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Knockdown of hepatoma-derived growth factor-related protein-3 induces apoptosis of H1299 cells via ROS-dependent and p53-independent NF-κB activation

Hong Shik Yun [a,b], Jeong-Hwa Baek [a,c], Ji-Hye Yim [a], Su-Jae Lee [b], Chang-Woo Lee [c], Jie-Young Song [a], Hong-Duck Um [a], Jong Kuk Park [a], In-Chul Park [a], Sang-Gu Hwang [a,*]

[a] Division of Radiation Cancer Biology, Korea Institute of Radiological & Medical Sciences, Seoul 139-706, Republic of Korea
[b] Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, Republic of Korea
[c] Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Republic of Korea

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ABSTRACT

We previously identified hepatoma-derived growth factor-related protein-3 (HRP-3) as a radioresistant biomarker in p53 wild-type A549 cells and found that p53-dependent induction of the PUMA pathway was a critical event in regulating the radioresistant phenotype. Here, we found that HRP-3 knockdown regulates the radioresistance of p53-null H1299 cells through a distinctly different molecular mechanism. HRP-3 depletion was sufficient to cause apoptosis of H1299 cells by generating substantial levels of reactive oxygen species (ROS) through inhibition of the Nrf2/HO-1 antioxidant pathway. Subsequent, ROS-dependent and p53-independent NF-κB activation stimulated expression of c-Myc and Noxa proteins, thereby inducing the apoptotic machinery. Our results thus extend the range of targets for the development of new drugs to treat both p53 wild-type or p53-null radioresistant lung cancer cells.

1. Introduction

Hepatoma-derived growth factor (HDGF) is the original member of a family of polypeptides designated HDGF-related proteins (HRPs). The HRP family consists of five proteins, which share a HATH domain at a well-conserved region of the N-terminus [1]. Very little is currently known regarding the function of the different HRP family members. HRP-1 exhibits testis-specific expression in mice and may play a pivotal role in the cell cycle phase around meiotic cell division during spermatogenesis [2]. HRP-2 influences several biological functions, including chromatin remodeling, DNA repair and ribosome biogenesis [3]. HRP-4 and lens epithelium-derived growth factor (LEDGF) have been confirmed to exhibit mitogenic activity in fibroblasts [1] and lens epithelial cells [4], respectively. LEDGF has been shown to function as a transcriptional activator and to potentiate the activity of human immunodeficiency virus integrase [5]. HRP-3 protein is expressed mainly in the testis and brain and to a lesser degree in the heart, ovaries, kidneys, spleen, and liver in humans [6]. Since HRP-3 in humans and mice contains a putative nuclear localization signal sequence in the self-specific region, it may function predominantly in the nucleus of cells in the brain and testis to play a role in cell proliferation. Consistent with this, overexpression of HRP-3 in human embryonic kidney cells has been shown to promote proliferative activity, whereas HRP-3 depletion in mantle cell lymphoma leads to proliferative arrest [7]. HRP-3 also plays a pivotal role during mouse embryonic neuronal development by interacting with the tubulin component of the neuronal cytoskeleton [8]. Because HRP-3 is expressed in both proliferative and differentiated cells of the brain, it might not be restricted to acting as a mitogenic factor [9]. The functional association of HRP-3 with tumor progression has been demonstrated in hepatocellular carcinoma (HCC) cells [10]. These studies showed that HRP-3 protected HCC cells from anoikis through a mechanism that was critically dependent on extracellular-signal-regulated kinase. We previously reported that HRP-3 acted through a signaling cascade involving regulation of reactive oxygen species (ROS)-dependent p53 activation and subsequent p53-dependent induction of PUMA (p53 upregulated modulator of apoptosis) expression to play an essential role in regulating the phenotype of p53 wild-type A549 cells [11]. However, the role and molecular mechanism of HRP-3 action in p53-null non-small-cell lung carcinoma (NSCLC) H1299 cells has remained completely unknown.

* Corresponding author. Address: Division of Radiation Cancer Biology, Korea Institute of Radiological & Medical Sciences, 75 Nowon-Ro, Nowon-Ku, Seoul 139-706, Republic of Korea.
E-mail address: sgh63@kcch.re.kr (S.-G. Hwang).

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In the present study, we demonstrated that silencing HRP-3 sensitizes H1299 cells to the induction of apoptotic cell death. Similar to the case in A549 cells, increased ROS generation through inhibition of nuclear factor erythroid 2 related factor 2 (Nrf2)/heme oxygenase 1 (HO-1) was a critical event during the apoptotic process in HRP-3-knockdown H1299 cells. However, in H1299 cells, unlike A549 cells, ROS induced nuclear factor-kappa B (NF-κB) activity, which acted as an upstream signal in the c-Myc and Noxa apoptotic pathway. Thus, we suggest that enhancement of the p53-independent ROS/NF-κB/c-Myc/Noxa cascade by HRP-3 knockdown plays an important role in promoting apoptosis in radioresistant, p53-null H1299 cells.

