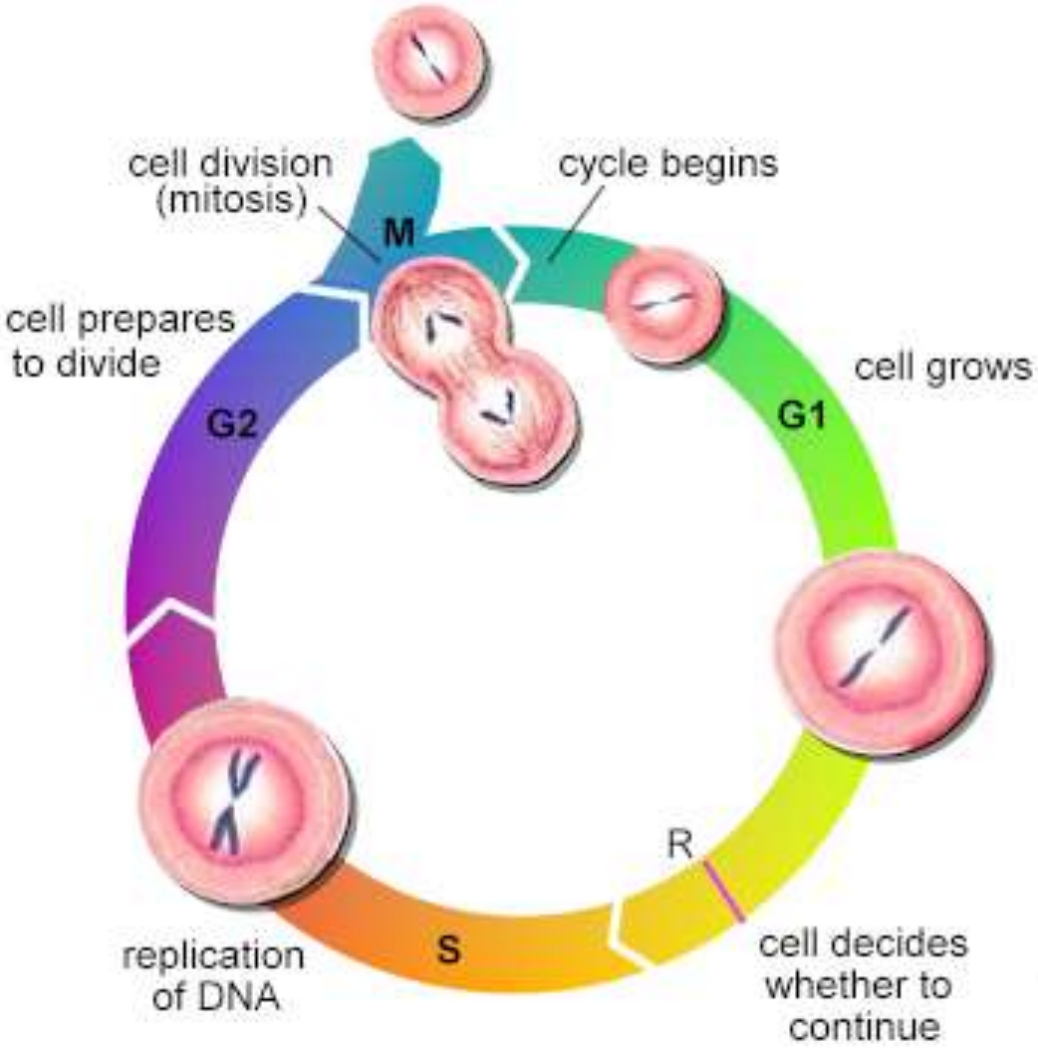
Selective killing of cancer cells by a small molecule targeting the stress response to ROS

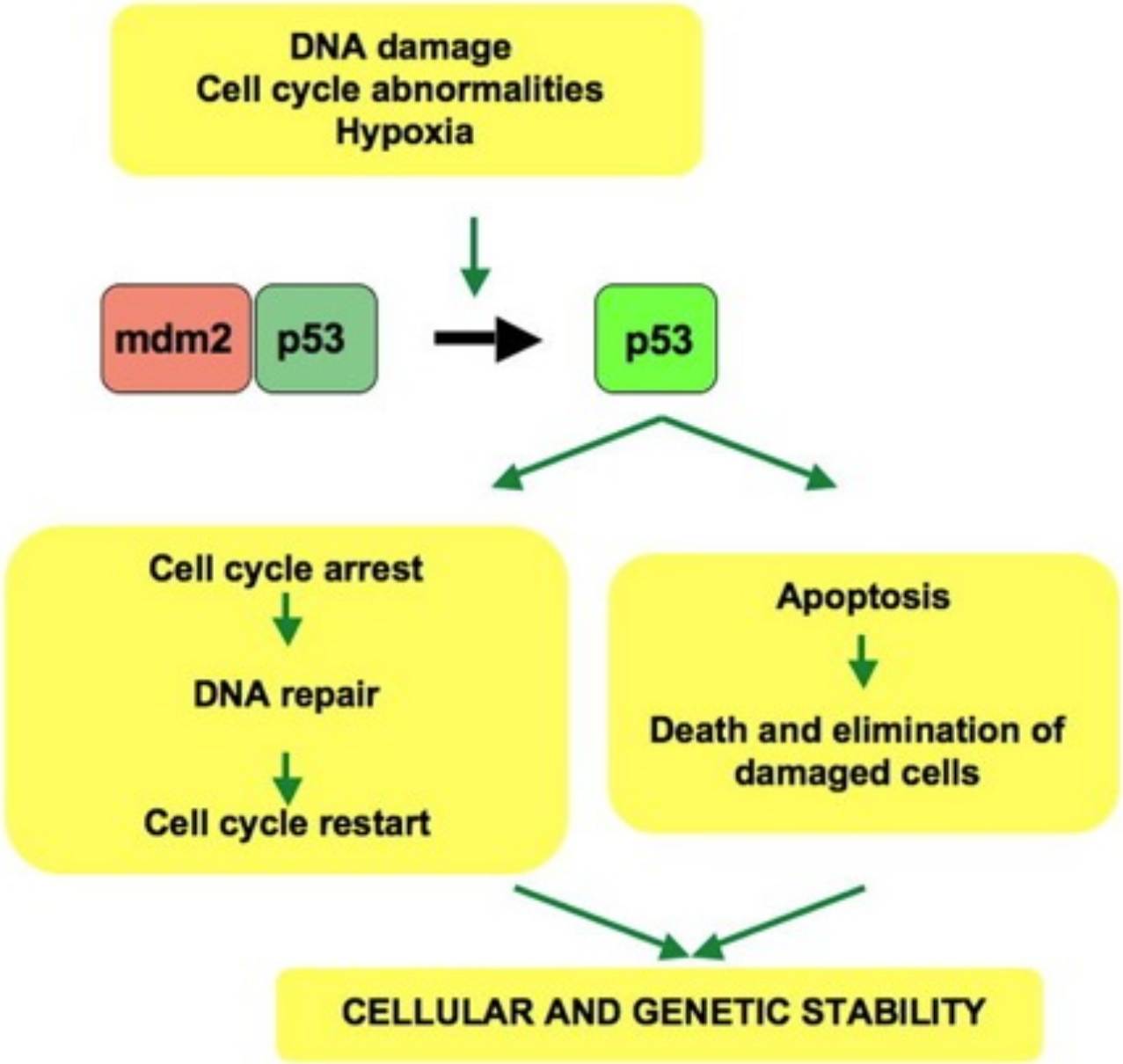
L Raj *et al*. *Nature* **475**, 231-234 (2011) doi:10.1038/nature10167

