Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

May 11-12, 2006

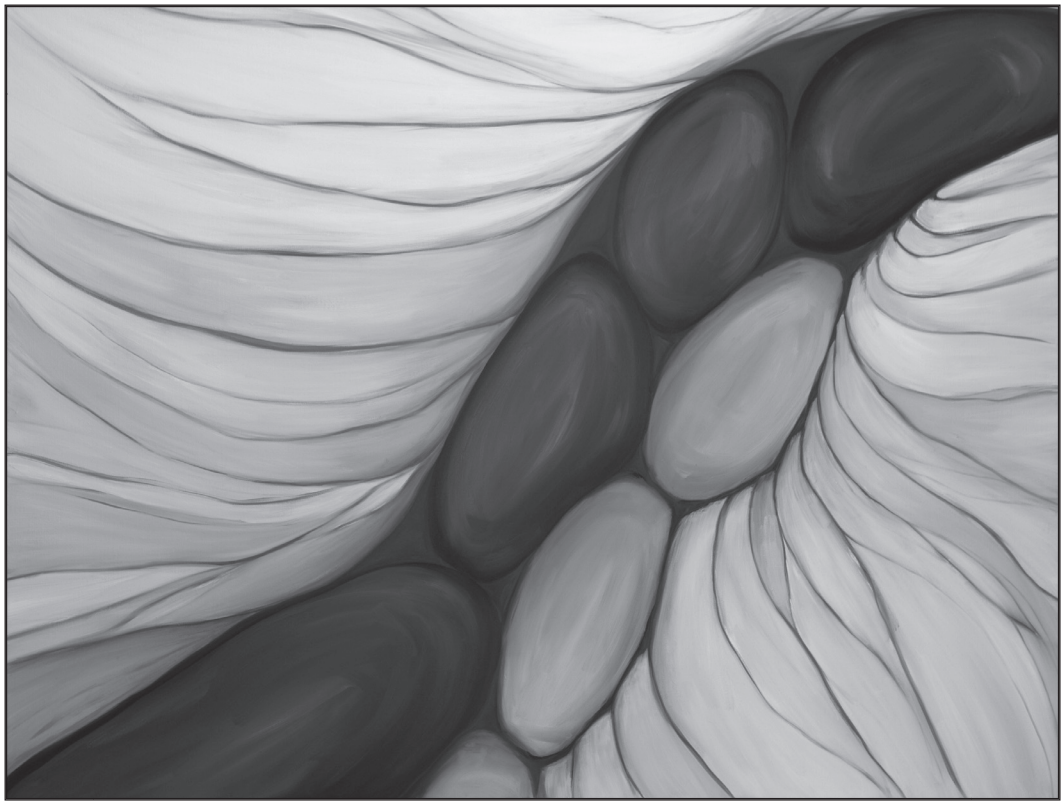
Lister Hill Auditorium
Bethesda, MD

Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes and Digestive and Kidney Diseases
National Cancer Institute
NIH Office of Rare Diseases

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

May 11-12, 2006

Lister Hill Auditorium
Bethesda, MD



Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes and Digestive and Kidney Diseases
National Cancer Institute
NIH Office of Rare Diseases

NIH Organizing Committee:

Kristin Abraham, Ph.D.
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 795
6707 Democracy Bouleavard
Bethesda, MD 20892-5460
Phone: (301) 451-8048
Email: abrahamk@extra.niddk.nih.gov

Carol Haft, Ph.D.
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institutes of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 793
6707 Democracy Boulevard
Bethesda, MD 20892
Phone: (301) 594-7689
Email: haftc@mail.nih.gov

Mary Ellen Perry, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 5034
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-7028
Email: perryma@mail.nih.gov

Betsy Wilder, Ph.D.
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 623
6707 Democracy Boulevard, MSC 5458
Bethesda, MD 20892-5458
Phone: (301) 594-1409
Email: betsywilder@nih.gov

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Contents

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Contents

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Agenda

Day 1: Thursday, May 11, 2006

Session I	Hamartoma Syndromes	
1:30 p.m.	**Opening Remarks** **Dr. Griffin Rodgers,** *Acting Director, National Institute of Diabetes and Digestive and Kidney Diseases* **Dr. Dinah Singer,** *Director, Division of Cancer Biology, National Cancer Institute* **Dr. Stephen Groft,** *Director, Office of Rare Diseases, National Institutes of Health*	
1:45 p.m.	**Keynote Address:** Tuberous Sclerosis From Mutations to Clinical Trials	**Dr. Sandra Dabora** *Brigham and Women's Hospital and Harvard Medical School*
2:30 p.m.	Phenomic Analysis of PTEN Hamartoma Tumor Syndrome	**Dr. Charis Eng** *Cleveland Clinic Genomic Medicine Institute*
3:00 p.m.	The LKB1 Kinase: Regulator of Tumorigenesis and Metabolism	**Dr. Reuben Shaw** *The Salk Institute*
3:30 p.m.	**Coffee Break**	

Session II	Insulin Signaling/mTOR	
4:00 p.m.	**Keynote Address:** Insulin/IGF-1 Receptor/mTOR Signaling Pathway Perturbation in Disease	**Dr. Tony Hunter** *The Salk Institute*
4:45 p.m.	Activation of mTOR Complex 1 and Stimulation of eIF3-eIF4G Association by Insulin	**Dr. John C. Lawrence** *University of Virginia*
5:15 p.m.	The Role of the Nutrient Input in TSC1/2-Rheb Mediated mTOR Signaling	**Dr. George Thomas** *University of Cincinnati*
5:45 p.m.	**Closing Remarks**	

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Agenda

Day 2: Friday, May 12, 2006

Session III	**Nutrient Sensing**	
8:00 a.m.	**Keynote Address:** Hamartoma Syndromes Result From Loss of Cell Intrinsic Sensors/Regulators of Bioenergetics	**Dr. Craig Thompson** *University of Pennsylvania School of Medicine*
8:45 a.m.	The Role of Akt in Energy Metabolism and the Genesis of Cancer	**Dr. Nissim Hay** *University of Illinois at Chicago*
9:15 a.m.	Regulation of Energy Metabolism and Growth of Prostate Cancer Cells by AMPK	**Dr. Zhijun Luo** *Boston University School of Medicine*
9:45 a.m.	**Coffee Break**	
10:15 a.m.	Insulin and Amino Acid Regulation of mTOR Signaling and Kinase Activity through the Rheb GTPase	**Dr. Joseph Avruch** *Massachusetts General Hospital*
10:45 a.m.	Aberrant Cell Survival and Proliferation Signaling in TSC Cells and Tumors	**Dr. Brendan Manning** *Harvard University, School of Public Health*
11:15 a.m.	**Late Breaking News:** Estradiol-Induced Activation of the Mammalian Target of Rapamycin Pathway Is Mediated by Phosphatidylinositol 3-Kinase/Akt and Tuberin	**Dr. Jane Yu** *Fox Chase Cancer Center*
11:35 a.m.	*Drosophila* Rheb, TOR, and TIF-IA	**Dr. Bruce Edgar** *Fred Hutchinson Cancer Research Center*
12:05 p.m.	**Lunch and Poster Viewing**	

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

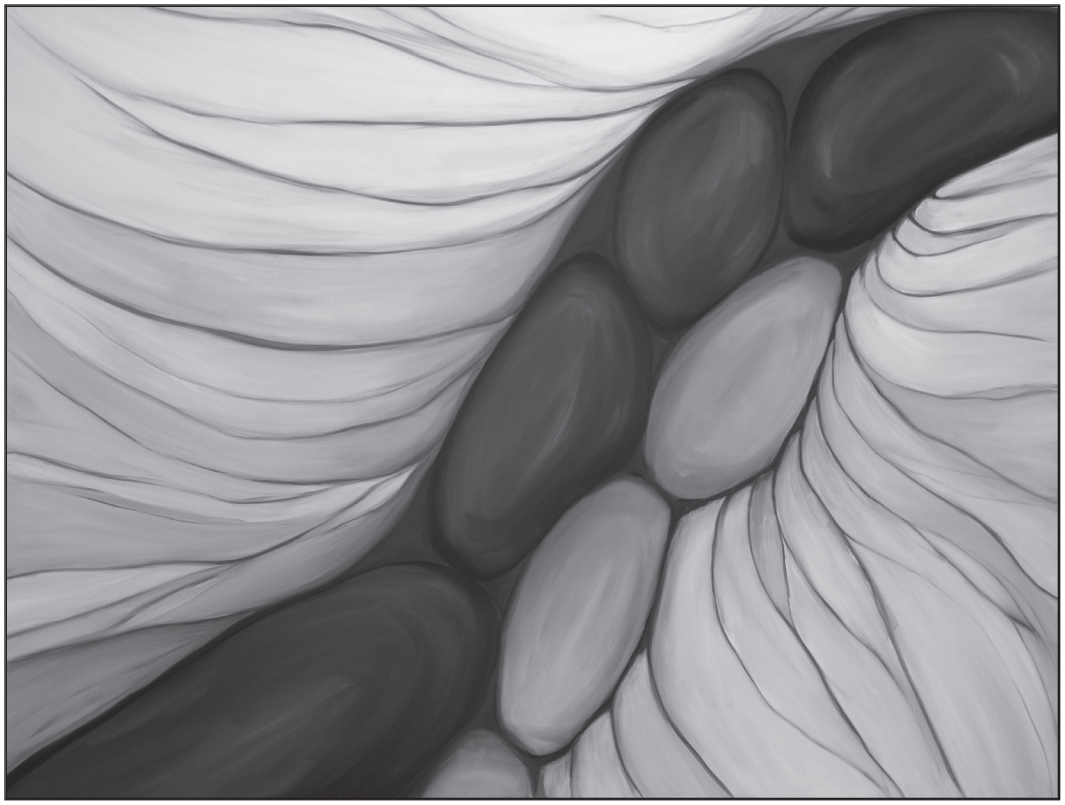
Agenda

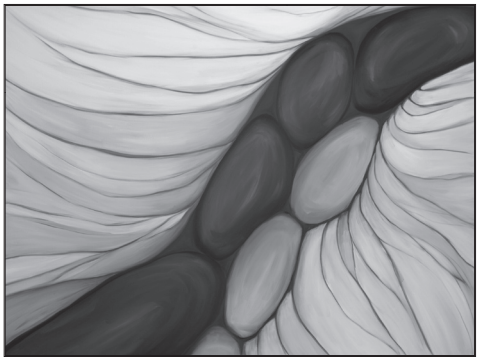
Session III	Nutrient Sensing (continued)	
2:05 p.m.	TORC1 Regulation by Hypoxia	**Dr. James Brugarolas** *University of Texas Southwestern Medical Center*
2:35 p.m.	Mechanisms of REDD1 Signaling to mTOR	**Dr. Leif Ellisen** *Harvard Medical School, Massachusetts General Hospital Cancer Center*
3:05 p.m.	**Coffee Break**	
3:35 p.m.	**Late Breaking News:** Insulin Receptor/TOR Signaling Controls Neuronal Differentiation by Regulating the Expression of the Ets1 Ortholog Pointed P2 in *Drosophila*	**Dr. Joseph Bateman** *King's College London*
3:55 p.m.	Regulation of and Signaling by the TORC1 Complex	**Dr. John Blenis** *Harvard Medical School*
4:25 p.m.	Control of Cell Growth and Insulin Sensitivity by the TSC Tumor Suppressor Proteins	**Dr. Duojia (DJ) Pan** *Johns Hopkins University School of Medicine*
4:55 p.m.	**Closing Remarks and Adjournment**	