2. Materials and methods

2.1. Cell culture and treatment

Human lung cancer cell lines (H1299, A549, and H460), cervical cancer cell lines (SiHa and HeLa), colon cancer cell lines (HCT-8 and HCT 116), and breast cancer cell lines (MCF-7 and MDA-MB-231) were purchased from the ATCC (Manassas, VA, USA). H1299, A549, H460, MCF-7, HCT-8 and HCT 116 cells were cultured in RPMI-1640 medium, and MDA-MB-231, SiHa and HeLa cells were cultured in DMEM medium. In all cases, media were supplemented with 10% fetal bovine serum. Cells were irradiated using a ^{137}Cs-ray source (Atomic Energy of Canada, Ltd., Mississauga, Canada) or treated with doxorubicin or vinblastine (Sigma, St. Louis, MO, USA) to induce apoptosis [11]. *N*-acetyl cysteine (NAC; Sigma) was used to scavenge ROS, and BAY 11-7082 (Calbiochem, San Diego, CA, USA) was used to block NF-κB activation [12].

2.2. Cell proliferation

Cells, with and without HRP-3 knockdown, were plated on culture dishes at a density of 3.5×10^5 cells/cm^2 and incubated for the indicated times. Cell proliferation was determined by directly counting surviving cells using a hemocytometer.

2.3. Assay for cell death

Cell death was quantified as described previously [12].

2.4. Oncomine data mining

Oncomine™ (Life Technologies, Ann Arbor, MI, USA) was used for data analysis and visualization. Datasets from the Oncomine cancer microarray database (https://www.oncomine.com/resource/main.html) were selected for use in determining changes in HRP-3 mRNA expression and DNA copy number. These datasets provide fold-change values of gene expression and statistical significance (determined by *P*-values) involving comparisons between cancer and normal samples or between different cancers, as well as gene expression changes related to clinical-pathological characteristics of patients. HRP-3 expression was compared in lung cancer and normal lung tissue extracts.

2.5. ROS assay

Cells were incubated with 10 nM 2′,7′-dichlorofluorescein diacetate (DCF-DA; Molecular Probes, Inc., Eugene, OR, USA) for 20 min to detect ROS, as described previously [12].

2.6. Quantitative real-time polymerase chain reaction (PCR)

HRP-3 mRNA levels were determined by quantitative real-time PCR using a Chromo 4 Cycler and SYBR Premix Ex Taq (Takara Bio, Shiga, Japan). Protocols and primer pairs used were as described previously [11], and reactions were preformed in triplicate.

2.7. Knockdown of HRP-3 by small interfering RNA (siRNA)

Cells were transfected with HRP-3 siRNA as described previously [11].

2.8. Western blot analysis

Western blot analyses were done as described previously [13] using primary antibodies against HRP-3 (Proteintech Group, Inc., Chicago, IL, USA); p53, Nrf2, Bcl-2, Bcl-xl, Bax, and Iκ-B (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA); Keap1 (R&D Systems, Inc., Minneapolis, MN, USA); cleaved-PARP (Asp214), cleaved caspase-3, phospho-p53 (Ser15), PUMA, and HO-1 (Cell Signaling Technology Inc., Beverly, MA, USA); and c-Myc and Noxa (Calbiochem, San Diego, CA, USA). β-Actin (Sigma) was used as a loading control.

2.9. Immunofluorescence confocal microscopy

Immunofluorescence staining for HRP-3 was performed as described previously [11].

2.10. Reporter gene assay

Cells were transfected with 0.5 μg plasmid DNA (pNF-κB-luc; Stratagene, Cambridge, UK) under the indicated experimental conditions using the Superfect transfection reagent (Qiagen, Crawley, UK), as described by the manufacturer. The cells were harvested and prepared for Dual-Reporter Luciferase Assays (Promega, Southampton, UK) according to the manufacturer's protocol. NF-κB transcriptional activity was quantified in triplicate using a luminometer and was normalized to the protein concentrations of the samples. NF-κB activity was also determined indirectly by analyzing the degradation of I-κB using Western blot analysis.

2.11. Statistical analysis

Cell culture experiments were repeated at least three times. Statistical differences between groups were assessed by Students *t*-test, and a p-value <0.05 was considered significant.

3. Results

3.1. Association of HRP-3 with the resistance phenotype of p53-null H1299 cells

We first examined the correlation between HRP-3 gene expression and lung cancer severity using the human genetic dataset analysis tool, Oncomine. As shown in Fig. 1A, HRP-3 mRNA levels were significantly higher in lung carcinoid tumors and small-cell lung carcinoma, marginally different in lung adenocarcinoma, and not different in squamous cell lung carcinoma compared to normal lung cells, demonstrating that increased expression of HRP-3 is correlated with increased tumorigenesis. In our previous report, we explored the role of HRP-3 as a radioresistance-related gene in p53 wild-type A549 cells [11]. To examine HRP-3 function in p53-null NSCLC H1299 cells, which are also radioresistant, we compared HRP-3 expression levels among lung cancer cells. Consistent with results obtained in A549 cells, HRP-3 transcript (Fig. 1B, top) and protein (Fig. 1B, bottom) levels were much higher in H1299 cells than in H460 cells, supporting the p53-independent association of HRP-3 with the radioresistance phenotype. HRP-3 protein was localized only in the nucleus of H1299 cells, consistent

