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Arsenic Trioxide Inhibits the Growth of Cisplatin-Resistant Cervical Cancer through the NPM1–p53 Signaling Axis

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Abstract

Background

Cisplatin resistance in advanced cervical cancer remains a serious clinical problem. Nucleophosmin 1 (NPM1) suppresses p53 and is associated with chemotherapy resistance. Arsenic trioxide (As_2O_3) may inhibit the growth of cisplatin-resistant cells through the NPM1–p53 pathway.

Methods

NPM1 expression was analyzed in TCGA data, and serum NPM1 levels were measured by ELISA in 323 cervical cancer patients and 200 healthy controls. NPM1 was overexpressed or knocked down in HeLa and cisplatin-resistant (HeLa/DDP) cells, followed by treatment with the p53 agonist TRAP-1 or the p53 inhibitor pifithrin-α. CCK-8, flow cytometry, qRT-PCR, Western blot, and immunofluorescence were used to assess cell proliferation, apoptosis, and NPM1–p53 signaling. BALB/c nude mouse xenografts (n = 3 per group) were administered As_2O_3 (7.5 mg/kg, i.p.), followed by immunohistochemistry and Western blotting.

Results

NPM1 was highly expressed in cervical cancer tissue and serum (103.43 ± 19.78 ng/mL vs. 66.32 ± 18.74 ng/mL; $P < 0.01$) and was associated with advanced FIGO stage and poor prognosis. HeLa/DDP cells expressed higher NPM1 than HeLa cells. NPM1 overexpression reduced p53 expression and promoted cell proliferation, whereas NPM1 knockdown or p53 activation increased p53 and inhibited cell growth (all $P < 0.01$). As_2O_3 decreased NPM1, upregulated p53, and promoted apoptosis; NPM1 overexpression attenuated, whereas NPM1 knockdown enhanced, these effects (all $P < 0.01$). As_2O_3 reduced the growth of HeLa/DDP and NPM1-overexpressing xenografts, decreasing NPM1 and increasing p53 ($P < 0.01$).

Conclusions

NPM1 is upregulated in cisplatin-resistant cervical cancer and suppresses p53. As_2O_3 downregulates NPM1 and promotes p53-mediated apoptosis in cisplatin-resistant cells and xenografts. These findings suggest that As_2O_3 may be a candidate agent against platinum-resistant cervical cancer; whether it can restore cisplatin sensitivity in combination settings warrants further investigation.

1. Introduction

Cervical cancer is still the fourth most frequent cancer among women globally; in 2020, there were about 604,000 new cases and 342,000 deaths [1]. Cisplatin-based systemic chemotherapy is the first-line

treatment for patients with locally advanced, recurrent, or metastatic disease who are not suitable for radical surgery [2]. However, primary or acquired cisplatin resistance occurs in 30–60% of patients, and the median overall survival in this group is only 7–12 months [2]. Addressing cisplatin resistance therefore remains an urgent unmet clinical need.

Cisplatin is a cytotoxic drug that forms platinum–DNA adducts and interstrand crosslinks, damaging DNA and inducing apoptosis via p53 [3]. The underlying mechanisms include increased drug efflux, glutathione-mediated detoxification, enhanced nucleotide excision repair, and loss of p53 function [3–5]. Persistent high-risk HPV infection causes the degradation of p53 by E6 in cervical cancer; however, the remaining p53 can be therapeutically restored, and inhibition of p53 is associated with chemoresistance [4, 2].

Nucleophosmin 1 (NPM1) is a multifunctional nucleolar transport protein involved in ribosome synthesis, centrosome duplication, and DNA damage repair. NPM1 is overexpressed in many solid tumours and hematologic malignancies, decreases the stability and activity of p53, promotes the development of cancer stem cells, and induces drug resistance [6, 7]. Whether NPM1 promotes cisplatin resistance in cervical cancer—and whether it can be targeted pharmacologically—remains unknown.

Arsenic trioxide (As_2O_3), known as pi-shuang in traditional Chinese medicine, has demonstrated anticancer activity through p53-related mechanisms [8, 9] and reduces the invasive and metastatic properties of cervical cancer cells [10]. A previous phase II trial showed that As_2O_3 plus carboplatin is safe and effective in locally advanced cervical cancer [11]. Network pharmacology identified NPM1 as a key hub target in the action of As_2O_3 against platinum-resistant cervical cancer. Here, we investigated whether As_2O_3 inhibits the growth of cisplatin-resistant cervical cancer by modulating the NPM1–p53 signaling pathway, using a large clinical cohort, genetically modified cell lines, and a xenograft model.

