Impact of p53 Truncation Mutants and Cyclophilin D Interactions on Three Types of Cancer

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p53 TRUNCATION MUTANTS

- *TP53* encodes the tumor suppressor p53 that keeps the cell cycle in check, and *TP53* mutations are extremely common in cancers
- Most mutations result in a loss of p53's tumor suppressor function
- However, there is a novel class of truncated p53 mutants, e.g., p53 R213* and R196*, that are gain-of-function and promote proliferation and migration

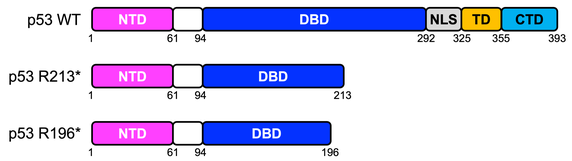

Figure 1. Structure of p53 and its truncation mutants. Full length, wildtype p53 (WT) contains an N-terminal domain (NTD), DNA binding domain (DBD), nuclear localization signal (NLS), tetramerization domain (TD), and C-terminal domain (CTD). The cancer-associated mutants R213* and R196* are truncated in the DBD and lack the NLS.

p53 MUTANTS AND CYCLOPHILIN-D

- While normal p53 translocates to the nucleus, p53 R213* and R196* translocate to mitochondria and interact with a protein called cyclophilin-D (CypD)
- CypD plays a role in mitochondrial metabolism and ATP production, and has been proposed to mediate the actions of the p53 truncation mutants
- However, whether CypD plays a role in the pro-tumor effects of these novel p53 mutants is unclear

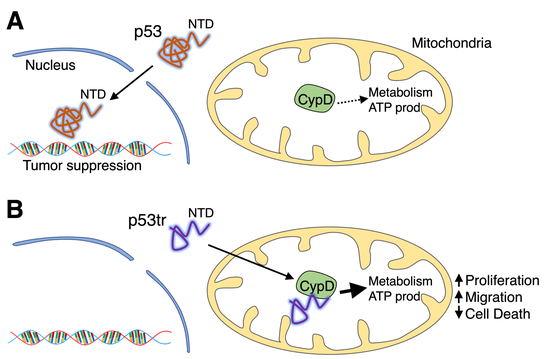

Figure 2. The proposed CypD-dependent mechanism for the pro-tumor effects of p53 truncation mutants. **A.** Normal full length p53 localizes to the nucleus to upregulate tumor suppressive anti-proliferative and pro-apoptotic genes. **B.** However, p53 truncation (p53tr) mutants instead localize to the mitochondria where they bind to the matrix protein CypD to upregulate mitochondrial metabolism and ATP production. This in turn promotes cell proliferation, migration, and cell survival.

HYPOTHESIS

- Expression of both p53 R213* and R196* mutants will increase expression of mitochondrial proteins, proliferation, and ATP, and reduce cell death

OBJECTIVE

- Examine whether CypD contributes to the mitochondrial and proliferative effects of truncated p53 proteins in three different kinds of cancers

METHODS

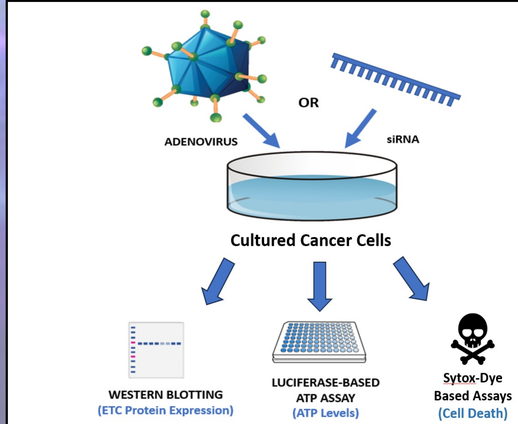

Figure 3. Schematic of methods. Human cancer cells (HCT116-colon, LNCAP-prostate, and MCF7-breast) were infected with wildtype (WT) p53, R213* and R196* expressing adenoviruses for 24-48hrs. A β-galactosidase adenovirus was used as a control. Western blotting, luciferase-based ATP assays, and Sytox exclusion dye were used to measure phospho-histone H3 and mitochondrial protein levels, ATP levels, and cell death, respectively.

p53TR EXPRESSION IN COLON, PROSTATE, & BREAST CANCER CELLS

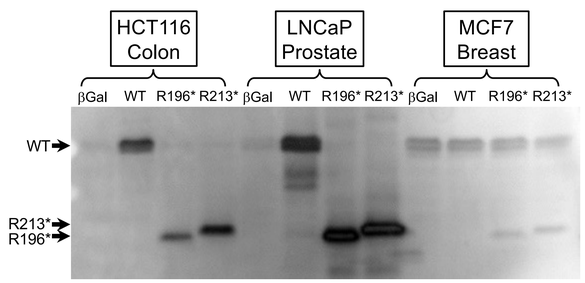

Figure 4. Expression of wildtype p53 and the R213* and R196* truncation mutants in colon, prostate, and breast cancer cells. HCT116-Colon, LNCaP-Prostate, and MCF7-Breast cells were infected with β-galactosidase (βGal), p53 wildtype (WT), R213*, and R196* expressing adenoviruses for 24hrs. Cell lysates were then Western blotted for p53.

