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Part I: P53 dysfunction by xeroderma pigmentosum group C defects enhances lung adenocarcinoma metastasis via increased MMP1 expression

ABSTRACT

Xeroderma pigmentosum group C (XPC) interacts with hHR23B to recognize DNA damage in global genomic repair. We previously demonstrated 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 did those with high XPC mRNA levels, suggesting that XPC defects may enhance tumor metastasis. However, the underlying mechanism is unclear. Attenuation of P53 function by XPC defect affects cell growth and capacity for migration/ invasion in XPC-knockdown- or –overexpression-lung cancer cells which had p53 wild-type or mutation, *in vitro* and *in vivo*. Expression of XPC mRNA was evaluated in 100 lung tumors by real-time RT-PCR to determine whether expression level was associated with the patients' clinico-pathological parameters. We show that p53 transcriptional activity is modulated by XPC, whereby XPC stabilizes hHR23B to form an hHR23B-p53 complex, which prevents p53 degradation. Over-expression of XPC also suppresses migration/invasion ability via repression of MMP1 transcription by p53. Tumors with low XPC mRNA had a higher prevalence of low MMP1 mRNA than did those with high XPC mRNA. Patients with low XPC mRNA level 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). P53 dysfunction by XPC defects may enhance lung cancer tumor progression and metastasis via increased MMP1 expression.

Key words: Xeroderma Pigmentosum Group C, lung tumor metastasis.

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 also were 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. Additionally, patients with lower XPC mRNA levels had a poorer prognosis than did 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 also may 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 they were in squamous cell carcinomas in smokers (68% *vs.* 36%, P = 0.02 for adenocarcinomas *vs.* squamous cell carcinomas; 62% *vs.* 28%, P = 0.034 for nonsmokers *vs*. smokers). Interestingly, XPC-methylated tumors that harbored wild-type p53 had lower levels of MDM2 mRNA than did

XPC-unmethylated tumors that harbored wild-type p53 (4.9452±2.32692 *vs*. 15.9463±2.7298, P=0.013). 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 in adenocarcinoma patients even though p53 mutations have not occurred.

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 over-expression 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 (MMPs), 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 up-regulated 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. Since MMP1 is apparently regulated by p53 (29), we therefore speculate that p53 dysfunction by XPC defects may up-regulate MMP1 transcription, leading to promotion of tumor metastasis. In the present study, we provide evidence

in both cell and animal models to demonstrate that p53 dysfunction by XPC defects may enhance tumor metastasis via up-regulation of MMP1 expression. More importantly, tumor progression and metastasis were also observed in lung cancer patients with XPC defects.

MATERIALS AND METHODS

Cells and media. Human lung cancer cell lines A427, H1299, H441, and A549 were obtained from the American Type Culture Collection (Manassas, VA, USA). A427 cells were grown in Minimum Essential Medium supplemented with 10% fetal bovine serum. H1299 and A549 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Lung adenocarcinoma cell lines CL1-5 are sublines that were selected from parental CL1-0 cultures with a Matrigel-coated polycarbonate membrane (Collaborative Biomedical, BD Biosciences, Bedford, MA) in a Transwell invasion chamber. H441 and CL1- cells were grown in RPMI-1640 medium with 10% fetal bovine serum. These cells were grown at 37°C in a 5% carbon dioxide atmosphere.

**Demethylation by 5-aza-2`-deoxycytidine.** A427 cells were seeded at a low density of $1x10^5$/100-mm dish 16 h before the treatment with 1 and 5 μM 5-aza-2`-deoxycytidine (5-AZA) (Sigma-Aldrich Co., St Louis, MO, USA) for 5 days. Treated and untreated cells from individual triplicate plates were harvested for analysis of their methylation status using the MSP-PCR assay, as described previously (5). XPC mRNA was analyzed by quantitative real-time RT–PCR.

Quantitative real-time RT-PCR. Total RNA was prepared from lung cancer cells using TRIZOL reagent (Invitrogen). Total RNA (5 μg) was used in cDNA synthesis with random primers using Superscript III reverse transcriptase (Applied Biosystems). The resulting cDNA (1:20 dilution) was used to detect the expression of endogenous XPC, p21, MDM2, MMP1, MMP2 and MMP9 mRNA by qPCR. The qPCR assays were performed at least in triplicate using the ABsolute qPCR SYBR Green ROX mix (Applied Biosystems, Foster City, CA) in a 7500HT real-time PCR system apparatus

(Applied Biosystems, Foster City, CA). No-reverse-transcription (no-RT) controls were performed with 100 ng of total RNA from each individual sample as a template, to ensure that amplification was not due to contamination with DNA. No signal could be detected in the no-RT control. Relative mRNA expression was calculated with the comparative C_t method ($\Delta\Delta C_t$). 18S rRNA was used for normalization. Primers as follow: XPC, forward primer 5`-ACCTGACCTGCCGTCTAGAA-3` and reverse primer 5`-TCCACCACCCTGTTGCTGTA-3`; p21, forward primer 5`-TCGCTTGTC ACCTTGCCAT-3` and reverse primer 5`-TGTGCTAGGAAGACC CGTCAT-3`; MDM2, forward primer 5`-GATCCAGGCAAATGTGCAATAC-3` and reverse primer 5`-GCTGGAATCTGTGAGGTGGTTAC-3`; MMP1, forward primer 5'-CCTTCTACCCGGAAGTTGAG-3' and reverse primer 5'-TCCGTGTAG CACATTCTGTC-3'; MMP2, forward primer 5`-ATAACCTGGATGCCGTCGT-3' and reverse primer 5'-AGGCACCCTTGAAGAAGTAGC-3'; MMP9, forward primer 5`-GAACCAATCTCACCGACAGG-3' and reverse primer 5'-GCCACCCG AGTGTAACCATA-3'; 18S rRNA, forward primer 5`-GTGAGCGATGGAACTTCG ACTT-3` and reverse primer 5`-GGCGTTTGGAGTGGTAGAAATC-3`.

Immunoblotting, immunoprecipitation, and ubiquitination assays. For cycloheximide-chase analysis, cells were treated with cycloheximide (40 μg/ml) for various times, washed in PBS containing cycloheximide, and then cells were lysed in a lysis buffer containing 50 mM Tris-HCl (PH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM $MgCl_2$ and 0.5% Triton X-100. Lysates were cleared by centrifugation at 13000g for 20 min at 4°C and analyzed by immunoblotting. Protein samples were separated by SDS–PAGE, transferred to a poly (vinylidene difluoride) membrane and probed with the indicated antibodies. Proteins were detected by chemiluminescence. For immunoprecipitation, lysates were incubated with antibodies coupled to protein

G-agarose (Oncogene) for 1 h at 4°C. The immunoprecipitates were washed four times with lysis buffer and then subjected to immunoblotting. For ubiquitination assays, cells were treated with MG132 (10 μM) for 4 h, and then cells were lysed by incubation for 10 min at 95°C with two volumes of TBS (10 mM Tris–HCl (pH 7.5), 150 mM NaCl) containing 2% (w/v) SDS. After adding eight volumes of 1% (w/v) Triton X-100 in TBS, the lysates were sonicated for 2 min and then incubated with protein G-agarose beads. The beads were removed by centrifugation, and the lysates were immunoprecipitated with protein G-agarose-coupled anti-p53 (DO7). The beads were first washed with 0.5M LiCl in TBS and then twice with TBS, and boiled. Proteins were separated by SDS–PAGE and analyzed by immunoblotting using an anti-ubiquitin antibody.

Antibodies. Primary antibodies used were as follows: monoclonal anti-p53 (DO7, DAKO), anti-hHR23B (cat. 611019, BD Transduction Lab), anti-XPC (generated in our lab) (5), anti-ubiquitin (cat. 13-1600, Zymed), anti-β-actin (AC-15, Sigma), MMP1 (GTX100534, Gene Tex), goat anti-rabbit IgG-HRP (sc-2005, Santa Cruz), and goat anti-rabbit IgG-HRP (sc-2054, Santa Cruz).

Plasmids and transfection. Plasmids containing the XPC expression construct were constructed by cloning the full-length human XPC cDNA (GenBank accession number NM_004628) into the pcDNA3.1 eukaryotic expression vector, which also expresses a neomycin (Neo) resistance gene. The XPC and MMP1 interference construct was cloned into the vector pCDNA-HU6 as described previously (5). Reporter plasmids p21 and MDM2 luciferase reporter constructs regulated by p21 and MDM2 promoters containing p53 response elements and were kindly provided by Dr. J.-L. Ko (Institute of Medical and Molecular Toxicology, Chung Shan Medical University, Taiwan). The wild-type p53 constructs and p53-knockdown plasmids

were kindly given by Dr. J.-L. Ko and Dr. J.-T. Chang, respectively (Institute of Medical and Molecular Toxicology, Chung Shan Medical University, Taiwan). All transfection experiments were performed with TransFast transfection reagents (Promega) in accordance with the manufacturer`s protocols.

Luciferase reporter assay. For the p53-mediated transactivation, treated cells were transfected with the indicated luciferase reporters, included p21 and MDM2, as indicated in the figure legends. Each dish also received 0.25 μg of β-galactosidase vector to normalize for differences in transfection. Luciferase activity was measured 48 h later, using a dual-luciferase kit (Promega, WI). The data represent relative luciferase activity and the values are means of triplicate experiments.

Chromatin immunoprecipitation (ChIP) assay. The native protein-DNA complexes were cross-linked by treatment with 1% formaldehyde for 15 min. The ChIP assay was carried out as reported earlier. Briefly, equal aliquots of isolated chromatin were subjected to immunoprecipitation with anti-p53 (DO7, DAKO), anti-c-Fos (sc-52, Santa Cruz), anti-c-Jun (sc-44, Santa Cruz), and IgG monoclonal antibodies (sc-2005, Santa Cruz). Primers as follow: p21, forward primer 5`-GTGGCTCTGATTGGCTTT CTG-3` and reverse primer 5`-CTGAAAACAGGCAGCCCAAG-3`; MDM2, forward primer 5`-TCGGGTCACTAGTGTGAACG-3` and reverse primer 5`-CACTGAACACAGCTGGGAAA-3; MMP1, forward primer 5`-GAAAGAAATG AATTGGAGAA-3` and reverse primer 5`-AGCTAGGAAGCTCCCTCTGT-3`.