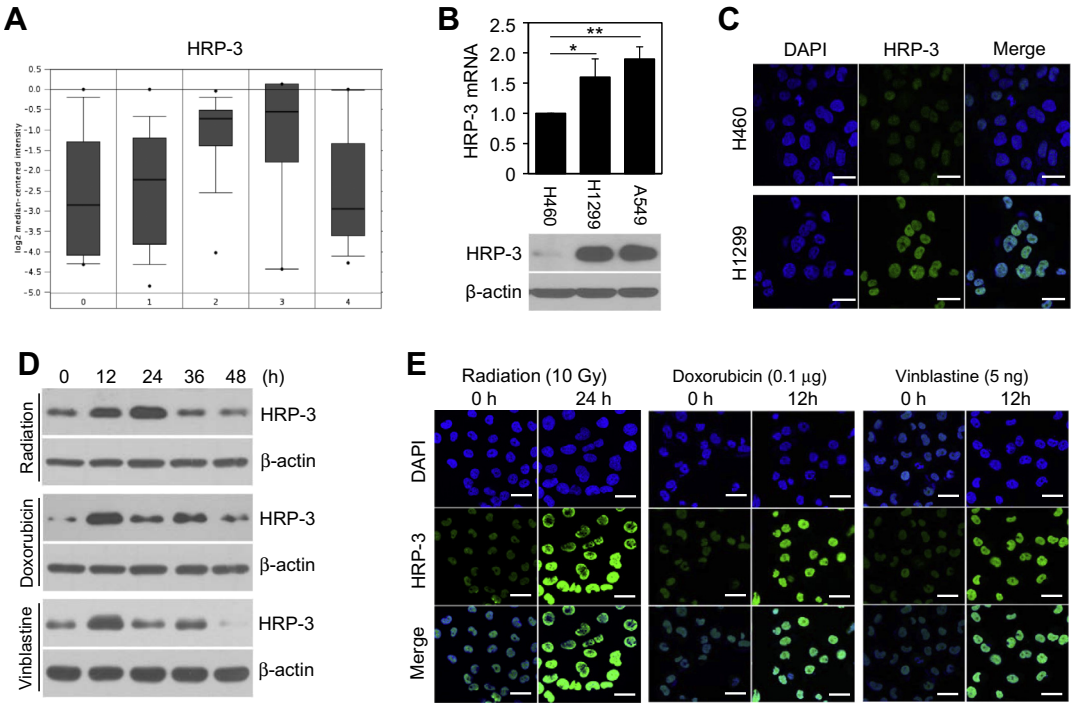

Fig. 1. HRP-3 is associated with the resistant phenotype of p53-null H1299 cells. (A) Available datasets in the Oncomine database were queried for HRP-3 expression with respect to cancer versus normal (threshold p-value = 1×10^{-4}; fold change $\geqslant 2$). 0, no value; 1, lung adenocarcinoma; 2, lung carcinoid tumor; 3, small cell lung carcinoma; 4, squamous cell carcinoma. (B) Transcript and protein levels of HRP-3 in lung cancer cells were determined by quantitative real-time PCR (top) and Western blotting (bottom), respectively. (C) Representative confocal images of HRP-3 localization in H460 and H1299 cells. Scale bar: 50 μm. (D and E) H1299 cells were treated with 10 Gy radiation, 0.1 μg/mL doxorubicin, or 5 ng/mL vinblastine for the indicated times. HRP-3 protein level and localization were determined by Western blotting (D) and laser scanning confocal microscopy (E), respectively. Scale bar: 50 μm.

with a presumptive primary role in nuclear signal transduction (Fig. 1C). We next determined whether HRP-3 mediates responses to radiation or anti-cancer drugs, such as doxorubicin or vinblastine, in H1299 cells. HRP-3 protein levels were increased by all stimuli, reaching a peak at about 12–24 h (depending on the stress) and then declining to basal levels (Fig. 1D), implicating HRP-3 as a radiation- and drug-responsive protein. Although the subcellular localization of HRP-3 changed rapidly from nucleus to cytoplasm in SMMC-7721 cells exposed to epidermal growth factor [10], its localization was unchanged by radiation or anti-cancer drugs in H1299 cells (Fig. 1E). Taken together, our data suggest that the radiation-responsive HRP-3 protein acts through a nuclear signal transduction mechanism to play a role in maintaining the resistance phenotype of p53-deficient H1299 cells.

