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14. ABSTRACT
TSC is a common inherited predisposition syndrome, affecting nearly 1 in 7,500 individuals. Individuals with TSC develop benign tumors in multiple organs, including the retina, skin, lung, kidney and brain. The identification of valid targets in TSC has been discouraging. In search of TSC targets, we recently identified NPM as a downstream effector of mTOR signaling in TSC cells, providing cells with an abundant supply of ribosomes necessary for supporting their increased growth rate. We now provide evidence that NPM forms a novel complex with DDX5 to drive TSC cell growth. Using the NCI diversity set of chemical compounds, we have now identified two compounds that potently inhibit split-luciferase activity in two TSC cells lines. Notably, these two compounds also inhibit the proliferation of TSC/p53-null and UMB1949 TSC cells while not altering the growth rates of p53-null cells that maintain TSC function, suggesting that these compounds might specifically target NPM-DDX5 complex formation when it is enhanced in TSC cells. We will now move these exciting data forward with a larger library screen while also continuing to validate these two chemical compounds.

15. SUBJECT TERMS
NPM, DDX5, TSC, chemical library, split-luciferase

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1. INTRODUCTION

TSC is a common inherited predisposition syndrome, affecting nearly 1 in 7,500 individuals. Individuals with TSC develop benign tumors in multiple organs, including the retina, skin, lung, kidney and brain. However, these lesions can present as malignant. TSC results from mutations in either the TSC1 or TSC2 gene, resulting in hyperactivation of the mTOR-signaling pathway. This pathway ultimately controls downstream effectors of cellular mRNA translation and proliferation. Multiple studies in mice have demonstrated the requirement of mTOR activity for aberrant TSC cell growth in vitro and in vivo and these initial findings have translated to human cells as well. However, the identification of other valid targets in TSC has been discouraging, leaving the field to concentrate on rapamycin and its analogues as the sole treatment for TSC. In search of additional TSC targets, we recently identified NPM as a downstream effector of mTOR signaling in TSC cells, providing cells with an abundant supply of ribosomes necessary for supporting their increased growth rate. We now provide evidence that NPM forms a novel complex with DDX5 to drive TSC cell growth. Targeting this interaction might provide a novel treatment strategy for TSC patients.

2. KEYWORDS

NPM, DDX5, TSC, chemical library, split-luciferase

3. ACCOMPLISHMENTS

Major Goals of the Project

There were three major goals for this past year in the grant proposal: 1) Screen 2,000 chemical library for split luciferase activity, 2) Screen 14,400 chemical library for split luciferase activity, and 3) validate that inhibitors block NPM-DDX5 complex.

Goals Accomplished

In the first year of this grant application, we have now successfully set up the high throughput split luciferase screening model and scaled it up to 96-well plate format using a non-automated screening method. Using *TSC1/p53*-null mouse embryonic fibroblasts (MEFs) and UMB1949 cells, we have screened the NCI Diversity Set of compounds for chemicals that inhibited luminescence by 3-fold or greater. We successfully used *p53*-null cells as a negative control on any resultant hits. Concentrations for each compound were 5 mM. Cells were incubated for 24 hours in each compound prior to incubation with luciferin. All cells were stably transduced with a GFP expression construct to account for cell viability as well as split-luciferase activity in each well of cells. Each plate contained a negative control where the ratio of GFP to photon flux was set at 100%. This allowed us to calibrate all 96 wells on each individual plate without the risk of plate-to-plate variance. An example of the results obtained for each plate is shown in Figure 1 that depicts plate 11. Samples #29 (75%), #77 (82%), and #92 (88%) resulted in significant decreases in luminescence.

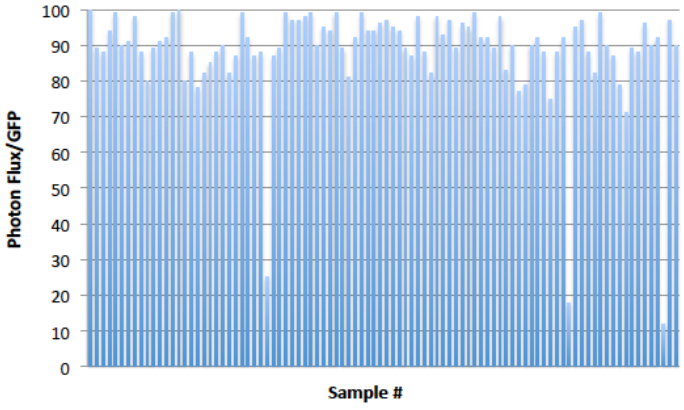

Figure 1. Analysis of Plate #11. 96-well plate #11 was seeded with TSC1/p53-null MEFs that had been transduced with GFP expression constructs and the NPM-DDX5 split-luciferase constructs. Cells were incubated for 24 hours in 5mM of each compound and analyzed for GFP fluorescence and luciferase luminescence. Lane 1 corresponds to DMSO control and set at 100%.

We next sought to determine whether any of these three compounds (tested at 5 mM concentration) could selectively inhibit the growth of TSC cell lines while not altering the proliferation of other non-TSC cells. Compound #29 and compound #77 were quite potent inhibitors of *TSC1/p53*-null MEFs and UMB1949 cells while leaving *p53*-null cells proliferation largely intact (Figure 2) over a three day period of growth. However, compound #92 inhibited the proliferation of both TSC cells and *p53*-null cells where TSC1 is competent (Figure 2), suggesting that its effects are largely toxic and not specific for any TSC-dependent biology.