Boyden chamber assays. A Boyden chamber with a pore size of 8 μm (Falcon) was used for in vitro migration and invasion assays. Invasion chambers were prepared by coating the membranes of 48-well inserts with 0.2 mg/ml collagen type I from calf skin (Sigma-Aldrich) and leaving them to set for 1 h at 37°C. A total of 10^5 cells per

well were added to the upper chamber, suspended in 50 μl of serum-free medium, with 50 μl of medium added to the lower chamber. After 36 hours (migration assay) or 40 hours (invasion assay), the cells on the upper surface were removed using a cotton bud. The remaining invaded cells were fixed in 95% ethanol and stained with 10% Giemsa dye for 1 h at room temperature. Finally, invaded cells were counted in 10 different fields of each filter by light microscopy (Olympus, Lake Success, NY). Experiments were repeated three times.

Collagen zymography. MMP1 protease activity was identified by collagen zymography. The polyacrylamide gel (10%) contained 5 mg/ml collagen type I from calf skin (Sigma-Aldrich). After electrophoresis, the gels were washed in 2.5 % Triton X-100 (Sigma-Aldrich, St. Louis, Missouri, USA) to remove the SDS, and the marker bands were indicated. The gels were then incubated in activation buffer, containing 50 mM Tris – HCl (pH 7.8), 5 mM $CaCl_2$, and 0.1 % Triton X-100, at 37°C for 43 hours. The gels were stained for 45 minutes with 2.5 g/l Coomassie Brilliant Blue R250 (Imperial Chemical Industries Plc, London, UK), 10% acetic acid, and 40% methanol in water, followed by destaining with 10% acetic acid and 40% methanol in water. The MMP1 appeared as bright bands within the stained gel as“partially active” (44 KDa) and “fully active” (41 kDa), as previously described by Rao et al. (43).

Stable transfected clone selection. The XPC knockdown expression vectors were transfected into CL1-0 cells, and XPC expression vectors were transfected into CL1-5 cells with TransFast transfection reagent (Promega). Twenty-four hours after transfection, stable transfectants were selected in geneticin (G418; Promega) at a concentration of 600 μg/mL. Thereafter, the selection medium was replaced every 3 days. After 2 weeks of selection in G418, clones of resistant cells were isolated and allowed to proliferate in medium containing G418 at 100 μg/mL. Integration of

transfected plasmid DNA was confirmed by reverse transcription–polymerase chain reaction and western blot analysis.

Effect of XPC on the cell proliferation of CL1-0 and CL1-5 lung cancer cells. Stable cells (10^3/ ml) were seeded into a 35-mm dish and cultured for 24, 48, 72, and 96 h, and then the cell number at each culture time point was counted for calculation of the doubling time.

Tumorigenesis and metastasis analysis. All animal studies were approved by the Institutional Animal Care and Use Committee at Chung Shan Medical University. In order 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 1-2 weeks while caged in groups of five. The mice were housed in pathogen-free conditions and fed a diet of animal chow and water throughout the experiment. Mice were randomized to one of four groups and were injected subcutaneously with CL1-0/shXPC cells (n = 10), CL1-5/XPC cells (n = 10), or CL1-0 transfected with non-specific 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, Elkhart, IN) 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.

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 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 were lifetime never smoked.

Statistical analysis. Statistical analysis was performed using the SPSS statistical software program (Version 11.0 SPSS Inc., Chicago, IL, USA). The Student's t-test and Fisher's exact test (two-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 down-stream gene (p21 and MDM2) by real-time RT-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 4~6 fold after the transfection of an XPC expression vector when compared with transfection with the empty vector (Fig. 1C, left panel). In contrast, transcriptional activity of p53 was significantly reduced in A549/shXPC cells (Fig. 1C, middle panel). 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. 1C, right panel). These results clearly indicate that the transcriptional function of wild-type p53 can be modulated by XPC defects in lung cancer cells.

To explore whether mutant p53 function could be restored by XPC, CL1-0 cells with a relatively higher XPC-expression were selected for XPC-knockdown (CL1-0/shXPC) and CL1-5 cells with lower XPC-expression which harbored p53 mutation at codon 248 were selected for XPC–transfection (CL1-5/XPC). The levels of hHR23B and p53 proteins were significantly reduced in CL1-0/shXPC and slightly elevated in CL1-5/XPC cells (Fig. 1D, left at top panel). Surprisingly, p21 and MDM2 expression levels were significantly reduced in CL1-0/shXPC cells compared with those of CL1-0/NC cells. Conversely, the mRNA levels of both genes were elevated more than 2-fold in CL1-5/XPC cells (Fig. 1D, middle at top panel). Luciferase reporter analysis showed that transcriptional function of p53 on p21 and MDM2 promoters decreased in CL1-0/shXPC cells and increased in CL1-5/XPC cells (Fig. 1D, right at top panel). The binding activity of p53 on p21 and MDM2 promoter regions disappeared in CL1-0/shXPC and appeared in CL1-5/XPC cells (Fig. 1D, left at bottom panel). The proliferation rate was also increased by XPC-knockdown in CL1-0 cells and decreased by XPC-overexpression in CL1-5 cells when compared with proliferation of NC and VC cells (Fig. 1D, right at bottom panel). These results clearly indicate that the mutant p53 function in CL1-0 and CL1-5 cells can be modulated by XPC status, resulting in changes in the cell proliferation rate.

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 above, 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 also was 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 up-regulated 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 above, MMP1 levels increased significantly in XPC-deficient cells when compared with normal dermal fibroblasts (28). Since MMP2 and MMP9 are representative proteases known to be involved in lung adenocarcinoma metastasis (30-35), we hypothesized that XPC

defects may promote the migration/ metastatic ability via up-regulation of these MMPs. Real-time RT-PCR assays showed that MMP1 expression was decreased by XPC over-expression in A427 and CL1-5 cells, and was increased by XPC knockdown in A549 and CL1-0 cells (Fig. 3A, left panel). However, MMP2 and MMP9 expression levels were not changed by XPC over-expression or knockdown in these cells (Fig. 3A, middle panel). Collagen zymography assays further confirmed that MMP1 expression modulated by XPC knockdown or over-expression in these cells was consistent with the protease enzyme activity (Fig. 3A, right panel). Collagen-Coated Boyden chamber assays further showed that the migration/invasion ability were significantly decreased by XPC over-expression in A427 and CL1-5 cells (Fig. 3B) and increased by XPC knockdown in A549 and CL1-0 cells 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 RNAi was transfected into A549/shXPC cells. The migration/invasion ability was significantly diminished in A549/shXPC/shMMP1 cells (Fig. 3B). These results strongly suggest that cell migration/invasion ability enhanced by XPC defects may be predominantly mediated through increased MMP1 expression.

P53 down-regulated 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 in A427 cells was mediated through the restoration of p53 function, we used either a p53 specific inhibitor, Pifithrin-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 to VC cells, but mRNA levels of both proteins in A427/XPC cells were reduced by Pifithrin-α treatment and p53 shRNA transfection (Fig. 3C, left at top panel). Western blotting

indicated that the decrease in MMP1 in A427/XPC cells was significantly restored by both treatments (Fig. 3C, right at top panel). 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 Pifithrin-α treatment or p53 shRNA transfection (Fig. 3C, bottom panel). Collagen zymography assays further confirmed that MMP1 protease enzyme activity in A427/XPC cells was elevated by either Pifithrin-α treatment or p53 shRNA transfection (Fig. 3D, left panel). The migration/invasion ability of A427/XPC cells was also increased by either treatment (Fig. 3D, right panel). However, restoration of the mutant p53 function by XPC transfection was not observed in H441 cells, which harbor a p53 mutation at codon 158 (supplementary Fig. 1A and 1B). Expression of MMP1 was also unaltered by XPC over-expression in H441 cells. Therefore, the migration/invasion ability in H441 cells was not influenced by XPC over-expression (Supplementary Fig. 1C and 1D). These results indicate that the migration/invasion enhancement due to MMP1 expression may be mediated through the suppression of p53 function by XPC defects.

Tumor growth and metastasis is 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 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 three 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 one mouse in the CL1-5/XPC group that had a small tumor

burden) (Fig. 4A, 4B). 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, 4B). 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, 4B). 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, CL-1-0/shXPC, CL1-5/VC and CL-1-5/XPC cells via the tail vein. After four months, the mice were sacrificed in order to count the lung tumor nodules in the mice from of each group (Fig. 4C, 4D). Among these four 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 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 cut-off point to categorize the tumors into two groups with respect to low or high XPC mRNA levels. Statistical analysis of the relationships of XPC mRNA levels with clinico-pathological 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 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 up-regulation 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 (36). The ubiquitin-associated (UBA) 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 demonstrated that XPC could prevent p53 deubiquitylation by stabilizing hHR23B protein. Further evidence for these observations is provided by that the reduction in 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 (37). Previous research has shown that PRIMA-1 can restore mutant p53 to its wild-type p53 function and can transactivate its downstream genes including p21, MDM2, and Bax (38). Thus, PRIMA-1 can induce *in vitro* and *in vivo* apoptosis (39). Recently, PRIMA-1 has been shown to reactivate mutant p53 (H175 and R248) via covalent binding to the p53 core domain (40). This work by Lambert et al. (40) and other groups (41, 42) raise the possibility that tumors that express mutant p53 may be effectively eradicated by blocking the gain-of-function effects of mutant p53. This could be effected either by molecules that directly disrupt the interaction of mutant p53 with other molecules or by restoring mutant p53 to its wild-type conformation. These results prompt us to speculate that XPC could restore mutant p53 to its wild-type conformation, thus preserving the DNA binding domain where wild-type

p53 interacts with hHR23B. Further studies using GST-pull down assays and/or X-ray crystallography analysis are needed to explore this possibility.

On the other hand, we have demonstrated in the present study that XPC defects promote metastatic ability and correlate with MMP1 expression through suppressed p53 function (Fig. 3). 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 (ECF) in choriocarcinoma cells (44). It also modulates the repression of the MMP9 promoter through NF-kB 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 knockdown in A549/shXPC cells (Fig. 3B). These results concur with a previous report that showed that MMP1 was over-expressed in XPC-deficient cells when compared with normal dermal fibroblasts (28). Thus, MMP1 appears to play an important role in lung tumor metastasis caused by XPC defects, especially in lung adenocarcinoma.

Lung adenocarcinoma is the most common histological 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.

