Project Information

**Project Title**

Small Molecule Targeting of the Hippo/YAP Pathway to Therapeutically Treat Cholangiocarcinoma

**Amount Requested**

$50,000

Applicant Information

**Applicant Name**

Sophia Shalhout

**eRA Commons Username**

sshalhout

**Degree (Select all that apply)**

- PhD

**Academic Level (Select all that apply)**

- Post-Doc Fellow

Institution Information

**Institution Name**

Harvard University and Children's Hospital Boston

**Institution City**

Boston

**Institution State**

Massachusetts

**Institution Country**

United States

**Email**

Sophia.Shalhout@childrens.harvard.edu

Mentor Information

**Mentor Name**
Cholangiocarcinoma (CC) is a highly fatal primary liver cancer and the second most common hepatic neoplasm worldwide. The incidence and mortality rates of CC are on the rise, with the vast majority of new cases detected at advanced stages. Therapeutic options directly targeting unresectable CC are non-existent, with radiation and chemotherapy ineffective. The current treatment modalities have resulted in overall survival rates below 10%. There is an immediate and critical need to focus on the development of the first small molecule therapies effective against CC by targeting the underlying causative mechanisms. Specifically, the Hippo/YAP-signaling pathway, which the Camargo lab has demonstrated to be a crucial regulator of liver organ size and liver cell fate, promotes the development of this cancer. Through its interaction with the TEAD transcription factors, YAP promotes proliferation and angiogenesis in CC, with YAP hyperactivation a common feature in human cholangiocarcinomas. Excitingly, we have recently discovered potent cell permeable small molecules that bind and inhibit the YAP-TEAD interaction. These molecules are able to robustly bind YAP and inhibit YAP-TEAD interaction at the biochemical and functional level. We will evaluate the activity of these novel molecules in CC cell lines and test their potency preclinically using CC mouse models. The overall aim of this project is to develop lead drug compounds targeted against the YAP-TEAD interaction for CC treatment.

The deregulation of YAP leads to disastrous effects in the liver, promoting the development of cholangiocarcinoma (CC) (9-11). Thus, the design and discovery of novel inhibitors of YAP activity and inhibition of the YAP-TEAD interaction has been proposed as a potential therapeutic strategy to treat patients with CC and to improve the current abysmal survival rates. We have discovered small molecules that bind YAP using an unbiased small molecule microarray (SMM) screening approach (FIGURE1). Ten promising hit series have emerged from this large scale HTS approach. Preliminary data from follow up cell-based evaluation of these molecules show that 12 compounds in Hit Series 1 (HS-1) augment the activity of a YAP-dependent transcriptional reporter. Furthermore, HS-1 compounds were shown to inhibit the YAP-TEAD interaction by a split luciferase reporter assay and a bioluminescence resonance energy transfer (BRET) protein interaction assay between YAP and TEAD (FIGURE2). Biochemical assays, namely YAP thermal shifts, SPR, and an Alpha Screen assay between YAP and TEAD further demonstrate the binding of these hits to the YAP-TEAD interaction site with EC50s between 0.09-70µM (FIGURE3). Thus, we have bona fide binders of YAP and inhibitors of YAP activity. We propose to determine in vitro potency, mechanism of action, selectivity, and in vivo profile of these molecules specifically using CC cell lines/mouse models and undergo lead compound development.
Our lab is a worldwide leader in the Hippo/YAP signaling pathway field, both in the context of normal liver function and liver cancer. The role of YAP in the development of bile duct cancer, or cholangiocarcinoma, and its association with poor survival rates, strongly supports targeting YAP therapeutically. We have carried out a large scale screen to discover potential compounds that can bind YAP and possibly inhibit its activity. Our current data shows that molecules discovered from our screening approaches do bind YAP and greatly reduce its interaction with its binding partner TEAD.

The experiments proposed here aim to test the activities of first-in-class inhibitors of a novel signaling pathway involved in the development of cholangiocarcinoma. The availability of these compounds puts us in a unique position to enable Hippo/YAP pathway manipulation as a tangible therapeutic strategy to treat cholangiocarcinoma. While our biochemical as well as functional cell-based data reveal promising candidates for drugs that can inhibit the YAP-TEAD interaction, we need to test the efficacy of our molecules in cholangiocarcinoma mouse models and cell lines.

