

Presenter: Yonghao Yu, PhD
Institution: Harvard Medical School
Presentation type: In person

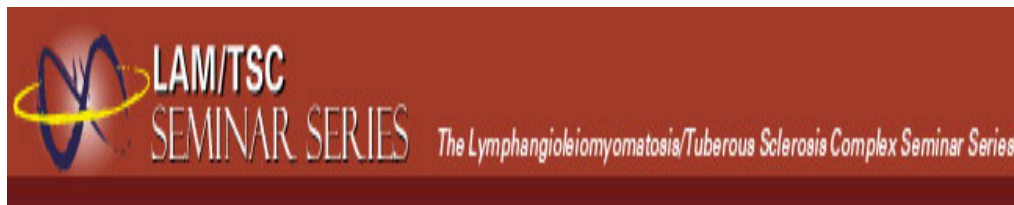
Identification and characterization of novel components within the mTORC1 signaling pathway using quantitative mass spectrometry

Yonghao Yu, PhD, Sang-Oh Yoon, PhD, Max Ma, PhD, Judit Villen, PhD, Steven P. Gygi, PhD, John Blenis, PhD

Department of Cell Biology, Harvard Medical School, Boston, MA

Abstract:

mTORC1 inhibitors, such as rapamycin and its analogs, are currently being clinically evaluated for the treatment of LAM. While these inhibitors have exhibited some promise, a number of fundamental aspects of the network remain poorly understood and very few physiological substrates of mTORC1 have been identified so far. This points to the critical need for a better understanding of the mTOR signaling network which could lead to additional therapeutic avenues in the treatment of the disease. We performed mass-spectrometry based profiling to identify protein kinase cascades involved in the mTORC1 signaling pathway. Specifically, we carried out a global, quantitative analysis of the phosphoproteome in rapamycin treated TSC2^{-/-} MEFs and identified hundreds of proteins that carried rapamycin-sensitive phosphorylation sites. In combination with in-depth characterization of the hits, we hope to provide novel mechanistic insights that will lead to the identification of novel therapeutic strategies for the treatment of LAM and related disorders.



LAM/TSC Seminar Series 2009-2010 Live/Virtual Poster Session Competition

Harvard Medical School
New Research Building, Room 350
Thursday, November 12th, 2009
5:00pm - 7:30pm
www.LAMTSCSeminarSeries.org

Welcome to the 2nd Annual LAM / TSC Graduate Student and Post-Doc Poster Session!

We are excited to have you join us as we highlight student and post-doc contributions to advancing LAM and TSC treatment research. Winners will be invited to present their work at our February 2010 seminar!

This evening's agenda:

- 4:45-5:00pm:** Poster set-up
- 5:00-5:15pm:** Introduction
- 5:15-6:45pm:** Poster Session and Judging (Judge's meet at 6:15pm to decide top 5 posters)
- 6:45-7:15pm:** Top 5 posters announced and each finalist gives a 5 minute oral presentation
- 7:15-7:25pm:** Final Judging; Winners announced and 3 cash prizes awarded
- 7:30pm:** Poster session ends

**light food and drinks provided throughout the session*

Presenter: Jane Yu, PhD

Institution: Brigham and Women's Hospital

Presentation type: In person

Development of Preclinical Models of LAM—Estrogen Promotes the Survival and Metastasis of Tuberin-Deficient Cells

Jane Yu, PhD, Tasha Morrison, BS, Faina Maychina, MS, Wei Qin, MD, David Kwiatkowski, MD, PhD, and Elizabeth Petri Henske, MD

Brigham and Women's Hospital, Boston, MA

Abstract:

Lymphangiomyomatosis (LAM) is an often fatal disease primarily affecting young women in which tuberin (TSC2)-null cells metastasize to the lungs. The mechanisms underlying the striking female predominance of LAM are not well understood. We report here that 17-beta-estradiol (E2) alters extracellular matrix architecture, decreases the type IV collagen deposit, and increases cellular matrix metalloproteinase-2 (MMP2) accumulation in Eker rat uterine leiomyoma-derived ELT3 xenograft tumors. We have discovered that estrogen induces a three to five-fold increase in pulmonary metastases of Tsc2-null ELT3 cells in male and female mice respectively. This enhanced metastasis is associated with elevated levels of circulating tumor cells and with activation of p42/44MAPK. Using a bioluminescence approach, we found that estrogen enhances the survival and lung colonization of intravenously injected Tsc2-null ELT3 cells by three-fold. CI-1040, an orally administered MEK1/2 inhibitor, blocks the lung colonization of intravenously injected cells, decreases the level of circulating tumor cells, and the metastasis of disseminated cells from the xenograft tumors. Taken together, these data reveal that the MEK pathway is a critical component of the estrogen-dependent metastatic potential of Tsc2-null cells and lead to a unique model of LAM pathogenesis with therapeutic implications in which E2 promotes the survival of disseminated tumor cells, thereby facilitating lung colonization and metastasis.

