NYSSEF/LISEF 2020 Research Plan

The Evaluation of the Therapeutic Potential of Orlistat in a Mouse Model of Hereditary hemorrhagic telangiectasia

Biomedical & Health Sciences

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a. RATIONALE

Hereditary hemorrhagic telangiectasia (HHT) is a genetic disorder characterized by the formation of blood vessels that do not include capillaries. This leads to the damage of the lungs, liver, skin, GI tract and the retina which may result in life threatening situations heightened by excessive bleeding. HHT is often associated with genetic mutations, specifically in the genes *ENG* (encoding endoglin) or *ACVRL1* (activin receptor-like kinase 1, Smad1/5/8 and AKT) [1,2]. While 1.4 million people are affected with this condition worldwide, there is no effective treatment or cure available. Additionally, in a recent study, an HHT patient who underwent liver transplantation with Arteriovenous Malformations (AVMs) was treated in a series of medical treatments, including Siro, and it was found to be an effective treatment in reducing the pathological symptoms of HHT [3]. For this reason, in the lab, I will determine whether certain FDA-approved drugs with known connections to other conditions could have disease-modifying properties in HHT mouse models and cellular models. I will primarily focus on Smad1/5/8 and AKT modulators for this experiment, most specifically Orlistat and Nintendanib [4]. Both are drugs that are already FDA approved and showed trends to be effective in restoring HHT pathology. I will conduct an experiment to determine whether therapeutic targeting of pathways associated (Smad1/5/8) with HHT and vascular malformations in the liver and retinas could rescue the signaling defects and vascular pathology of HHT.

b. HYPOTHESIS & RESEARCH QUESTIONS

Hypothesis:

I hypothesize that Orlistat, a drug that is FDA approved, would be efficient in ameliorating the HHT vascular pathology in a mouse model with BMP 9/10 inhibition.

Research Question:

On the basis that the BMP signaling pathway was identified as the most potent Smad1/5/8 and AKT activator, there lies several questions.

- Will the Orlistat efficiently work in the transmammary model of BMP9 - immunoblocking?
- What are the mechanisms by which Smad1/5/8 and AKT pathways are controlled?
- What is the potential of a VEGF modulator Nintendanib in combination with Orlistat?

Predicted Outcome:

I expect that Orlistat or Nintendanib will efficiently work in the trans-mammary model of BMP9/10 immunoblocked mouse in order to investigate the mechanisms by which signaling pathways are controlled.

c. RESEARCH METHODS

Procedure:

Transmammary-delivered immunoblocking of BMP9 and Orlistat treatments, and retinal vasculature analyses in mice: Retinal whole-mount histochemistry will be performed in mice [5]. The retinas of BMP9 and Orlistat intraperitoneally injected mice will be fixed in 4% paraformaldehyde for 20 min on ice and retinas will be isolated and analyzed by histochemistry.

Immunohistochemistry and AVM measurement: The retina will be stained with anti-isolectin B4 antibody. Then images will be captured for AVM width analysis, AVM count, and vascular density. As for immunohistochemistry analysis, images for the analysis of the vascular network density will be acquired using a laser confocal microscopy. Quantification will be done by using the measure particles tool and measuring the area occupied by the vasculature in a region of interest.

Western Blot: To determine altered BMP signaling, followed by Orlistat or Nintendanib treatment, I will perform SDS-PAGE electrophoresis and Western blot using anti-Smad, anti-AKT or Actin antibodies. Proteins will be extracted from livers.

Risk and Safety: I will be working with relatively strong and toxic chemicals which may be harmful when swallowed or when directly exposed to the skin or eyes. Therefore, I will wear lab gloves, goggles and a lab coat at all times. I will conduct safety training through the Feinstein Institute program. The handling of hazardous chemicals will be done under the supervision of qualified lab personnel.

Data Analysis: Quantifications will be performed using ImageJ. The analyses will be performed using GraphPad Prism 7 and P value <0.05 will be considered to be statistically significant between the experimental and control groups.

d. DISCUSSION OF RESULTS

AVM Formation in Retina: The retinal model of a mouse can be studied to determine pathological angiogenesis during different stages of vascular development [6]. In the same context, the *in vivo* potential of Orlistat and Orlistat+Nitendonib to modulate HHT vascular pathology will be assessed. If the irregular arrangement of blood vessels formerly in the retina is no longer present, this would demonstrate the effectiveness of Orlistat in which the blood vessels have returned to their normal arrangement. The number of AVMs will be measured using an ImageJ software and will be analyzed to test the effectiveness of Orlistat in reducing the number of AVMs.

Different Smad1/5/8 and AKT Modulators: In order to see if Orlistat can reduce BMP9-specific Smad1/5/8 signaling, I will conduct a Western Blot as mentioned above. If the Orlistat along with Nitendanib alters vascular pathology, I may see the different activation/ phosphorylation of Smad and AKT. Actin will be used for the loading control in each condition.

Alternative methods to measuring vascular malformation: HHT pathogenesis requires the

overactivation of the mTOR and VEGFR2 pathways [7]. I will test to determine the effect of Orlistat is through the use of a bleeding assay. The effectiveness of the drug can be analyzed through measuring the amount of blood that is caused by vascular malformations.

This drug repurposing strategy has the potential to fast-track new clinical investigations for HHT by furthering the knowledge on the effectiveness of Orlistat along with other modulators.

e. BIBLIOGRAPHY

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[4] Sakaro AI, Marotta PJ and McAlister VC (2006) Regression of cutaneous and gastrointestinal telangiectasia with sirolimus and aspirin in a patient with hereditary hemorrhagic telangiectasia. *Ann. Intern. Med.* 144(3):226–227.

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Item #4. Hazardous chemicals, activities & devices:

I will conduct safety training through the Feinstein program which includes an assembly along with several videos. The handling of all hazardous chemicals will be conducted under the supervision of qualified lab personnel. Potentially hazardous agents will include Triton X-100, Sodium Dodecyl Sulfate (SDS), Acrylamide, Methanol, and paraformaldehyde. *In vivo* treatment of Dimethyl Sulfoxide (DMSO), Orlistat and Nintendanib will be performed by a supervisor or lab personnel. Hazardous chemicals may be harmful when swallowed or when directly exposed to the skin or eyes. Methanol and paraformaldehyde are flammable liquid and vapour. Therefore, I will need to wear lab gloves, goggles and a lab coat at all times, store containers in a well-ventilated place and use appropriate equipment. Containers will always be tightly closed as well. All materials will be disposed at a specific container labeled in the lab. Organic wastes will be placed in a separate tank and will be appropriately disposed of by a lab member other than myself.

- Triton X-100 -Sigma-Aldrich
  1% Triton-X-100 in PBS solution will be used for cell lysate preparation.
- Sodium Dodecyl Sulfate (SDS)-Sigma-Aldrich
  10% SDS solution will be used for SDS-PAGE/Western blot.
- Acrylamide-Sigma-Aldrich
  30% Acrylamide solution will be used for SDS-PAGE/Western blot.
- Methanol-Sigma-Aldrich
  20% Methanol will be used for transferring membrane during Western blot.
- Paraformaldehyde-Sigma-Aldrich
  4% ready-to-use solution (neutralized in PBS) will be used for tissue fixing under laminar hood.