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Enhancing the invasive traits of breast cancers by CYP1B1 via regulation of p53 to promote uPAR expression

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ABSTRACT

Human cytochrome P450 1B1 (CYP1B1) catalyzes estrogen metabolism to produce metabolites that promote the progression of breast cancer. Since the invasive properties of cancer cells cause cancer relapse, which dramatically reduces patient survival, we investigated the new pro-invasive mechanism involving CYP1B1 in breast cancer. Exploring clinical data from invasive breast cancer patients revealed that CYP1B1 exhibits a potential correlation with urokinase-type plasminogen activator receptor (uPAR). Interestingly, uPAR mRNA expression was elevated in invasive breast cancer patients carrying *TP53* genes with driver mutations, and our results showed that CYP1B1 activates the uPAR pathway following regulation of p53 according to its mutant status. CYP1B1 suppressed wild-type (WT) p53 whereas it induced the oncogenic gain-of-function mutant $p53^{R280K}$, not only via transcriptional regulation but also the protein stabilization and activation following phosphorylation on Ser15 residue of $p53^{R280K}$. Intriguingly, results from *CYP1B1* polymorphic gene study and 4-hydroxyestradiol (4-OHE_2) treatment showed that CYP1B1 regulates p53s and uPAR through its enzymatic activity. Furthermore, effects of DMBA and TMS on uPAR expression disappeared in $HCT116^{p53-/-}$ cells, indicating that p53 is critical for uPAR induction by CYP1B1. Collectively, our results demonstrate that CYP1B1 may reduce the relapse-free survival rate of breast cancer patients by inducing invasive traits in cancer cells via p53 regulation based on the mutation status of *TP53* genes and further activation of the uPAR pathway. The elucidation of the previously unknown molecular mechanism of CYP1B1 may provide evidence for the development of effective anti-cancer therapeutic strategies that target the progression of cancer invasion.

1. Introduction

Cancer remains one of the world's major health issues, with approximately 19.3 million new cases and nearly 10.0 million related deaths worldwide in 2020 [1]. The number of cases of female breast cancer, in particular, was reported as the highest among newly diagnosed cases, and the number of deaths by female breast cancer was the fifth highest worldwide in 2020 [1]. Breast cancer is the leading cause of cancer-related death (in 110 countries) and the most frequently diagnosed cancer (in 159 countries) in female populations [1]. Patient mortality of cancer has a significant correlation with metastasis status, and numerous studies have stated that 90 % of cancer deaths are caused by metastases [2,3]. Therefore, identifying the key regulators of breast cancer metastasis is critical for developing therapeutic approaches with advanced clinical outcomes for breast cancer, which is the most common and lethal type of cancer.

Since estrogen and its metabolites have been identified as critical factors in the development of breast cancer, researchers have focused on the molecular mechanisms of key regulators of estrogen metabolism, which produces carcinogenic metabolites [4,5]. In particular, catechol estrogen metabolites, 4-hydroxyestradiol (4-OHE_2) and 2-hydroxyestradiol (2-OHE_2), play crucial roles as carcinogens in various ways, including induction of DNA mutations and activation of proteases for extracellular matrix (ECM) degradation, which eventually promote breast cancer cell invasion and metastasis [6–8]. Cytochrome P450 1B1 (CYP1B1) is the key enzyme for producing 4-OHE_2 and 2-OHE_2 and has tumor-specific expression patterns [8,9]. The role of CYP1B1 in cancer metastases has been partially demonstrated; it promotes the epithelial-

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mesenchymal transition (EMT) process by regulating EMT-related factors [10,11]. Furthermore, several clinical studies have recently revealed that CYP1B1 expression is significantly higher in metastatic tumors than in normal tissues and primary tumors [12,13]. The findings of the aforementioned studies reveal a strong link between CYP1B1 hyperexpression in tumors and cancer metastasis. However, the precise molecular mechanism is still unknown.

MCF-7 and MDA-MB-231 cells are the most commonly used cell lines for breast cancer research [14] and previous reports have described that these cell lines exhibit different levels of invasiveness: MDA-MB-231 cells have been reported as a more aggressively invasive breast cancer cell line than MCF-7 cells [15,16]. In this study, we employed these two cell lines to investigate how the tumor-specific expression of CYP1B1, which increases with the metastatic level of tumors, is associated with the invasive properties of breast cancer and revealed the detailed molecular mechanism underlying it. By exploring the role of CYP1B1 in breast cancer cell lines with different invasiveness, our study aimed to determine the correlation between CYP1B1 expression level and the basal invasiveness of breast cancer cells through identifying the molecular mechanisms of CYP1B1 for regulation of invasive cell signaling in breast cancer.

2. Materials and methods

2.1. Reagents

7,12-Dimethylbenz[α]anthracene (DMBA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dr. Sanghee Kim generously provided the 2,2′,4,6′-tetramethoxystilbene (TMS) (Seoul National University, Seoul, Korea). Moloney murine leukemia virus (M-MLV) reverse transcriptase and RNase inhibitor were obtained from Promega (Madison, WI, USA). SYBR green was purchased from QIAGEN (Hilden, Germany). Rabbit polyclonal antibodies for CYP1B1 and GAPDH, and Ultra Cruz™ Mounting Medium were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal antibody for p53 were purchased from Millipore (Bedford, MA, USA). Rabbit polyclonal antibodies for p-p53 (Ser15), Integrin α5, and Integrin β1 were purchased from Cell Signaling Technology (Beverly, MA, USA). HRP-conjugated goat anti-rabbit IgG, Texas Red-conjugated mouse anti-rabbit IgG, and DyLight® 594-conjugated goat anti-mouse IgG were obtained from Bethyl (Montgomery, TX, USA). HRP-conjugated goat anti-mouse IgG and newly synthesized rabbit polyclonal antibodies for uPAR were purchased from Abclon (Guro, Seoul, South Korea).

2.2. Cell lines and cell culture

The human breast cancer cells MCF-7, MDA-MB-231, MDA-MB-468, and immortalized normal mammary epithelial cell line MCF-10A were obtained from the American Type Culture Collection (Manassas, VA, USA), and T47D, BT-20 were obtained from the Korean Society Cell Bank (KCLB). HCT116$^{p53+/+}$ (p53 wild-type) and HCT116$^{p53-/-}$ (p53 null-type) isogenic colon cancer cell lines were provided by Dr. Bert Vogelstein (Johns Hopkins University, USA). MCF-7, MDA-MB-231, T47D, BT-20 cells were cultured in RPMI medium (Hyclone, Marlborough, MA, USA), and MDA-MB-468, HCT116$^{p53+/+}$ and HCT116$^{p53-/-}$ cells were cultured in DMEM medium (Hyclone), supplemented with 10 % (*v*/v) heat-inactivated fetal bovine serum (FBS) (Hyclone), 100 μg/mL streptomycin, and 100 U/mL penicillin (Thermo Fisher Scientific, Waltham, MA, USA). MCF-10A cells were cultured in monolayers, as reported previously [17]. The cell lines were validated by short tandem repeat (STR) profiling analysis. Every six months, all cell lines were checked for the presence of *Mycoplasma* using the polymerase chain reaction (PCR) Mycoplasma Detection Set (Takara Bio Inc., Shiga, Japan). After thawing, the cells were assessed and stabilized for four passages of cell culture.

2.3. Adenovirus infection

The infection of MCF-7 cells with adenovirus carrying CYP1B1-ORF genes (ViGene Biosciences Inc., Rockville, MD, USA) was conducted in serum-free media at a multiplicity of infection (MOI) of 750 virus particles (vp)/cell. After 24 h, the media was replaced with fresh media containing serum. Cells were maintained in a humidified atmosphere at 37 °C with 5 % CO_2 for 24 h before being fixed or harvested for further studies. Under these conditions, the transduction efficiency of the CYP1B1 gene-carrying adenovirus was nearly 100 %.

2.4. Construction and transduction of CYP1B1 short hairpin RNA (shRNA) lentivirus

The plasmid pLKO.1 that encodes a human immunodeficiency virus (HIV)-derived lentiviral vector adequate for the insertion of shRNA constructs driven by an upstream U6 promoter was cloned with the optimal shRNA sequence targeting human CYP1B1 (5′-GCGACATGATGGACGCCTTTAT-3′). This modified plasmid or empty vector was then co-transfected into HEK293T cells with plasmids for lentiviral packaging to produce control or CYP1B1 shRNA lentivirus. The constructed control or CYP1B1 shRNA lentivirus were added to cells for virus infection and gene transduction, and after 24 h, infected cells were treated with puromycin for selection. After 24 h, the media was replaced with growth media without puromycin, and cells were cultured for further CYP1B1 knockdown studies.

2.5. Transient transfection of plasmid DNA and siRNA

The plasmid pcDNA 3.1/Zeo carrying the CYP1B1-encoding sequence for CYP1B1 overexpression and CYP1B1-specific siRNA (target sequence: CAGCATGATGCGCAACTTCTT, QIAGEN) and PLAUR-specific siRNA (target sequence: AAGCCGTTACCTCGAATGCAT, QIAGEN) were used in transfections. Cells (1×10^6 cells/dish) were transfected with 3 μg plasmid or 37.5 nM siRNA at room temperature with the Neon Transfection System (Invitrogen, Carlsbad, CA, USA) and cultured in 60- or 100-mm dishes in RPMI containing 10 % FBS without antibiotics. Cells were harvested or treated with agents after 48 h for further studies.

2.6. Real-time PCR (quantitative PCR, qPCR)

Total RNA was extracted using Ribospin™ vRD (GeneAll, Songpa, Seoul, South Korea). At 37 °C for 1 h, the reverse transcription for total RNA (500 ng) was conducted in 20 μL of total volume containing 5× RT buffer, 100 pmol oligo-dT primer, 40 U RNase inhibitor, 10 mM dNTPs, and 200 U M-MLV reverse transcriptase. The Rotor-Gene SYBR® PCR Kit (QIAGEN) was used for quantitative PCR (qPCR), and QIAGEN Rotor-Gene Q Series software was used for analyzing the results. Each reaction contained 1 μM oligonucleotide primers, 2 μL cDNA, and 12.5 μL 2× SYBR® Green PCR Master Mix in a final volume of 25 μL. PCR conditions were set as follows: one cycle at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 5 s, and annealing/extension at 60 °C for 10 s. Data is representative of experiments in triplicate. Primer sequences are listed in Supplementary Table S1.

2.7. Western blot analysis

Whole cells were scrapped for harvest and lysed for 30 min in 50 mM Tris-HCl (pH 8.0) with 1 mM PMSF, 1 μg/mL leupeptin, 1 % nonidet P-40, 1 μg/mL aprotinin, and 150 mM NaCl, and subsequently centrifuged at 4 °C for 15 min at 22000 g. The bicinchoninic acid (BCA) Protein Assay Reagents (Thermo Fisher Scientific) were used for determination of protein concentrations. 20 μg of extracted proteins were separated using SDS-PAGE on 10–15 % polyacrylamide gels and transferred onto PVDF membranes electrophoretically. Incubation with specific

antibodies was performed overnight for membranes following blocking process with 5 % nonfat milk in Tris-buffered saline containing 0.1 % Tween-20 (TBS-T) for 1 h at 4 °C. Proteins were visualized by enhanced chemiluminescence reagents after 2 h of incubation with secondary antibodies (Thermo Fisher Scientific). Quantitative data was obtained using Quantity One software (Bio-Rad, Hercules, CA, USA).

2.8. Confocal microscopic analysis

Cells were cultured on coverslips after transfection or virus infection or treated with the indicated reagent concentrations, rapidly washed with PBS, and fixed with 3.7 % (*w/v*) paraformaldehyde (Sigma-Aldrich) for 30 min at room temperature. After washing with PBS, the cells were blocked for 30 min using PBS containing 5 % goat serum and 0.2 % Triton X-100 before being incubated overnight with specific primary antibodies. The cells were then thoroughly washed before being stained for 2 h with Texas Red-conjugated goat anti-rabbit IgG or DyLight® 594-conjugated goat anti-mouse IgG (1:500) for 2 h. Following additional washes, the coverslips were mounted onto glass slides using Ultra Cruz™ Mounting Medium containing 4′,6-diamidino-2-phenylindole (DAPI). Fluorescence signals were detected using a LSM700 Confocal Laser Scanning Microscope (Carl Zeiss, Jena, Germany).

