

**Presenter:** Jane Yu, PhD

**Institution:** Fox Chase Cancer Center/ Brigham and Women's Hospital/  
Harvard Medical School

**Lab/Department/Division:** Lisa Henske Lab

**Presentation type:** Virtual

### **Estrogen Promotes the Survival and Pulmonary Metastasis of Tuberin-Deficient Cells**

**Jane Yu**<sup>1</sup>; Chunrong Wang<sup>1</sup>; Tasha Morrison<sup>1,2</sup>; Lisa Hernandez-Cuebas<sup>3</sup>; Aris Astrinidis<sup>3</sup>; Magdalena Karbowiczek<sup>1</sup>; Laura Seeholzer<sup>1</sup>; Eric Ariazi<sup>1</sup>; Craig Jordan<sup>1</sup>; Cheryll Walker<sup>4</sup>; Elizabeth Petri Henske<sup>1,2</sup>

<sup>1</sup>Fox Chase Cancer Center, Philadelphia; <sup>2</sup>Brigham and Women's Hospital/Harvard Medical School, Boston; <sup>3</sup>Drexel University College of Medicine, Philadelphia; <sup>4</sup>University of Texas MD Anderson Cancer Center, Houston

#### **Abstract:**

Lymphangioleiomyomatosis (LAM) is a devastating disease affecting young women. To determine whether estrogen promotes the metastasis of TSC2-null cells, we established a xenograft mouse model of LAM. TSC2-deficient rat uterine leiomyoma (ELT3) cells were injected into male or female ovariectomized CB17-scid mice implanted with estrogen or placebo pellets. All mice developed tumors. E2-treated primary tumors higher levels of nuclear phospho-p42/44 MAPK compared with placebo. These results suggest that the MEK/MAPK pathway contributes to the E2-induced metastatic potential of Tsc2-null ELT3 cells. We treated the mice with the MEK1/2 inhibitor, CI-1040 beginning one day post cell inoculation. CI-1040 reduced the number of circulating tumor cells, and decreased the number of lung metastases by 100%. When ELT3 cells were injected intravenously, E2 enhanced their survival, and CI-1040 blocked the lung colonization. In vitro, E2 inhibited their anoikis, which was associated with enhanced MAPK phosphorylation and decreased levels of the pro-apoptotic Bim-1. Both the increased survival and resistance to anoikis were reduced by the MEK inhibition. These data indicate that the activation of MEK/MAPK signaling pathway might contribute to the elevated metastatic phenotype of ELT3 cells. To investigate the role of the estrogen receptor and mTOR pathway, we treated the mice with Fulvestrant or RAD001. RAD001 completely blocked both the primary tumor development and the estrogen-induced metastasis. Fulvestrant did not inhibit the primary tumors, but completely blocked estrogen-promoted lung metastases. This animal model may have relevance to both LAM pathogenesis and to the development of targeted therapeutic strategies for LAM.



The Lymphangioleiomyomatosis/Tuberous Sclerosis Complex Seminar Series

# LAM/TSC

## Seminar Series 2008-9 Live/Virtual Poster Session

Harvard Medical School  
New Research Building, Room 350  
Thursday, November 6th, 2008  
5pm-7pm

[www.LAMTSCSeminarSeries.org](http://www.LAMTSCSeminarSeries.org)

# Welcome to the inaugural LAM/TSC Graduate Student and Post- Doc Poster Session!

We are excited to have you join us as we highlight contributions of graduate students and post-docs throughout the globe in advancing LAM and TSC treatment. Stay with us for the entire evening to be a part of probing Q&A interviews with judges, presenters and members of the research community and final awards of cash prizes. Winners will be invited to present their work at our January 2009 seminar!

