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p53 Dysfunction by Xeroderma Pigmentosum Group C Defects Enhance Lung Adenocarcinoma Metastasis via Increased Mmp1 Expression

Yi-Hui Wu¹, Tzu-Chin Wu², Jiunn-Wang Liao³, Kun-Tu Yeh⁴, Chih-Yi Chen⁵, and Hwei Lee^{1,2,6}

Abstract

Xeroderma pigmentosum group C (XPC) interacts with hHR23B to recognize DNA damage in global genomic repair. We previously showed that XPC is predominantly affected by its hypermethylation and is associated with an increased occurrence of p53 mutation in lung cancer. Tumors with low XPC mRNA levels had a poorer prognosis than those with high XPC mRNA levels, suggesting that XPC defects may enhance tumor metastasis. However, the underlying mechanism is unclear. Here, we show that p53 transcriptional activity is modulated by XPC, whereby XPC stabilizes hHR23B to form an hHR23B-p53 complex that prevents p53 degradation. In addition, in lung cancer cells and xenograft tumors in nude mice, overexpression of XPC suppresses cell/tumor metastatic ability via repression of matrix metalloproteinase-1 (MMP1) transcription by p53. Among tumors from lung cancer patients, those with low XPC mRNA also tended to have low expression of MMP1 mRNA compared with those with high XPC mRNA. Patients with low XPC mRNA levels also more commonly had tumors with late-stage, distant metastasis (M1), nodal metastasis, and *T* value ($P < 0.001$ for tumor stage, distant metastasis, and nodal metastasis; $P = 0.006$ for *t* value). In conclusion, p53 dysfunction caused by XPC defects in lung cancers may enhance tumor metastasis via increased MMP1 expression. *Cancer Res*; 70(24): 10422–32. ©2010 AACR.

Introduction

The xeroderma pigmentosum group C (*XPC*) gene is a critical component of the DNA damage recognition system required for global genomic repair. Accumulated evidence indicates that XPC defects are associated with an increased risk of cancer (1–3). In an animal knockout model, a higher incidence of spontaneous testicular tumors in *XPC*^{-/-} *p53*^{-/-} double mutant mice was observed when compared with *XPC*^{+/+} *p53*^{-/-} mice. Lung adenomas were also spontaneously induced in XPC knockout mice and lung adenocarcinomas developed when *GADD45*, a *p53* downstream gene, was

deleted from these knockout mice (4). Therefore, XPC defects, combined with the inactivated p53 pathway, may initiate lung adenocarcinoma development.

Our previous report indicated that XPC was predominantly affected by promoter hypermethylation and that it may contribute to the occurrence of p53 mutation in lung tumors (5). We also observed XPC defects to be more common in nonsmokers and in early-stage tumors and that XPC mRNA altered by XPC hypermethylation may confer nodal metastasis and tumor recurrence. In addition, patients with lower XPC mRNA levels had a poorer prognosis than those with higher XPC mRNA levels, particularly with respect to adenocarcinomas (6). Therefore, we hypothesize that XPC defects may not only be associated with initiation of adenocarcinoma development but may also promote tumor metastasis. In our previous population-based study, XPC-methylated lung tumors that harbored wild-type p53 were more common in adenocarcinomas in nonsmokers than in squamous cell carcinomas in smokers (68% vs. 36%, $P = 0.02$ for adenocarcinomas vs. squamous cell carcinomas; 62% vs. 28%, $P = 0.03$ for nonsmokers vs. smokers). Interestingly, XPC-methylated tumors that harbored wild-type p53 had lower levels of MDM2 mRNA than XPC-unmethylated tumors that harbored wild-type p53 (4.94 ± 2.32 vs. 15.94 ± 2.72 , $P = 0.01$). These observations in lung tumors have led us to speculate that p53 function may be altered by XPC hypermethylation. Therefore, p53 dysfunction by XPC hypermethylation may play a more important role in lung cancer development among nonsmokers and adenocarcinoma patients, even though p53 mutations have not occurred.

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The hHR23B protein is incorporated with XPC to recognize DNA damage sites during the process of nucleotide excision repair (NER; 7, 8), and hHR23B is considered to play an essential role in XPC protein stability (9). Interestingly, depletion of hHR23B with small interference RNA (siRNA) results in a more rapid degradation of p53 by proteasome whereas overexpression of hHR23B induces the accumulation of ubiquitinated p53 (10, 11). Accumulated evidence indicates that p53 modulates cancer progression by regulating tumor metastasis (12–15). Therefore, we hypothesize that p53 could be inactivated by XPC defects via hHR23B degradation and, in this way, enhance tumor metastasis.

Matrix metalloproteinases (MMP), a family of human zinc-dependent endopeptidases, are responsible for degradation of the extracellular matrix, which is a crucial step for tumor invasion and metastasis (16–19). Among the MMPs, MMP1 has been identified as one of the most highly upregulated proteins in a variety of cancers, including colorectal, esophageal, pancreatic, gastric, breast, malignant melanoma, and prostate cancer (20–27). Fréchet et al. (28) indicated that MMP1 protein and its mRNA are significantly increased in XPC-deficient cells when compared with normal dermal fibroblasts. Because MMP1 is apparently regulated by p53 (29), we therefore speculate that p53 dysfunction by XPC defects may upregulate MMP1 transcription, leading to promotion of tumor metastasis. In the present study, we provide evidence in both cell and animal models to show that p53 dysfunction by XPC defects may enhance tumor metastasis via upregulation of MMP1 expression. More important, tumor progression and metastasis were also observed in lung cancer patients with XPC defects.

Materials and Methods

Cells culture

Human lung cancer cell lines A427 and A549 were obtained from the American Type Culture Collection (ATCC). CL1-0 and CL1-5 cells were kindly provided by Dr. P.C. Yang (Department of Internal Medicine, National Taiwan University Hospital). A427 cells were grown in Minimum Essential Medium supplemented with 10% FBS. A549 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS. CL1-0 and CL1-5 cells were grown in RPMI-1640 medium with 10% FBS. All these cell lines were grown at 37°C in a 5% carbon dioxide atmosphere. Cells were cultured and stored according to the supplier's instructions and used at passages 5 to 20. Once resuscitated, cell lines are routinely authenticated (once every 6 months, cells were last tested in December 2009) through cell morphology monitoring, growth curve analysis, species verification by isoenzymology and karyotyping, identity verification using short tandem repeat profiling analysis, and contamination checks.