2.9. cBioPortal

The cBioPortal for Cancer Genomics (http://www.cbioportal.org) is a depository of cancer genomics data [18,19]. Using TCGA Pan-Cancer Atlas dataset, we investigated the basal expression level of CYP1B1 in cancers, collecting data from 32 studies for human cancers (10,967 samples in total). The correlation between CYP1B1 and EMT-related factors and uPAR and the correlation between mutation status of *TP53* and the expression level of uPAR was studied using TCGA Pan-Cancer Atlas dataset with 994 breast invasive carcinoma samples. The alteration frequency of *PLAUR* was presented by analyzing the data from 22 studies of different types of human breast cancer with samples from 8108 patients.

2.10. GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is a web-based interactive tool for analyzing gene expression in 9736 tumors and 8587 normal samples [20]. GEPIA was used to analyze the data for expression of uPAR in 1085 breast invasive tumor samples comparing to 291 normal samples from TCGA and the Genotype-Tissue Expression (GTEx) datasets (Log_2FC cutoff: 1; *p*-value cutoff: 0.01). Analysis of variance (ANOVA) was used to compare tumor sample to normal samples.

2.11. Generation of a xenograft model using stable CYP1B1-suppressed MDA-MB-231 cells to measure tumor growth

CYP1B1-knocked down or control MDA-MB-231 cells (2.5×10^6 cells/injection) were injected subcutaneously into the backs of Balb/c nude mice (female, 6 weeks) obtained from Japan SLC, Inc. (Shizuoka, Japan), in a mixture with Matrigel (Sigma-Aldrich) (1:1). All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Chung-Ang University (2017–00096, 2019–00003) and were performed according to the IACUC guidelines. Once every two days, tumor volume was measured around the injected area. Twenty-nine days post-injection, all xenograft tumors were larger than 50 mm^3. After six weeks from injection, tumor volumes in three mice were measured to analyze the effect of CYP1B1 knockdown. Using calipers, volume was calculated on the basis of width (x) and length (y) using the following formula: $x^2y/2$.

2.12. Immunohistochemistry for tissue samples

The xenograft model mice were sacrificed when tumor growth measurements were complete, and the tumors were immunohistochemically processed. Immunostaining was performed on formalin-fixed paraffin-embedded tissue sections. Following incubation with specific antibodies for detection, liquid diaminobenzidine (DAB) + substrate chromogen system (Agilent Technologies, Santa Carla, CA, USA) was added before counterstaining with hematoxylin. Each stained tissue section was scanned using Pannoramic MIDI II (3DHISTECH Ltd., Budapest, Hungary) and visualized using the Pannoramic Viewer v.1.15.4 program (3DHISTECH Ltd.).

2.13. Invasion assay

Cancer cell invasion was examined using the QCM™ 24-well Cell Invasion Assay Kit (Millipore), according to the manufacturer's protocol. Briefly, 300 μL of cell suspension in serum free media (1×10^6 cells/mL) were seeded onto insert chambers with an 8 μm collagen-coated polycarbonate membrane. The ECM layer-invaded cells were stained with DAPI. Invading cells were visualized and observed under a LSM700 Confocal Laser Scanning Microscope (Carl Zeiss) for counting of cell numbers. Each experiment was repeated at least ten times.

2.14. Microarray for comparing the gene expression patterns of MCF-7 and MDA-MB-231 cells

Cells (5×10^5) were seeded onto 60 mm cell culture plates. After 48 h, the cells were harvested, and total RNA was isolated using Ribospin™ vRD (GeneALL Biotechnology Co.). Microarray hybridization was performed using the human GE 4 × 44 K v2 Microarray Kit (Agilent). Total RNA was prepared using Low RNA Input Linear Amplification Kit Plus (Agilent) according to manufacturer's instructions, and its quality was determined using an Agilent 2100 Bioanalyzer. Microarray data analysis was performed using GeneSpring 7.3.1 software (Agilent). The gene expression levels in MDA-MB-231/MCF-7 cells were quantified to identify and compare the changes in their gene expression patterns.

2.15. Dual luciferase reporter assay

Using the Neon™ transfection system (Invitrogen), cells (2×10^4 cells/well) were seeded onto 96 well plates after co-transfection with 200 ng of uPAR reporter plasmids and pRL-renilla (Promega), as a control, according to the manufacturer's protocol. After 24 h, cells were treated with 5 μM of DMBA with or without designated concentrations of TMS for 48 h. The cells were treated with TMS prior to DMBA treatment for 1 h. Subsequently, cells were lysed using passive lysis buffer at room temperature for 15 min and luciferase activities were measured with using the Dual Luciferase Assay System (Promega) on FilterMax F3 (Molecular Devices, LLC, San Jose, CA, USA). Each experiment was repeated at least three times.

2.16. Statistical analysis

Statistical analyses were performed using one-way analysis of variance and Dunnett's multiple comparison *t*-test using the GraphPad Prism 9 Software (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at $P \leq 0.05$.

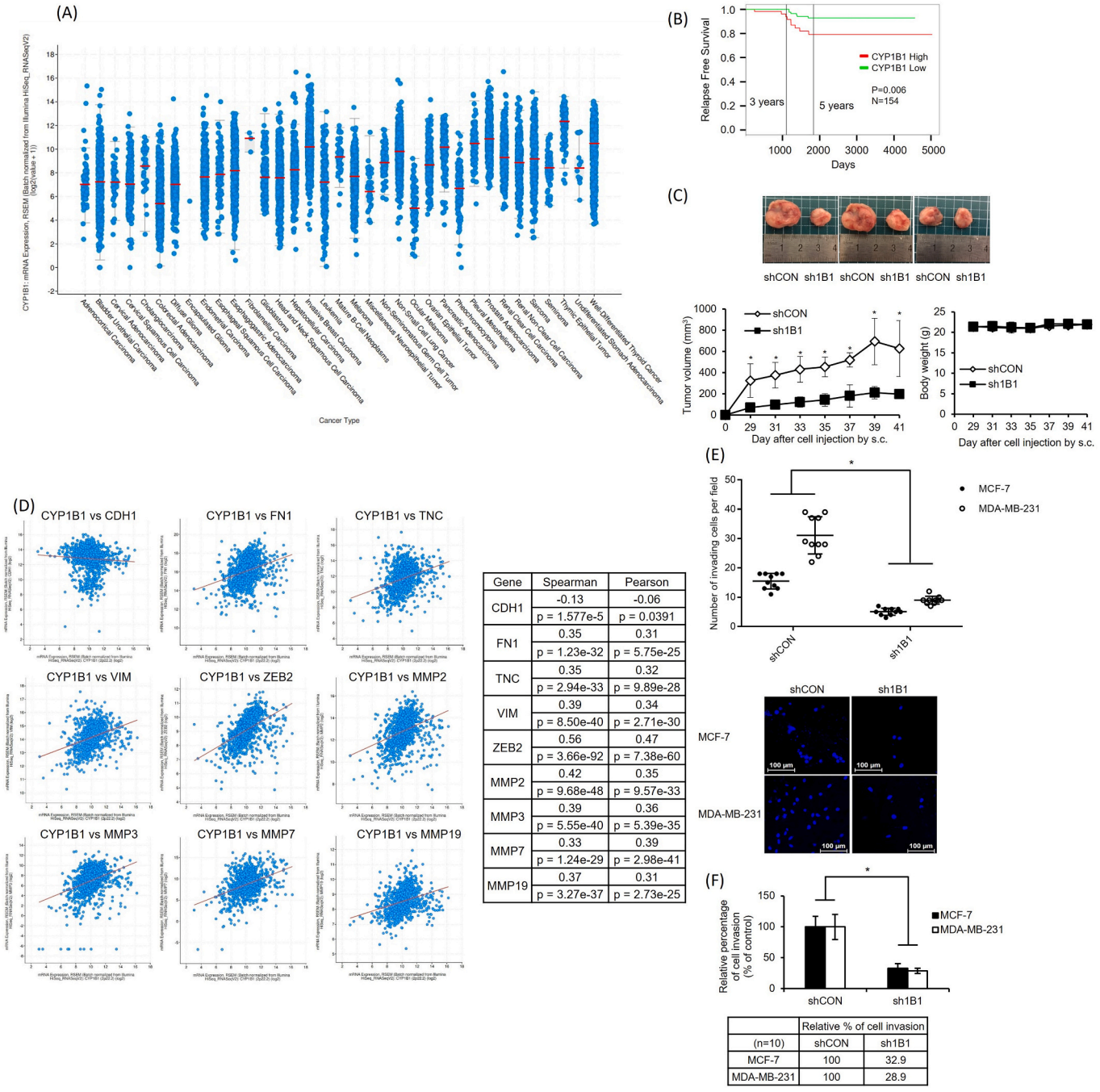
3. Results

3.1. CYP1B1 induces tumor progression and cancer cell invasion, which may cause the reduction of relapse-free survival of breast cancer patients with relatively high expression level of CYP1B1

To identify the expression pattern of CYP1B1 in various types of

cancers, we used the cBioPortal online database to collect and analyze the clinical data of 10,071 patients with cancer from 32 different The Cancer Genome Atlas (TCGA) datasets. Most types of invasive cancers, such as invasive breast cancer, had higher levels of mRNA expression of CYP1B1 (Fig. 1A).

We investigated the effect of CYP1B1 on tumor growth, survival rate of patients with cancer, and cancer cell invasion to further determine the correlation between CYP1B1 and the invasive properties of breast cancer. A Kaplan–Meier plot illustrating the clinical microarray dataset of patients with breast cancer obtained from PROGgeneV2 (GSE9893; $n =$



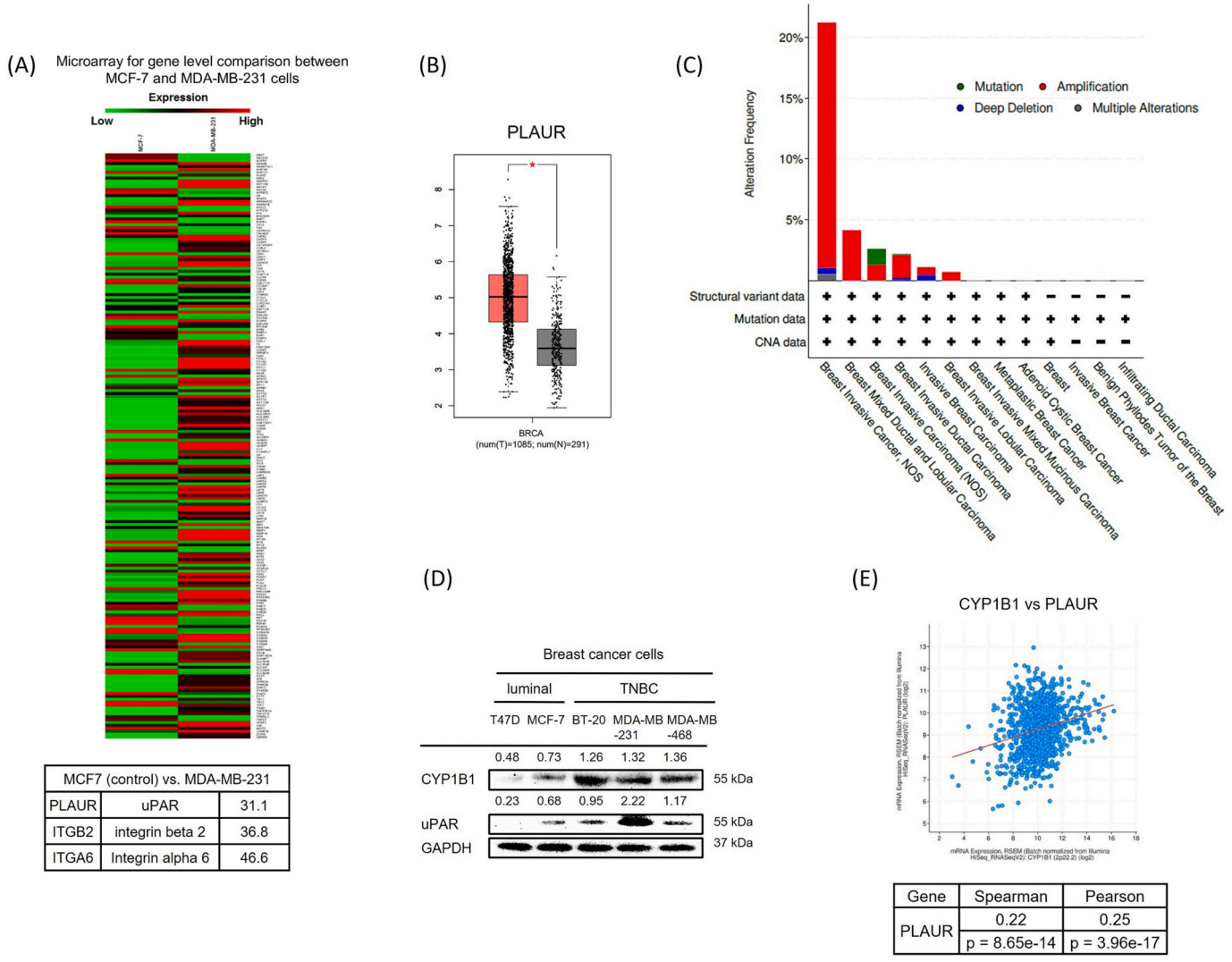
Gene	Spearman	Pearson
CDH1	-0.13	-0.06
	p = 1.577e-5	p = 0.0391
FN1	0.35	0.31
	p = 1.23e-32	p = 5.75e-25
TNC	0.35	0.32
	p = 2.94e-33	p = 9.89e-28
VIM	0.39	0.34
	p = 8.50e-40	p = 2.71e-30
ZEB2	0.56	0.47
	p = 3.66e-92	p = 7.38e-60
MMP2	0.42	0.35
	p = 9.68e-48	p = 9.57e-33
MMP3	0.39	0.36
	p = 5.55e-40	p = 5.39e-35
MMP7	0.33	0.39
	p = 1.24e-29	p = 2.98e-41
MMP19	0.37	0.31
	p = 3.27e-37	p = 2.73e-25

