIS), utilizing ECIS model ZΘ (Applied Biophysics, Troy, NY, USA). All the experiments were conducted on confluent cells which had reached a steady-state resistance of at least 800 Ω (Barabutis et al., 2015).

2.5. Densitometry and statistical analysis

Image J software (National Institute of Health) was used to perform densitometry of immunoblots. All data are expressed as mean values ± SEM (standard error of mean). A value of $P < 0.05$ was considered significant. GraphPad Prism 5.01 from GraphPad (CA, USA) was used for data analysis. The letter n represents the number of experimental repeats.

3. Results

3.1. 17-DMAG induces P53 and suppresses APE1/Ref1 expression in BPAEC

BPAEC were treated with either vehicle (DMSO) or 1 μM 17-DMAG for 8, 16 and 48 h. This Hsp90 inhibitor significantly induced p53, and suppressed APE1/Ref1 expression after 16 and 48 h of treatment. Treatment of BPAECs for a shorter period, did not significantly affected both proteins. The results appear in Fig. 1A.

3.2. 17-AAG induces P53 and suppresses APE1/Ref1 expression in BPAEC

The endothelial cells were treated with the Hsp90 inhibitor 17-AAG or vehicle (DMSO) for 8, 16 and 48 h. 17-AAG elevated P53 expression levels in all treatments. The most prominent effect occurred after only 48 h of incubation. On the other hand, APE1/Ref1 levels were lessened

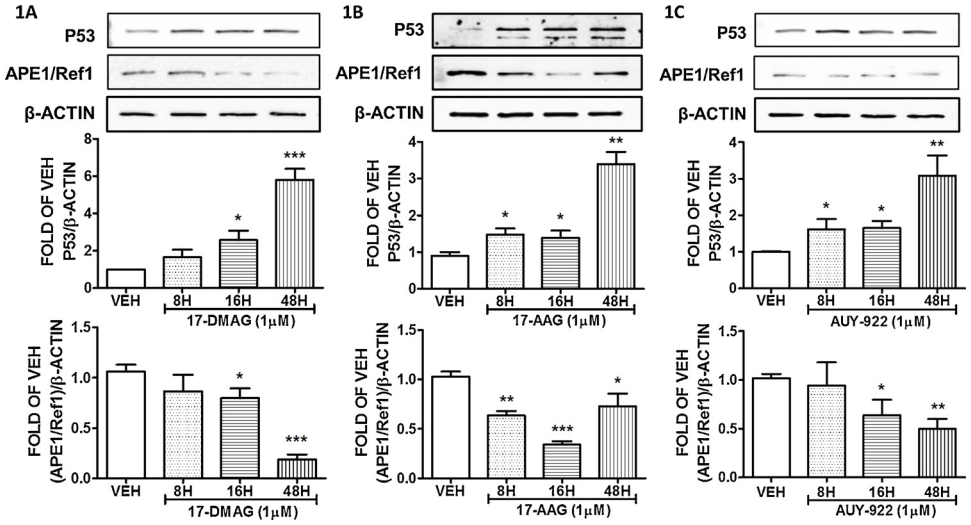

Fig. 1. Hsp90 inhibition induces P53 and suppresses APE1/Ref1 expression in BPAEC. Western Blot analysis of P53, APE1/Ref1 and β actin after treatment of BPAEC with vehicle (DMSO), 17-DMAG (1 μM) **(A),** 17-AAG (1 μM) **(B)**, and AUY-922 (1 μM) **(C)** for 8, 16 and 48 h. The blots shown are representative of 3 independent experiments. The signal intensity of the P53 and APE1/Ref1 bands were analyzed by densitometry. Protein levels were normalized to β actin. *P < 0.05, **P < 0.01, ***P < 0.001 vs vehicle. Means ± SEM.

due to that treatment in all time points (8, 16 and 48 h). The results are shown in Fig. 1B.

3.3. AUY-922 induces P53 and suppresses APE1/Ref1 expression in BPAEC

BPAEC were treated with either vehicle (DMSO) or 1 μM of AUY-922 for 8, 16 and 48 h. The results indicate that this Hsp90 inhibitor significantly induced p53 expression levels after 16 and 48 h of treatment. Indeed, APE1/Ref1 expression was reduced after 16 and 48 h of AUY-922 exposure (Fig. 1C).

