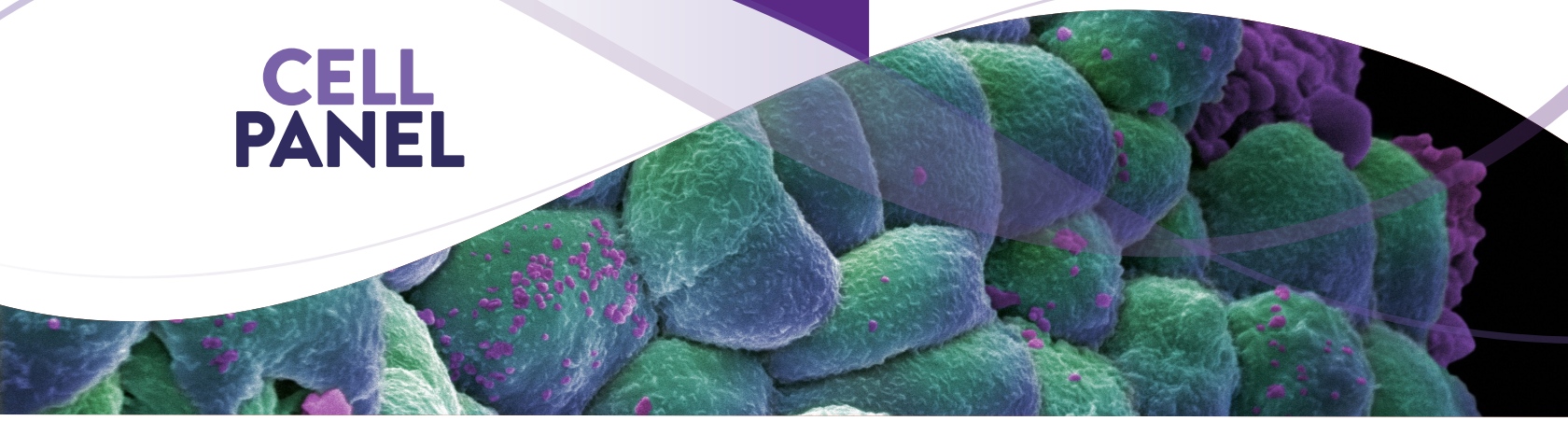
ATCC

CELL PANEL



BREAST CANCER P53 HOTSPOT MUTATION CELL PANEL

p53 is a tumor suppressor protein encoded by the TP53 gene that responds to DNA damage by regulating cell-cycle arrest, apoptosis, and senescence. At least 50 % of human tumors contain mutations or deletions of the TP53 gene. The Breast Cancer p53 Hotspot Mutation Cell Panel (ATCC® TCP-2010™) comprises 8 select cell lines derived from breast cancer that have been sequenced and validated by ATCC. This panel includes WT p53 cell lines as well as cultures with p53hotspot mutations at codons 175, 248, 249, or 273. The panel is useful for anti-cancer drug targeting or reactivation of mutant p53, as well as studies related to p53 molecular mechanisms.

ATCC® No.	Name	Tissue	Histology	Tumor Source	TP5status	Zygosity	CDS mutation	AA mutation
HTB-25™	MDA-MB-175-VII	breast	ductal carcinoma	metastasis (pleural effusion)	WT			
HTB-27™	MDA-MB-361	breast	adenocarcinoma	metastasis (brain)	WT			
CRL-2351™	AU565	breast	adenocarcinoma	metastasis (pleural effusion)	MUT	homozygous	c.524G>A	p.R175H
HTB-30™	SK-BR-3	breast	adenocarcinoma	metastasis (pleural effusion)	MUT	homozygous	c.524G>A	p.R175H
CRL-2315™	HCC70	breast	ductal carcinoma	primary	MUT	homozygous	c.743G>A	p.R248Q
HTB-122™	BT-549	breast	ductal carcinoma	primary	MUT	homozygous	c.747G>C	p.R249S
CRL-2314™	HCC38	breast	ductal carcinoma	primary	MUT	homozygous	c.818G>T	p.R273L
HTB-132™	MDA-MB-468	breast	adenocarcinoma	metastasis (pleural effusion)	MUT	homozygous	c.818G>A	p.R273H

