

Full-length article

Over-expression of nm23-H1 in HeLa cells provides cells with higher resistance to oxidative stress possibly due to raising intracellular p53 and GPX1¹

Run AN^{2,3}, Yong-lie CHU², Chan TIAN³, Xiao-xia DAI², Jing-hong CHEN², Qi SHI³, Jun HAN³, Xiao-ping DONG^{3,4}²School of Medicine, Xi'an Jiao-Tong University, Xi'an 710061, China; ³State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 100052, China

Key words

nm23-H1; oxidative stress; reactive oxygen species; cell viability; p53; GPX1

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⁴ Correspondence to Prof Xiao-ping DONG.
Phn 86-10-8353-4616.
Fax 86-10-6353-2053.
E-mail dongxp238@sina.com

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Abstract

Aim: To determine whether the antitumor factor nm23 is related with antioxidation. **Methods:** Full-length human nm23-H1 was cloned into a mammalian-expressing vector and transiently introduced into HeLa cells. **Results:** A remarkably low level of reactive oxygen species (ROS) was detected in the cells overexpressing nm23-H1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and trypan blue assays found that the cells transfected with a nm23-H1-expressing plasmid had higher viability and stronger resistance to oxidative stress. Immunoprecipitation tests revealed that endogenous nm23-H1 formed a protein complex with p53. Furthermore, the intracellular levels of p53 and p53-regulated gene *GPX1* were obviously increased in the cells overexpressing nm23-H1. The downregulation of p53 in the cells overexpressing nm23-H1 resulted in a higher cellular ROS level and lower cell viability. **Conclusion:** The findings suggest that nm23-H1 may act as a cellular protector against oxidative stress, possibly triggering the p53-related antioxidative pathway.

Introduction

Oxidative stress is known for its involvement in the pathophysiology of many human diseases, including, but not restricted, to cancer. Reactive oxygen species (ROS) changes have been implicated at all stages of the carcinogenic process^[1]. Oxidative DNA damage can trigger tumor initiation. In terms of cell proliferation, ROS have been shown to modulate the cell cycle through the modulation of various cell cycle proteins, including p53^[2] and the ataxia telangiectasia-mutated protein^[3]. Metastasis is an integral part of tumor progression, during which ROS have been documented to play a major role^[4,5]. A previous study reported that the long-term exposure of mouse NMuMG mammary epithelial cells to H₂O₂, which mimics chronic inflammation, showed a cellular phenotypic conversion with striking similarities to malignant transformation, accompanied by the induction of genes associated with cell adhesion and migratory behavior, together with the activation of the small G protein Rac1 and

mitogen-activated protein (MAP) kinases^[6]. Importantly, following these changes, the epithelial cells ultimately acquire the potential to invade a reconstituted basement membrane in the presence of normal fibroblasts. In fact, various studies have shown that metastatic tumor cells produce higher levels of ROS than primary malignant cells, which greatly reduce metastasis, together with increasing levels of ROS metabolizing enzymes and antioxidant compounds^[7-9].

nm23 is an antitumoral metastatic gene that was first reported in murine melanoma. In humans, 8 related genes, *nm23-1* to *-8*, have been described^[10]. The first 2 genes (*nm23-H1* and *nm23-H2*), as well as their respective counterparts in rats (*nm23-β* and *nm23-α*) and mice (*nm23-M1* and *nm23-M2*), have been shown to encode different isoforms of the enzyme, named nucleoside diphosphate kinase (NDPK). Extensive studies have revealed that the nm23 protein participates in many biological activities, including development, differentiation, proliferation, endocytosis, and apoptosis^[11]. Recently, nm23-H1 was

confirmed to have been involved in the regulation of the p53-induced signaling pathway, in which nm23-H1 acts as a positive regulator^[12]. Additionally, mouse nm23-M2 can activate endogenous transcription of the *nm23-M1* gene, and the over-expression of nm23-M1 and -M2 prevents the cells from oxidative stress-induced death^[13].