3.4. 17-DMAG induces P53 and suppresses APE1/Ref1 in HULEC-5a

The endothelial cells were subjected to treatment with either vehicle (DMSO), or 1 μM of the Hsp90 inhibitor 17-DMAG for 8, 16 and 48 h. Our observations (Fig. 2A) reveal that this compound reduced the expression of APE1/Ref1 in a time dependent manner, while it induced P53 abundance.

3.5. 17-AAG induces P53 and suppresses APE1/Ref1 in HULEC-5a

Human Lung Microvascular Endothelial Cells were treated with vehicle (DMSO) or 17-AAG for 8, 16 and 48 h. This Hsp90 inhibitor significantly induced P53, and suppressed APE1/Ref1 expression levels in all treatments. The results are shown in Fig. 2B.

3.6. AUY-922 induces P53 and suppresses APE1/Ref1 in HULEC-5a

Commercially available human lung microvascular endothelial cells were exposed to vehicle (DMSO) or AUY-922 (1 μM) for 8, 16 and 48 h. Those treatments significantly upregulated P53 expression, while suppressed APE1/Ref1 levels. The results are shown in Fig. 2C.

3.7. Suppression of P53 by Pifithrin induces APE1/Ref1 in both BPAEC and HuLEC-5a

Bovine Pulmonary Aortic Endothelial Cells and Human Lung Microvascular Endothelial Cells were treated with vehicle (DMSO), or Pifithrin (20 μM) to reduce the P53 expression levels. The results depicted in Fig. 3A and B shows the significant efficiency of this inhibitor towards the P53 reduction. Fig. 3A demonstrates the elevation of APE1/Ref1 expression due to P53 suppression in bovine cells, and similar effects are shown in the case of the human cells (Fig. 3B).

3.8. Silencing of P53 by siRNA induces APE1/Ref1 in HULEC-5

Human Lung Endothelial Cells were exposed to either irrelevant siRNA (siCTR) or siRNA that specifically targets P53 (siP53) for 48 and 72 h. The results shown in Fig. 3C, are in line with those observations

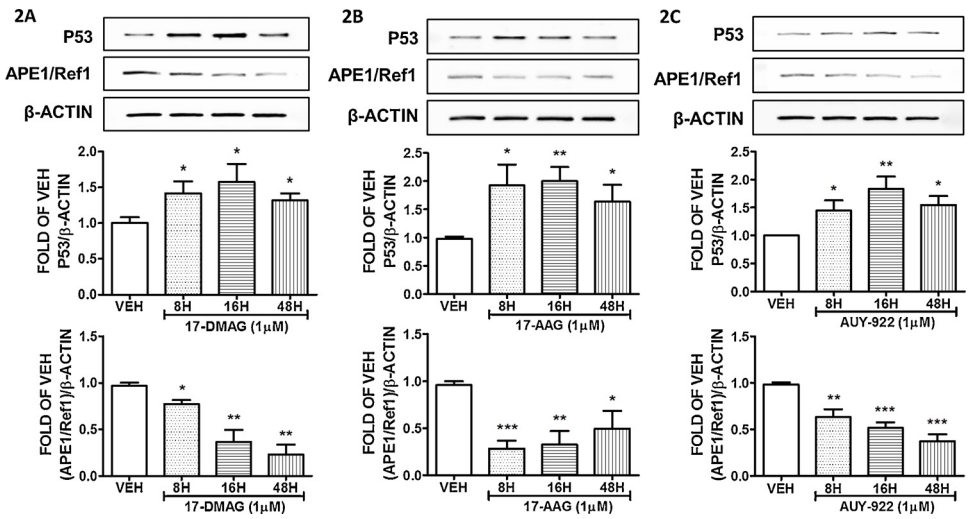

Fig. 2. Hsp90 inhibition induces P53 and suppresses APE1/Ref1 expression in HuLEC-5a.
Western Blot analysis of P53, APE1/Ref1 and β actin after treatment of HULEC-5a with vehicle (DMSO), **(A)** 17-DMAG (1 μM), **(B)** 17-AAG (1 μM) and **(C)** AUY-922(1 μM) for 8, 16 and 48 h. The blots shown are representative of 3 independent experiments. The signal intensity of the P53 and APE1/Ref1 bands were analyzed by densitometry. Protein levels were normalized to β actin. *P < 0.05, **P < 0.01, ***P < 0.001 vs vehicle. Means ± SEM.