**Presenter:** Chelsey Woodrum

**Institution:** Brigham and Women's/Harvard Medical School

**Lab/Department/Division:** Sandra Dabora Lab

**Presentation type:** In person

## Treatment Strategies for TSC and LAM

Nancy Lee<sup>1</sup>; Alison Nobil<sup>1</sup>; **Chelsey Woodrum**<sup>1</sup>; Elizabeth Thiele<sup>2</sup>; David Franz<sup>3</sup>; Stephen Ashwal<sup>4</sup>; Francis DiMario<sup>5</sup>; Daniel Miles<sup>6</sup>; Arthur Sagalowski<sup>7</sup>; Judi Manola<sup>8</sup>; Judy Garber<sup>9</sup>; Sandra Dabora<sup>1</sup>

<sup>1</sup>Division of Translational Medicine, Brigham and Women's Hospital; <sup>2</sup> Massachusetts General Hospital; <sup>3</sup>Cincinnati Children's Hospital; <sup>4</sup>Loma Linda University Medical Center; <sup>5</sup>Connecticut Children's Medical Center; <sup>6</sup>NYU School of Medicine; <sup>7</sup>UT Southwestern Medical Center; <sup>8</sup>Dana-Farber/Harvard Cancer Center; <sup>9</sup>Dana-Farber Cancer Institute

### Abstract:

Tuberous sclerosis complex (TSC) is a multi-organ autosomal dominant tumor disorder that causes significant morbidity. Hamartin and tuberin, the TSC1 and TSC2 gene products respectively, form a complex that inhibits mTOR kinase activity in a conserved cellular signaling pathway that regulates growth and protein translation. Rapamycin, an mTOR kinase inhibitor, has been shown to normalize dysregulated mTOR signaling suggesting that mTOR kinase inhibition may be a useful approach to systemic therapy for TSC. Preclinical studies using Tsc2+/- mice demonstrate the efficacy of a prolonged maintenance dose of rapamycin as a single agent and in combination with IFN-g. We have also used a Tsc2-/- subcutaneous tumor model to evaluate sorafenib, atorvastatin, and doxycycline as single agents and in combination with rapamycin. This preclinical work will help guide the optimal design of future clinical trials for TSC tumors and related disorders. We and our collaborators (Drs. Elizabeth Thiele, David Franz, Steve Ashwal, Arthur Sagalowsky, Fran DiMario, Daniel Miles, Judy Garber, Judith Manola) have recently implemented a related multi-center phase II trial for patients with kidney angiomyolipomas associated with TSC. An overview of our preclinical studies and ongoing TSC clinical trial will be presented.

### **This evening's agenda:**

- 5:00-5:45pm:** Introduction/Judging begins\*  
\*Abstracts circulated in advance and posters available for viewing at 4:30pm
- 5:45-6:00pm:** Judges convene and select top 5 posters
- 6:00-6:30pm:** 5 minute presentations of top 5 posters by presenters
- 6:30-6:45pm:** Judges convene to choose winners  
Food is served  
Networking Opportunity
- 6:45-7:00pm:** Winners announced, 3 cash prizes awarded

**Presenter:** Shomit Sengupta  
**Institution:** Massachusetts Institute of Technology  
**Lab/Department/Division:** Department of Biology  
**Presentation type:** In person

### **Transgenic Mice Overexpressing Rheb2 Develop Tumors with Similarities to Angiomyolipomas**

**Shomit Sengupta**<sup>1</sup>; Stephanie E. Oh<sup>1</sup>; Michael Brown<sup>2</sup>; David Sabatini<sup>1,3,4,5</sup>

<sup>1</sup>MIT, Department of Biology; <sup>2</sup>Koch Institute, MIT; <sup>3</sup>Whitehead Institute; <sup>4</sup>Broad Institute; <sup>5</sup>HHMI

#### **Abstract:**

Tuberous sclerosis is an autosomal dominant syndrome characterized by the presence of benign tumors in multiple organs. Tuberous sclerosis is caused by mutations in either Tsc1 or Tsc2- proteins that form a heterodimeric complex with GAP activity towards the mTORC1 activating GTPase Rheb. As a result, it is thought that hyper-activation of mTORC1 signaling is central to the development of tumors in patients with tuberous sclerosis. A large majority of patients with TSC develop angiomyolipomas (AMLs)- neoplasms containing fat, vascular, and smooth-muscle elements. In addition, AMLs are also found in patients with Lymphangiomyomatosis (LAM) which is a disorder found either sporadically or associated with TSC. Given the heterogeneous nature of AMLs, mouse models would be useful to research how such tumors arise, progress, and could be treated. We generated mice overexpressing Rheb2 in multiple tissues under administration of doxycycline. Overexpression of Rheb2 mimics the loss of Tsc1 or 2 by hyperactivating mTORC1. Upon overexpression of the transgene, mice exhibit a wide array of phenotypes including multi-focal growths originating from the mesentery. These growths are composed of primarily fatty tissue as well as blood vessels, gas and blood filled cysts, and inflammatory cells. Histological analysis reveals subsets of cells with increased phospho-S6 levels, HMB-45 and MITF positivity. The size and severity of growths are dependent on duration of transgene expression, and removal of doxycycline after tumor formation leads to regression. Further work is needed to confirm the initial histological and pathological findings.