Current literature
Presented by Zhuzhu WANG
9/3/11

The cell cylce



→ G1/S checkpoint by p53

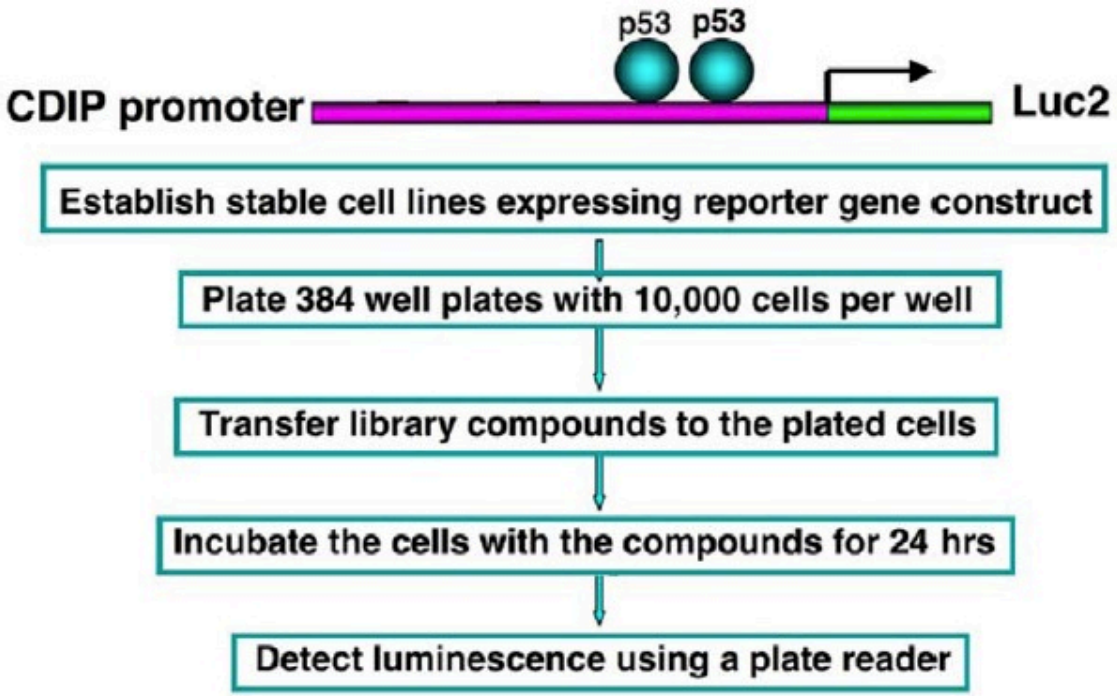

DNA damage
Cell cycle abnormalities
Hypoxia
mdm2
p53
p53
Cell cycle arrest
DNA repair
Cell cycle restart
Apoptosis
Death and elimination of damaged cells
CELLULAR AND GENETIC STABILITY

Wikipedia

3

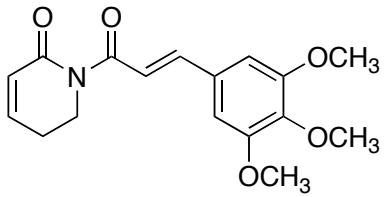
➢ CDIP (Cell Death Involved p53-target) is an important p53 apoptotic effector.

The EMBO journal (2007) 26, 3410-3422



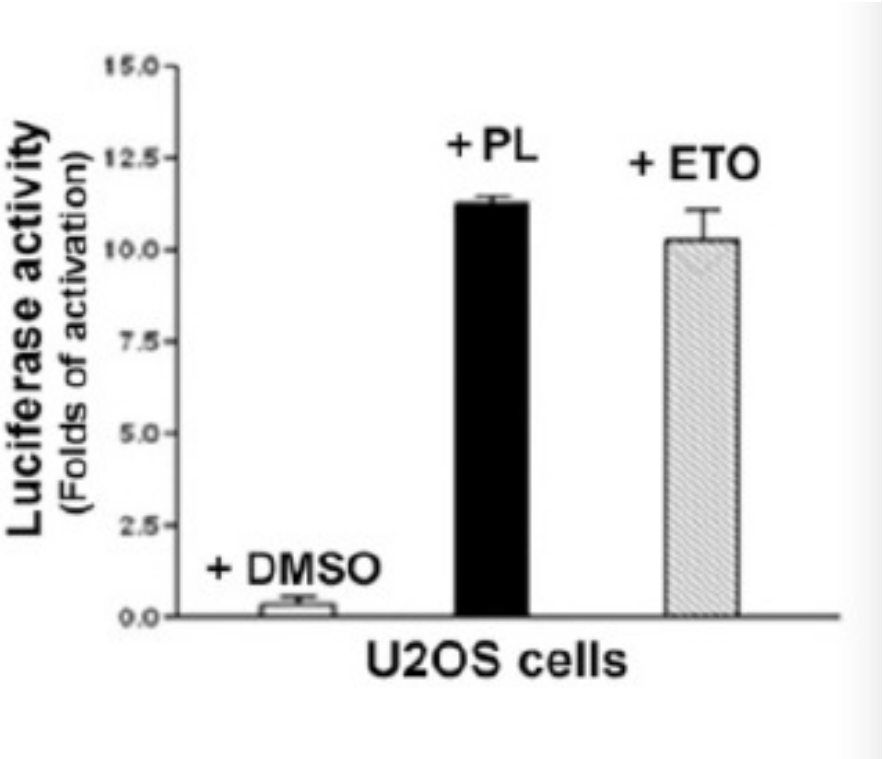
Supplementary fig 1. An overview of the screening method

- Luciferase Reporter vectors: Human CDIP promoter carrying p53-responsive elements Operatively linked to the firefly luc2 reporter gene
- U2OS: is a human osteosarcoma cell line expressing wild type p53.



Piperlongumine is isolated from the plant species Piper longum L and was shown to have cytotoxic effect.

Fig. 1a Structure of piperlongumine



Supplementary fig. 2b, PL compound (10 μM) stimulates luciferase activity of CDIP promoter containing p53-binding sites in U2OS cells. Etoposide (ETO, 25 μM) was used as a positive control.

