RESEARCH INFORMATION

Describe your preparation and desire for performing undergraduate research with your chosen mentor

I have worked with Professor for several semesters and was awarded UROP funding last summer. This research will serve as my senior capstone project.

Research Project Title

Stk25-mediated activation of Hippo signaling

Describe the research problem

This section should be about 100-500 words. It should describe background information of the previous work by your mentor and other scientists underlying the scientific question and its significance to the field of biology. There should be a clear sentence near the end that states the hypothesis you plan to test. Be sure to cite any statements of fact that are not common knowledge (see Cited Sources section below).

It has been reported that Stk25 is significantly focally deleted in solid tumors (1). However, the significance of this deletion remains unknown. In accordance with our hypothesis, we believe that Stk25 deletions represent one of major ways by which cancer cells inactivate the Hippo pathway.

In a broader context, the Hippo pathway is an important field to study as it is crucial in maintaining tissue homeostasis. Deregulation of the Hippo pathway has been reported in a large number of human cancers, and it often correlates with poor clinical prognosis. This deregulation has been observed mostly from immunohistochemical detection of YAP overexpression and increased nuclear levels in human tumors (2). Importantly, YAP overexpression might be a prominent inducer of metastasis, as it stimulates the epithelial-mesenchymal transition (EMT) of cells before separating from the original tumor. Given these clinical realities we believe that a better understanding of the Hippo pathway will reveal new therapeutic opportunities that are more cancer-specific in nature. In the context of this proposal, we believe that pharmacologic stabilization of Stk25 represents a promising therapeutic strategy.

The Hippo tumor suppressor pathway is a highly conserved signaling pathway that is essential in regulating organ sizes, cell proliferation, and apoptosis (3). Initially discovered in *Drosophila melanogaster*, the Hippo pathway is of great interest in the field of cancer biology as its inactivation has been reported in a large number of human cancers (4). The ultimate goal of this pathway is to halt cell proliferation by regulating the oncoprotein YAP and TAZ. In proliferating cells, YAP and TAZ translocate to the nucleus where they bind to TEAD transcription factors. This binding promotes the transcription of various genes essential for cellular growth and division (5). When the Hippo pathway is active, YAP and TAZ get phosphorylated by their upstream kinases LATS1/2. Phosphorylation of YAP and TAZ results in their interaction with 14-3-3 proteins, leading to their cytoplasmic retention, and eventual degradation via the ubiquitin-proteosome pathway (5).

Numerous stimuli such as contact inhibition, cytoskeletal deformation (6), serum starvation, and glucose deprivation (7) have been shown to activate the Hippo pathway. Further, our lab recently reported that tetraploidy functions as a potent inducer of the Hippo pathway (3). However, the precise molecular mediators responsible for the Hippo pathway activation under these conditions remain poorly understood. Mst1/2, which are members of the mammalian Sterile-20 like kinases (Stk25), have been described as canonical regulators of LATS1/2, as they phosphorylate and activate these downstream kinases. However, recent reports have found that Hippo pathway activation by the aforementioned physiologic stimuli appears to be entirely independent of Mst1/2 (1). Likewise, our lab discovered that tetraploidy activated the Hippo pathway in an Mst1/2 independent fashion (6).

In order to identify critical molecular mediators of the Hippo pathway that may be responsible for these Mst1/2 independent phenomena, our lab recently performed a focused kinome screen, which identified kinase members of STRIPAK complex as potential regulators of Hippo signaling. Of these kinase members, Mst4 and Stk25 were the most prominent hits. Since then, we have followed up and validated the role that these kinases play in regulating the Hippo pathway in response to actin cytoskeleton dynamics, contact inhibition, and serum starvation. This grant will be a continuation of this larger project to comprehensively identify the specific molecular interactions and mechanisms underlying Mst4 and Stk25 mediated activation of the Hippo pathway. In particular, I will focus on elucidating the relationship between Stk25 and LATS1/2, as loss of Stk25 was found to reduce phosphorylation of both LATS and YAP in vitro.

Is Stk25-mediated activation of Hippo signaling mediated by LATS1/2?

What methods will be used?

This section should be about 100-500 words. It should begin with a clear "if-then" sentence as follows: "If (restate the hypothesis) is true, then (state what will happen if you perform this experiment)." This is followed by a brief discussion of the methods being employed, needed controls, etc. Be sure to cite any sources for methods or reagents previously developed.

If Stk25-mediated activation of Hippo signaling is mediated by LATS1/2, then LATS1/2 must be expressed for Stk25-dependent Hippo signaling up-regulation.
And, If Stk25-mediated activation of Hippo signaling is mediated by LATS1/2, then Stk25 may directly interact with Lats1 and/or Lats2.
In our prior studies, we observed that Stk25 increased phosphorylation of YAP, as well as LATS1/2. This suggests that Stk25 may cause YAP phosphorylation through LATS1/2 activation. We will evaluate the presence of this Stk25-LATS1/2-YAP axis by using MEF (mouse embryonic fibroblast) cells from transgenic mice that have had LATS1/2 stably deleted (LATS1/2 KO). These LATS1/2 KO MEF cells will be used to generate cell lines that either stably overexpress FLAG-Stk25 or GFP as control. We will then evaluate the level of Hippo pathway activation in these cells via quantitative immunoblotting for Hippo pathway components, including phospho-LATS, LATS1, LATS 2, phospho-YAP, YAP, and TAZ. We will also utilize immunofluoresnce to examine YAP and TAZ nuclear-to-cytoplasmic ratios of these cell lines. All experiments will be performed in triplicate. These series of experiments will allow us to answer whether LATS 1/2 is indispensable for Stk25-mediated Hippo pathway activation.

