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TITLE: Integration of Genomic, Biologic, and Chemical Approaches to Target p53 Loss and Gain-of-Function in Triple Negative Breast Cancer

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1. INTRODUCTION

This is the first annual progress report for DoD Award W81XWH-13-1-0287 / BC123219, investigating biochemical states resulting from alterations in the p53 signaling pathway in triple negative breast cancer (TNBC). Development of therapies for TNBC is a clinical and scientific challenge due to the heterogeneity of the disease and the lack of recurrent, drug-targetable molecular alterations¹⁻³. Our research focuses on the p53 tumor suppressor pathway, which is altered in the majority of TNBC cases and produces two adaptive states: loss of function (LOF) of wild-type p53 through mutation, gene silencing, or amplification of negative p53 regulators, and gain of function (GOF) displayed by some “hotspot” p53 mutant proteins that accumulate to high levels within the cell and drive oncogenic phenotypes including growth, migration, and drug resistance⁴⁻⁷. We hypothesize that targeting these adaptive biochemical states will provide candidate therapeutic targets for a large fraction of TNBC, a cancer for which there are no molecular targets to date. We are pursuing two specific aims: 1) to identify which signaling pathways, in either adaptive state, are required for TNBC cell viability, and 2) to test validated targets for “druggability” by fragment-based screening and develop small molecular inhibitors against targets that are both valid and druggable.

2. KEYWORDS

Listed in original application with emphasis on the following in this renewal:

p53
triple negative breast cancer
subtypes
gene expression
somatic cell genetics
CRISPR/Cas

3. OVERALL PROJECT SUMMARY

Specific Aim 1: We will verify which clinically characterized p53 mutants are oncogenic in TNBC lines and model systems. TNBC cell lines representative of the different adaptive states will be subjected to high-throughput siRNA-based synthetic lethality screens as a primary search for signaling pathways that, when targeted, can impact viability under a given adaptive state. One sub-aim is to target the p53 LOF adaptive state, and the other is to identify pathways that provide insight to how select high frequency gain of function p53 mutants can confer an oncogenic state. The intent for siRNA screening is to identify key pathway components that can be advanced as candidate targets for “druggability.”

We have made significant progress on the characterization and generation of TNBC lines harboring high-frequency p53 missense and loss-of-function mutants. Using gene knockdown approaches, we have screened our highly annotated and comprehensive TNBC cell line panel⁸ to identify which gain-of-function missense mutants, when lost, result in viability decrease through reduced proliferation and/or apoptosis *in vitro*, suggesting a dependence of the cell on the GOF adaptive state. These cell lines and mutant p53 states represent key model systems for the next stage of experimentation which involves synthetic lethality screening. As an ongoing objective within these studies, we have optimized siRNA transfection conditions for each cell line, which will contribute to the efficacy of the synthetic lethality screen.

In addition, we have optimized the use of CRISPR/Cas, a novel somatic cell recombination technology, to generate isogenic mutants within our TNBC cell lines (**Figure 1**). In addition to serving as a valuable cell context-identical reagent for transcriptional assays, the comparison of these artificially engineered p53 LOF and GOF states to p53 mutations that were selected for during tumorigenesis should provide insight to the role of tumor evolution in the acquisition of these biochemical adaptive states.

Specific Aim 2: To avoid expending valuable time and resources on targets for which there is a low probability of success for therapeutic intervention, we will clone, express, and purify potential target proteins (identified and validated through synthetic lethal screening in Aim 1) and screen them against a molecular fragment library (~15,000 compounds, <300 M.W.) using two-dimensional heteronuclear single quantum correlation (HSQC) NMR (of uniformly ¹⁵N-labeled proteins) or saturation transfer difference (STD) NMR (of unlabeled proteins). Proteins that exhibit binding to >0.1% of the molecules in this screen will be considered druggable and will be candidates for further discovery efforts including modifying the hits obtained in fragment-based screens to produce molecules that bind more tightly to the target protein. These efforts will be guided by NMR and/or X-ray crystal structures of protein-ligand complexes using iterative structure-based design. Compounds will also be optimized for their ability to block the biochemical and cellular functions of the target, leading to an inhibition of growth of cancer cell lines.