# Special Thank You to:

## Our Judges:

**Joe Avruch, MD, PhD**

Massachusetts General Hospital/ Harvard Medical School

**Myles Brown, MD**

Dana-Farber Cancer Institute/ Harvard Medical School

**Lew Cantley, PhD**

Beth Israel Deaconess Medical Center/ Harvard Medical School

**Augustine Choi, MD**

Brigham and Women's Hospital/ Harvard Medical School

**Elizabeth (Lisa) Henske, MD**

Brigham and Women's Hospital/ Harvard Medical School

**David Kwiatkowski, MD, PhD**

Brigham and Women's Hospital/ Harvard Medical School

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# Our Presenters:

## **Roberta Beauchamp**

Massachusetts General Hospital/ Harvard Medical School  
Center for Human Genetic Research

## **Manuela Funke, MD**

Massachusetts General Hospital/ Harvard Medical School  
Department of Rheumatology

## **Anne Gallagher, PhD**

Massachusetts General Hospital/ Harvard Medical School  
Herscot Center for TSC

## **Greg Hoffman, PhD**

Harvard Medical School  
Blenis Lab

## **Jingxiang Huang**

Harvard School of Public Health  
Department of Genetics and Complex Disease  
Manning Lab

## **Jun Kawasaki**

Children's Hospital Boston/ Harvard Medical School  
Department of Surgery and Vascular Biology  
Chan Lab

## **Anu Krishnan, MD MRCP**

St. Vincent's Hospital, Sydney, Australia  
Department of Thoracic Medicine

**Presenter:** Yakov Peter, PhD

**Institution:** Brigham and Women's/ Harvard Medical School

**Lab/Department/Division:** Pulmonary and Critical Care Medicine

**Presentation type:** In person

## **Evaluation of the Lung Repair Process in LAM/TSC**

**Yakov Peter**<sup>1</sup>; Simon D. Spivack<sup>2</sup>; Edward P. Ingenuito<sup>3</sup>; Steven D. Shapiro<sup>4</sup>  
<sup>1</sup>Department of Cell Biology, Yeshiva University, New York, NY 10033; <sup>2</sup>Department of Pulmonary Medicine, Albert Einstein College of Medicine, Bronx, NY 10461; <sup>3</sup>Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 022115; <sup>4</sup>Department of Medicine, University of Pittsburgh, PA 15261

### **Abstract:**

Advances in regenerative medicine will enhance our understanding of tissue repair with the goal to alleviate syndromes such as lymphangioliomyomatosis and tuberous sclerosis complex (LAM/TSC). To dissect out the repair process and isolate cells involved in lung regeneration we inflict alveolar damage and collect a unique population of viable cells that expand and differentiate in culture. We refer to these cells as the lung reparative population (LRP). Cells of the LRP express high levels of alpha smooth muscle actin, commonly found in smooth muscle, and surfactant protein C, a marker of alveolar type II cells. *In vitro* the LRP can migrate and self assemble to spontaneously form both polyhedral and spherical shaped tissue-like structures, but not cysts. Sheep lung extracts show similar morphological, structural and biochemical properties to those of mice and engraftment of the LRP into a host mouse lung results in integration and generation of new tissue. We are currently collecting human lung tissue to isolate and study the properties of the human LRP. These can then be compared to those from distinct organisms, genders, and even diseased specimens. Elucidation into the cells and pulmonary repair mechanisms will open new avenues and contribute to the development of novel cell-based therapies for LAM/TSC.