Speaker Abstracts

Session I

Hamartoma Syndromes





Keynote Address Abstract:
Tuberous Sclerosis From Mutations to Clinical Trials

Sandra L. Dabora

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

Tuberous sclerosis complex (TSC) is a multi-organ autosomal dominant tumor disorder that causes significant disability. The incidence of TSC is 1:6000, there are approximately 50,000 TSC patients in the United Sates, and an estimated 1-2 million are affected worldwide. Similar to other tumor suppressor gene disorders, the majority (60-70%) of new cases are due to spontaneous mutations. Although the hamartomatous growths that occur in TSC do not generally become malignant, the morbidity associated with TSC is significant because 80-90 percent of affected individuals have epilepsy, 50-70 percent have cognitive impairment, many have behavior disorders, 80-90 percent have brain abnormalities (tubers or subependymal nodules), 10 percent develop brain tumors (subependymal giant cell astrocytomas), 60-80 percent develop kidney tumors (angiomyolipomas), 25 percent have kidney cysts (including some with polycystic kidney disease), 75 percent have skin tumors (facial angiofibromas), 50 percent of infants have cardiac tumors (rhabdomyomas), and 30 percent of adult females have pulmonary abnormalities, including lymphangioleiomyomatosis (LAM).[1-3] Sporadic pulmonary LAM is a progressive pulmonary disorder that is genetically related to TSC because somatic mutations in the *TSC1* or *TSC2* gene have been identified in abnormal lung tissue from LAM patients.[4]

It is known that 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 nutrient uptake, growth, and protein translation. Rapamycin, an mTOR kinase inhibitor that is an approved drug for immunosuppression following organ transplantation, has been shown to normalize disregulated mTOR signaling in cells that lack normal hamartin or tuberin, suggesting that mTOR kinase inhibition may be a useful approach to systemic therapy for TSC and/or LAM. Interferon-gamma (IFN-gamma) is another promising therapeutic agent because it reduces the frequency of kidney tumors in a TSC mouse model and a high expressing allele of IFN-gamma is associated with a decreased frequency of kidney angiomyolipomas in *TSC2* patients.[5] Recently, we have completed preclinical studies using TSC mouse models demonstrating the efficacy of both a rapamycin analog (CCI-779) and IFN-gamma.[6] Based on the efficacy and safety of CCI-779 in our preclinical studies using TSC mouse models and the work of other groups demonstrating the potential utility of treating TSC related tumors with rapamycin, we are now implementing a multi-center phase II trial for patients with TSC and/or LAM and kidney angiomyolipomas. An overview and update on genetype-phenotype studies, preclinical studies, and TSC clinical trials will be covered.

References:

1. Dabora, S.L., S. Jozwiak, D.N. Franz, P.S. Roberts, A. Nieto, J. Chung, Y.S. Choy, M.P. Reeve, E. Thiele, J.C. Egelhoff, J. Kasprzyk-Obara, D. Domanska-Pakiela, and D.J. Kwiatkowski. Mutational analysis in a cohort of 224 tuberous sclerosis patients indicates increased severity of *TSC2*, compared with *TSC1*, disease in multiple organs. *Am J Hum Genet*, 2001;68(1):64-80.

(continued on next page)

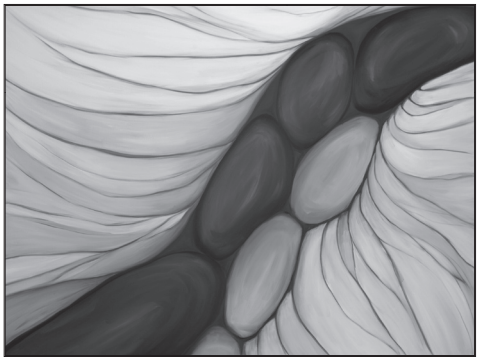
2. Franz, D.N., A. Brody, C. Meyer, J. Leonard, G. Chuck, S. Dabora, G. Sethuraman, T.V. Colby, D.J. Kwiatkowski, and F.X. McCormack. Mutational and radiographic analysis of pulmonary disease consistent with lymphangioleiomyomatosis and micronodular pneumocyte hyperplasia in women with tuberous sclerosis. *Am J Respir Crit Care Med*, 2001;164(4):661-8.

3. Gomez, M., J. Sampson, and V. Whittemore, eds. *The Tuberous Sclerosis Complex*. Third Ed. 1999, Oxford University Press: Oxford, England.

4. Carsillo, T., A. Astrinidis, and E.P. Henske. Mutations in the tuberous sclerosis complex gene *TSC2* are a cause of sporadic pulmonary lymphangioleiomyomat osis. *Proc Natl Acad Sci U S A*, 2000;97:6085-90.

5. Dabora, S.L., P. Roberts, A. Nieto, R. Perez, S. Jozwiak, D. Franz, J. Bissler, E.A. Thiele, K. Sims, and D.J. Kwiatkowski. Association between a high-expressing interferon-gamma allele and a lower frequency of kidney angiomyolipomas in TSC2 patients. *Am J Hum Genet*, 2002;71(4):750-758.

6. Lee, L., P. Sudentas, B. Donohue, K. Asrican, A. Worku, V. Walker, Y. Sun, K. Schmidt, M.S. Albert, N. El-Hashemite, A.S. Lader, H. Onda, H. Zhang, D.J. Kwiatkowski, and S.L. Dabora. Efficacy of a rapamycin analog (CCI-779) and IFN-gamma in tuberous sclerosis mouse models. *Genes Chromosomes Cancer*, 2005;42(3):213-27.



Phenomic Analysis of the PTEN Hamartoma Tumor Syndrome

Charis Eng

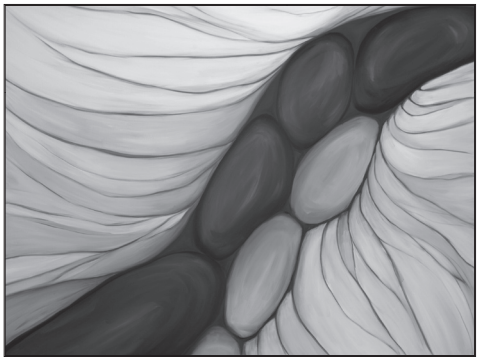
Genomic Medicine Institute, Cleveland Clinic Lerner Research Institute, and Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH; Cancer Research UK Human Cancer Genetics Research Group, University of Cambridge, Cambridge, United Kingdom

Phenomics refers to meticulous clinical phenotyping and documentation, which are the cornerstones of human genetics studies that are successfully translated into the clinical routine. Phenomic analysis of *PTEN* and its related disorders over a decade have resulted in rapid translation to routine clinical cancer genetics care. The first recognized *PTEN*-related disorder is Cowden syndrome (CS), an autosomal dominantly inherited disorder characterized by multiple hamartomas and a high risk of breast, thyroid, and endometrial cancers. It is difficult to recognize because many of the features when taken singly are common in the general population, and thus, is underdiagnosed. Using 12 extended CS families, we mapped the susceptibility locus to 10q23, and subsequently identified germline mutations in *PTEN* in CS. The 9-exon *PTEN* encodes a 403-amino acid lipid and protein phosphatase, which signals down the phosphoinositol-3-kinase (PI3K)/Akt pathway resulting in G1 cell cycle arrest and/or apoptosis. In a single series of 37 classic CS probands, 30 (81%) were found to harbor germline *PTEN* mutations. Genotype-phenotype analyses revealed that mutation-positive CS had an increased risk of developing breast cancer compared to mutation negative CS (P=0.004). Mutations occurring in the exon 5-encoded phosphatase core motif and 5' of it, and missense mutations were associated with multi-organ involvement, a surrogate of disease severity (P=0.1). These observations have implications for genetic counseling and organ-specific surveillance and/or prophylaxis. Interestingly, we have also found that 60 percent of a seemingly unrelated autosomal dominant syndrome, Bannayan-Riley-Ruvalcaba syndrome (BRRS), characterized by megencephaly, lipomatosis, and speckled penis, also carried germline *PTEN* mutations. Genotype-phenotype analyses also showed that breast tumors are associated with mutation positive BRRS. Subsequently, up to 20 percent of Proteus ("Elephant Man") syndrome and 50 percent of a Proteus-like syndrome have been found to have germline *PTEN* mutations. The finding of *PTEN* mutations in a subset of these three syndromes not previously associated with cancer suggests that irrespective of clinical diagnosis, germline *PTEN* mutation confers an increased risk of breast, thyroid, and endometrial cancer. Overall, our data suggest that a molecular-based diagnosis, that is, *PTEN* Hamartoma Tumor Syndrome (PHTS), would be more useful clinically than clinical syndrome name with regard to cancer risks and surveillance. Because finding a *PTEN* mutation dictates cancer risk and surveillance, we then proceeded to find the etiology of the 20 percent of CS and 40 percent of BRRS without apparent intragenic mutation. Amongst 95 CS and 27 BRRS, 3 BRRS probands were found to have deletions encompassing all or part of *PTEN*. Nine of 95 and none of 27 BRRS were found to have germline variants in the promoter region of *PTEN*. These variants were not found in 200 normal control chromosomes and were shown to be associated with decreased *PTEN* protein levels by Western blot and by increased P-Akt, proving that these promoter variants are pathogenic mutations. Interestingly, the individuals with promoter mutations had "mild" phenotype (few organs involved) but 8 of the 9 had breast cancer. Our data suggest that *PTEN* deletion and promoter analysis be added to the routine clinical armamentarium.

(continued on next page)

Recently, the signal transduction cross-talk between the protein products of *PTEN* and the susceptibility genes for tuberous sclerosis (TSC) and Peutz-Jeghers syndrome (PJS, LKB1) have been elucidated. Although all three syndromes comprise hamartomas, the clinical manifestations are quite distinct. However, central nervous system (CNS) and the gastrointestinal (GI) tract are organ systems affected (differently) in these three disorders. Lhermitte-Duclos disease (LDD), dysplastic gangliocytoma of the cerebellum, has been described in isolation and together with CS. To determine what proportion of LDD is associated with germline *PTEN* mutations, we ascertained 18 individuals (15 adults and 3 children) with the pathologic diagnosis of LDD. All 15 adults but no children with LDD were found to have *PTEN* mutations. The PTEN mutations were associated with decreased *PTEN* expression and increased P-Akt expression in LDD compared to normal cerebellum. Thus, we conclude that all presentations of adult-onset LDD, irrespective of family history or associated signs, be offered *PTEN* testing in the setting of genetic counseling. Because autistic features have been described intermittently in case reports of BRRS and a subset of TSC, we accrued 18 individuals with autism spectrum disorder (ASD) and macrocephaly. Exclusion criteria included known CS/BRRS or known *PTEN* mutation. Among these 18 ASD subjects, 3 (17%) were found to have germline missense mutations in *PTEN*. From these preliminary observations, we conclude that *PTEN* could be a susceptibility gene for ASD in the subset with macrocephaly.