Presenter: Mary Wertz, BS (presenting for Alessia DiNardo, PhD)

Institution: Children's Hospital Boston

Presentation type: In person

Tuberous Sclerosis Complex Activity Is Required to Control Neuronal Stress Responses in an mTOR-Dependent Manner

Alessia DiNardo, PhD¹, Ioannis Kramvis¹, Namjik Cho¹, Abbey Sadowski¹, Lynsey Meikle, Ph.D², David J. Kwiatkowski, MD, PhD², and Mustafa Sahin, MD, PhD¹

¹The F. M. Kirby Neurobiology Center, Department of Neurology, Children's Hospital Boston, Harvard Medical School; and ²Division of Translational Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School

Abstract:

Tuberous sclerosis complex (TSC) is a neurogenetic disorder caused by loss-of-function mutations in either the TSC1 or TSC2 genes and frequently results in prominent CNS manifestations, including epilepsy, mental retardation, and autism spectrum disorder. The TSC1/TSC2 protein complex plays a major role in controlling the Ser/Thr kinase mammalian target of rapamycin (mTOR), which is a master regulator of protein synthesis and cell growth. In this study, we show that endoplasmic reticulum (ER) stress regulates TSC1/TSC2 complex to limit mTOR activity. In addition, Tsc2-deficient rat hippocampal neurons and brain lysates from a Tsc1-deficient mouse model demonstrate both elevated ER and oxidative stress. In Tsc2-deficient neurons, the expression of stress markers such as CHOP and HO-1 is increased, and this increase is completely reversed by the mTOR inhibitor rapamycin both in vitro and in vivo. Neurons lacking a functional TSC1/TSC2 complex have increased vulnerability to ER stress-induced cell death via the activation of the mitochondrial death pathway. Importantly, knockdown of CHOP reduces oxidative stress and apoptosis in Tsc2-deficient neurons. These observations indicate that ER stress modulates mTOR activity through the TSC protein complex and that ER stress is elevated in cells lacking this complex. They also suggest that some of the neuronal dysfunction and neurocognitive deficits seen in TSC patients may be attributable to ER and oxidative stress and therefore potentially responsive to agents moderating these pathways.

Special Thank You to:

Our Judges:

Joe Avruch, MD, PhD

Harvard Medical School/ Massachusetts General Hospital

Lew Cantley, PhD

Harvard Medical School/Beth Israel Deaconess Medical Center

Kiran Chada, PhD

Columbia University,
UMDNJ—Robert Wood Johnson Medical School

Jeanine D'Armiento, MD, PhD

Columbia University College of Physicians and Surgeons/
Columbia University Medical Center

Elizabeth (Lisa) Henske, MD

Harvard Medical School/ Brigham and Women's Hospital

Vera Krymskaya, PhD

University of Pennsylvania School of Medicine/ Abramson
Cancer Center

David Kwiatkowski, MD, PhD

Harvard Medical School/ Brigham and Women's Hospital

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Our Presenters:

Christian C. Dibble, BS

Harvard School of Public Health
Mechanisms of mTORC2 inhibition in cells deficient for the TSC1-TSC2 complex

Katrin Duevel, PhD

Harvard School of Public Health
Activation of a metabolic gene regulatory network by mTORC1 signaling

Patrick Geraghty, PhD

Columbia University-UMDNJ Robert Wood Johnson Medical School
Activation of HMGA2 pathway in benign mesenchymal tumors

June Goto, PhD

Brigham and Women's Hospital
Regulable loss of Tsc1 in neural progenitor cells yields a model of tuberous sclerosis complex (TSC) with giant cells and other features replicating cortical tubers

Sangyeul Han, PhD

Massachusetts General Hospital
Aberrant hyperactivation of Akt and mammalian target of rapamycin complex 1 (mTORC1) signaling in sporadic and TSC-associated chordomas

Sean Hasso, PhD

Children's Hospital Boston
Tissue specific requirements for TSC1 and TSC2 during zebrafish development

Neil Kubica, PhD

Harvard Medical School
Pathway-driven pharmacogenomic analysis identifies miR-195 as a critical downstream effector of mTORC1 repression in a subset of PTEN^{-/-} and TSC^{-/-} cell lines.