2. Materials and Methods

2.1 Clinical cohort and bioinformatics

2.1.1 Patients and samples

In total, 323 patients with primary cervical cancer who had not previously undergone therapy were treated at our hospital between 2020 and 2024. All diagnoses were confirmed histologically according to the CSCO Guidelines for Cervical Cancer (2024). The age range of the patients was 25–85 years (mean = 58.93 ± 13.30). Histological subtypes: squamous cell carcinoma (81.1%), adenocarcinoma (13.9%), adenosquamous carcinoma (3.1%), and other rare types (1.9%). Serum samples from 200 healthy women served as controls. The study protocol was approved by the Ethics Committee of The First Affiliated Hospital of Guangzhou University of Chinese Medicine (approval valid from 1 November 2023), and it was carried out in line with the Declaration of Helsinki.

2.1.2 ELISA

Peripheral blood was centrifuged at 1000 × g for 20 minutes in the dark at 4°C, and the serum was stored at − 80°C. For the assay, each sample was diluted 1:1, and the measured concentration was multiplied by the dilution factor. Serum NPM1 concentration was determined using a commercial ELISA kit according to the manufacturer's instructions. The standard curve range was 0–100 ng/mL; absorbance was measured at 450 nm. Samples with concentrations above the upper limit of the standard curve were re-assayed at a higher dilution.

2.1.3 Network pharmacology and TCGA data analysis

Potential therapeutic targets related to cervical cancer were retrieved from disease-related databases, and drug-related targets were collected from corresponding compound databases. After removing duplicate targets, the intersection of disease-associated and drug-associated targets was obtained. The protein–protein interaction (PPI) network of intersecting targets was constructed using the STRING database and visualized with Cytoscape software. Topological parameters including degree, betweenness centrality and closeness centrality were calculated; targets with high degree values were defined as hub targets. RNA-seq data and clinical annotations of cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) were obtained from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/). Differential expression between tumours and normal tissues was determined by DESeq2 and edgeR ($P < 0.05$). Kaplan–Meier curves and the log-rank test were used to compare overall survival (OS) and disease-free survival (DFS) between patients with high and low NPM1 expression (median cut-off).

2.2 Cell culture

HeLa cells (BNCC342189) were cultured in high-glucose DMEM, and cisplatin-resistant HeLa/DDP cells were purchased from Henan Beina Biological Testing Co., Ltd. (Zhengzhou, Henan, China) and cultured in RPMI-1640. Both media were supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin, and the cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

2.3 Lentiviral transduction

Lentiviral vectors overexpressing NPM1 (NPM1-OE), expressing NPM1-targeting shRNA, or the corresponding negative controls were packaged in HEK293T cells with psPAX2 and pMD2.G via PEI transfection. At 48 h and 72 h, the viral supernatants were collected, filtered, and added to the target cells in the presence of Polybrene. Puromycin (4–5 μg/mL) was used to select stable cell lines. After transduction, efficiency exceeded 95%, as confirmed by fluorescence microscopy, qRT-PCR and Western blot.

2.4 Proliferation and apoptosis assays

2.4.1 CCK-8 assay

Cells (5×10^3/well) were seeded in 96-well plates and treated with cisplatin, As_2O_3, the p53 agonist TRAP-1 (MedChemExpress, Monmouth Junction, NJ, USA), or the p53 inhibitor pifithrin-α

(MedChemExpress, Monmouth Junction, NJ, USA) for 24–96 h. CCK-8 reagent (10 μL) was added, and after 2 h, absorbance was measured at 450 nm. The half-maximal inhibitory concentration (IC_{50}) was calculated from the dose–response curve.

2.4.2 Flow cytometry for apoptosis

Cells were resuspended in Annexin V-binding buffer and stained with Annexin V-APC and 7-aminoactinomycin D (7-AAD) in the dark for 15 minutes. Flow cytometry and FlowJo software were used to quantify the apoptotic population.

2.5 Molecular analyses

2.5.1 Quantitative real-time PCR

Total RNA was extracted with TRIzol and reverse-transcribed. NPM1 and TP53 mRNA levels were quantified by SYBR Green qPCR using the 2^(−ΔΔCt) method; GAPDH served as the reference gene.

2.5.2 Western blotting

PMSF was added to RIPA buffer, and total protein was quantified by BCA assay. Equal amounts of protein were separated by SDS-PAGE, transferred to PVDF membranes, and probed overnight at 4°C with primary antibodies against NPM1, p53 and GAPDH, followed by HRP-conjugated secondary antibodies. Bands were visualized by ECL and quantified with ImageJ.

2.5.3 Immunofluorescence

Cells were fixed, permeabilized, blocked with 3% BSA, and incubated with primary antibodies and fluorophore-conjugated secondary antibodies. Nuclei were stained with DAPI, and images were acquired by confocal laser scanning microscopy.