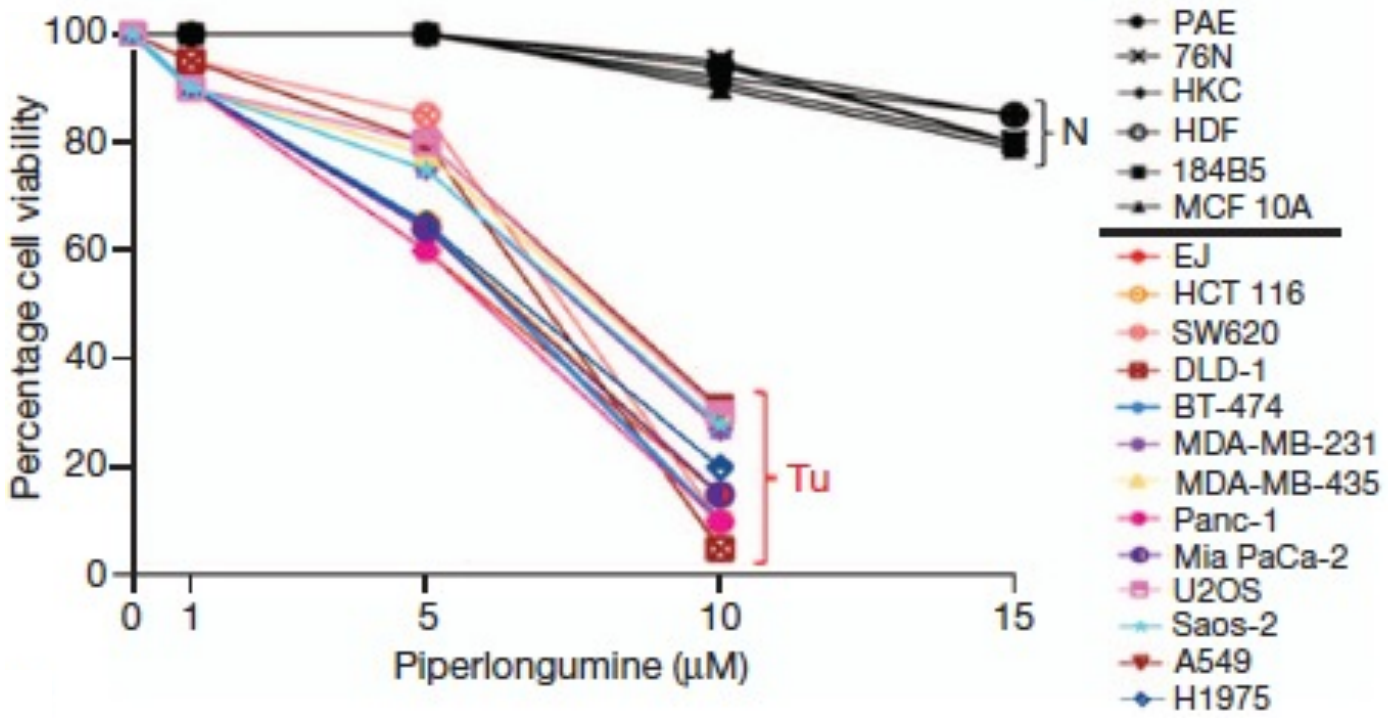


Fig. 1b, Piperlongumine treatment induces cell death in cancer cells but not in normal cells.

- Conclusion: Piperlongumine have a cancer-cell-selective killing property
- Hypothesis: Sensitivity to piperlongumine result from the process of malignant transformation ?

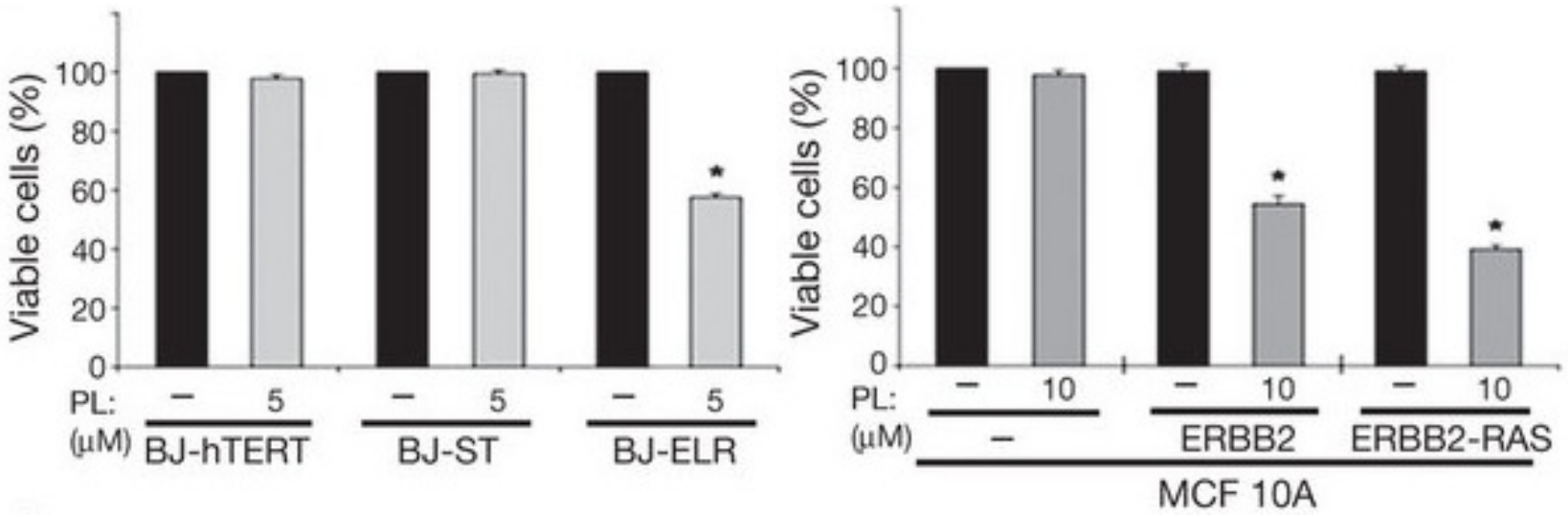


Fig. 1c, Selective cell death caused by piperlongumine (PL) in oncogenically transformed human BJ skin fibroblasts (left panel) and MCF 10A cell lines (right panel)

- Ectopic expression of the telomerase catalytic subunit (hTERT) in combination with the simian virus 40 large-T oncoprotein (ST) results in tumorigenic conversion of normal human BJ skin fibroblast cells.

Hahn, W. C. et al, Nature **400**, 464-468 (1999)

- Oncogenes Erbb2 and Ras can induce mammary tumorigenesis.

Ryo, A, et al. Mol. Cell. Biol. **22**, 5281-5295 (2002)

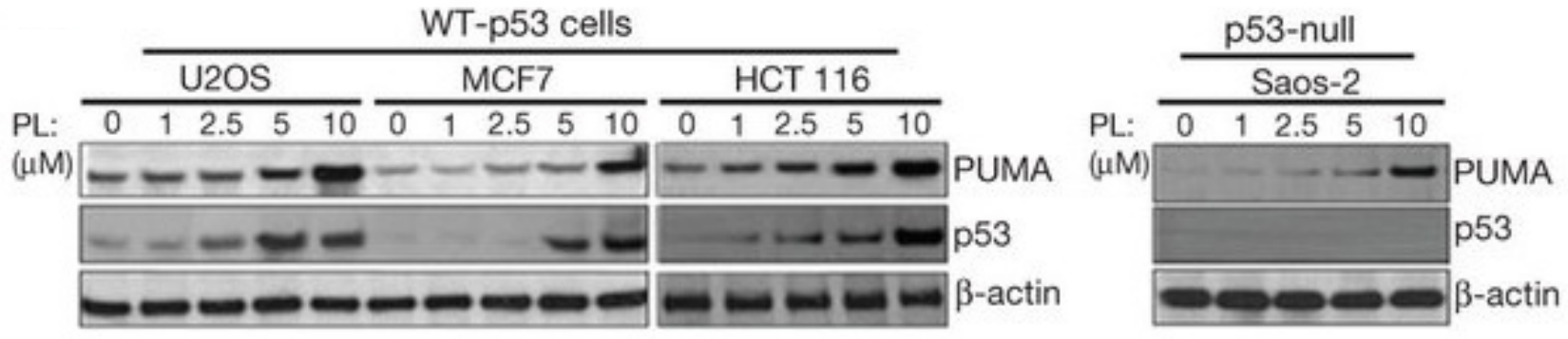


Fig. 1d, The effects of piperlongumine on p53 and its target PUMA were measured by western blot analyses in several cancer cell lines. β-actin expression was used as a loading control.