Presenter: Du-yu Nie, MD, PhD

Institution: Children's Hospital Boston

Presentation type: In person

TSC2-Rheb Signaling Regulates EphA-Mediated Axon Guidance

Du-yu Nie¹, MD, PhD, Alessia Di Nardo¹, PhD, Juliette M. Han¹, Mustafa Sahin¹, MD, PhD, Sandra Debora², MD, Pier Paolo Pandolfi³, MD, PhD, Elena B. Pasquale⁴, PhD

¹Children's Hospital Boston, Boston, MA; ²Brigham and Women's Hospital, Boston, MA; ³Beth Israel Deaconess Cancer Center, Boston, MA; ⁴University of San Diego, LaJolla, CA

Abstract:

Tuberous sclerosis complex (TSC) is a disease caused by mutations in either the TSC1 or the TSC2 genes, which encode a protein complex that regulates mTOR kinase signaling by inactivating the Rheb GTPase. The mechanisms by which TSC1/TSC2 deficiency results in epilepsy and autism remain largely unknown. Here, we report that Tsc2 haploinsufficiency causes aberrant retinogeniculate projections, suggesting defects in Eph/ephrin-mediated axon guidance. Furthermore, we show that EphA receptor activation leads to inhibition of ERK1/2 kinase activity and decreased inhibition of Tsc2 by ERK1/2. Thus, ephrin stimulation inactivates the mTOR pathway by stimulating Tsc2 activity. In Tsc2-deficient or Rheb-hyperactive neurons, mTOR is constitutively activated, and ephrin-induced growth cone collapse is reduced. Our results demonstrate that TSC2-Rheb-mTOR signaling cooperates with the ephrin-Eph receptor system to control axon guidance in the visual system.

Presenter: Tasha Morrison, BS

Institution: Brigham and Women's Hospital

Presentation type: In person

Estrogen Enhances Resistance to Anoikis in Tuberin-Deficient Cells

Tasha A. Morrison, BS, Jane Yu, PhD, and Elizabeth P. Henske, MD

Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Abstract:

Introduction

Genetic evidence indicates that lymphangioleiomyomatosis (LAM) is the result of benign metastasis of tuberin (Tsc2)-null cells. The molecular mechanisms underlying LAM pathogenesis is not well known. Metastatic cancer cells have the ability to resist anoikis—apoptosis due to loss of extracellular matrix attachment. In a xenograft model of LAM, we found that 17-beta-estradiol (E2) causes a significant increase in circulating tumor cells and promotes lung metastasis. The metastatic phenotypes are associated with the activation of p42/44 MAPK and are inhibited by MEK1/2 inhibitor, CI-1040. We hypothesize that E2 promotes survival of Tsc2-null cells placed in circulation. To determine the components that mediate estrogen-enhanced survival of Tsc2-null cells, we analyze the pro-apoptotic protein Bim (Bcl-2 interacting mediator of cell death), a critical activator of anoikis. Bim is phosphorylated by p42/44 MAPK, leading to proteasomal-mediated degradation.

Results

We found that estrogen decreases levels of cleaved caspase-3 and DNA fragmentation, indicating that E2 promotes resistance to anoikis. We also found that Bim accumulation is reduced at 1 hour of detachment, which is associated with enhanced cell survival. PD98059 treatment blocks E2-reduced Bim and increases levels of cleaved caspase-3 and DNA fragmentation, suggesting that E2-induced resistance to anoikis is MAPK-dependent. In adherent cells we found that estrogen decreases Bim transcripts at 24 hours of E2 stimulation, measured by real-time RT-PCR. E2 also reduces Bim protein levels. Pre-incubation of cells with MG132 and PD98059 blocks estrogen's reduction of Bim. In conclusion, we found that E2 regulates pro-apoptotic protein Bim, in Tsc2-null cells. Inhibition of MEK1/2 and proteasomal activity block E2-regulated Bim accumulation and cell survival. We anticipate that targeting signaling pathways contributing to Bim activation and proteasome activity may have clinical specificity and significance in treating LAM.