We are prepared to begin the experiments outlined in the original Specific Aim 2 after the completion of our synthetic lethal screen proposed in Aim 1. The timeline for completing these experiments is outlined in the conclusion section below.

4. KEY RESEARCH ACCOMPLISHMENTS

No publications to report at this time; we are in the reagent and verification stage of this project.

5. CONCLUSION

Specific Aim 1: We will finalize our analysis of GOF dependence in our TNBC cell line panel and complete the generation of isogenic cell lines as models for target validation and transcriptional comparative analyses. Using our optimized transfection conditions, we will conduct the synthetic lethality siRNA screen outlined in our original proposal, using cell lines representative of the LOF and GOF biochemical adaptive states. We will rank hits based on a combination of the magnitude of the viability change, Z-score and sensitivity index, and the number of siRNAs scoring per gene. After validating targets in independent cell lines and our isogenic models and characterizing the nature of any viability decreases identified (e.g. apoptosis vs. cell cycle arrest), we will select candidates for further “druggability” screening. We anticipate completing this stage of the research within the upcoming Year 2 of the award period.

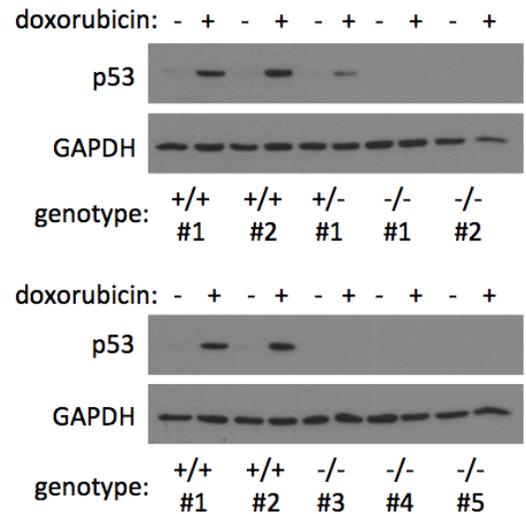


Figure 1. CRISPR/Cas-mediated *in vitro* somatic cell recombination was used to generate isogenic p53 mutants in TNBC cell lines. A diploid, p53 wild-type TNBC cell line (+/+) was targeted with a plasmid encoding *S. pyogenes*-derived Cas9 protein⁹. Resulting single cell colonies were sequenced and identified to contain heterozygous (+/-) or homozygous (-/-) frameshift mutations in *TP53* exons. Whole cell lysates were prepared from cell lines with the indicated genotypes and Western blot analyses performed with antibodies specific to p53. The results show the status of p53 protein (reduced or eliminated), after a doxorubicin treatment (1 μM, 8 hr) which is well established to induce cellular stress and elevation in p53 protein levels in the +/+ or +/- state.

Specific Aim 2: The most promising candidates from each sub-aim of Specific Aim 1 (LOF vs. GOF adaptive states) will be advanced to small molecule screening to evaluate their potential for druggability and to identify promising lead molecules for pharmaceutical development. Using a “fragment library” of 10,000 small molecules with a molecular weight of 280 or less, we will conduct NMR screens to assess protein-ligand interactions. Proteins with hit rates >0.1% will undergo a fragment-based drug design protocol, as outlined in our original proposal, in addition to screening with more traditional chemical libraries. Lead optimization and cellular evaluation will be conducted on an ongoing basis once candidate protein-ligand partners are identified. We anticipate entering this stage of the proposal by the latter half of the upcoming Year 2 of the award period, with the majority of Year 3 dedicated to completing this aim and manuscript generation.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Poster Presentation:

Shaver TM, Tang L, Pietenpol JA. “Targeting Gain-of-Function p53 Missense Mutants.” 16th International p53 Workshop, Stockholm, Sweden. June 2014.

7. INVENTIONS, PATENTS, AND LICENSES

Nothing to report at this time.

8. REPORTABLE OUTCOMES

Nothing to report at this time.

9. OTHER ACHIEVEMENTS

Nothing to report at this time.

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10. APPENDICES – N/A