Bat-derived partially cytoplasmic p53 mutant does not increase DNA damage repair rate

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Abstract

p53 is most widely recognized for the role it plays in oncogenesis, as it is the most frequently mutated gene in human cancer. However, the function of p53 remains incompletely understood. p53 has well described stress-activated transcription factor activity but has less well described cytoplasmic functions as well. Bats have evolved non-canonical amino acid changes in the nuclear localization signal (NLS) of p53, which result in partial cytoplasmic location. Bats' intrinsic ability to more rapidly repair various forms of DNA damage suggests that p53 may have unknown and beneficial novel cytoplasmic functions. In order to investigate these functions, our lab generated the $p53^{K316P}$ (PKK) mutant mouse, which mimics the NLS mutation and partial cytoplasmic localization seen in bat p53. We hypothesized that partially cytoplasmic p53 may increase the rate of DNA damage repair in $p53^{K316P}$ mouse embryonic fibroblast (MEF) cells. We tested this hypothesis by treating wild type and $p53^{K316P}$ MEF cells with DNA damage conditions and measuring the rate of DNA damage repair using immunofluorescent staining of γH2AX, a marker of DNA damage.

Background

- Prior to experimentation the Zhang Lab generated a $p53^{K316P}$ mouse model, which has an amino acid change in a nuclear localization signal (NLS), resulting in partial cytoplasmic localization of p53.
- DNA has multiple pathways by which to facilitate damage repair, the type of pathway used is dependent on the type of damage induced
  - Nucleotide excision repair (NER): Used to repair types of damage that distort the DNA double helix such as thymine dimers
    - Induced using cisplatin
  - Base excision repair (BER): Used to detect and remove single damaged bases
    - Induced using fluorouracil (5FU) and hydrogen peroxide
  - Double strand break (DSB) repair: Used to repair double stranded breaks vis non-homologous or homologous end joining
    - Induced via exposure to ionizing radiation
- DAPI is a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA and is used as a control to visualize all nuclear DNA.
- γH2AX is formed from the phosphorylation of H2AX following alteration to chromatin structures as induced by DNA damage.

Methodology

- Following the induction of DNA damage, the DNA damaging agents were washed away and the MEF cells were left to repair the DNA damage for periods 12, 24, 36, and 48 hours.
- Cells were fixed and immunofluorescent staining was used to determine the levels of γH2AX relative to the control DAPI.
- Images were processed using ImageJ to subtract background and enhance overall image quality.
- The strength of the γH2AX signal was compared to the strength of the DAPI signal to quantify the amount of DNA damage present in the cell culture.

Example Immunofluorescent Images

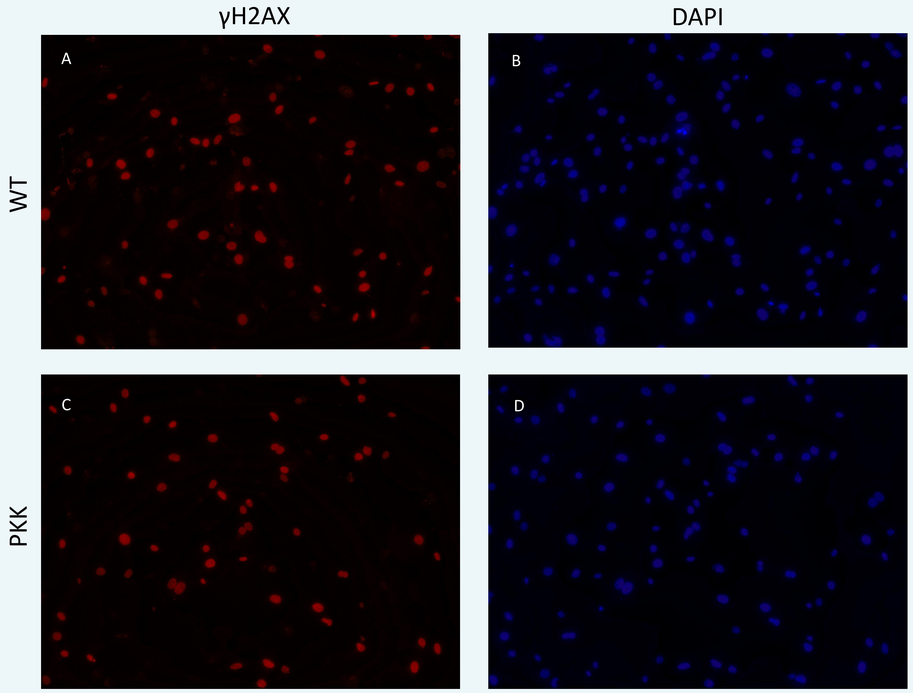

Figure 1. DAPI depicts all nuclear DNA and serves as a control for γH2AX which depicts the amount of DNA damage. Wild type and $p53^{K316P}$ (PKK) MEF cells were treated with 10μM cisplatin for 1hr. Cisplatin was then washed away and cells were allowed to repair DNA damage for 24hr. Cells were then stained for γH2AX (red) and DAPI (blue).

Results

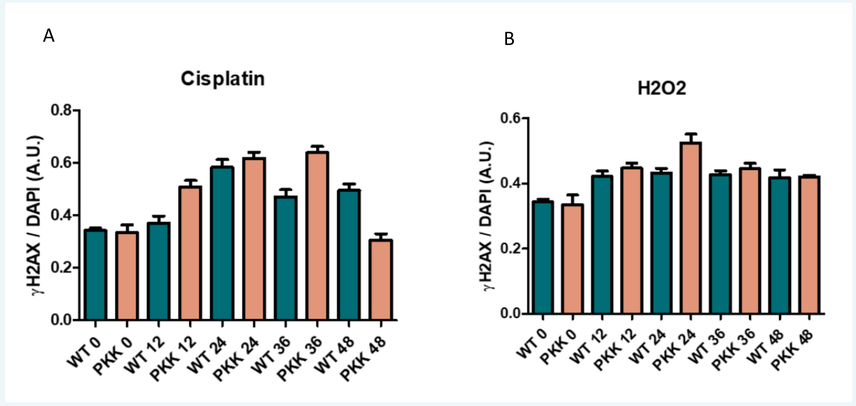

Figure 2. PKK mutants do not upregulate the rate of DNA damage repair relative to WT. Wild type and $p53^{K316P}$ (PKK) MEF cells were pulsed with 10μM cisplatin (A) or 300μM H_2O_2 (B) for 1hr. The damaging agent was then washed away and cells were incubated for 0 hours, 12 hours, 24 hours, 36 hours, or 48 hours before fixation and immunofluorescent staining. Representative images were then taken and the ratio of γH2AX signal to DAPI signal was quantified from 3 representative images per well.

Conclusion

The results depicted do not support the hypothesis that $p53^{K316P}$ mutation upregulates the rate of DNA damage response. In response to DNA damage, there was no consistent correlation between more rapid DNA damage repair rate in mutant cell lines compared to wild-type. This also contradicts preliminary evidence that revealed that $p53^{K316P}$ MEF cells had more rapid clearance of γH2AX foci in response to UV radiation.

Future Directions

Due to inconsistent results DNA damage experiments will no longer be performed until optimization of the IF protocol has occurred. Project directionality will be newly focused on examining a metabolic phenotype of the $p53^{K316P}$ mouse.

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