Hamartomas in the GI tract are also common to PHTS, PJS, and to a lesser extent TSC. The major genetic differential diagnosis for hamartomatous polyposis include PHTS, PJS, and juvenile polyposis syndrome (JPS caused by *SMAD4* or *BMPR1A* mutations). Thus, we prospectively accrued subjects, without regard to associated disorders or family history, who presented with a minimum of five polyps in a lifetime, of which at least one must be hamartomatous. Of 49 individuals meeting these clinical criteria, we found that 22 percent carried an unexpected germline mutation in *PTEN*, *BMPR1A*, *SMAD4*, *LKB1*, and *Endoglin*. Based on these observations, it may be suggested that individuals presenting with at least five polyps, one of which must be an hamartoma, be offered genetic testing in the setting of genetic counseling, given that each of these genes does lend susceptibility to cancers in different organs with implications for surveillance.



The LKB1 Kinase: Regulator of Tumorigenesis and Metabolism

Reuben Shaw

Molecular and Cell Biology Laboratory, The Salk Institute, La Jolla, CA

The serine/threonine kinase LKB1 is a tumor suppressor gene mutated in the familial cancer condition Peutz-Jeghers syndrome, as well as a substantial percentage of sporadic lung adenocarcinomas. To elucidate its tumor suppressor function, we sought to identify its substrates and define critical downstream effector pathways. Using a proteomic and bioinformatics approach, we identified the AMP-activated protein kinase (AMPK) as an LKB1 substrate. AMPK is a highly conserved sensor of cellular energy status found in all eukaryotic cells that is activated by stresses that increase the cellular AMP/ATP ratio, including oxygen and glucose deprivation, as well as exercise. We then went on to show that AMPK inhibits the mTOR pathway, a key regulator of cell growth and protein synthesis, by directly phosphorylating the TSC2 tumor suppressor. Importantly, we found that LKB1 is required for repression of mTOR under low ATP conditions in an AMPK- and TSC2-dependent manner. Moreover, hamartomatous gastrointestinal polyps from Lkb1+/- mice show hyperactivated mTOR signaling, similar to hamartomas from germline mutations in TSC2 or PTEN. To examine whether LKB1 also plays a role in regulating AMPK in metabolic tissues, we made a liver-specific deletion of LKB1 in adult mice. Deletion of LKB1 in the adult murine liver results in a near complete loss of AMPK activity. Hepatic loss of LKB1 function results in dramatic hyperglycemia with upregulated gluconeogenic and lipogenic gene expression. In LKB1-deficient liver, TORC2, a transcriptional co-activator of CREB, is hypophosphorylated and enters the nucleus, driving expression of PGC1α, which in turn drives gluconeogenesis. Importantly, adenoviral shRNA for TORC2 significantly reduces PGC1α expression and normalizes blood glucose levels in LKB1 liver-deleted mice, indicating that TORC2 is a prime target of the LKB1/AMPK signaling pathway regulating gluconeogenesis. Finally, we demonstrate that metformin, one of the most widely prescribed type 2 diabetes therapeutics, requires hepatic LKB1 to lower blood glucose levels.

Notes

Notes

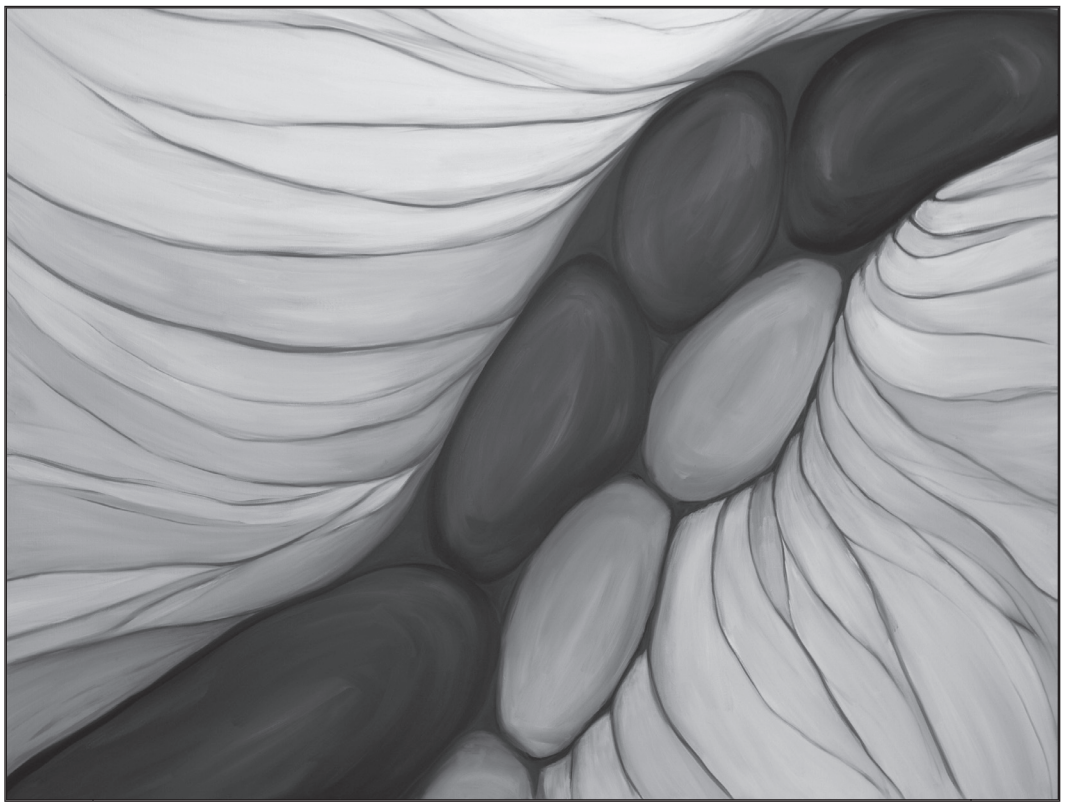
Notes

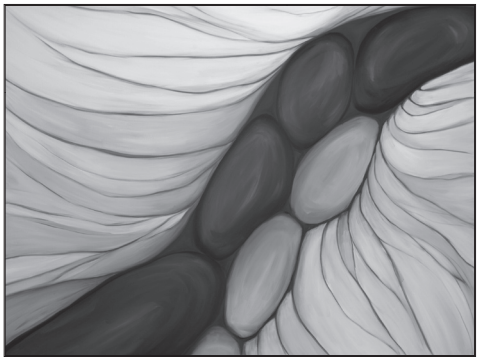
Notes

Speaker Abstracts

Session II

Insulin Signaling/mTOR



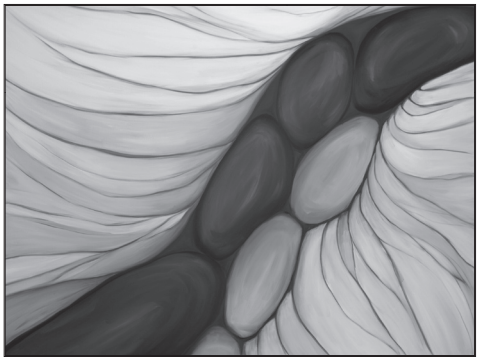


Keynote Address Abstract: Insulin/IGF-1 Receptor/mTOR Signaling Pathway Perturbation in Disease

Tony Hunter

Molecular and Cell Biology Laboratory, The Salk Institute, La Jolla, CA

Tuberous sclerosis is a largely benign tumor syndrome derived through acquisition of somatic lesions in genes encoding the tumor suppressor products, TSC1 or TSC2. Loss of function of the TSC1-TSC2 complex, which acts as a GAP for the Rheb small G protein, results in constitutive, unrestrained signaling from the cell growth pathway comprised of Rheb, mTOR, and S6K. We have found that constitutive activation of the Rheb/mTOR/S6K cassette, whether by genetic deletion of TSC1 or TSC2 or by ectopic expression of Rheb, induces insulin resistance, as a result of downregulation of the insulin receptor substrates, IRS1 and IRS2, which become limiting for signal transmission from the insulin receptor to PI3K. Downstream of PI3K, the survival kinase, Akt, is completely refractory to activation by IRS-dependent growth factor pathways, such as insulin or IGF-I, in TSC1- or TSC2-deficient cells, but not to activation by IRS-independent pathways, such as those utilized by PDGF. The antiapoptotic program induced by IGF-I is severely compromised in TSC2-null cells, but is resorted by rapamycin inhibition of mTOR signaling. We conclude that inappropriate activation of the Rheb/mTOR/S6K pathway imposes a negative feedback program to attenuate IRS-dependent processes, such as cell survival. We have also shown that TSC deficiency and Rheb expression induce mTOR and S6K1-dependent phosphorylation of IRS1 at several Ser residues, and that S6K1 phosphorylates IRS1 on multiple Ser/Thr residues in vitro, showing strong preference for ArgxArgxxSer/Thr sites over Ser/Thr.Pro sites. Localization of IRS1 to the cytosolic compartment is increased in TSC1/2-deficient cells or in 293 cells overexpressing Rheb. Redistribution of IRS1 to the cytosol is sensitive to rapamycin, which inhibits mTOR, and is abrogated by collective mutation of six mTOR-regulated IRS1 Ser phosphorylation sites. Our studies suggest that, through Ser phosphorylation, mTOR directs inappropriate subcellular compartmentalization of IRS1 in pathological states of insulin resistance and in tumors associated with the related familial hamartoma disorders tuberous sclerosis, Peutz-Jeghers syndrome, and PTEN-related tumor predisposing syndromes.
We have also found that DNA damage induction of NF-κB and downstream survival genes is reduced in TSC-null cells and in cancer cells in which TSC2 is depleted by siRNA, and that rapamycin restores NF-κB induction. In consequence these cells are highly sensitive to chemotherapeutics, such as adriamycin. Our results suggest that the effectiveness of rapamycin in cancer therapy may be compromised by its ability to upregulate Akt and NF-κB survival signaling.

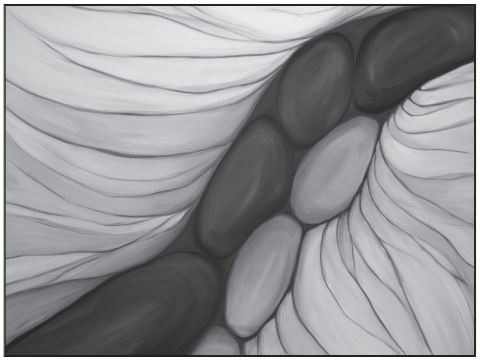


Activation of mTOR Complex 1 and Stimulation of eIF3-eIF4G Association by Insulin

John C. Lawrence

Pharmacology, University of Virginia, Charlottesville, VA

Insulin stimulates protein synthesis by promoting phosphorylation of the eIF4E binding protein, 4EBP1. This effect is rapamycin-sensitive and mediated by mTORC1, a signaling complex containing the protein kinase mTOR, mLst8, and a substrate-binding subunit, raptor. Recent findings in our laboratory indicate that the activation of mTORC1 by insulin involves an increase in substrate binding to raptor. Incubating 3T3-L1 adipocytes with insulin markedly increased 4EBP1 binding to raptor *in vitro*. This response was accompanied by a stable increase in the 4EBP1 kinase activity of mTORC1. The effects of insulin on binding and kinase activity were abolished by disrupting either the TOS motif in 4EBP1 or the interaction between mTOR and raptor. Both effects of insulin occurred rapidly at physiological concentrations of the hormone and required an intact mTORC1. Size fractionation experiments indicated that the form of mTORC1 activated by insulin was dimeric mTORC1 (mTOR:raptor:mLst8)2. In another study, we attempted to identify new proteins that interacted with mTOR by conducting yeast two hybrid screens of a 3T3-L1 adipocyte library. eIF3f was one of three hits that survived a stringent screen using a fusion protein containing the COOH terminal third of mTOR as bait. Subsequent experiments confirmed that mTOR interacted directly with eIF3f. In addition, mTOR was found to control the association of eIF3 and eIF4G. Activating mTOR signaling with insulin increased by as much as fivefold the amount of eIF4G bound to eIF3. This novel effect was blocked by rapamycin and other inhibitors of mTOR, and it required neither eIF4E binding to eIF4G nor eIF3 binding to the 40S ribosomal subunit. The increase in eIF4G associated with eIF3 occurred rapidly and at physiological concentrations of insulin. Moreover, the magnitude of the response was similar to the increase in eIF4E binding to eIF4G produced by insulin. Thus, increasing eIF4G association with eIF3 represents a potentially important mechanism by which insulin, as well as amino acids and growth factors that activate mTOR, stimulate translation.