3.2. HRP-3 depletion synergizes with radiation to induce enhanced apoptosis in a cell-type dependent manner

Apoptotic stimuli marginally induced cell death in H1299 cells (approximately 20%, 23%, and 26% for radiation, doxorubicin, and vinblastine, respectively) and markedly induced cell death in H460 cells (approximately 41%, 39%, and 43%, respectively) (Fig. 2A), demonstrating that H1299 cells possess a resistance phenotype compared with H460 cells. To determine whether HRP-3 is associated with the tolerance of H1299 cells, we depleted endogenous HRP-3 using siRNA. HRP-3 depletion in H1299 cells strongly reduced cell proliferation in a time-dependent fashion (Fig. 2B) and elevated the levels of cleaved PARP and active caspase-3 (Fig. 2C) compared to control cells. To better understand the function of HRP-3 in the resistance phenotype, we analyzed the effect of HRP-3 loss-of-function in H1299 cells treated with the same stimuli as shown in Fig. 2A. Whereas transfection of H1299 cells with siHRP-3 alone induced approximately 21% cell death compared to 10% in controls, co-stimulation of siHRP-3-transfected cells with radiation, doxorubicin, or vinblastine promoted cell death by approximately 42%, 45%, and 47%, respectively (Fig. 2D), indicating that HRP-3 knockdown enhances the cell death sensitivity of H1299 cells. To examine whether the correlation between HRP-3 and radiosensitivity is universal, we stimulated other cancer cell lines with siHRP-3 alone or in combination with radiation. Although siHRP-3 alone induced apoptosis to different degrees in SiHa, HCT-8 and MCF-7 cells, combined stimulation with radiation resulted in synergistic induction of apoptosis (Fig. 2E). siHRP-3 alone did not induced apoptosis in HeLa or MDA-MB-231 cells and did not synergize with radiation in these cells (Fig. 2E). Therefore, our results suggest that the contribution of HRP-3 to the regulation of radiosensitivity is cell-type dependent.

3.3. Depletion of HRP-3 induces apoptosis of H1299 cells via ROS generation, independent of PUMA and Bax signaling pathways

Consistent with the result presented in section 3.2, both cleaved PARP and active caspase-3 levels were significantly increased by HRP-3 knockdown, alone or in combination with radiation, compared to control cells (Fig. 3B). These patterns of apoptotic markers were paralleled with morphological changes characteristic of apoptosis (Fig. 3A, left) and an increased cell death rate (Fig. 3A, right). To identify the mechanisms by which HRP-3 depletion induces cytotoxicity in H1299 cells, we focused on the transcription factor Nrf2 and its target HO-1 antioxidant coordinators involved in regulating ROS production. Knockdown of HRP-3 expression in H1299 cells resulted in a marked reduction in Nrf2 and HO-1 protein levels without any change in the expression of Keap-1, a repressor of Nrf2 activation (Fig. 3C), suggesting that a p53-independent Nrf2/HO-1 signaling mechanism, as a downstream target of HRP-3, is responsible for the induction of ROS.