Human p53 is a 393 amino acid nuclear phosphoprotein and transcription factor. Evidence highlights that p53 works in a more positive way, participating in the maintenance of intracellular redox homeostasis and the protection of the genome from oxidative damage. Several p53-regulated genes, such as *GPX1*, *SOD2*, and aldehyde dehydrogenase 4 family member A1 (*ALDH4A1*), encode products as antioxidants^[14,15]. The reactivation of over-oxidized Prx is mediated by 2 p53-regulated sestrins, namely PA26 and Hi95 (encoded by *SESN1* and *SESN2*, respectively), which are required for the regeneration of Prx containing cysteine sulfuric acid^[16,17]. In the absence of stress or after mild stress, a relatively low level of p53 is sufficient to upregulate several antioxidant genes that decrease ROS levels and protect cells from DNA damage^[18].

To address the possibility that the antitumoral activity of nm23-H1 is related with antioxidation, a recombinant plasmid encoding the full-length human *nm23-H1* gene was constructed and transiently induced into HeLa cells in this study. A remarkably low level of ROS and strong resistance to oxidative stress were observed in the cells overexpressing nm23-H1. Furthermore, we found that the levels of p53 and p53-regulated gene *GPX1* were markedly increased in the cells receiving the nm23-H1-expressing plasmid. The downregulation of p53 in the cells overexpressing nm23-H1 resulted in higher cellular ROS and lower cell viability; the cellular level of GPX1 also decreased. This suggests that nm23-H1 as a metastasis suppressor may act as a protector against oxidative stress, possibly triggering the p53-related antioxidative pathway.

Materials and methods

Plasmid construction The full-length human nm23-H1 (X73066) sequence was amplified using PCR. The primers used were human nm23-H1-P1 (GGATCC ATG GCC AAC TGT GAG CG, with the *Bam*HI site underlined) and human nm23-H1-P2 (GAATTC CCT CCT GTC ATT CAT AGA T, with an *Eco*RI site underlined). The PCR product was cloned into a pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA), generating recombinant plasmid pcDNA-nm23-H1.

Cell culture and transient transfection Human cervical carcinoma cell line HeLa was maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, USA). The cells were plated into 6-, 24-, and 96-well plates (Falcon, Japan) 1 d before transfection. The recombinant plasmid pcDNA-nm23-H1, as well as vector pcDNA3.1, was transfected into the monolayer cells with Lipofectamine 2000 transfection reagent (Invitrogen, USA). Twenty-four hours after transfection 5, 10, 20, or 40 $\mu\text{mol/L}$ H_2O_2 was added to DMEM and maintained for another 12 h. The cells were then harvested in phosphate-buffered saline (PBS), pelleted by centrifugation, and suspended in lysis buffer (10 mmol/L Tris-HCl, pH 7.8, 0.5% sodium deoxycholate, 0.5% Nonidet P-40, 100 mmol/L NaCl, and 10 mmol/L EDTA) supplemented with a complete protease inhibitor mixture. The protein content of each lysate was determined using the BCA assay kit (Beyotime Biotechnology, Haimen, Jiangsu, China).

Western blotting Various cell lysates were separated by 12% SDS-PAGE and electrotransferred onto nitrocellulose membranes. After blocking with 5% non-fat milk in PBST (PBS, pH 7.6, containing 0.05% Tween-20) overnight at 4 °C, the membranes were incubated with 1:100 nm23-H1, 1:100 p53 antibody (Boston, China), or 1:1000 GPX1 antibody (Cell Signaling Technology, Danvers, MA, USA) for 2 h at room temperature, and then incubated with 1:2000 horseradish peroxidase-conjugated antimouse immunoglobulin G (IgG) or anti-rabbit IgG (Santa Cruz, Santa Cruz, CA, USA). The protein bands were visualized by an ECL kit (PE Applied Biosystems, Foster City, CA, USA).

Measurement of intracellular oxidative activity Determination of the level of intracellular reactive oxygen species was carried out with a ROS assay kit (Beyotime Biotechnology, Haimen, China). Briefly, the cells were treated with dihydrodichloro-fluorescein diacetate (DCF-DA) in serum-free medium at 37 °C for 30 min and harvested in PBS. The fluorescence of each well was then measured on a flow cytometer with 480 nm excitation and 530 nm emission wavelengths.