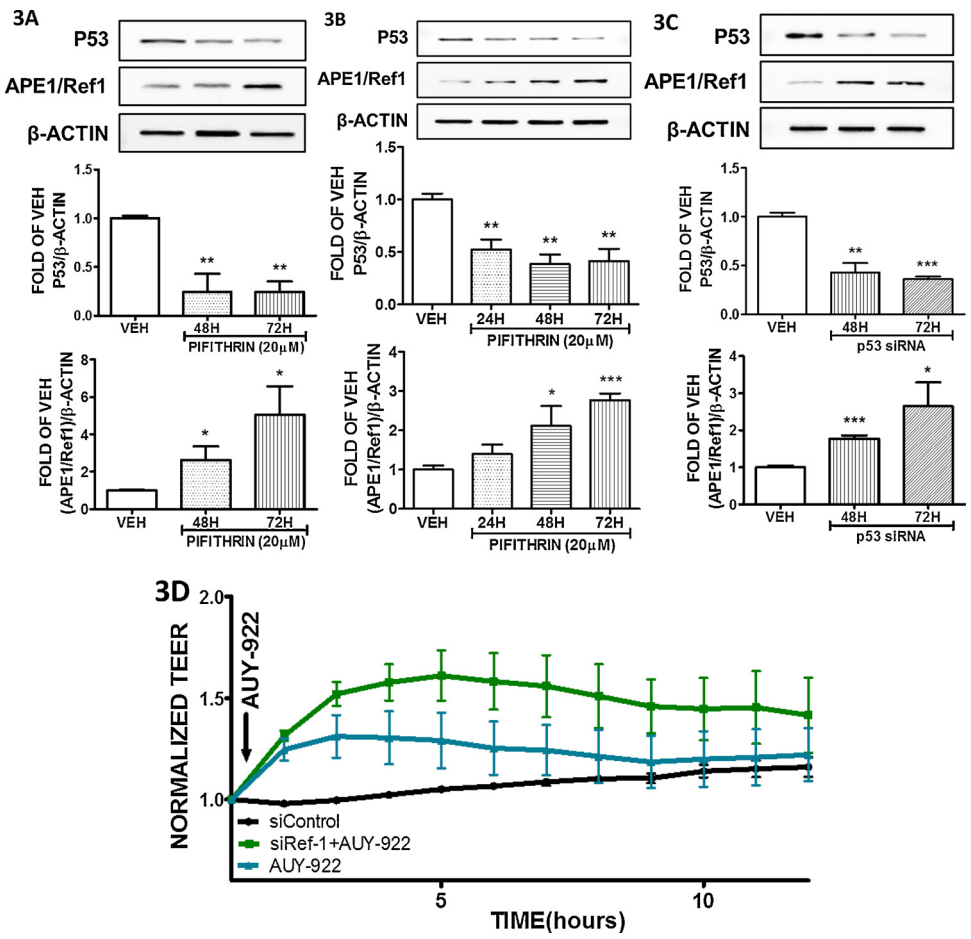

Fig. 3. Effects of APE1/Ref1 modulation on endothelial permeability.
(A) Western blot analysis of P53, APE1/Ref1 and β actin levels in BPAEC treated with vehicle (DMSO) or Pifithrin (20 μM) for 48 and 72 h. **(B)** Western blot analysis of P53, APE1/Ref1 and β actin levels in HULEC-5a treated with vehicle (DMSO) or Pifithrin (20 μM) for 24, 48 and 72 h. **(C)** Western blot analysis of P53, APE1/Ref1 and β actin levels in HULEC-5a treated with si-CTR or si-P53 for 48 and 72 h. The blots shown are representative of 3 independent experiments. The signal intensity of the P53 and APE1/Ref1 bands were analyzed by densitometry. Protein levels were normalized to β actin. *P < 0.05, **P < 0.01, ***P < 0.001 vs vehicle. Means ± SEM. **(D)** AUY-922 was added to the media of the si-APE1/Ref1 transfected HULEC-5 cells. A gradual decrease in endothelial permeability (increased TEER) was observed in all the AUY-922 - treated cells. However, this Hsp90 inhibitor exerted a stronger effect in the cells exposed to si-APE1/Ref1 than those treated with siCTR. n = 3 per group. Means ± S.E.

shown in Fig. 3A and B, and support our hypothesis that APE1/Ref1 is negatively regulated by P53.