2.6 Xenograft model

Twenty-four female BALB/c nude mice (4–6 weeks old) were subcutaneously injected with 7×10^6 HeLa, HeLa/DDP, or NPM1-overexpressing cells (HeLa-NPM1-OE or HeLa/DDP-NPM1-OE). When the tumour volume reached approximately 100 mm^3, the mice were randomly divided into eight groups (n = 3 per group; Table 1) and administered As_2O_3 (7.5 mg/kg, i.p.) or saline daily for 14 days. Tumour length, width and volume were recorded every two days. Tumours were subjected to Western blotting and immunohistochemistry (IHC), and staining intensity was quantified by the H-score. The animal experiments were approved by the Laboratory Animal Ethics Committee of Lai'an Technology (Guangzhou) Co., Ltd. (approval No. G2024130, dated 25 October 2024) and were carried out in line with the ARRIVE 2.0 guidelines.

Table 1
Grouping of nude mice in the in vivo experiment

Group	Cell type / Treatment
1	HeLa / Saline
2	HeLa / As_2O_3 (7.5 mg/kg, i.p.)
3	HeLa-NPM1-OE / Saline
4	HeLa-NPM1-OE / As_2O_3 (7.5 mg/kg, i.p.)
5	HeLa/DDP / Saline
6	HeLa/DDP / As_2O_3 (7.5 mg/kg, i.p.)
7	HeLa/DDP-NPM1-OE / Saline
8	HeLa/DDP-NPM1-OE / As_2O_3 (7.5 mg/kg, i.p.)

2.7 Statistical analysis

SPSS 23.0 and GraphPad Prism 8.0 were used for statistical analysis. Data are presented as mean ± standard deviation. A t-test or Mann–Whitney U test was used for two-group comparisons, and one-way ANOVA was employed for multiple-group comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1 NPM1 upregulation is associated with advanced stage and poor prognosis

According to the analysis of TCGA-CESC data, NPM1 mRNA expression is higher in cervical cancer tissues than in normal tissues ($P < 0.05$; Fig. 1B). Patients with high NPM1 expression had shorter OS and DFS than those with low expression (both $P < 0.05$; Fig. 1C, D). Network pharmacology identified NPM1 as a high-degree hub target (Fig. 1A).

The serum NPM1 level in the clinical cohort was significantly higher in cervical cancer patients than in healthy individuals (103.43 ± 19.78 vs. 66.32 ± 18.74 ng/mL; $t = 21.50$, $P < 0.01$; Fig. 1E, Table 2). Serum NPM1 levels rose from 87.36 ± 10.99 ng/mL at stage IA to 131.20 ± 14.22 ng/mL at stage IVA, with a slight decrease at stage IVB (123.41 ± 23.61 ng/mL) ($P < 0.01$; Table 2). Because the increase was not strictly monotonic across adjacent stages and the subgroup sizes for stages IIIA and IVA were small ($n = 2$ each), these data support an overall association between serum NPM1 and disease extent rather than a strictly stage-graded trend.

Table 2
Serum NPM1 levels in cervical cancer patients and healthy controls

Group	n	NPM1 (ng/mL, mean ± SD)
Healthy controls	200	66.32 ± 18.74
Cervical cancer (all stages)	323	103.43 ± 19.78*
FIGO IA	39	87.36 ± 10.99*
FIGO IB	106	95.66 ± 15.73*
FIGO IIA	57	102.18 ± 13.65*
FIGO IIB	28	109.90 ± 20.15*
FIGO IIIA	2	109.01 ± 23.44*
FIGO IIIB	13	113.18 ± 15.98*
FIGO IIIC	64	117.63 ± 20.23*
FIGO IVA	2	131.20 ± 14.22*
FIGO IVB	12	123.41 ± 23.61*
Note: * $P < 0.01$ vs. healthy controls.		

3.2 NPM1 promotes cisplatin resistance and proliferation by suppressing p53

The IC_{50} of cisplatin in HeLa/DDP cells was 4.37- to 4.44-fold higher than that in parental HeLa cells (at 72 h: 6.305 vs. 1.443 μg/mL; at 96 h: 3.586 vs. 0.808 μg/mL), confirming cisplatin resistance. Basal NPM1 protein was significantly higher in HeLa/DDP than in HeLa cells ($P < 0.01$; Fig. 2B).

NPM1 overexpression significantly decreased both TP53 mRNA and p53 protein levels ($P < 0.01$), whereas NPM1 knockdown increased p53 expression ($P < 0.01$) (Fig. 2C–E). The p53 agonist TRAP-1 reduced NPM1 expression ($P < 0.01$), whereas the p53 inhibitor pifithrin-α increased it. Confocal microscopy showed that nuclear NPM1 and p53 were inversely expressed ($P < 0.05$; Fig. 2F, G). Collectively, these results indicate that NPM1 promotes cell proliferation and platinum resistance by suppressing p53.