Trypan blue and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays The cells were cultured in 24-well tissue culture plates and transiently transfected with nm23-H1-expressing plasmids or pcDNA3.1. Forty-eight hours after transfection, the cells were harvested and stained with the DNA-binding dye trypan blue (Sigma, St Louis, MO, USA) for 30 min. The number of trypan blue positive-stained cells in each well was counted under a microscope. To evaluate the cell

viability, the cells were plated as normal in 96-well trays and exposed to 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, USA) in culture medium for 4 h. After being lysed, the formazan product was dissolved in DMSO. Absorbance was measured at 540 nm in a spectrophotometer.

siRNA experiment To interfere with the expression of the p53 protein in the cultured cells, a commercial p53 siRNA kit (SignalSilence p53 siRNA kit; Cell Signaling Technology, Danvers, MA, USA) was used for generating a status of downregulated p53. In brief, the HeLa cells were plated into 6-well tissue culture plates 1 d before transfection. pcDNA-nm23-H1 was cotransfected into the cells with transfection reagent and p53 siRNA or control siRNA. Forty-eight hours after transfection, the cellular expression of p53 and other cytokines were evaluated by Western blotting.

Statistical analysis Statistical analysis was performed using the *t*-test. Probabilities of less than 0.05 were considered statistically significant.

Results

Cells overexpressing nm23-H1 showed more active growth capacity Recombinant plasmid pcDNA-nm23-H1 was transiently transfected into the HeLa cells, and the expression of nm23-H1 was evaluated by nm23-H1-specific Western blotting. Figure 1A shows that the cells receiving pcDNA-nm23-H1 produced a remarkably higher level of nm23-H1 signals, compared with the cells receiving pcDNA3.1. To observe the influence of the over-expression of the nm23-H1 protein on cell growth activities, the viabilities of the transfected cells were

measured 48 h after transfection with trypan blue and MTT assays. The MTT analyses showed that more dead cells in the cells with pcDNA3.1 than those with pcDNA-nm23-H1 (Figure 1B). The mean optical density (*OD*) value of the cells overexpressing nm23-H1 was 1.411, while that of the cells transfected with pcDNA3.1 was 0.978, with statistical differences ($P=0.007$). The trypan blue assays revealed that the viabilities of the HeLa cells overexpressing nm23-H1 were repeatedly higher than that of the cells transfected with plasmid pcDNA3.1 (Figure 1C), showing statistical differences ($P=0.003$). The results suggest that the over-expression of nm23-H1 helps the cells to maintain more active growth ability.

Cells overexpressing nm23-H1 possessed low levels of intracellular free radicals To address the influence of nm23-H1 on the intracellular oxidation process, the generation of free radicals in the cells receiving pcDNA-nm23-H1 and pcDNA3.1 was measured by staining with fluorescent dye DCF-DA. Compared with the preparation transfected with pcDNA3.1, the ROS level in the cells overexpressing nm23-H1 was obviously lower (Figure 2A). The average ROS level in the pcDNA3.1 groups was 1.33-fold higher than that of nm23-H1 (Figure 2B), revealing statistical difference ($P=0.013$).

To test the antioxidant role of nm23-H1 under oxidative stress, the mock and nm23-H1 overexpressed cells were challenged with 10 $\mu\text{mol/L}$ H_2O_2 for 12 h and the ROS levels were measured. An obviously high level of intracellular free radicals was seen in all preparations after treatment with 10 $\mu\text{mol/L}$ H_2O_2 , compared with those without H_2O_2 challenge. As expected, a stronger decrease of intracellular free radicals was identified in the cells expressing nm23-H1, showing statistical difference