Vincent Lemaitre, PhD

Columbia University College of Physicians and Surgeons
Inhibition of the mTOR/p70S6K signaling pathway with Rapamycin induces collagenase expression in endothelial cells

Izabela Malinowska-Kolodzie, MD, PhD

Brigham and Women's Hospital

Lymphangioleiomyomatosis - experience with analysis and culture of 27 chylous pleural effusions

Alessandra L. Moore

Massachusetts General Hospital

Microfluidic detection of circulating LAM cells for the non-invasive detection, monitoring and genotyping of LAM disease

Tasha Morrison, PhD

Brigham and Women's Hospital

Estrogen Enhances Resistance to Anoikis in Tuberin-Deficient Cells

Du-yu Nie, MD, PhD

Children's Hospital Boston

TSC2-Rheb Signaling Regulates EphA-Mediated Axon Guidance

Mary Wertz (presenting for Alessia DiNardo)

Children's Hospital Boston

Tuberous Sclerosis Complex Activity Is Required to Control Neuronal Stress Responses in an mTOR-Dependent Manner

Jane Yu, PhD

Brigham and Women's Hospital

Development of Preclinical Models of LAM - Estrogen Promotes the Survival and Metastasis of Tuberin-Deficient Cells

Yonghao Yu, PhD

Harvard Medical School

Identification and characterization of novel components within the mTORC1 signaling pathway using quantitative mass spectrometry

Presenter: Christian Dibble, BS
Institution: Harvard School of Public Health
Presentation type: In person

Mechanisms of mTORC2 inhibition in cells deficient for the TSC1-TSC2 complex

Christian C. Dibble¹, Jingxiang Huang¹, PhD, John M. Asara², PhD, Brendan D. Manning¹, PhD

¹Harvard School of Public Health, Boston, MA; ² Beth Israel Deaconess Medical Center, Department of Medicine, Harvard Medical School, Boston, MA

Abstract:

While there has been great progress in understanding the regulation of mTORC1 and its inhibition by the TSC1-TSC2 complex, little is known about the signaling mechanisms that regulate mTORC2 and whether the TSC1-TSC2 complex is involved. Recent studies from our lab have revealed new mTORC1-dependent and -independent mechanisms by which the TSC1-TSC2 complex regulates mTORC2. We have found that in cells lacking a functional TSC1-TSC2 complex, the intrinsic kinase activity of mTORC2 is impaired and signaling to both PI3K-dependent and -independent mTORC2 substrates is attenuated. Importantly, this defect is separable from Rheb and mTORC1-driven inhibitory feedback mechanisms. To better understand mTORC2 regulation we have also identified 21 phosphorylation sites on the essential mTORC2 component Rictor and found that one of these sites, T1135, is phosphorylated by S6K1 downstream of mTORC1. Although Rictor-T1135 phosphorylation does not affect the kinase activity of mTORC2, expression of a non-phosphorylatable Rictor-T1135A mutant in cells causes increased mTORC2-dependent phosphorylation of Akt-S473 in response to insulin. Therefore, phosphorylation of Rictor-T1135 by S6K1 likely constitutes a new negative feedback mechanism affecting Akt. Consistent with it being a substrate of S6K1, Rictor-T1135 is constitutively phosphorylated in TSC1-TSC2-deficient cells and this could contribute to the misregulation of Akt. However, additional mechanisms must account for the severe impairment of mTORC2 kinase activity and comprehensive attenuation of mTORC2 substrates we observe in these cells. Our studies add to the understanding of mTORC2 regulation under both physiological and pathological conditions and help implicate mTORC2 inactivation in the pathogenesis of tuberous sclerosis complex and lymphangioleiomyomatosis.

Presenter: Alessandra L. Moore, BS
Institution: Massachusetts General Hospital
Presentation type: In person

Microfluidic detection of circulating LAM cells for non-invasive detection, monitoring and genotyping of LAM disease

Sunitha Nagrath¹, PhD, **Alessandra L. Moore**¹, **BS**, Mahnaz Zenali¹, BS, Elizabeth Peterson², BSN, RN, Elizabeth Henske^{2,4}, MD, Mehmet Toner^{1,3}, PhD

¹Center for Engineering in Medicine, Massachusetts General Hospital; ²Center for LAM Research and Clinical Care, Brigham and Women's Hospital; ³Department of Surgery, Massachusetts General Hospital; ⁴Department of Medicine, Brigham and Women's Hospital, Boston, MA

Abstract:

Lymphangioleiomyomatosis (LAM) is a tragic disease characterized by the aberrant growth of smooth muscle-like cells in the lungs and kidneys of reproductive age women. To date, no effective methods of molecular characterization exist due to the unavailability of LAM specific cells. The result of lacking molecular information on the disease is that there is currently no effective therapy for the treatment of LAM, making prognoses grim. However, it is theorized that metastasis may be a mechanism by which LAM cells disseminate, as circulating LAM cells (CLCs) are found in blood, chyle, and urine. We propose the modification of the current "CTC-chip", (a break-through microfluidic based technology used for the isolation of circulating tumor cells from whole blood), to capture and characterize CLCs. By exchanging the "CTC-chip" capture antibody of epithelial cell adhesion molecule (EpCAM) for one of the proposed biomarkers of LAM, we may specifically target CLCs, creating a high throughput, point-of-care device. The creation of this novel technology may lead to early diagnoses and reliable monitoring of patients throughout the course of their disease. Our current efforts led us to the cell surface antigens, CD44, its variant, CD44v6, and GPMNB as potential capture antibodies. The current identification criteria holds that cells captured using a CD44 antibody and stained positive for CD44v6 and DAPI, while stained negative for CD45, a common leukocyte marker, are CLCs. In several patients, we were able to capture high numbers (several thousand cells per milliliter of blood) of what we believe to be CLCs via this system, however, at the expense of cell purity. With modifications, including the possible use of other sarcoma markers for capture, using a clear polydimethylsiloxane (PDMS) platform, tumor cell culture, and a variety of cell staining techniques, we believe a successful CLC capture device may be developed.

Presenter: Izabela Malinowska-Kolodziej, MD, PhD

Institution: Brigham and Women's Hospital

Presentation type: In person

Lymphangioliomyomatosis - experience with analysis and culture of 27 chylous pleural effusions

Izabela Malinowska-Kolodziej¹, MD, PhD; Geraldine Finlay², MD, PhD, Cheryl Doughty¹, PhD, David Kwiatkowski¹, MD, PhD

¹Translational Medicine Division, Brigham and Women's Hospital, Boston, MA;

²Department of Pulmonary, Critical Care and Sleep Medicine, Tufts Medical Center, Boston, MA

Abstract:

Lymphangioliomyomatosis (LAM) is a rare cystic disease with progressive lung destruction and development of small nodules containing smooth-muscle like cells and lymphatic channels. LAM occurs at high frequency in adult women with Tuberous Sclerosis Complex (TSC). It also occurs in women without TSC, so-called sporadic LAM. Sporadic LAM is typically more severe than TSC-associated LAM. Chylous pleural effusions are seen in about 20% of LAM patients.

We have had the opportunity to study thoracentesis fluids from 19 patients with sporadic LAM, a total of 27 samples as some patients have provided multiple samples. Chylous pleural fluids contain a variable proportion of LAM cell clusters, as originally described by Seyama et al. (Am J Surg Pathol. 2005 29:1356-66). In our experience 12 samples contained relatively abundant LAM clusters, 5 fluids had a few clusters, and 10 samples had no or minimal clusters. LAM cell clusters consist of a central core of smooth muscle like cells that express melanocytic markers (HMB45, MART-1) and a surface layer of lymphatic endothelial cells that are LYVE+ and VEGFR3+. Some pleural fluid samples (4 of 27) contained irregularly shaped groups of cells that were of a different morphology, not true LAM cell clusters, and of uncertain origin.

In culture, we obtain Short-Term-Cultured LAM cell clusters (STC-LCC) which consist of large elongated smooth muscle-like cells and nests of lymphatic endothelial cells (LEC). Smooth muscle media supports attachment of smooth muscle like cells (smooth muscle actin+, pS6(S240/244)+), but promotes growth of numerous smaller cells of myofibroblast type from these fluids, while LEC are suppressed. This observation argues against a hypothesis that LAM cells secrete VEGF-D that supports LEC survival and growth. On the other hand, in endothelial media with VEGF-A both large SMC-like cells and LEC cells attach and spread initially. However, in all conditions tested, there is no appreciable growth of the LAM cells (Ki67-), suggesting that current in vivo conditions do not match with the growth needs of LAM cells.