In parallel with our studies utilizing LATS1/2 KO MEF cells, we will address whether Stk25 directly binds and phosphorylates LATS1/2. There is substantial evidence that would suggest the presence of this interaction: Mst3 (a closely related protein to Stk25) has been found to bind and directly activate NDR1/2 kinases which belong to the same kinase family as LATS1/2 (6). We hypothesize that one way Stk25 may activate the Hippo pathway is by directly phosphorylating the hydrophobic motif of LATS1/2 kinases.

To validate this hypothesis, we will generate stable cell lines of HEK293A (human embryonic kidney) cells that overexpress both Hemagglutinin (HA) tagged versions of LATS1/2 and either FLAG epitope-tagged version of either wild-type Stk25 (Stk 25 WT) or kinase dead Stk25 (Stk25 KD). Cell lysates of these cell lines will be subjected to immunoprecipitation with antibodies targeting either the FLAG or HA epitope, to assess if the converse partner kinase co-precipitates. Further, we will assess the phosphorylation status of these overexpressed HA- LATS kinases by using phos-tag gels, in which a manganese based reagent causes slower migration for proteins based on their relative degrees of phosphorylation. Western blot analysis of these phos-tag gels will allow us to visualize the degree to which a specific protein is phosphorylated. We anticipate that LATS1/2 will remain unphosphorylated in cells overexpressing the kinase dead version of Stk25, while the opposite will be true in cells that overexpress Stk25WT.

How will the data be collected and analyzed?

This section should be about 100-500 words. It will require you to describe the format the data will be collected in (e.g., measurements taken through an electron microscope). This could include a description of the expected result if your hypothesis is supported. Be sure to describe how your data will be analyzed so as to confirm or reject any of your hypotheses. This section should end with a description of the next steps for each outcome. Be sure to cite sources for any proposed analytical methods previously developed by other scientists in the field.

The LATS1/2 KO MEF cells that overexpress FLAG-Stk25 (or GFP as control) will show changes in the level of Hippo-pathway components, including phospho-LATS, LATS1, LATS 2, phospho-YAP, YAP, and TAZ on western blots. Nuclear-to-cytoplasmic ratios from immuno fluorescence of YAP and TAZ in these cell lines will indicate whether the Stk25 is interacting with YAP and/or TAZ. If Stk25 directly binds and phosphorylates LATS1/2, the stable cell lines of HEK293A that express HA-LATS1/2 and FLAG-Stk 25 (or a kinase dead Stk25) should show co-immunoprecipitation with antibodies targeting either the FLAG or HA epitope.

Cited Sources

The three previous sections (Problem, Methods, Data Analysis) should be well cited for all statements of fact that are not common knowledge and any previously developed methods and reagents. There is no limit to this section. This section should NOT be a simple bibliography list, but rather the citations should be connected to the aforementioned statements in the sections

above. The style can be either footnotes, citations numbers, or author-date. You may view citation examples here.

(1) Zhou, D., et al. (2009). Mst1 and Mst2 Maintain Hepatocyte Quiescence and Suppress Hepatocellular Carcinoma Development through Inactivation of the Yap1 Oncogene. Cell. 16, 425–438.
(2) Harvey, K. F., Zhang, X. and Thomas, D. M. (2013) The Hippo pathway and human cancer. Nature Reviews Cancer. 13, 246-257.
(3) Yu, F. X., and Guan, K. L. (2013) The Hippo pathway: regulators and regulation. Genes Dev. 27, 355-371.
(4) Moroishi, T. et al. (2015) A YAP/TAZ-induced feedback mechanism regulates Hippo pathway homeostasis. Genes Dev. 29,1271-1284.
(5) Halder , G., Dupont, S., Piccolo, S. (2012) Transduction of mechanical and cytoskeletal cues by YAP and TAZ. Nature Reviews Mollecular Biology. 6, 591-600.
(6) Stegert, M. R. et al. (2005) Regulation of NDR Protein Kinase by Hydrophobic Motif Phosphorylation Mediated by the Mammalian Ste20-Like Kinase MST3. Mol. Cell. Biol. 25, 11019-11029
(7) Wada, K.-I., Itoga, K., Okano, T., Yonemura, S. & Sasaki, H. (2011) Hippo pathway regulation by cell morphology and stress fibers. Development 138, 3907–3914.
(8) Ganem, N. J., Cornils, H., Chiu, S. Y., O'Rourke, K. P., Arnaud, J., Yimlamai, D., Théry, M., Camargo, F. D., Pellman, D. (2014) Cytokinesis failure triggers suppressor pathway activation. Cell. 158, 833-848.