The Role of the Nutrient Input in TSC1/2-Rheb Mediated mTOR Signaling

George Thomas

Strauss Professor of Cancer Research, Genome Research Institute, University of Cincinnati, Cincinnati, OH

Counter to prevailing views, recent studies from our laboratory showed that nutrient, such as amino acids (AAs), input into the mTOR/S6K1 signaling pathway is not mediated by either the tumor suppressor TSC1/TSC2 or its target, the protooncogene Rheb. In the absence of TSC1/TSC2, we found that S6K1 activation is elevated and refractile to mitogen stimulation, such as insulin, but can still be regulated by AAs. However, this is not the case for Rheb as siRNA knock-down of Rheb protein levels blocks both the insulin and AA input to S6K1. Nonetheless, withdrawal of AAs, which triggers S6K1 inactivation, has no effect on elevated Rheb-GTP levels, leading to the hypothesis that Rheb-GTP is necessary but not sufficient to drive S6K1 activation in the absence of AAs. These findings suggested that the AA input to S6K1 resided on a parallel pathway to that of the TSC1/2-Rheb axis. As earlier studies demonstrated that wortmannin, a class 1 PI3K inhibitor, blocks AA-induced S6K1 activation and AAs do not induce PKB activation, this suggested that a novel wortmannin sensitive signaling component was responsible for mediating the AA input to S6K1. These observations led us to class 3 PI3K, or hVps34, as the novel target by which these responses were mediated. In brief, ectopic expression of hVps34 drives S6K1 activation, but only in the presence of AAs, and this effect is blocked by siRNAs directed against hVps34. Moreover, stimulation of cells with AAs increases hVps34 activity as measured by the production of PI3P, the product of hVps34. PI3P mediates the recruitment of proteins containing FYVE or Px domains to endosomal membranes, with PI3P rich micro-domains acting as signaling platforms. Consistent with hVps34 mediating the AA input to S6K1, this response is attenuated by expression of a cDNA containing two FYVE domains, which bind to PI3P and block binding of proteins having either FYVE or PX domains, preventing S6K1 activation.

Notes

Notes

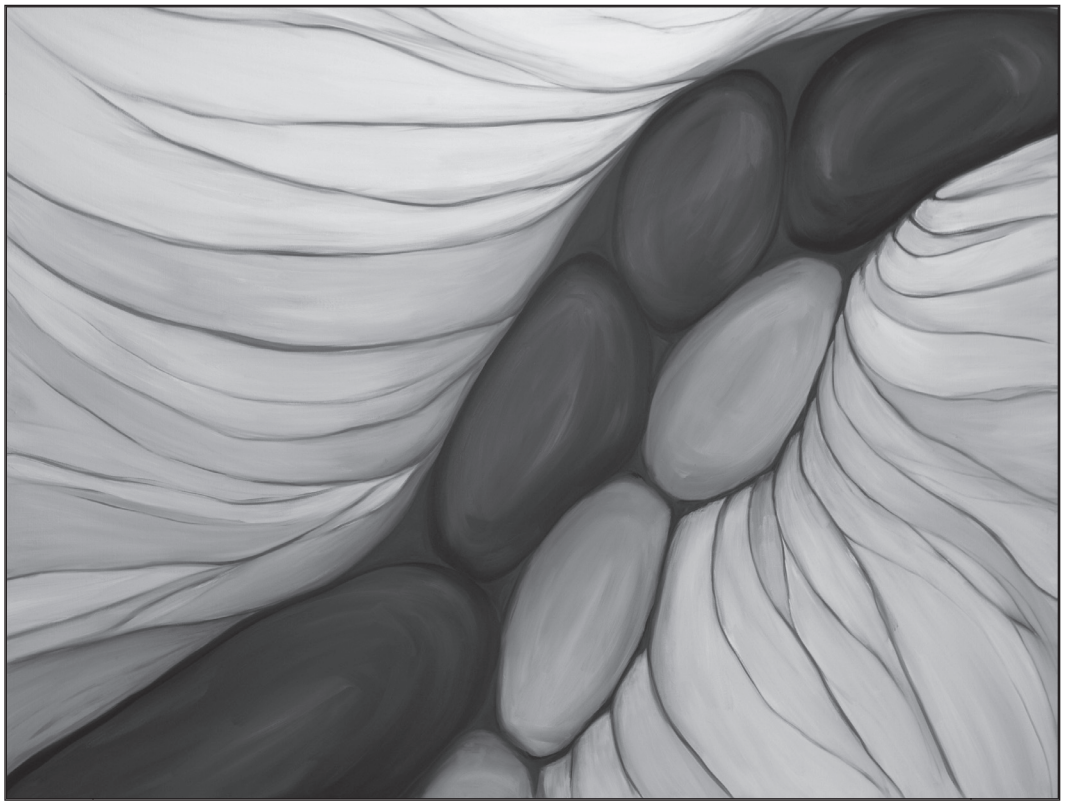
Notes

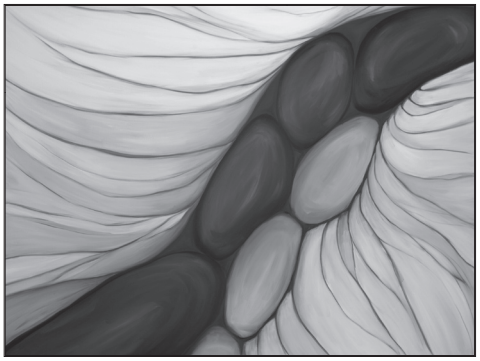
Notes

Speaker Abstracts

Session III

Nutrient Sensing



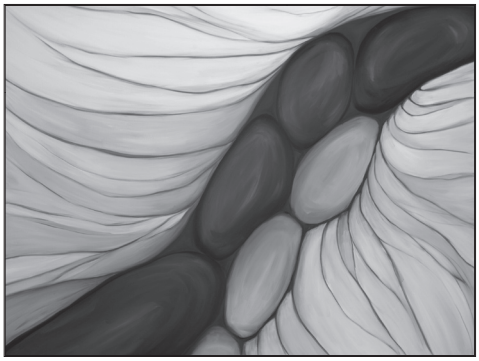


Keynote Address Abstract:
Hamartoma Syndromes Result From Loss of Cell Intrinsic Sensors/Regulators of Bioenergetics

Craig B. Thompson

Abramson Family Cancer Research Institute, Department of Cancer Biology, University of Pennsylvania School of Medicine, Philadelphia, PA

Genetic loss of the tumor suppressors PTEN, LKB, Tsc1/2, or VHL results in hamartoma syndromes. All of the genes involved play critical but distinct roles in coordinating cell growth with intracellular nutrient availability. PTEN acts as a negative regulator of nutrient uptake, preventing cells from taking up nutrients in excess of the need for bioenergetic and synthetic substrates. LKB, in association with AMPK, functions as a sensor of glucose availability and acts to suppress cell growth and proliferation in response to glucose limitation. Tsc1 and Tsc2 function as a complex to coordinate the activity of TOR with the activity of the signaling pathways that control carbohydrate and amino acid uptake. Finally, VHL acts as a tumor suppressor by serving as a critical component of oxygen sensing, suppressing cell growth and proliferation under times of oxygen limitation. Thus, hamartoma syndrome-associated tumor suppressors function as a metabolic control network to coordinate cellular bioenergetics with cell growth control. Together these data support that mammalian cells lack a cell intrinsic ability to take up nutrients from their environment and that this presents a barrier to cellular transformation. Potential therapeutic implications of this hypothesis will be discussed.



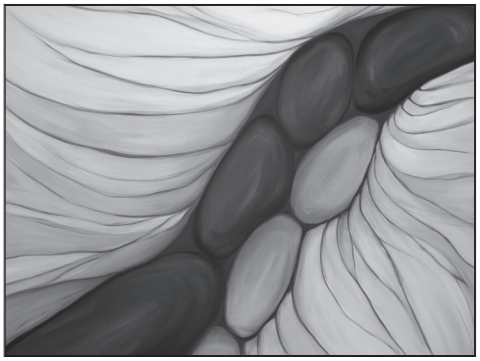
The Role of Akt in Energy Metabolism and the Genesis of Cancer

Nissim Hay

Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL

Hyperactivation of Akt is one of the most common lesions occurring in human cancers. However, the most evolutionarily conserved role of Akt is in energy metabolism. Evidence will be provided that in mammalian cells the role of Akt in energy metabolism was recruited to the apoptotic cascade, cell proliferation, and susceptibility to tumorigenesis.

Inhibition of Akt activity could be an attractive therapeutic approach for cancer. However, because Akt is a multipotent kinase it is not clear whether it is possible to ablate Akt activity without severe physiological consequences. Using mouse genetics, we were able to show that partial inhibition of Akt or selective inhibition of Akt1 is sufficient to inhibit tumor development without other severe physiological consequences. The role of the different Akt isoforms in cancer and diabetes will be discussed.

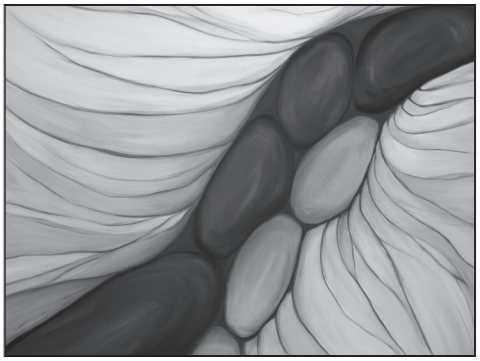


Regulation of Energy Metabolism and Growth of Prostate Cancer Cells by AMPK

Zhijun Luo

Diabetes Research Unit, Boston University School of Medicine, Boston, MA

AMPK is a fuel sensing enzyme that is activated by hormones, cytokines, exercise, and stresses that diminish cellular energy state (e.g., glucose deprivation). In addition, metformin and thiozolidinediones, agents used to diminish insulin resistance in type 2 diabetes, have been shown to activate AMPK. Activation of AMPK increases processes that generate ATP (e.g., fatty-acid oxidation) and restrains others such as fatty acid-, glycerolipid- and protein synthesis that consume ATP, but are not acutely necessary for survival. Conversely, when cells are presented with a sustained excess of glucose, AMPK activity diminishes and these synthetic processes are enhanced. In light of the observations that many cancer cells require high rates of fatty acid and protein synthesis for their invasive growth and survival and that treatment of type 2 diabetes with metformin reduces the risk of cancer, we investigated if AMPK activators play an inhibitory role in the growth of prostate cancer cells. We have found an apparently selective inhibition of the growth of prostate cancer cells by three distinct AMPK activators, AICAR, rosiglitazone, and metformin. We have also found that these effects are associated with inhibition of fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), and mTOR.