3.9. Suppression of APE1/Ref1 by siRNA potentiates the barrier enhancement effect of AUY-922 in HULEC-5a

HULEC-5a cells were transfected with either irrelevant siRNA or siRNA for APE1/Ref1 and consequently transfected with AUY-922 inhibitor. The results shown in Fig. 3D suggest that the suppression of APE1/Ref1 enhanced the beneficial effects of AUY-922 towards the enhancement of the vascular barrier structure.

3.10. Induction of P53 suppresses APE1/Ref1 in BPAECs and HuLEC-5a

Bovine Pulmonary Aortic Endothelium cells were exposed to 10 μM of Nutlin, to induce p53 expression. The treatment was efficient, since P53 levels were elevated after 48 and 72 h of treatment. That P53 upregulation resulted to APE1/Ref1 suppression (Fig. 4A). A similar effect was exerted in HULEC-5a cells, as it appears on Fig. 4B. When those cells were treated with 10 μM of Nutlin for 24, 48 and 72 h, elevated their P53 levels and reduced APE1/Ref1 expression. When BPAECs (Fig. 4C) and HULEC-5a (Fig. 4D) were treated with the unfolded protein response element inductor Tunicamycin, they induced their P53 levels and suppressed their APE1/Ref1 levels; in a similar manner to Nutlin and Hsp90 inhibitors.

4. Discussion

The “Endothelium Defender” P53, has long been associated with anti-cancer activities (Uddin and Barabutis, 2019). When it was first discovered, the investigators concluded that it was a cancer promoter, since they had isolated and focused their studies to its mutated form. However, it was soon became an undisputable fact that the “Guardian of the genome” was counteracting malignancies (Levine, 2018). Those P53 activities occur in conjunction with anti-inflammatory responses, since cancer and inflammation are highly interrelated processes. Sites of chronic inflammation exert the highest potential to develop cancers (Barabutis et al., 2018a).

Hsp90 inhibitors block the maturation of proteins strongly involved in both metastatic and inflammatory phenomena. These Hsp90 clients are elements crucial for cell survival, proliferation and adaption to diverse stimuli (Zuehlke et al., 2018). However, when those carefully orchestrated cellular processes are compromised due to severe inflammatory and carcinogenetic stimuli, they convert to “Trojan horses” of severe pathological manifestations. In those instances, Hsp90 inhibition blocks the progression of those anomalies, and virtually assist the cells to counteract those challenges and recover to the prior non-pathological state (Echeverria et al., 2019).

Hsp90 inhibitors have been shown to protect the endothelium against inflammatory insults, by employing a variety of mechanisms. Our laboratory has shown that those effects are exerted by suppressing the src-mediated tyrosine phosphorylation of Hsp90 (Barabutis et al., 2013), sabotaging the LPS-induced P53 phosphorylation (Barabutis et al., 2019), and corrupting the MLCII activation (Barabutis et al., 2015), which in turn forms the F actin fibers. Moreover, Hsp90 inhibitors induced P53 to deactivate the actin - severing activity of cofilin (Barabutis et al., 2018b). Indeed, Hsp90 inhibition induced the abundance of the Hsp90/P53 complexes in the intracellular niche, preventing the proteasomal degradation and elimination of P53 by LPS (Barabutis et al., 2015). Both in vivo and in vitro studies in advanced models of ALI, indicate that Hsp90 inhibitors are holding a great potential to fight lung inflammation (Barabutis et al., 2016).