Furthermore, we aim to discover and develop sister compounds found to be more effective and more potent than the initial ones discovered in our screens. Our work will lay the foundation for the clinical application of targeting the Hippo/YAP-signaling pathway as a therapeutic strategy in the fight against cholangiocarcinoma.

Specific Aims (2500 Max Characters)

We successfully completed large scale HTS SMM screening campaigns against purified recombinant YAP protein, as well as YAP and TEAD in protein complexes from cell lysates (FIGURE1). YAP and YAP-TEAD focused biochemical, kinetic, and thermodynamic binding assays as well as cell-based reporter testing were carried out as secondary screens and have resulted in several series of compounds to follow up on. Compounds HS102 and HS110 from “Hit-series 1” (HS-1) are the most promising hits and are the focus of this proposal.

Specific Aim 1: To test HS102 and HS110 in vitro, and in vivo using human CC cell lines and CC murine models. A CC line previously established as YAP-dependent, HUCCT-1, will be assessed for sensitivity to compounds HS102 and HS110. YAP-independent lines will also be tested in parallel. A ‘dead analog’ that has a similar structure to HS102 and HS110 but shows no biochemical or functional effect, HS000, will serve as a control for molecular characterization. Briefly, gene-profiling signatures, proliferation assays, and anti-metastatic effects based on migration wound healing assays and cell motility/invasion assays will be assessed in vitro. HS102 and HS110 will then be extensively tested using nude mice and CC tumor xenograft models to determine effects on CC cancer in vivo (1, 2).

Specific Aim 2: To validate mechanism of action and develop lead compounds for targeted therapy against YAP in CC. HS102 and HS110 will be tested for structure-activity relationship (SAR) and pull-down assays will be used to determine any off-target binding. Lysates from CC cell lines treated with compounds will be used in YAP pull-down experiments followed by blotting against YAP and TEAD to assess interaction inhibition. Compounds will undergo specificity testing including SILAC studies as well as linking compounds to affinity resins and determining interacting proteins via MS. Tanimoto clustering will be used for SAR-by-purchase of commercially available analogs for evaluation in biochemical/cell based assays to establish whether the appendages or the backbone moiety confer specificity. Synthetic approaches and NMR may be used to fully define SAR. Optimizing leads will require continued evaluation of binding as we attempt to establish the true pharmacophore.

Background and Significance (3000 Max Characters)
Our lab has conducted pioneer work on the Hippo/YAP-signaling pathway as a crucial regulator of liver organ size and liver cell fate(1, 6, 7). Activation of this growth suppressive pathway leads to phosphorylation, cytoplasmic retention, and inactivation of the transcriptional coactivator YAP. At its core are two kinases MST1/2 that interact with their substrates LATS1/2. Phosphorylation of YAP by LATS1/2 leads to its cytoplasmic retention and degradation. However, when the Hippo pathway is not activated, YAP remains unphosphorylated and is recruited to the nucleus to interact with the DNA binding transcription factor TEAD (FIGURE 4) (8, 9). This activates a complex program of gene expression that results in potent growth (6, 10) (FIGURE 4).

We recently identified the TEAD-positive enhancers that YAP occupies in CC cells driving high transcription levels via mediator (11) and we have demonstrated that YAP changes liver cell fate towards a cholangiocellular or progenitor-cell-like phenotype (7). Using a Doxycycline-inducible hepatocyte-specific activated YAP (YAP S127A) overexpressing mouse model, we show that YAP+ hepatocytes have massive proliferative expansion. YAP+ hepatocytes transform to develop liver tumors (FIGURE 5A) (2).