3.3 As_2O_3 remodels the NPM1–p53 axis, inhibits proliferation, and induces apoptosis

As_2O_3 reduced the viability of both HeLa and HeLa/DDP cells in a dose-dependent manner ($P < 0.01$; Fig. 3A–E). Flow cytometry showed that As_2O_3 increased early and late apoptosis in both cell lines ($P < 0.01$; Fig. 3F, G).

As shown in Fig. 3H, I, at 25 μmol/L, As_2O_3 downregulated NPM1 and upregulated p53 ($P < 0.01$). NPM1 overexpression attenuated the p53 induction and growth inhibition by As_2O_3 ($P < 0.01$), whereas NPM1 knockdown enhanced these effects ($P < 0.01$; Fig. 3I), indicating that As_2O_3 acts at least partly by suppressing NPM1.

3.4 As_2O_3 suppresses cisplatin-resistant xenograft growth and restores p53 in vivo

NPM1 overexpression was associated with accelerated tumour growth. Intraperitoneal As_2O_3 (7.5 mg/kg/day for 14 days) significantly reduced the volume and weight of both HeLa and HeLa/DDP xenografts (Fig. 4A). Compared with untreated HeLa xenografts, HeLa/DDP and NPM1-overexpressing tumours showed upregulated NPM1 and downregulated p53 ($P < 0.01$); conversely, As_2O_3 reduced NPM1 and elevated p53 ($P < 0.01$), with significant differences from the corresponding NPM1-overexpressing groups ($P < 0.01$) (Fig. 4B). By immunohistochemistry, untreated HeLa/DDP tumours showed numerically higher NPM1 staining than HeLa tumours (H-score 243.45 ± 15.54 vs. 232.43 ± 16.24), although this difference was modest; p53 staining showed the opposite pattern. As_2O_3 significantly reduced NPM1 ($P < 0.01$) and increased nuclear p53 ($P < 0.01$), consistent with the in vitro results (Fig. 4C, D).

4. Discussion

NPM1 is highly expressed in cervical cancer and is associated with advanced FIGO stage and poor prognosis; it promotes cisplatin resistance by inhibiting p53. As_2O_3 downregulates NPM1 and increases p53 expression, and thereby inhibits the growth of cisplatin-resistant cervical cancer in vitro and in vivo.

Persistent high-risk HPV infection (types 16 and 18) underlies the development of cervical cancer, and over one-third of patients with advanced or recurrent disease are platinum-resistant, with relatively poor OS and DFS [1, 2, 12, 13]. As_2O_3, also known as pi-shuang in traditional Chinese medicine, has demonstrated anticancer efficacy in cervical cancer models [14, 15], and a previous phase II trial found that As_2O_3 plus carboplatin is effective in locally advanced cervical cancer [11]. Based on these findings, the present study investigated the role of the NPM1–p53 axis in the action of As_2O_3 against platinum resistance.

Cisplatin causes DNA crosslinks and double-strand breaks, and thus induces p53-dependent apoptosis [3, 16]. Functional suppression of p53 is a typical mechanism of platinum resistance [4, 17, 18]. We found that NPM1 inhibits p53 in cervical cancer cells: NPM1 overexpression decreased both p53 mRNA and protein, promoted cell proliferation, and NPM1 reduction increased p53 and suppressed growth. Similar crosstalk has been observed in other cancers; for instance, NPM1 can recruit MDM2 to promote the ubiquitination and degradation of p53 [7]. Because NPM1 overexpression in our study also reduced TP53 mRNA, transcriptional repression may operate alongside post-translational mechanisms; the relative contribution of each remains to be defined. Among the 323 patients in our cohort, those with high serum NPM1 had a later FIGO stage, and TCGA analysis showed that high NPM1 is associated with

shorter OS and DFS. Although these data link NPM1 to more aggressive disease, multivariate analysis adjusting for FIGO stage and histological type is required to determine whether it is an independent prognostic factor.

As_2O_3 reduced NPM1 and increased p53 at 25 μmol/L in vitro and 7.5 mg/kg in vivo, thereby inhibiting the proliferation of both parental and cisplatin-resistant cells and inducing apoptosis. NPM1 overexpression attenuated these effects, whereas NPM1 knockdown enhanced them, supporting on-target specificity. In xenografts, As_2O_3 reduced tumour burden, decreased NPM1, and increased nuclear p53. Together with previous reports that As_2O_3 activates p53 and inhibits HPV E6/E7 in cervical cancer cells [19, 20] and can restore p53 function in mutant settings [21], these findings support modulation of the NPM1–p53 axis as a candidate mechanism of action of As_2O_3.