Comparison of 137 tumor tissues and their normal counterparts showed that reduced XPC mRNA levels were prevalent in patients who were nonsmokers and whose tumors showed nodal metastasis, who had advanced stage disease, or who had disease relapse after surgical therapy. By Kaplan-Meier analysis, patients with reduced XPC mRNA levels, and especially nonsmokers, also had a shorter progression to disease relapse (manuscript in submission). Thus, reduction in XPC mRNA expression could serve as an independent molecular marker for predicting disease relapse in NSCLC, especially in nonsmokers. If alterations in XPC expression are involved in lung adenocarcinoma progression, this might provide new opportunities for therapeutic intervention.

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 hHR23B degradation. Through cell and animal models, we demonstrate that p53 dysfunction by XPC defects may enhance tumor metastasis by the up-regulation of MMP1 expression.

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FIGURE LEGENDS

Figure 1. XPC positive regulates p53 transcriptional function.

(A) XPC, p21 and MDM2 mRNA expressions in A549, A427, CL1-0 and CL1-5 cells. (B) Top panel: A427 cells treated with 1 and 5 μM 5-AZA for 5 days. Cells were collected and harvested for MSP assay or mRNA expression. U: unmethylated band; M: methylated band. Levels of relative expression of XPC, p21 and MDM2 were determined using real-time PCR. Bottom panel: XPC, p21 and MDM2 mRNA expressions in A427 cells transiently transfected with the indicated amounts of XPC gene cDNA expression vector as indicated and A549 cells stably transfected with a vector encoding a small interfering hairpin RNA targeted against XPC mRNA (shXPC) or a non-specific control (NC). Immunoblotting was performed to evaluate the p53 and XPC protein expressions in A427 cells transiently transfected with the indicated amounts of XPC gene cDNA expression vector and A549 cells stably transfected with a vector encoding a small interfering hairpin RNA targeted against XPC mRNA (shXPC) or a non-specific control (NC). β–actin was the loading control. (C) Left panel at bottom: A427 cells were co-transfected with XPC gene cDNA expression vector or vector control (VC) along with 1 μg of reporter plasmid for 48 h. XPC-RNAi knockdown A549 cells were transfected with 1 μg of reporter plasmid for 48 h. Relative luciferase units and standard deviations were derived from triplicate experiments. Right panel at bottom: ChIP assay was performed to evaluate the binding activity of p53 on p21 and MDM2 promoters in A427 cells transfected with XPC gene cDNA expression vector and XPC-RNAi knockdown A549 cells. (D) Left panel at top: XPC, hHR23B and p53 protein expressions in XPC-RNAi knockdown CL1-0 cells (shXPC) or NC and CL1-5 cells were stably transfected with XPC gene cDNA expression vector or VC. β-actin served as the loading control. Middle panel at

top: XPC, p21 and MDM2 mRNA expressions in CL1-0/shXPC, CL1-0/NC, CL1-5/VC and CL1-5/XPC cells. Right panel at top: CL1-0/shXPC, CL1-0/NC, CL1-5/VC and CL1-5/XPC cells transfected with 1 μg of reporter plasmid for 48 h. Relative luciferase units and standard deviations were derived from triplicate experiments. Left panel at bottom: ChIP assay was performed to evaluate the binding activity of p53 on p21 and MDM2 promoters in CL1-0/shXPC, CL1-0/NC, CL1-5/VC and CL1-5/XPC cells. Right panel at bottom: Cell count for cell proliferation in CL1-0/NC, CL1-0/shXPC, CL1-5/VC and CL1-5/XPC cells.

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 μM) 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 μg/ml) and analyzed by immunoblotting. (C) A549 and CL1-0 cells transfected with shXPC were treated with MG132 for 4 h and then cell lysates were immunoprecipitated with anti-p53 antibodies. The resulting immunoprecipitates (IPs) 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 h and then cell lysates were immunoprecipitated with anti-hHR23B, anti-p53, anti-XPC and anti-IgG antibodies, and the resulting immunoprecipitates (IPs) were analyzed by immunoblotting.

Figure 3. Over-expression of XPC suppresses cell metastatic ability via repression of MMP1 transcription by p53.

(A) MMP1, MMP2 and MMP9 mRNA expressions were evaluated by real-time

RT-PCR assay in A427 cells transiently transfected with the indicated amounts of XPC gene cDNA expression vector, A549/NC, A549/shXPC, CL1-0/NC, CL1-0/shXPC, CL1-5/VC and CL1-5/XPC cells. Western blotting was performed to evaluate the MMP1 protein expressions in A427 cells transiently transfected with the indicated amounts of XPC gene cDNA expression vector, A549/NC, A549/shXPC, CL1-0/NC, CL1-0/shXPC, CL1-5/VC and CL1-5/XPC cells. β–actin was the loading control. Collagen zymography assay was performed to evaluate the MMP1 protease activity in A427 cells transiently transfected with the indicated amounts of XPC gene cDNA expression vector, A549/NC, A549/shXPC, CL1-0/NC, CL1-0/shXPC, CL1-5/VC and CL1-5/XPC cells.. (B) *In vitro* migration and invasion activity of A427 cells transiently transfected with the indicated amounts of XPC gene cDNA expression vector, A549/NC, A549/shXPC and A549/shXPC/shMMP1 cells and CL1-0/NC, CL1-0/shXPC, CL1-5/VC and CL1-5/XPC cells. All data represent the mean ± the standard deviation of 3 separate experiments. Levels of relative expression of MMP1 were evaluated by real-time RT-PCR. (C) A427 cells were transiently transfected with 5 μg XPC gene cDNA expression vector for 24 h, and then treated with 20 μM PFT-α or 5 μg shp53 plasmids for 24 h. Cells were collected and harvested for protein and mRNA expressions. Left panel: MMP1, p53 and XPC protein expressions were evaluated by immunoblotting. β–actin was the loading control. Right panel: Levels of relative expression of p21 and MDM2 were determined using real-time RT-PCR. ChIP assay was performed to evaluate the binding activity of c-Fos and c-Jun on MMP1 promoters in A427 cells transiently transfected with XPC gene cDNA expression vector after both treatments. (D) Left panel: Collagen zymography assay was performed to evaluate the MMP1 protease activity in A427 cells transiently transfected with XPC gene cDNA expression vector after both treatments. Right panel: *In vitro* migration and invasion activity of A427

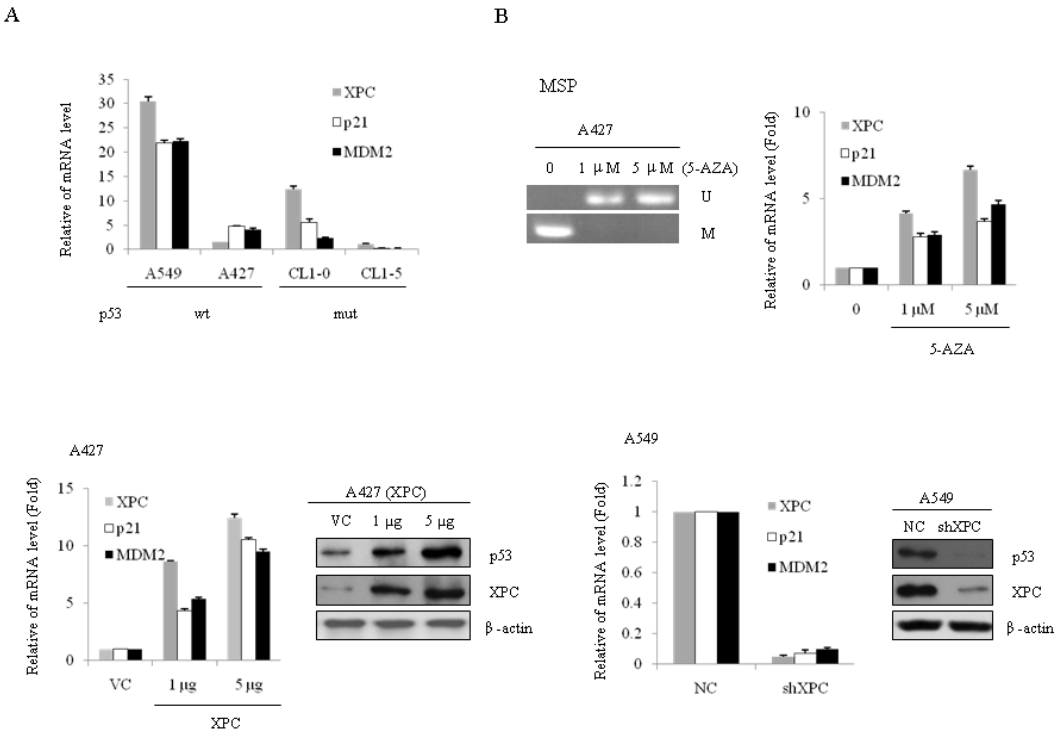
cells transiently transfected with XPC gene cDNA expression vector after both treatments. All data represent the mean ± the standard deviation of 3 separate experiments. Levels of relative expression of MMP1 were evaluated by real-time RT-PCR.

Figure 4. XPC suppresses metastasis.

(A, B) Growth patterns of xenograft tumors formed subcutaneously by CL1-0/NC, CL1-0/shXPC, CL1-5/VC and CL1-5/XPC cells. Each data point represents the mean ± SD of ten primary tumors. (C) Lungs were excised and photographed after experimental metastasis assay. (D) Histological analyses of lung metastatic tumors.