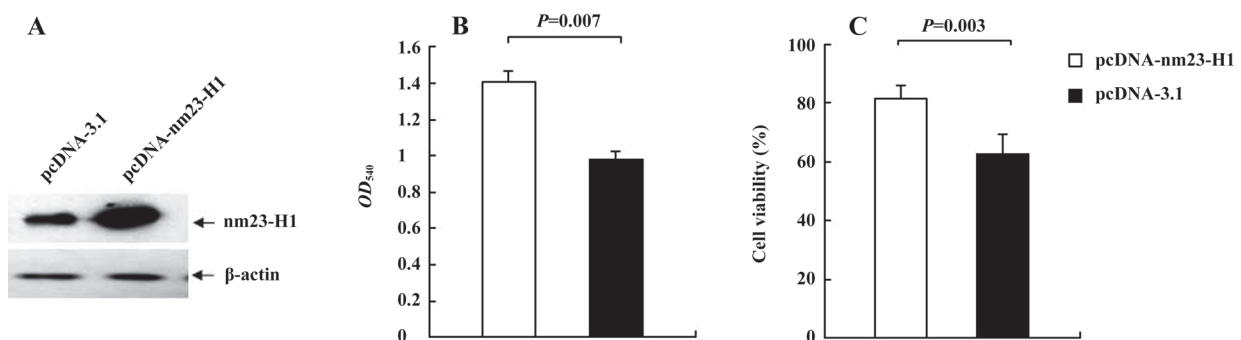


Figure 1. Over-expression of nm23-H1 in HeLa cells increased the cell viability. Cells were analyzed 48 h after transfection. (A) analysis of the expression of nm23-H1 in HeLa cells by Western blotting. Preparations of transfections of recombinant plasmids pcDNA-nm23-H1 and pcDNA3.1 are indicated at the top. Specific signals of nm23-H1 and β -actin are shown with arrows. (B) MTT. Average *OD* values are indicated left of the panels. (C) trypan blue staining. Average cell viabilities (%) are illustrated left of the panels. Average data of each preparation was calculated based on 3 independent experiments and represented as mean \pm SD. Statistical differences between groups are shown at the top.

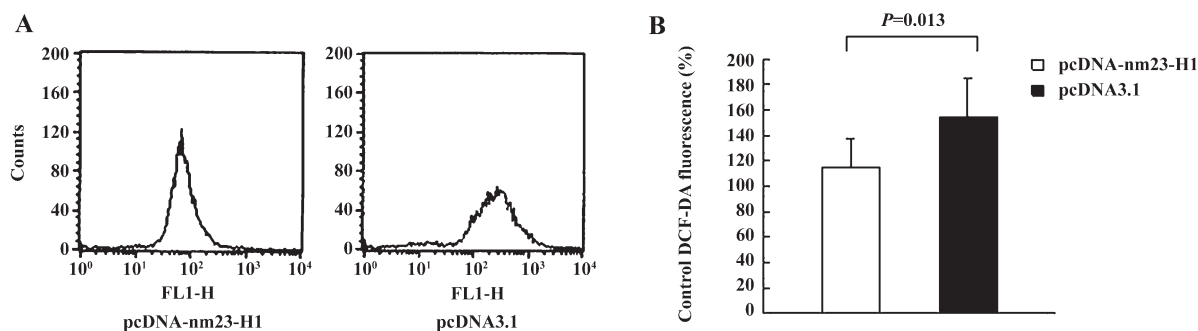


Figure 2. Evaluations of the levels of intracellular ROS after transfection of pcDNA-nm23-H1 and pcDNA3.1. Cells were analyzed 48 h after transfection. (A) measurements of the cells stained with fluorescent dye DCF-DA on a flow cytometer with 480 nm excitation and 530 nm emission wavelengths. (B) average ROS values calculated based on 3 independent experiments and represented as mean \pm SD. ROS values are indicated left of the panel. Statistical differences between groups are shown at the top.

compared with the cells with pcDNA3.1 ($P<0.05$, data not shown). The results presented suggest that nm23-H1 over-expression in the HeLa cells may provide a protective function by participating in antioxidant defense.