	Relative % of cell invasion	
(n=10)	shCON	sh1B1
MCF-7	100	32.9
MDA-MB-231	100	28.9

Fig. 1. CYP1B1 shows significant correlation with invasive types of cancers and reduces the relapse-free survival rates of breast cancer patients via induction of tumor growth and cancer cell invasion. (A) The mRNA expression levels of CYP1B1 in 10,071 cancer patients with 35 types of cancers. Data were obtained from cBioPortal. (32 TCGA datasets) (B) Kaplan-Meier plot for the relapse-free survival rates of breast cancer patients with high or low CYP1B1 levels, were analyzed. (GSE9893; $n = 154$, $P = 0.006$) (C) The control and stable CYP1B1-knocked down MDA-MB-231 cells were injected into the backs of Balb/c nude mice as a mixture with Matrigel (1:1), and the size of xenograft tumor was measured once every two days along with the body weight ($n = 3$). (D) The correlation between CYP1B1 and key factors for cancer cell invasion was analyzed based on the Spearman's rank-order correlation coefficient and Pearson correlation coefficient. *P* values for every correlation coefficient were presented together. CDH1: *E*-cadherin. FN1: Fibronectin 1. TNC: Tenascin C. VIM: Vimentin. ZEB2: Zinc Finger E-Box Binding Homeobox 2. MMP: Matrix metalloproteinase. (E-F) Cell invasion was measured using the transwell invasion assay in the control or stable CYP1B1-knocked down MCF-7 and MDA-MB-231 cells. Data were from 10 independently quantified experiments (*, indicated $P \leq 0.05$ via Dunnett's *t*-test). (E) The number of invading cells were plotted and the confocal microscopic analysis for invaded cells were conducted using DAPI staining, and (F) the number of invading shCON cells was compared to the number of invading sh1B1 cells and the relative percentage of cell invasion was calculated for MCF-7 and MDA-MB-231 cells, respectively.

154, $P = 0.006$) [21] showed that higher levels than the mean value of CYP1B1 expression result in earlier deaths of patients with breast cancer due to cancer relapse, which is deeply correlated with cancer metastasis (Fig. 1B) [22,23]. Following xenograft model with MDA-MB-231 cells, an aggressive and invasive breast cancer cell line, knockdown of *CYP1B1* by stably expressing lentiviral shRNA significantly inhibited tumor growth without affecting body weight (Fig. 1C and S1).

The gene expression data of carcinoma samples from 1082 patients with invasive breast cancer were analyzed for the Spearman's rank-order correlation coefficient (SROCC) and Pearson correlation coefficient (PCC) between CYP1B1 and each factor using cBioPortal tools, and the results showed a significant positive correlation between CYP1B1 and cancer metastasis-inducing factors like matrix metalloproteinases (MMPs) and EMT-inducing factors (Fig. 1D). The factors in Fig. 1D were selected by criteria with Spearman's and Pearson's correlation coefficients >0.3 and p value <0.05. In case of *CDH1* (E-cadherin), we selected the gene with p value <0.05 and the negative correlation coefficient values. Based on these findings, we performed an invasion assay using *CYP1B1*-knocked down MCF-7 and MDA-MB-231 breast cancer cells, which are approximately two times more invasive than MCF-7 cells as shown in data from control groups (Fig. 1E), and found that *CYP1B1* suppression strongly inhibited cell invasion in both cell lines (Fig. 1E, F, S1). Taken together, these results suggest that high expression level of CYP1B1 may reduce the survival rate of patients with breast cancer by inducing tumor progression and invasion.



MCF7 (control) vs. MDA-MB-231		
PLAUR	uPAR	31.1
ITGB2	integrin beta 2	36.8
ITGA6	Integrin alpha 6	46.6

Gene	Spearman	Pearson
PLAUR	0.22	0.25
	p = 8.65e-14	p = 3.96e-17

Fig. 2. CYP1B1 exhibits a positive correlation with uPAR, a critical signaling receptor for cancer cell invasion. (A) Microarray hybridization was performed to analyze the basal expression levels of genes, including *PLAUR*, in MCF-7 and MDA-MB-231 cells. Red dots represent genes that were >2-fold upregulated in MDA-MB-231 cells compared to MCF-7 cells, while green dots indicate genes that were >2-fold downregulated in MDA-MB-231 cells compared to MCF-7 cells. Relative expression levels of *PLAUR*, *ITGB2*, and *ITGA6* in MDA-MB-231 cells were calculated and compared to the gene expression levels observed in MCF-7 cells as the control. (B) The mRNA expression of *PLAUR*, which encodes uPAR, was assessed comparing tumor samples of breast cancer patients and normal samples from TCGA and GTEx datasets on the GEPIA database. The RNA-Seq data were normalized using transcripts per kilobase million (TPM) method. TPM values in 1085 tumor samples from breast invasive cancer patients and 291 normal samples were plotted following conversion to $\log_2$-normalized transcripts per million [$\log_2$(TPM + 1)] ($P < 0.01$). (C) The gene alteration frequency of *PLAUR* in 8108 cancer patients with 13 types of breast cancers, including invasive and non-invasive types. Data were obtained from cBioPortal. (22 datasets) (D) The basal protein expression levels of CYP1B1 and uPAR were determined by Western blot analysis using naive T47D, MCF-7, BT-20, MDA-MB-231, and MDA-MB-468 cells. (E) The correlation between *CYP1B1* and *PLAUR* was analyzed based on the Spearman's rank-order correlation coefficient and Pearson correlation coefficient. P values for each correlation coefficient were also presented.

3.2. CYP1B1 exhibits a correlation with urokinase-type plasminogen activator receptor (uPAR), which is a key receptor for metastatic signaling in patients with invasive breast cancer and such cells

To reveal the detailed molecular mechanism of CYP1B1 for breast cancer metastasis, we searched for a candidate that has a potential correlation with CYP1B1 and plays a critical role in breast cancer cell invasion. First, we performed microarray analysis with MCF-7 and MDA-MB-231 cells because they are both breast cancer cell lines with different properties, particularly in terms of their invasiveness. Among 323 genes involved in cell-cell signaling, 32 genes that were overexpressed the most (at least over 30 times higher) in MDA-MB-231 cells compared to MCF-7 cells were identified (Fig. S2). From our list of 32 genes, we selected 3 genes previously reported as invasive signaling receptors in cancers for further studies: *PLAUR*, *ITGB2*, and *ITGA6* (Fig. 2A) [24–26]. Clinical TCGA data from Gene Expression Profiling Interactive Analysis (GEPIA) showed that *PLAUR* and *ITGB2* were significantly overexpressed in tumor samples from patients with invasive breast cancer (n = 1085) compared with levels in normal samples (n = 291), whereas *ITGA6* exhibited an opposite expression pattern (Fig. 2B and S3). In addition, the overexpression level of *PLAUR* in breast tumor samples compared to that in normal samples was significantly greater than that of *ITGB2*. Based on these data, *PLAUR*, which encodes uPAR, a critical signaling receptor for inducing EMT and cancer cell invasion via regulation of various types of metastasis mechanisms, such as the p38-MAPK pathway in breast cancer cells [27,28], was selected as a candidate key regulator of breast cancer invasion. Interestingly, cancer type summary data from the cBioPortal database of 8108 patients with several types of breast cancer using 22 datasets showed that samples from patients with invasive breast cancer had a significantly higher frequency of oncogenic alterations in PLAUR, including amplification, compared to samples from patients with non-invasive breast cancer (Fig. 2C). This indicates that overexpression of uPAR may be related to the invasive traits of breast cancer. To further confirm the potential correlation between CYP1B1 and uPAR in breast cancer, especially in terms of invasiveness, we measured the basal expression levels of CYP1B1 and uPAR in five different breast cancer cell lines. Intriguingly, the results demonstrated that triple-negative breast cancer cells, including MDA-MB-231 cells, which are known to be highly invasive and more aggressive than other subtypes of breast cancer cell lines, showed higher basal expression levels of uPAR along with CYP1B1 than luminal breast cancer cells, including MCF-7 cells (Fig. 2D) [29,30]. Furthermore, clinical TCGA data of 994 breast invasive carcinoma samples from cBioPortal were analyzed, revealing a positive correlation between CYP1B1 and uPAR (Fig. 2E). These findings suggest that tumor-specific CYP1B1 overexpression may lead to more aggressive and invasive traits in breast cancer cells by increasing the basal uPAR expression level.

3.3. CYP1B1 induces uPAR expression by enhancing the promoter activity of uPAR

Previously, we discovered that 4-OHE$_2$-induced specificity protein 1 (Sp1) serves as a key regulator of the CYP1B1-induced EMT process in breast cancer cell lines via transcriptional activation of EMT-inducing factors, including zinc finger *E*-box binding homeobox 2 (ZEB2) and zinc finger protein SNAI1 [10]. Interestingly, the Sp1 binding site was discovered in the uPAR promoter region, and it has been reported that uPAR expression and Sp1 DNA-binding activity are both elevated in patients with breast carcinoma [31,32]. Based on these findings, we investigated whether CYP1B1 induces uPAR expression by increasing uPAR promoter activity. To this end, we altered the expression levels of CYP1B1 in cells using the inducer, 7,12-dimethylbenz[α]anthracene (DMBA), and specific inhibitor, 2,2′,4,6′-tetramethoxystilbene (TMS), of CYP1B1 and measured the promoter activity of uPAR [33,34]. Surprisingly, CYP1B1 significantly elevated uPAR promoter activity not only in breast cancer cells but also in MCF-10A, a normal mammary epithelial cell line (Fig. 3A). Following transcriptional regulation, CYP1B1 increased the production of uPAR protein in breast cancer cells and MCF-10A cells via transcriptional regulation (Fig. 3B-E). *In vivo* studies with xenograft models using control or *CYP1B1*-knocked down MDA-MB-231 cells revealed a significant decrease in uPAR protein expression levels due to CYP1B1 suppression (Fig. 3F). As these results indicate that CYP1B1 induces uPAR expression, we further investigated whether CYP1B1 upregulates breast cancer cell invasion through induction of uPAR expression. Intriguingly, the levels of cancer cell invasion were significantly increased by CYP1B1 overexpression in both MCF-7 and MDA-MB-231 cells, and suppression of uPAR expression using siRNA in CYP1B1-overexpressed cells strongly decreased the cancer cell invasion (Fig. 3G, H, S4). Taken together, these results elucidate that CYP1B1 may increase the invasiveness of breast cancer cells through elevation of uPAR expression by inducing uPAR promoter activity.

3.4. CYP1B1 transcriptionally regulates wild-type (WT) p53, whereas the oncogenic mutant R280K p53 is additionally promoted by CYP1B1 via a post-translational modification (PTM) for protein stabilization

In addition to Sp1, several transcription factors, including WT p53, target uPAR for the regulation of cancer invasion [35,36]. p53 is a well-known tumor suppressor that is expressed as various mutant forms in cancer cells, and numerous studies have reported that mutant p53 genes are present in more than half of cancers [37,38]. Gain-of-function (GOF) mutant p53 genes have been reported to perform various oncogenic functions, including cancer cell invasion and migration [39,40]. According to the cBioPortal analysis of TCGA data from 994 breast invasive carcinoma samples, the mRNA expression level of uPAR in *TP53*-mutated samples was higher than that in WT *TP53* samples (Fig. 4A). Furthermore, the majority of the mutations were driver mutations, which cause cancer (Fig. 4B) [41,42]. These findings suggest that CYP1B1 may promote the invasive properties of breast cancer by inducing uPAR expression, which may be associated with p53 and its mutant status.