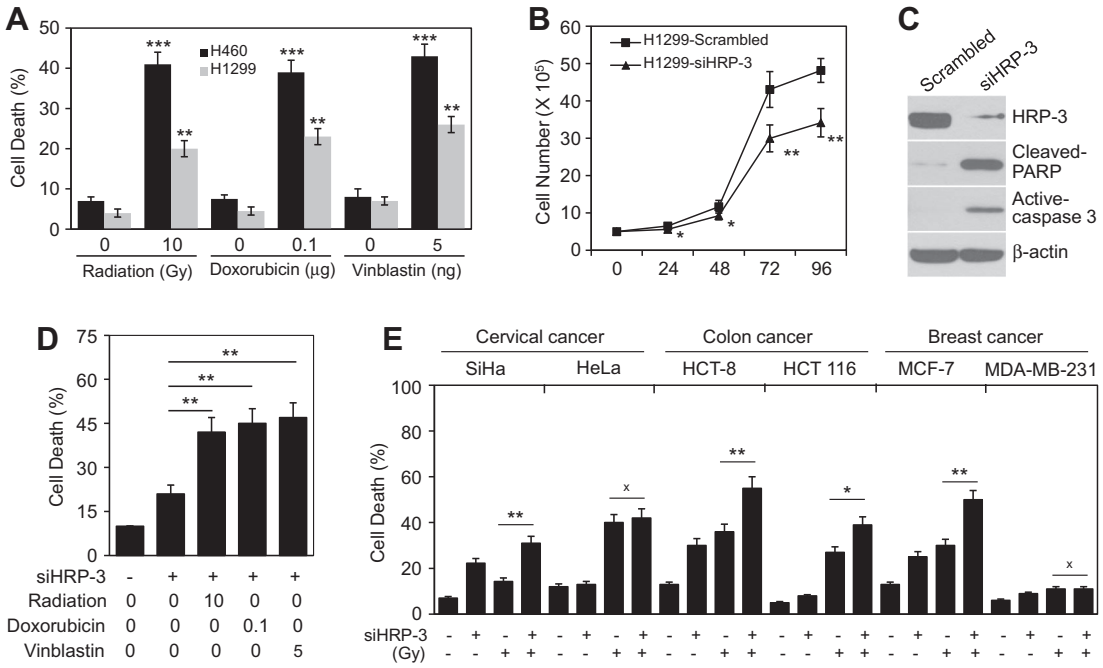

Fig. 2. Synergistic effects of HRP-3 depletion and treatment with radiation or anti-cancer drugs on cytotoxicity toward H1299 cells. (A) H460 and H1299 cells were treated as in Fig. 1E for 48 h. Cell death was determined by FACS analysis, and data are expressed as means ± SD (**$p < 0.005$ and ***$p < 0.0005$ compared with untreated controls). (B and C) H1299 cells were transfected with 100 nM scrambled or HRP-3 siRNA for 5 h and then cultured for an additional 4 d (B) or 2 d (C). Proliferation was determined by cell counting, and data are expressed as means ± SD (*$p < 0.05$ and **$p < 0.005$ compared with controls) (B) and protein levels of two apoptosis markers were determined by Western blotting (C). (D) H1299 cells were transfected with 100 nM scrambled or HRP-3 siRNA and then treated as in Fig. 1E for 48 h. Cell death was determined by FACS analysis, and data are expressed as means ± SD (**$p < 0.005$ compared with cells treated with siHRP-3 only). (E) Cells were transfected with 100 nM scrambled or HRP-3 siRNA and then treated without or with 10 Gy radiation for 48 h. FACS analysis data are expressed as means ± SD (*$p < 0.05$ and **$p < 0.05$ compared with cells treated with radiation alone; x, not significant).

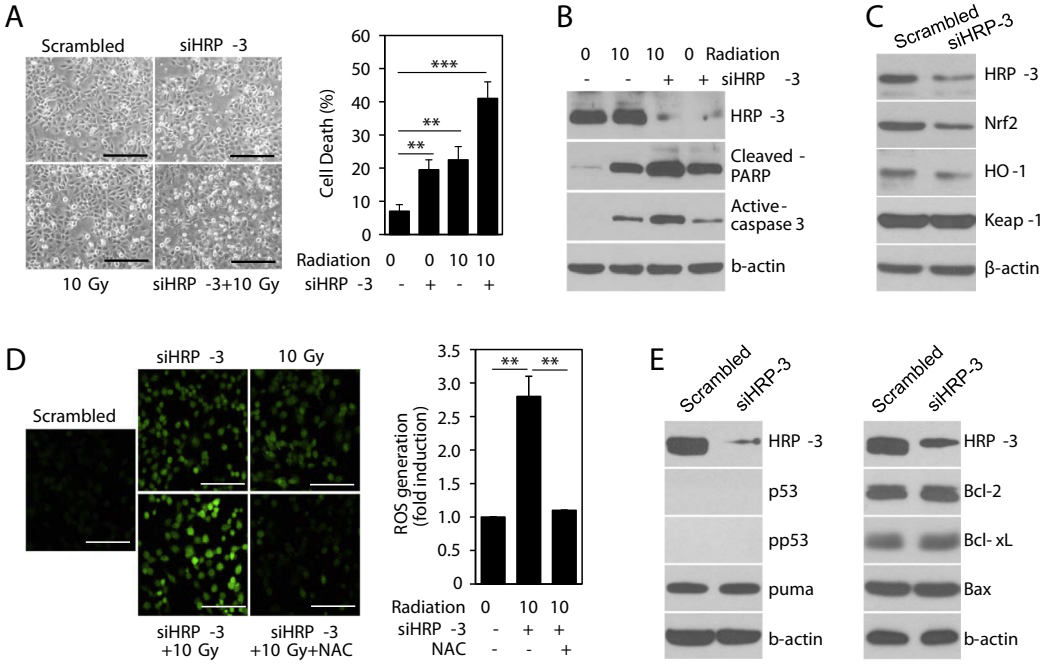

Fig. 3. HRP-3 knockdown-induced apoptosis is associated with ROS generation, but not Bax family modulation, in H1299 cells. (A and B) H1299 cells were stimulated as in Fig. 2E. Morphological changes were observed by light microscopy (A, left; scale bars: 1 mm) and cell death was determined by FACS analysis (A, right). Data are expressed as means ± SD (**$p < 0.005$ and ***$p < 0.0005$ compared with untreated controls). Levels of HRP-3 and apoptosis-related proteins were determined by Western blotting (B). (C) H1299 cells were transfected with 100 nM scrambled or HRP-3 siRNA and incubated for 48 h. Levels of ROS-related proteins were determined by Western blotting. (D) H1299 cells were stimulated as in Fig. 2E in the absence or presence of 3 mM NAC. Fluorescence in cells stained with 10 nM DCF-DA was analyzed by a laser-scanning confocal microscopy (D, left; scale bar, 1 mm), and ROS production was assessed by FACS analysis of DCF-DA-stained cells (D, right). Data are expressed as means ± SD (**$p < 0.05$ compared with controls). (E) H1299 cells were transfected as in C. Levels of cell death-related proteins were determined by Western blotting.

Laser scanning confocal microscopy also showed that HRP-3 interference alone or in combination with radiation markedly induced ROS generation in H1299 cells (Fig. 3D, left). In siHRP-3-treated H1299 cells exposed to radiation, ROS was induced by approximately 2.8-fold compared to control cells. This induction was reduced to normal values by the ROS scavenger NAC (Fig. 3D, right), demonstrating that HRP-3 plays a pivotal role in the regulation of ROS independent of p53. We previously reported that the p53 and Bcl-2 family proteins, Bax and Bcl-2, as well as PUMA acted as downstream signaling effectors of ROS to induce apoptosis of A549 cells [11,12]. However, none of these signal transduction pathways were involved in H1299 cell death induced by HRP-3 interference (Fig. 3E), confirming the association of ROS-dependent and p53-independent mechanisms with other signaling pathways.