Presenter: Katrin Duevel, PhD

Institution: Harvard School of Public Health

Presentation type: In person

Activation of a metabolic gene regulatory network by mTORC1 signaling

Katrin Duevel¹, Pichai Ramen², Jessica Yecies¹, Suchithra Menon¹, Alex Kipovski¹, Stephen Clever², Leon Murphy², Brendan Manning¹

¹Department of Genetics and Complex Disease, Harvard School of Public Health, Boston, MA; ² Novartis Institute for Biomedical Research, Cambridge, MA

Abstract:

We are using cell and tumor models affecting the TSC1-TSC2 complex to understand the cellular and molecular effects of elevated mTORC1 activity, which can be detected in the majority genetic tumor syndromes and sporadic cancers. Loss of function of the TSC1-TSC2 complex results in constitutive mTORC1 signaling that is no longer sensitive to perturbations in cellular growth conditions. Using a combination of TSC gene disruption and rapamycin to, respectively, activate and inhibit mTORC1, we have identified a set of transcripts that are strictly regulated by mTORC1 signaling. Through this approach, we have characterized a metabolic gene regulatory network stimulated by mTORC1 that alters the bioenergetic properties of cells to promote anabolic processes underlying cell growth and proliferation. Metabolic assays and metabolomic profiling of TSC-deficient cells and their response to rapamycin support these genomic findings. These alterations in basic cellular metabolism offer novel points of therapeutic intervention for the many tumors exhibiting uncontrolled mTORC1 activation, such as those arising in the TSC and LAM diseases.

Presenter: Patrick Geraghty, PhD

Institution: Columbia University, UMDNJ-Robert Wood Johnson Medical School

Presentation type: Virtual

Activation of HMGA2 pathway in benign mesenchymal tumors

Patrick Geraghty, PhD, Devipriya Sankarasharma, PhD, Jeanine D'Armiento, MD, PhD, Kiran Chada, PhD

Columbia University, UMDNJ-Robert Wood Johnson Medical School, New York, NY

Abstract:

High mobility group A2 (HMGA2) is an architectural transcription factor and primarily expressed in the undifferentiated mesenchyme. We previously demonstrated that HMGA2 is misexpressed in benign mesenchymal tumors of a differentiated phenotype, and subsequently that HMGA2 is also misexpressed in pulmonary lymphangiomyomatosis (LAM). To investigate the relationship between HMGA2 and TSC expression we examined Tsc2^{+/-} mice, which have a high incidence of tumors in a number of organs (including kidneys, liver, lungs, foot and eye). Hmga2 expression was absent from normal tissue but was expressed in all tumor tissue in the Tsc2^{+/-} mice. Surprisingly, tuberlin was still expressed in 100% of the mesenchymal tumors, as detected by PCR and immunohistochemistry and no mesenchymal tumors had increased active mTOR and S6K. In order to approach the question of causality, Tsc2^{+/-} mice were bred on to the Hmga2^{-/-} genetic background. There was a complete absence of mesenchymal tumors formed in the Tsc2^{+/-}-Hmga2^{-/-} mice background.

Analysis of human renal angiomyolipomas (AML) and LAM demonstrated that the HMGA2 pathway is activated in both groups of these mesenchymal tumors (100% of samples). Interestingly, 71% of the AML expressed tuberlin and just over 53% of the AML samples have increased mTOR activity. Intriguingly, all the LAM samples expressed tuberlin but 46% had increased mTOR activity. Therefore, the mouse and human studies suggest the likelihood of multiple tumorigenesis pathways being responsible for the mesenchymal tumors in tuberous sclerosis and LAM. This data suggests that activation of HMGA2 is necessary in this TSC2-associated tumorigenesis pathway.

Presenter: Vincent Lemaitre, PhD

Institution: Columbia College of Physicians and Surgeons

Presentation type: Virtual

Inhibition of the mTOR/p70S6K signaling pathway with Rapamycin induces collagenase expression in endothelial cells

Vincent Lemaitre, PhD; Abdoulaye J. Dabo, MSc; Kiran Chada*, PhD; Jeanine D'Armiento, MD, PhD

Columbia University College of Physicians and Surgeons, Department of Medicine, New York, NY; *University of Medicine and Dentistry of New Jersey, Department of Biochemistry, Piscataway, NJ

Abstract:

Progressive lung destruction develops in LAM secondary to increased protease production by LAM cells. Although trials have been initiated with the use of the drug rapamycin in the treatment of LAM, an examination of the effect of rapamycin on protease production has not been undertaken. Our laboratory has established that the protease MMP-1, expressed in the parenchymal cells of emphysema patients, can lead to tissue destruction when overexpressed in the lung of transgenic mice.