Insulin and Amino Acid Regulation of mTOR Signaling and Kinase Activity through the Rheb GTPase

Xiaomeng Long[1], Yenshou Lin[1], Matthew Liu[1], Sara Ortiz-Vega[1], and Kazuyoshi Yonezawa[2*], and Joseph Avruch[1]

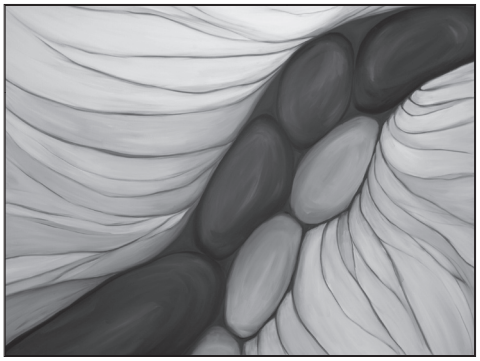
*[1]Diabetes Research Laboratory, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; [2]Biosignal Research Center, Kobe University, Nada-ku, Kobe, Japan (*deceased)*

TOR, the target of rapamycin, is a giant protein kinase that controls cell size in response to nutrient sufficiency and to signals generated by insulin/IGF and other growth factor receptors. TOR signals through two physically separate multiprotein complexes; TOR regulation of cell size is effected through the ability of TOR complex 1 (which also contains the polypeptides raptor and mLST8) to control a subset of translational regulators, such as the eIF-4E binding proteins and the p70 S6 kinases (which are direct substrates), the transcription of ribosomal and other genes, and to negatively regulate proteasomal protein degradation and autophagy. Genetic and biochemical evidence has established that the receptor-initiated inputs control mTOR primarily through the ability of activated Type 1 Ptd Ins-3 kinase and its effector PKB to inhibit the GTPase activating function of the Tuberous Sclerosis Complex (TSC1/TSC2) toward the ras-like small GTPase Rheb. In turn, Rheb-GTP is a positive regulator of TOR signaling. Cells lacking either TSC1 or TSC2 exhibit nearly complete conversion of endogenous Rheb to the GTP-charged state and constitutive activation of TOR complex 1 signaling, as reflected by activation of the p70 S6 kinase and hyperphosphorylation of 4E-BP. In TSC null cells, mTOR signaling is largely unaffected by addition or withdrawal of serum or polypeptide growth factors, as well as by AICAR, a relatively selective activator of the AMP-activated protein kinase. Nevertheless, in TSC-null as in normal cells, withdrawal of extracellular amino acids, or just leucine, causes a complete, reversible inhibition of TOR complex 1 signaling, without significantly altering the state of Rheb-GTP charging. Thus, whereas insulin and AMPK control TORC1 by regulating Rheb GTP charging, amino acid/leucine sufficiency appears to regulate the ability of Rheb-GTP to control TORC1 signaling.

We have examined the mechanisms by which Rheb regulates the activity of TOR complex 1. Transient overexpression of Rheb can restore TOR complex 1 signaling in amino acid deficient cells, although massive Rheb overexpression is required. Rheb rescue of TOR complex 1 signaling is eliminated by mutation of the Rheb switch 1 segment, and by mutations that eliminate the ability of Rheb to bind guanyl nucleotides. Levels of recombinant Rheb that enable the rescue of TORC1 activity from amino acid depletion *in vivo* coprecipitate endogenous mTOR and raptor, as well as a small fraction of the endogenous TSC complex. Recombinant Rheb binds endogenous or recombinant mTOR in amounts far greater than that retrieved with the most closely related small GTPases, Ha-Ras or Rap-1. The inactive Rheb switch 1 mutants, however, also bind mTOR and the nucleotide-deficient Rheb mutants retrieve much larger amounts of mTOR than does wild-type Rheb. Nevertheless, the mTOR polypeptides associated with nucleotide-deficient Rheb mutants are entirely devoid of kinase activity *in vitro*, as is the small amount of mTOR that coprecipitates with val12-Ha-Ras. Reciprocally, mTOR polypeptides bound to Rheb(Q64L), a mutant that is almost completely GTP charged, exhibit twice-higher kinase activity *in vitro* than mTOR bound to wild-type Rheb, which is about 50 percent GTP-charged. Thus, the ability of Rheb-GTP to bind directly to TORC1 is critical to the acquisition of TORC1 kinase activity *in vivo*. Rheb binds to the upper lobe of the mTOR catalytic domain, and although Rheb GTP charging is critical for TORC1 activation, it is not required for the ability of Rheb to bind mTOR, either during coexpression or *in vitro* using purified polypeptides. Rheb also binds the WD propeller domains of raptor and mLST8; however, the functional significance of these interactions is unknown.

(continued on next page)

The ability of Rheb to bind to the mTOR catalytic domain *in vivo* is inhibited by amino acid or leucine withdrawal, whereas the binding to raptor and mLST8 is unaffected. This inhibition of the Rheb-TOR interaction is not due to changes in Rheb-GTP charging, but is due to an action mediated through the larger lobe of the mTOR catalytic domain; deletion of this segment does not disturb the binding of recombinant Rheb to the upper lobe of the mTOR catalytic domain, but renders the interaction insensitive to inhibition by amino acid/leucine withdrawal. The molecular basis for amino acid regulation of the Rheb-mTOR interaction is currently under investigation.

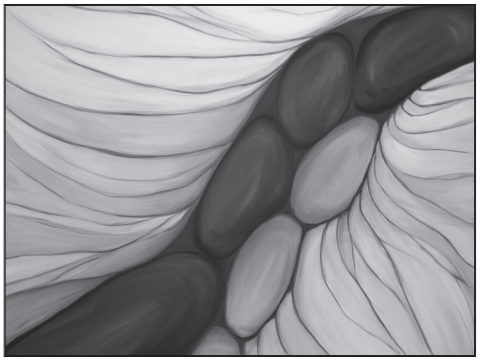


Aberrant Cell Survival and Proliferation Signaling in TSC Cells and Tumors

Brendan Manning

Genetics and Complex Diseases, Harvard University, School of Public Health, Boston, MA

We have recently shown that there is a strong connection between the slow growth of tumors in a mouse model of TSC and a negative feedback loop active in TSC-deficient cells and tumors. In the absence of *Tsc1* or *Tsc2*, the PI3K-Akt pathway is inhibited in an mTOR-dependent manner, and this has tumor suppressive effects in this setting. We now have additional evidence that Akt attenuation extends to TSC-associated kidney angiomyolipomas in humans. Interestingly, this mTOR-dependent inhibition of PI3K-Akt signaling has also been implicated in insulin resistance underlying type 2 diabetes. To gain further insight into the pathophysiological consequences of this feedback regulation, we are characterizing survival and proliferation events downstream of PI3K in the absence of the TSC genes. Surprisingly, we find that Akt targets, such as FOXO1 and GSK3, are differentially regulated in TSC null cells and tumors. For instance, while FOXO1 is constitutively dephosphorylated and nuclear, consistent with Akt inhibition, GSK3 is constitutively phosphorylated and inhibited. We have demonstrated that GSK3 phosphorylation is mediated by aberrantly high S6K1 activity and contributes to the serum-free proliferation properties of TSC null cells. Therefore, under conditions of mTOR-dependent Akt attenuation, elevated S6K1 activity can take the place of Akt for regulation of GSK3. In addition, we find that TSC-deficient cells have a proliferation and survival advantage under some conditions (e.g., serum withdrawal), while these cells are more prone to apoptosis under other conditions (e.g., glucose deprivation, endoplasmic reticulum stress, etc).



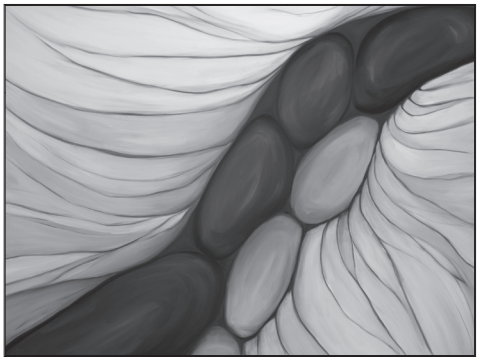
Late Breaking News:
Estradiol-Induced Activation of the Mammalian Target of Rapamycin Pathway Is Mediated by Phosphatidylinositol 3-Kinase/Akt and Tuberin
(Also Presented as Poster #20)

*** Jane Yu and Elizabeth Petri Henske**

Fox Chase Cancer Center, Philadelphia, PA

Tuberous Sclerosis Complex (TSC) results from loss of TSC1 or TSC2. The TSC1 and TSC2 gene products, hamartin and tuberin, associate as a functional complex to inhibit mTOR via Rheb. Lymphangioleiomyomatosis is a TSC-linked pulmonary smooth muscle cell proliferation that occurs almost exclusively in women, suggesting that estrogen is linked to the function of tuberin. However, the pathways through which estrogen signals to tuberin are not well understood. To investigate tuberin's role in regulating cytoplasmic estrogen signaling, we first determined whether estrogen activates mTOR activity by examining the phosphorylation of downstream targets of mTOR, p70 ribosomal S6 kinase (p70S6K) and S6, in MCF-7 cells. 17-beta estradiol rapidly increased the phosphorylation of p70S6K (3-fold) and S6 (6-fold) within 15 minutes. The estrogen-induced activation of S6K and S6 was blocked by Rapamycin, Wortmannin, and PD28095, suggesting that the PI3K/mTOR and MEK/MAPK pathways co-mediate estrogen signaling to mTOR. Because Rheb is known to activate mTOR and S6K, we next asked whether estrogen regulates Rheb activation. Estradiol increased the active GTP-fraction of endogenous Rheb by fourfold within 10 minutes in MCF-7 cells. Tuberin is a substrate of protein kinases Akt and RSK1, and both Akt and RSK1 can be activated by estrogen. Estradiol stimulated endogenous tuberin phosphorylation at T1462 (a site of phosphorylation by both Akt and RSK1) approximately fourfold. Expression of wild-type tuberin or tuberin carrying alanine mutations at the two Akt phosphorylation sites (S939A and T1462A) inhibited the estradiol-induced phosphorylation of S6. To investigate tuberin's role in regulating genomic estrogen signaling, we confirmed that expression of tuberin resulted in significant inhibition (fivefold) of ERE-luciferase activity compared with vector control, as has been shown previously by the Noonan laboratory. We also tested patient-derived TSC2 mutants: N1643K, P1675L, N1651S, and R611Q. The mutant forms of tuberin did not inhibit luciferase activity to the same extent as wild-type tuberin. These results suggest for the first time the potential disease-relevance of tuberin's transcriptional repression. In summary, we demonstrate for the first time that estradiol activates the mTOR/S6K/S6 pathway in a PI3K/Akt and MAPK/RSK1 dependent manner. Estradiol's activation of mTOR is inhibited by tuberin, and is associated with an increase in GTP-Rheb and phospho-tuberin. These data imply that tuberin integrates estrogen signals from multiple pathways to regulate mTOR activity. We also demonstrate that wild-type tuberin, but not mutant forms, inhibits ERE-luciferase activity. These signaling pathways may have relevance to both the pathogenesis and therapy of LAM.