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

Fig. 1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B
Relative of mRNA level
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MDM2
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A427
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p53
wt
mut
MSP
A427
0
1 μM
5 μM
(5-AZA)
U
M
Relative of mRNA level (Fold)
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VC
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5 μg
p53
XPC
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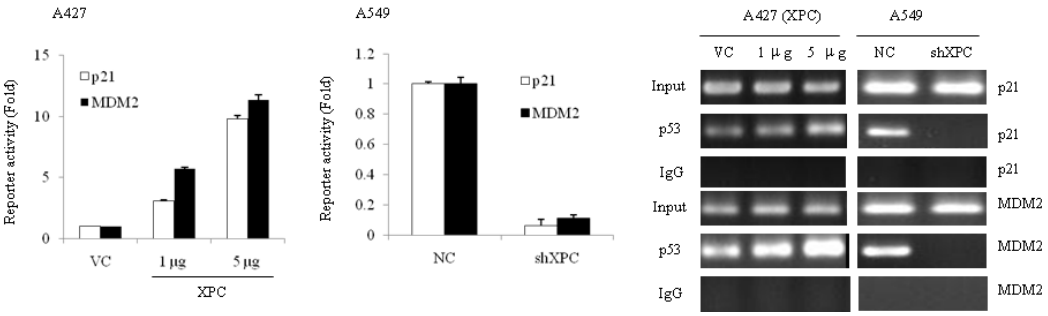
NC
shXPC

Fig. 1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A427
A549
A427 (XPC)
Reporter activity (Fold)
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VC
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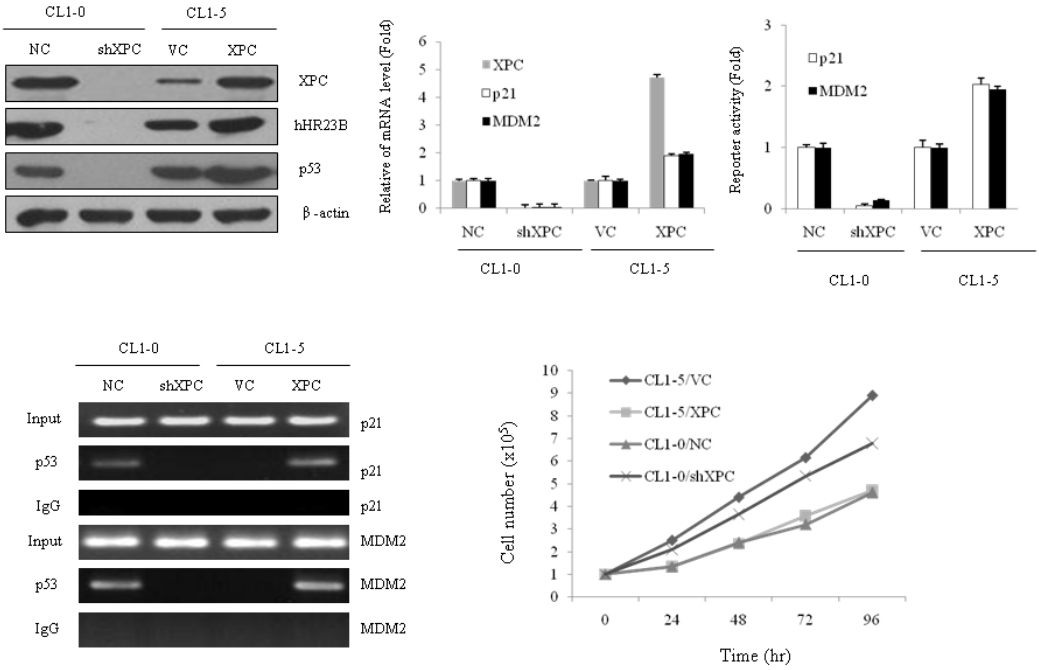
XPC
NC
shXPC
Input
p53
IgG

Fig. 1

D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VC
XPC
XPC
hHR23B
p53
β-actin
Relative of mRNA level (Fold)
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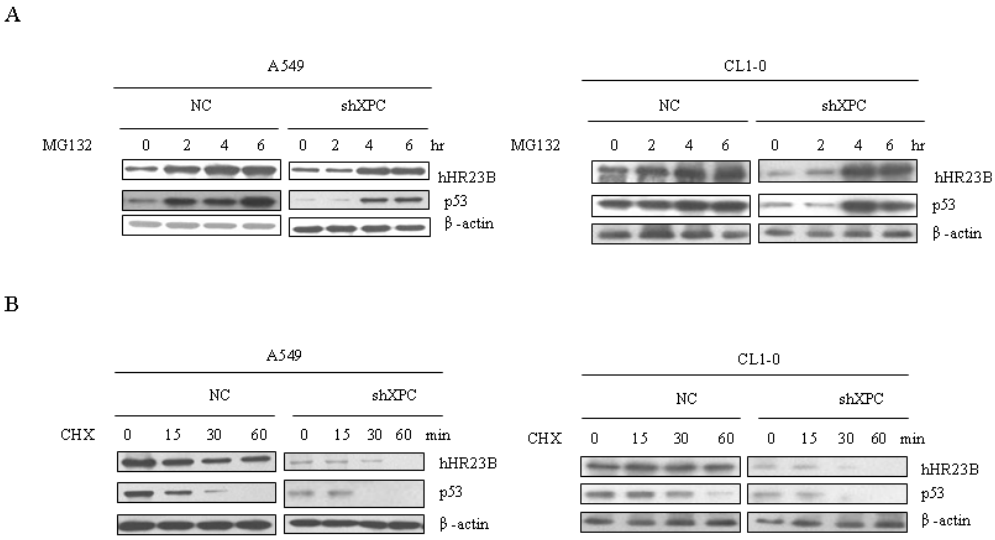
p21
MDM2
Reporter activity (Fold)
p21
MDM2

CL1-0
CL1-5
NC
shXPC
VC
XPC
Input
p53
IgG
p21
MDM2

CL1-5/VC
CL1-5/XPC
CL1-0/NC
CL1-0/shXPC
Cell number (x10⁵)
Time (hr)

Fig. 2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0 2 4 6 0 2 4 6 hr
hHR23B
p53
β-actin
B
A549
NC
shXPC
CHX
0 15 30 60 0 15 30 60 min
hHR23B
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CHX
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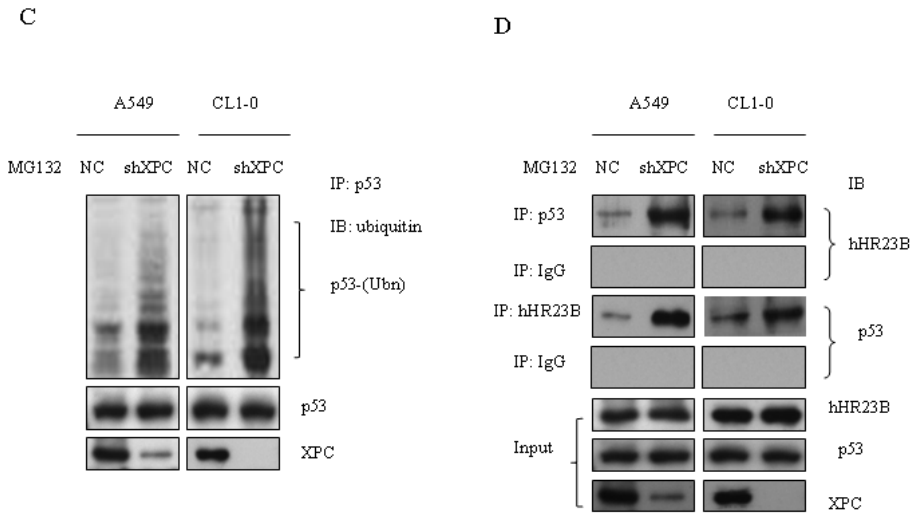
hHR23B
p53
β-actin

Fig. 2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CL1-0
MG132
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A549
CL1-0
MG132
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IB
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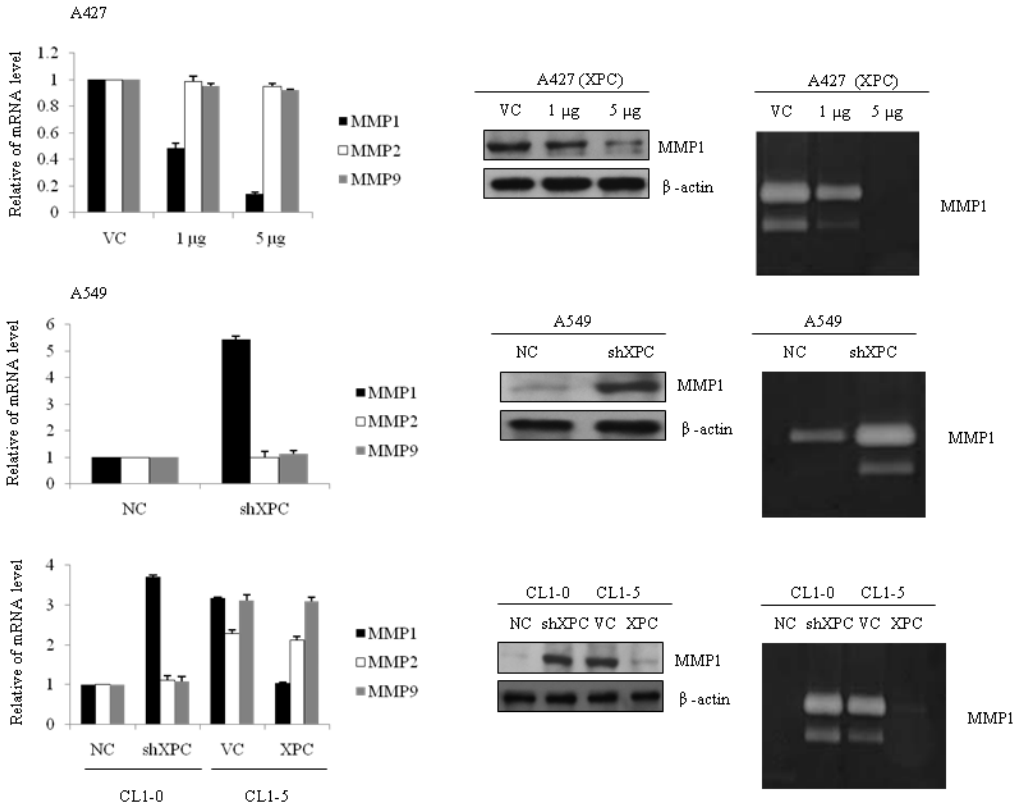
p53
XPC

Fig. 3

A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Relative of mRNA level
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1
0.8
0.6
0.4
0.2
0
VC
1 μg
5 μg
MMP1
MMP2
MMP9
A427 (XPC)
VC 1 μg 5 μg
MMP1
β -actin
A427 (XPC)
VC 1 μg 5 μg
MMP1
A549
Relative of mRNA level
6
5
4
3
2
1
0
NC
shXPC
MMP1
MMP2
MMP9
A549
NC shXPC
MMP1
β -actin
A549
NC shXPC
MMP1
Relative of mRNA level
4
3
2
1
0
NC shXPC VC XPC
CL1-0 CL1-5
MMP1
MMP2
MMP9
CL1-0 CL1-5
NC shXPC VC XPC
MMP1
β -actin
CL1-0 CL1-5
NC shXPC VC XPC
MMP1