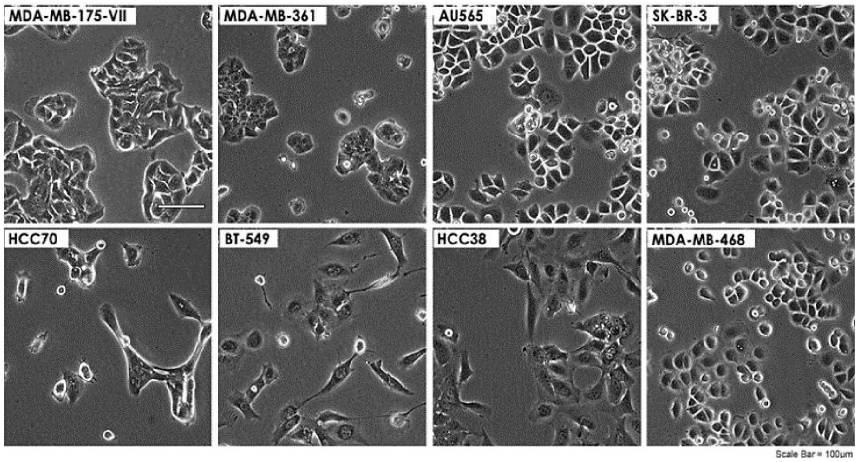


Figure 1: Cell morphology of eight cell lines in the Breast Cancer p53 Hotspot Mutation Cell Panel. Two p53 wild-type breast cancer cell lines, MDA-MB-175-VII and MDA-MB-361, and six p53 hotspot mutation breast cancer cell lines, AU565, SK-BR-3, HCC70, BT-549, HCC38 and MDA-MB-468, were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon'" microscopy, and images of the indicated cell lines were captured by an Olympus® digital camera. Scale bar represents 100 µm.

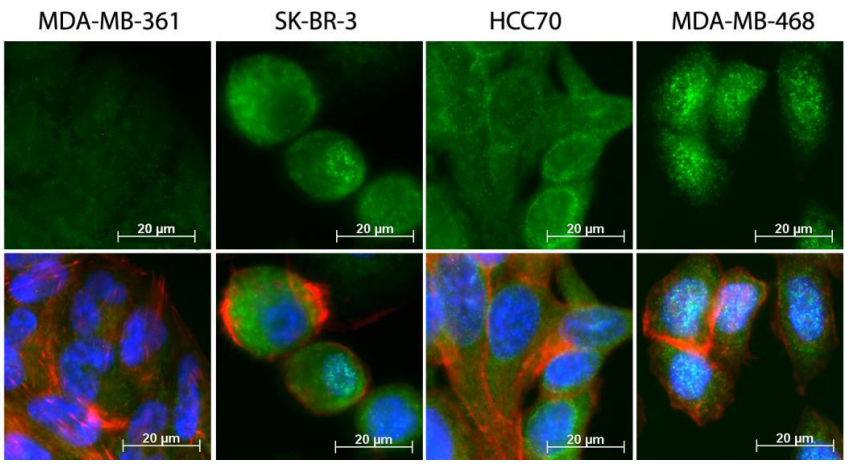


Figure 2: Immunofluorescence staining of p53. The indicated p53 wild-type and p53 mutation cells were grown on collagen-coated coverslips. Cells were fixed with 4% paraformaldehyde. p53 was stained with p53 primary antibody and Alexa Fluor 488 secondary antibody (green). F-actin was visualized with phalloidin Alexa Fluor 594 (red). Nuclei of the cells were visualized with Hoechst 33342 (blue). Single florescence channel images of p53 staining are shown in the upper row, and multichannel merged images are shown in the bottom row.

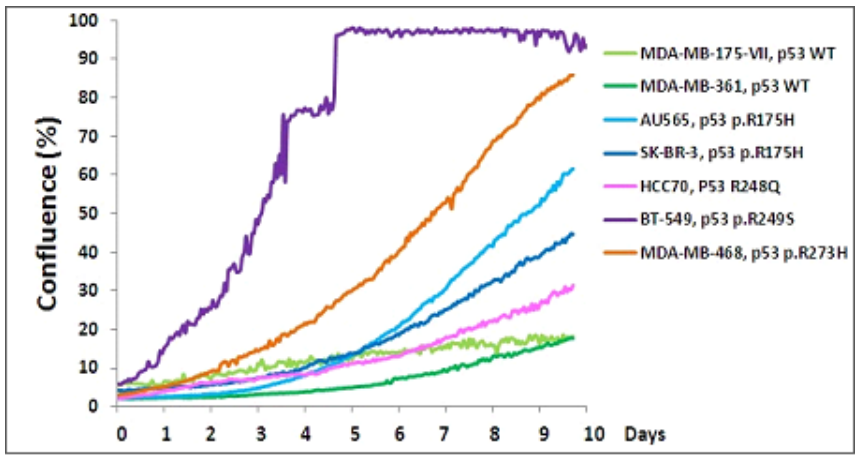


Figure 3: Cell growth kinetics. The indicated p53 mutation cells were cultured in ATCC recommended media, and plated at 3000 cells/well in 96-well plates. Cell growth kinetics were constantly monitored for 10 days using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).