**Presenter:** Melika Mozzafari

**Institution:** Erasmus Medical Center, Rotterdam, The Netherlands

**Lab/Department/Division:** Department of Clinical Genetics

**Presentation type:** Virtual

**Functional Analysis of TSC1 Missense Mutations Identified in Individuals with Tuberous Sclerosis Complex**

**Melika Mozzafari:** Marianne Hoogeveen-Westerveld; Mark Nellist

Department of Clinical Genetics, Erasmus Medical Center, 3015 GE Rotterdam, The Netherlands

**Abstract:**

We have investigated the effects of putative 13 TSC1 missense mutations identified in individuals with signs and/or symptoms of TSC on the TSC1-TSC2 complex and mTOR signalling. We show that specific amino acids substitutions close to the N-terminal of TSC1 reduce steady state levels of TSC1, resulting in the activation of mTOR signalling.

**Xin Lu, MD**

Massachusetts General Hospital/ Harvard Medical School  
Department of Pathology

**Jian Ma, PhD**

Brigham and Women's Hospital/ Harvard Medical School  
Kwiatkowski Lab

**Sarah Mahoney**

Harvard Medical School  
Department of Cell Biology  
Blenis Lab

**Melika Mozzafari**

Erasmus Medical Center, Rotterdam, The Netherlands  
Department of Clinical Genetics

**Yakov Peter, PhD**

Brigham and Women's Hospital/ Harvard Medical School  
Pulmonary and Critical Care Medicine

**Shomit Sengupta**

Whitehead Institute/ Massachusetts Institute of Technology

**Chelsey Woodrum**

Brigham and Women's Hospital/ Harvard Medical School  
Dabora Lab

**Jane Yu, PhD**

Fox Chase Cancer Center, Philadelphia, Pennsylvania  
Henske Lab

**Presenter:** Roberta Beauchamp

**Institution:** Massachusetts General Hospital/ Harvard Medical School

**Lab/Department/Division:** CHGR-Simches 5<sup>th</sup> floor

**Presentation type:** In person

### **MED28 Functions as a Repressor of Smooth Muscle**

#### **Differentiation: Implications for Development and Disease**

**Roberta L. Beauchamp**<sup>1</sup>; Kim S. Beyer<sup>1</sup>; James F. Gusella<sup>1</sup>; Anders M. Näär<sup>2</sup>; Vijaya Ramesh<sup>1</sup>

<sup>1</sup>Massachusetts General Hospital, Center for Human Genetic Research, Boston, MA

<sup>2</sup>MGH Cancer Center, Department of Cell Biology, Harvard Medical School, Charlestown, MA

#### **Abstract:**

We previously isolated MED28 (magacin) as an interactor of the neurofibromatosis 2 (NF2) tumor suppressor protein merlin. MED28 is also a subunit of the mammalian Mediator complex. Mediator is a conserved transcriptional cofactor, playing an essential role in positive/negative regulation of transcription. Distinct Mediator subunit composition, and its interaction with subsets of transcription factors is thought to contribute to gene regulation specificity. In this study, downregulation of MED28 in NIH3T3 and C2C12 cells results in a significant induction of several genes associated with smooth muscle cell (SMC) differentiation. Conversely, MED28 overexpression represses SMC genes. More importantly, multipotent mesenchymal murine precursors (C2C12) can transdifferentiate into SMCs when Med28 is downregulated. Our data also show that MED28 functions as a negative regulator of SMC differentiation in concert with other Mediator head module subunits, as well as the negative regulatory subunit CDK8. Smooth muscle cells arise from multiple types of progenitors throughout development. They are highly plastic, readily switching between proliferative and differentiated states in response to extracellular cues. Med28 is expressed in mouse embryonic stem cells and in developing mouse and human tissues enriched in smooth muscle including intestine, heart, and lung. In addition, MED28 is highly expressed in a subset of estrogen-responsive human tumors including breast carcinoma and uterine leiomyomas. We believe that MED28 plays a critical role in smooth muscle development in both heart and lung, having potential implications for disorders associated with abnormalities in SMC growth and differentiation including atherosclerosis, asthma, hypertension as well as smooth muscle tumors. benefit in lung cancer patients with inactivation of TSC1/TSC2 and TNS signaling.