Fig. 3

B

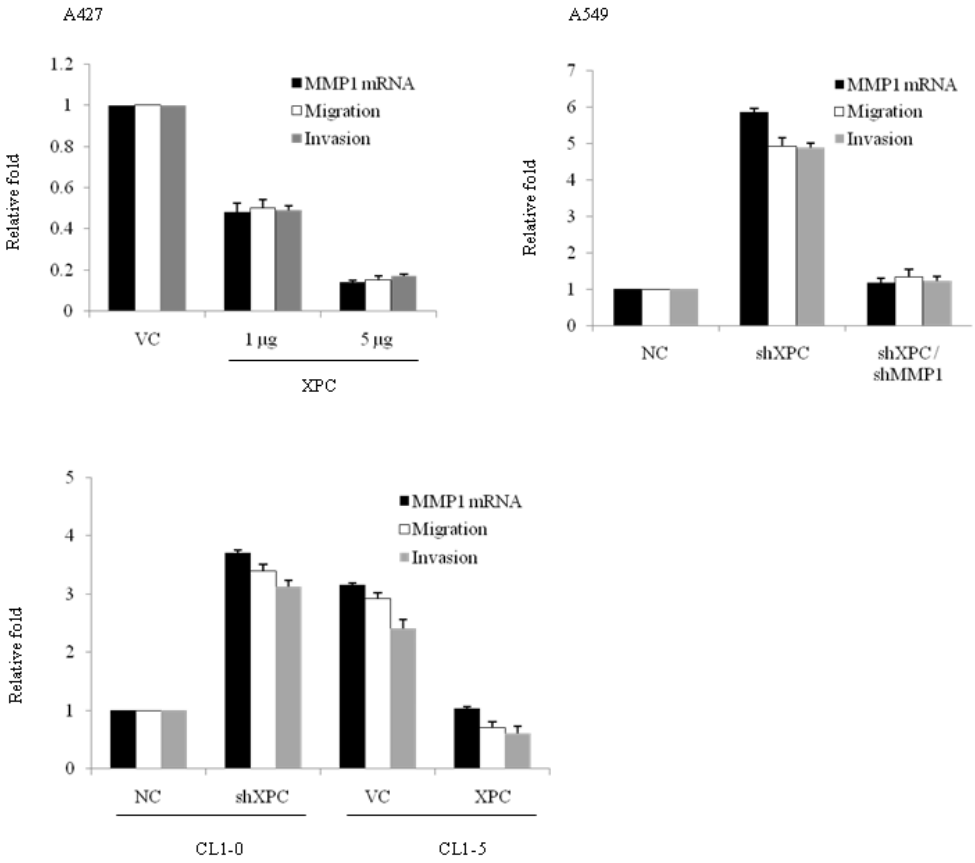
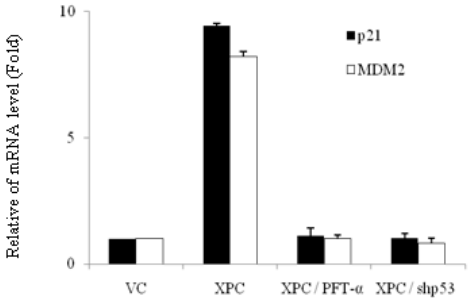
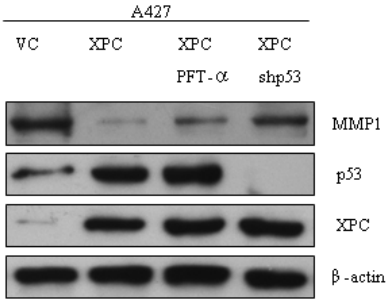

A427
Relative fold
1.2
1
0.8
0.6
0.4
0.2
0
MMP1 mRNA
Migration
Invasion
VC
1 μg
5 μg
XPC
A549
Relative fold
7
6
5
4
3
2
1
0
MMP1 mRNA
Migration
Invasion
NC
shXPC
shXPC / shMMP1
Relative fold
5
4
3
2
1
0
MMP1 mRNA
Migration
Invasion
NC
shXPC
VC
XPC
CL1-0
CL1-5

Fig. 3

C


10
5
0
Relative of mRNA level (Fold)
p21
MDM2
VC
XPC
XPC / PFT-α
XPC / shp53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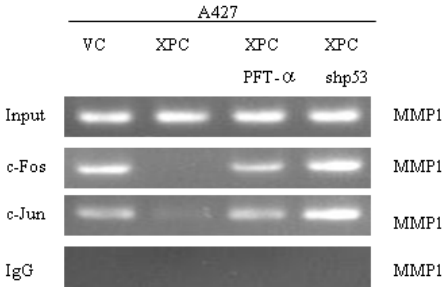
VC
XPC
XPC
PFT-α
XPC
shp53
MMP1
p53
XPC
β -actin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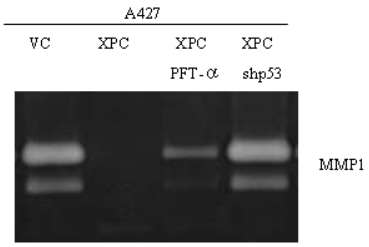
XPC
XPC
PFT-α
XPC
shp53
Input
c-Fos
c-Jun
IgG
MMP1
MMP1
MMP1
MMP1

Fig. 3

D


A427
VC
XPC
XPC
PFT-α
XPC
shp53
MMP1

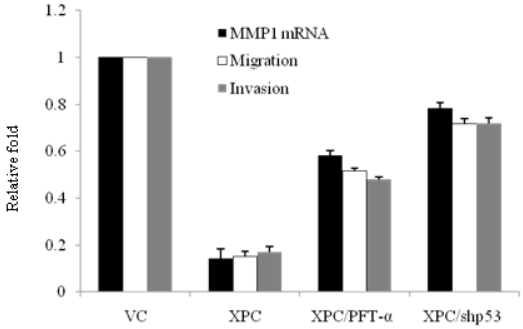

1.2
1
0.8
0.6
0.4
0.2
0
Relative fold
MMP1 mRNA
Migration
Invasion
VC
XPC
XPC/PFT-α
XPC/shp53

Fig. 4

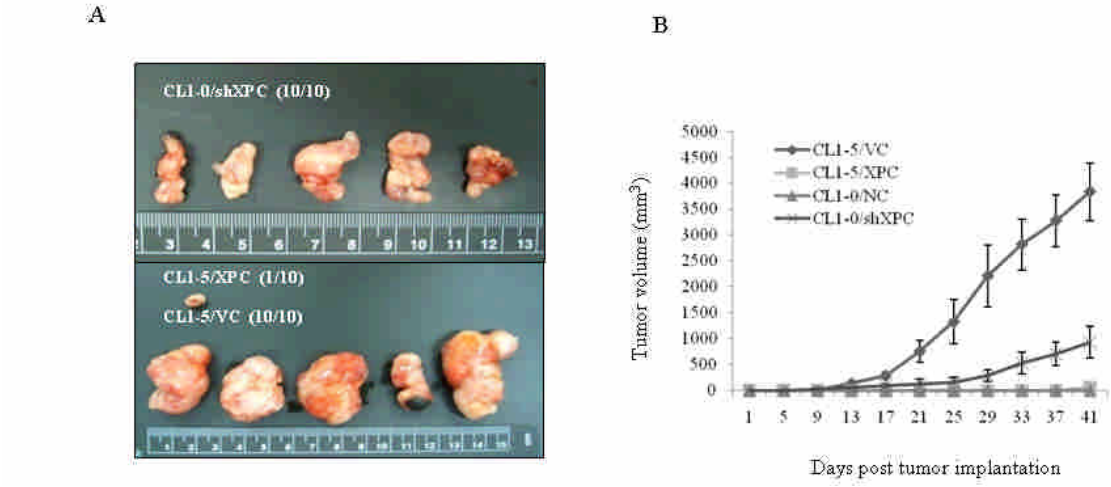

A
CL1-0/shXPC (10/10)
CL1-5/XPC (1/10)
CL1-5/VC (10/10)
B
CL1-5/VC
CL1-5/XPC
CL1-0/NC
CL1-0/shXPC
Tumor volume (mm3)
Days post tumor implantation

Fig. 4

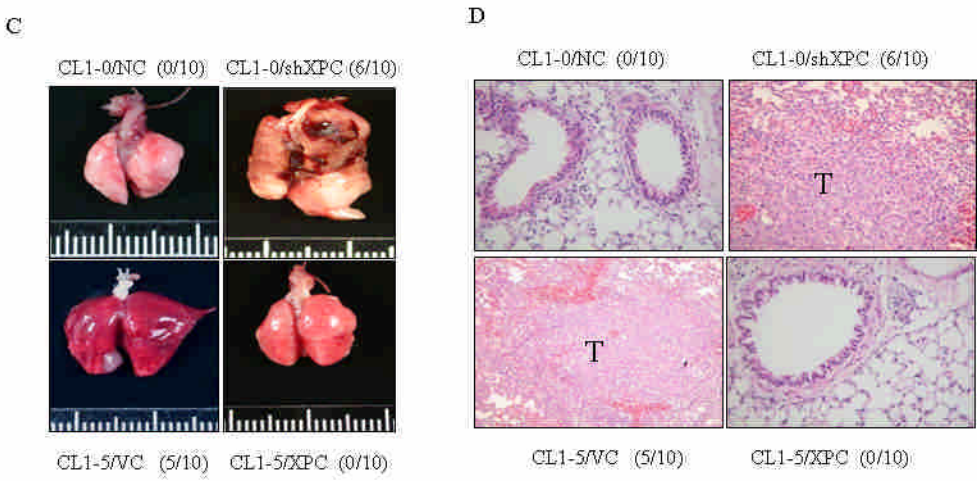

C
CL1-0/NC (0/10)
CL1-0/shXPC (6/10)
CL1-5/VC (5/10)
CL1-5/XPC (0/10)
D
CL1-0/NC (0/10)
CL1-0/shXPC (6/10)
T
T
CL1-5/VC (5/10)
CL1-5/XPC (0/10)

Fig. 5

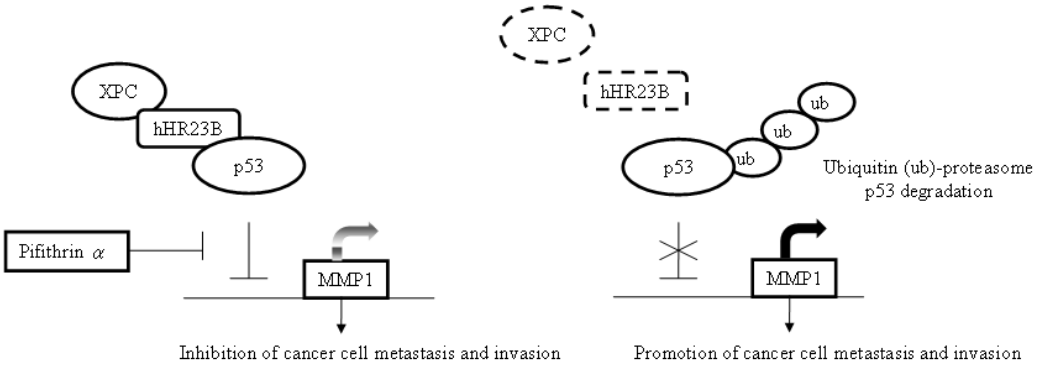

XPC
hHR23B
p53
Pifithrin α
MMP1
Inhibition of cancer cell metastasis and invasion
XPC
hHR23B
ub
ub
ub
p53
Ubiquitin (ub)-proteasome
p53 degradation
MMP1
Promotion of cancer cell metastasis and invasion

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

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

* P value of CL1-0/NC *vs.* CL1-0/shXPC and CL1-5/VC *vs.* CL1-5/XPC was calculated by One-Way ANOVA test. *P*-value <=0.05 was considered significant.