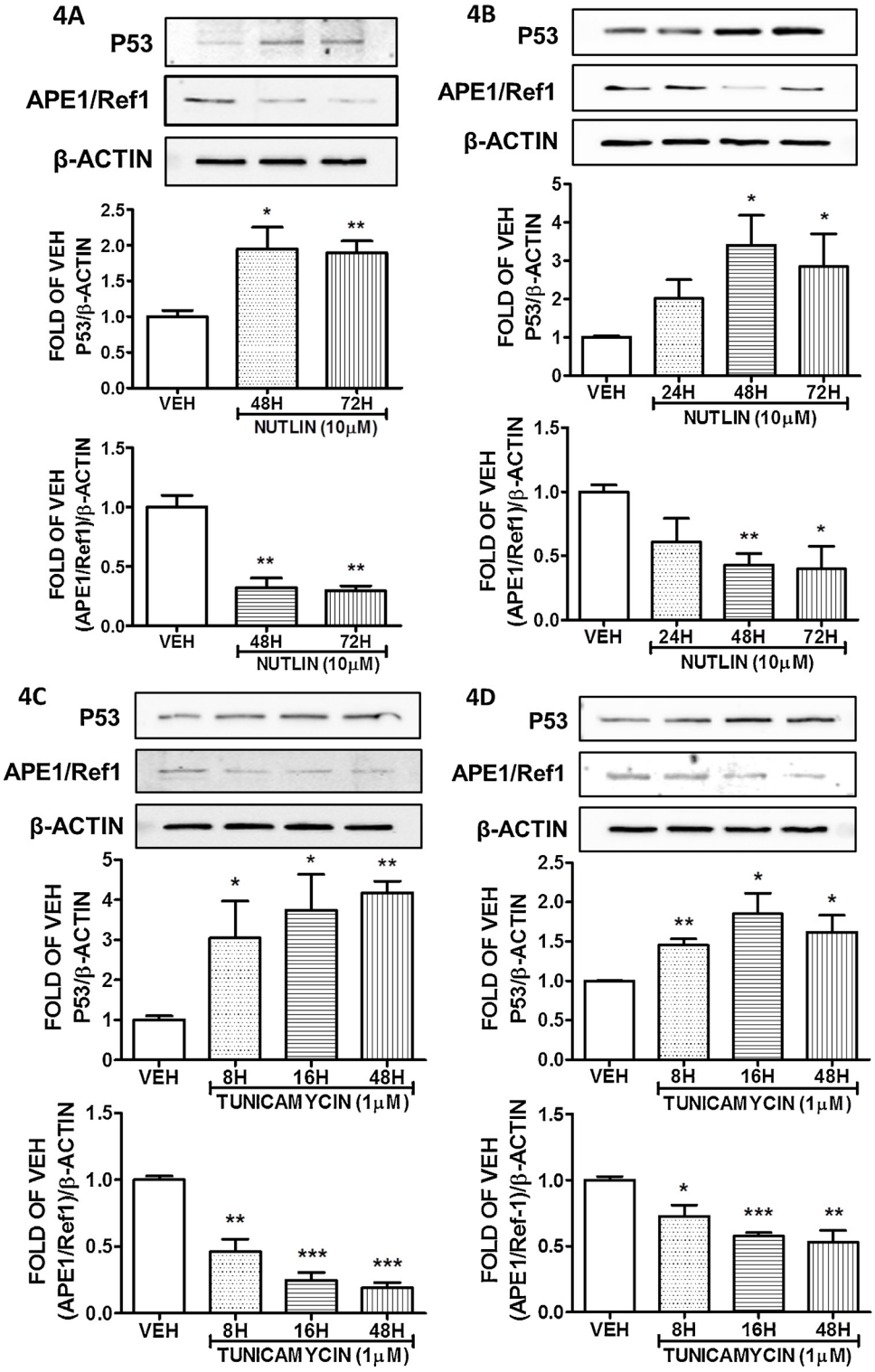

Fig. 4. Induction of P53 suppresses APE1/Ref1 in lung endothelium cells.
(A) Western Blot analysis of P53, APE1/Ref1 and β actin after treatment of BPAEC with vehicle (DMSO) or Nutlin (10 μM) for 48 and 72 h. **(B)** Western Blot analysis of P53, APE1/Ref1 and β actin after treatment of HULEC-5a with vehicle (DMSO) or Nutlin (10 μM) for 24, 48 and 72 h. **(C)** Western Blot analysis of P53, APE1/Ref1 and β actin after treatment of BPAEC with vehicle (DMSO) or Tunicamycin (1 μM) for 8, 16 and 48 h. **(D)** Western Blot analysis of P53, APE1/Ref1 and β actin after treatment of HULEC-5a with vehicle (DMSO) or Tunicamycin (1 μM) for 8, 16 and 48 h. The blots shown are representative of 3 independent experiments. The signal intensity of the P53 and APE1/Ref1 bands were analyzed by densitometry. Protein levels were normalized to β actin. *P < 0.05, **P < 0.01, ***P < 0.001 vs vehicle. Means ± SEM.

P53 suppress both cancer and inflammation, by eliminating Reactive Oxygen and Nitrogen species. In response to low levels of oxidative stresses, p53 plays primarily antioxidant roles (Safdar et al., 2016). P53 affected proteins, such as sestrin, glutathione peroxidase (GPX), and aldehyde dehydrogenase (ALDH), are involved in reducing oxidative stresses (Gambino et al., 2013). In particular, sestrin protects the cells from hydrogen peroxide–induced damage by generation of peroxiredoxins. GPX scavenges hydrogen peroxide or organic hydroperoxides. ALDH has also shown to be a contributor in the antioxidant function of p53 (Liu and Xu, 2011). Moreover, P53 has been associated with the anti-inflammatory activities of the anti-cancer compounds Growth Hormone Releasing Hormone antagonists (Barabutis and Schally, 2008b; Barabutis et al., 2011; Barabutis and Schally, 2010).