**Presenter:** Sarah Mahoney

**Institution:** Harvard Medical School

**Lab/Department/Division:** John Blenis Lab

**Presentation type:** In person

### **Phosphopeptide Analysis Yields Potentially Novel Targets of S6 Kinase**

**Sarah Mahoney**, Judit Villen; Jill Sylvester; Steve Gygi; John Blenis

#### **Abstract:**

The S6 Kinases are known to function downstream of PI3 Kinase and mTOR signaling, becoming activated through growth factor and nutrient stimulation.

Along with eIF4E-binding protein, the S6 Kinases are amongst the best-studied substrates of mTOR. The S6 Kinases play a role in cap-dependent translation, feedback inhibition of mTOR signaling through IRS-1, and cell growth. However knockout models show that these may not be the only functions of S6 Kinases.

The known targets of S6 Kinases do not fully explain their role in ribosomal biogenesis and determining cell size, highlighting the need to identify the full complement of kinase targets.

In this study, we used a SILAC mass spectrometry method in combination with immunoprecipitation with an antibody to the S6 Kinase substrate consensus phosphosite, to identify novel targets of S6 Kinase. We identified 190 peptides containing phosphorylations at the S6 Kinase substrate motif, 60 of which were at least twice as abundant in insulin-stimulated cells as compared to cells that were also treated with rapamycin. These 60 candidates for novel

S6 Kinase substrates have wide varieties of cellular functions. We plan to follow up on the mechanisms of one or more of these novel targets to elucidate how S6 Kinase may be involved in ribosomal biogenesis and other cellular functions

**Presenter:** Jian Ma, PhD

**Institution:** Brigham and Women's Hospital/ Harvard Medical School

**Lab/Department/Division:** David Kwiatkowski Lab

**Presentation type:** In person

### **TSC1 Loss Synergizes with KRAS Activation in Lung Cancer Development and Confers Rapamycin Sensitivity**

**Jian Ma**<sup>1</sup>; Mei-Chih Liang<sup>2,5</sup>; Liang Chen<sup>2,5</sup>; Piotr Kozlowski<sup>1</sup>; Wei Qin<sup>1</sup>; Danan Li<sup>2,5</sup>; Takeshi Shimamura<sup>2</sup>; Roman Thomas<sup>3</sup>; D. Neil Hayes<sup>4</sup>; Matthew Meyerson<sup>2,6</sup>; Kwok-Kin Wong<sup>2,7</sup>; David J. Kwiatkowski<sup>1</sup>

<sup>1</sup>Division of Translational Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA; <sup>2</sup>Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; <sup>3</sup>Max Planck Institute for Neurological Research and Department for Internal Medicine, University of Cologne, Cologne, Germany; <sup>4</sup>Department of Medicine, The Lineberger Comprehensive Cancer Center, The University of North Carolina School of Medicine, Chapel Hill, NC; <sup>5</sup>Ludwig Center at Dana-Farber/Harvard Cancer Center, Boston, MA; <sup>6</sup>Broad Institute of Harvard University and Massachusetts Institute of Technology, Cambridge, MA; <sup>7</sup>Department of Medicine, Brigham and Women's Hospital, Boston, MA

#### **Abstract:**

Germline TSC1/ TSC2 mutations cause Tuberous Sclerosis Complex (TSC), a hamartoma syndrome with lung involvement. To explore the potential interaction between TSC1/TSC2 and KRAS activation in lung cancer, mice were generated in which Tsc1 loss and KrasG12D expression occur in lung epithelial cells. Mice with combined Tsc1-KrasG12D mutation had dramatically reduced tumor latency (median survival 11.6 ± 15.6 weeks) in comparison to KrasG12D alone mutant mice (median survival 27.5 weeks). Tsc1-Kras G12D tumors showed consistent activation of mTORC1, and responded to treatment with rapamycin leading to significantly improved survival, while rapamycin had minor effects on cancers in KrasG12D alone mice. TSC1/TSC2 genomic loss was found in 22% of 86 human lung cancer specimens. Six of 80 lung cancer cell lines showed low TSC1/TSC2 expression and TSC null signaling (TNS), indicative of constitutive mTORC1 activation. TSC1/TSC2 reconstitution reversed this pattern and reduced the growth of the cell lines. TNS lines also showed significant sensitivity to rapamycin in soft agar growth assays, in contrast to control lines. These data indicate Tsc1/Tsc2 loss synergizes with Kras mutation to enhance lung tumorigenesis in the mouse, and suggests that mTOR inhibitors may have unique therapeutic benefit in lung cancer patients with inactivation of TSC1/TSC2 and TNS signaling.

