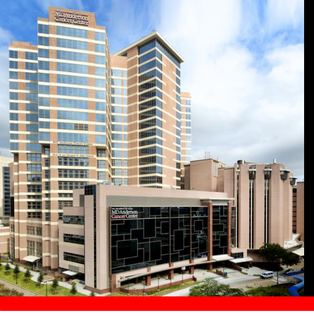

Deciphering how mutant p53 suppresses innate immune response by Toll-like receptors in a triple-negative breast cancer model.

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Introduction

- The immune system initiates immune responses by pattern recognition receptors (PRRs).
- Pathogen associated molecular patterns (PAMPs) are recognized by mechanisms such as:
  - The retinoic acid-inducible gene I (RIG-I)-like receptor (RLR) family.
  - The toll-like receptor (TLR) family.
  - The DNA sensor cyclic GMP-AMP (cGAMP) synthase (cGAS).
- PAMPs induce activation of IRF3/7 and NF-kB signaling pathway, which results in induction of type I interferons (IFNs) and cytokines.
- Bulk RNA sequencing comparing tumors with mutant p53 and mutant p53 deletion suggest that mutant p53 deletion impacts all three mechanisms, however only the cGAS mechanism has been described in literature (*Gosh at al*).

Materials and Methods

Table 1. Agonists of TLRs used and their Pathogen Associated Molecular Patterns.

PRR	PAMPs	Agonists
TLR1/2	Gram – bacteria	Pam3CSK4
TLR2	Gram + bacteria	HKLM
TLR3	viral dsRNA	Poly I:C HMW
TLR3	viral dsRNA	Poly I:C LMW
TLR4	LPS Gram - bacteria	LPS-EK
TRL5	bacterial flagellin	ST-FLA
TLR6/2	Gram +	FSL1
TLR7	viral ssRNA	ssRNA-40
TLR9	bacterial DNA	ODN1826

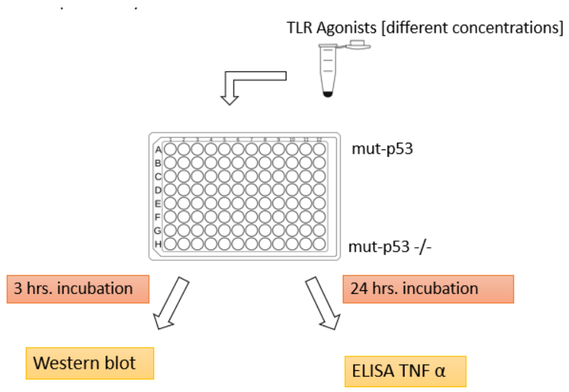

Fig. 1. Overall workflow. The experiment was conducted using Western blot and TNFα ELISA. The incubation periods were 3 and 24 hours respectively after TLR agonist treatment.

Results

- Western blot results indicate that there is lower phosphorylation of TBK1 in mutant p53 cells compared to deleted mutant p53 under treatment of TLRs 9 and 3 (Fig.2).
- Induction of phosphorylation of –IKKα/β in mutant p53 cells under treatment of TLR3 (Fig.3)
- TNF α production below detection levels under treatment of TLRs 1-9 using ELISA (Fig.4).

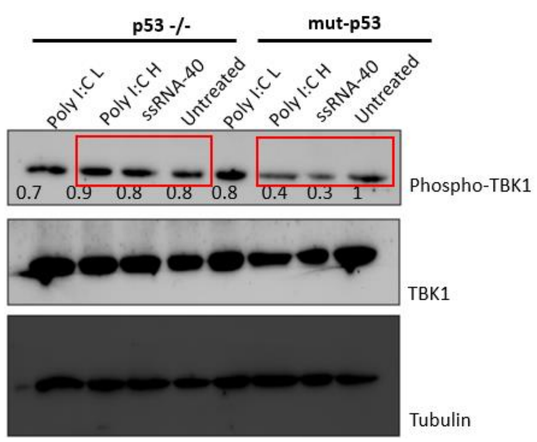

Fig. 2 Higher phospho-TBK1 signaling in p53 -/- in comparison to mut-p53.

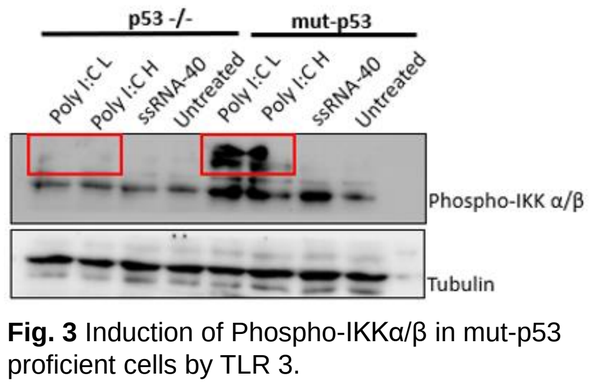

Fig. 3 Induction of Phospho-IKKα/β in mut-p53 proficient cells by TLR 3.

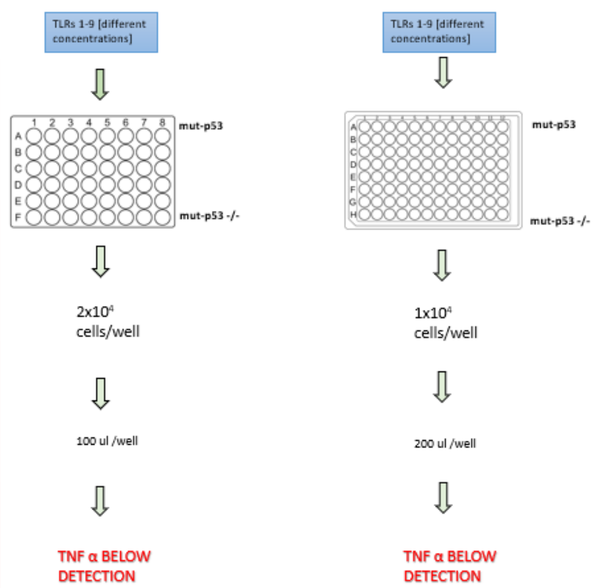

Fig. 4. TNFα below detection levels after TLR agonist treatment via ELISA.

Conclusions

- TLR3 induces phospho-IKK a/b in mutant p53 proficient cells but not in mutant p53 deleted cells suggesting higher levels of NF-kB.
- Higher phospho-TBK1 signaling in cells without mut-p53 compared to cells with mut-p53.
- Levels of secreted TNF-α in the supernatant in both cell lines were below detection level via ELISA, suggesting that more cells per well are needed or longer incubation time after TLR treatment.

Future Work

- We are currently testing cytokine expression of TNF-α, IFN-β,IFN-α and IL6 using RT-PCR.
- ELISA and Western will be conducted again to ensure reproducibility of the data.
- Longer of incubation period for ELISA
- Increase the number of cells per well to $3e^4$ as well as increasing the volume to 300ul per well.

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