Given that MCF-7 mainly expresses WT p53, whereas MDA-MB-231 predominantly expresses the R280K mutant of p53 (p53^{R280K}), an oncogenic driver mutant of p53 implicated in tumor metastasis [43–45], we attempted to investigate the influence of the p53 status on CYP1B1-induced uPAR expression and breast cancer cell invasiveness using these two cell lines. To determine the correlation between p53 and CYP1B1, we measured p53 mRNA and protein expression levels following *CYP1B1* overexpression or knockdown. Interestingly, CYP1B1 significantly suppressed WT p53 in MCF-7 cells at both the mRNA and protein levels, whereas the oncogenic mutant p53^{R280K} in MDA-MB-231 cells was strongly induced by CYP1B1 (Fig. 4C-H, S5). *In vivo* xenograft models using stably *CYP1B1*-knocked down MDA-MB-231 cells also showed that CYP1B1 suppression significantly reduced protein expression of the oncogenic mutant p53^{R280K} (Fig. 4I).

Since the strong induction of mutant p53^{R280K} protein expression following transient *CYP1B1* overexpression in MDA-MB-231 cells was markedly downregulated by a specific inhibitor of CYP1B1, TMS (Fig. 4G and S5), and previous data described mutant p53^{R280K} overexpression in MDA-MB-231 cells [46], we assumed that CYP1B1 may regulate the protein levels of WT p53 and mutant p53^{R280K} through PTM in addition to gene regulation. Moreover, accumulation of stabilized mutant p53 protein is important for its oncogenic GOF [47,48]. Therefore, we investigated whether CYP1B1 regulates the phosphorylation of mutant p53^{R280K} at Ser15, which is critical for p53 stabilization and activation [49]. Surprisingly, *CYP1B1* overexpression significantly increased phosphorylation at Ser15 of mutant p53^{R280K}, which was significantly reduced by TMS, while the level of phosphorylation at Ser15 of WT p53 was markedly reduced by CYP1B1 (Fig. 4J). Stable *CYP1B1* suppression strongly downregulated the phosphorylation at Ser15 of mutant p53^{R280K}, whereas it elevated the phosphorylation at Ser15 of WT p53 (Fig. 4K).

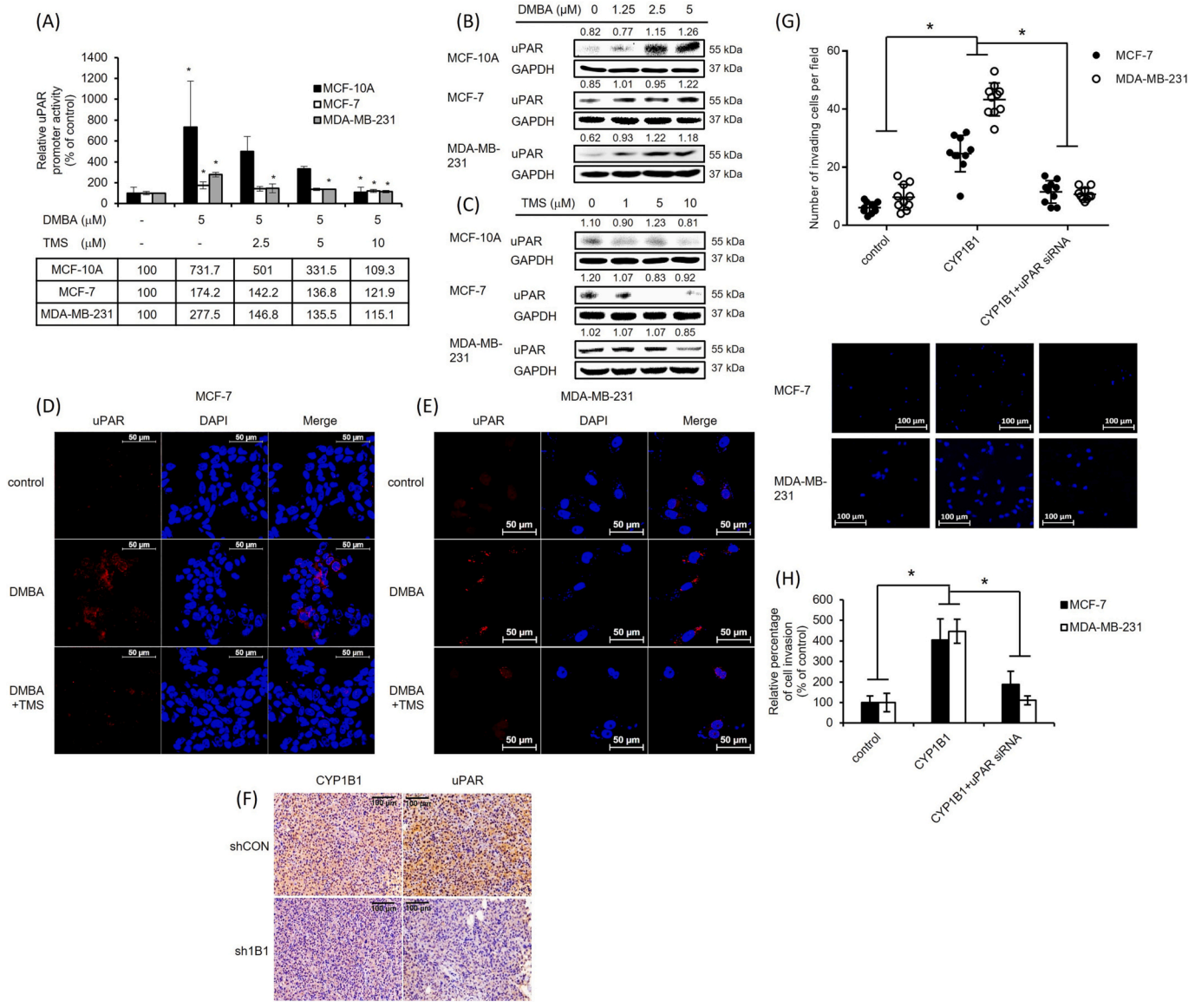


Fig. 3. CYP1B1 induces the expression level of uPAR via enhancing the promoter activity of uPAR. (A) The promoter activity of uPAR was determined via a dual luciferase assay following treatment with 5 μM DMBA in the presence or absence of treatment with designated concentrations of TMS for 48 h in MCF-10A, MCF-7, and MDA-MB-231 cells. The cells were treated with TMS prior to DMBA treatment for 1 h. Data are representative of experiments performed in triplicate (*, indicated $P \leq 0.05$ via Dunnett's *t*-test). (B-C) The protein expression levels of uPAR were measured with Western blotting in MCF-10A, MCF-7, and MDA-MB-231 cells following treatment in cells with (B) designated concentrations of DMBA for 24 h, and (C) designated doses of TMS for 48 h. (D-E) Confocal microscopic analyses for uPAR were conducted following treatment with 5 μM DMBA in the presence or absence of treatment with 10 μM of TMS for 48 h in MCF-7 and MDA-MB-231 cells. The cells were treated with TMS prior to DMBA treatment for 1 h. (D) MCF-7 cells, and (E) MDA-MB-231 cells. (F) Immunohistochemistry was performed using the xenograft tumor tissues, and the protein expression levels of CYP1B1 and uPAR were determined (n = 3). (G-H) MCF-7 and MDA-MB-231 cells were co-transfected with control or CYP1B1-overexpression vector and control or *PLAUR*-specific siRNA. After co-transfection, transwell invasion assay was conducted and the results were analyzed following confocal microscopic analysis for invaded cells using DAPI staining. Data were from 10 independently quantified experiments (*, indicated $P \leq 0.05$ via Dunnett's *t*-test). (G) The number of invading cells were plotted and the confocal microscopic analysis for invaded cells stained with DAPI were conducted, and (H) the relative percentage of cell invasion was calculated using the number of invading cells.

In summary, CYP1B1 regulates p53 expression based on mutation status to induce invasive traits in breast cancer; CYP1B1 inhibits WT p53 and promotes oncogenic mutant $p53^{R280K}$ through transcriptional regulation. Furthermore, CYP1B1 modulates additional protein stabilization and activation mechanisms to increase $p53^{R280K}$ protein levels and decrease WT p53 protein levels through regulation of phosphorylation at Ser15 of p53 protein.

3.5. WT p53 represses the promoter activity of uPAR, whereas $p53^{R280K}$ exerts a stimulating action on uPAR expression, resulting in the opposite effects on breast cancer cell invasion

Because CYP1B1 regulates p53 based on its mutation status, we investigated whether CYP1B1 induces uPAR via p53 regulation and how p53 mutation status affects the outcome. To determine whether CYP1B1 regulates uPAR expression via p53, we knocked down *TP53* in MCF-7 and MDA-MB-231 cells. Knockdown of WT *TP53* resulted in drastic promotion of uPAR expression, whereas knockdown of $TP53^{R280K}$ strongly reduced uPAR expression (Fig. 5A and B). To rule out the