Table 2. Relationships between XPC mRNA level and clinico-pathological parameters in lung cancer patients.

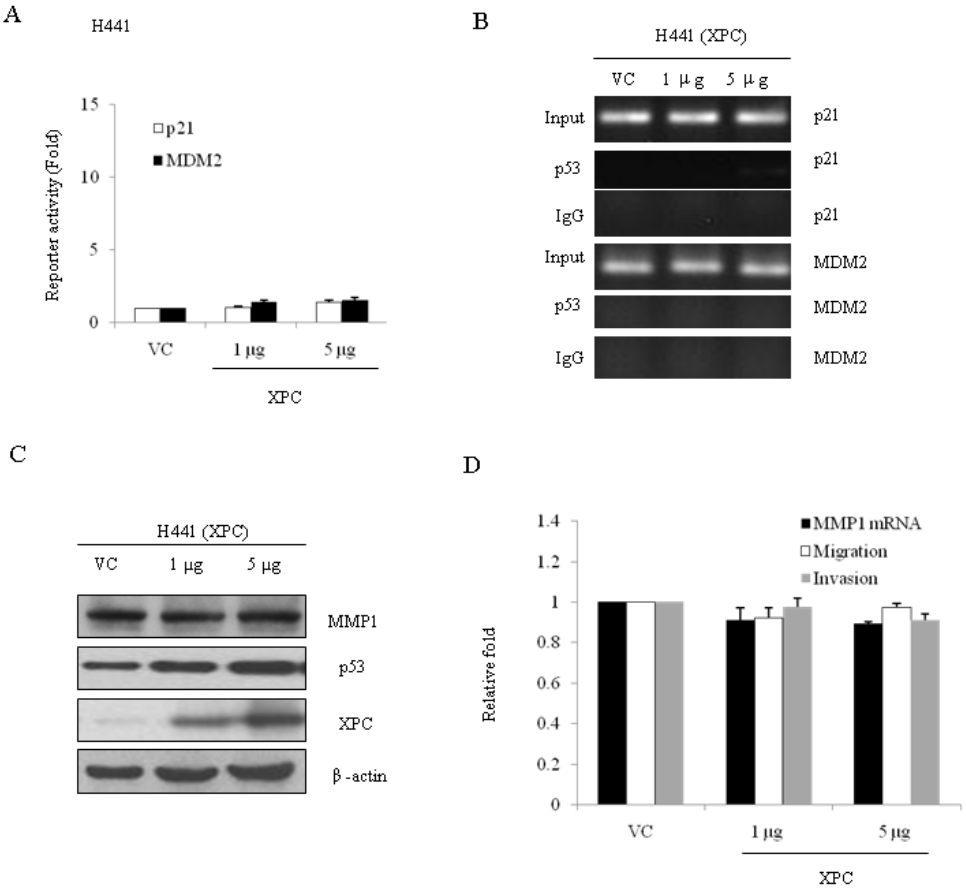
Parameter	Patient no.	XPC mRNA		P value
		Low	High	
Age				0.840
<70	43	21 (49)	22 (51)	
>=70	57	29 (51)	28 (49)	
Gender				0.841
Female	45	22 (49)	23 (51)	
Male	55	28 (51)	27 (49)	
Smoking				0.391
Nonsmoker	68	36 (53)	32 (47)	
Smoker	32	14 (44)	18 (56)	
Type				0.673
AD	66	34 (52)	32 (48)	
SQ	34	16 (47)	18 (53)	
Stage				< 0.001
I	26	4 (15)	22 (85)	
II	10	1 (10)	9 (90)	
III	25	11 (44)	14 (56)	
IV	39	34 (87)	5 (13)	
T				0.006
1	20	5 (25)	15 (75)	
2	40	17 (43)	23 (57)	
3	11	8 (73)	3 (27)	
4	29	20 (69)	9 (31)	
N				< 0.001
0	40	11 (28)	29 (72)	
1	11	3 (27)	8 (73)	
2	26	17 (65)	9 (35)	
3	23	19 (83)	4 (17)	

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. XPC regulates p53 transcriptional function.

(A) H441 cells were co-transfected with XPC gene cDNA expression vector along with 1 μg of reporter plasmid for 48 h. Relative luciferase units and standard deviations were derived from triplicate experiments. (B) ChIP assay was performed to evaluate the binding activity of p53 on p21 and MDM2 promoters in H441 cells transfected with XPC gene cDNA expression vector. (C) MMP1, p53 and XPC protein expressions were evaluated by immunoblotting of H441 cells transiently transfected with XPC gene cDNA expression vector. β-actin was the loading control. (D) *In vitro* migration and invasion activity of H441 cells transiently transfected with XPC gene cDNA expression vector after both treatments. All data represent the mean ± the standard deviation of 3 separate experiments. Levels of relative expression of MMP1 were evaluated by real-time RT-PCR.

Fig. S1


A
H441
Reporter activity (Fold)
15
10
5
0
p21
MDM2
VC
1 μg
5 μg
XPC
B
H441 (XPC)
VC
1 μg
5 μg
Input
p53
IgG
Input
p53
IgG
p21
p21
p21
MDM2
MDM2
MDM2
C
H441 (XPC)
VC
1 μg
5 μg
MMP1
p53
XPC
β-actin
D
Relative fold
1.4
1.2
1
0.8
0.6
0.4
0.2
0
MMP1 mRNA
Migration
Invasion
VC
1 μg
5 μg
XPC

Part II: Reduced XPC mRNA Level Predicts Survival and Relapse in Non-Small Cell Lung Cancer

ABSTRACT

Background Lung tumors with low levels of mRNA for the Xeroderma pigmentosum group C (XPC) gene has been shown to associate with poorer survival in patients with non-small cell lung cancer (NSCLC). In the present study, we investigate whether XPC mRNA levels in lung tumors could predict survival and relapse in patients with resected NSCLC.

Methods Lung tumors and adjacent normal lung tissues were surgically resected from 137 NSCLC patients. The XPC mRNA levels were evaluated by real-time polymerase chain reaction (PCR). A tumor with a ratio of XPC mRNA expression of less than 1 compared to a normal tissue counterpart was defined as having a reduced XPC mRNA level.

Results Reduced XPC mRNA levels were more prevalent in patients who were nonsmokers or who had advanced stage disease, nodal metastasis, or disease relapse. Patients with reduced XPC mRNA levels had hazard ratios (HR) of 1.891 for OS and 6.334 for disease-free survival (DFS) compared with those with non-reduced XPC mRNA levels ($P = 0.016$ for OS; $P < 0.001$ for DFS). This prognostic significance was only observed in nonsmokers, not in smokers. More surprisingly, the HR value for predicting DFS was significantly higher for reduced XPC mRNA level than was tumor stage (6.334 vs. 1.232).

Conclusion Reduced XPC mRNA expression may act an independent molecular marker to predict DFS in resected NSCLC, especially in nonsmokers. Thus, we suggest that XPC may be a potential target for demethylation agents to improve clinical outcomes in NSCLC patients.

Key words: XPC mRNA, disease relapse, lung cancer.

INTRODUCTION

Lung cancer is the leading cause of cancer death around the world and non-small cell lung cancer (NSCLC) accounts for about 80% of all lung cancers.[1] Current management of NSCLC is largely guided by tumor stage. Patients with early stage (I and II) tumors are treated by complete surgical resection, with or without adjuvant chemotherapy, while stage III patients require combined modality approaches that may include neo-adjuvant chemotherapy, radiation, and surgery. The overall 5-year survival rates of these patients remain relatively poor, ranging from 70% for stage I patients to 25 % for stage III patients whose tumors are surgically resected.[2] Most patient deaths are caused by disease recurrence. Consequently, there is considerable interest in searching for molecular markers that could identify patients whose disease is more likely to recur after surgery.[3] The most clinically relevant of these markers currently include carcinoembryonic antigen (CEA),[4] cancer antigen 125 CA-125,[5] tumor M2-pyruvate kinase,[5] cytokeratin 19 fragment (CYFRA 21-1),[5,6] C-reactive protein (CRP),[7] and *EGFR* mutation and *EGFR* copy number.[8] Although these biomarkers are clinically available in some areas, their use in determining disease recurrence of NSCLC is not presently recommended or encouraged.[9]

Recent molecular epidemiological studies have indicated that lung cancer arises through different molecular mechanisms in nonsmokers compared to smokers.[10-15] In general, the disease mechanisms are more complicated for smokers with non small cell lung cancer (NSCLC) than they are for nonsmokers. However, several reports now indicate that survival rates are significantly increased for nonsmokers compared to smokers.[16-20] Smoking is thought possibly to induce resistance of lung cancers to chemotherapeutic agents by induction of cytochrome P450 (CYP)[21] and alteration of the capacity for metabolism of these drugs. Tsao et al.[22] reported that chemotherapy

outcomes were better for nonsmokers than for smokers, although continued smoking during the chemotherapy did not affect overall survival. Regarding the smoking status with respect to radiation therapy for NSCLC, poorer prognosis after radiation therapy was predicted for current smokers with stage I and II disease than for nonsmokers.[23] Similarly, when treated with inhibitors of the epidermal growth factor receptor (EGFR)-associated tyrosine kinase, such as gefitinib and erlotinib, nonsmokers respond better and have better survival outcomes than do those with a history of tobacco smoking.[24, 25] Thus, understanding the molecular biology of lung cancer in nonsmokers represents an important research priority because of the growing epidemiological relevance of cancer in nonsmokers and in light of the potential need for different therapeutic approaches. Our expectation in the current study is that the establishment of molecular markers for disease recurrence and/or metastasis that can predict disease-free survival (DFS) of NSCLC may be more successful in nonsmokers than in smokers.