Several limitations should be acknowledged. First, this was a single-center retrospective study with potential selection bias; the prognostic value of NPM1 requires validation in prospective, multicenter cohorts. Second, only HeLa (HPV18+) cells were used, and confirmation in other HPV-type models and patient-derived organoids has not yet been performed. Third, the precise biochemical mechanism by which NPM1 inhibits p53—for example, via MDM2-mediated ubiquitination—has not been experimentally elucidated. Fourth, combination experiments with cisplatin were not performed; therefore, our data demonstrate growth-inhibitory activity of As_2O_3 against cisplatin-resistant cells rather than reversal of cisplatin resistance, and synergy analyses will be required before clinical translation. Fifth, the biochemical origin of circulating NPM1 (passive release from necrotic tumour cells versus active secretion) and its diagnostic performance, including ROC analysis, remain to be validated in independent cohorts. Sixth, the long-term toxicity of As_2O_3 was not assessed in this study. Combination strategies of As_2O_3 with platinum agents, or with cisplatin-sensitizing phytochemicals such as curcumin [22], may deserve further study. Future work will examine the combined effect of As_2O_3 and platinum agents in patient-derived xenograft models and determine whether serum NPM1 is a useful prognostic biomarker.

5. Conclusions

NPM1 is upregulated in cisplatin-resistant cervical cancer and suppresses p53. As_2O_3 downregulates NPM1 and promotes p53-mediated apoptosis, inhibiting the growth of cisplatin-resistant cervical cancer in vitro and in vivo. Whether As_2O_3 can restore cisplatin sensitivity or synergize with platinum agents requires further investigation in combination-treatment models. These findings indicate that As_2O_3 may benefit patients with platinum-resistant cervical cancer and may warrant further clinical investigation.

Declarations

Ethics approval and consent to participate:

The study protocol was approved by the Ethics Committee of The First Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangzhou, China) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment.

The animal experiments were approved by the Laboratory Animal Ethics Committee of Lai'an Technology (Guangzhou) Co., Ltd. (approval No. G2024130) and were carried out in line with the ARRIVE 2.0 guidelines.

Consent for publication:

Not applicable.

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Conflict of interest: The authors have no competing interests to declare. All authors have completed the ICMJE uniform disclosure form.

Author contributions:

Conceptualisation: Li Sijin and Luo Songping; Methodology: Li Sijin, Yu Chan, Xie Linlin; Formal analysis and investigation: Li Sijin, Xu Haixia, Li An; Writing (original draft): Li Sijin, Yu Chan; Writing (review and editing): Luo Songping, Zhou Ying, Liang Feimei; Funding acquisition, resources and supervision: Luo Songping. All authors read and approved the final manuscript.

Data availability:

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. TCGA data are publicly available at https://portal.gdc.cancer.gov/.