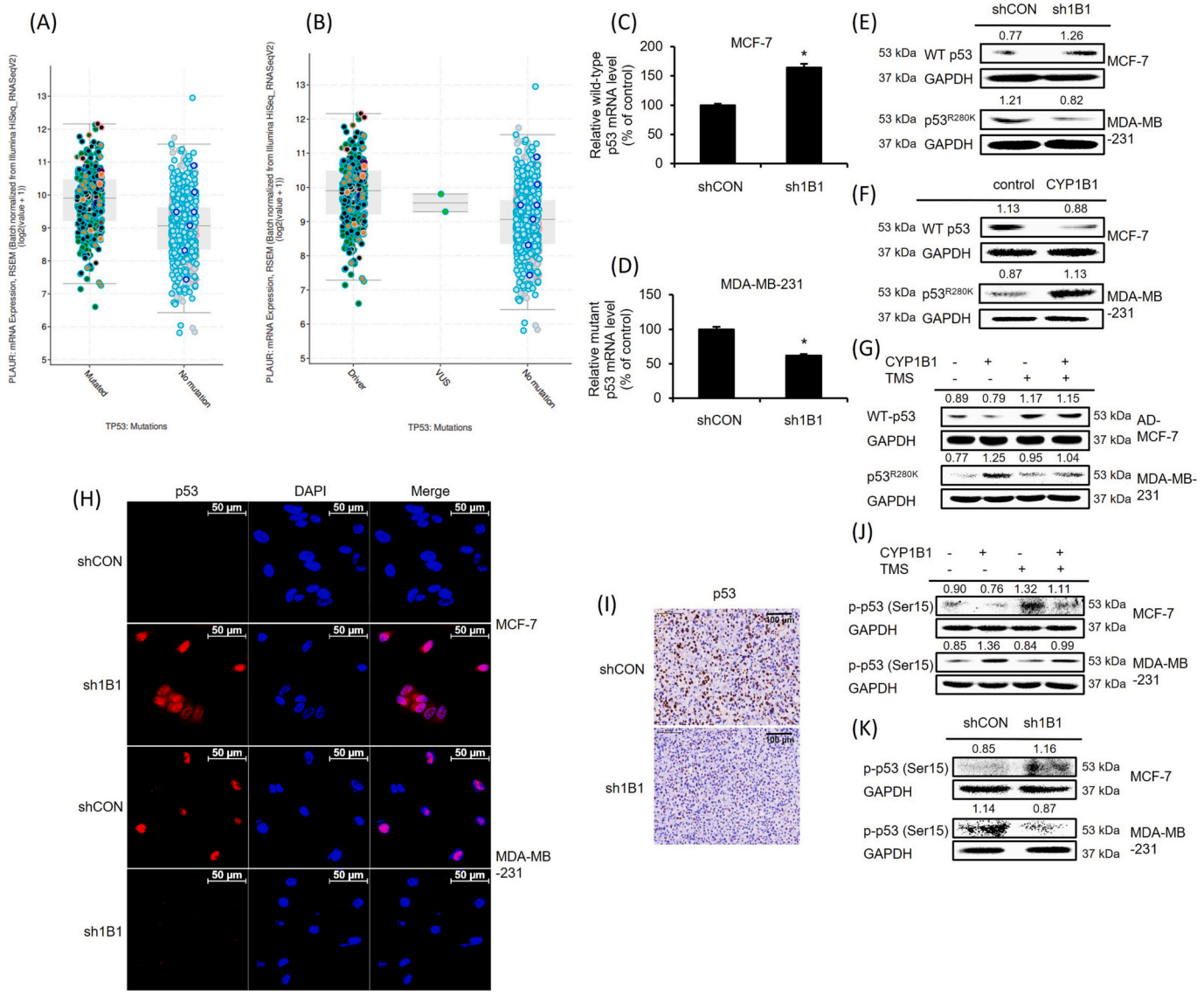


Fig. 4. CYP1B1 suppresses the mRNA and protein expression levels of wild-type p53 in MCF-7 cells, whereas the oncogenic mutant $p53^{R280K}$ in MDA-MB-231 cells is promoted by CYP1B1 through phosphorylation on Ser15 for additional stabilization and activation. (A-B) The mRNA expression levels of *PLAUR* in breast cancer patients were compared based on (A) the mutation status of p53 in samples, and (B) the type of mutations of p53 in samples. Driver represents driver mutations for cancer, and VUS represents a variant of unknown significance. Data were obtained from TCGA Pan-Cancer Atlas dataset with 994 breast invasive carcinoma samples in cBioPortal. (C-D) The mRNA expression levels of WT p53 or $p53^{R280K}$ were measured using qPCR following stable CYP1B1 knockdown by lentiviral shRNA transduction in (C) MCF-7, and (D) MDA-MB-231 cells. Results are representative of experiments performed in triplicate (*, indicated $P \leq 0.05$ via Dunnett's *t*-test). (E-G) The protein expression levels of WT p53 in MCF-7 cells and $p53^{R280K}$ in MDA-MB-231 cells were determined using Western blotting following (E) stable CYP1B1 knockdown by lentiviral shRNA transduction, (F) transient CYP1B1 overexpression by gene transfection, and (G) treatment with 10 μM of TMS for 48 h in control or CYP1B1-overexpressed cells by adenoviral transduction in MCF-7 cells or transient gene transfection in MDA-MB-231 cells. (H) Confocal microscopic analyses for p53 were conducted in stably CYP1B1-knocked down MCF-7 and MDA-MB-231 cells with lentiviral shRNA transduction. (I) Immunohistochemistry was performed using the xenograft tumor tissues, and the protein expression levels of p53 were determined (n = 3). (J-K) The level of phosphorylation on Ser15 residue of $p53^{R280K}$ was measured by Western blotting in MCF-7 and MDA-MB-231 cells following (J) treatment with 10 μM of TMS for 48 h in control or transiently CYP1B1-overexpressed cells, and (K) stable CYP1B1 knockdown with lentiviral shRNA transduction.

possibility that changes in uPAR expression by genetic regulation of *TP53* were due to characteristic differences between the two cell lines, we directly overexpressed WT *TP53* and $TP53^{R280K}$ in p53 null cell line, $HCT116^{p53-/-}$. Interestingly, Fig. 5C demonstrated that the main reason for the alteration of uPAR expression according to the genetic regulation of *TP53* was the regulatory action of p53 on uPAR depending on the p53 mutant status, not the cellular characteristics. Furthermore, we overexpressed WT *TP53* in MDA-MB-231 cells and $TP53^{R280K}$ in MCF-7 cells and compared the effects of each p53 mutation status on uPAR expression and breast cancer cell invasion. The basal promoter activity level of uPAR was considerably higher in MDA-MB-231 cells than in MCF-7 cells, but it was drastically reduced to almost the same level as in MCF-7 cells by overexpression of WT *TP53* (Fig. 5D and S6). Moreover, the promoter activity of uPAR in MCF-7 cells was significantly induced by overexpression of mutant $TP53^{R280K}$ (Fig. 5D and S6). uPAR protein expression levels revealed a similar pattern; the basal protein level of uPAR was much higher in MDA-MB-231 cells than in MCF-7 cells. However, overexpression of WT *TP53* in MDA-MB-231 cells significantly downregulated uPAR protein expression to near-basal levels in MCF-7 cells, whereas overexpression of $TP53^{R280K}$ in MCF-7 cells markedly increased uPAR protein expression (Fig. 5E, F, S6). Consequently, the level of cancer cell invasion in basal MDA-MB-231 cells, which was over

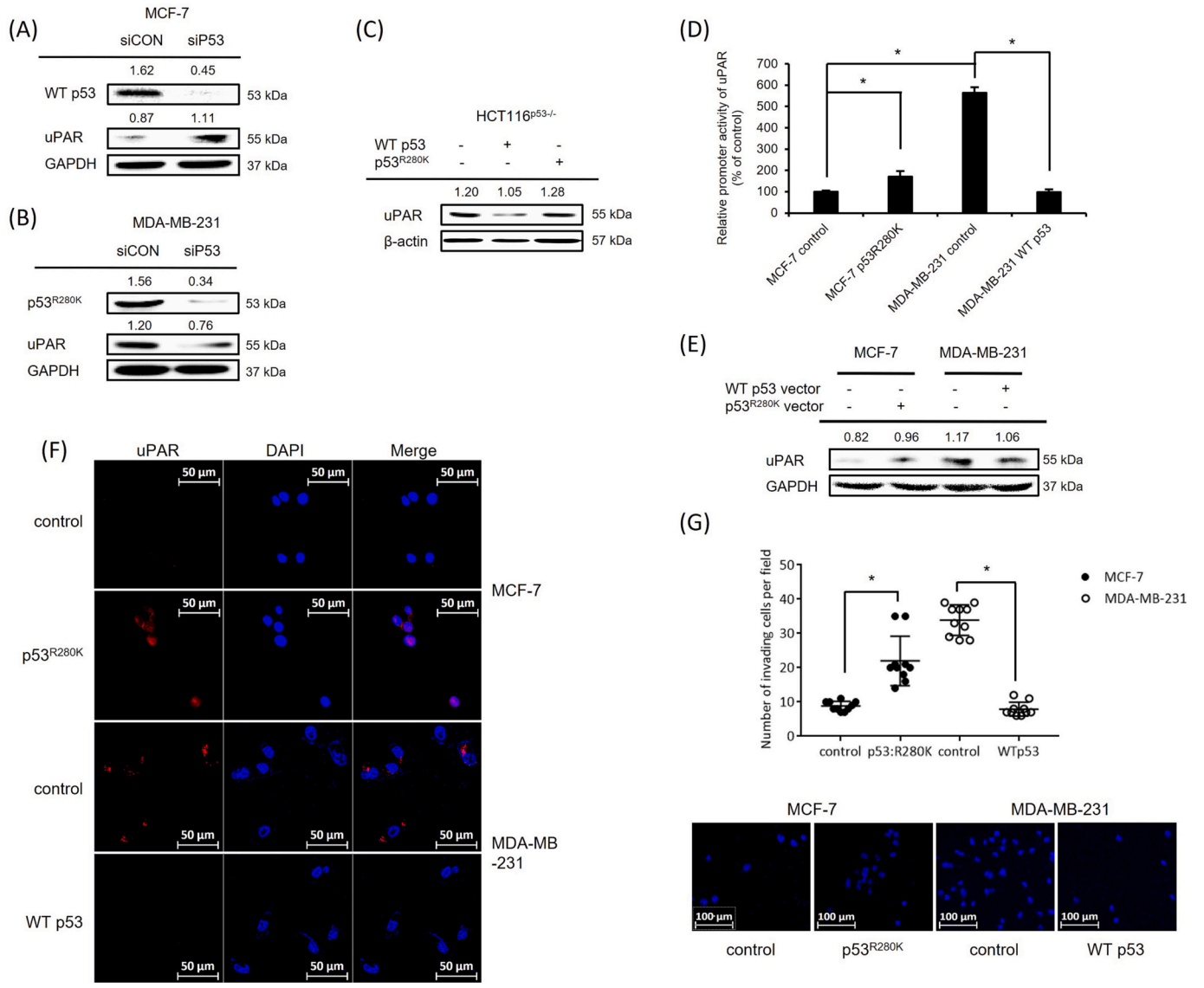


Fig. 5. WT p53 inhibits the level of breast cancer cell invasion through suppression of uPAR expression via reducing the promoter activity of uPAR, while the oncogenic mutant $p53^{R280K}$ enhances the promoter activity and protein expression of uPAR to induce the breast cancer cell invasion. (A-B) The protein expression levels of uPAR were detected using Western blot analyses following knockdown of p53 by siRNA in (A) MCF-7 cells, and (B) MDA-MB-231 cells. (C) The protein expression levels of uPAR were determined using Western blot analyses in $HCT116^{p53-/-}$ cells after transient transfection of control or WT p53/mutant $p53^{R280K}$ genes. (D) The promoter activity of uPAR was measured via a dual luciferase assay in MCF-7 and MDA-MB-231 cells with transient transfection of control or WT p53/mutant $p53^{R280K}$ genes. Data were from 5 independently quantified experiments and normalized by values of MCF-7 control group (*, indicated $P \leq 0.05$ via Dunnett's *t*-test). (E-F) The protein expression levels of uPAR were detected in MCF-7 cells following transient transfection of control or $p53^{R280K}$ gene or in MDA-MB-231 cells following transient transfection of control or WT p53 gene, using (E) Western blot analyses, and (F) confocal microscopic analysis. (G) Cell invasion was determined using the transwell invasion assay in the control or $p53^{R280K}$-overexpressed MCF-7 cells and control or WT p53-overexpressed MDA-MB-231 cells. Data were from 10 independently quantified experiments (*, indicated $P \leq 0.05$ via Dunnett's *t*-test). The confocal microscopic analysis was conducted to detect the invaded cells stained with DAPI.

three times higher than that in basal MCF-7 cells, was significantly suppressed by overexpression of WT *TP53* to almost the same level as in basal MCF-7 cells, whereas overexpression of $TP53^{R280K}$ in MCF-7 cells strongly promoted the invasion of cells (Fig. 5G and S6). These results suggest that p53 can alter the invasive properties of breast cancer cells through the regulation of uPAR expression based on the mutation status of p53.

3.6. CYP1B1 enhances uPAR expression by regulating p53 depending on its mutation status through its enzymatic activity to generate 4-OHE_2

As the experimental system for transient overexpression of CYP1B1 in Fig. 4 was proved to significantly enhance the enzymatic activity of CYP1B1 for 4-OHE_2 production in our previous paper [10], we investigated whether CYP1B1 regulates p53 and uPAR via its enzymatic activity. Polymorphic genetic variants of *CYP1B1* with different enzymatic activities were transiently overexpressed and their effects on WT p53, $p53^{R280K}$, and uPAR expression were determined. L432V, an allelic variant of *CYP1B1* with higher enzymatic activity, significantly reduced the expression of WT p53 and induced the uPAR expression, while the N203S variant form of *CYP1B1* with lower activity exhibited opposite results in MCF-7 cells (Fig. 6A) [50,51]. On the contrary, $p53^{R280K}$ and uPAR expression in MDA-MB-231 cells were upregulated by L432V *CYP1B1* and downregulated by N203S *CYP1B1* (Fig. 6B). In addition, when the cells were directly treated with the enzyme product of CYP1B1, 4-OHE_2, the expression of WT p53 was strongly suppressed, but