The Xeroderma pigmentosum group C (XPC) gene is a critical component of global genomic repair that is required for DNA damage recognition. Accumulated evidence indicates that XPC defects are associated with an increased risk of cancer.[26-30] Our previous report indicated that XPC level was predominantly altered by promoter hypermethylation and that it may contribute to the occurrence of p53 mutations in lung tumors.[31] We also observed XPC defects to be more common in nonsmokers, and that reduced levels of XPC mRNA caused by XPC promoter hypermethylation may confer nodal metastasis and tumor recurrence. Additionally, patients with lower XPC mRNA levels had a poorer prognosis than did those with higher XPC mRNA levels.[32] In the present study, we further questioned whether comparison of XPC mRNA levels in lung tumors to levels in adjacent normal lung tissues could be used to

predict disease recurrence in NSCLCs, particularly in nonsmokers.

MATERIALS AND METHODS

Study population. Lung cancer tissues and adjacent normal lung tissues were obtained surgically from patients with pathologically proven NSCLC at Department of Chest Surgery, Taichung Veteran General Hospital in Taichung, Taiwan, between June, 1994, and December, 2005. 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. Smokers were defined as active or previous smokers and nonsmokers as those who had never smoked. Tumor types and stages were histologically determined according to the WHO classification. Pathology samples were processed for conventional histological procedures. No tumors were included if they contained elements of small cell carcinoma. The study was approved by the Medical Research Ethics Committee of the Chung Shan Medical University Hospital. The study number is CS04063.

Quantitative real-time RT-PCR. Total RNA was prepared from lung tumor and adjacent normal lung tissues using TRIZOL reagent (Invitrogen). Total RNA (5 μg) was used in cDNA synthesis with random primers using Superscript III reverse transcriptase (Applied Biosystems). The resulting cDNA (1:20 dilution) was used to detect the expression of endogenous XPC mRNA by qPCR. The qPCR assays were performed at least in triplicate using the ABsolute qPCR SYBR Green ROX mix (Applied Biosystems, Foster City, CA) in a 7500HT real-time PCR system apparatus (Applied Biosystems, Foster City, CA). The primers used were as follows: (a) XPC, forward primer 5`-ACCTGACCTGCCGTCTAGAA-3` and reverse primer 5`-TCCACCACCCTGTTGCTGTA-3`; (b) 18S rRNA, forward primer 5`-GTGAGCGATGGAACTTCGACTT-3` and reverse primer 5`-GGCGTTTGGAGTG -GTAGAAATC-3`. No-reverse-transcription (no-RT) controls were performed with

100 ng of total RNA from each individual sample as a template to ensure that amplification was not due to contamination with DNA. No signal could be detected in the no-RT control. Relative mRNA expression was calculated with the comparative C_t method ($\Delta\Delta C_t$). The quantity of XPC mRNA was normalized to 18S rRNA. The expression ratio of XPC mRNA in tumors was defined as the corrected density in tumor tissues divided by that of paired normal lung tissues. Reduced expression was considered if the expression ratio was < 1. This defined method was performed according to a previous report. [33]

Statistical analysis. All statistical analyses were conducted using SPSS 15.0 statistical software. The associations between expression of XPC mRNA and clinicopathologic features were analyzed by Student *t* test and the X^2 test. The survival curves were estimated by the Kaplan-Meier method and compared by the log-rank test. The Cox-regression model was used to perform univariate and multivariate analyses, including use of all clinicopathologic features as covariates. In all tests, a P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Patient demographics

Between 1994 and 2005, 137 patients (35 females and 102 males) with median age of 67 ± 9.303 years (range, 26 to 83 years) were enrolled. None of the subjects had received radiation therapy or chemotherapy before surgery. Patients and tumor characteristics are presented in Table 1. Fifty-eight percent of patients (n = 79) were nonsmokers and 42% of patients (n = 58) were smokers. Fifty-one percent of patients (n = 70) had stage III tumors, 11 % (n = 15) of tumors were stage II, and 38 % (n = 52) of tumors were stage I. The median follow-up for all patients was 919 days, with a range of 114 to 4061 days. Fifty-two of 137 (38 %) patients were confirmed to have disease relapse, including 11 with local recurrence, 27 with distant metastasis, and 14 with both events.

Reduced XPC mRNA level in lung tumors as compared with normal counterparts is common in tumors with nodal metastasis and advanced stage

XPC mRNA levels in 137 lung tumors and adjacent normal lung tissues were measured by real-time RT-PCR. In 81 of 137 (59 %) lung tumors, XPC mRNA levels were lower than in their adjacent normal counterparts (Table 2). Reduced XPC mRNA levels were more common in nonsmokers than in smokers (72 % vs. 41 %, $P < 0.001$, Table 2). Surprisingly, XPC mRNA levels decreased significantly in patients with tumors in advanced stages with lymph node metastasis as compared to patients with early stage disease and no lymph node metastasis ($P < 0.001$ for stage; $P < 0.001$ for N value, Table 2). No other clinical parameters, including gender, tumor size, and tumor type, were associated. Most importantly, the prevalence of reduced XPC mRNA levels was significantly higher in patients with disease relapse than in those

without disease relapse after surgical therapy (87 % vs. 42 %, P < 0.001, Table 2).

XPC mRNA level may act as an independent predictor of OS and DFS for NSCLC

We followed up all patients for 4061 days to verify whether patients with reduced XPC mRNA had a higher risk of disease relapse than those without reduced XPC mRNA. The metastatic sites of these patients with disease relapse are presented in Table 1. Univariate logistic regression analysis further indicated that the parameters of age, gender, smoking, and tumor type were not associated with overall survival (OS) or disease-free survival (DFS) (Table 3). As expected, tumor stage had a strong impact on OS and DFS (HR = 2.182, 95 % CI, 1.409 ~ 3.379, P < 0.001 for OS; HR = 1.613, 95 % CI, 1.174 ~ 2.216, P = 0.003 for DFS, Table 3). More importantly, reduced XPC mRNA levels appeared to predict poorer OS and DFS in NSCLC (HR = 2.160, 95 % CI, 1.363 ~ 3.424, P = 0.001 for OS; HR = 4.367, 95 % CI, 2.620~7.184, P < 0.001 for DFS, Table 3). Multivariate logistic regression analysis further revealed that reduced XPC mRNA level not only acted as an independent prognostic factor of OS (HR = 1.891, 95 % CI, 1.126~3.177, P = 0.016, Table 4), but it also was an independent predictor of DFS in NSCLC (HR = 6.334, 95 % CI, 2.861 ~ 8.845, P < 0.001, Table 4). When study cases were divided into the two categories of nonsmoker and smoker for multivariate logistic regression analysis, reduced XPC mRNA level was found to be an independent predictor of disease relapse for nonsmokers (HR = 11.406, 95 % CI, 1.630 ~ 19.999, P = 0.009, Table 5). However, no prognostic value of reduced XPC mRNA level was observed for smokers (HR = 3.515, 95 % CI, 0.789 ~ 9.336, P = 0.176, Supplementary Table 1). These findings suggest that reduced XPC mRNA level may be a valuable marker for the identification of resected NSCLC with high risk of disease recurrence, particularly in nonsmokers.

DISCUSSION

Lung cancer is the leading cause of cancer death in the world and cure rates are less than 15%.[34] Tobacco smoking is considered to be the predominant risk factor for development of lung cancer, however a distinct group of patients develops the disease without a history of tobacco smoking. The percentage of nonsmokers suffering from lung cancer is increasing throughout the world. Differences in lung cancer biology between nonsmokers and smokers have been illustrated in findings from several studies.[35-39] One of the most striking distinctions is the observed differential response to drugs that target the epidermal growth factor receptor (EGFR). Compared with current or former smokers diagnosed with lung cancer, nonsmoker patients treated with these agents have higher response rates to treatment and better survival.[35, 36] Other analyses have demonstrated distinct mutational or expression patterns in K-ras, p53, and nitrotyrosine in tumors of nonsmokers compared with smokers.[37-39] Thus, the establishment of molecular markers for lung cancer in nonsmokers is important because of the implications for therapeutic trials for lung cancer. In this study, we demonstrated a significantly reduced XPC mRNA levels in lung cancer patients with disease relapse (Table 2). Multivariate logistic regression analysis revealed that a reduced XPC mRNA level was an independent predictor of DFS in NSCLC (Table 4). In addition, we also demonstrated that the finding of reduced XPC mRNA levels was more prevalent in nonsmokers than in smokers ($P < 0.001$, Table 2). These results concur with previous reports showing that smokers with lung cancer and healthy controls tend to have more proficient DNA repair capacity than do nonsmokers.[32, 40] Kaplan-Meier analysis further demonstrated that nonsmoker patients with reduced XPC mRNA levels had a shorter duration for disease recurrence than did patients without reduced XPC mRNA levels ($P = 0.001$). These high-risk patients with

reduced XPC mRNA levels may benefit from strict surveillance or from early adjuvant chemotherapy.

In the present study, lung tumors with reduced XPC mRNA levels had a higher prevalence of XPC promoter hypermethylation (31/41, 76%) than did those with non-reduced XPC mRNA levels (10/41, 24%；P = 0.010). Moreover, XPC promoter hypermethylation was more common in nonsmokers than in smokers (41 % vs. 16 %, P = 0.002). These results concur with our previous report showing that XPC was predominantly affected by promoter hypermethylation and was related to occurrence in lung tumors.[23] In our previous population-based study, XPC-methylated lung tumors harboring wild-type p53 were more common in adenocarcinomas and nonsmokers than in squamous cell carcinomas and smokers (68% *vs.* 36%, P = 0.02 for adenocarcinomas *vs.* squamous cell carcinomas; 62% *vs.* 28%, P = 0.034 for nonsmokers *vs*. smokers). Among tumors with wild-type p53, MDM2 mRNA levels in XPC-methylated tumors were lower than in unmethylated tumors (4.9452±2.32692 *vs*. 15.9463±2.7298, P = 0.013). In cell and animal models, we demonstrated that XPC promoter hypermethylation may promote lung tumor progression and metastasis via p53 dysfunction (manuscript in submission). Therefore, we strongly suggest that reduced XPC mRNA levels arising from promoter hypermethylation may enhance tumor progression and metastasis, resulting in patients with poor OS and DFS.