Quantitative real-time RT-PCR and Boyden chamber assays

These assays were performed according to a previously published report (6).

Immunoblotting, immunoprecipitation, and ubiquitination assays

These assays were performed according to a previously published report (30).

Chromatin immunoprecipitation assay

This assay was conducted according to a previously published report (31).

Demethylation by 5-aza-2'-deoxycytidine and luciferase reporter assay

These assays were conducted according to a previously published report (5).

Reporter plasmids, XPC expression, XPC knockdown, and MMP1 knockdown constructs

Detailed plasmids were presented in the Supplementary Methods section.

Transfection

All transfection experiments were conducted with TransFast transfection reagents (Promega) in accordance with the manufacturer's protocols.

Collagen zymography

MMP1 protease activity was identified as described previously (32).

Tumorigenesis and metastasis analysis

All animal studies were approved by the Institutional Animal Care and Use Committee at Chung Shan Medical University. To measure tumorigenicity, the models of lung adenocarcinoma were 6-week-old female BALB/c nude mice ($n = 40$; supplied by the National Laboratory Animal Center, Taiwan) that were acclimated for 1 to 2 weeks while caged in groups of 5. The mice were housed in pathogen-free conditions and fed a diet of animal chow and water throughout the experiment. Mice were randomized to 1 of 4 groups and were injected subcutaneously with CL1-0/shXPC cells ($n = 10$), CL1-5/XPC cells ($n = 10$), or CL1-0 transfected with nonspecific control (NC), and CL1-5 cells transfected with vector controls (VC; $n = 10$, each; 10^7 cells in 0.1 mL of PBS). Tumors were measured with calipers every other day, starting on day 13 after the injection, when they had become palpable and visible. Tumor volumes were calculated using the equation: $\text{width}^2 \times \text{length} \times 0.5$. Subcutaneous tumors were surgically excised, weighed, and photographed, and a portion of each tumor was placed in 10% formalin for paraffin embedding or was snap-frozen in optimum cutting temperature solution (Miles) in preparation for subsequent immunohistochemical analysis. For metastasis models, mice were injected with CL1-0/NC, CL1-0/shXPC, CL1-5/VC, and CL1-5/XPC cells ($n = 10$, each group) via the tail vein (10^6 cells in 0.1 mL of PBS). After 4 months, mice were sacrificed by an overdose with anesthetic and all organs were examined for metastasis formation. The lungs were removed, weighed, and fixed in 10% formalin. The number of lung tumor metastases was counted under a dissecting microscope.