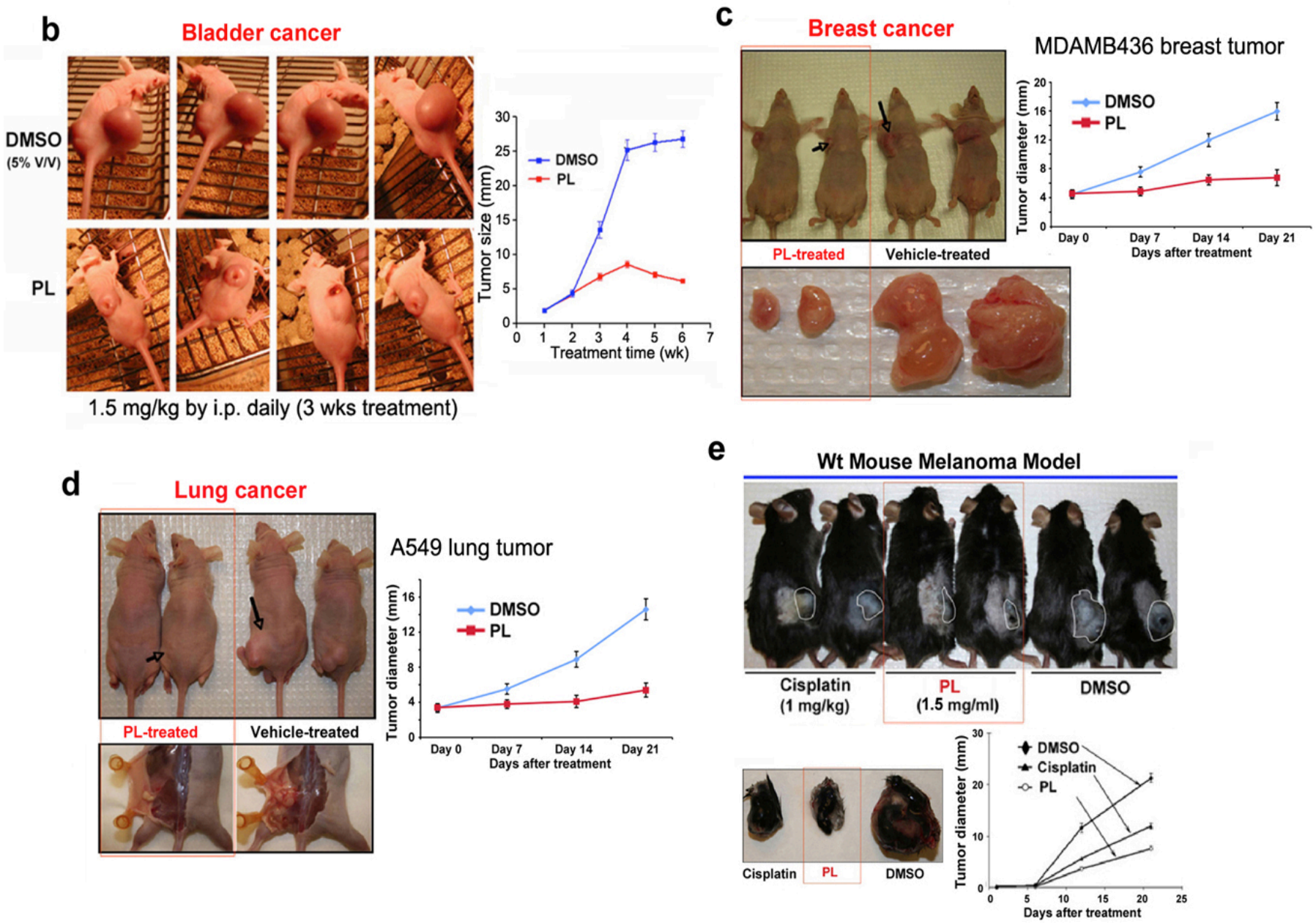
- Wild-type p53 expression was significantly enhanced.
- The p53 proapoptotic target BCL2 binding component 3 (BBC3, also known as PUMA) was enhanced in both WT-p53 cancer cells and p53-null cancer cells.
- Apoptotic transcripts levels ↑, pro-survival transcripts levels ↓ only in cancer cells once treated with PL. (Supplemental Fig. 8)

Conclusion: Piperlongumine induces cell death or apoptosis in cancer cells by modulating the expression of members of apoptotic and survival pathways.

In vivo antitumor effect of piperlongumin in established tumor xenografts in mice

Cancer	Cell line	Treatment with PL	# animals
Bladder (Nu/Nu mice)	EJ (p53 inactivating mutation)	1.5 mg/kg i.p. injections at 3-5 mm tumor size; every day for 21 days	14
Breast (Nu/Nu mice)	MDA-MB436 (p53 inactivating mutation)		14
Lung (Nu/Nu mice)	A549 (p53 wild type)		14
Melanoma (wt-mice)	F10-B16 (p53 wild type)		14

Supplementary Fig. 9a, Tumor models used in this study



Supplementary Fig. 9bcde. Anti-tumor effect of PL in bladder cancer, breast cancer, lung cancer and melanoma xenograft mouse models

In vivo antitumor effect of piperlongumin transgenic mouse model of spontaneous breast cancer, MMTV-PyVT