The importance of Hippo signaling in cancer is emerging. The deregulation of YAP leads to spectacular effects in the liver, promoting the development of cholangiocarcinoma (CC) and hepatocellular carcinoma (HCC) (12-14). Transgenically activated YAP in the liver (1, 2), co-delivery of activated YAP and β-catenin (13), and liver-specific deletion of the core kinases of the Hippo pathway lead to HCC/CC tumors in mice (3-5). Murine models for CC have been developed with constitutively active AKT and YAP as well as with PIK3CA and YAP (14, 15). Discoveries of alternative signal inputs to YAP suggest that Hippo signaling is just one of the pathways to regulate YAP-driven carcinogenesis, and rather, YAP processes several signaling inputs that may lead to cancer (16), proliferation, EMT, and tumor survival (17).

Clinically, studies show nuclear YAP is elevated in human CC (17). High YAP levels in CC significantly correlate with tumor size, liver cirrhosis, vascular invasion, and intrahepatic metastasis (12). Recurrence-free, disease-specific, and overall survival rates are significantly poorer in CC patients with high YAP levels (FIGURE 5B). The eventual translation to the clinic will require small molecule inhibitors of YAP activity. Targeting YAP for the treatment of CC is a promising therapeutic strategy and we have discovered first-in-class candidate molecules able to bind YAP and inhibit YAP-TEAD interaction. Our lab has pioneered work on the Hippo pathway in mammals and has created a number of animal models that put us in a leading position to effectively evaluate therapies aimed at CC preclinically. We aim to test the activity of these molecules in multiple CC mouse models and develop more effective lead compounds.

**Project Timeline (2500 Max Characters)**
The proposed project is scheduled for one year, starting June 2017 to May 2018. By June 2017, all secondary biochemical and cell-based screens determining binding to YAP, and inhibition of the YAP-TEAD interaction will be completed for all compounds in the HS-1 series. Kd, koff/kon, and IC50s/EC50s will be determined and may lead to expansion of testing to include other molecules in addition to HS102 and HS110.

**JUNE- AUGUST 2017**

Dose response curves and IC50s for HS102 and HS110 will be determined for the YAP-dependent human CC cell lines from a panel of already characterized CC lines. The most sensitive cell line will be chosen for continued assessment alongside a less sensitive line as a control. In vitro testing of the sensitivity of these CC lines to our compounds alongside YAP-independent non-CC line testing, will occur during the summer of 2017 in a high throughput manner.

**SEPTEMBER 2017**

HS102 and HS110 will undergo PK/PD data acquisition to determine how to design the in vivo mouse model studies.

**OCTOBER 2017- FEBRUARY 2018**

Compounds will undergo short term in vivo studies as well as long term in vivo studies in CC mouse models during this time frame. Mechanism of action (MOA) studies and structure-activity relationship (SAR) studies will also begin in OCTOBER 2017 alongside in vivo work. Specificity/on-targets and off-targets will be evaluated during this time period. SAR-by-purchase will also be conducted and these compounds will be reevaluated with SPR, the split-luciferase assay and alpha screen between YAP- and TEAD. More dead analogs will be generated which give information regarding efficacy based on appendages or core moieties.

**MARCH- MAY 2018**

Synthetic approaches and NMR may be used to fully define SAR. Optimizing leads and establishing the true pharmacore will commence during this period for lead development.

Less than successful efforts will lead me to shift priorities toward synthetic chemistry for probe optimization of molecules that show inhibitory effects but do not meet the cut off criteria. I can also re-evaluate other HS-1 compounds as well as HS-2 through HS-10 from the hit lists.

**Literature Cited**


Supporting Files - Please upload any supporting files such as charts or graphs in JPG or PDF format.

- SHALHOUT-CC-FOUNDATION-FIGURES-GRANT.pdf

## Budget

### Impact or Collab Award

I want to apply for an Impact Award (up to $50,000)

### Impact Applicant Name

Sophia Shalhout

### Impact App Title

Postdoctoral Research Fellow

### Impact App Institution

Harvard University and Children's Hospital Boston

### Impact App Personnel 1 Name

Sophia Shalhout

### Impact App Per 1 Effort

100

### Impact Per 1 Base Salary

50000

### Impact Per 1 Salary Requested

45000

### Impact App Personnel 2 Name

Not applicable Not applicable

### Impact App Per 2 Effort

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### Impact Per 2 Base Salary

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### Impact Per 2 Salary Requested

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**Impact Total Requested**

50000