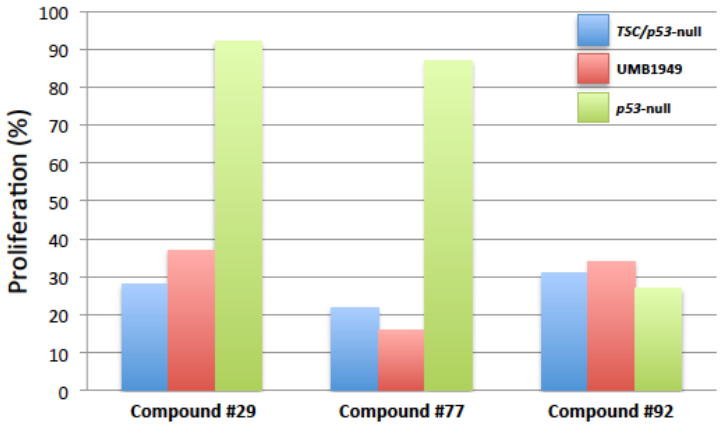

Figure 2. Three test compounds result in inhibition of cell proliferation. We plated three cell lines: *TSC1/p53*-null, UMB1949, and *p53*-null cells in 6-well dishes and incubated each well with DMSO control or 5 mM of each compound (#29, #77 and #92). Cells were counted three days later and normalized to DMSO control wells.

Our next goal is to determine whether compound #29 and #72 inhibit the formation of NPM-DDX5 complexes by using immunoprecipitation assays with NPM and DDX5-recognizing antibodies. We will also conduct a more thorough dose response for both cell proliferation and split-luciferase inhibition with each compound.

Training Opportunities

Nothing to Report

Results Disseminated to the Community

I participated this past year in disseminating our initial findings to three independent groups of large donors to the American Cancer Society. These donors visited my laboratory at Washington University where I discussed the research in this grant proposal and how our results were moving the field of TSC research and treatments forward. We engaged in a question and answer session where the donors queried me on the clinical impact of this work. I anticipate doing this laboratory tour again next year and have already been asked by the American Cancer Society to do so.

Plans for Next Reporting Period

Our first set of experiments for the second and final year of this grant will be to initiate and compete the proposed 14,400 Maybridge chemical libraries screen. We believe that we can perform this screen rapidly since we have spent much time working out the necessary conditions for a robust luciferase signal in our split NPM-DDX5 complex split luciferase construct. Once that is completed, we will quickly move into the validation stage where we will test whether the identified compounds inhibit the formation of NPM-DDX5 complexes. This will initially be performed using the same split-luciferase construct, but eventually we will perform co-immunoprecipitation experiments to validate the formation of complexes. Finally, we will measure cell cycle progression and proliferation of TSC cells in the presence of each identified compound that we have validate in the complex formation assays. Furthermore, assays to measure cell apoptosis will conclude our analysis of the validated compounds.

4. IMPACT

Impact on Principal Discipline

Our current work will be incredibly impactful for those researchers involved in identifying novel compounds that might provide evidence as to a novel approach to treat TSC clinically. Additional, our data now provide evidence that the split luciferase approach can work when looking for compounds that block the interaction of two proteins. Additionally, we have proven the utility of studying the NPM-DDX5 interaction in the context of TSC.

Impact on Other Disciplines

Nothing to Report

Impact on Technology Transfer

Nothing to Report

Impact on Society

We have disseminated the data and ideals from this grant proposal to several groups in the St. Louis community. They were encouraged by our progress and excited about the future clinical impact our work might provide.

5. CHANGES/PROBLEMS

Changes in Approach

Nothing to Report

Anticipated Problems or Delays

We were delayed in our ability to test the 14,400 Maybridge chemical libraries for split luciferase activity. This was both a product of our inability to rapidly obtain the library and our difficulties in acquiring a robust luciferase signal that was readable above the observed noise in the system. We believe that we have now cleared the latter hurdle and are ready to immediately begin our screen of the 14,400 compound libraries this year without further delay.

Changes in Human, Animal Biohazards and/or Selective Agents

Nothing to Report

6. PRODUCTS

Publications, Conference Papers and Presentations

Nothing to Report

Internet Sites

Nothing to Report

Technologies or Techniques

Nothing to Report

Inventions, Patents and/or Licenses

Nothing to Report

7. PARTICIPANTS

Individuals That Have Worked on Project

Name:	Jason D. Weber
Project Role:	PI
Nearest person month worked:	1.2
Contribution to Project:	Dr. Weber served as the mentor for Ms. Yaddanapudi in planning all experiments and overseeing the final data analysis.
Funding Support:	NIH R01CA190986, NIHR01CA174743, W81XWH-15-1-0528

Name:	Sree Yaddanapudi
Project Role:	Graduate Student
Nearest person month worked:	6
Contribution to Project:	Ms. Yaddanapudi performed all of the experiments outlined in specific aim1 for year 1

Changes in Active Other Support for PD/PI
Nothing to Report

Other Organizations Involved as Partners
Nothing to Report