Innovative therapeutic strategies are urgently needed for lung cancer treatment. DNA methyltransferase (DNMT) not only plays a pivotal role in lung tumorigenesis, but also appears to be a promising molecular biomarker for early diagnosis and treatment of NSCLC.[41] Among DNA demethylating agents, the most widely used in experimental and clinical scenarios is the nucleoside DNMTi, which essentially is comprised of cytosine analogs and cytidine deaminase analogs including

5-Aza-cytidine (5-Aza-CR) and 5-Aza-2'-deoxycytidine (5-Aza-CdR).[42,43] The antitumor effect of 5-Aza-CR for murine lung cancer has been demonstrated by Belinsky et al.[44], who showed that a low dose of 5-Aza-CR could decrease lung tumor incidence by 30%, while a 50% decrease might be achieved by 5-Aza-CR combined with HDACi sodium phenylbutyrate. Thus, reductions in hypermethylation and in histone deacetylase activities can block epigenetically-mediated gene silencing and may provide a novel clinical strategy for lung cancer prevention.[44] Several clinical trials have now used DNA demethylation or histone deacetylation agents to improve clinical outcome of lung cancer.[45-48] Based on our present study and on these previous reports, we strongly suggest that lung cancer patients with XPC promoter hypermethylation, and especially nonsmokers, can benefit from the treatment with demethylation agents alone or in combination with other regimens.

In conclusion, we provide evidence for the first time to indicate that reduced XPC mRNA level in lung tumors compared with levels in normal counterpart tissues may act as a potential predictor of OS and DFS in NSCLC, particularly in nonsmokers. Therefore, XPC may be a highly useful target for demethylation therapy to improve clinical outcome via prevention of disease relapse, especially in nonsmokers with NSCLC.

ACKNOWLEDGEMENTS

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Table 1. Patient characteristics.

	Patients (n=137)	
Characteristics	**No.**	**%**
Age		
Median (range)	67 (26 ~ 83)	
≦67	74	54
>67	63	46
Gender		
Female	35	26
Male	102	74
Smoking		
No	79	58
Yes	58	42
Stage		
I	52	38
II	15	11
IIIA	61	45
IIIB	9	6
Type		
ADC	67	49
SCC	70	51

ADC: adenocarcinomas; SCC: squamous cell carcinomas.

Table 1. (Continued)

Relapse	
Local recurrence	11
Distant metastases	27
Single site distant metastasis	
Adrenal gland	1
Bone	3
Brain	4
Liver	6
Lung	9
Multiple site distant metastasis	
Brain and liver	1
Bone and brain	2
Bone and liver	1
Local recurrence & distance metastases	14
Single site distant metastasis	
Bone	2
Brain	2
Liver	1
Rectum	1
Adrenal gland	1
Chest wall	1
Lung	3
Multiple site distant metastasis	
Bone and liver	1
Brain and liver	2

Table 2. Relationships between XPC mRNA level and clinicopathological parameters in NSCLC.

		XPC		
	N	**Reduced**	**Non-reduced**	**P value**
Age				0.011
≦67	74	51 (69)	23 (31)	
> 67	63	30 (48)	33 (52)	
Gender				0.086
Female	35	25 (71)	10 (29)	
Male	102	56 (55)	46 (45)	
Smoking				< 0.001
No	79	57 (72)	22 (28)	
Yes	58	24 (41)	34 (59)	
Stage				< 0.001
I	51	19 (37)	32 (63)	
II	16	10 (63)	6 (37)	
III	70	52 (74)	18 (26)	
Type				0.061
ADC	67	45 (67)	22 (33)	
SCC	70	36 (52)	34 (48)	

ADC: adenocarcinoma; SCC: squamous cell carcinoma.

Table 2. (Continued)

T value				0.089
1	4	0 (0)	4 (100)	
2	98	58 (59)	40 (41)	
3	25	16 (64)	79 (36)	
4	10	7 (70)	3 (30)	
N value				< 0.001
0	68	28 (41)	40 (59)	
1	26	17 (66)	9 (34)	
2	42	35 (83)	7 (17)	
3	1	1 (100)	(0)	
Disease relapse				< 0.001
No	85	36 (42)	49 (58)	
Yes	52	45 (87)	7 (13)	

NSCLC: non-small cell lung cancer; ADC: adenocarcinoma; SCC: squamous cell carcinoma.

Densitometry quantification of XPC mRNA was normalized to 18S RNA, and the corrected density in lung tumors was divided by that of adjacent normal counterparts and presented as an expression ratio. Reduced XPC mRNA level was defined as an expression ratio of < 1, while ≧ 1 was defined as a non-reduced XPC mRNA level.

Table 3. Univariate analysis for the prognostic potential of XPC mRNA level on overall survival and disease-free survival in NSCLC.

Variables	N	Overall survival			Disease free survival		
		HR	95% CI	P	HR	95% CI	P
Age							
≦67	74	Ref.			Ref.		
> 67	63	1.027	0.671~1.573	0.902	0.732	0.419~1.280	0.274
Gender							
Female	35	Ref.			Ref.		
Male	102	1.364	0.810~2.296	0.242	1.167	0.611~2.227	0.640
Smoking							
No	79	Ref.			Ref.		
Yes	58	1.008	0.656~1.548	0.971	0.496	0.273~0.899	0.021
Stage							
I+II	67	Ref.			Ref.		
III	70	2.182	1.409~3.379	< 0.001	1.613	1.174~2.216	0.003
Type							
ADC	67	Ref.			Ref.		
SCC	70	0.744	0.486~1.140	0.175	0.500	0.282~0.887	0.018
XPC							
Reduced	81	2.160	1.363~3.424	0.001	4.367	2.620~7.184	< 0.001
Non-reduced	56	Ref.			Ref.		

NSCLC: non-small cell lung cancer; ADC: adenocarcinoma; SCC: squamous cell carcinoma; CI: confidence interval; HR: hazard ratio; Ref.: reference.

Table 4. Multivariate logistic regression analysis for the prognostic potential of XPC mRNA level on overall survival and disease-free survival in NSCLC.

Variables	N	Overall survival			Disease free survival		
		HR	95% CI	P	HR	95% CI	P
Age							
≦67	74	Ref.			Ref.		
> 67	63	1.186	0.768~1.831	0.442	1.020	0.578~1.799	0.946
Gender							
Female	35	Ref.			Ref.		
Male	102	1.612	0.887~2.929	0.117	2.091	0.940~4.202	0.138
Smoking							
No	79	Ref.			Ref.		
Yes	58	1.132	0.657~1.953	0.655	0.654	0.313~1.368	0.259
Stage							
I+II	67	Ref.			Ref.		
III	70	1.409	1.083~1.833	0.011	1.232	1.159~3.832	0.015
Type							
ADC	67	Ref.			Ref.		
SCC	70	0.677	0.415~1.103	0.118	0.691	0.361~1.320	0.263
XPC							
Reduced	81	1.891	1.126~3.177	0.016	6.334	2.861~8.845	< 0.001
Non-reduced	56	Ref.			Ref.		

NSCLC: non-small cell lung cancer; ADC: adenocarcinomas; SCC: squamous cell carcinomas; CI: confidence interval; HR: hazard ratio; Ref.: reference.

Table 5. Results of univariate and multivariate logistic regression analysis in nonsmokers with NSCLC.

Variables	N	Univariate Analysis			Multivariate Analysis		
		HR	95% CI	P	HR	95% CI	P
Age							
≦67	43	Ref.			Ref.		
>67	36	0.780	0.399~1.526	0.469	0.968	0.490~1.909	0.924
Gender							
Female	35	Ref.			Ref.		
Male	44	1.885	0.842~3.773	0.073	2.017	1.000~4.070	0.050
Stage							
I+II	42	Ref.			Ref.		
III	37	3.583	1.776~7.229	0.001	2.268	1.088~4.731	0.029
Type							
AD	54	Ref.			Ref.		
SQ	25	0.514	0.233~1.134	0.099	0.708	0.314~1.596	0.405
XPC							
Reduced	57	12.519	2.280~20.732	0.002	11.406	1.630~19.999	0.009
Non-reduced	22	Ref.			Ref.		

NSCLC: non-small cell lung cancer; CI, confidence interval; HR: hazard ratio; ref., reference.

Supplementary Table 1. Results of univariate and multivariate logistic regression analysis in smoking patients with NSCLC.

Variables	N	Univariate Analysis			Multivariate Analysis		
		HR	95% CI	P	HR	95% CI	P
Age							
≦67	31	Ref.			Ref.		
>67	27	0.731	0.262~2.038	0.549	1.124	0.368~3.274	0.831
Stage							
I+II	25	Ref.			Ref.		
III	33	1.607	0.591~4.371	0.353	0.726	0.218~2.412	0.601
Type							
AD	13	Ref.			Ref.		
SQ	45	1.072	0.299~3.844	0.915	1.283	0.311~5.288	0.730
XPC							
Reduced	24	3.116	0.885~8.115	0.166	3.515	0.789~9.336	0.176
Non-reduced	34	Ref.			Ref.		

NSCLC: non-small cell lung cancer; CI, confidence interval; HR: hazard ratio; ref., reference.

無研發成果推廣資料

96 年度專題研究計畫研究成果彙整表

計畫主持人：李輝	計畫編號：96-2628-B-040-023-MY3
計畫名稱：XPC 基因在肺腫瘤化之角色（II）	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	1	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	2	4	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	2	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

本研究室發現 XPC 發生甲基化可能會引起 p53 基因的突變，進而參與肺癌之形成(Oncogene,26:4761-73,2007)。XPC 表現量可做為肺癌之獨立之預後臨床指標(Cancer,110:215-23,2007)。本計畫進一步證實 XPC 會增加 hHR23B 與 p53 的結合能力，進而增加 p53 蛋白的穩定性，促進 p53 的轉錄功能，透過抑制 MMP1 表現量，降低細胞侵襲與轉移的能力。由動物實驗證實，XPC 會抑制腫瘤生長與轉移的能力。肺組織檢體顯示 XPC 與 MMP1 的基因表現呈現負相關趨勢。綜合上述結果，XPC 缺失會造成 p53 失去功能導致 MMP1 表現量增加，進而促進轉移能力(Cancer Research, potentially accepted,2010)。同時分析肺癌病患其腫瘤與腫瘤周邊正常組織之 XPC 表現量，顯示：不抽菸者、晚期與淋巴結轉移的肺癌病患其 XPC 表現量在腫瘤組織低於腫瘤周邊正常組織。XPC mRNA 在腫瘤組織降低的病患具有 1.891 倍的危險性；發生復發的時間也早於表現量高之肺癌病患，因此 XPC 表現量可做為預測腫瘤復發的指標(Chest, under review,2010)。