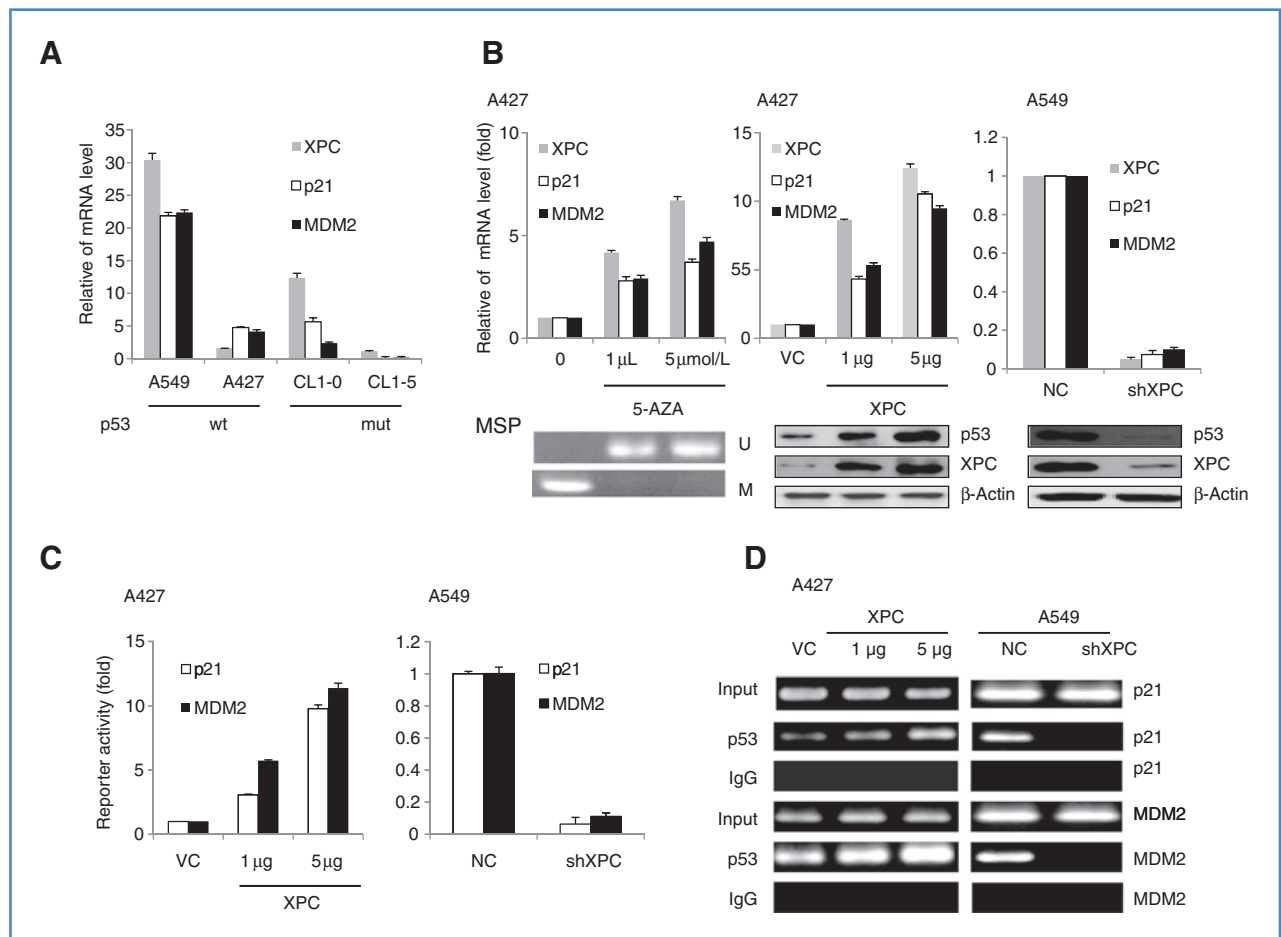


Figure 1. XPC positive regulates p53 transcriptional function. A, XPC, p21, and MDM2 mRNA expression levels in A549, A427, CL1-0, and CL1-5 cells. B, Left, A427 treated with 1 and 5 μ mol/L 5-AZA for 5 days. MSP assay: U, unmethylated band; M, methylated band. Levels of relative expression of XPC, p21, and MDM2 were determined using real-time RT-PCR. Middle and right, XPC, p21, and MDM2 mRNA expression levels, p53 and XPC protein expression levels in A427 transiently transfected with XPC gene cDNA expression vector (1 and 5 μ g), A549/shXPC, and A549/NC. C, A427 (1 and 5 μ g) and A549/shXPC transfected with 1 μ g of reporter plasmid for 48 hours. D, ChIP assay was conducted to evaluate the binding activity of p53 on p21 and MDM2 promoters in A427 (1 and 5 μ g) and A549/shXPC.

Lung tumor specimens

The Institutional Review Board at Chung Shan Medical University Hospital approved the study, and all participants gave written informed consent. A total of 100 lung cancer patients (International Classification of Diseases, 9th Revision; ICD code 162), including 45 females and 55 males, were enrolled in this study through the collection of specimens by a computed tomography (CT)-guided biopsy at Chung Shan Medical University Hospital. All cases also underwent a series of examinations at various pathologic stages by board-certified pathologists. Demographic data, which included age, gender, and smoking status, were collected from each patient by an interview and a review of the hospital charts with informed consent. Smoking status of patients was defined as smokers who had smoked more than 100 cigarettes, and nonsmokers who, in their lifetime, never smoked.

Statistical analysis

Statistical analysis was conducted using the SPSS statistical software program (Version 11.0 SPSS Inc.). Student's *t* test and Fisher's exact test (2-tailed) were used for statistical analyses.

Results

Wild-type or mutant p53 function is attenuated by XPC status

To verify whether p53 function could be modulated by XPC defects, p53 wild-type lung cancer cells (XPC-methylated A427 and XPC-unmethylated A549 cells) were selected to evaluate mRNA expression levels of p53 downstream gene (*p21* and *MDM2*) by real-time reverse transcriptase (PCR). As expected, p21 and MDM2 mRNA were highly expressed in the XPC-unmethylated A549 cells when compared with the XPC-methylated A427 cells (Fig. 1A). However, when treated with

a demethylating agent, 5-AZA-dC (AZA), or when an XPC expression vector was transiently transfected, the A427 cells showed significantly elevated p21 and MDM2 mRNA expression levels following either treatment (Fig. 1B). Conversely, p21 and MDM2 mRNA levels were almost completely suppressed when the *XPC* gene of A549 cells was knocked down by its RNAi (Fig. 1B). The levels of p53 proteins were also significantly increased in A427/XPC and decreased in A549/shXPC cells (Fig. 1B). To verify whether the p53 transcriptional function is elevated by XPC, both cell types were transfected with an XPC expression vector or XPC RNAi vector, along with a luciferase reporter construct controlled by the p53-response elements of the p21 or by the MDM2 promoter. Transcriptional activity of p53 on p21 and MDM2 promoters in A427 cells increased about 4- to 6-fold after the transfection of an XPC expression vector when compared with transfection with the empty vector. In contrast, transcriptional activity of p53 was significantly reduced in A549/shXPC cells (Fig. 1C). Chromatin immunoprecipitation (ChIP) analysis further indicated that the binding ability of p53 on the p21 and MDM2 promoters in A427 cells was significantly increased by the transfection of an XPC expression vector whereas the binding activity of p53 on the p21 and MDM2 promoters was completely suppressed in A549/shXPC cells (Fig. 1D). In addition, the mutant p53 function in CL1-0 and CL1-5 cells, which harbored p53 mutation at codon 248, can be modulated by XPC status (Supplementary Fig. S1). The transcriptional function of p53 proteins with different mutation sites including V143A, S240R, R248W, R249S, and E286Q were also restored by transfection of XPC expression vector in H1299 cells (Supplementary Fig. S2). These results clearly indicate that the transcriptional function of wild-type or mutant p53 can be modulated by XPC defects in lung cancer cells.

Stabilization of p53 protein by XPC occurs via increased interaction of p53 with hHR23B

The hHR23B protein has been shown to interact with XPC and p53 to protect both proteins from deubiquitylation and degradation (9–11). As mentioned earlier, hHR23B and p53 protein levels were changed by XPC status. We therefore hypothesized that the deubiquitylation and degradation of p53 and hHR23B proteins could be modulated by XPC status. To test this hypothesis, MG132, a specific inhibitor of the 26S proteasome, was used to treat both A549 and CL1-0 cells, with or without shXPC transfection. The levels of p53 and hHR23B proteins were then determined by Western blotting at different time intervals. The p53 and hHR23B protein levels in A549/shXPC and CL1-0/shXPC cells were lower than those of NC cells, indicating that the degradation of both proteins in both shXPC cell types was more rapid than that occurring in NC cells (Fig. 2A). Cycloheximide pulse-chase experiment indicated that the half-lives of p53 and hHR23B proteins were significantly reduced in both shXPC cells when compared with the half-lives in NC cells (Fig. 2B). The ubiquitination pattern of p53 protein in both shXPC cell types was also more extensive than in NC cells after MG132 treatment (Fig. 2C). Immunoprecipitation assays indicated that the interaction between p53 and hHR23B in both shXPC cell types was

elevated by MG132 treatment in the presence of the same amounts of both proteins (Fig. 2D). Collectively, these results suggest that stabilization of p53 protein by XPC is mediated through increased interaction of p53 with hHR23B.