Cells overexpressing nm23-H1 were more resistant to the challenge of H₂O₂ To observe the possible effectiveness of the antioxidation capacity produced by the expression of exogenous nm23-H1 on cell growth, HeLa cells transfected with pcDNA-nm23-H1 and pcDNA3.1 were challenged with different amounts of H₂O₂ for 12 h and the cell viabilities were evaluated by MTT assays. It showed that the number of living cells was reduced, along with increases in the amount of H₂O₂ in all preparations. Compared with the mock cells, more living cells were observed in that of nm23-H1 (Figure 3). To address the difference in the reduction extent of living cells, the relative cell viability of each sample was calculated by dividing the OD value at various concentrations of H₂O₂ with that of the

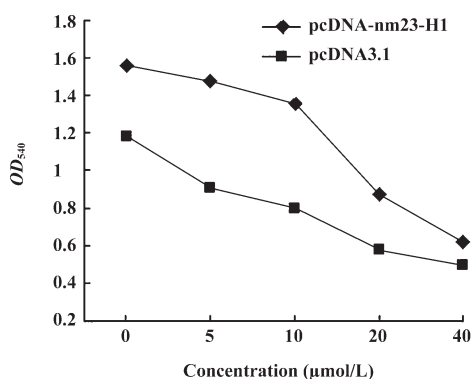


Figure 3. Cells overexpressing nm23-H1 possessed higher antioxidative activity against H₂O₂ to a certain extent. Average OD value of each reaction was calculated with 3 independent tests.

same preparation without treatment of H₂O₂. More alive cells were observed in the groups of pcDNA-nm23-H1 at 5, 10, and 20 μmol/L ($P<0.05$ data not shown), but not at 40 μmol/L. These results indicate that the over-expression of nm23-H1 in the monolayer cells protects the cells against oxidative damage to a certain extent.

Over-expression of nm23-H1 upregulated the expressions of p53 and p53-regulated gene GPX1 A previous study reported that the nm23-H1 tumor suppressor could activate p53 function. To observe the possible influence of nm23-H1 on the expression of p53, recombinant plasmid pcDNA-nm23-H1 was transfected into the cultured HeLa cells. p53-specific Western blotting found that the level of cellular p53 was clearly increased in the cells receiving pcDNA-nm23-H1, but remained unchanged in the cells receiving pcDNA3.1 compared with the mock cells (Figure 4A). As with the expression of p53, the level of GPX1, a p53-regulated gene product, was markedly increased in the cells transfected with pcDNA-nm23-H1 (Figure 4A). This indicates that the expression of nm23-H1 increases the intracellular levels of p53 and p53-regulated GPX1.

To observe whether nm23-H1 formed a molecular complex with p53, total HeLa extracts were prepared and co-immunoprecipitation experiments were carried out. Using the p53 antibody or glutathione *S*-transferase (GST) antibody as the capturing antibody and the nm23-H1 antibody as the detecting antibody, obvious nm23-H1 signaling was detected in the preparations precipitated with the p53 antibody, but not in those with the GST antibody (Figure 4B). In contrast, the HeLa extracts were precipitated with nm23-H1 or GST antibodies, and subsequently detected by p53-specific Western blotting. As expected, specific p53 signals were observed in the preparations precipitated

