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14. ABSTRACT

The goals of this Application are to utilize our new knowledge of the mechanism by which loss of Merlin induces tumorigenesis to identify small molecule compounds that block YAP/TEAD-dependent transcription by acting at any step of our newly identified pathway, and to test the preclinical efficacy of lead compounds in xenograft models of NF2. During the first year of this grant, we have generated *NF2* mutant cel lines expressing two different types of YAP reporters and tested their suitability for high throughput screening.

15. SUBJECT TERMS

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Introduction

The tumor predisposition syndrome Neurofibromatosis type 2 (NF2) arises from inactivating mutations of the tumor suppressor gene *NF2*, which encodes the FERM domain-containing protein Merlin. Children and young adults, who inherit an *NF2* mutation, develop Schwannomas, usually of the VIII cranial nerve, but also meningiomas and ependymomas. The only effective current therapy for NF2 involves repeated and debilitating surgeries. As a monogenic disease, NF2 should be eminently sensitive to targeted medical therapies. In fact, NF2 should be eradicated by the appropriate targeted therapy in the same way Chronic Myelogenous Leukemia is cured by *Gleevec*. The prospect of resistance is minimal, as Schwannoma cells do not seem to possess other signaling alterations except those caused directly by loss of *NF2*. Yet, progress in this area has been hampered by the lack of a clear mechanism of Merlin's tumor suppressor function. Our studies have shown that inactivation of Merlin/NF2 de-regulates the E3 ubiquitin ligase $CRL4^{DCAF1}$, which promotes tumorigenesis by inhibiting Lats1/2 and thereby activating YAP/TEAD-dependent transcription. The goals of this Application are to utilize our new knowledge of the mechanism by which loss of Merlin induces tumorigenesis to identify small molecule compounds that block YAP/signaling by acting at any step of our newly identified pathway, and to test the preclinical efficacy of lead compounds in xenograft models of NF2.

Keywords

NF2, E3 ubiquitin ligase, high throughput small molecule screening, targeted therapy.

Accomplishment

Major goals and objectives: The overall goal of this grant is to conduct high-throughput cell-based screens to identify lead compounds that inhibit YAP/signaling. Task I. The FC1801 will be stably transduced with a Firefly luciferase TEAD reporter in combination with a control Renilla luciferase construct. Initial screens will be performed with a library comprising all compounds that have undergone Phase 1 trial for any indication (~4,000) and then with a library comprising a diversity set of compounds (~17,600). Hits will be prioritized by using selective inhibition of proliferation of NF2-mutant cells as the major criterium (year 1). Task 2. Subsequent screens will involve a large library comprising more than 178,000 small molecules. Hits will be prioritized by using selective inhibition of proliferation of NF2-mutant cells as the major criterium (year 2). Task 3. Cell biological and biochemical experiments will be conducted to determine if any of the lead compounds inhibits $CRL4^{DCAF1}$ or YAP and by what mechanism (year 3).

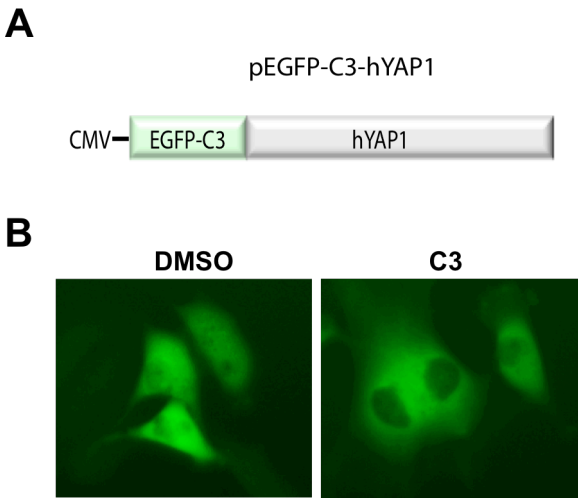

Figure 1. Generation of MDA-MB-231cells expressing the YAP reporter EGFP-YAP. MDA-MB-231 cells were transiently transfected with pEGFP-C3 encoding human YAP1 (addgene #17843) (**A**) and positively transduced cells were selected with neomycin. Cells expressing high levels of the reporter protein were sorted by FACS and found to exhibit YAP in both the cytosol and nucleus. To test EGFP-YAP localization, EGFP-YAP-MDA-MB-231 cells were plated and then treated with vehicle (DMSO) or 2 μg/ml C3 (Rho inhibitor I). As shown by imaging, C3 treatment blocked Yap nuclear localization, indicating that the recombinant EGFP-YAP protein behaves like endogenous YAP protein (**B**).