Hsp90 inhibitors have been shown to exert similar anti-oxidant effects in a diverse variety of tissues. HSP90 inhibition by 17-DMAG reduced oxidative stress in experimental atherosclerosis. Treatment of ApoE(-/-) mice with 17-DMAG reduced the generation of ROS in the aortic plaques, as well as the activation of the extracellular signal-regulated kinase (ERK). Vascular smooth muscle cells treated with that compound exerted increased levels of HSP27 and HSP70 (marker of

Hsp90 inhibition) and inhibited ERK activation. A significant reduction in NADPH oxidase dependent ROS production was observed when Hsp90 was silenced by transfection of specific siRNA. On the other hand, the suppression of the HSP70 exerted the opposite effects (Madrigal-Matute et al., 2012). However, in cancer cells, a strong Hsp90 inhibition in combination with combined glutaminase triggers cellular death. The severe blocking of Hsp90 function, activated the unfolded protein response that extent, which in turn triggered cellular death due to elevated endoplasmic reticulum stress (Li et al., 2015). Indeed, another study reported that Hsp90 inhibitors selectively decrease superoxide production, thus are predominantly involved in the regulation of reactive oxygen species in COS-7 and HEK293 cells (Chen et al., 2015).

In light of those events, we decided to investigate whether P53 expression, which is protective against LPS-induced violations of endothelial integrity, is associated with APE1/Ref1. The latter molecule is an upstream effector of VEGF, and it is strongly involved in the pathogenesis of a plethora of inflammatory conditions (Kelley et al., 2012; Choi et al., 2016). The activation of APE1/Ref1 may be due to radiation, ROS and RNS, hypoxia, ischemia, as well as intracellular increases of Ca^{+2} (Thakur et al., 2014). It activates TFs including c-Jun, activator protein-1 (AP-1), nuclear factor kappa B (NF-κB), and hypoxia-inducible factor 1α (HIF-1α). All those proteins are involved in various cellular processes such as cell survival, growth, and inflammation (Bapat et al., 2009; Ema et al., 1999; Xanthoudakis and Curran, 1992). APE1/Ref-1 reduces the cysteine (Cys) residues of these TFs by activating their DNA-binding activity (Bhakat et al., 2009). Currently there are intense efforts to develop molecules targeting the redox function of the DNA repair/redox protein APE1/Ref-1 in various states of human pathology (Laev et al., 2017).

We employed bovine pulmonary aortic endothelial cells and human lung microvascular endothelial cells to investigate whether the induction of P53 due to Hsp90 inhibition, would influence the expression levels of APE1/Ref1. Figs. 1–3 indicate that 17-DAG, 17-AAG and AUY-922 Hsp90 inhibitors induced P53 expression, in line with previous observations in human lung microvascular endothelial cells (Barabutis et al., 2015, 2018b, 2019). Furthermore, the APE1/Ref1 levels were reduced. Similar effects were observed upon Nutlin-induced P53 augmentation (Fig. 4A, B), as well as after treatment of the cells with the P53 inductor Tunicamycin (Fig. 4C, D). On the other hand, when the P53 levels were suppressed by Pifithrin (Fig. 3A, B) and siP53 (Fig. 3C), the expression of APE1/Ref1 was elevated.

To access the direct effect of APE1/Ref1 silencing to human cells, we employed the ECIS system, in order to monitor the transendothelial resistance of the confluent endothelium monolayers. After the cells were transfected with si RNA that specifically targets this transcription factor, we treated the cells with the P53 inductor and Hsp90 inhibitor AUY922. The results indicate that the suppression of APE1/Ref1 potentiated the effects of Hsp90 inhibition (Fig. 3D), suggesting the negative impact of that molecule/redox factor on the vascular barrier integrity.

5. Conclusions

The current study supports our ongoing investigations on the crucial role of P53 towards the vascular endothelium, and suggests that it exerts its effects via the downregulation of the APE1/Ref1 protein. Further studies will further substantiate our findings, by employing genetically modified rodents and barrier disruptive elements that cause lethal pulmonary pathologies, such as ALI/ARDS.

Declarations of interests

None.

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