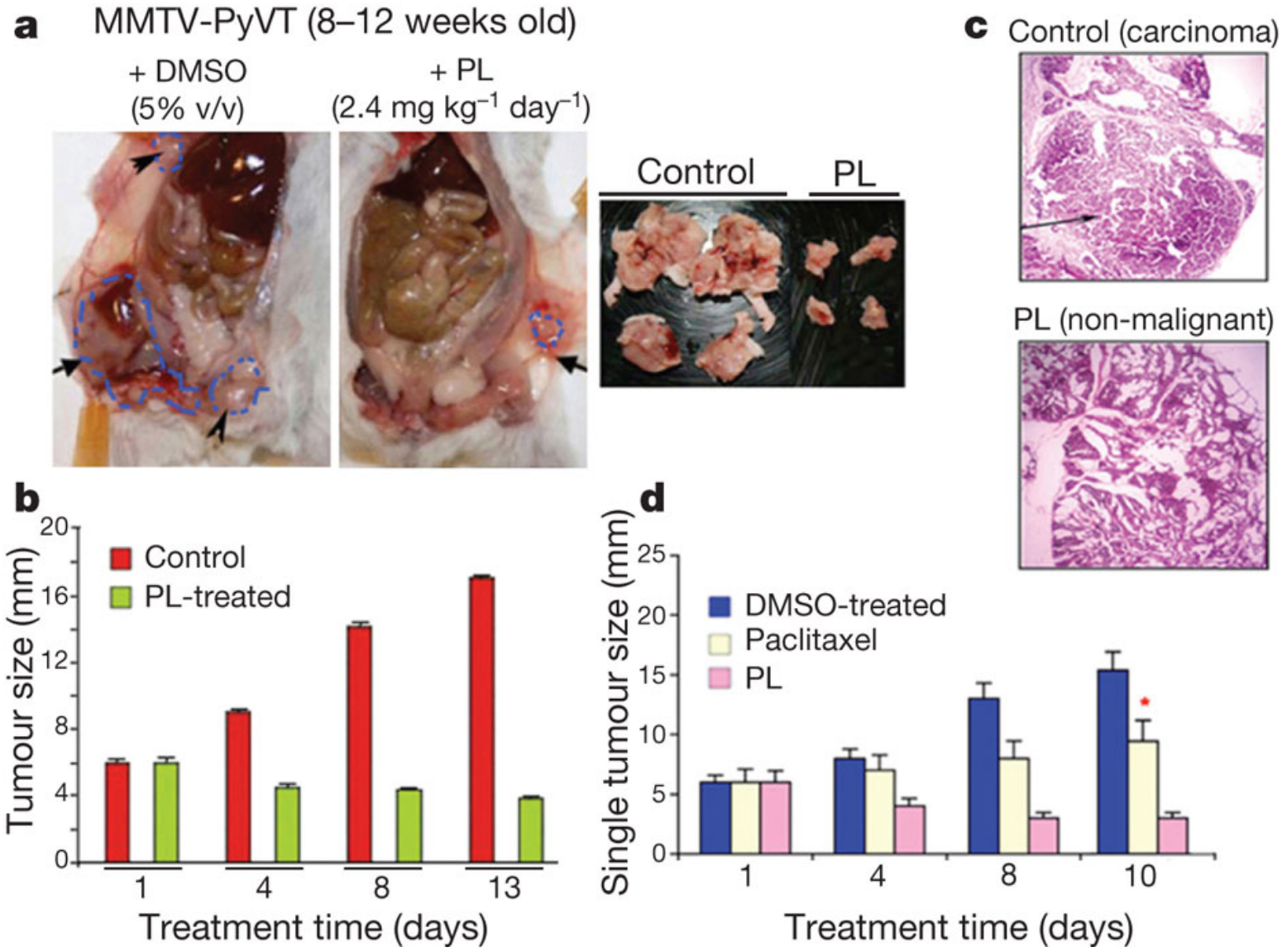
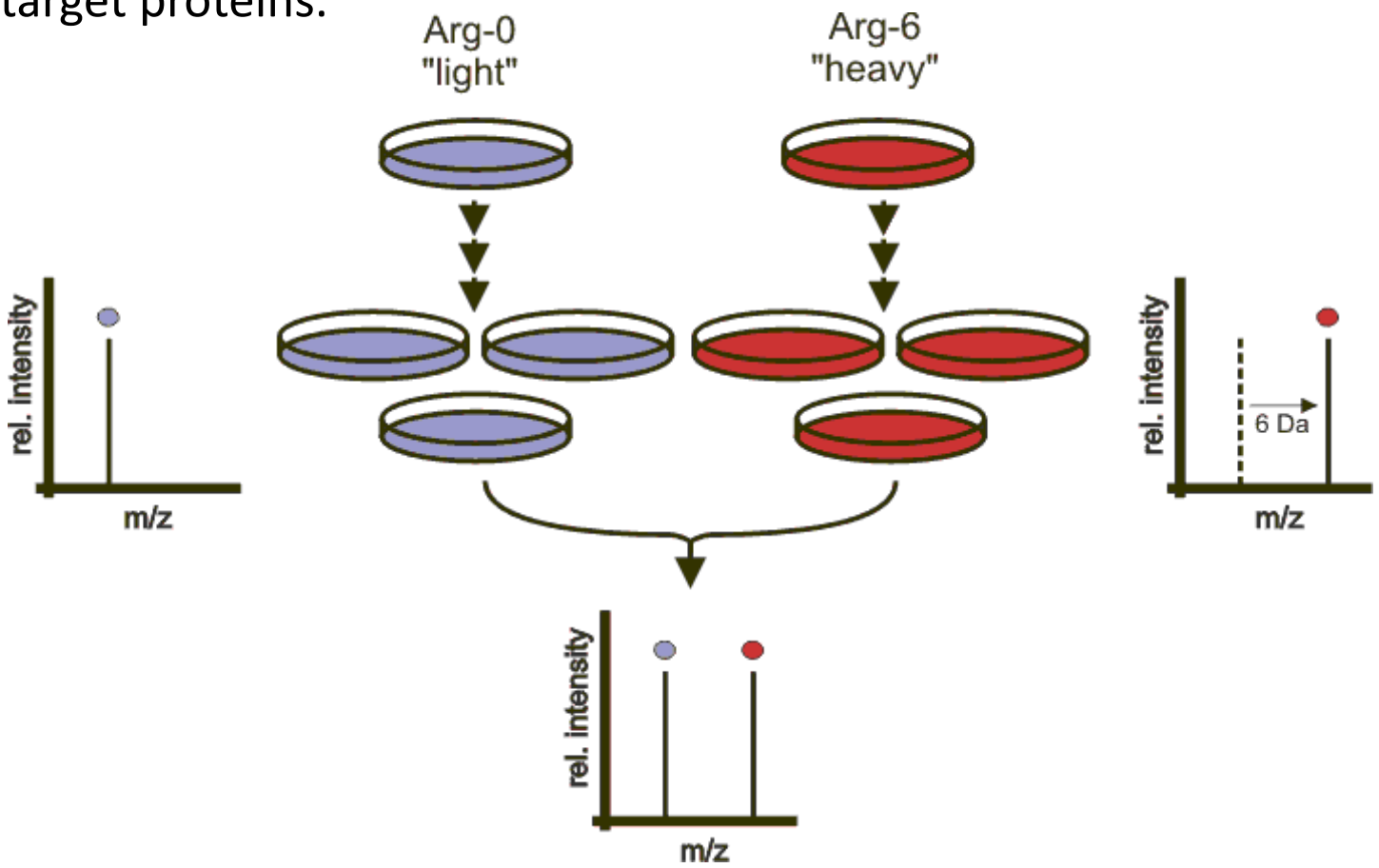


Fig. 2.

Which protein is targeted by piperlongumin ?