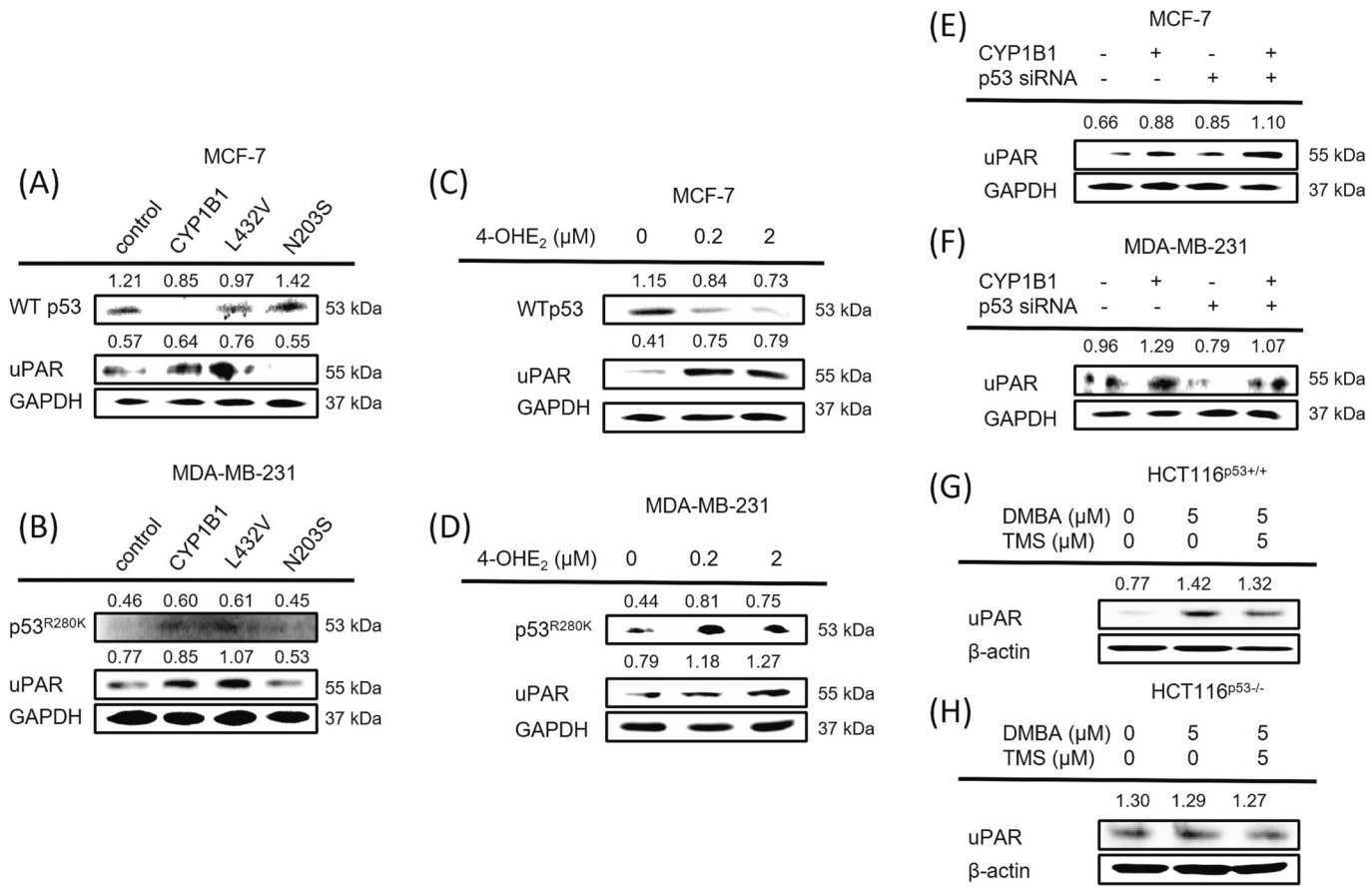


Fig. 6. CYP1B1 promotes uPAR expression via reduction of WT p53 and induction of $p53^{R280K}$ through its enzymatic activity to generate 4-OHE_2 in breast cancer cells. (A-B) Allelic variants of *CYP1B1* genes with higher (L432V) or lower (N203S) enzymatic activity to generate 4-OHE_2 were overexpressed along with normal *CYP1B1* gene and the protein expression levels of uPAR were determined using Western blot analyses in (A) MCF-7 cells, and (B) MDA-MB-231 cells. (C-D) MCF-7 and MDA-MB-231 cells were treated with designated concentrations of 4-OHE_2 in the charcoal-stripped FBS-containing phenol red free RPMI media. After 48 h, cells were harvested and Western blot analyses for detection of uPAR were performed. (C) MCF-7 cells, and (D) MDA-MB-231 cells. (E-F) MCF-7 and MDA-MB-231 cells were co-transfected with control or CYP1B1-overexpression vector and control or *TP53*-specific siRNA. After 48 h of post-transfection stabilization, cells were harvested and the protein expression levels of uPAR were measured using Western blot analyses. (E) MCF-7 cells, and (F) MDA-MB-231 cells. (G-H) $HCT116^{p53+/+}$ or $HCT116^{p53-/-}$ cells were treated with 5 μM DMBA in the presence or absence of 5 μM of TMS for 48 h and the protein expression levels of uPAR were determined using Western blot analyses. The cells were treated with TMS prior to DMBA treatment for 1 h. (G) $HCT116^{p53+/+}$ cells, and (H) $HCT116^{p53-/-}$ cells.

the expression of $p53^{R280K}$ and uPAR was remarkably increased (Fig. 6C and D). These data elucidated that CYP1B1 regulates WT p53, $p53^{R280K}$, and uPAR through its enzymatic activity to generate 4-OHE_2.

To clarify whether CYP1B1 induces uPAR expression through regulation of WT or mutant p53, we examined changes in uPAR protein expression levels following genetic regulation of *TP53* and *CYP1B1*. Both *CYP1B1* overexpression and knockdown of WT *TP53* in MCF-7 cells significantly promoted uPAR expression, which resulted in a synergistic effect on uPAR expression when WT *TP53* was knocked down in *CYP1B1*-overexpressed cells (Fig. 6E). However, repression of $TP53^{R280K}$ strongly reduced uPAR expression and even diminished the promoting effect of CYP1B1 on uPAR in MDA-MB-231 cells (Fig. 6F). Additionally, in $HCT116^{p53+/+}$ cells exhibiting WT p53, DMBA markedly induced uPAR expression but TMS inhibited DMBA action, indicating that uPAR induction was mediated by CYP1B1 (Fig. 6G). Interestingly, the action of DMBA on uPAR did not occur in $HCT116^{p53-/-}$ cells in Fig. 6H, suggesting that upregulation of uPAR by CYP1B1 could not be executed without p53. Collectively, these results revealed that p53 is crucial for uPAR induction by CYP1B1.

Subsequently, we investigated whether CYP1B1 action for cancer cell invasion is dependent on uPAR induction by p53. Interestingly, nutlin-3a, an inhibitor of murine double minute 2 (MDM2), significantly blocked the promotion effects of CYP1B1 on uPAR expression (Fig. 7A and B) and the consequent activation of the uPAR pathway in MCF-7 cells, but had no significant effects on MDA-MB-231 cells; the activation of uPAR pathway was proven by the elevated protein levels of integrin α5β1 [52] (Fig. 7C and D) and the increased level of cancer cell invasion (Fig. 7E and F). The lack of impact of nutlin-3a on the CYP1B1-induced uPAR pathway in MDA-MB-231 cells can be explained by a previously published study, which demonstrated that mutant p53 proteins are stable in tumor cells, as their negative feedback loop with MDM2 is defective due to several signaling pathways, including Hsp90-mediated stabilization, which counteract and diminish the action of MDM2 as an E3 ligase on mutant p53 [44,53]. Therefore, we concluded that the different effects of nutlin-3a on DMBA-induced uPAR and breast cancer cell invasion in MCF-7 and MDA-MB-231 cells were due to the distinct mutation status of p53 in each cell line.

In summary, our findings show that p53 can change the invasive levels of breast cancer cells based on their mutation status by regulating uPAR expression and that CYP1B1 can enhance the invasive traits of breast cancer cells using the ability of p53 to regulate uPAR.

4. Discussion

In this study, we established the molecular mechanism of CYP1B1 for the induction of invasive breast cancer traits; CYP1B1 activated the uPAR pathway by enhancing uPAR promoter activity via regulation of p53. These novel findings regarding the role of CYP1B1 may help

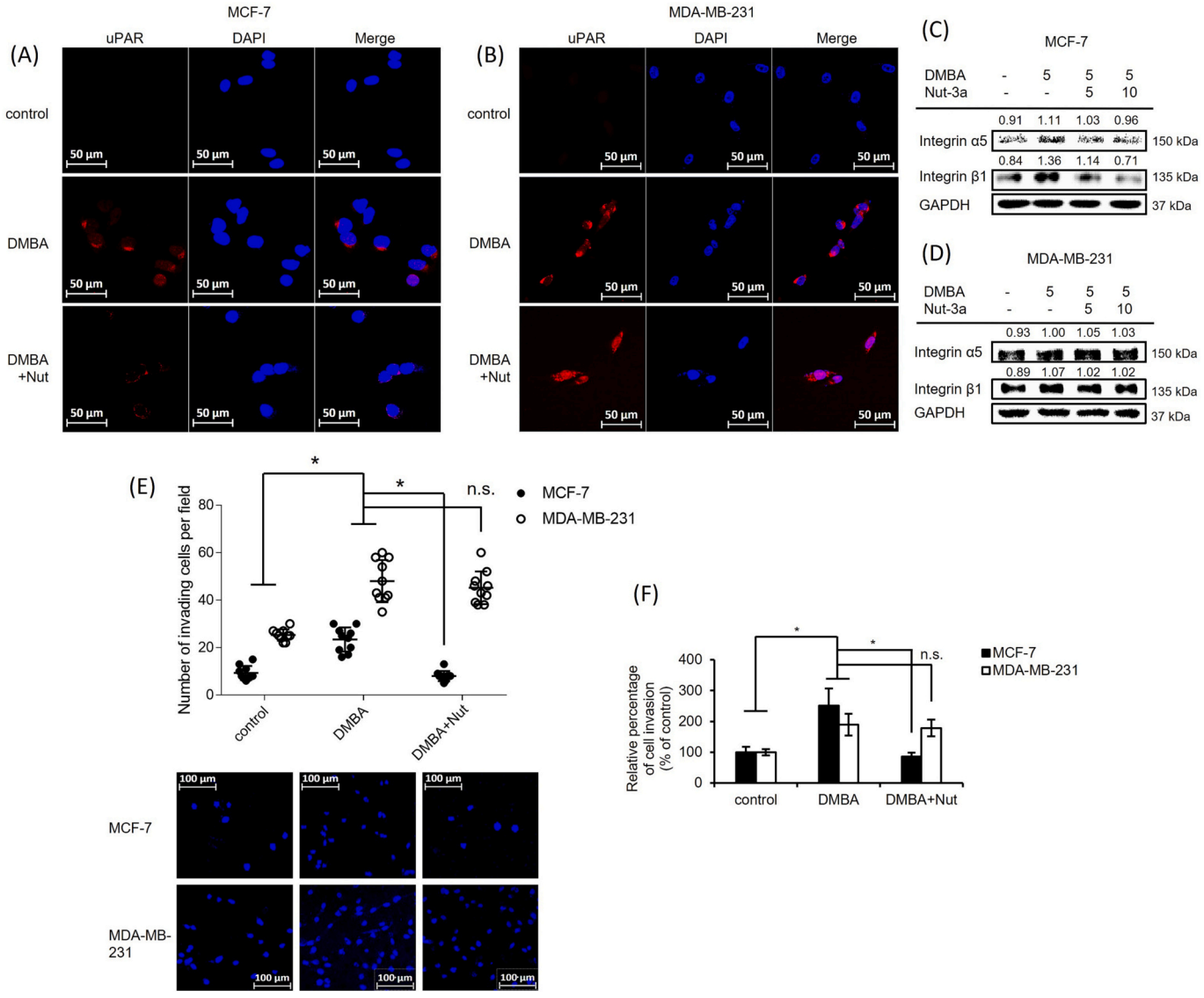


Fig. 7. CYP1B1 induces breast cancer cell invasion through activation of uPAR-integrin pathway following regulation of p53 according to the mutant status of p53 in breast cancer cells. (A-B) Confocal microscopic analyses were conducted to detect the protein expression levels of uPAR following treatment with 5 μM DMBA in the presence or absence of 10 μM nutlin-3a for 24 h in (A) MCF-7 cells, and (B) MDA-MB-231 cells. Cells were treated with nutlin-3a prior to DMBA treatment for 1 h. (C-D) The protein expression levels of integrin α5β1 were determined by Western blotting following treatment with 5 μM DMBA in the presence or absence of 5, 10 μM nutlin-3a for 24 h in (C) MCF-7 cells, and (D) MDA-MB-231 cells. Cells were treated with nutlin-3a prior to DMBA treatment for 1 h. (E-F) Cell invasion was measured using the transwell invasion assay in MCF-7 cells and MDA-MB-231 cells after treatment with 5 μM DMBA in the presence or absence of 10 μM nutlin-3a for 24 h. Cells were treated with nutlin-3a prior to DMBA treatment for 1 h. Data were from 10 independently quantified experiments (*, indicated $P \leq 0.05$ via Dunnett's *t*-test). (E) The number of invading cells were plotted and the confocal microscopic analysis for invaded cells stained with DAPI were conducted, and (F) the relative percentage of cell invasion was calculated using the number of invading cells.

explain the correlation between high levels of CYP1B1 expression and the low relapse-free survival rate of patients with breast cancer. Relapse of metastatic breast cancer is the leading cause of morbidity and mortality and is often associated with drug resistance in cancer cells, impairing the accuracy of diagnosis and the efficacy of therapeutic treatment [54]. Besides our findings regarding the role of CYP1B1 in inducing basal invasive properties in breast cancer, CYP1B1 has been widely described as one of the major factors making cancer cells tolerant to chemotherapies owing to its enzymatic ability to degrade or inactivate drugs and the significant contribution of *CYP1B1* polymorphic genes to anti-cancer drug resistance [55–57]. Since oncogenic mutant p53 genes have been shown to enhance chemoresistance in several ways [58,59], our findings regarding the correlation between CYP1B1 and oncogenic mutant $p53^{R280K}$ may be able to demonstrate the molecular mechanism of CYP1B1 for chemoresistance in invasive breast cancer.

