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PII: S0014-4827(19)30347-7

DOI: https://doi.org/10.1016/j.yexcr.2019.111505

Article Number: 111505

Reference: YEXCR 111505

To appear in: *Experimental Cell Research*

Received Date: 20 May 2019

Revised Date: 14 July 2019

Accepted Date: 18 July 2019

Please cite this article as: J. Xu, Z. Sheng, F. Li, S. Wang, Y. Yuan, M. Wang, Z. Yu, NEDD4 protects vascular endothelial cells against Angiotensin II-induced cell death via enhancement of XPO1-mediated nuclear export, *Experimental Cell Research* (2019), doi: https://doi.org/10.1016/j.yexcr.2019.111505.

NEDD4 protects vascular endothelial cells against Angiotensin II-induced cell death via enhancement of XPO1-mediated nuclear export

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Abstract

NEDD4 is an E3 ubiquitin ligase containing the HECT domain, which regulates various cellular processes, but its role in vascular endothelial cells is unknown. In the present study, we found that NEDD4 bound directly to XPO1 by co-immunoprecipitation screening. In HUVECs (human umbilical vein endothelial cells), overexpression of NEDD4 reduced Ang II-induced ROS level and cell apoptosis. Ang II stimulation led to nuclear accumulation of cargoes, while overexpression of NEDD4 enhanced the XPO1-dependent nuclear export of its cargoes. KPT185, an inhibitor of XPO1, can abolished the protective effect of NEDD4 under Ang II treatment. In addition, NEDD4 could promote the interaction between XPO1 and RanBP3 via K63-linked ubiquitination of XPO1. These results suggested that NEDD4 played a protective role in vascular endothelial cell injury through regulating XPO1-mediated nuclear export.

Key words: NEDD4, XPO1, ubiquitination, nuclear expor

1. Introduction

Dysfunction of endothelial cells plays a critical role in the development of vascular pathogenesis and disorders, including hypertension, thrombosis and atherosclerosis, etc.[1,2]. Angiotensin II (Ang II), the major vasoactive factor, is able to promote vascular pathological remodeling via leading to endothelial cell damage. Evidence showed that oxidative stress, DNA damage and caspase activation contribute to endothelial cell apoptosis, which is involved in Ang II-mediated endothelial cells injury[3,4].

Exportin 1 (XPO1), a transport receptor, is one vital component of the nuclear transporter complex embedded within the nuclear envelope, which mediates the nuclear-to-cytoplasmic export of variety proteins and certain RNAs[5]. XPO1 contacts with its cargo via recognition of the specific leucine-rich nuclear export signals (NES), which is rely on many scaffold molecules and the small GTPase Ran to facilitate this process[6–8]. So far, many tumor suppressors or anti-apoptotic regulators, such as P53, BRCA1, and survivin, were identified as the cargos interacting with XPO1[9–11]. Overexpression of XPO1 is responsible for cell survival, which has been reported to promote the tumorigenesis[12–14]. Recently, we find that XPO1-mediate nuclear export of RNF146, an important repressor in poly (ADP-ribose) polymerase dependent cell death, plays a role in Ang II-induced endothelial cell injury[15]. However, how XPO1 is regulated in this situation remains elusive.

Homologous to the E6-AP carboxyl terminus (HECT) E3 ligases found in all eukaryotic organisms directly catalyze target for ubiquitination, are involved in the regulation of various physiological processes[16]. In *Saccharomyces cerevisiae*, the family members of HECT E3 ligase can regulate nucleocytoplasmic export. Rsp5, one of the main HECT E3 ligase in yeast, has been reported to be required for proper mRNA, tRNA and rRNA export[17–19]. Another HECT E3 ligase Tom1 contributes

to nucleocytoplasmic transport of heterogeneous nuclear ribonucleoproteins and mRNA[20,21]. NEDD4 (neuronal precursor cell-expressed, developmentally downregulated 4), also known as NEDD4-1, containing an C2 domain, three WW domains, and a C-terminal catalytic HECT domain, is one major homologue of yeast HECT E3 ubiquitin ligase widely expressed in mammalian cells. NEDD4 exerts anti-apoptotic effect via multiple mechanisms. NEDD4 can block cell apoptosis by targeting PTEN for proteasomal degradation[22]. Recent study has shown that NEDD4 reduced ischemia/reperfusion induced apoptosis of cardiomyocytes through activation of PI3K/Akt signaling[23]. In addition, NEDD4 is expressed in vascular endothelial cells and is involved in endocytosis by vascular endothelial growth factor receptor-2 (VEGF-R2) degradation[24]. However, the role of NEDD4 in vascular endothelial cells under the apoptotic stress is unknown.

In the present work, we demonstrated NEDD4 is only HECT E3 ligase that interacted with XPO1 in HUVECs (human umbilical vein endothelial cells). NEDD4 protected HUVEC against Ang II-induced cell death through enhancing XPO1-mediated nuclear export. XPO1 was directly ubiquitinated by NEDD4 in K63-linked fashion, which facilitated the interaction of XPO1 and RanBP3 (Ran-binding Protein 3), a scaffold factor in the nuclear-export complex.