Cell migration/invasion ability is enhanced by XPC defects via upregulated MMP1 transcription due to p53 dysfunction

XPC defects may enhance the migration/invasion capability in lung cancer cells (6); however, the underlying mechanism remains unclear. As mentioned earlier, MMP1 levels increased significantly in XPC-deficient cells when compared with normal dermal fibroblasts (28). Because MMP2 and MMP9 are representative proteases known to be involved in lung adenocarcinoma metastasis (33–38), we hypothesized that XPC defects may promote the migration/invasion ability via upregulation of these MMPs. Real-time RT-PCR assays showed that MMP1 expression was increased by XPC knockdown in A549 cells and was decreased by XPC overexpression in A427 cells. However, MMP2 and MMP9 expression levels were not changed by XPC overexpression or knockdown in these cells (Fig. 3A). Collagen zymography assays further confirmed that MMP1 expression modulated by XPC knockdown or overexpression in these cells was consistent with the protease enzyme activity (Fig. 3B, top). Collagen-coated Boyden chamber assays further showed that the migration/invasion ability was significantly increased by XPC knockdown in A549 cells and decreased by XPC overexpression in A427 cells (Fig. 3B, bottom) when compared with VC and NC cells. To test whether MMP1 increased by XPC knockdown was responsible for the increase in migration/invasion ability, MMP1 shRNA was transfected into A549/shXPC cells. The migration/invasion ability was significantly diminished in A549/shXPC/shMMP1 cells (Fig. 3B, bottom). Soft agar colony number and size increased significantly after transfection of A549 cells with shXPC. However, the colony number and size were significantly reduced when the A427 cells were transfected with XPC expression vector (Supplementary Fig. S3). These results clearly indicate that the XPC defects not only promote cell migration/invasion ability but also enhance oncogenic potential through increased MMP1 expression.

p53 downregulated MMP1 transcription by inhibiting the activity of the AP1 transcription factor that binds to the MMP1 promoter (29). To determine whether the increase in MMP1 by XPC transfection of A427 cells was mediated through the restoration of p53 function, we used either a p53-specific inhibitor, pifithrin- α (PFT- α), or p53 knockdown by p53 shRNA to treat A427/XPC cells. As expected, p21 and MDM2 mRNA levels were elevated in A427/XPC cells when compared with VC cells, but mRNA levels of both proteins in A427/XPC cells were reduced by PFT- α treatment and p53 shRNA transfection (Fig. 3C, left top). Western blotting indicated that the decrease in MMP1 in A427/XPC cells was significantly restored by both treatments (Fig. 3C, left bottom). As indicated by ChIP analysis, the binding of c-Fos and c-Jun to the MMP1 promoters in XPC-overexpressing A427 cells was increased by either PFT- α treatment or p53 shRNA transfection (Fig. 3C, right). Collagen zymography assays further

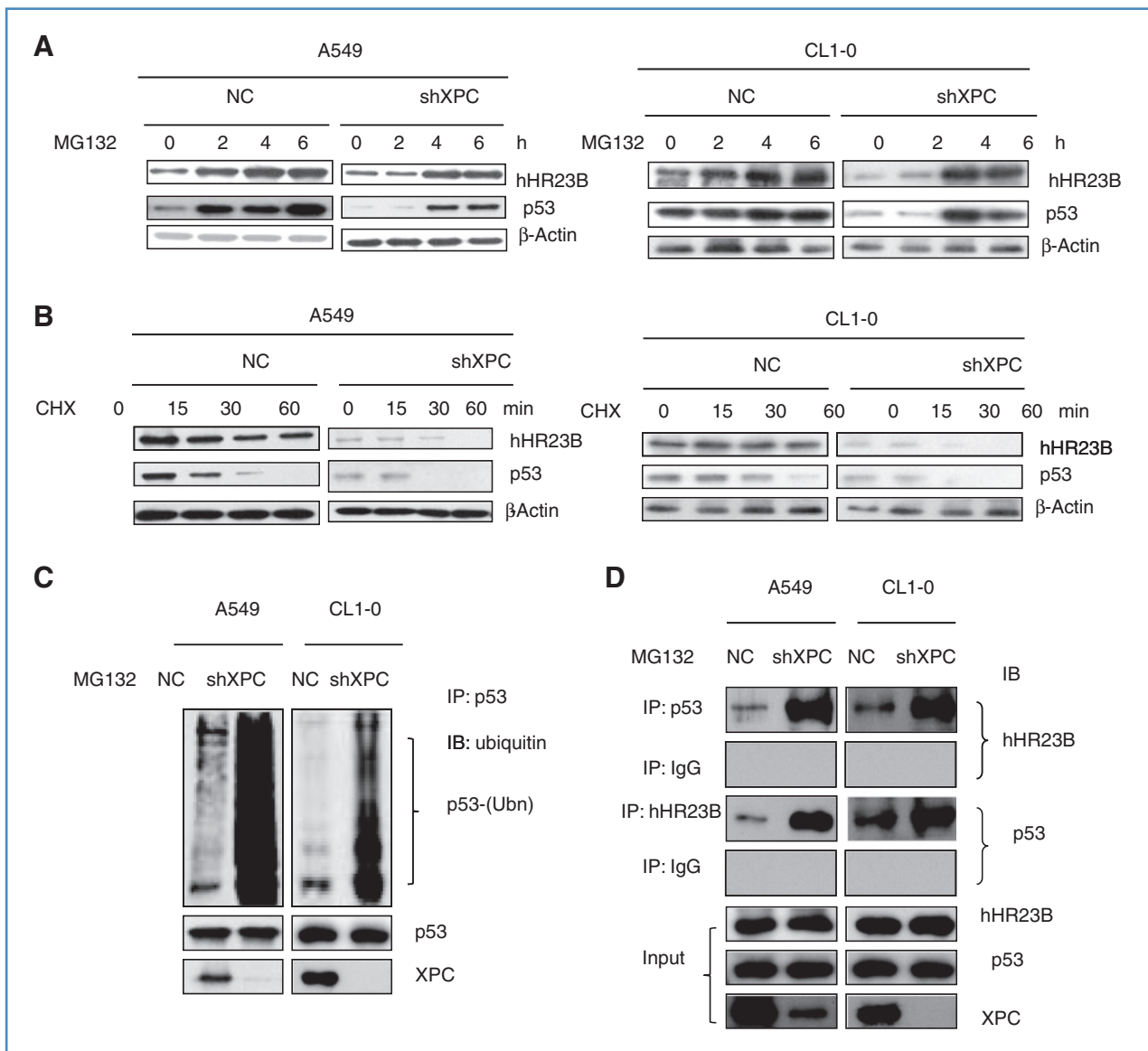


Figure 2. Stabilization of p53 protein by XPC occurs via increased interaction of p53 with hHR23B. A, A549 and CL1-0 cells transfected with shXPC were treated with MG132 (10 μ mol/L) for the indicated times and analyzed by immunoblotting. B, A549 and CL1-0 cells transfected with shXPC were incubated for the indicated times with cycloheximide (CHX; 40 μ g/mL) and analyzed by immunoblotting. C, A549 and CL1-0 cells transfected with shXPC were treated with MG132 for 4 hours and then cell lysates were immunoprecipitated with anti-p53 antibodies. The resulting immunoprecipitates (IP) were analyzed by immunoblotting (IB), using an anti-ubiquitin antibody. D, A549 and CL1-0 cells transfected with shXPC were treated with MG132 for 4 hours and then cell lysates were immunoprecipitated with anti-hHR23B, anti-p53, and anti-IgG antibodies, and the resulting IPs were analyzed by immunoblotting.