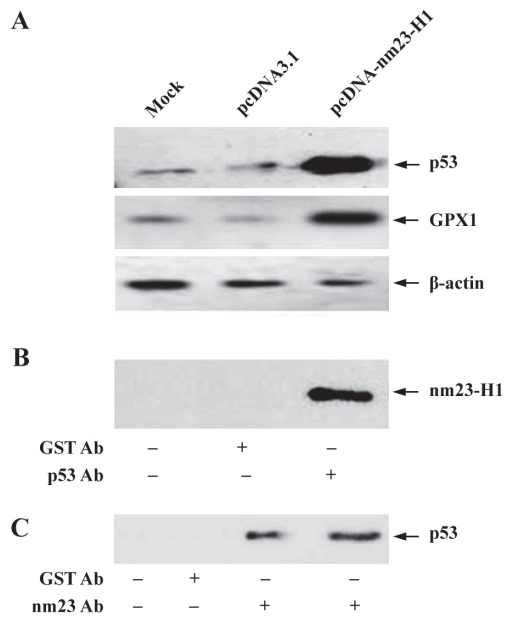


Figure 4. Overexpressing nm23-H1 upregulated the intracellular levels of p53 and p53-regulated gene *GPX1*, and analyses of the molecular interaction between endogenous p53 and nm23-H1 in HeLa cells by co-immunoprecipitation. (A) cells were analyzed 48 h after transfection, and the expressions of p53 and *GPX1* were evaluated by Western blotting. Preparations of mock HeLa cells and transfections of recombinant plasmids pcDNA-nm23-H1 and pcDNA3.1 are shown at the top. (B) precipitation with the p53 antibody and illustrated with the nm23-H1 antibody, respectively. (C) precipitation with the nm23-H1 antibody and illustrated with the p53 antibody, respectively. Precipitations with the GST antibody were used as control. Additions of the capturing antibodies in each preparation are shown at the bottom. Specific signals of p53, *GPX1*, β-actin, and nm23-H1 are shown with arrows.

with the nm23-H1 antibody, but not in those with the GST antibody (Figure 4C). These results suggest that nm23-H1 may form a physical protein complex with p53.

Downregulation of cellular p53 level elevated intracellular ROS and decreased viability in the cells overexpressing nm23-H1 In order to assess the detailed contribution of p53 to the antioxidation of nm23-H1, the cultured HeLa cells were cotransfected with pcDNA-nm23-H1 and p53 siRNA. In contrast, cells were cotransfected with pcDNA-nm23-H1, and unrelated control siRNA was supplied as the negative control. Forty-eight hours after transfection, the cells were harvested and the expressions of p53 and other agents were evaluated by Western blotting. Figure 5 shows that the p53 protein level was markedly decreased in the cells receiving p53 siRNA and pcDNA-nm23-H1, compared with the mock cells transfected with pcDNA-nm23-H1 alone and the cells transfected with a

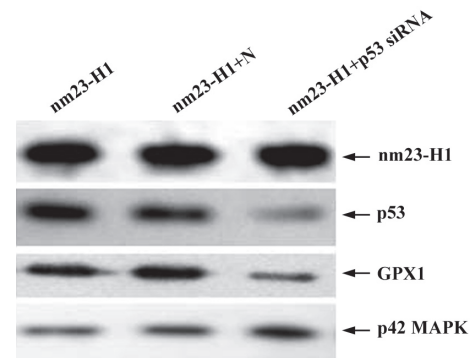


Figure 5. Transfection of p53 siRNA efficiently downregulated the expressions of cellular p53 and *GPX1* in the cells overexpressing nm23-H1. HeLa cells were cotransfected with pcDNA-nm23-H1 and p53 siRNA (illustrated as nm23-H1+p53 siRNA), pcDNA-nm23-H1 and negative control siRNA (illustrated as nm23-H1+N), or pcDNA-nm23-H1 (illustrated as nm23-H1). Forty-eight hours after transfection, the expressions of nm23, p53, *GPX1*, and p42 MAP kinase were evaluated by Western blotting. Various preparations are indicated at the top. Specific signals of nm23, p53, *GPX1*, and p42 MAP kinase are shown with arrows.

combination of pcDNA-nm23-H1 and control siRNA. In contrast to p53, the levels of nm23 and p42 MAP kinase, which were used as a control in various preparations, were almost comparable. This indicates that the introduction of p53 siRNA successfully reduces the expression of p53, while the expression of nm23-H1 after the transfection of pcDNA-nm23-H1 is not affected. Moreover, the level of *GPX1* in the cells receiving p53 siRNA was notably decreased (Figure 5), highlighting that the expression of *GPX1* was highly repressed after p53 knockdown, even in the cells overexpressing nm23-H1.

To observe the influences of p53 knockout on the features of antioxidation and growth of the cells, the over-expression of nm23-H1, cellular ROS level, and viability were measured 48 h after transfection. Compared with the cells receiving pcDNA-nm23-H1 and control siRNA, the ROS level in the cells receiving pcDNA-nm23-H1 and p53 siRNA were markedly increased (Figure 6A), 1.52-fold higher than that of the control (Figure 6B), revealing statistical difference ($P=0.031$). The MTT assays revealed that the viability of the cells transfected with pcDNA-nm23-H1 and p53 siRNA was obviously lower than that of the cells transfected with a combination of pcDNA-nm23-H1 and control siRNA (Figure 6C), with statistical differences ($P=0.004$). These data suggest that the increased resistance to oxidative stress in the cells after the over-expression of nm23-H1 is due to the p53-related antioxidant pathway.