3.4. ROS-dependent NF-κB activation and c-Myc/Noxa expression are essential for siHRP-3-induced apoptosis of H1299 cells

To dissect the effectors involved in regulating HRP-3 depletion-induced apoptosis, we focused on activation of NF-κB, a transcription factor activated by oxidant mechanisms [12]. Transfection of H1299 cells with siHRP-3 led to degradation of Iκ-B protein (Fig. 4A, left) and this phenomenon was associated with an increase (1.8-fold) in NF-κB activity compared to control cells (Fig. 4A, right). These findings are supported by the observed increases in the expression of c-Myc, a transcriptional target of NF-κB, and Noxa, a transcriptional target of c-Myc (Fig. 4A, left). To more firmly establish the apoptotic role of NF-κB in H1299 cells, we monitored changes in NF-κB after treating HRP-3-silenced cells with radiation. Although treatment of H1299 cells with radiation alone caused degradation of Iκ-B and induction of c-Myc/Noxa expression compared to control cells, these phenomena were enhanced in HRP-3-knockdown H1299 cells treated with radiation (Fig. 4B, left). Specifically, whereas radiation alone increased NF-κB activity by approximately 1.8-fold, it increased NF-κB by 3.1-fold in HRP-3-depleted H1299 cells (Fig. 4B, right). As shown in Fig. 4C, Iκ-B degradation and NF-κB activation induced by siHRP-3 were completely restored to control levels by scavenging ROS with NAC. Consistent with this, NAC dramatically attenuated the increase in c-Myc and Noxa levels induced in H1299 cells by transfection of siHRP-3 (Fig. 4C, left). Finally, we confirmed that accumulation of c-Myc and Noxa proteins was due to NF-κB using the inhibitor of NF-κB activation, BAY 11-7082 (BAY). As expected, treatment of H1299 cells with BAY blocked siHRP-3-induced Iκ-B degradation (Fig. 4D, left) and NF-κB activation (Fig. 4D, right). In line with these results, the expression of c-Myc and Noxa proteins, which were highly induced by siHRP-3, were down-regulated by BAY in parallel with the decrease in NF-κB activity (Fig. 4D, left). These results demonstrate that ROS-induced NF-κB activity contributes to the apoptotic commitment of H1299 cells through a cascade involving the NF-κB downstream targets, c-Myc and Noxa.

4. Discussion

The tumor suppressor gene *p53* is frequently somatically mutated or deleted in various human NSCLC cells, and this status usually leads to elevated cellular resistance to anticancer drugs or radiation. In keeping with this, A549 and H460 cells carrying wild-type copies of p53 are more sensitive to resveratrol-induced apoptosis than p53-deficient H1299 cells [14]. In the present study, H460 cells also showed greater sensitivity to anti-cancer drugs and radiation than H1299 cells. Because combining radiotherapy with gene therapy may be an effective strategy for treating resistant lung cancers, understanding the molecular mechanisms responsible for radiosensitivity could prove very helpful in improving cancer treatment. In our previous report, we found that depletion of the HRP-3 gene in A549 cells, which normally exhibit a distinct