confirmed that MMP1 protease enzyme activity in A427/XPC cells was elevated by either PFT- α treatment or p53 shRNA transfection (Fig. 3D, top). The migration/invasion ability of A427/XPC cells was also increased by either treatment (Fig. 3D, bottom). In addition, MMP1 expression, enzyme activity, and cell migration/invasion ability decreased significantly in CL1-5/XPC cells and increased markedly in CL1-0/shXPC cells. The increased migration/invasion ability in CL1-0/shXPC cells was eliminated when the cells were transfected with shMMP1. The MMP1 transcription was de-repressed in CL1-5/XPC cells after PFT- α treatment (Supplementary

Fig. S4). These results indicated that the migration/invasion enhancement due to MMP1 expression may be mediated through the suppression of p53 function by XPC defects, even though CL1-0 and CL1-5 cells harbored R248W mutant p53.

Tumor growth and metastasis are enhanced by XPC defects in nude mice models

To examine whether p53 dysfunction by XPC defects could enhance tumor growth, a xenograft tumor model in nude mice was examined in which 10 mice from each group were subcutaneously injected with the stable clones (CL1-0/shXPC

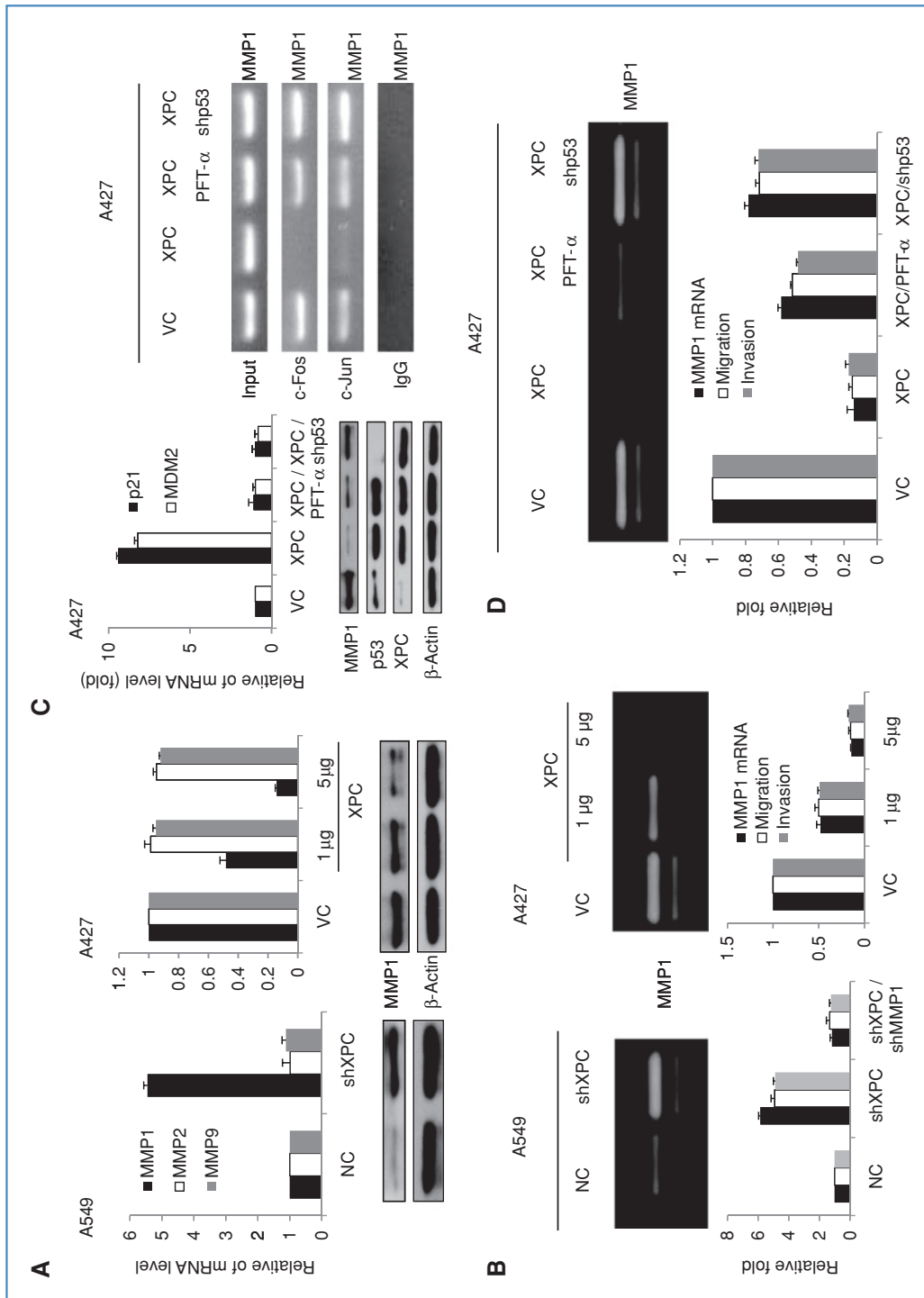


Figure 3. Overexpression of XPC suppresses cell metastatic ability via repression of MMP1 transcription by p53. A, MMP1, MMP2, and MMP9 mRNA expression levels were evaluated by real-time RT-PCR assay and MMP1 protein expression was evaluated by immunoblotting in A549/NC, A549/shXPC, A427/VC, and A427 (1 and 5 μg). B, Top, collagen zymography assay was performed to evaluate the MMP1 protease activity in A549/NC, A549/shXPC, A427/VC, and A427 (1 and 5 μg). Bottom, *in vitro* migration and invasion activity of A549/NC, A549/shXPC, A549/shXPC/shMMP1, A427/VC, and A427 (1 and 5 μg). Levels of relative expression of MMP1 were evaluated by real-time RT-PCR. C, A427 transiently transfected with 5 μg of XPC gene cDNA expression vector for 24 hours and then treated with 20 μM of PFT-α or 5 μg of shp53 plasmids for 24 hours. Cells were collected and harvested for mRNA and protein expression. Left top, levels