2. Materials and methods

2.1. Cell culture and reagents

HUVECs (human umbilical vein endothelial cells), Hela cell line and HK-2 cells were purchased from Type Culture Collection Committee of Chinese Academy of Science (Shanghai, China), cultured in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2 incubator. Angiotensin II (Ang II) was obtained from Sigma-Aldrich and dissolved in sterile, ultrapure water. KPT-185 and cycloheximide (CHX) were purchased from Selleck Chemicals and dissolved in Dimethylsulfoxide (DMSO).

2.2. Plasmids preparation and transfection

Full-length XPO1 cDNA were amplified from a human cDNA library using standard

PCR techniques and subcloned into p3XFlag-CMV(TM)-14 expression vector (Sigma). Full-length NEDD4, AIP4, WWP1 and SMURF1 were amplified and inserted into pcDNA3.1-Myc vector, respectively (Invitrogen). Each construct used was confirmed by direct sequencing. Cultured cells were transfected with the plasmids by Lipofectamine® 2000 regent according to manufacturer's instructions (Invitrogen).

2.3. Cellular Reactive Oxygen Species (ROS) Detection

Intracellular ROS level was measured with Cellular Reactive Oxygen Species Detection Assay Kit (Abcam, ab113851). Briefly, HUVECs were seeded in a 96-well plate before treatment. After treated with Ang II, HUVECs were collected and washed with PBS twice. After that 20 μM 2',7'–dichlorofluorescin diacetate (DCFDA) was used to stain the cells in serum-free DMEM for 30 min at 37°C. Then, Flow cytometry was performed to quantify the levels of ROS, the mean fluorescence was determined by counting 10,000 events.

2.4. Nuclear–cytoplasmic fractionation separation and Western Blot

Nuclear and cytoplasmic fractionations were separated with the NE-PER Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Fisher scientific) according to the manufacturer’s protocol. Western blot analysis for protein expression was performed using standard methods. The antibodies against specific proteins used in this study were as follows: NEDD4 (proteintech, 21698-1-AP), AIP4 (Abcam, ab108515), WWP1 (proteintech, 13587-1-AP), SMURF1 (Abcam, ab38866), XPO1 (proteintech, 66763-1-Ig), RNF146 (Abcam, ab201212), Apoptosis-inducing factor (AIF) (proteintech, 17984-1-AP), Cleaved Caspase-3 (Abcam, ab208003), Caspase-3 (Abcam, ab197202), TP53 (proteintech, 10442-1-AP), RanBP3 (CST, #93706), alpha-Tubulin (proteintech, 11224-1-AP) and Histone-H3 (proteintech, 17168-1-AP). The signals of blots were quantified and analyzed using the ImageJ software.

2.5. Co-immunoprecipitation and in vivo ubiquitination assay

Briefly, cell lysates were extracted with mild cell lysis buffer and cleared by centrifugation. Then the supernatants were immunoprecipitated with appropriate

antibodies and protein A/G Plus-agarose for overnight at 4°C. The immunocomplexes were then washed with lysis buffer three times, and then used for immunoblotting analysis with the indicated antibodies.

For the *in vivo* ubiquitination assay, NEDD4 overexpression or knockdown HUVECs were pretreated with MG132 for 4 hours before harvesting. Then, the cells were lysed and immunoprecipitated with anti-XPO1 antibody. Subsequently, the ubiquitination level of XPO1 was tested with K48-ubiquitination or K63-ubiquitination antibody.

2.6. Cell apoptosis analysis

The apoptosis rate of HUVECs was tested by Apoptosis Detection Kits (BD, USA) following the manufacturer's instructions. Briefly, 5×10^5 cells were harvested and resuspended in 200 μL binding buffer, followed by a 15 min incubation with 5 μL Annexin V-FITC and 5μL propidium iodide (PI) in the dark at 37°C. Then, the flow cytometry analysis was employed for detecting apoptotic events.

2.7. Statistical analysis

All data are shown as means ± SEM and analyzed with GraphPad prism 6 software (San Diego, United States). The unpaired Student t test was used to determine the statistical significance of differences between two groups. One-way ANOVA analysis was used for more than two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. NEDD4 is the only human Rsp5 homolog that interacts with XPO1 in vascular endothelial cells

Rsp5 is the major HECT domain-containing ubiquitin ligase encoded by the *Saccharomyces cerevisiae* genome. NEDD4, AIP4, WWP1, and SMURF1 are four obvious homologs of Rsp5 in human cells with similar sequence and domain arrangement (Fig. 1A). To identify which HECT E3 ligase was the potential regulator of XPO1-mediated nuclear export, we exogenously co-expressed XPO1 with NEDD4, AIP4, WWP1, and SMURF1 and detected the interaction between XPO1 and the four

Rsp5-homologs by co-immunoprecipitation in the proximal tubule epithelial cell line (HK-2 cells). The results showed that only NEDD4 can interact with XPO1, whereas AIP4, WWP1, and SMURF1 could not bind to XPO1 (Fig. 1B). To confirm this interaction between XPO1 and NEDD4, we further performed co-immunoprecipitation by XPO1 antibody or NEDD4 antibody in HUVECs. The data indicated that NEDD4 indeed interacted with XPO1 in HUVECs (Fig. 1C and D). Collectively, these data demonstrated that XPO1 was a substrate of the HECT ubiquitin ligase, NEDD4, in HUVECs.