In this study, the effect of rapamycin on the modulation of MMP-1 expression was determined in human aortic endothelial cells (HAECs). Treatment of HAECs with rapamycin (100 to 300 nM for 24 hours) significantly up regulated MMP-1 mRNA and protein expression and down regulated tissue inhibitor of metalloproteinases-3, a critical MMP inhibitor and regulator of angiogenesis. Interestingly, cigarette smoke extract (CSE) also induces MMP-1 expression in endothelial cells through inhibition of mTOR. After treatment with CSE, MMP-1 mRNA expression was increased 25 times compared to non-treated cells, while TIMP-3 was down regulated (P<0.05). Treatment with CSE resulted in decreased phosphorylated p70S6K, a specific target of mTOR. Our study demonstrates that inhibition of mTOR modulates MMP-1 and TIMP-3 expression in aortic endothelial cells and warrants further examination as it may have implications in patients treated with rapamycin. Although rapamycin blocks smooth muscle cell proliferation effects on other cell types could lead to unexpected lung destruction. Additionally, the rapamycin-induced side effect of pneumonitis could potentially be secondary to the up regulation of this degradative protease.

Presenter: Neil Kubica, PhD
Institution: Harvard Medical School
Presentation type: In person

Pathway-driven pharmacogenomic analysis identifies miR-195 as a critical downstream effector of mTORC1 repression in a subset of PTEN^{-/-} and TSC^{-/-} cell lines

Neil Kubica, PhD and John Blenis, PhD

Department of Cell Biology, Harvard Medical School, Boston, MA

Abstract:

The mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase that is found in at least two distinct protein complexes with unique biological functions. The mTOR complex 1 (mTORC1) integrates numerous upstream inputs (e.g. growth factors, amino acids and intracellular energy) to regulate the balance between cellular anabolism and catabolism. Genetic lesions upstream of mTORC1 lead to chronic pathway hyperactivation and enhanced cellular anabolism, giving malignant cells a growth advantage that contributes to tumor formation and progression. It is estimated that ~80-90% of cancers exhibit mTORC1 hyperactivation, making mTORC1 a promising drug target. Unfortunately, clinical trials using analogues of the mTORC1 inhibitor rapamycin have been largely unsuccessful in the treatment of cancer. One strategy to overcome rapamycin resistance is to elucidate events downstream of mTORC1 in order to design novel diagnostics to better predict rapamycin efficacy and/or design entirely new therapeutics. One class of potential effectors of mTORC1 inhibition are microRNAs (miRNAs), a class of endogenous functional small RNAs that regulate gene expression by repressing mRNA translation. miRNA expression is widely downregulated in human cancer, suggesting that most miRNAs act as tumor suppressors. While there are several examples of inhibitory interactions between specific miRNAs and particular oncogenes, approaches designed to elucidate a comprehensive view of the relationship between critical tumorigenic cell signaling pathways and miRNAs are lacking. We will present work designed to identify and characterize miRNAs that act as functional players downstream of mTORC1 signaling network repression in cell-based models of cancer.

Presenter: June Goto, PhD
Institution: Brigham and Women's Hospital
Presentation type: In person

Regulable loss of Tsc1 in neural progenitor cells yields a model of tuberous sclerosis complex (TSC) with giant cells and other features replicating cortical tubers

June Goto, PhD¹, Wei Qin, PhD¹, Roderick T. Bronson, DVM², Delia Talos, MD³, Jennifer Chan, MD, PhD⁴, Mustafa Sahin, MD, PhD³, David Kwiatkowski, MD, PhD¹

¹Brigham and Women's Hospital, Boston, MA; ²Harvard Medical School, Boston, MA; ³Children's Hospital Boston, Boston, MA; ⁴University of Calgary, Canada

Abstract:

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder in which the hallmark clinical manifestation is the cortical tuber, seen in 90% of patients. Cortical tuber extent correlates, roughly, with the severity of TSC neurological symptoms including epilepsy, mental retardation, and autistic spectrum disorder. However, the pathogenesis of cortical tubers and how they cause morbidity in TSC are not well understood. Here we report a new mouse model of TSC due to focal loss of Tsc1 in neural progenitor cells. We have combined our conditional Tsc1 allele (Tsc1cc) with tet-inducible alleles. Pregnant dams bearing Tsc1cc Nestin-rtTA+ tet-OP-cre+ pups are treated with varying doses of doxycycline at various time points from embryonic day 8 (E8) to E16. Mice treated at E13 developed pathologic features most closely resembling cortical tubers at age 6 months. The Rosa26-LacZ allele was used to demonstrate that doxycycline administration induced recombination in neural progenitor cells in the ventricular zone and cortical plate of embryos, resulting in a decrease in Tsc1 protein levels and activation of mTOR pathway with hyperphosphorylation of ribosomal protein S6. Enlarged cells are seen predominantly in layers II-IV by P14, and progressively develop into highly vacuolated balloon-like giant cells, similar to those seen in TSC cortical tubers. The mutant mice display spontaneous seizures, and macrocephaly with subsequent failure to thrive and median survival of 40 days. These results provide support for the two hit model of tuber development, and will enable insight into the pathogenesis of epilepsy and autistic behavior in patients with TSC. .