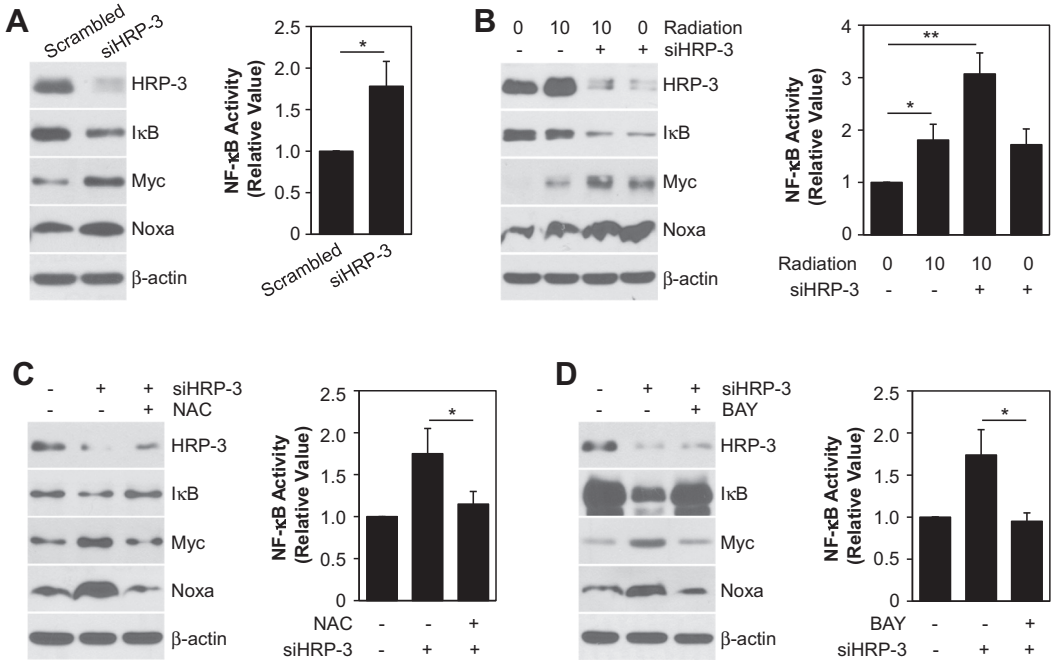

Fig. 4. ROS-dependent NF-κB activation and subsequent Myc/Noxa expression are involved in the apoptosis of HRP-3-depleted H1299 cells. (A) H1299 cells were transfected as in Fig. 3C. Protein levels were determined by Western blotting (left), and NF-κB transcriptional activity was assessed using a reporter gene assay (right). Data are expressed as means ± SD (*$p < 0.05$ compared with controls). (B-D) H1299 cells were transfected with 100 nM scrambled or HRP-3 siRNA and then left untreated or treated with 10 Gy radiation (B), 3 mM NAC (C), or 2 μM BAY 11-7082 (D) for 48 h. Protein levels were determined by Western blotting (left), and NF-κB transcriptional activity was assessed by reporter gene assay. Data are expressed as means ± SD (*$p < 0.05$ and **$p < 0.005$ compared with untreated controls in B; *$p < 0.05$ compared with cells treated with siHRP-3 alone in C and D).

radioresistant phenotype, conveyed pro-apoptotic properties through upregulation of p53-dependent PUMA expression [11]. However, HRP-3 functions and apoptotic signaling pathways in p53-null lung cancer cells have remained unknown.

Here, we demonstrated that HRP-3 knockdown strongly promoted cell death in H1299 cells via an ROS-dependent apoptotic process. The mechanism of ROS induction in H1299 cells, which included a marked decrease in the antioxidant enzyme HO-1, was completely consistent with the results obtained in A549 cells (Fig. 3), indicating that HRP-3 depletion promotes ROS induction through both p53-dependent (A549) and -independent (H1299) mechanisms. Although radiation is used extensively to treat cancer and has been shown to generate ROS in a variety of cells [13], we found that the synergistic effects of combined therapy with siHRP-3 was cell-type dependent. Notably, we found that HRP-3-knockdown H1299 cells exhibited enhanced activation of NF-κB (Fig. 4), which has dual functions as an inhibitor or activator of apoptosis according to circumstances [15]. Although several lines of evidence point to NF-κB subunits being recruited to the p53 pathway, our observation that inhibition of ROS generation suppressed siHRP-3-induced NF-κB activation suggests that NF-κB activation in p53-deficient H1299 cells is ROS dependent. Thus, NF-κB activation is capable of serving a pro-apoptotic function in H1299 cells, as amply illustrated in a recent review [15]. Although we previously established that ROS is an upstream signaling element in HRP-3-depleted A549 cells that acted through the p53/PUMA cascade to promote apoptosis, we found that the PUMA pathway was not involved in the siHRP-3-mediated apoptosis of H1299 cells. Moreover, the linkage of mitochondrial dysfunction to the NF-κB pathway targets Bax and Bcl-2 and subsequent release of cytochrome c from mitochondria- key aspects of cell death in HRP-3-knockdown A549 cells [12]-were also not involved in siHRP-3-induced apoptosis in H1299 cells (Fig. 3). Notably, we found that HRP-3 depletion induced an increase in the protein levels of the transcription factor c-Myc and the pro-apoptotic Noxa in H1299 cells. In contrast to the function of c-Myc as a promoter of cancer cell growth and survival, we found here that c-Myc participated in the apoptotic response. Generally, sensitization to or triggering of apoptosis by c-Myc can be either p53-dependent or p53-independent, depending on the cell type and apoptotic trigger [16]. Our results provide additional evidence for the association of p53-independent, c-Myc-activated apoptosis in a lung cancer cell type. Increased expression of Noxa by p53 is also fundamentally important, causing apoptosis predominantly by triggering cytochrome c release from mitochondria into the cytosol [16]. Although the genes for c-Myc and Noxa are known to be targets of p53, both proteins played a pivotal role in amplifying the apoptotic pathway in p53-deficient H1299 cells. Thus, our data strongly suggest that c-Myc is a target of activated NF-κB in siHRP-3-transfected H1299 cells, and the subsequent increase in c-Myc expression may directly induce Noxa gene expression to induce cytotoxicity. These findings are fully consistent with the p53-independent and c-Myc-dependent transactivation of the *Noxa* promoter observed in melanoma cancer cells stimulated with the anticancer agent bortezomib [17].

In conclusion, NF-κB activation is required for the siHRP-3-induced signaling pathways upstream of c-Myc and Noxa in the absence of functional p53. In addition, siHRP-3 can mediate both p53-dependent and -independent apoptotic signals and intersects with the radiation-response pathway in a cell-type dependent manner. Therefore, our results suggest that the application of HRP-3 knockdown, in combination with other methods of radiotherapy or chemotherapy, is a promising new therapeutic strategy for the treatment of both p53 wild-type and p53-null lung cancers.