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Figures

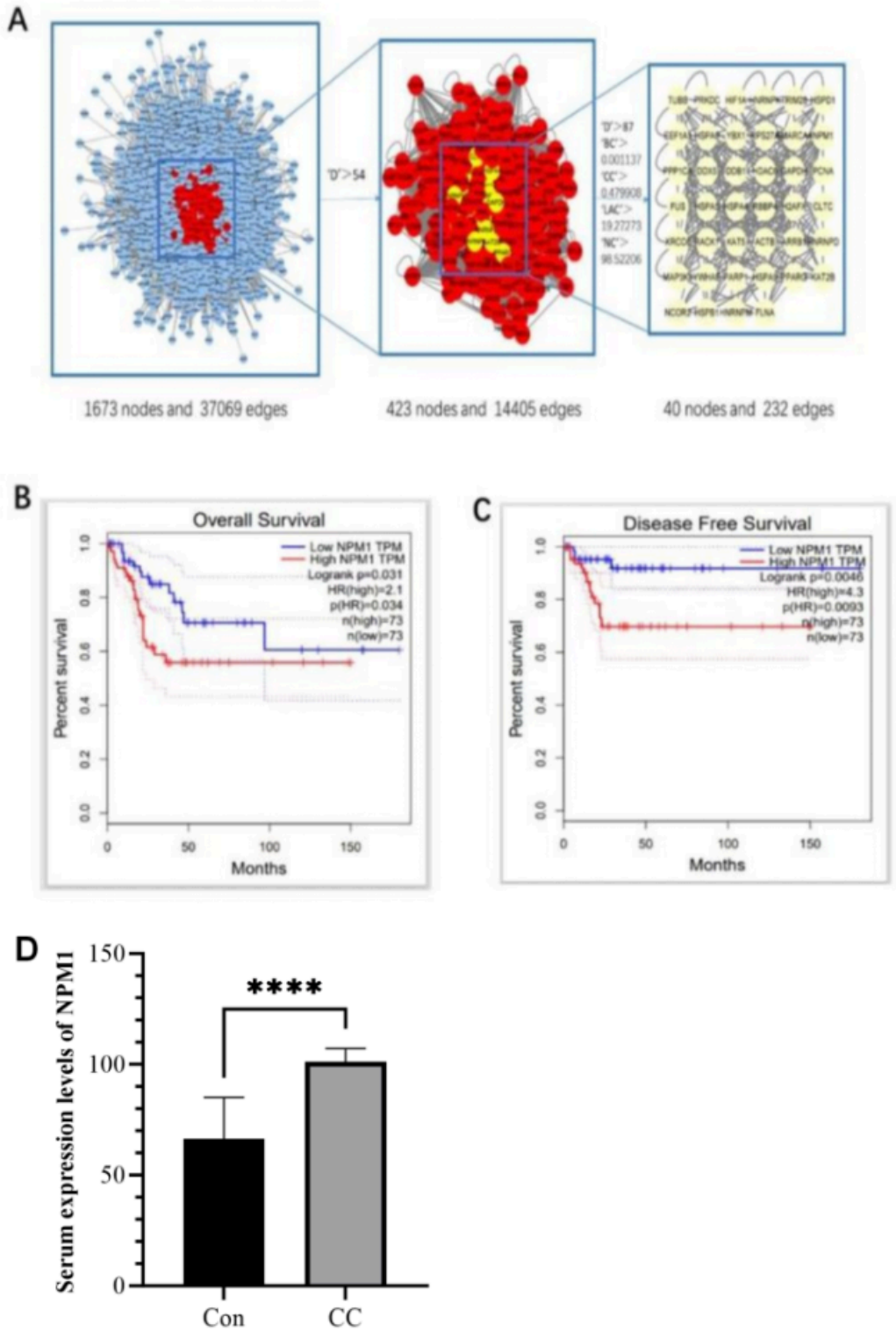


Figure 1

NPM1 is upregulated in cervical cancer and is associated with poor prognosis. (A) *Network pharmacology target network for As_2O_3 against cisplatin-resistant cervical cancer. (B) NPM1 mRNA expression in TCGA-CESC tumors vs. normal tissues (P < 0.05). (C, D) Kaplan–Meier OS and DFS curves stratified by median NPM1 expression (P < 0.05). (E) Serum NPM1 in cervical cancer patients (n = 323) and healthy controls (n = 200) by ELISA (P < 0.01).*