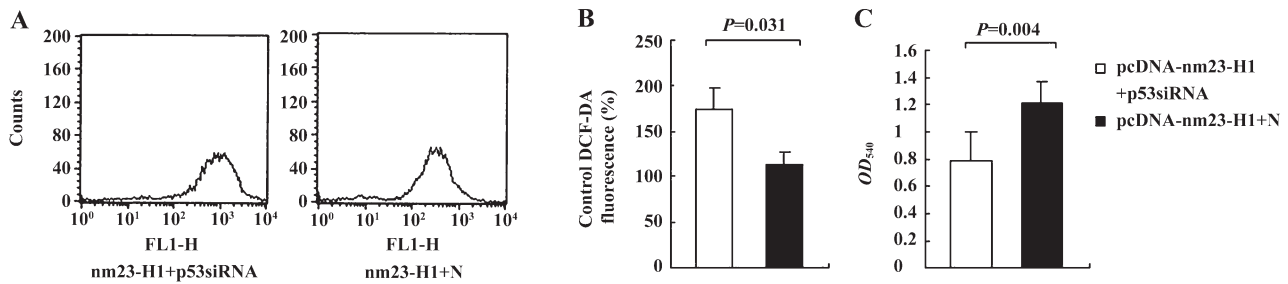


Figure 6. Downregulation of p53 in the cells overexpressing nm23-H1 decreased the cell viability and elevated the intracellular ROS. (A) measurements of the cells stained with fluorescent dye DCF-DA on a flow cytometer with 480 nm excitation and 530 nm emission wavelengths 48 h after transfection. nm23-H1+p53 siRNA, transfection of pcDNA-nm23-H1 and p53 siRNA; nm23-H1+N, transfection of pcDNA-nm23-H1 and control siRNA. (B) average ROS values calculated based on 3 independent experiments and represented as mean \pm SD. ROS values are indicated left of the panels. Statistical differences between groups are shown at the top. (C) MTT assays. Average OD values are indicated left of the panels. Average data of each preparation was calculated based on 3 independent experiments and represented as mean \pm SD. Statistical differences between groups are shown at the top.

Discussion

In this report, we provide evidence that the transient expression of human nm23-H1 in HeLa cells endows the cells not only higher viability, but also a lower level of ROS and stronger resistance against oxidative stress *in vitro*. As a potential tumor repressor, *nm23* family products are involved in many cellular biological processes. The expression of *nm23* products in malignant tumors is believed to be a marker, representing relatively good prognosis^[17]. It has been reported that cells overexpressing either mouse *nm23-M1* or *-M2* products, NDPKA or NDPKB, could protect the cells from oxidative stress-induced death^[13]. Transferring the orthologous *NDPK2* gene into *Arabidopsis thaliana* has been shown to induce plant tolerance against several environmental stresses, such as cold conditions, salt, and H₂O₂ by decreasing the level of ROS. The excessive production of ROS as a result of oxidative stress may cause cell death, or sometimes induce chronic inflammation, which could be due to severely damaged cell DNA^[18]. Both NDPKA and NDPKB may act as DNA glycosylase lyases^[19], which are involved in the mechanisms of oxidative DNA damage repair^[20]. Therefore, the nm23-reduced intracellular ROS level may be involved in cell protective activity.

We have addressed the molecular interaction between nm23-H1 and p53 in HeLa cells. *nm23* products are active proteins that interact with other cellular biological products. It has been shown that nm23-H1 interacts with STRAP, a transforming growth factor (TGF) receptor-interacting protein that inhibits TGF-signaling^[21]. nm23-H2 has been shown to interact with the unique A/B domain of estrogen receptor (ER)- β ^[22]. Jung *et al* found that nm23-H1 and its interacting partner STRAP were directly involved in p53

activation through a physical interaction with p53, and the over-expression of wild-type nm23-H1 or STRAP results in the upregulation of p53, as well as its targets, including p21 and Bax^[12]. Our data also confirm that nm23 can form a protein complex with p53 and up-regulate p53 in cells.

