Investigating the role of p53 in caspase-2-mediated cell cycle regulation and apoptosis in the prevention of genomic instability.

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Introduction

The protease caspase-2 and the transcription factor p53 are both involved in the induction of apoptosis and cell cycle arrest and are both classified as tumor suppressors. Despite this, unlike p53, the mechanisms underlying caspase-2 activation and function remain poorly understood.

Gap in knowledge: While studies have shown that both caspase-2 and p53 can activate each other, what determines what protein is upstream and in what conditions caspase-2 can function in a p53-independent manner are not well understood

Hypothesis: p53 determines how caspase-2 is activated in response to arrest resulting in apoptosis or cell cycle regulation to protect against accumulation of DNA damage.

Cell lines with different p53 status activate caspase-2 at different levels according to the stage of the cell cycle

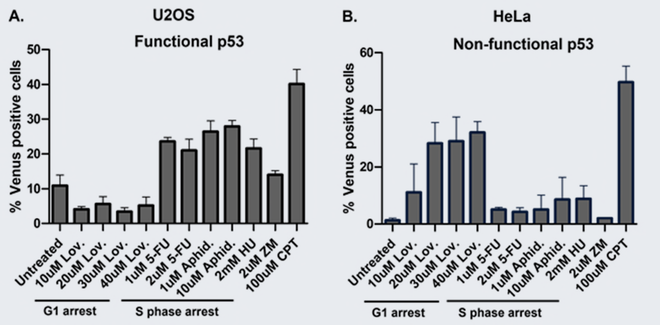

Figure 1 A. HeLa cells B. U2OS cells stably expressing C2 Pro-VC-2A-C2 Pro-VN-2AmCherry treated with lovastatin (Lov), 5-fluorouracil (5-FU), aphidicolin (Aphid), hydroxyurea (HU), camptothecin (CPT), and ZM447439 (ZM). After 24 hours, the percentage of Venus positive cells was determined for at least 30 images per treatment. The results are the mean of three independent experiments.

Caspase-2 and p53-deficient cells bypass a cell cycle checkpoint

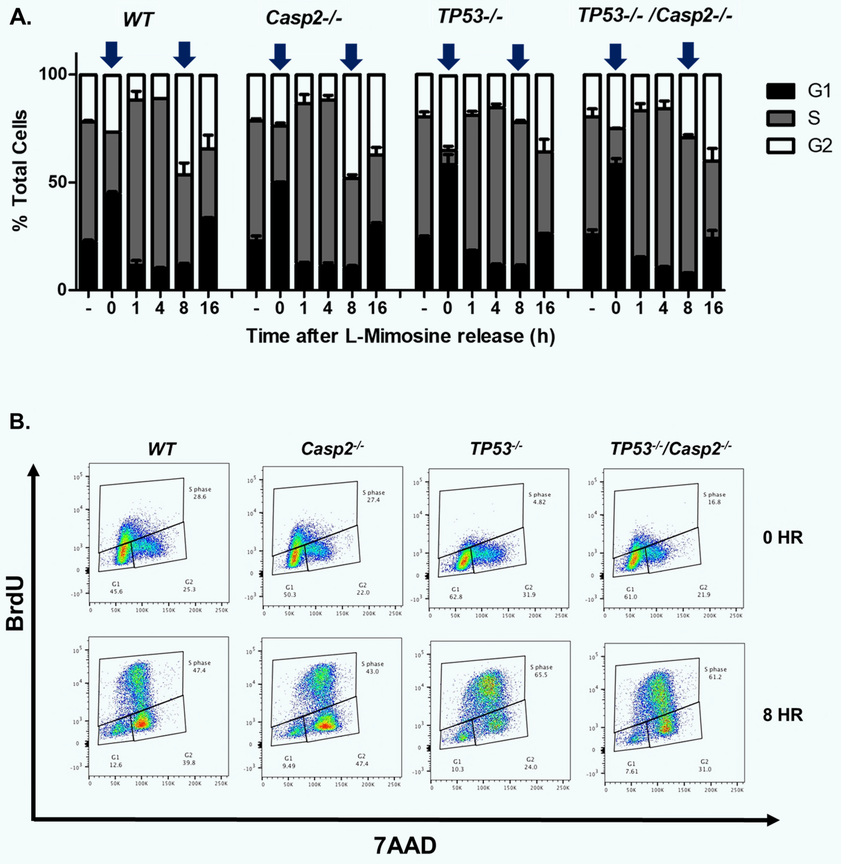

Figure 2 A. Wild-type, Casp2-/- , Tp53-/-, and Casp2-/- /Tp53-/- HCT116 cells were untreated (-) or treated with the 0.5 mM L-mimosine. After 22 hours, the L-mimosine treatment was washed out and fresh medium was added. At various times after L-mimosine release, the cells were pulsed for 30 minutes with 10μM BrdU and stained with anti-BrdU-FITC/7-AAD. The cell cycle profile was then determined by flow cytometry. B. Representative flow plots of the 0HR, and 8HR timepoints from A.

Loss of caspase-2, and p53 impairs activation of the DNA damage response

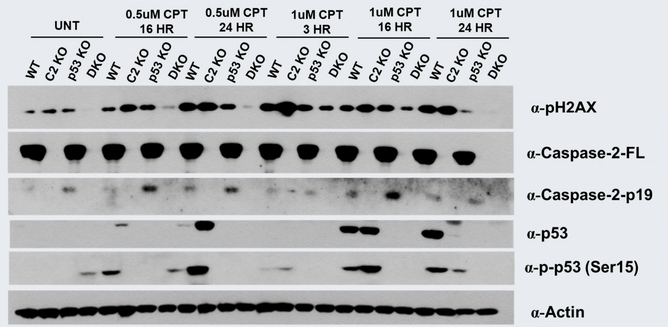

Figure 3. HCT116, HCT116 Casp2-/- (C2 KO), HCT116 TP53 -/- (p53 KO), and HCT116 Casp2-/-/TP53 -/- (DKO) were left untreated or treated with 0.5uM or 1uM camptothecin (CPT) for 3 hours followed by replacement with fresh media. Cells were harvested 3 hours, 16 hours, or 24 hours following the removal of CPT.

Conclusions

- p53 might be regulating the activation of caspase-2 according to the cell cycle
- Caspase-2, and p53 might be working in independent pathways to regulate the DNA damage response, which are activated to compensate for the absence of each other

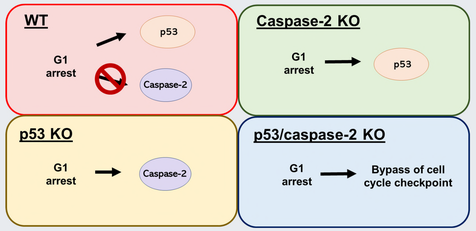

Figure 4. Summary of caspase-2, and p53 activation following G1 arrest