EFFECTS OF p53TR ON MITOCHONDRIAL PROTEINS

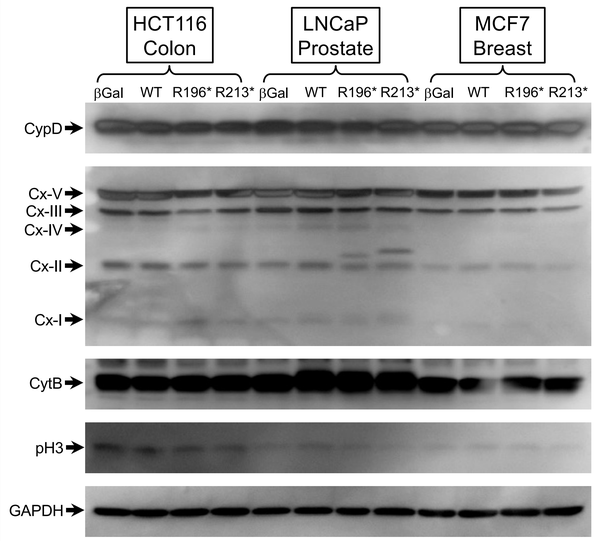

Figure 5. Effects of p53 wildtype and truncation mutants on various mitochondrial proteins and phospho-histone H3. HCT116-Colon, LNCaP-Prostate, and MCF7-Breast cells were infected with β-galactosidase (βGal), p53 wildtype (WT), R213*, and R196* expressing adenoviruses for 24hrs. Cell lysates were then Western blotted for CypD, OXPHOS complexes (Cx), Cytochrome-B (CytB), and Phospho-Histone H3 (pH3). Glyceraldehyde-6-Phosphate Dehydrogenase (GAPDH) was used as a loading control.

EFFECTS OF p53TR ON ATP LEVELS

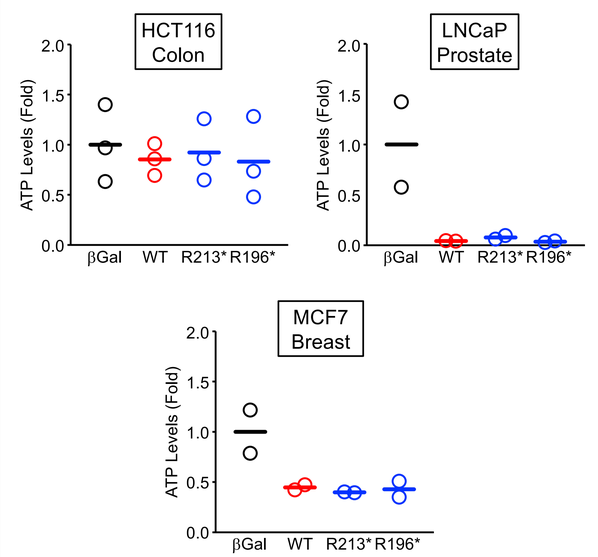

Figure 6. Effects of p53 wildtype and truncation mutants on cellular ATP levels. HCT116-Colon, LNCaP-Prostate, and MCF7-Breast cells were infected with β-galactosidase (βGal), p53 wildtype (WT), R213*, and R196* expressing adenoviruses for 24hrs. Cells were then harvested, lysed, and ATP measured using a luciferase-based assay.

EFFECTS OF p53TR ON CELL DEATH

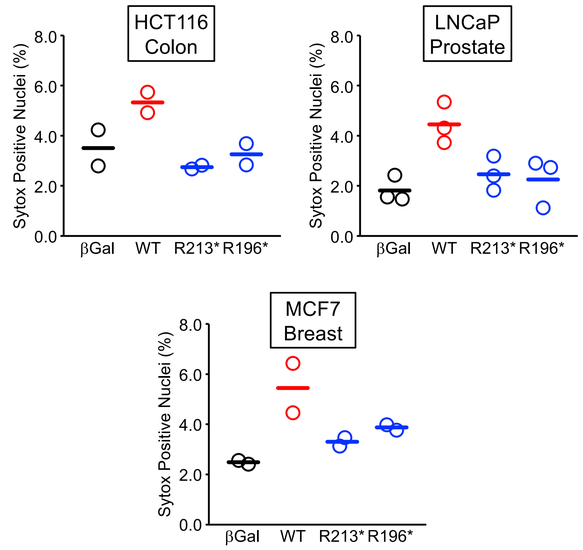

Figure 7. Effects of p53 wildtype and truncation mutants on cell death. HCT116-Colon, LNCaP-Prostate, and MCF7-Breast cells were infected with β-galactosidase (βGal), p53 wildtype (WT), R213*, and R196* expressing adenoviruses for 48hrs. Cells were then co-stained with Sytox (dead cells) and DAPI (all cells) to determine the number of dead cells.

SUMMARY & FUTURE DIRECTIONS

- p53 wildtype and R213* and R196* truncation mutants were successfully overexpressed in each of the cancer cell types
- No obvious changes were seen in the various mitochondrial protein expressions at this point
- Opposite to what we expected, ATP levels remained the same in the HCT116 cells but decreased in both the MCF7 and LNCaP cells
- We will continue studies evaluating the effects of the mutants on mitochondria, proliferation, and cell death
- We will knockdown CypD using siRNA to investigate the role it plays in these effects

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