3.2. NEDD4 protects HUVECs against Ang II-induced cell death

To investigate the biological role of NEDD4 in Ang II-induced HUVECs damage, we first assessed the expression level of NEDD4 after Ang II treatment. The HUVECs was subjected to Ang II (1 μM) treatment for 24 hours, and the qRT-PCR and Westernblot analysis showed that the expression of NEDD4 was decreased both in transcriptional and translational level (Fig. 2A and B).

Accordingly, we assumed that NEDD4 lost its function in this model. Then we used the overexpression experiment to determine the effect of NEDD4 to the HUVECs damage. The NEDD4 was overexpressed by transfection of Myc-NEDD4 plasmids (Fig. 2C). Compared with the vector transfected HUVECs, overexpression of NEDD4 significantly reduced the ROS level caused by Ang II treatment (Fig. 2D). Flow cytometry also showed that overexpression attenuated the cell death induced by Ang II (Fig. 2E). Moreover, overexpression of NEDD4 resulted in marked decreases in cleaved-caspases 3 and AIF (Fig. 2F-H). Overall, these results suggested a directly protective role of NEDD4 against Ang II-stimulated HUVECs death.

3.3. The protective effect of NEDD4 is dependent on XPO1-mediated nuclear export

Maintenance of XPO1-mediated nuclear export of specific cargoes has been proved to play a protective role against stress-induced cell death. RNF146, whose nuclear-export plays a key role in Ang II-induced HUVECs injury, has been recently reported as a cargo of XPO1[15]. TP53, the classic cell apoptosis inducer, was also

reported as the XPO1's cargo [25]. Therefore, we addressed whether the nuclear export of RNF146 and TP53 was affected by NEDD4. Ang II treatment significantly induced nuclear accumulation of TP53 and RNF146, while overexpression of NEDD4 promoted the nuclear export of both TP53 and RNF146 in Ang II treated HUVECs (Fig. 3A). This observation suggested that the nuclear export of XPO1's cargoes might be regulated by NEDD4.

In order to further investigate whether XPO1-mediated nuclear export is required for the protective effect of NEDD4, we used the XPO1 inhibitor (KPT-185) to block XPO1's function[15,26]. Addition of KPT-185 aggravated the intracellular ROS level induced by Ang II, and further abolished the beneficial effect of NEDD4-overexpression against Ang II-induced cell damage (Fig. 3B and C). In keeping with these findings, treatment of KPT-185 also withdrew the down-regulation of cleaved-caspases 3 caused by NEDD4-overexpression under the Ang II-mediated cellular pro-death response (Fig. 3D-F). Moreover, we found that KPT-185 efficiently inhibited the nuclear-export of RNF146 and TP53 in the present of NEDD4-overexpression (Fig. 3G). These results indicated that XPO1-mediated nuclear export of cell death regulators was a required molecular event for the beneficial effect of NEDD4 against Ang II-induced HUVECs death.

3.4. K63-linked ubiquitination by NEDD4 affects XPO1-RanBP3 interaction

To better understand how NEDD4 regulates XPO1-mediated nuclear export, we examined the effect of NEDD4 on the expression level of XPO1. Unexpectedly, the overall protein level of XPO1 was not affected by NEDD4-overexpression in HUVECs (Fig. 4A). Therefore, we speculated that NEDD4 did not mediate the proteasomal degradation of XPO1. To confirm this, the HUVECs was treated with CHX, the inhibitor of protein synthesis, for the indicated times, which showed that overexpression of NEDD4 did not regulate the degradative rate of XPO1 (Fig. 4B and C). Different types of polyubiquitinated modification may exert definitive function. K48-linked polyubiquitination usually functions as the signal for proteasomal degradation, whereas K63-linked polyubiquitination has been shown to have

non-degradative functions[27]. Accordingly, we used the specific antibodies recognizing the two different types of polyubiquitinated chain to test which kind of polyubiquitinated modification can be catalyze by NEDD4. Only the K63-linked polyubiquitination level of XPO1 was increased by overexpression of NEDD4 compared to that through K48 (Fig. 4D).

K63-linked polyubiquitin chains may serve as a scaffold for a protein complex assembling[28]. RanBP3 (Ran-binding Protein 3) was reported to interact with XPO1 functions as a vital scaffold factor in the nuclear-export complex[29]. According to this, we subsequently investigated whether K63-linked polyubiquitin of XPO1 impacted the interaction between XPO1 and RanBP3. Interestingly, further co-immunoprecipitation experiments showed that the interaction between XPO1 and RanBP3 was significantly enhanced by overexpression of NEDD4 in HUVECs (Fig. 4E). These results indicated that NEDD4 could catalyze the K63-linked polyubiquitination of XPO1, which facilitated the interaction between XPO1 and RanBP3.