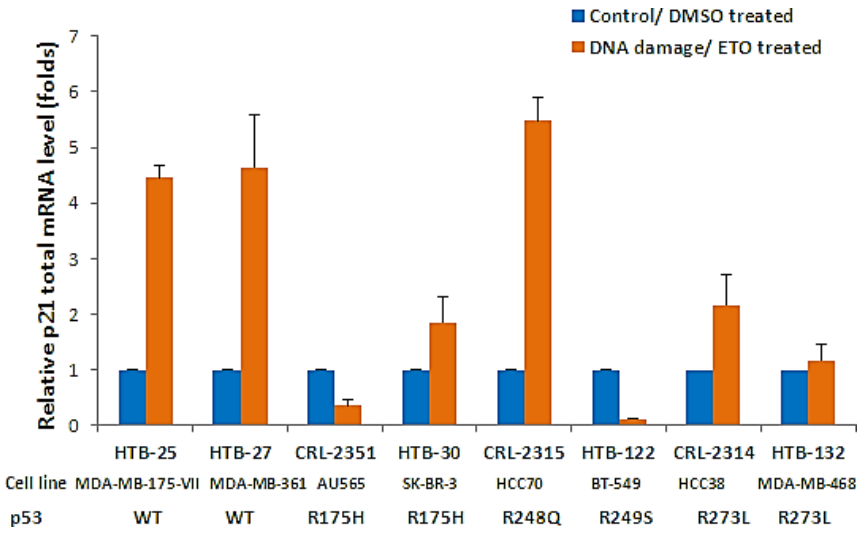


Figure 4: Real-time PCR analysis of total mRNA levels of p21, a downstream target of p53, in the indicated p53 wild-type and p53 mutation cell lines. Cells were treated with 20 µm etoposide (ETO) for 6 hours to induce DNA damage, or treated with DMSO as a control. Total mRNA levels of p21 and 36B4 were determined by real time quantitative PCR. Relative p21 total mRNA changes were normalized to the housekeeping gene 36B4.

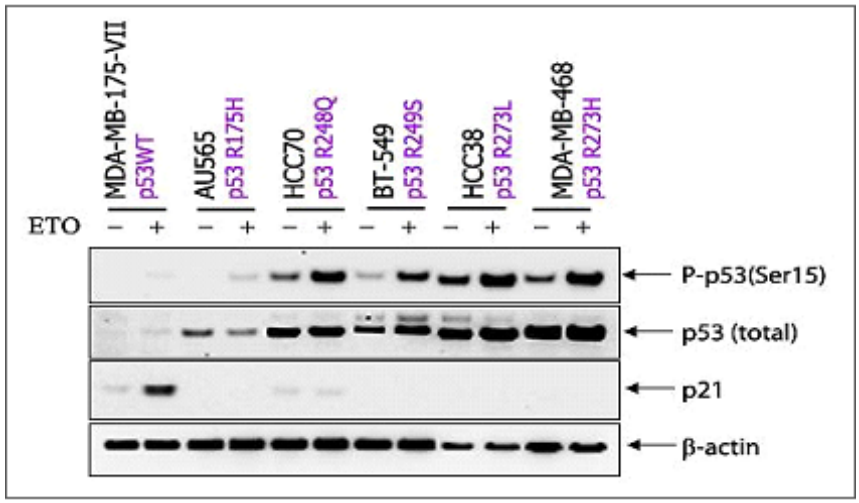


Figure 5: The indicated p53 wild-card and p53 mutation cells were treated with 20 µM etoposide (ETO) for 8 hours to induce DNA damage, or treated with DMSO as a control. Western blotting assay was used to examine phosphorylation of p53 at Serine 15, total protein express of p53, and expression of p21, a downstream target of p53. β-actin protein was also examined as a control.

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