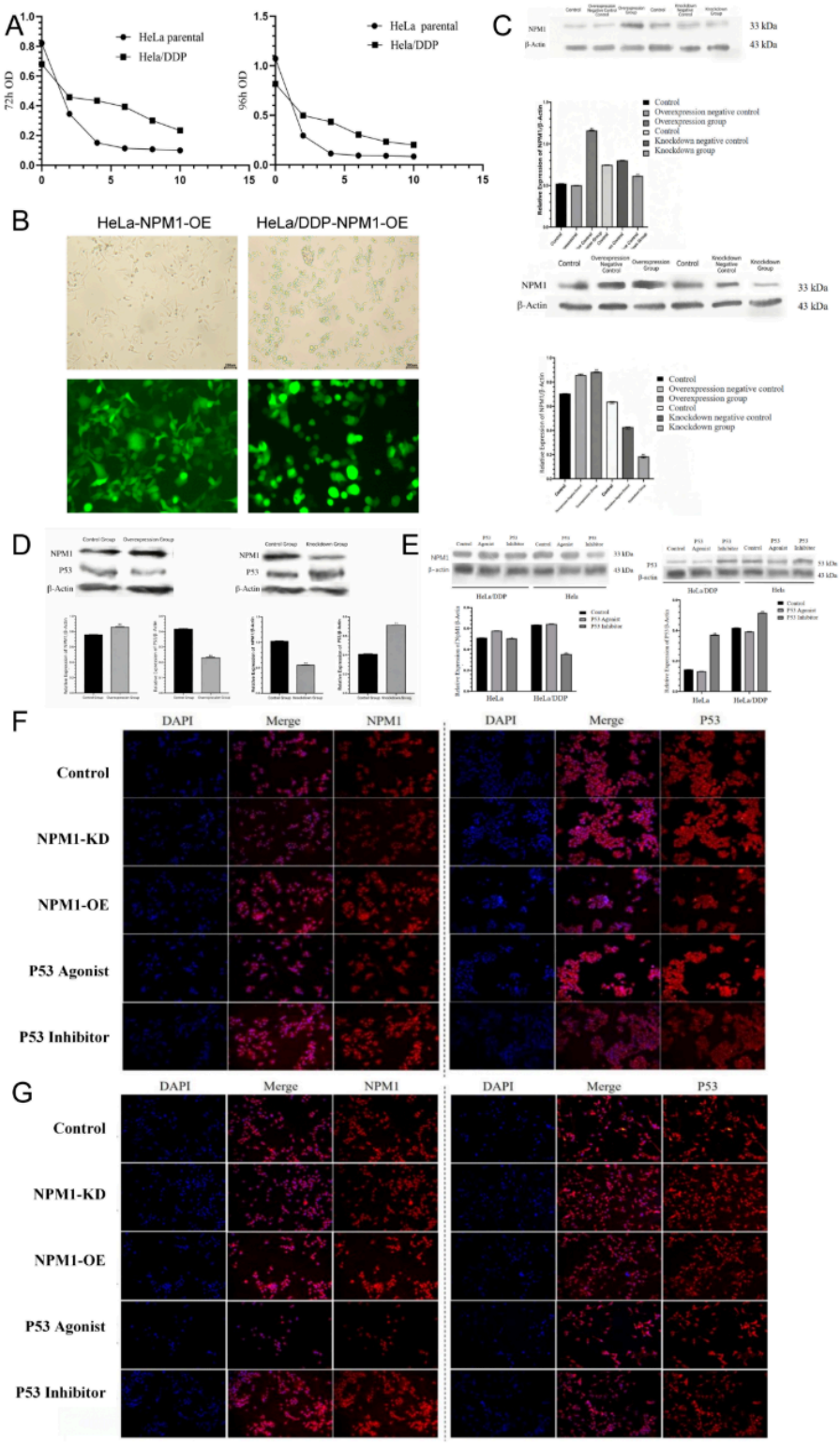


Figure 2

NPM1 promotes cisplatin resistance and proliferation by suppressing p53. (A) *Cisplatin IC_{50} in HeLa and HeLa/DDP cells (72 h: 1.443 vs. 6.305 μg/mL; 96 h: 0.808 vs. 3.586 μg/mL). (B) NPM1 immunofluorescence in HeLa and HeLa/DDP cells. (C–E) Effects of NPM1 overexpression or knockdown on NPM1 and p53 expression. (F, G) Representative confocal immunofluorescence images and quantitative analysis showing the inverse nuclear expression of NPM1 and p53. Data are mean ± SD. P < 0.01.*

Notes: Control, untreated cells; overexpression negative control, cells stably transduced with empty NC lentivirus; knockdown negative control, cells stably transduced with non-targeting shRNA control lentivirus; knockdown, cells stably transduced with NPM1-targeting shRNA lentivirus; overexpression, cells stably transduced with NPM1 overexpression lentivirus. P < 0.01 vs. Control.

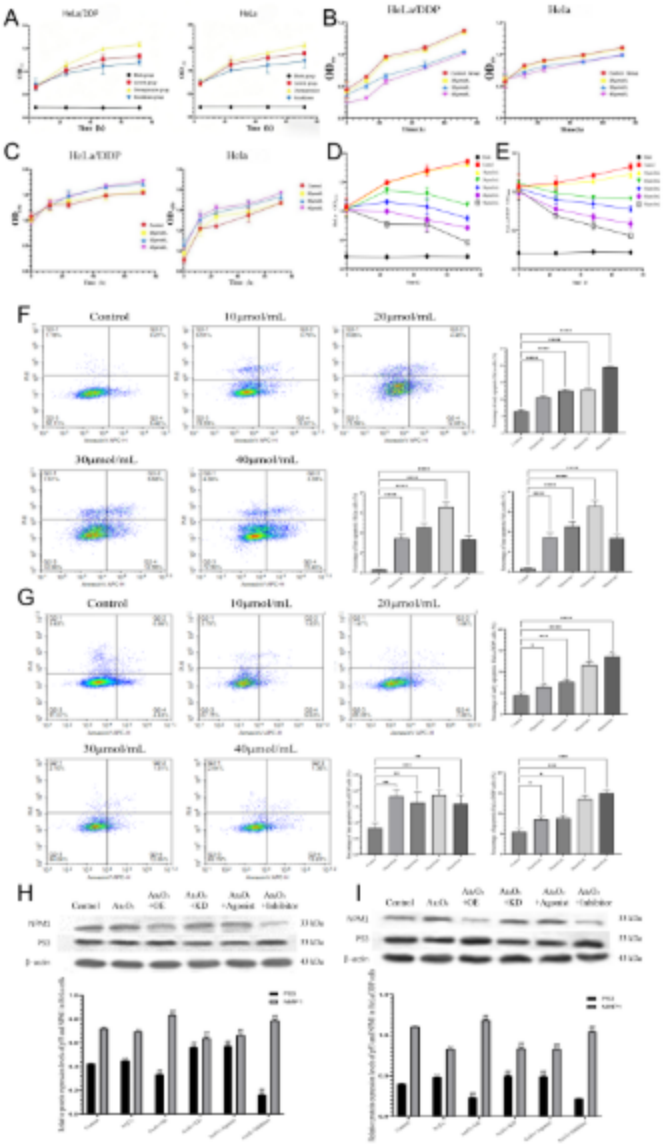


Figure 3

As_2O_3 remodels the NPM1–p53 axis to inhibit proliferation and induce apoptosis. *(A–E) CCK-8 dose–response curves for HeLa and HeLa/DDP cells. (F, G) Apoptosis analysis by Annexin V/7-AAD flow cytometry. (H, I) NPM1 and p53 protein levels after treatment with 25 μmol/L As_2O_3; rescue experiments with NPM1 overexpression or knockdown. P < 0.01.*