**Presenter:** Manuela Funke, MD

**Institution:** Massachusetts General Hospital/Harvard Medical School

**Lab/Department/Division:** Department of Rheumatology

**Presentation type:** In person

### **Endothelin and Endothelin Receptors in Lymphangioliomyomatosis**

**M. Funke, MD**<sup>1</sup>; A. Gazdhar, MD, PhD<sup>1</sup>; L.-P. Nicod, MD<sup>2</sup>; T. Geiser, MD<sup>1</sup>

<sup>1</sup>Clinic of Pulmonology, Bern University Hospital, Switzerland

<sup>2</sup>Pulmonary Division, Lausanne University Hospital, Switzerland

#### **Abstract:**

Lymphangioliomyomatosis (LAM) is a rare and progressive disease of young women characterized by proliferation of immature smooth muscle cells (LAM cells). Cystic destruction and remodeling of lung parenchyma result in airway obstruction and impaired diffusion capacity, finally leading to respiratory insufficiency. No effective treatment is available so far - novel therapeutic strategies are therefore urgently needed. Since endothelin (ET-1) is known to be a potent growth factor for smooth muscle cells, we hypothesized that the endothelin pathway may be involved in the proliferation of LAM cells. We performed immunohistochemical staining in 3 patients with histologically proven LAM and consistently detected ET-1, and its receptors, ERA and ERB positive staining, mainly in the nodular lesions typically for LAM. One LAM patient was treated with the endothelin receptor antagonist bosentan during 12 months and clinically improved, supporting our hypothesis of a possible involvement of the endothelin pathway in patients with LAM. In conclusion, ET-1 and its receptors, ERA and ERB, are expressed in lung tissue obtained from patients with LAM, mainly in LAM lesions. ERB is even predominantly expressed in LAM lesions. We therefore suggest that the endothelin pathway is involved in the pathogenesis of LAM. The endothelin antagonist bosentan possibly represent a novel therapeutic strategy in patients with LAM.

**Presenter:** Anne Gallagher, PhD

**Institution:** Massachusetts General Hospital/Harvard Medical School

**Lab/Department/Division:** Herscot Center for TSC

**Presentation type:** In person

### **Tuberous Sclerosis Complex: MRI Findings Reveal 3 Different Types of Tuber Complexes in Children**

**Anne Gallagher, PhD<sup>1</sup>**; Delma Y Jarrett, MD<sup>2</sup>; Elizabeth A Thiele, MD, PhD<sup>1</sup>; P Ellen Grant, MD<sup>2</sup>

<sup>1</sup>Department of Neurology, Massachusetts General Hospital, Boston MA

<sup>2</sup>Department of Radiology, Massachusetts General Hospital, Boston, MA

#### **Abstract:**

Identification of tuber subtypes in Tuberous Sclerosis Complex (TSC) would allow searching for genotype and clinical associations which may help stratify patients for treatment trials. This study aimed to determine if cortical tubers and adjacent white matter abnormalities (tuber complexes, TC) in TSC could be classified based on MRI signal. 3D SPGR, T2, FLAIR images and ADC maps of 29 children with TSC were evaluated for increased or decreased signal intensity of cortical tubers and TC, compared to normal brain parenchyma, and to determine ADC range in a subset of lesions. Results showed that TC can be divided into three types: TC(A) are isointense on 3D SPGR and subtly hyperintense on T2 and FLAIR. TC(B) lesions are hypointense on 3D SPGR and homogeneously hyperintense on T2 and FLAIR. Their borders are not well circumscribed; they exert little mass effect and minimally disrupt the gyral pattern. ADC values are <1700 but >1300 x10<sup>-6</sup>mm<sup>2</sup>/s. TC(C) lesions are hypointense on 3D SPGR and homogeneously hyperintense on T2 but heterogeneous on FLAIR with a hypointense central region surrounded by a hyperintense rim. They exert mass effect on the surrounding brain and distort the gyral pattern. ADCs are >1900x10<sup>-6</sup>mm<sup>2</sup>/s. These different types of TC could be either the result of the same molecular process under the influence of different modifiers, or the result of three distinct molecular processes. Further work must be done to correlate these findings with the genotype and phenotype and to explore the different histopathology.