3.5. RanBP3 was essential for the protective effect of NEDD4

To further confirm that RanBP3 was required for the protective effect of NEDD4, RanBP3 was knocked down by interference RNA (si-RNA) transfection. The western blot revealed that both si-RNAs effectively repressed the protein expression level of RanBP3 in HUVECs (Fig. 5A). Knockdown of RanBP3 could cancel the ROS-repressing effect of overexpression of NEDD4 in Ang II-treated HUVECs (Fig. 5B). The result of flow cytometry showed that knockdown of RanBP3 repressed the anti-apoptotic effect of NEDD4 overexpression (Fig. 5C). In addition, knockdown of RanBP3 re-upregulated the expression of the apoptotic markers, cleaved-caspase 3 and AIF, at the present of NEDD4 overexpression in Ang II treated HUVECs (Fig. 5D-F). To see whether the cargos of XPO1 were affected by RanBP3 deficiency in this condition, we tested the level of RNF146 and TP53 in the nuclear and cytoplasmic fractions, respectively. The results demonstrated that knockdown of RanBP3 efficiently inhibited the nuclear export of RNF146 and TP53 in spite of

overexpressed NEDD4 in Ang II treated HUVECs (Fig. 5G). The above analyses suggested that RanBP3 was essential for the protective effect of NEDD4 and XPO1 dependent nuclear export of apoptosis related proteins.

4. Discussion

The nuclear transporter XPO1 plays an important role in regulating tumor cell death. We recently report that XPO1 can protect vascular endothelial cell against Ang II induced cell death[15]. However, the mechanism by which XPO1 is regulated remains unclear. In this study, we screen four major HECT E3 ubiquitin ligases in human cells and identify NEDD4 as the one that functionally regulates XPO1 in vascular endothelial cells. NEDD4 reduces Ang II-induced endothelial cell apoptosis by promoting XPO1-mediated nuclear export. Mechanistically, NEDD4 facilitates the binding of XPO1 to the accessory protein RanBP3 by mediating the K63-linked polyubiquitination of XPO1, which results in maintaining the stability of the XPO1 nuclear transport complex (Fig.6). Therefore, our first finding provides the evidence indicating that NEDD4 plays a pivotal role in attenuating Ang II-induced endothelial damage by regulating XPO1-dependent nuclear export.

There has been much evidence that XPO1 plays an important role in promoting cell survival and inhibiting cell death [30]. We recently present that RNF146 is a cargo of XPO1 in vascular endothelial cells. Overexpression of XPO1 can export RNF146 from nucleus to cytoplasm for AIF degradation, which in turn reduces Ang II- mediated endothelial cell death[15]. In addition, another well-known apoptosis-inducing factor, which is also a transcription factor, TP53, is also regulated by XPO1[31,32]. Inhibition of XPO1 can cause TP53 to accumulate in the nucleus and produce an apoptotic response[25]. In the present study, we find that Ang II can induce the aggregation of RNF146 and TP53 in nucleus. And promotion of XPO1-mediated nuclear export enhances the translocation of RNF146 and TP53 out of the nucleus, which exerts beneficial effect against Ang II-induced cell damage. These results suggest that RNF146 and TP53 accumulated in nucleus by XPO1

dysregulation are involved in Ang II-induced endothelial cell injury.

Saito et al. have reported that XPO1 can be modified by NEDD8, a ubiquitin-like protein, which lead to proteasome-dependent degradation[33]. Some proteomic studies have also implied that XPO1 can be modified by ubiquitination[34–37]. We first discover XPO1 as a substrate of ubiquitination, and it can be modified by both K48 and K63 linked ubiquitination. In this report, we mainly find that NEDD1 directly interacts with XPO1 and mediates its K63-linked ubiquitination. In general, K63-linked ubiquitination plays a role in promoting protein-protein interactions. Our results also show that NEDD4-mediated ubiquitination of XPO1 promotes its interaction with RanBP3, which involves in maintenance of the structure and function of nuclear exporter complex. RanBP3 serves as a scaffold to prompt the efficient assembly of export complex and stabilize XPO1-cargo interaction[8,29]. Our data suggest that the interaction between XPO1 and RanBP3 promoted by NEDD4 can efficiently increase the nuclear transport of the substrates, despite the decrease of XPO1's expression by Ang II stimuli. Collectively, we speculate that the ubiquitinated site may be near the interaction region between XPO1 and RanBP3, which needs to be confirmed by further studies.