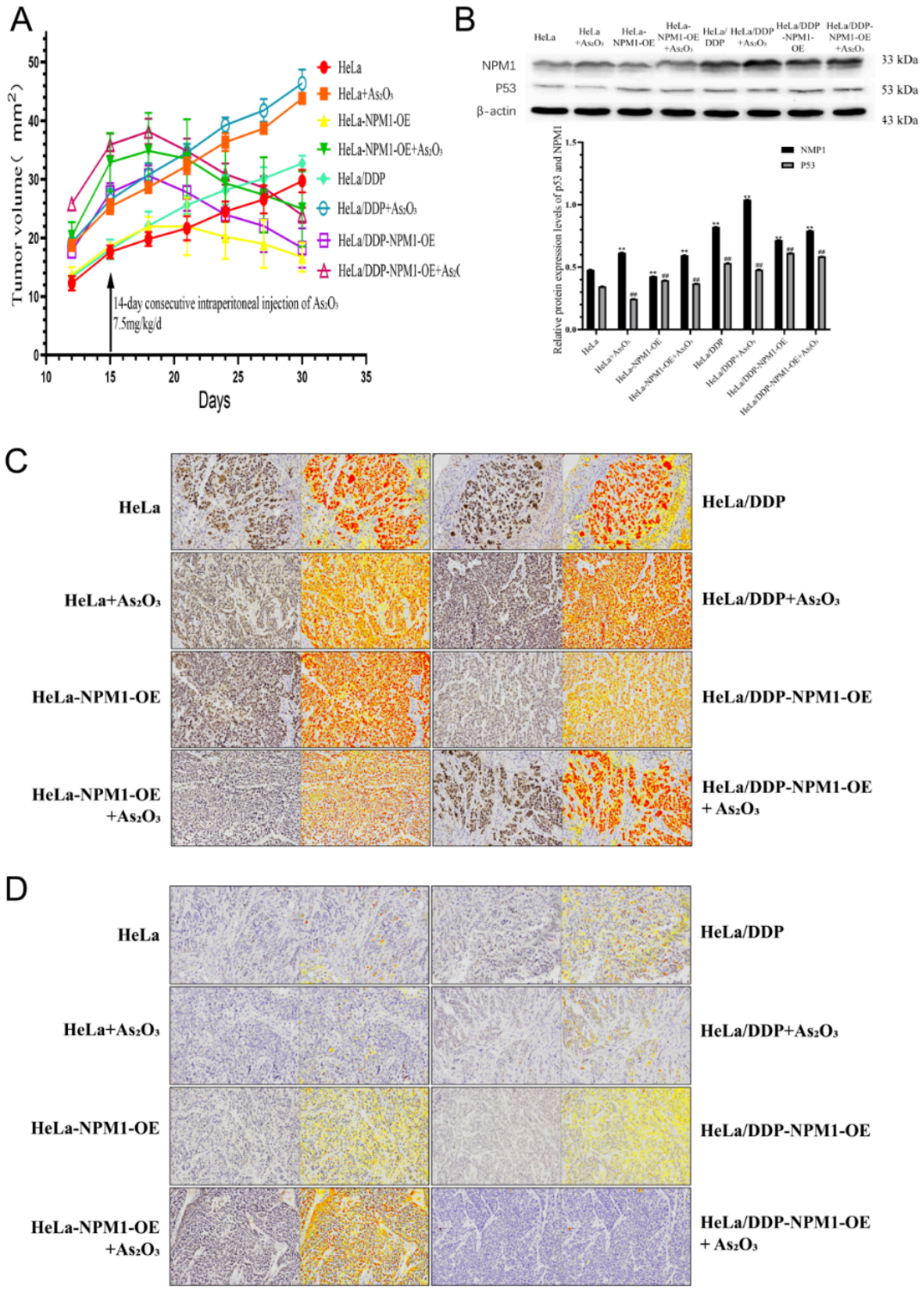


Figure 4

As_2O_3 suppresses xenograft growth and restores p53 in vivo.

(A) Tumour growth curves, representative images and tumour weights in the different groups. (B) Protein levels of p53 and NPM1 in tumours from the different groups. (C, D) IHC and H-score quantification of NPM1 and p53 in xenograft tumours. P < 0.01 vs. control; ‡‡P < 0.01 vs. corresponding untreated group.