Surprisingly, we found that CYP1B1 regulated the level of phosphorylation at Ser15 of p53 differently depending on the mutation status of p53; CYP1B1 inhibited phosphorylation at Ser15 of WT p53, whereas the level of phosphorylation at Ser15 of mutant $p53^{R280K}$ was significantly induced by CYP1B1 (Fig. 4J and K). PTMs of WT p53 are possible at >15 % of all amino acid residues and alter the functional activity of WT p53 upon different types of stimuli by modulating various interactions with >300 proteins rather than causing structural changes [60]. PTMs of mutant p53 are relatively less known than those of WT p53, but all PTMs identified in mutant p53s so far also occur in WT p53 [58]. Although most PTMs of p53 do not discriminate between WT and mutant p53, the patterns of PTMs of mutant p53 may be different from those of WT p53 because the conformational changes caused by mutations can expose or cover the target residues for PTMs and trigger the sequential alterations of PTM patterns due to the interdependent nature

of p53 PTMs [47,60,61]. Compared to DNA-bound WT p53, structural differences in mutant $p53^{R280K}$ were primarily identified in the DNA-binding domain region, including several target residues for p53 PTM, such as Lys120 and Ser183 [62]. These conformational changes in mutant $p53^{R280K}$ may modify the phosphorylation patterns at Ser15 by CYP1B1, which can be further investigated in a follow-up study.

Phosphorylation of Ser15, one of the most common PTMs of p53, stabilizes p53 proteins through inhibition of proteasomal degradation of p53 by MDM2 and stimulates the transcription activity of p53 via inducing nuclear accumulation of p53 protein [63–65]. Ser15 phosphorylation of p53 is known to be a key point of p53 activation because it induces a series of additional PTMs, such as phosphorylation at Thr18 and Ser20, which triggers recruitment of factors for C-terminal acetylation of p53 and prevents proteasomal degradation of p53 [65–67]. Phosphorylation at Ser15 of mutant p53 by DNA-PK, ERK1/2 MAP kinase, and ATM was identified to be critical for oncogenic GOF of mutant p53, and inhibition of Ser15 phosphorylation of mutant p53 partially restored the function of WT p53 [68–71]. Interestingly, several p53 mutants, including $p53^{R280K}$ in breast cancer cell lines, were found to be constitutively phosphorylated at Ser15, and overexpression of MDM2 failed to affect Ser15 phosphorylation levels of mutant p53s [72]. This previous report demonstrated that constitutive phosphorylation of mutant p53s at Ser15 could be associated with highly activated ERK1/2 MAP kinase in tumor cells compared to normal cells [72]. Based on these findings and those in Fig. 6, we suggest that the promoting effects of CYP1B1 mediated by $p53^{R280K}$ on key factors in uPAR signaling could not be diminished by nutlin-3a, possibly because the mutant $p53^{R280K}$ in MDA-MB-231 cells is constitutively phosphorylated at Ser15 by CYP1B1, thus avoiding the negative regulation by MDM2. To prove this hypothesis and identify the key kinases for Ser15 phosphorylation by CYP1B1, further follow-up study is needed.

In conclusion, our findings provide evidence that CYP1B1 significantly contributes to induction of basal invasive properties in breast cancer and may induce chemoresistance, which in turn reduces the survival rate of patients with breast cancer but without relapse of disease. These findings suggest that targeting CYP1B1 may be useful to establish novel anti-cancer strategies to ameliorate the morbidity and mortality of patients with invasive breast cancer. Since the importance of oncogenic mutant p53 in cancers has been recognized, targeting oncogenic mutant p53 for cancer therapy using small molecules, RNAi, immunotherapy, and noncoding RNAs has emerged as an enticing and promising strategy [38,73]. Based on our findings, which demonstrated a correlation between CYP1B1 and oncogenic mutant p53 in breast cancer, combination therapy with anti-cancer agents targeting CYP1B1 or oncogenic mutant p53 will provide a synergistic effect for cancer treatment that prevents relapse. Furthermore, precise medical strategy for patients with breast cancer can be established based on the personal genomic information about the status of CYP1B1 polymorphism and p53 mutation.

Compliance with ethical standards

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Chung-Ang University (2017-00096, 2019-00003) and were performed according to the guidelines of an approved protocol by IACUC.

CRediT authorship contribution statement

Yeo-Jung Kwon: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft, Review & Editing, Visualization Young-Jin Chun: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition Tae-Uk Kwon: Validation, Investigation, Data Curation Sangyun Shin: Validation, Formal analysis, Visualization Boyoung Lee: Formal analysis, Data Curation Hyein Lee: Formal analysis, Visualization Hyemin Park: Formal analysis, Data Curation Donghak Kim: Conceptualization, Writing – Review & Editing Aree Moon: Conceptualization, Writing – Review & Editing.

All authors have read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets for our findings can be found in this article and supporting information. Additional information about our data and materials can be provided by the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2023.166868.

References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 71 (3) (2021) 209–249, https://doi.org/10.3322/caac.21660.
[2] K. Ganesh, J. Massagué, Targeting metastatic cancer, Nat. Med. 27 (1) (2021) 34–44, https://doi.org/10.1038/s41591-020-01195-4.
[3] R.L. Siegel, K.D. Miller, H.E. Fuchs, A. Jemal, Cancer statistics, 2022, CA Cancer J. Clin. 72 (1) (2022) 7–33, https://doi.org/10.3322/caac.21708.
[4] H. Samavat, M.S. Kurzer, Estrogen metabolism and breast cancer, Cancer Lett. 356 (2) (2015) 231–243, https://doi.org/10.1016/j.canlet.2014.04.018.
[5] J.N. Sampson, R.T. Falk, C. Schairer, et al., Association of estrogen metabolism with breast cancer risk in different cohorts of postmenopausal women, Cancer Res. 77 (4) (2017) 918–925, https://doi.org/10.1158/0008-5472.CAN-16-1717.
[6] H. Seeger, D. Wallwiener, E. Kraemer, A.O. Mueck, Comparison of possible carcinogenic estradiol metabolites: effects on proliferation, apoptosis and metastasis of human breast cancer cells, Maturitas. 54 (1) (2006) 72–77, https://doi.org/10.1016/j.maturitas.2005.08.010.
[7] S.V. Fernandez, I.H. Russo, J. Russo, Estradiol and its metabolites 4-hydroxyestradiol and 2-hydroxyestradiol induce mutations in human breast epithelial cells, Int. J. Cancer 118 (8) (2006) 1862–1868, https://doi.org/10.1002/ijc.21590.
[8] B. Starek-Swiechowicz, B. Budziszewska, A. Starek, Endogenous estrogens-breast cancer and chemoprevention, Pharmacol. Rep. 73 (6) (2021) 1497–1512, https://doi.org/10.1007/s43440-021-00317-0.
[9] G.I. Murray, M.C. Taylor, M.C. McFadyen, et al., Tumor-specific expression of cytochrome P450 CYP1B1, Cancer Res. 57 (14) (1997) 3026–3031.
[10] Y.J. Kwon, H.S. Baek, D.J. Ye, et al., CYP1B1 enhances cell proliferation and metastasis through induction of EMT and activation of Wnt/beta-catenin signaling via Sp1 upregulation, PLoS One 11 (3) (2016), e0151598, https://doi.org/10.1371/journal.pone.0151598.
[11] W. Lanxiang, W. Bin, X. Ge, et al., Long-term exposure of 4-hydroxyestradiol induces the cancer cell characteristics via upregulating CYP1B1 in MCF-10A cells, Toxicol. Mech. Methods 29 (9) (2019) 686–692, https://doi.org/10.1080/15376516.2019.1650146.
[12] Y.M. Al-Saraireh, F.O.F.O. Alshammari, A.M.M. Youssef, et al., Profiling of CYP4Z1 and CYP1B1 expression in bladder cancers, Sci. Rep. 11 (1) (2021) 5581, https://doi.org/10.1038/s41598-021-85188-4.
[13] L. Gu, Y. Liu, C. Jiang, L. Sun, H. Zhou, Identification and clinical validation of metastasis-associated biomarkers based on large-scale samples in colon-adenocarcinoma, Pharmacol. Res. 160 (2020), 105087, https://doi.org/10.1016/j.phrs.2020.105087.
[14] Y. Shi, P. Ye, X. Long, Differential expression profiles of the transcriptome in breast cancer cell lines revealed by next generation sequencing, Cell. Physiol. Biochem. 44 (2) (2017) 804–816, https://doi.org/10.1159/000485344.