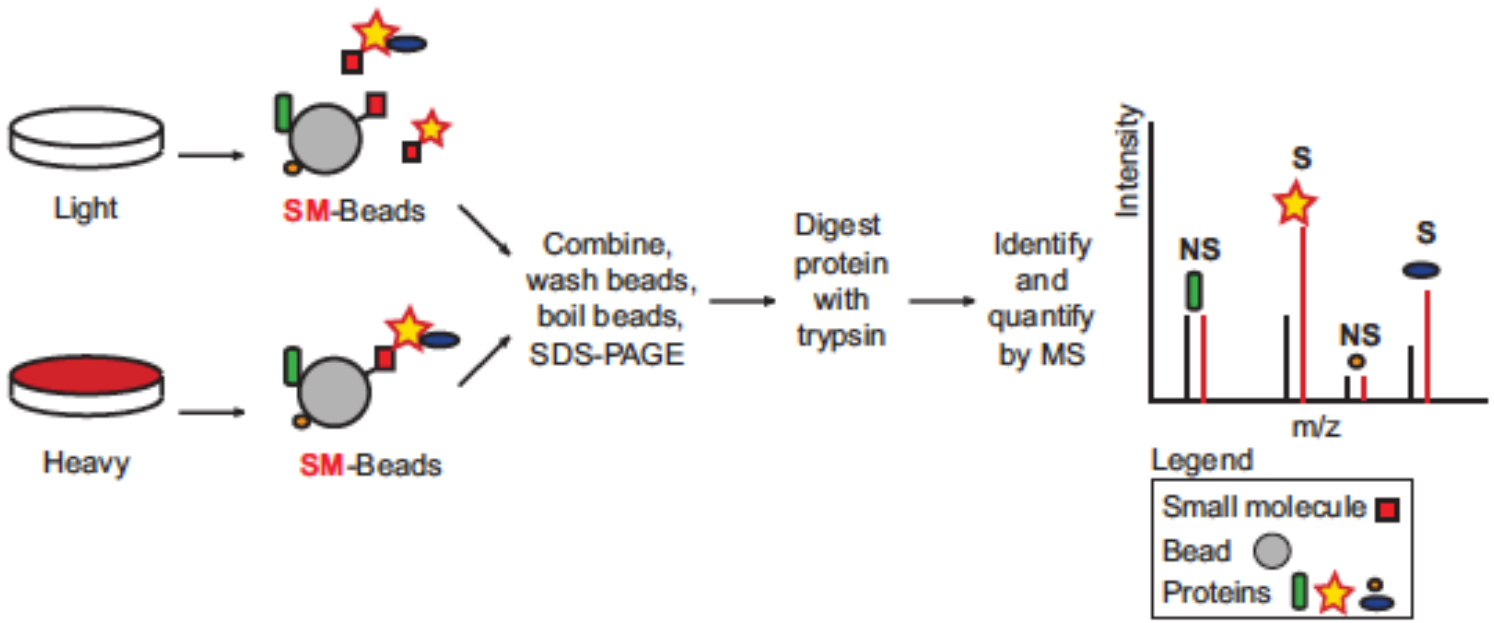
- Method: Combing affinity enrichment with stable-isotope labeling with amino acids in cell culture (SILAC) and quantitative proteomics to identify the target proteins.



The principle of SILAC. Cells are differentially labeled by growing them in light medium with normal arginine (Arg-0, blue colour) or medium with heavy arginine (Arg-6, red colour).

Wikipedia

SILAC identifies specific protein interactions with SM baits



Ong, S.E. et al. Proc. Natl Acad. Sci. USA **106**, 4617-4622 (2009)

- Result: Glutathione S-transferase pi 1 (GSTP1) and carbonyl reductase 1 (CBR1). (Supplementary Fig. 16b)
- GSTP1 and CBR1 are proteins known to regulate oxidative stress.
- Hypothesis: Piperlongumine modulate redox and ROS homeostasis ?

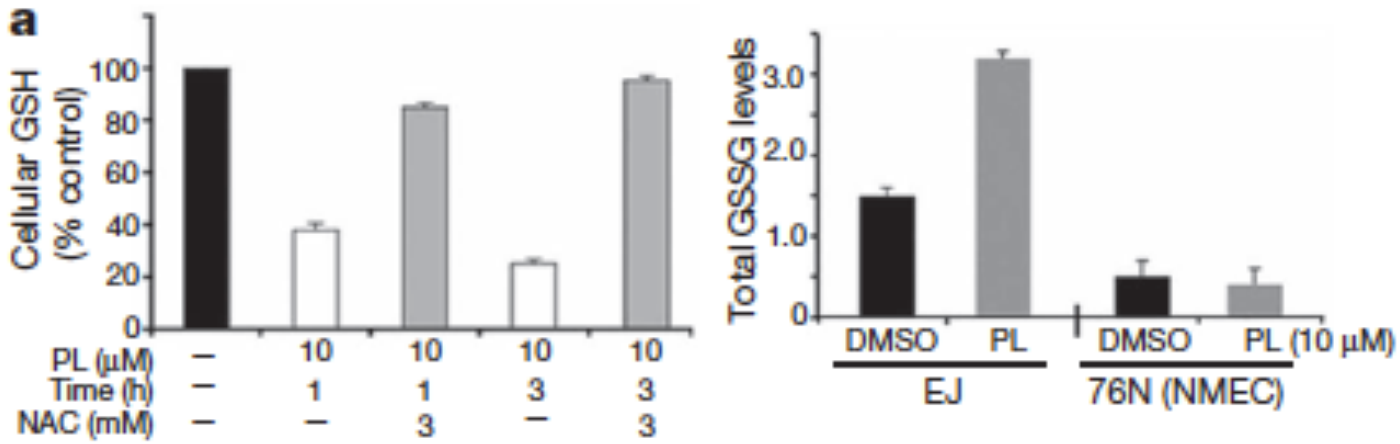
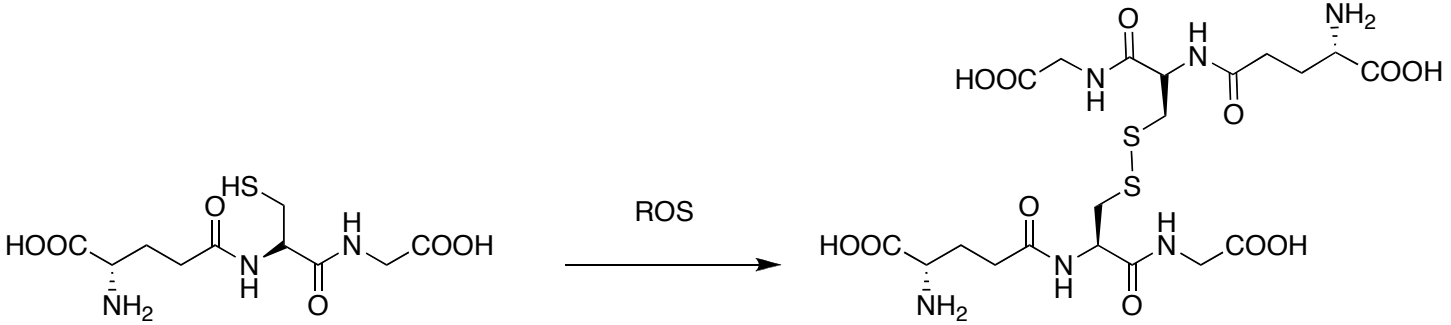


Fig. 3a, Piperlongumine-mediated modulation of GSH and GSSG. GSH levels were determined after EJ cells were either treated with piperlongumine or pretreated withNAC for 1 h, followed by piperlongumine treatment for 1 h or 3 h (left panel). GSSG levels were also determined after EJ cells and 76N (NMEC) cells were treated with piperlongumine for 3 h (right panel)

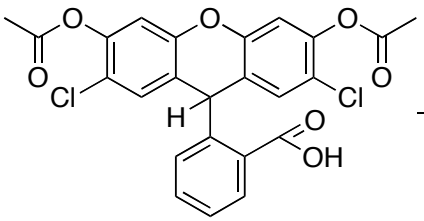


Glutathione (GSH)

Glutathione disulfide (GSSH)

- ↑ (GSSH/GSH): indicative of oxidative stress.
- NAC: N-acetyl-L-cysteine, bioavailable precursor of glutathione.