**Presenter:** Xin Lu, MD

**Institution:** Massachusetts General Hospital/Harvard Medical School

**Lab/Department/Division:** Department of Pathology

**Presentation type:** In person

### **Mutational Analysis of TSC1/2 Genes in Renal Cell Carcinoma**

**Xin Lu;** Wei Qin; Chin-Lee Wu; David Kwiatkowski

Departments of Urology and Pathology, Massachusetts General Hospital; Department of Medicine, Brigham and Women's Hospital

#### **Abstract:**

Tuberous sclerosis complex (TSC) is an autosomal dominant condition in which affected individuals develop benign tumors (hamartomas) in many organs and associated with germline mutations of the TSC1 and TSC2 genes. Slightly increased risk of renal malignancy is reported in TSC patients. However, TSC gene mutation has not been reported in renal cell carcinoma (RCC). We analyzed 50 sporadic RCCs for TSC1/2 mutations by MLPA covering the entire coding regions of the TSC genes. We found 2 mutations, including one TSC1 LOH and one TSC2 duplication in these samples. These data suggest that mutation of TSC1 or TSC2 is not frequent in sporadic RCC and that the molecular mechanisms of renal carcinogenesis in TSC patients are likely to be distinct.

**Presenter:** Anu Krishnan, MD MRCP  
**Institution:** St. Vincent's Hospital, Sydney, Australia  
**Lab/Department/Division:** Department of Thoracic Medicine  
**Presentation type:** Virtual

### **Correlation between Quantitative CT Scans, Quality of Life and Functional Parameters in LAM**

**Anu Krishnan**<sup>1</sup>; Elizabeth Silverstone<sup>2</sup>; Deborah H Yates<sup>1</sup>  
<sup>1</sup>Department of Thoracic Medicine; <sup>2</sup>Medical Imaging, St. Vincent's Hospital, Sydney, Australia

#### **Abstract:**

Background: CT scans are essential for diagnosing cystic lung diseases such as LAM. Quantitative CT scanning has previously been used in LAM and good correlation has been found between lung function and extent of air trapping as measured by emphysema software. However, little information is available regarding correlation with quality of life and other functional capacity indicators. Aim: To correlate quantitative score of cystic lesions obtained by volumetric CT software with quality of life data and functional capacity in LAM as well as pulmonary function. Methods: 17 patients with LAM underwent quantitative CT scans and lung function tests as well as six minute walk tests. Quality of life was assessed using the St. Georges Respiratory Questionnaire and breathlessness estimated using a visual analogue score (VAS). Functional capacity was assessed using a 6 minute walk test. Results: There was a significant correlation between the total lung capacity as measured by CT and TLC on lung function testing ( $r = 0.84$ ). Residual volume also correlated with CT lung volume ( $r=0.77$ ). There was no correlation with other parameters such as FEV1, FVC, diffusion capacity, blood gases or quality of life indices. There was also no correlation between functional capacity as measured by 6 minute walk distance. Conclusions: In this preliminary study, quantitative CT was useful for estimating some physiological parameters but did not correlate with quality of life or other functional information.

**Presenter:** Greg Hoffman, PhD  
**Institution:** Harvard Medical School  
**Lab/Department/Division:** Department of Cell Biology, John Blenis Lab  
**Presentation type:** In person