of relative expression of p21 and MDM2 were determined using real-time RT-PCR. Left bottom, MMP1, p53, and XPC protein expression was evaluated by immunoblotting. Right, ChIP assays were conducted to evaluate the binding activity of c-Fos and c-Jun on MMP1 promoters in A427 transiently transfected with 5 μg of XPC gene cDNA expression vector after both treatments. D, Top, collagen zymography assay was conducted to evaluate the MMP1 protease activity in A427 transiently transfected with 5 μg of XPC gene cDNA expression vector after both treatments. Bottom, *in vitro* migration and invasion activity of A427 transiently transfected with 5 μg of XPC gene cDNA expression vector after both treatments. Levels of relative expression of MMP1 were evaluated by real-time RT-PCR.

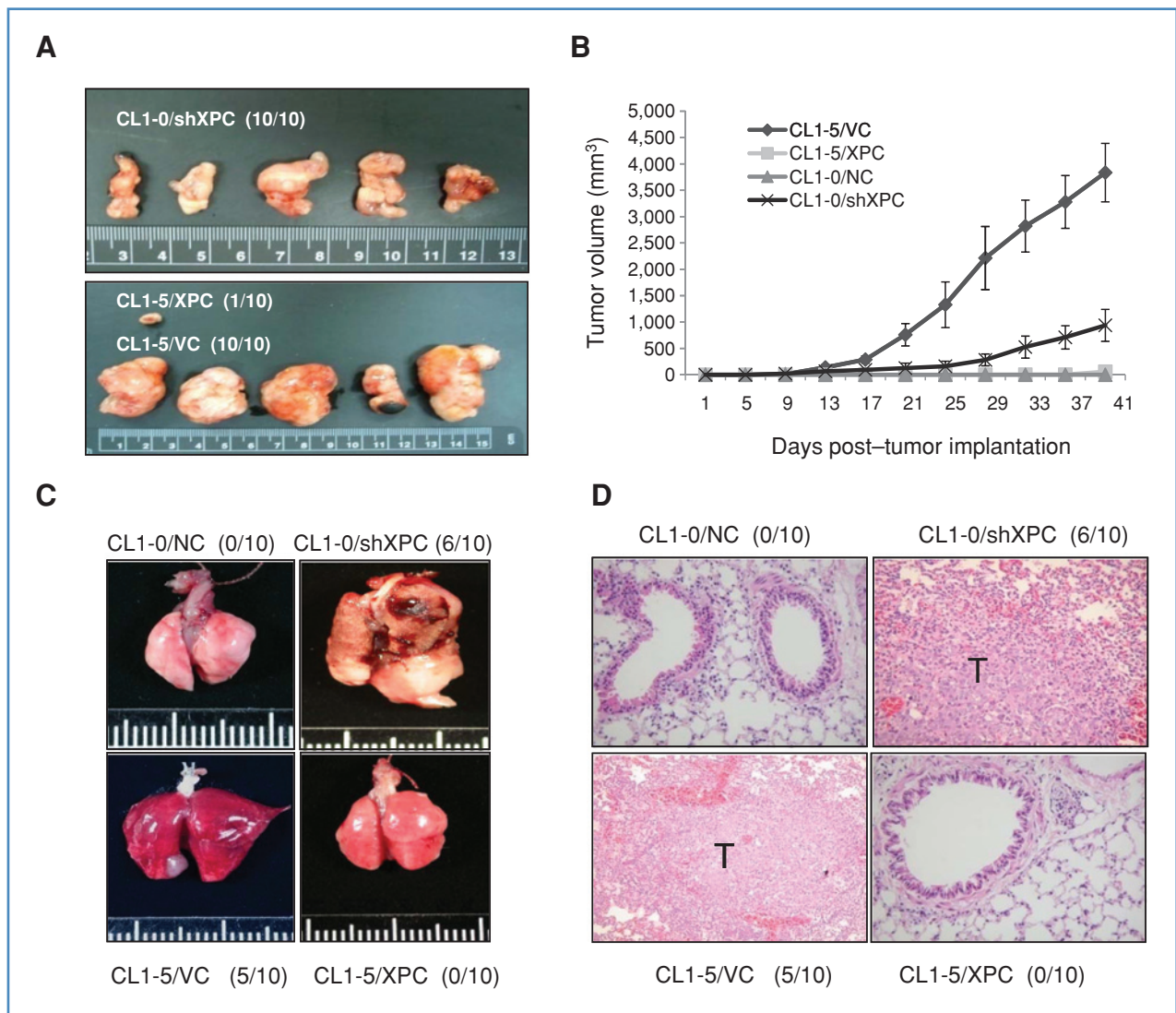


Figure 4. XPC suppresses metastasis. A and B, growth patterns of xenograft tumors formed subcutaneously by CL1-0/NC, CL1-0/shXPC, CL1-5/VC, and CL1-5/XPC. Each data point represents the mean \pm SD of 10 primary tumors. C, lungs were excised and photographed after experimental metastasis assay. D, histologic analyses of lung metastatic tumors.

and CL1-5/XPC) and their counterparts of control cells (CL1-0/NC and CL1-5/VC). Palpable tumors were found in the CL1-5/VC group on day 13, but no tumors were found in the mice of the other 3 groups from the outset. To estimate the tumor volume, all mice were sacrificed on day 41; all mice in the CL1-5/VC and CL1-0/shXPC groups harbored tumor burdens, but mice in the CL1-5/XPC and CL1-0/NC groups had no tumor burdens (except for 1 mouse in the CL1-5/XPC group that had a small tumor burden; Fig. 4A and B). The tumor volume of the CL1-5/VC mice was significantly larger than that of the CL1-5/XPC group ($P = 0.001$, Fig. 4A and B). No tumors were found in the mice in the CL1-0/NC group, even though the tumor volume of the CL1-0/shXPC mice was significantly lower than in the CL1-5/VC mice ($P < 0.001$, Fig. 4A and B). These results clearly indicate that p53 dysfunction by XPC defects may enhance tumor growth in subcutaneously injected nude mice.