Accomplishments: *Prior screens for inhibitors of dysregulated Hippo-YAP signaling were conducted using 293T cells overexpressing YAP and a TEAD reporter (Liu-Chittenden et al., 2012). Since this approach can only lead to the identification of agents that disrupt the YAP-TEAD interaction or the binding of the complex to DNA, limiting the target space of the screen, we have proposed to rely on upstream dysregulation of the pathway and conduct our screens in NF2 mutant cells, which do not overexpress YAP. This approach is much more powerful, as it can yield compounds that interfere with all of the upstream components of dysregulated Hippo-YAP signaling.*

We initially tested a classical TEAD reporter in 293T cells. Preliminary experiments indicated that this reported functions as anticipated in response to overexpressed YAP. We therefore generated several reporter-expressing *NF2* mutant cell lines and tested them. FC1801 and MB231 cells were stably transduced with a Firefly luciferase TEAD reporter in combination with a control Renilla luciferase construct and several clones of each derivative were isolated. TEAD-dependent expression of Firefly luciferase and background expression of Renilla luciferase was monitored by bioluminescence to identify clones expressing low levels of Renilla luciferase and high levels of Firefly luciferase. Control experiments were conducted to verify that expression from the TEAD reporter is inhibited by re-expression of Merlin, silencing of DCAF1, and overexpression of Lats1/2. Verteporfin, which inhibits the interaction of YAP with TEAD (Liu-Chittenden et al., 2012), was used as positive control. *However, we could not discern a clear activation of the TEAD reporter in NF2 mutant cells that could be reversed by expression of Merlin or treatment with Verteporfin, suggesting that this reporter system is not sensitive enough in physiopathologically relevant cell types. In parallel, it also became evident*

that YAP can affect not only TEAD but also other transcriptional factors (Manderfield et al., 2015; Zanconato et al., 2015), which further limits the ability of TEAD reporters to monitor dysregulated Hippo-YAP signaling.

We reasoned that using nuclear translocation of YAP as a criterium for YAP activation would have been more sensitive and physiopathologically relevant than using a TEAD reporter. In addition, this approach would have also allowed us to perform in parallel high content imaging screens with genome wide shRNA libraries to uncover novel and potentially targetable mechanisms underlying activation of YAP in *NF2* mutant cells. We generated an EGFP-YAP construct (Fig. 1A), stably transduced FC1801 and MB231 cells, and selected several clones expressing EGFP-YAP. Clonal populations expressing homogeneously high levels of EGFP-YAP were then selected through repeated cycles of FACS sorting. *Imaging experiments were conducted to verify that EGFP-YAP accumulates in the nucleus of NF2 mutant cells (Fig. 1B, left). As anticipated, treatment of the cells with the Rho inhibitor C3 blocked nuclear accumulation of the reporter (Fig. 1, right). Control experiments were performed to verify that re-expression of Merlin, silencing of DCAF1, and overexpression of Lats1/2 induces extrusion of GFP-YAP from the nucleus. We are currently optimizing this assay for high-throughput screening of chemical libraries at our High Throughput Screening (HTS) Core Facility.*

Opportunities for training and professional development: The postdoctoral fellow and graduate student involved in this project have learned general principles of high throughput screening from Ralph Garippa, who heads our HTS Core Facility. Furthermore, they had the opportunity to discuss their project with me on a weekly basis and to attend to several seminars and work-in-progress meetings at MSKCC.

Dissemination: Nothing to report.

Plans: In the next reporting period, we plan to conduct high throughput screens with a library comprising all compounds that have undergone Phase 1 trial for any indication (~4,000) and then with a library comprising a diversity set of compounds (~17,600). Hits will be prioritized by using selective inhibition of proliferation of *NF2*-mutant cells as the major criterium.

Reference:

Liu-Chittenden, Y., Huang, B., Shim, J.S., Chen, Q., Lee, S.J., Anders, R.A., Liu, J.O., and Pan, D. (2012). Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. Genes Dev *26*, 1300-1305.

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Zanconato, F., Forcato, M., Battilana, G., Azzolin, L., Quaranta, E., Bodega, B., Rosato, A., Bicciato, S., Cordenonsi, M., and Piccolo, S. (2015). Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nat Cell Biol *17*, 1218-1227.

Impact

- Development of cell lines for the screens.

What were the major goals of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report.

Changes/Problems

Nothing to Report.

Products

Nothing to Report.

Participants & Other Collaborating Organizations

- What individuals have worked on the project?

Name: Young-Mi Kim
Project Role: Research Associate
Nearest person month worked: 6
Contribution to Project: Dr. Kim has performed experiments with the *NF2* mutant NB231 cells.

Name: Mayur Gadiya
Project Role: Graduate Student
Nearest person month worked: 2.4
Contribution to Project: Mr. Gadiya has performed experiments with the *NF2* mutant FCI801 cells.

- Has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?

  - Wei Li has been replaced by Young-Mi Kim.

- What other organizations have been involved as partners?
  - Nothing to Report.

Special Reporting Requirements & Appendices

None.