***Recipient of Travel Award**

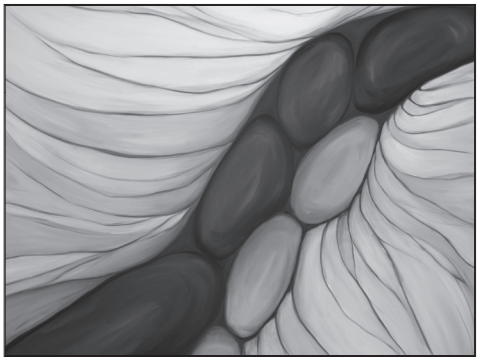


Drosophila Rheb, TOR, and TIF-IA

Bruce A. Edgar, Savraj S. Grewal, Dayna Hall, and Leslie Saucedo

Fred Hutchinson Cancer Research Center, Seattle, WA

The TSC1/2 complex mediates many or perhaps all of its effects on cell growth through the Rheb small GTPase, which it inhibits, and the *target-of-rapamycin* (TOR) protein kinase, which requires Rheb for activity. To address how Rheb and TOR control cell growth, we used RNAi to inhibit each of the approximately 20 components of the Insulin/TOR growth regulatory network in *Drosophila* S2 cells. Assays on the affected cells ruled out the regulation of glucose and amino acid import as a major mode of growth control by Rheb and TOR, and indicated that the control of protein synthesis is key. *In vivo* experiments showed that loss of TOR function leads to a shutdown of ribosome synthesis in larvae, similar to that observed when these animals are starved of dietary protein. Conversely, overexpression of Rheb, which hyperactivates TOR, is sufficient to increase rRNA synthesis, stimulate ribosome supply, and increase bulk protein synthesis. Moreover, overexpressed Rheb reverses the effects of starvation on ribosome production and protein synthesis. Our data from S2 cells, as well as genetic data from *Drosophila*, indicate that the effects of TOR on ribosome biogenesis cannot be attributed solely to the well-characterized targets, S6K and 4E-BP. Accordingly, we have begun to characterize TIF-IA, a critical regulator of RNA Polymerase I activity (and rRNA transcription), as potential TOR target. In both yeast and mammalian cells, TIF-IA associates with Pol I and stimulates rRNA transcription in a growth-dependent manner, and TIF-IA has been reported to be regulated by TOR in mammalian cells. We identified *Drosophila* TIF-IA mutants and found that they phenocopy nutrient starvation, or loss of TOR or Rheb. Using a TIF-IA-DAM-methylase fusion protein expressed *in vivo*, we have determined that TIF-IA localization to the rDNA is TOR-dependent. Overexpression of an activated variant TIF-IA is sufficient to stimulate rDNA transcription, but appears not to increase ribosome production or rates of cell growth. This indicates that Rheb must control other targets coordinately to upregulate protein synthesis. Exactly how TIF-IA and Pol I activity are regulated by TOR signaling remains an open question, which we are currently investigating.

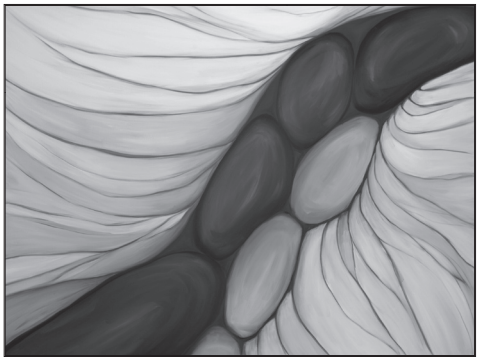


TORC1 Regulation by Hypoxia

James Brugarolas

Center for Developmental Biology, University of Texas Southwestern Medical Center, Dallas, TX

Tuberous Sclerosis Complex is a disease characterized by hamartoma tumors in multiple tissues resulting from mutations in either the *TSC1* or the *TSC2* genes. TSC1 and TSC2 form a tumor suppressor protein complex that functions as a GAP towards Rheb, an activator of TORC1. TORC1 is a heterotrimeric protein complex with S/T kinase activity that regulates translation initiation. TORC1 integrates signals from nutrients, growth factors, oxygen and energy stores with the protein translation machinery. TORC1 regulation by growth factors and energy stores, but not by nutrients, requires the TSC1/TSC2 complex. We have discovered that TSC1/TSC2 is also essential for TORC1 regulation by hypoxia. Disruption of the TSC1/TSC2 complex through loss of TSC1 or TSC2 blocks hypoxia-induced TORC1 inhibition. Notably, while signals from energy stores are relayed to TORC1 through a pathway involving the serine/threonine kinases LKB1 and AMPK, TORC1 inhibition by hypoxia requires neither LKB1 nor AMPK. Hypoxia-induced TORC1 inhibition requires *de novo* transcription and the expression of the hypoxia-inducible *REDD1* gene. Using both loss-of-function as well as gain-of-function studies, we have determined that REDD1 is both necessary and sufficient for TORC1 inhibition by hypoxia. Notably, inhibition of TORC1 by REDD1 requires an intact TSC1/TSC2 complex. The importance of this pathway is illustrated by the observation that failure to inhibit TORC1 in response to hypoxia (such as in TSC1/TSC2-deficient cells) leads to inappropriate cell proliferation and might predispose to tumor growth.

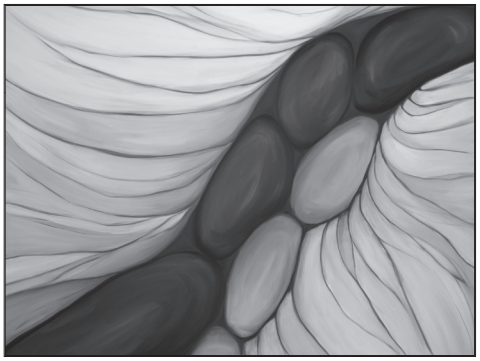


Mechanisms of REDD1 Signaling to mTOR

Leif Ellisen

Harvard Medical School, Massachusetts General Hospital Cancer Center, Boston, MA

Appropriate cellular responses to nutrient, energy, and hypoxic stress are critical for normal growth and development, while dysregulation of these stress response pathways contributes to common diseases, including diabetes and cancer. We and others have identified a novel, phylogenetically conserved pathway that is required for appropriate regulation of the mTOR kinase in response to energy stress, hypoxia, and other cell stress conditions. This pathway involves activation of the REDD1 family of proteins, which function to inhibit mTOR activity via the tuberous sclerosis tumor suppressor proteins TSC1 and TSC2. Despite its essential role in regulating mTOR activity, the mechanism by which REDD1 inhibits mTOR is poorly understood. We find that REDD1 regulates mTOR through a convergent pathway involving the AMP-activated kinase AMPK, which normally phosphorylates TSC2 leading to mTOR inhibition. In the absence of REDD1, stress-induced activation of AMPK as well as AMPK-dependent TSC2 phosphorylation are unperturbed, despite severely defective mTOR regulation. In contrast, overexpression of REDD1 is sufficient to downregulate mTOR activity in the absence of AMPK activation. These findings suggest a model in which AMPK-mediated phosphorylation facilitates REDD1-dependent TSC1/2 activation and mTOR inhibition. These findings provide an explanation for the observed timing of mTOR regulation following energy stress and hypoxia. Potential mechanisms and implications of this model will be discussed.



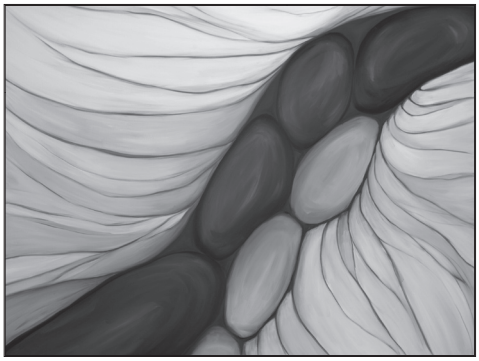
Late Breaking News:
Insulin Receptor/TOR Signaling Controls Neuronal Differentiation by Regulating the Expression of the Ets1 Ortholog Pointed P2 in *Drosophila* (Also Presented as Poster #3)

***Joseph M. Bateman[1] and Helen McNeill[2]**

[1]Wolfson Centre for Age-Related Disease, King's College London, London, United Kingdom; [2]Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada

A fundamental challenge during the development of any complex organism is the coordination of proliferation and differentiation. The *Drosophila* retina is a highly tractable model for studying the relationship between growth and neuronal differentiation. We have shown that the insulin receptor (InR)/TOR pathway plays a key role in controlling the timing of neuronal differentiation in *Drosophila* (Bateman and McNeill, 2004). By using mutants in various components of the InR/TOR pathway, we showed that activation of this pathway causes precocious differentiation of neurons. Conversely, inhibition of InR/TOR signalling significantly delays neurogenesis. Correct temporal control of neuronal differentiation is essential for tissue pattern formation, and consequently mutations in components of the InR/TOR pathway cause pattern defects in the adult. We will present data that suggest a mechanism by which InR/TOR signalling controls neuronal differentiation. Our data show that activation or inhibition of the InR/TOR pathway regulates the expression of the epidermal growth factor receptor (EGFR) ligand Argos. Activation of the InR/TOR pathway, by loss of pten or tsc1, results in increased Argos transcription. Argos transcription is a readout EGFR/mitogen activated protein kinase (MAPK) signalling levels and so these data indicate that control of differentiation by the InR/TOR pathway is achieved though regulation of EGFR/MAPK signalling. Furthermore, our recent data suggest that the mechanism by which InR/TOR signalling elicits this response is by regulation of the expression of the ETS transcription factor Pointed P2 (pntP2). pntP2 is orthologous to the proto-oncoprotein Ets1. Ets1 promotes invasive behaviour in many cell types, and Ets1 expression in tumours is indicative of a poorer prognosis. Our findings demonstrate that the InR/TOR pathway is able to regulate EGFR/MAPK signalling and that this regulation may be exerted through the transcriptional control of pntP2.

***Recipient of Travel Award**

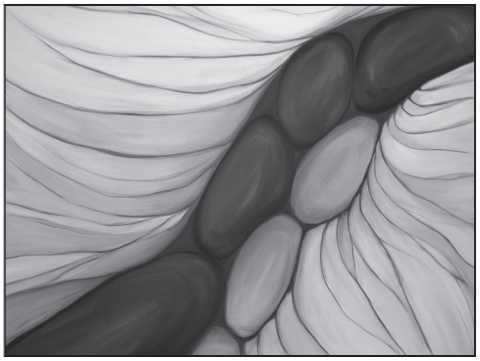


Regulation of and Signaling by the TORC1 Complex

John Blenis

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

Growth factor and oncogene-regulated PI3 kinase- and Ras-activated signaling pathways converge on the nutrient- and energy-sensing mTOR pathway to modulate cell growth, survival and proliferation in many cell types. In human cancer, components of these pathways are often amplified or mutated resulting in inappropriate cell growth. Rapamycin, a specific inhibitor of mTOR, has emerged as a drug with potential anticancer therapeutic efficacy alone or in combination therapy. The molecular basis of how mTOR-raptor (mTORC1) signaling is initiated and how its effector, S6 kinase 1, regulates cell growth through translation initiation is poorly understood. How components of the eIF3 translation initiation complex act as a scaffold for the mTORC1 pathway to coordinate a series of sequential protein-protein interactions regulated by ordered phosphorylation events will be discussed.