To verify whether XPC may enhance lung tumor metastasis, 10 mice from each group were injected with CL1-0/NC, CL1-0/shXPC, CL1-5/VC, and CL1-5/XPC cells via the tail vein. After 4 months, the mice were sacrificed to count the lung tumor nodules in the mice from each group (Fig. 4C and D). Among these 4 groups, 5 of the 10 (50%) mice in the CL1-5/VC group had lung tumor burdens, but tumors were not seen in mice in the CL1-5/XPC or the CL1-0/NC groups (Table 1). Interestingly, 6 of the 10 mice (60%) in the CL1-0/shXPC group had lung tumor nodules (Table 1). The mean number of lung tumor nodules in the CL1-0/shXPC mice was higher than in the CL1-5/VC mice (17 for CL1-0/shXPC vs. 12 for CL1-5/VC). The lung weight of the CL1-0/shXPC mice was greater than in the CL1-0/NC mice (470 mg vs. 373 mg; $P < 0.001$; Table 1), but there was no difference between the CL1-0/shXPC and CL1-5/VC mice (470 mg vs. 441 mg). More interestingly, the

Table 1. Comparison of lung tumor metastatic nodules and lung weight among the 4 groups of nude mice injected with CL1-0/NC, CL1-0/shXPC, CL1-5/VC, and CL1-5/XPC cells

Cell lines	Lung weight, mg		Lung metastasis	
	Mean \pm SD Median (range)	<i>P</i>	No. of mice with lung metastasis/total no. of mice	Median lung nodules (range)
CL1-0/NC	373.90 \pm 3.07 374 (370–379)	<i>P</i> < 0.001 ^a	0/10	0
CL1-0/shXPC	470.20 \pm 52.31 504 (406–520)		6/10	17 (0–19)
CL1-5/VC	441.70 \pm 34.09 441 (405–477)	<i>P</i> < 0.001 ^a	5/10	12 (0–13)
CL1-5/XPC	383.40 \pm 8.99 384 (370–395)		0/10	0

^a*P* values of CL1-0/NC vs. CL1-0/shXPC and CL1-5/VC vs. CL1-5/XPC were calculated by 1-way ANOVA. *P* \leq 0.05 was considered significant.

lung weight of the CL1-5/XPC mice was significantly lower than in the CL1-5/VC mice (383 mg vs. 441 mg, *P* < 0.001). These results strongly suggest that p53 dysfunction by XPC defects may promote lung tumor metastasis.

Reduced XPC mRNA level is associated with distant metastasis in lung cancer patients

To clarify which of the observations from the cell and animal models might be found in lung cancer patients, XPC, p21, MDM2, and MMP1 mRNA expression levels were determined by real-time RT-PCR from 37 early-stage (I + II) and 63 late-stage (IIIb + IV) tumors, collected by CT-guided biopsy. The median value of XPC mRNA levels was 1.299 among these tumors (0–119.24), and this value was used as a cutoff point to categorize the tumors into 2 groups with respect to low or high XPC mRNA levels. Statistical analysis of the relationships of XPC mRNA levels with clinicopathologic parameters showed that a low XPC mRNA level was more common in tumors with late-stage, distant metastasis (M1), nodal metastasis, and *T* value (*P* < 0.001 for tumor stage, distant metastasis, and nodal metastasis; *P* = 0.006 for *T* value; Table 2). Interestingly, lung tumors with low XPC mRNA had a higher prevalence of low MDM2 and p21 expression levels than did those with high XPC mRNA (*P* < 0.001; Table 2). In contrast, XPC mRNA expression in tumors was negatively correlated with MMP1 mRNA expression (*P* < 0.001; Table 2). These *in vivo* results from patients are consistent with the *in vitro* findings from cells and *in vivo* xenograft tumors. Therefore, XPC defects enhance tumor progression and metastasis via upregulation of MMP1, and this may be associated with a poor prognosis for patients with lung adenocarcinoma.

Discussion

Ubiquitination of p53 is generally considered as an inhibitory negative signal that leads to p53 dysfunction (39). The ubiquitin-associated domain of hHR23B has been associated

with binding to polyubiquitin chains formed on p53 and protecting them from deubiquitylation (10). In the present study, we showed that XPC could prevent p53 deubiquitylation by stabilizing hHR23B protein. Further evidence for these observations is provided by the reduction in the expression of p53 and hHR23B proteins in XPC-knockdown A549 and CL1-0 cells, the marked increase in both proteins in the presence of the 26S proteasome inhibitor MG132, and the degradation of both proteins by 26S proteasomes under XPC defects (Fig. 2). We also observed that the binding ability of p53 on the p21 and MDM2 promoters was significantly increased by XPC via an increased interaction of hHR23B with p53. These results concur with a previous report showing that hHR23B proteins are required for p53 activation via enhancement of hHR23B and p53 binding on the p21 promoter (31). Our results clearly indicate that the mutant p53 function in CL1-0 and CL1-5 cells can be attenuated by XPC status (Supplementary Fig. S1). Reactivation of mutant p53 is a promising strategy for novel cancer therapy. The small molecule RPIMA-1 (p53 reactivation and induction of massive apoptosis) can restore mutant p53 (H175 and H273) to the wild-type conformation and allow it to inhibit the growth of various tumor cells in a p53-dependent manner and to slow the *in vivo* growth of tumors (40, 41). The overall tertiary folds of R248 mutant p53 have been shown to be similar to those of the wild-type p53 (42). Lambert et al. further indicated that the ability of R248 mutant p53 to bind covalently onto the p53 core domain can be activated by RPIMA-1 (43). Therefore, we speculated that XPC could restore R248W mutant p53 to the wild-type conformation and thereby preserve the DNA-binding domain for interaction with hHR23B, which, in turn, would result in increased stability of the R248 mutant p53 protein. Further studies using GST pull-down assays and/or X-ray crystallography analysis are needed to explore this possibility.

On the contrary, we have showed in the present study that XPC defects promote metastatic ability and correlate with MMP1 expression through suppressed p53 function (Fig. 3).