NEDD4 is a widely expressed E3 ligase in many cell types with numerous substrates. NEDD4 has been shown to protected cardiomyoblast cells against ischemia/reperfusion injury[23]. Furthermore, deficiency of NEDD4 could be involved in vascular calcification of vascular smooth muscle cells[38]. In line with those studies, our findings suggest a protective role of NEDD4 in cardiovascular system. Our results demonstrated that NEDD4 regulates nuclear protein XPO1. Some studies have also found that NEDD4 is expressed in the nucleus and regulates nuclear substrates' function such as histone H3 and heat shock transcription factor 1[39–42]. Our data reveal that NEDD4 directly ubiquitinates XPO1 for K63-linked form without altering its expression level. Indeed, other research has shown the non-proteolytic role of NEDD4 in the nucleus[39]. Additionally, NEDD4 mediates phospho-AKT for K63-linked poly-ubiquitination at the plasma membrane to promote

nuclear trafficking of AKT[43].

In summary, we found that HECT E3 ligase NEDD4 protected vascular endothelial cell against Ang II-induced cell death. Mechanistically, NEDD4 directly ubiquitinated XPO1 via K63-linked form, enhancing the interaction between XPO1 and RanBP3, and thereby increased the XPO1-mediated nuclear export capacity. Thus, our data provide a novel perspective on the role of NEDD4 in Ang II-induced vascular endothelial cell injury and new insight for the treatment of vascular disorders.

Acknowledgments

This study was supported by grants from the Natural Science Foundation of Jiangxi (No. 2016BBG70193).

Conflict of Interest

The authors declare no conflict of interest.

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Figure Legends

Figure 1. NEDD4 is the only human Rsp5 homolog that interacts with XPO1 in vascular endothelial cells.

(A) Domain structure of four human Rsp5 homologs. The positions of the C2 domain (yellow), WW domain (green), and the HECT domain (red) are shown. (B) The interaction between XPO1 and the four Rsp5 homologs (NEDD4, AIP4, WWP1, and SMURF1) by co-immunoprecipitation in the HK-2 cells. Cell extracts were IP with anti-Flag Ab. (C and D) The co-immunoprecipitation assay of XPO1 and NEDD4 in HUVECs. Data are representative of means ± SEM of three assays.

Figure 2. Overexpression of NEDD4 reduces Ang II-induced HUVECs injury and apoptosis.

(A) qRT-PCR analysis of NEDD4 expression in HUVECs treated with Ang II. (B) Western Blot analysis of NEDD4 in HUVECs treated with Ang II. (C) The expression

level of NEDD4 in HUVECs was significantly increased by transfection p-NEDD4, compared with vector group, respectively. (D) Flow cytometry assay was used to examine the intracellular ROS levels of HUVECs cells transfected with vector or p-NEDD4 and then treated with or without Ang II (1 μM) for 24 h. (E) Measurement of apoptotic cells following overexpression of NEDD4 in HUVECs. Results are expressed as scatter diagram (left) and calculated percentage of annexin-V-positive cell population (right). (F) Western Blot analysis of apoptotic markers (cleaved-caspases 3 and AIF) in in Ang II treated HUVECs after NEDD4 overexpression. (G and H) Quantitative analysis of Western blot data. Data represented mean ± s.e.m., n =3; $^{*}p<0.05$.

Figure 3. The protective effect of NEDD4 is dependent on XPO1-mediated nuclear export.

(A) Western blot analysis of RNF146 and TP53 in cytoplasmic and nuclear fraction of HUVECs after Ang II treatment in the presence of overexpressed NEDD4 (p-NEDD4). (B) Measurement of ROS level from Ang II-treated HUVECs after KPT-158 exposure with the presence of overexpressed NEDD4. (C) The data of flow cytometry experiment in Ang II-treated HUVECs after KPT-158 exposure with the presence of overexpressed NEDD4. (D) Western Blot analysis of apoptotic markers (cleaved-caspases 3 and AIF) in Ang II-treated HUVECs after KPT-158 exposure with the presence of overexpressed NEDD4. (E and F) Quantitative analysis of Western blot data. Data represented mean ± s.e.m., n =3; $^{*}p< 0.05$. (G)Western blot analysis of RNF146 and TP53 in cytoplasmic and nuclear fraction in Ang II-treated HUVECs after KPT-158 exposure with the presence of overexpressed NEDD4.

Figure 4. K63-linked ubiquitination by NEDD4 affects XPO1-RanBP3 interaction.

(A) Western Blot analysis of XPO1 in HUVECs transfected with NEDD4 plasmids. (B-C) The effect of NEDD4 overexpression on the protein degradative rate of XPO1. (D) *in vivo* ubiquitination assay showed the K48-linked and K63-linked ubiquitination status of XPO1 after NEDD4 overexpression in HUVECs. (E)

Co-immunoprecipitation showed the effect of NEDD4 overexpression on the interaction between XPO1 and RanBP3. Data are representative of means ± SEM of three assays.

Figure 5. RanBP3 was essential for the protective effect of NEDD4.