Our data reveal that not only does the level of p53 increase when overexpressing nm23-H1 in HeLa cells, but also that of GPX1. In intracellular glutathione peroxidase, GPX is one of the primary antioxidant enzymes that scavenge hydrogen peroxide and organic hydroperoxides with glutathione as the hydrogen donor. There are 3 other members of the selenium-dependent GPX family, in which cytosolic GPX (GPX1) is the predominant form^[23]. Because GPX decomposes hydrogen peroxide and organic hydroperoxides produced during normal metabolism and after oxidative insults, GPX prevents peroxide-induced DNA damage, lipid peroxidation, and protein degradation. Even very low levels of p53 present in normal tissues in the absence of stress are sufficient enough to drive the expression of several antioxidant genes^[16]. Comparative analyses of the p53 protein levels among human papillomavirus (HPV)-positive cervical carcinoma cell lines have revealed that the SiHa cell line has the highest steady-state level of p53, exhibits the highest expressions of *GPX1*, *SESN1* and *SESN2*, while possessing a higher resistance to H₂O₂ damage. When the expression of p53 is knocked down, the expressions of GPX1, SESN1, and SESN2 are also inhibited, and furthermore, the levels of ROS were raised^[24]. In fact, direct evidence of a lower GPX1 level, higher ROS level, and lower viability were obtained in the cells overexpressing nm23-H1 after the downregulation of p53 in this study. Although the exact molecular mechanism of how overexpressed nm23-H1 reduces the intracellular ROS level remains unknown, together with these data, we

can speculate that a low level of ROS and higher resistance to oxidative stress by the over-expression of nm23-H1 in HeLa cells is due to the subsequently raised expressions of p53 and GPX1.

Interestingly, the influence of p53 on the metabolism seems to be bidirectional, in that it regulates the expression of antioxidant enzymes resulting in a reduced ROS level, while occasionally inducing ROS accumulation^[25]. Sablina *et al* proposed that in the absence of stress or after mild stress, p53 induced the expression of antioxidant genes that decrease ROS levels and protected cells from DNA damage, while severe or extended stress led to stronger activation of p53, and thus, induced the pro-oxidant genes, resulting in elevated ROS levels and cell death^[16]. It lays out a cellular self-protective pathway that helps cells survive under mild stress, for example, a lower amount of H₂O₂, but promotes cell death when encountering severe stress in order to avoid the accumulation of malformed cells^[2,26]. The transient expression of nm23-H1, which increases cell survival, has been observed only with lower concentrations of H₂O₂ (<40 μmol/L) in this study. Certainly, the effectiveness of the H₂O₂ treatment on the cultured cells involves many components, besides changes of p53, but the increases of p53 and GPX should be key factors in the protective activity of nm23-H1.

Reduced nm23 expression has been correlated with poor survival or higher frequency for metastasis in human breast, gastric, cervical, ovarian, and hepatocellular carcinoma and melanoma, although it does not represent an independent prognostic factor^[17]. The over-expression of transfected *nm23* reduced the *in vivo* tumor metastatic potential of melanoma and breast carcinoma cell lines. The pathogenesis of cancer has not yet been clarified, but substantial evidence suggests that free radicals, particularly oxygen radicals, play an important role in the complex course of multistep carcinogenesis. Much of the evidence has come from the fact that antioxidants that scavenge free radicals directly or that interfere with the generation of free radical-mediated events inhibit the neoplastic process^[27]. Thereby decreasing ROS and higher resistance against oxidative stress due to the over-expression of nm23 might possibly play some role during tumor formation and metastasis.

Author contribution

Run AN, Yong-lie CHU, and Xiao-ping DONG designed research; Run AN, Chan TIAN, Xiao-xia DAI, Jing-hong CHEN, and Qi SHI performed research; Run AN, Chan TIAN, and Jun HAN analyzed data; Run AN,

Chan TIAN, Xiao-ping DONG wrote the paper.

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