Table 2. Relationships of XPC mRNA level with clinicopathologic parameters and p21, MDM2, and MMP1 expression in NSCLC

Parameter	N	XPC		P	Parameter	N	XPC		P
		Low	High				Low	High	
Age				0.840	N				<0.001
<70	43	21 (49)	22 (51)		0	40	11 (28)	29 (72)	
70	57	29 (51)	28 (49)		1 + 2 + 3	60	39 (65)	21 (35)	
Gender				0.841	M				<0.001
Female	45	22 (49)	23 (51)		–	61	16 (26)	45 (74)	
Male	55	28 (51)	27 (49)		+	39	34 (87)	5 (13)	
Smoking				0.391	p21				<0.001
No	68	36 (53)	32 (47)		Low	50	40 (80)	10 (20)	
Yes	32	14 (44)	18 (56)		High	50	10 (20)	40 (80)	
Type				0.673	MDM2				<0.001
AD	66	34 (52)	32 (48)		Low	50	38 (76)	12 (24)	
SQ	34	16 (47)	18 (53)		High	50	12 (24)	38 (76)	
Stage				<0.001	MMP1				<0.001
I + II	36	5 (14)	32 (86)		Low	50	12 (24)	38 (76)	
III + IV	64	45 (71)	18 (29)		High	50	38 (76)	12 (24)	
T				0.001					
1 + 2	60	22 (37)	38 (63)						
3 + 4	40	28 (70)	12 (30)						

These findings are consistent with previous studies that showed an increased incidence of lung tumors in mice lacking both XPC and *GADD45a*, a *p53* downstream gene (4). These results suggest that XPC defects, combined with an inactivated *p53* pathway, may not only initiate lung tumor development but can also promote tumor metastasis. *p53* mediates the induction of MMP2 transcription by epidermal growth factor in choriocarcinoma cells (44). It also modulates the repression of the MMP9 promoter through NF- κ B inactivation in human soft tissue sarcoma (45). In addition, loss of *p53* function leads to changes in integrin-mediated MMP9 transcriptional activation during the progression of squamous cell carcinoma (46). However, we did not observe any effect of XPC on *p53*-mediated processes involving either MMP2 or MMP9 in our study (Fig. 3A). This discrepancy may be due to the use of different cancer cell types in the different reports. In present study, we further provide evidence to show that the migration/invasion ability was remarkably suppressed by MMP1 knock-down in A549/shXPC cells (Fig. 3B). These results concur with a previous report that showed that MMP1 was overexpressed in XPC-deficient cells when compared with normal dermal fibroblasts (28). Thus, MMP1 seems to play an important role in lung tumor metastasis caused by XPC defects, especially in lung adenocarcinoma.

Lung adenocarcinoma is the most common histologic type of NSCLC and is common in nonsmokers and in women (47). Therefore, establishing molecular markers to predict disease relapse of this adenocarcinoma is urgently needed. Previous studies have shown that smokers with lung cancer tend to have more proficient DNA repair

capacity than nonsmokers (48). This result suggests that DNA repair capacity in smokers may be an adaptive response to DNA damage induced by chronic tobacco carcinogen exposure (48). Among nonsmokers, it is likely that either an inadequate response of the DNA repair machinery to DNA damage or inaccurate repair contributes to the risk of developing lung cancer. Our previous reports indicated that DNA adduct levels in lung tissues from nonsmoking lung cancer patients were not statistically different from those in smoking lung cancer patients (49). More interestingly, the adduct levels in female nonsmokers were significantly higher than those of male nonsmokers (50). To understand whether DNA repair gene alteration could be responsible for the different susceptibility to DNA damage, our previous reports indicated that XPC defects through promoter hypermethylation were more common in nonsmokers than in smokers with lung cancer (5, 6). This seemed to at least partially explain why no differences in adduct levels were observed in lung tissues between smokers and nonsmokers. In the present study, we further showed that XPC defects may alter wild-type *p53* function, resulting in promotion of tumor aggressiveness via upregulation of MMP1 (Fig. 3C). Therefore, XPC defects may offer a different molecular mechanism for lung tumorigenesis, particularly in nonsmokers.

In summary, we show a mechanism by which XPC suppresses cancer cell invasion through the *p53*-mediated negative regulation of MMP1, an invasion-promoting factor (Fig. 5). We provide evidence to confirm that XPC defects may alter *p53* protein stability and its transcriptional activity via an increase in

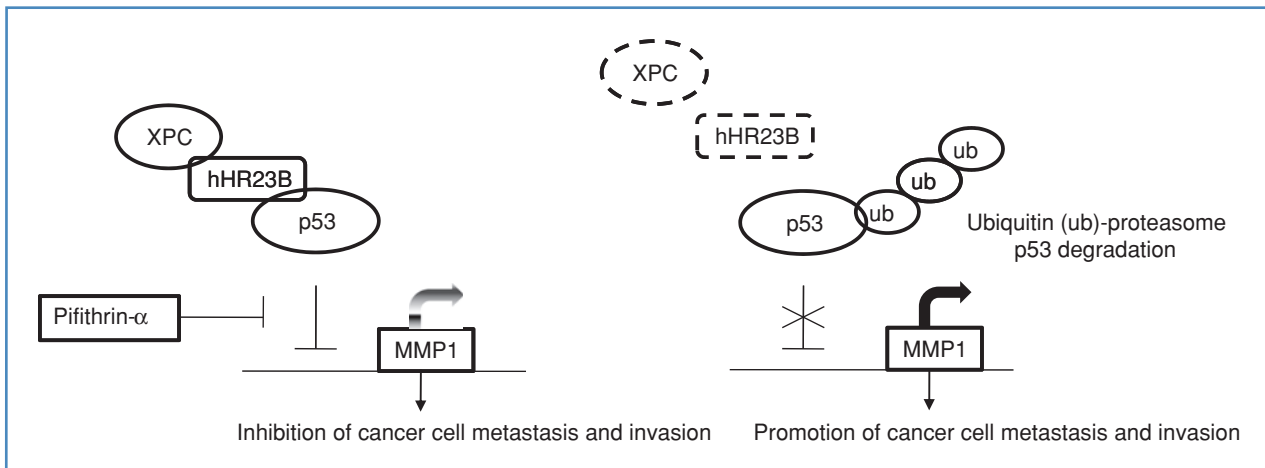


Figure 5. Proposed model illustrating the regulatory influences of XPC on p53 degradation and cancer cell invasion.

hHR23B degradation. Using cell and animal models, we show that p53 dysfunction by XPC defects may enhance tumor metastasis by the upregulation of MMP1 expression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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