### **A functional siRNA screen identifies novel regulators of mTORC1 signaling**

**Greg Hoffman**; Max Hsia; John Blenis  
Harvard Medical School, Department of Cell Biology, Boston, MA

#### **Abstract:**

Several fundamental aspects of the signaling network responsible for mTORC1 activation remain poorly understood including the mechanism of Rheb activation and how mTORC1 kinase activity is regulated by cellular amino acid levels. In order to address these questions, we developed a high throughput, cell-based assay for measuring phosphorylation of rpS6 at Ser235/236 in mammalian tissue culture cells and performed a large scale siRNA screen in human cancer cells to identify likely targets for pharmacological inhibition of the pathway. As both growth factor and amino acid inputs are required for full activation of mTORC1 and subsequent phosphorylation of rpS6, knockdown of components required for both the growth factor and amino acid branches of the mTORC1 signaling network lead to a reduction in the phospho-rpS6 signal and score as hits in the primary screen. In addition to many known pathway components, a number of uncharacterized genes scored as strong hits in our screen. Bioinformatics analysis of the siRNA data set identifies a number of conserved pathways that play previously unappreciated roles in regulation of mTORC1 activity. Importantly, a siRNA mediated knockdown of many of these targets blocks mTORC1 signaling in TSC2-/- MEFs, suggesting that may serve as novel targets for therapeutic intervention for the treatment of LAM/TSC and other diseases associated with deregulated mTORC1 signaling

**Presenter:** Jingxiang Huang

**Institution:** Harvard School of Public Health

**Lab/Department/Division:** Department of Genetics and Complex Diseases, Brendan Manning Lab

**Presentation type:** In person

**Presenter:** Jun Kawasaki

**Institution:** Children's Hospital Boston/ Harvard Medical School

**Lab/Department/Division:** Joanne Chan Lab, Department of Surgery and Vascular Biology

**Presentation type:** In person

### The TSC1-TSC2 Complex is Required for Proper Activation of mTOR Complex 2

**Jingxiang Huang;** Christian C. Dibble; Mika Matsuzaki; Brendan D. Manning

Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts, USA

#### **Abstract:**

The mammalian target of rapamycin (mTOR) is a protein kinase that forms two functionally distinct complexes important for nutrient and growth factor signaling. Both complexes phosphorylate a hydrophobic motif on downstream protein kinases, which contributes to the activation of these kinases. mTOR complex 1 (mTORC1) phosphorylates S6K1, while mTORC2 phosphorylates Akt. The TSC1-TSC2 complex is a critical negative regulator of mTORC1. However, how mTORC2 is regulated and whether the TSC1-TSC2 complex is involved are unknown. We find that mTORC2 isolated from a variety of cells lacking a functional TSC1-TSC2 complex is impaired in its kinase activity toward Akt. Importantly, the defect in mTORC2 activity in these cells can be separated from effects on mTORC1 signaling and known feedback mechanisms affecting insulin receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI3K). Our data also suggest that the TSC1-TSC2 complex positively regulates mTORC2 in a manner independent of its GTPase-activating protein (GAP) activity toward Rheb. Finally, we find that the TSC1-TSC2 complex can physically associate with mTORC2 but not mTORC1. These data demonstrate that the TSC1-TSC2 complex inhibits mTORC1 and activates mTORC2, which through different mechanisms, promotes Akt activation.

### Vascular Consequences of Enhanced TOR Signaling in a Zebrafish Model

**Jun Kawasaki;** Sandrine Aegerter; Daniel Reed; Sean Hasso; Joanne Chan

Vascular Biology Program, Children's Hospital and the Dept. of Surgery, Harvard Medical School, Boston, MA 01225, USA

#### **Abstract:**

The PI3K-Akt-TOR pathway impacts a number of receptor signaling networks affecting cell-to-cell communication within a whole organism. In the vasculature, it can be activated upon ligand stimulation of the various receptor tyrosine kinases. Using the transparent zebrafish embryo, we developed a model for enhanced TOR signaling in the vasculature through down-regulation of its upstream regulators. In this model, vascular defects occurred consistently and rapidly. By 2 days of embryonic development, the caudal cardinal vein became massively enlarged, with aberrant migration of intersegmental veins along the trunk. Circulation is disrupted, leading to poor perfusion. As the zebrafish model is amenable to chemical biology and genetics, we demonstrated that this phenotype is augmented in a PTEN-deficient background. We have also used small molecule inhibitors against PI3K and TOR kinases to determine whether these vascular abnormalities could be ameliorated. Specifically, we have found incubations with rapamycin, PI-103, GDC-0941, or BEZ235, to generate different degrees of vascular rescue or toxicity. These data suggest that the zebrafish model can provide a versatile system to examine the TOR signaling pathway, as it can be activated through genetic mutations in upstream regulators or by aberrant ligand-receptor signaling levels, in diseases such as LAM and TSC.