Acknowledgments

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References

[1] F. Dietz, S. Franken, K. Yoshida, H. Nakamura, J. Kappler, V. Gieselmann, The family of hepatoma-derived growth factor proteins: characterization of a new member HRP-4 and classification of its subfamilies, Biochem. J. 366 (2002) 491–500.
[2] T. Kuroda, H. Tanaka, H. Nakamura, Y. Nishimune, T. Kishimoto, Hepatoma-derived growth factor-related protein (HRP)-1 gene in spermatogenesis in mice, Biochem. Biophys. Res. Commun. 262 (1999) 433–437.
[3] K. Thakar, I. Votteler, D. Kelkar, T. Shidore, S. Gupta, S. Kelm, F. Dietz, Interaction of HRP-2 isoforms with HDGF: chromatin binding of a specific heteromer, FEBS J. 279 (2012) 737–751.
[4] D.P. Singh, N. Ohguro, T. Kikuchi, T. Sueno, V.N. Reddy, K. Yuge, L.T. Chylack, T. Shinohara, Lens epithelium-derived growth factor: effects on growth and survival of lens epithelial cells, keratinocytes, and fibroblasts, Biochem. Biophys. Res. Commun. 267 (2000) 373–381.
[5] G. Maertens, P. Cherepanov, W. Pluymers, K. Busschots, E. De Clercq, Z. Debyser, Y. Engelborghs, LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells, J. Biol. Chem. 278 (2003) 33528–33539.
[6] K. Ikegame, M. Yamamoto, Y. Kishima, H. Enomoto, K. Yoshida, M. Suemura, T. Kishimoto, H. Nakamura, A new member of a hepatoma-derived growth factor gene family can translocate to the nucleus, Biochem. Biophys. Res. Commun. 266 (1999) 81–87.
[7] E. Ortega-Paino, J. Fransson, S. Ek, C.A. Borrebaeck, Functionally associated targets in mantle cell lymphoma as defined by DNA microarrays and RNA interference, Blood 111 (2008) 1617–1624.
[8] H.M. El-Tahir, M.M. Abouzied, R. Gallitzendoerfer, V. Gieselmann, S. Franken, Hepatoma-derived growth factor-related protein-3 interacts with microtubules and promotes neurite outgrowth in mouse cortical neurons, J. Biol. Chem. 284 (2009) 11637–11651.
[9] M.M. Abouzied, S.L. Baader, F. Dietz, J. Kappler, V. Gieselmann, S. Franken, Expression patterns and different subcellular localization of the growth factors HDGF (hepatoma-derived growth factor) and HRP-3 (HDGF-related protein-3) suggest functions in addition to their mitogenic activity, Biochem. J. 378 (2004) 169–176.
[10] Q. Xiao, K. Qu, C. Wang, Y. Kong, C. Liu, D. Jiang, H. Saiyin, F. Jia, C. Ni, T. Chen, Y. Zhang, P. Zhang, W. Qin, Q. Sun, H. Wang, Q. Yi, J. Liu, H. Huang, L. Yu, HDGF-related protein-3 is required for anchorage-independent survival and chemoresistance in hepatocellular carcinomas, Gut 62 (2013) 440–451.
[11] H.S. Yun, E.H. Hong, S.J. Lee, J.H. Baek, C.W. Lee, J.H. Yim, H.D. Um, S.G. Hwang, Depletion of hepatoma-derived growth factor-related protein-3 induces apoptotic sensitization of radioresistant A549 cells via reactive oxygen species-dependent p53 activation, Biochem. Biophys. Res. Commun. 439 (2013) 333–339.
[12] M.J. Kim, H.S. Yun, E.H. Hong, S.J. Lee, J.H. Baek, C.W. Lee, J.H. Yim, J.S. Kim, J.K. Park, H.D. Um, S.G. Hwang, Depletion of end-binding protein 1 (EB1) promotes apoptosis of human non-small-cell lung cancer cells via reactive oxygen species and Bax-mediated mitochondrial dysfunction, Cancer Lett. 339 (2013) 15–24.
[13] E.H. Hong, S.J. Lee, J.S. Kim, K.H. Lee, H.D. Um, J.H. Kim, S.J. Kim, J.I. Kim, S.G. Hwang, Ionizing radiation induces cellular senescence of articular chondrocytes via negative regulation of SIRT1 by p38 kinase, J. Biol. Chem. 285 (2010) 1283–1295.
[14] D.C. Ferraz da Costa, F.A. Casanova, J. Quarti, M.S. Malheiros, D. Sanches, P.S. Dos Santos, E. Fialho, J.L. Silva, Transient transfection of a wild-type p53 gene triggers resveratrol-induced apoptosis in cancer cells, PLoS One 7 (2012) e48746.
[15] N.D. Perkins, T.D. Gilmore, Good cop, bad cop: the different faces of NF-kappaB, Cell Death Differ. 13 (2006) 759–772.
[16] B. Hoffman, D.A. Liebermann, Apoptotic signaling by c-MYC, Oncogene 27 (2008) 6462–6472.
[17] M.A. Nikiforov, M. Riblett, W.H. Tang, V. Gratchouck, D. Zhuang, Y. Fernandez, M. Verhaegen, S. Varambally, A.M. Chinnaiyan, A.J. Jakubowiak, M.S. Soengas, Tumor cell-selective regulation of NOXA by c-MYC in response to proteasome inhibition, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 19488–19493.