[15] R. Chiotaki, H. Polioudaki, P.A. Theodoropoulos, Differential nuclear shape dynamics of invasive and non-invasive breast cancer cells are associated with actin cytoskeleton organization and stability, Biochem. Cell Biol. 92 (4) (2014) 287–295, https://doi.org/10.1139/bcb-2013-0120.
[16] H. González-King, S. Tejedor, M. Ciria, et al., Non-classical notch signaling by MDA-MB-231 breast cancer cell-derived small extracellular vesicles promotes malignancy in poorly invasive MCF-7 cells, Cancer Gene Ther. 29 (7) (2022) 1056–1069, https://doi.org/10.1038/s41417-021-00411-8.
[17] J. Debnath, S.K. Muthuswamy, J.S. Brugge, Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures, Methods. 30 (3) (2003) 256–268, https://doi.org/10.1016/s1046-2023(03)00032-x.
[18] E. Cerami, J. Gao, U. Dogrusoz, et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Discov. 2 (5) (2012) 401–404, https://doi.org/10.1158/2159-8290.CD-12-0095.
[19] J. Gao, B.A. Aksoy, U. Dogrusoz, et al., Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (269) (2013), https://doi.org/10.1126/scisignal.2004088 pl1.
[20] Z. Tang, C. Li, B. Kang, et al., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res. 45 (W1) (2017) W98–W102, https://doi.org/10.1093/nar/gkx247.
[21] C.P. Goswami, H. Nakshatri, PROGgeneV2: enhancements on the existing database, BMC Cancer 14 (2014) 970, https://doi.org/10.1186/1471-2407-14-970.
[22] S. Uda, M. Mukai, K. Kishima, et al., Pelvic local recurrence as first relapse predicts prognosis for clinical stage II/III lower rectal cancer: a clinicopathological investigation, Mol. Clin. Oncol. 14 (2) (2021) 33, https://doi.org/10.3892/mco.2020.2195.
[23] L.R. Yates, S. Knappskog, D. Wedge, et al., Genomic evolution of breast cancer metastasis and relapse, Cancer Cell 32 (2) (2017) 169–184, https://doi.org/10.1016/j.ccell.2017.07.005.
[24] N. Mahmood, C. Mihalcioiu, S.A. Rabbani, Multifaceted role of the urokinase-type plasminogen activator (uPA) and its receptor (uPAR): diagnostic, prognostic, and therapeutic applications, Front. Oncol. 8 (2018) 24, https://doi.org/10.3389/fonc.2018.00024.
[25] A. Benedicto, J. Marquez, A. Herrero, et al., Decreased expression of the β2 integrin on tumor cells is associated with a reduction in liver metastasis of colorectal cancer in mice, BMC Cancer 17 (1) (2017) 827, https://doi.org/10.1186/s12885-017-3823-2.
[26] R.L. Stewart, K.L. O'Connor, Clinical significance of the integrin α6β4 in human malignancies, Lab. Investig. 95 (9) (2015) 976–986, https://doi.org/10.1038/labinvest.2015.82.
[27] L. Tang, X. Han, The urokinase plasminogen activator system in breast cancer invasion and metastasis, Biomed. Pharmacother. 67 (2) (2013) 179–182, https://doi.org/10.1016/j.biopha.2012.10.003.
[28] R.D. Lester, M. Jo, V. Montel, S. Takimoto, S.L. Gonias, uPAR induces epithelial-mesenchymal transition in hypoxic breast cancer cells, J. Cell Biol. 178 (3) (2007) 425–436, https://doi.org/10.1083/jcb.200701092.
[29] L. Yin, J.J. Duan, X.W. Bian, S.C. Yu, Triple-negative breast cancer molecular subtyping and treatment progress, Breast Cancer Res. 22 (1) (2020) 61, https://doi.org/10.1186/s13058-020-01296-5.
[30] X. Dai, H. Cheng, Z. Bai, J. Li, Breast cancer cell line classification and its relevance with breast tumor subtyping, J. Cancer 8 (16) (2017) 3131–3141, https://doi.org/10.7150/jca.18457.
[31] A. Zannetti, S.D. Vecchio, M.V. Carriero, et al., Coordinate up-regulation of Sp1 DNA-binding activity and urokinase receptor expression in breast carcinoma, Cancer Res. 60 (6) (2000) 1546–1551.
[32] E. Soravia, A. Grebe, P.D. Luca, et al., A conserved TATA-less proximal promoter drives basal transcription from the urokinase-type plasminogen activator receptor gene, Blood. 86 (2) (1995) 624–635.
[33] Y.S. Park, Y.J. Kwon, Y.J. Chun, CYP1B1 activates Wnt/beta-catenin signaling through suppression of Herc5-mediated ISGylation for protein degradation on beta-catenin in HeLa cells, Toxicol. Res. 33 (3) (2017) 211–218, https://doi.org/10.5487/TR.2017.33.3.211.
[34] Y.J. Chun, Y.K. Oh, B.J. Kim, et al., Potent inhibition of human cytochrome P450 1B1 by tetramethoxystilbene, Toxicol. Lett. 189 (1) (2009) 84–89, https://doi.org/10.1016/j.toxlet.2009.05.005.
[35] S. Shetty, T. Velusamy, S. Idell, et al., Regulation of urokinase receptor expression by p53: novel role in stabilization of uPAR mRNA, Mol. Cell. Biol. 27 (16) (2007) 5607–5618, https://doi.org/10.1128/MCB.00080-07.
[36] Y.P. Bhandary, S.K. Shetty, A.S. Marudamuthu, et al., Regulation of lung injury and fibrosis by p53-mediated changes in urokinase and plasminogen activator inhibitor-1, Am. J. Pathol. 183 (1) (2013) 131–143, https://doi.org/10.1016/j.ajpath.2013.03.022.
[37] F. Mantovani, L. Collavin, G. Del Sal, Mutant p53 as a guardian of the cancer cell, Cell Death Differ. 26 (2) (2019) 199–212, https://doi.org/10.1038/s41418-018-0246-9.
[38] G. Zhu, C. Pan, J.X. Bei, et al., Mutant p53 in cancer progression and targeted therapies, Front. Oncol. 10 (2020), 595187, https://doi.org/10.3389/fonc.2020.595187.
[39] P.A. Muller, K.H. Vousden, p53 mutations in cancer, Nat. Cell Biol. 15 (1) (2013) 2–8, https://doi.org/10.1038/ncb2641.
[40] M. Nakayama, C.P. Hong, H. Oshima, et al., Loss of wild-type p53 promotes mutant p53-driven metastasis through acquisition of survival and tumor-initiating properties, Nat. Commun. 11 (1) (2020) 2333, https://doi.org/10.1038/s41467-020-16245-1.
[41] F. Supek, B. Miñana, J. Valcárcel, T. Gabaldón, B. Lehner, Synonymous mutations frequently act as driver mutations in human cancers, Cell. 156 (6) (2014) 1324–1335, https://doi.org/10.1016/j.cell.2014.01.051.
[42] J.R. Pon, M.A. Marra, Driver and passenger mutations in cancer, Annu. Rev. Pathol. 10 (2015) 25–50, https://doi.org/10.1146/annurev-pathol-012414-040312.
[43] S.A. Mehta, K.W. Christopherson, P. Bhat-Nakshatri, et al., Negative regulation of chemokine receptor CXCR4 by tumor suppressor p53 in breast cancer cells: implications of p53 mutation or isoform expression on breast cancer cell invasion, Oncogene. 26 (23) (2007) 3329–3337, https://doi.org/10.1038/sj.onc.1210120.
[44] D. Walerych, M. Napoli, L. Collavin, G. Del Sal, The rebel angel: mutant p53 as the driving oncogene in breast cancer, Carcinogenesis. 33 (11) (2012) 2007–2017, https://doi.org/10.1093/carcin/bgs232.
[45] F. Vogiatzi, D.T. Brandt, J. Schneikert, et al., Mutant p53 promotes tumor progression and metastasis by the endoplasmic reticulum UDPase ENTPD5, Proc. Natl. Acad. Sci. U. S. A. 113 (52) (2016) E8433–E8442, https://doi.org/10.1073/pnas.1612711114.
[46] Y.H. Bae, J.H. Ryu, H.J. Park, et al., Mutant p53-Notch1 signaling axis is involved in curcumin-induced apoptosis of breast cancer cells, Korean J. Physiol. Pharmacol. 17 (4) (2013) 291–297, https://doi.org/10.4196/kjpp.2013.17.4.291.
[47] R. Vijayakumaran, K.H. Tan, P.J. Miranda, S. Haupt, Y. Haupt, Regulation of mutant p53 protein expression, Front. Oncol. 5 (2015) 284, https://doi.org/10.3389/fonc.2015.00284.
[48] X. Yue, Y. Zhao, Y. Xu, et al., Mutant p53 in cancer: accumulation, gain-of-function, and therapy, J. Mol. Biol. 429 (11) (2017) 1595–1606, https://doi.org/10.1016/j.jmb.2017.03.030.
[49] H. Zhao, F. Traganos, Z. Darzynkiewicz, Phosphorylation of p53 on Ser15 during cell cycle caused by topo I and topo II inhibitors in relation to ATM and Chk2 activation, Cell Cycle 7 (19) (2008) 3048–3055, https://doi.org/10.4161/cc.7.19.6750.
[50] J. Trubicka, E. Grabowska-Kłujszo, J. Suchy, et al., Variant alleles of the CYP1B1 gene are associated with colorectal cancer susceptibility, BMC Cancer 10 (2010) 420, https://doi.org/10.1186/1471-2407-10-420.
[51] F. Pasutto, G. Chavarria-Soley, C.Y. Mardin, et al., Heterozygous loss-of-function variants in CYP1B1 predispose to primary open-angle glaucoma, Invest. Ophthalmol. Vis. Sci. 51 (1) (2010) 249–254, https://doi.org/10.1167/iovs.09-3880.
[52] T. Tarui, N. Andronicos, R.P. Czekay, et al., Critical role of integrin alpha 5 beta 1 in urokinase (uPA)/urokinase receptor (uPAR, CD87) signaling, J. Biol. Chem. 278 (32) (2003) 29863–29872, https://doi.org/10.1074/jbc.M304694200.
[53] U.M. Moll, O. Petrenko, The MDM2-p53 interaction, Mol. Cancer Res. 1 (14) (2003) 1001–1008.
[54] V. Gote, A.R. Nookala, P.K. Bolla, D. Pal, Drug resistance in metastatic breast cancer: tumor targeted nanomedicine to the rescue, Int. J. Mol. Sci. 22 (9) (2021) 4673, https://doi.org/10.3390/ijms22094673.
[55] Z. Zhu, Y. Mu, C. Qi, et al., CYP1B1 enhances the resistance of epithelial ovarian cancer cells to paclitaxel in vivo and in vitro, Int. J. Mol. Med. 35 (2) (2015) 340–348, https://doi.org/10.3892/ijmm.2014.2041.
[56] M.C. McFadyen, H.L. McLeod, F.C. Jackson, et al., Cytochrome P450 CYP1B1 protein expression: a novel mechanism of anticancer drug resistance, Biochem. Pharmacol. 62 (2) (2001) 207–212, https://doi.org/10.1016/s0006-2952(01)00643-8.
[57] A. Laroche-Clary, V. Le Morvan, T. Yamori, J. Robert, Cytochrome P450 1B1 gene polymorphisms as predictors of anticancer drug activity: studies with in vitro models, Mol. Cancer Ther. 9 (12) (2010) 3315–3321, https://doi.org/10.1158/1535-7163.MCT-10-0673.
[58] E. Alvarado-Ortiz, K.G. de la Cruz-López, J. Becerril-Rico, et al., Mutant p53 gain-of-function: role in cancer development, progression, and therapeutic approaches, Front. Cell Dev. Biol. 8 (2020), 607670, https://doi.org/10.3389/fcell.2020.607670.
[59] Y. Huang, N. Liu, J. Liu, et al., Mutant p53 drives cancer chemotherapy resistance due to loss of function on activating transcription of PUMA, Cell Cycle 18 (24) (2019) 3442–3455, https://doi.org/10.1080/15384101.2019.1688951.
[60] T.A. Nguyen, D. Menendez, M.A. Resnick, C.W. Anderson, Mutant TP53 posttranslational modifications: challenges and opportunities, Hum. Mutat. 35 (6) (2014) 738–755, https://doi.org/10.1002/humu.22506.
[61] D.W. Meek, C.W. Anderson, Posttranslational modification of p53: cooperative integrators of function, Cold Spring Harb. Perspect. Biol. 1 (6) (2009), a000950, https://doi.org/10.1101/cshperspect.a000950.
[62] A.S. Gomes, F. Trovão, B.A. Pinheiro, et al., The crystal structure of the R280K mutant of human p53 explains the loss of DNA binding, Int. J. Mol. Sci. 19 (4) (2018) 1184, https://doi.org/10.3390/ijms19041184.
[63] J. Loughery, M. Cox, L.M. Smith, D.W. Meek, Critical role for p53-serine 15 phosphorylation in stimulating transactivation at p53-responsive promoters, Nucleic Acids Res. 42 (12) (2014) 7666–7680, https://doi.org/10.1093/nar/gku501.
[64] A. Ito, C.H. Lai, X. Zhao, et al., p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2, EMBO J. 20 (6) (2001) 1331–1340, https://doi.org/10.1093/emboj/20.6.1331.
[65] M.F. Lavin, N. Gueven, The complexity of p53 stabilization and activation, Cell Death Differ. 13 (6) (2006) 941–950, https://doi.org/10.1038/sj.cdd.4401925.
[66] M. Li, J. Luo, C.L. Brooks, W. Gu, Acetylation of p53 inhibits its ubiquitination by Mdm2, J. Biol. Chem. 277 (52) (2002) 50607–50611, https://doi.org/10.1074/jbc.C200578200.

[67] E. Appella, C.W. Anderson, Post-translational modifications and activation of p53 by genotoxic stresses, Eur. J. Biochem. 268 (10) (2001) 2764–2772, https://doi.org/10.1046/j.1432-1327.2001.02225.x.
[68] H. Song, M. Hollstein, Y. Xu, p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM, Nat. Cell Biol. 9 (5) (2007) 573–580, https://doi.org/10.1038/ncb1571.
[69] M. Sonego, M. Schiappacassi, S. Lovisa, et al., Stathmin regulates mutant p53 stability and transcriptional activity in ovarian cancer, EMBO Mol. Med. 5 (5) (2013) 707–722, https://doi.org/10.1002/emmm.201201504.
[70] R.A. Frum, I.M. Love, P.K. Damle, et al., Constitutive activation of DNA damage checkpoint signaling contributes to mutant p53 accumulation via modulation of p53 ubiquitination, Mol. Cancer Res. 14 (5) (2016) 423–436, https://doi.org/10.1158/1541-7786.MCR-15-0363.
[71] E. Sugikawa, N. Yazaki, S. Tsunoda, N. Nakanishi, M. Ohashi, Inhibition of mutant p53 phosphorylation at serine 15 or serine 315 partially restores the function of wild-type p53, Biochem. Biophys. Res. Commun. 261 (2) (1999) 256–263, https://doi.org/10.1006/bbrc.1999.1019.
[72] V. Melnikova, A.B. Santamaria, S.V. Bolshakov, H.N. Ananthaswamy, Mutant p53 is constitutively phosphorylated at serine 15 in UV-induced mouse skin tumors: involvement of ERK1/2 MAP kinase, Oncogene. 22 (38) (2003) 5958–5966, https://doi.org/10.1038/sj.onc.1206595.
[73] J. Hu, J. Cao, W. Topatana, et al., Targeting mutant p53 for cancer therapy: direct and indirect strategies, J. Hematol. Oncol. 14 (1) (2021) 157, https://doi.org/10.1186/s13045-021-01169-0.