(A) Western Blot analysis of RanBP3 in HUVECs transfected with siRNA of RanBP3. Data represented mean ± s.e.m., n =3; $^{*}p< 0.05$. (B) Measurement of ROS level showed the effect of RanBP3 knock-down in Ang II-treated HUVECs with the presence of overexpressed NEDD4. (C) The data of flow cytometry experiment showed the apoptotic rate of HUVECs. (D) Western Blot analysis of apoptotic markers (cleaved-caspases 3 and AIF). (E and F) Quantitative analysis of Western blot data. Data represented mean ± s.e.m., n =3; $^{*}p< 0.05$. (G) Western blot analysis of RNF146 and TP53 in cytoplasmic and nuclear fraction.

Figure 6. Proposed model for regulation of NEDD4 on XPO1-mediated nuclear export. The mechanism by which NEDD4 plays a protective role in endothelial cell. In nucleus, NEDD4 promotes XPO1-RanBP3 interaction via K63-linked polyubiquitination of XPO1, which facilitates XPO1-dependent nuclear export of the cargo.

Figure 1

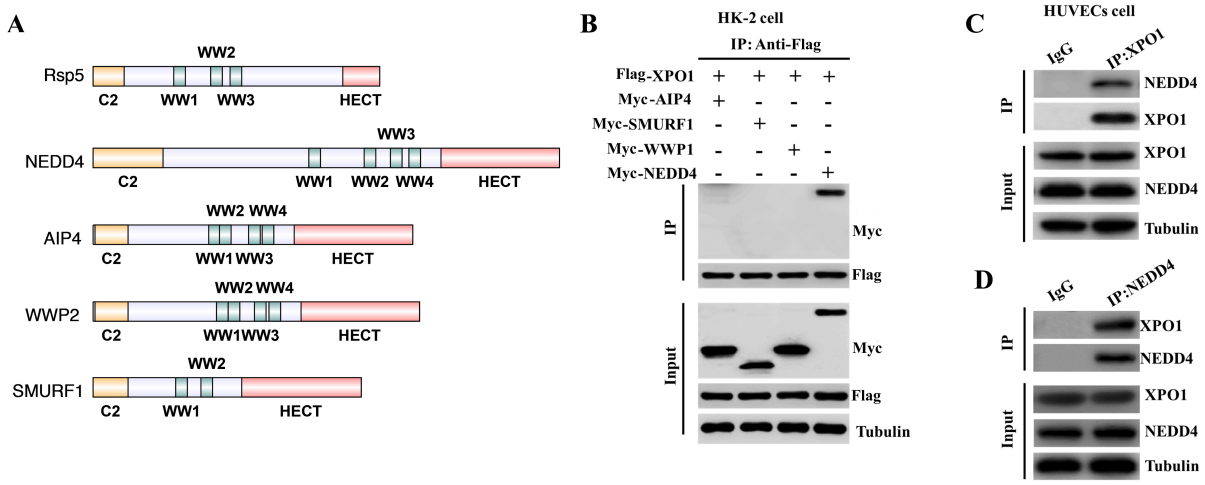


Figure 2

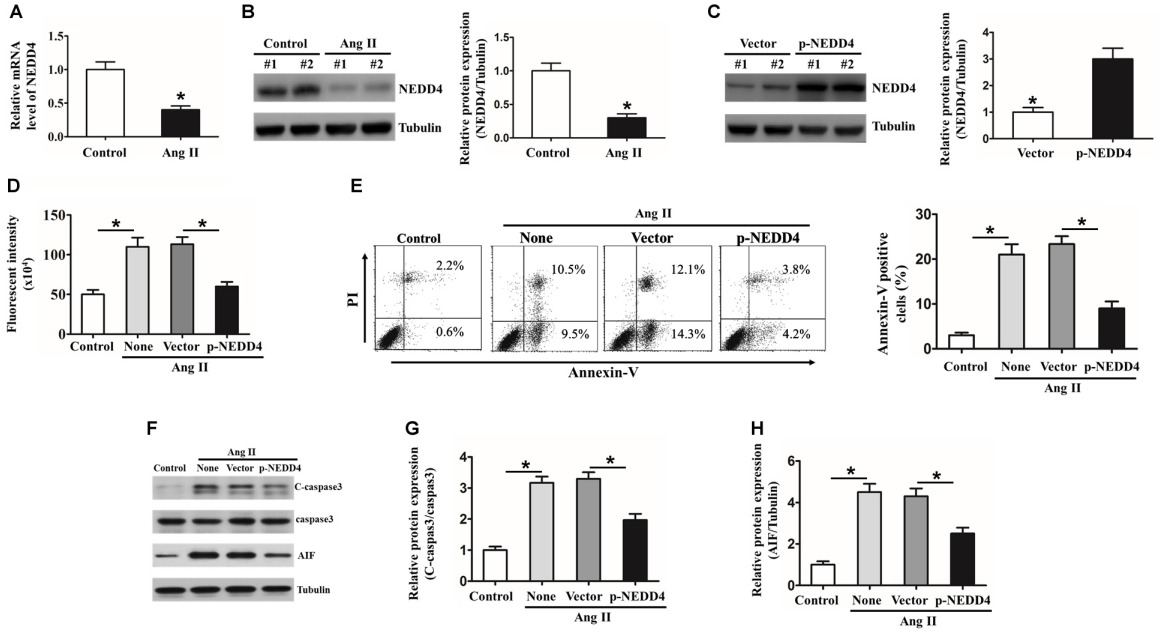


Figure 3