Control of Cell Growth and Insulin Sensitivity by the TSC Tumor Suppressor Proteins

Duojia Pan

Johns Hopkins University School of Medicine, Baltimore, MD

Cell growth is a fundamental cellular process that is tightly regulated by hormonal and nutritional cues. Using *Drosophila* as a model system, we and others have previously defined a nutrient-sensitive growth regulating pathway that involves the tuberous sclerosis tumor suppressors Tsc1 and Tsc2, the small GTPase Rheb and the protein kinase TOR. We further showed that the TSC-Rheb-TOR pathway acts in parallel to, and converges on, the canonical insulin pathway to regulate protein synthesis in cell growth. Thus, the TSC-insulin signaling network provides an excellent example in which hormonal and nutritional signals are coordinately sensed to control cell growth.

The canonical insulin signaling pathway is known to regulate diverse physiological processes, including cell growth, metabolism, reproduction, and longevity. Although numerous studies have implicated the TSC tumor suppressors as negative regulators of cell growth, less is known about their involvement in animal metabolism. I will discuss our recent studies implicating TSC tumor suppressors as negative regulators of insulin sensitivity in animal metabolism.

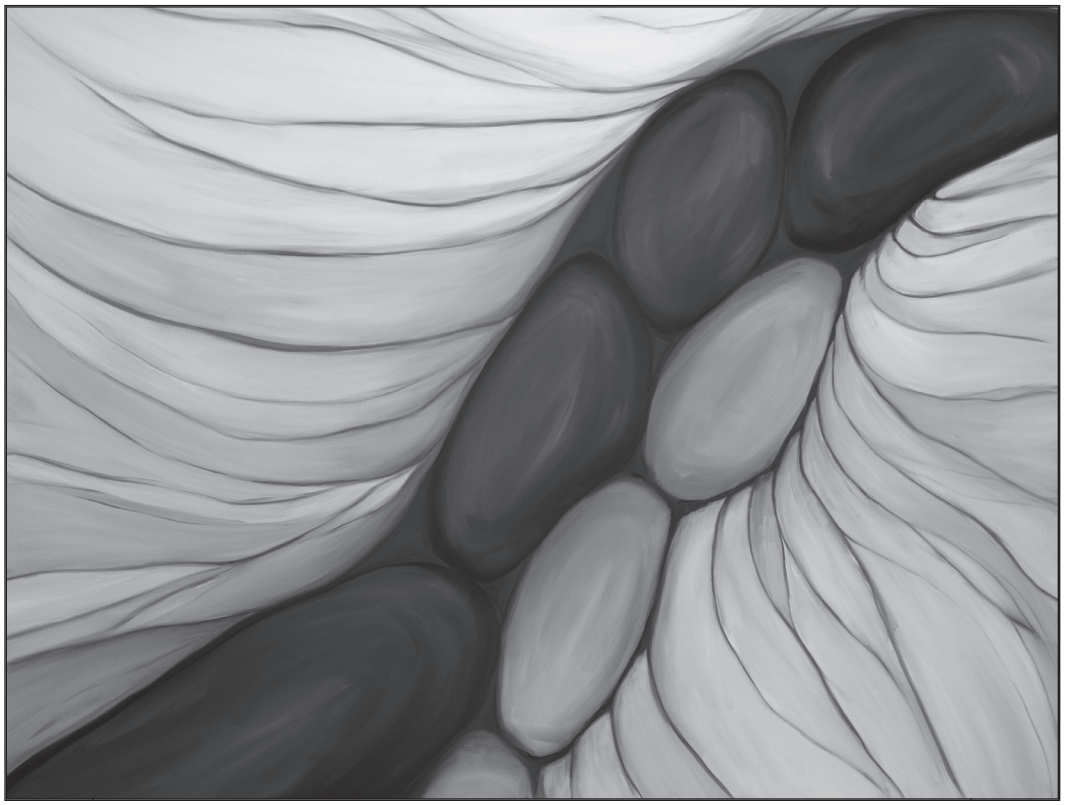
Notes

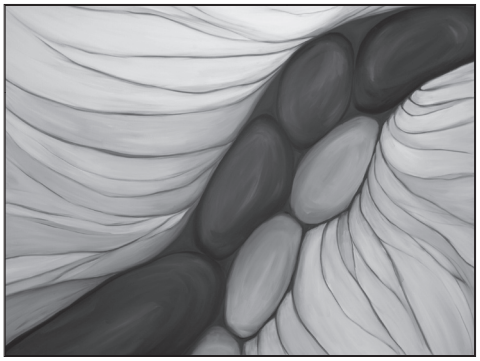
Notes

Notes

Notes

Poster Abstracts





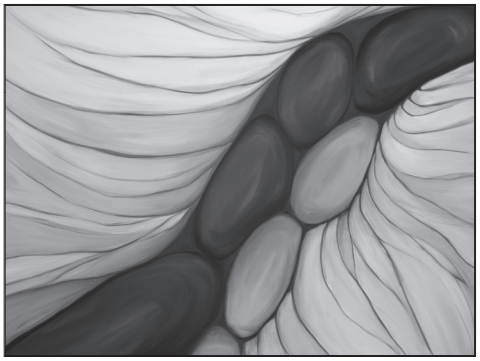
Poster #1: Functional Interaction Between Polo-Like Kinase 1 (PLK1) and Hamartin, the Tuberous Sclerosis Complex 1 (TSC1) Gene Product

***Aristotelis Astrinidis[1], William T. Senapedis[1,2], and Elizabeth P. Henske[1]**

[1]Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA; [2]Harvard Medical School, Boston, MA

Tuberous Sclerosis Complex (TSC) is a tumor suppressor gene syndrome caused by mutations in TSC1 and TSC2, which encode hamartin and tuberin, respectively. Hamartin and tuberin form heterodimers and inhibit growth factor and energy signal transduction cascades to the mammalian target of rapamycin (mTOR), a key regulator of protein synthesis and cell growth. Previously, we showed that hamartin is phosphorylated by CDC2/cyclin B1 during the G2/M phase of the cell cycle. Here, we report that hamartin localizes to the centrosomes and that phosphorylated hamartin and tuberin co-immunoprecipitate with the mitotic kinase polo-like kinase 1 (Plk1), forming a tertiary complex. Plk1 interacts with the NH2-terminus part of hamartin (first 880 amino acids), which contain two potential binding sites for the polo-box domain (residues T310 and S332). A non-phosphorylatable hamartin mutant with an alanine substitution at residue T310 does not interact with Plk1, while alanine mutations in other potential CDC2/cyclin B1 sites, including S332, do not abolish the hamartin-Plk1 interaction. Tsc1–/– mouse embryonic fibroblasts have increased the number of centrosomes and increased DNA content, compared to Tsc1+/+ cells. Both phenotypes are rescued after pretreatment with the mTOR inhibitor rapamycin. Finally, hamartin negatively regulates the protein levels of Plk1, and RNAi-mediated downregulation of Plk1 reduces the phosphorylation of component proteins of the mTOR pathway (p70S6K, S6, and 4E-BP1). These data reveal a novel subcellular localization and a novel interaction partner for hamartin, and implicate hamartin in the regulation of centrosome duplication and mitosis, in part through the aberrant activation of mTOR.

***Recipient of Travel Award**



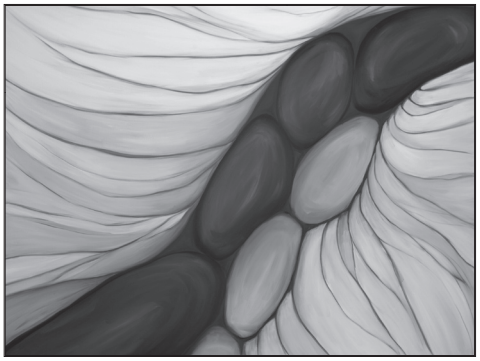
Poster #2: Development of a Kidney-Specific *BHD* Gene Knockout Mouse Model

Masaya Baba[1,6], Mutsuo Furihata[4], Seung-Beom Hong[1,6], Lino Tessarollo[2], Eileen Southon[2,3], Michelle B. Warren[1,6], Peter Igarashi[6], Berton Zbar[6], W. Marston Linehan[1], and Laura S. Schmidt[1,3,6]

[1]Urologic Oncology Branch, NCI Frederick, Frederick, MD; [2]Mouse Cancer Genetics Program, NCI Frederick, Frederick, MD; [3]LASP and BRP, SAIC-Frederick, Inc., NCI Frederick, Frederick, MD; [4]Department of Tumor Biology, Kochi Medical School, Kochi, Japan; [5]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX; [6]Laboratory of Immunobiology, NCI Frederick, Frederick, MD

The Birt-Hogg-Dube' syndrome (BHD), a hamartoma disorder with an increased risk for renal neoplasia, is caused by germline mutations in the *BHD* gene predicted to prematurely truncate the BHD protein, folliculin. Somatic second "hit" mutations identified in BHD-associated renal tumors suggest a tumor suppressor function for this novel gene. Animal models for BHD have been described in the dog and rat, which are caused by inherited mutations in BHD orthologs, and are embryonic lethal when present in the homozygous state. To study *BHD* gene function *in vivo* and avoid embryonic lethality, we have developed a conditional *BHD* gene targeting vector by recombineering methodology, and targeted the BHD gene in mouse embryonic stem (ES) cells. Correctly targeted mouse ES cells were injected into C57BL/6 blastocysts, and resulting chimeras were backcrossed to C57BL/6 mice. F1 mice with germline transmission of the *BHD floxed (f)* allele were interbred and also bred with a strong Cre deletor transgenic mouse to generate *BHD f/f* and *BHD delete (d)/+* mice for further study. In an effort to mimic the renal tumor phenotype seen in BHD patients, we have targeted BHD deletion to the mouse kidney using breeding strategies with Ksp (kidney specific promoter)-Cre transgenic mice. *BHD f/d*, *Ksp-Cre* mice developed enlarged kidneys that were tenfold heavier than control *BHD f/+ Ksp-Cre* littermates. The *BHDf/d*, *Ksp-Cre* mice died by 3 weeks of age due to renal failure as indicated by blood urea nitrogen levels tenfold higher than littermate controls. Histopathological examination of the *BHDf/d*, *Ksp-Cre* kidneys revealed extremely dilated renal tubules and multiple cysts. The presence of atrophic, compressed glomeruli suggested the cause of renal failure. Most of the dilated tubules and cysts showed a monolayer of epithelial cells, but some areas contained multiple layers of epithelial cells and/or cells projecting into the lumen. These morphological changes suggest that homozygous *BHD* inactivation may cause loss of growth control in tubular epithelial cells, consistent with the notion that *BHD* may function as a tumor suppressor gene. We will describe our strategy for generation of the mouse model for *BHD* and report progress in analysis of *BHD* inactivated kidneys in the kidney-targeted conditional *BHD* knockout mice.

This research was funded by NCI-Contract N01-CO-12400.