Presenter: Sangyeul Han, PhD
Institution: Massachusetts General Hospital
Presentation type: In person

Aberrant hyperactivation of Akt and mammalian target of rapamycin complex 1 (mTORC1) signaling in sporadic and TSC-associated chordomas

Sangyeul Han¹, PhD, Carolyn Polizzano¹, BcS, Gunnlaugur P. Nielsen², MD, Francis J. Hornicek³, MD, Andrew Rosenberg², MD, Vijaya Ramesh¹, PhD

¹Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA; ²Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; ³Orthopaedic Oncology Service, Center for Sarcoma and Connective Tissue Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Abstract:

Chordomas are rare, malignant bone neoplasms in which the pathogenic mechanisms remain unknown. Interestingly, Tuberous Sclerosis Complex (TSC) is the only syndrome where the incidence of chordomas has been described. We previously reported the pathogenic role of the TSC genes in TSC-associated chordomas. In this study, we investigated whether aberrant TSC/mTORC1 signaling pathway is associated with sporadic chordomas. We assessed the status of mTORC1 signaling in primary tumors/cell lines of sacral chordomas and further examined upstream of mTORC1 signaling, including PTEN (phosphatase and tensin homologue deleted on chromosome ten) tumor suppressor. We also tested the efficacy of the mTOR inhibitor rapamycin on signaling and growth of chordoma cell lines. Sporadic sacral chordoma tumors and cell lines examined commonly displayed hyperactivated Akt and mTORC1 signaling. Strikingly, expression of PTEN, a negative regulator of mTORC1 signaling, was not detected or significantly reduced in chordoma-derived cell lines and primary tumors. Furthermore, rapamycin inhibited mTORC1 activation and suppressed proliferation of chordoma-derived cell line. Our results suggest that loss of PTEN as well as other genetic alterations which result in constitutive activation of Akt/mTORC1 signaling may contribute to the development of sporadic chordomas. More importantly, a combination of Akt and mTORC1 inhibition may provide clinical benefits to chordoma patients.

Presenter: Sean Hasso, PhD
Institution: Children's Hospital Boston
Presentation type: In person

Tissue specific requirements for TSC1 and TSC2 during zebrafish development

Sean M. Hasso, Daniel D. Reed, Jun Kawasaki, Juliette M. Han, Mustafa Sahin and Joanne Chan

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Abstract:

Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder linked to mutations in either the Hamartin (*Tsc1*) or Tuberin (*Tsc2*) genes, and is characterized by tumor-like lesions in kidney, brain, heart, lungs, and other organs. While it is known that the *Tsc1/2* gene products function as a regulatory complex upstream of RHEB (ras homolog enriched in brain) and the mammalian target of rapamycin (mTOR), the exact roles of these genes in TSC and LAM remains unclear. Using the zebrafish embryo as a model, we have investigated the role of *Tsc1/2* function during development. We have found that morpholino mediated knockdown of these genes results in distinct defects including loss of *islet-1* expressing spinal neurons, abnormal kidney morphogenesis, and cardiovascular anomalies. Molecular analysis revealed increased expression of the *Vegf* ligands; similar to the elevated VEGF-A levels in mouse *Tsc1/2* models. Treatment of *Tsc1/2*-MO injected embryos with the mTORC1 inhibitor, rapamycin, is sufficient to rescue some, but not all of the observed phenotypes. These data demonstrate a tissue specific requirement for *Tsc1/2* function during embryonic development, and further suggest mTOR independent functions for *Tsc1/2* in specific cell types and organ systems. Our observed phenotypes, the induction of VEGF expression, and the ability to rescue aspects of *Tsc1/2* deficiency with rapamycin closely mirror mammalian models, supporting the notion that this signaling cascade can be dissected using the zebrafish model system. We are currently pursuing the tissue specific requirements of *Tsc1/2*-mTOR function using transgenes to drive an activated form of RHEB. These transgenic tools will facilitate the investigation of the multi-organ effects of *Tsc1/2* deficiency and provide insights into progression of TSC and LAM.