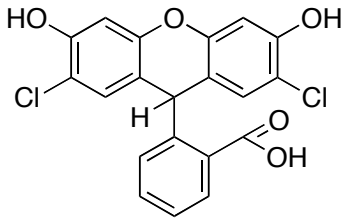
Identification of ROS using Oxidized DCF and Flow-cytometry



Uptake by cells

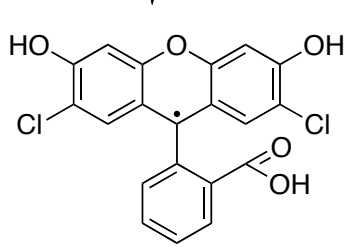
De-acetylation by esterases

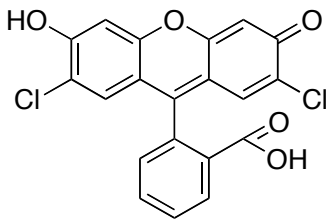
2', 7' -Dichlorodihydrofluorescein diacetate
DCFH-DA



2', 7' -Dichlorodihydrofluorescein

ROS





2',7' -Dichlorofluorescein (DCF)

$\lambda_{exitation}$= 485 nm

$\lambda_{emission}$ = 530 nm

Eruslanov E, Kusmartsev S., Methods Mol Biol. 2010;594:57-72.

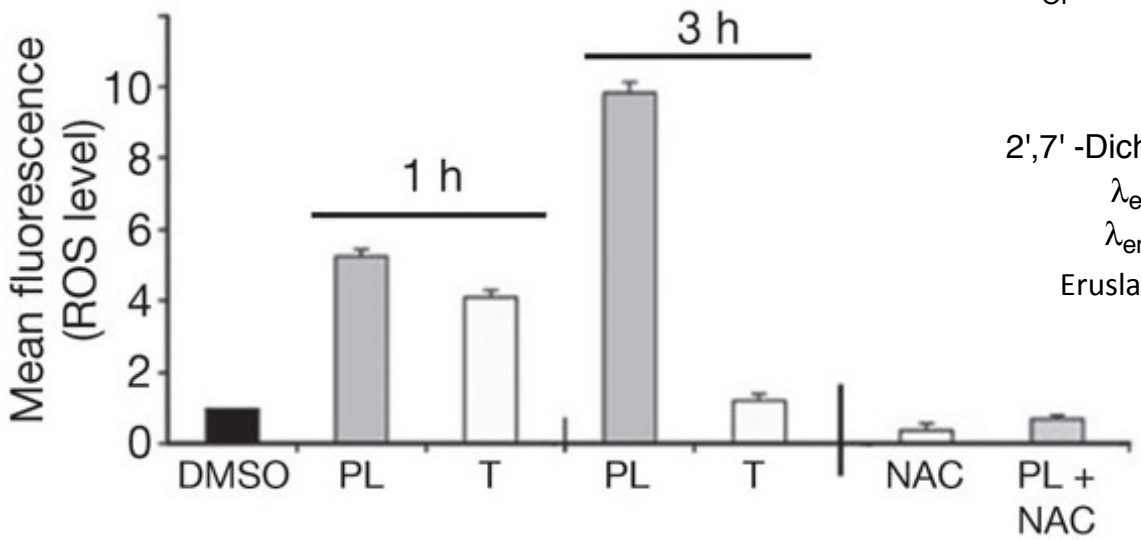


Fig. 3b, Piperlongumine-induced ROS elevation and reversion by NAC. EJ cells were treated with piperlongumine (PL, 10 µM), paclitaxel (T, 25 nM) or DMSO for 1 h and 3 h. Cells were also pretreated with 3 mM NAC for 1 h, followed by 10 µM piperlongumine for 3 h.

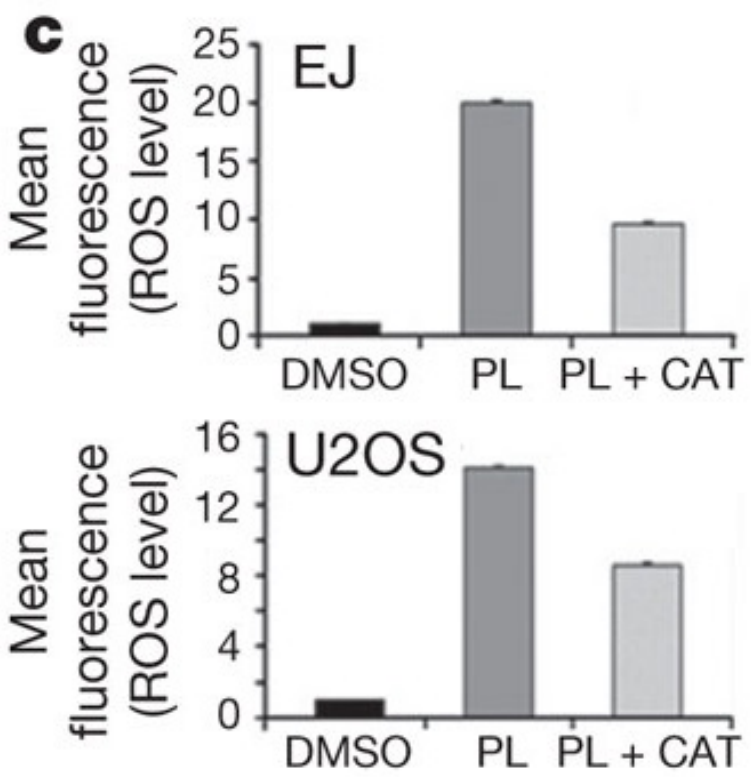


Fig. 3c. Reversion of piperlongumine-induced ROS accumulation by catalase. EJ or U2OS cells were pretreated with catalase (CAT, 2,000 U ml^{-1}) for 2 h, followed by 10 µM piperlongumine for 3 h.

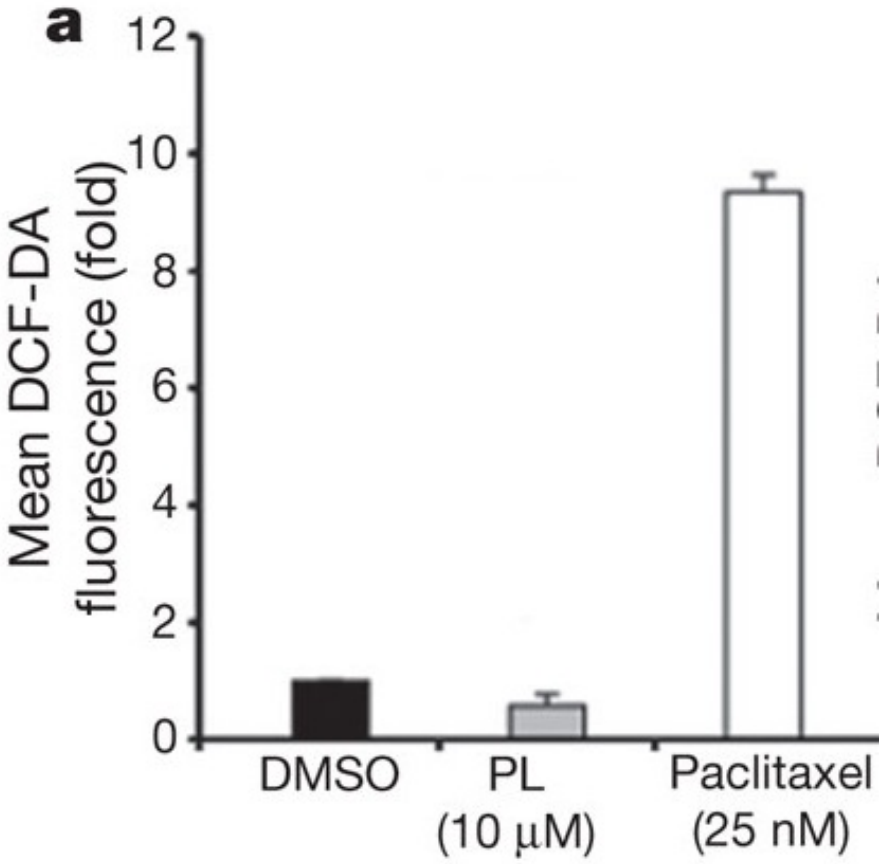


Fig. 4a. Piperlongumine does not increase ROS levels in normal cells (16N). ROS levels were measured by flow cytometry and shown by quantitative bar graph measured as the fold change over DMSO-treated levels