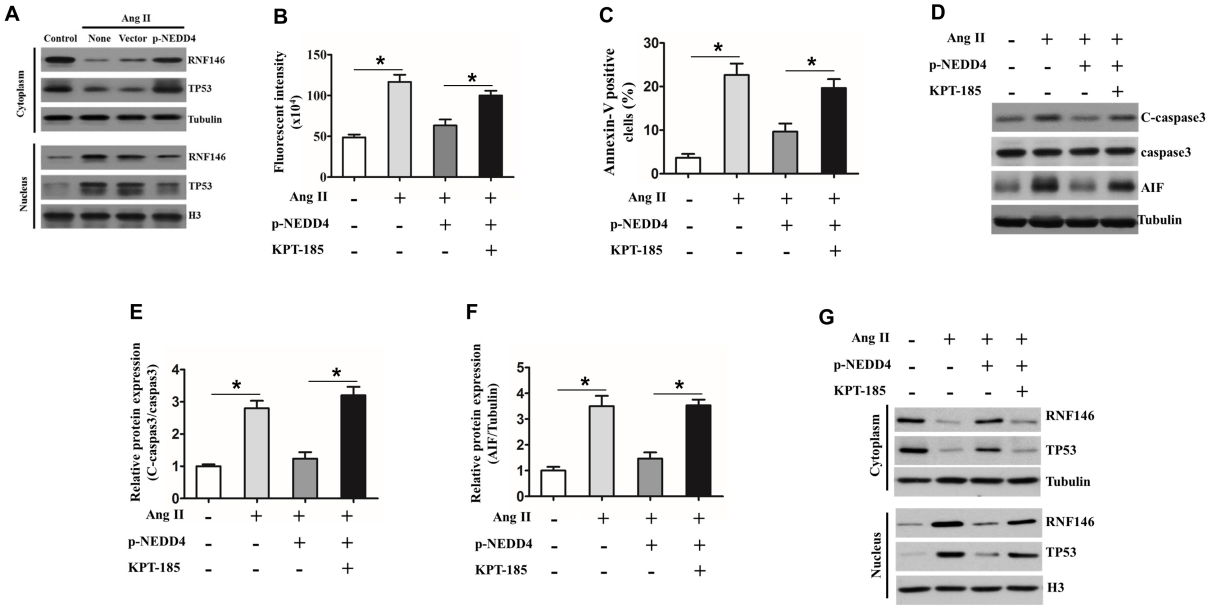


Figure 4

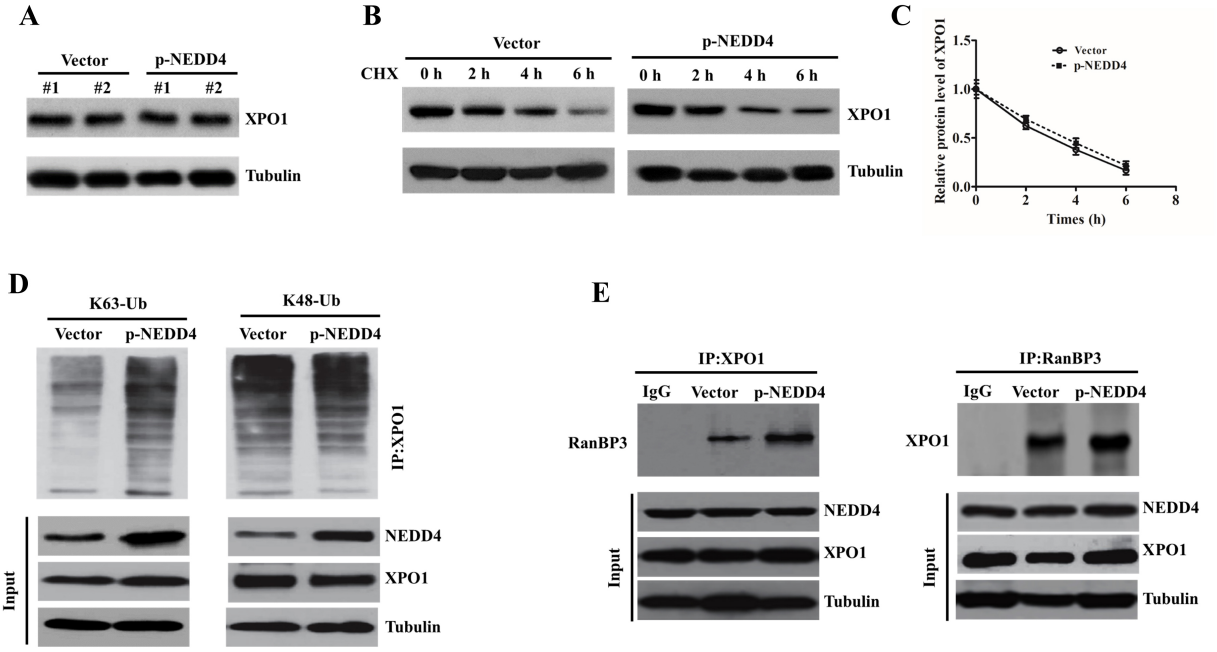


Figure 5

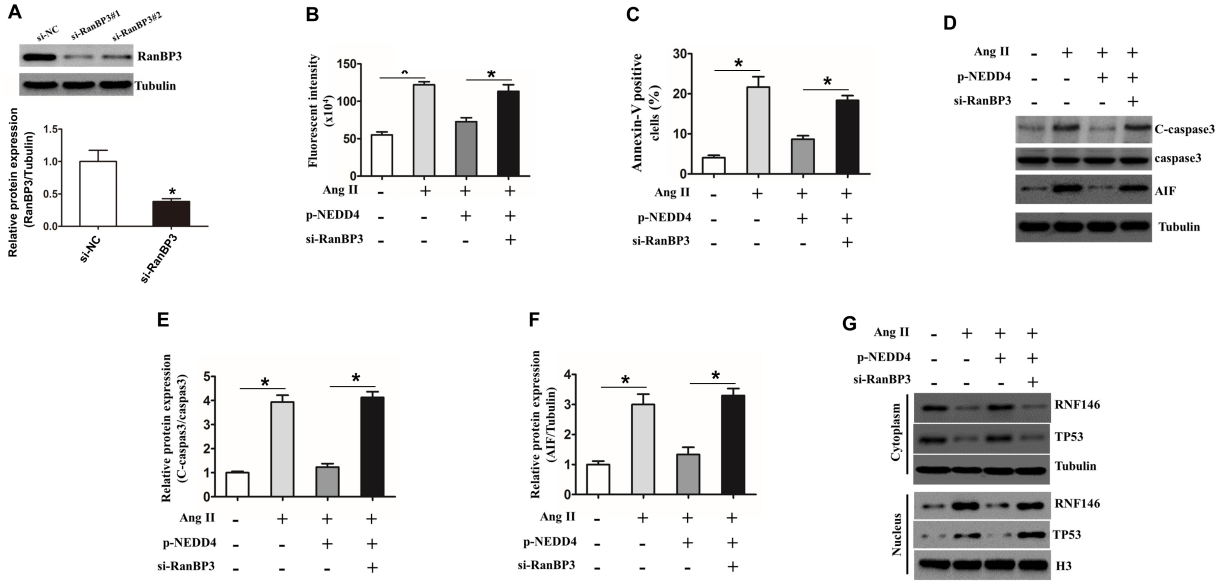
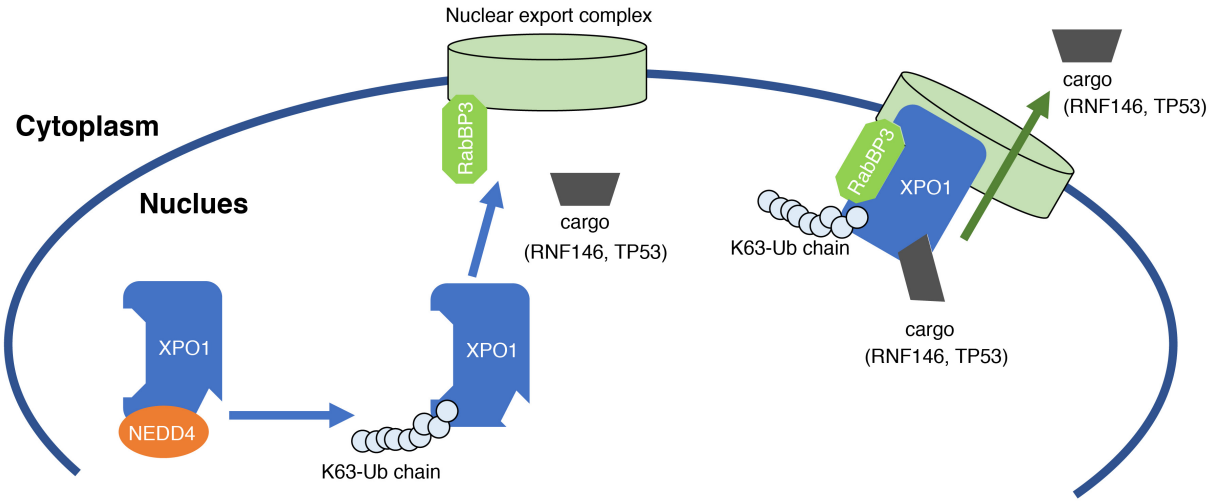


Figure 6



Highlights

- HECT E3 ligase NEDD4 interacts directly with XPO1 and mediates XPO1 for K63-linked ubiquitination
- Overexpression of NEDD4 protects HUVECs against Ang II-induced cell death
- The protective effect of NEDD4 is dependent on XPO1-mediated nuclear export
- K63-linked ubiquitination of XPO1 enhances its interaction with RanBP3