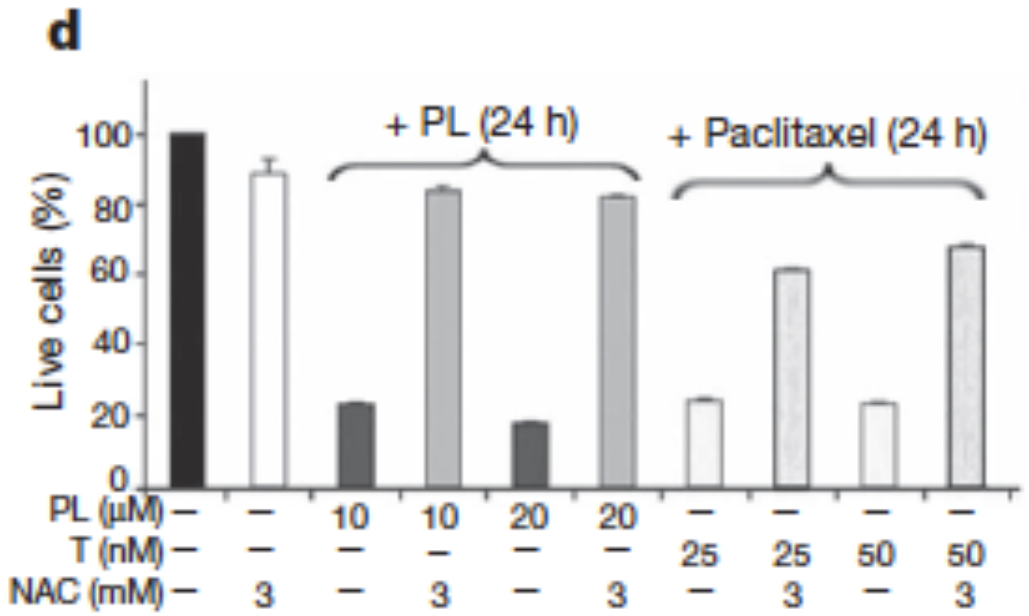


Fig. 3d, Piperlongumine-induced cell death can be rescued by NAC on EJ cells

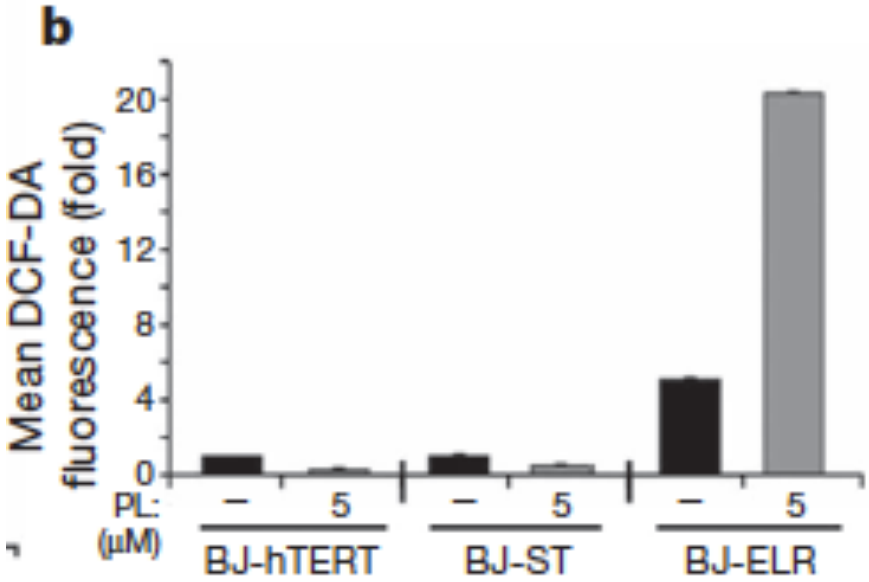
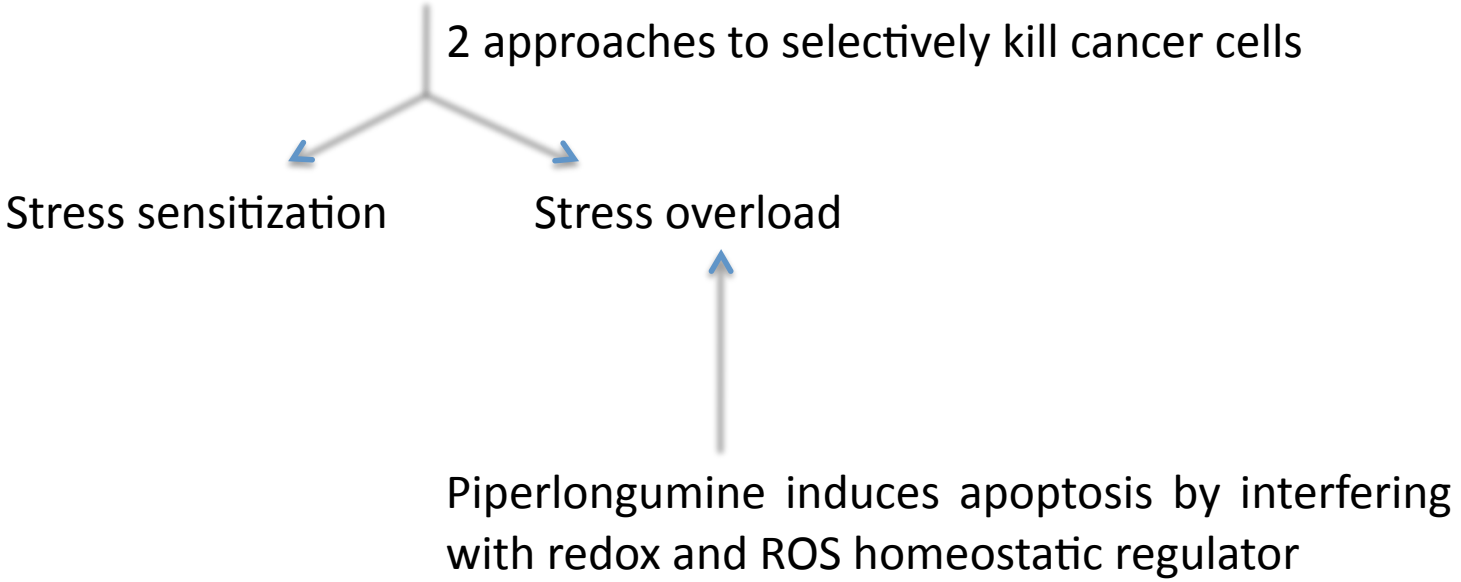


Fig. 4b, Selective induction of ROS by piperlongumine in oncogenically transformed BJ human fibroblasts (BJ-ELR), but not in non-transformed BJ fibroblasts (BJ-hTERT and BJ-ST)

Conclusion

- Normal cells: low levels of ROS

  Cancer cells: high levels of ROS



- Novel strategy for cancer therapy: targeting the ROS stress-response pathway
- Possible future extension: Chemical modification of this small molecule?