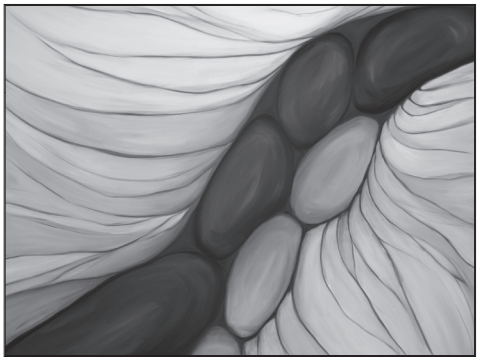
Poster #3: Insulin Receptor/TOR Signaling Controls Neuronal Differentiation by Regulating the Expression of the Ets1 Ortholog Pointed P2 in *Drosophila* (Also Presented as a Late Breaking News Topic)

***Joseph M. Bateman[1] and Helen McNeill[2]**

[1]Wolfson Centre for Age-Related Disease, King's College London, London, United Kingdom; [2]Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada

A fundamental challenge during the development of any complex organism is the coordination of proliferation and differentiation. The *Drosophila* retina is a highly tractable model for studying the relationship between growth and neuronal differentiation. We have shown that the insulin receptor (InR)/TOR pathway plays a key role in controlling the timing of neuronal differentiation in *Drosophila* (Bateman and McNeill, 2004). By using mutants in various components of the InR/TOR pathway, we showed that activation of this pathway causes precocious differentiation of neurons. Conversely, inhibition of InR/TOR signalling significantly delays neurogenesis. Correct temporal control of neuronal differentiation is essential for tissue pattern formation, and consequently mutations in components of the InR/TOR pathway cause pattern defects in the adult. We will present data that suggest a mechanism by which InR/TOR signalling controls neuronal differentiation. Our data show that activation or inhibition of the InR/TOR pathway regulates the expression of the epidermal growth factor receptor (EGFR) ligand Argos. Activation of the InR/TOR pathway, by loss of pten or tsc1, results in increased Argos transcription. Argos transcription is a readout EGFR/mitogen activated protein kinase (MAPK) signalling levels and so these data indicate that control of differentiation by the InR/TOR pathway is achieved though regulation of EGFR/MAPK signalling. Furthermore, our recent data suggest that the mechanism by which InR/TOR signalling elicits this response is by regulation of the expression of the ETS transcription factor Pointed P2 (pntP2). pntP2 is orthologous to the proto-oncoprotein Ets1. Ets1 promotes invasive behaviour in many cell types, and Ets1 expression in tumours is indicative of a poorer prognosis. Our findings demonstrate that the InR/TOR pathway is able to regulate EGFR/MAPK signalling and that this regulation may be exerted through the transcriptional control of pntP2.

***Recipient of Travel Award**



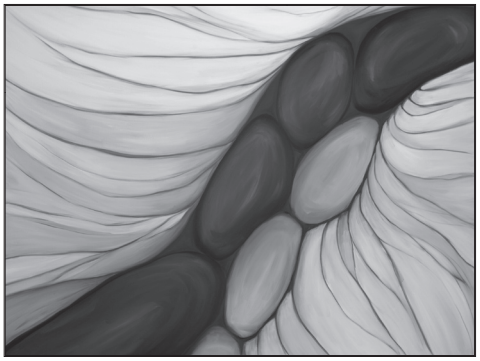
Poster #4: Identification of Translationally Regulated mRNAs in TSC1/2 Null Cells

***B. Bilanges[1], M. Kolesnishenko[1], R. Argonza-Barrett[2], M. Chen, [2], and D. Stokoe[1]**

[1]University of California – San Francisco Cancer Research Institute, San Francisco, CA; [2]Agilent Technologies Inc., Palo Alto, CA

The PI3-K/mTOR pathway integrates signals from nutrients, energy status, and growth factors to regulate many processes, including cell growth and division, autophagy, ribosome biogenesis and metabolism. The mammalian target of rapamycin (mTOR) controls multiple cellular functions in response to amino acids and growth factors, in part by regulating two regulators of protein translation, p70 S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). Tuberous sclerosis (TSC) is an autosomal dominant genetic disorder caused by mutations in either TSC1 or TSC2, and characterized by benign hamartoma growth. TSC1 and TSC2 function as a complex to suppress signaling in the mTOR/S6K/4E-BP pathway controlling protein translation and cell growth in response to nutrient and growth factor stimuli. Importantly, TSC mutant cells commonly exhibit a constitutive activation of mTOR leading to an increase of protein translation. However, the regulation of protein translation via TSC/mTOR pathway is still unclear, and more specifically the identity of the mRNAs translationally regulated by this pathway is unknown. In this study, we show that Mouse Embryonic Fibroblasts (MEFs) lacking TSC1 or TSC2 raises the basal level of mTOR activity such that these cells are resistant to either serum or nutrient starvation. We show that, in WT MEFs, serum starvation inhibits mTOR activity pathway and causes mRNA redistribution between polysomes and subpolysomes. On the other hand, TSC1 null or TSC2 null MEFs show constitutive mTOR activity and eIF4F complex assembly upon serum deprivation, and no changes in the polysome/subpolysome ratio. Using microarray analysis on polysome- and subpolysome-associated mRNAs, we have identified specific mRNAs that are the most dramatically affected by the loss of either TSC1 or TSC2. Interestingly, almost all of the mRNAs that are translationally regulated by serum in WT MEFs show constitutive polysome association in TSC1 or TSC2 null MEFs. About 35 percent of the mRNAs translationally regulated by serum encode terminal oligopyrimidine (TOP) mRNAs (such as ribosomal proteins, initiation and elongation factors). Non-TOP mRNAs are also regulated by serum, suggesting that inhibition of mTOR/S6K/4EBP1 signaling causes translation shut off of both TOP and non-TOP mRNAs. We next show that rapamycin treatment strongly inhibits mTOR/S6K/4EBP1 signaling and induces a rapid change in the association of mRNAs associated with polysomes. About 50 percent of the serum-regulated mRNAs are rapamycin-insensitive, suggesting an mTOR-independent (or rapamycin-resistant) pathway controlling translation. We are currently analyzing sequences in UTR regions required for serum, TSC and rapamycin sensitivity, as well as UTR binding proteins that are important for this. This study, therefore, increases our understanding of how signal transduction pathways impact on protein translation in response to changes in the extracellular environment.

***Recipient of Travel Award**



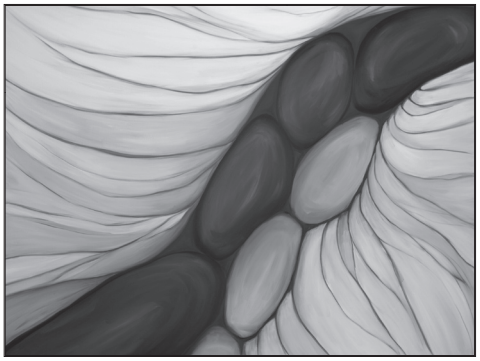
Poster #5: Control of the mTOR Target Lipin by Insulin and Epinephrine

Thurl E. Harris[1], An Chi[2], Todd A. Huffman[1], Anil Laxman[1], Jeffrey Shabanowitz[2], Donald F. Hunt[2], and John C. Lawrence Jr.[1]

[1]Department of Pharmacology, University of Virginia, Charlottesville, VA; [2]Department of Chemistry, University of Virginia, Charlottesville, VA

Lipin has been independently discovered three times: first as the protein product of the Lpn1 gene, which is disrupted in fatty liver dystrophy (fld) mice; next as an adipocyte protein that is phosphorylated in a rapamycin-sensitive manner in response to insulin; and most recently as Mg-dependent phosphatidic acid (PA) phosphatase (PAP), which not only catalyzes the penultimate step in triacylglycerol (TAG) synthesis but also would be expected to remove phospholipase D-generated PA, an activator of mTOR signaling. The present experiments were conducted to identify phosphorylation sites in lipin, and to investigate the control of PAP activity. By mass spectrometry analyses of lipin isolated from 3T3-L1 adipocytes, we identified as many as 23 phosphorylation sites. Seven sites conform to an (S/T)P motif, four sites fit the consensus for phosphorylation by CK2, and two are potential targets of GSK3. Results of experiments involving peptide mapping and mutagenesis identified S106 as a major insulin-stimulated and rapamycin-sensitive phosphorylation site, which affects the electrophoretic mobility of lipin. The S106 site is conserved in all lipin isoforms and in species from *Saccharomyces cerevisiae* to man. Results obtained with a phosphospecific antibody confirmed that S106 was phosphorylated in response to insulin, not only in 3T3-L1 adipocyte but also in skeletal muscle, heart, liver, and adipose tissue *in vivo*. Lipin immunoprecipitated from extracts of 3T3-L1 adipocytes exhibited PAP activity, as did overexpressed lipin immunoprecipitated from HEK293 cells. PAP activity was abolished by mutating Gly84 to Arg to mimic the substitution found in fld2j mice, which exhibit lipodystrophy. Thus, the loss of PAP activity may explain the failure to accumulate TAG in adipose tissue in these mice. PAP activity was also abolished when Glu was substituted for a putative catalytic Asp in the haloacid dehalogenase (HAD) domain, which is located in the COOH terminal region of lipin. Interestingly, mutating either of two phosphorylation sites in the HAD domain markedly reduced PAP activity, suggesting that these sites might be involved in controlling the activity of lipin. In contrast, mutating S106 to Ala did not directly affect PAP activity, in either the presence or absence of insulin. The total PAP activity of lipin in 3T3-L1 adipocytes was not significantly changed by insulin, although the hormone caused a redistribution of lipin, moving the protein from a particulate fraction to the soluble fraction. This response was blocked by rapamycin. In contrast, epinephrine caused translocation of lipin out of the soluble fraction and into a pellet fraction that contained nuclei, mitochondria, and plasma membranes. Our findings suggest that PAP activity is controlled via phosphorylation-mediated relocalization of the lipin protein to different cellular compartments.

This work was supported in part by DK28312 and DK52753.

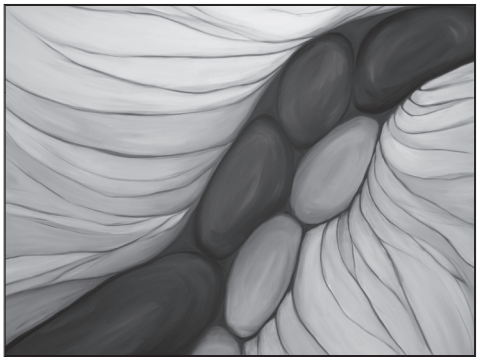


Poster #6: Small Molecule Enhancers of mTOR Inhibition

Mariam Aghajan, Fei Fu, and Jing Huang

Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA

The function of the TOR (target of rapamycin) protein kinase pathway is central to cell growth control in eukaryotes. In animals, growth signals (e.g., nutrients and insulin-like growth factors) impinge on TOR activities through an intricate network of proteins involving the oncoproteins LKB1, PI3K, and Akt, and the tumor suppressor proteins TSC1, TSC2, and PTEN. As a result, rapamycin or other drugs that can effectively modulate this network have great potential for treating relevant diseases involving the dysregulation of the network, including Tuberous Sclerosis, Peutz-Jeghers Syndrome, and Cowden's Syndrome. Using a chemical genetic approach, we have identified a number of novel small molecules that enhance mTOR inhibition in cell culture systems (not yet tested in animal models). We will present our preliminary studies of the mechanisms of these molecules in the context of mTOR signaling and cell growth control. Such small molecules offer a unique opportunity for better understanding the molecular regulation of the TOR network as well as for developing novel therapeutic interventions in this area.



Poster #7: An Algorithm for Identifying Novel Targets of Transcription Factors: Application to Hypoxia-Inducible Factor 1

Yue Jiang[1], Bojan Cukic[1], Donald A. Adjeroh[1], Heath D. Skinner[2], and Bing-Hua Jiang[2]

[1]Lane Department of Computer Science and Electrical Engineering, West Virginia University, Morgantown, WV; [2]Mary Babb Randolph Cancer Center, Department of Microbiology, Immunology and Cell Biology, West Virginia University, Morgantown, WV

Motivation

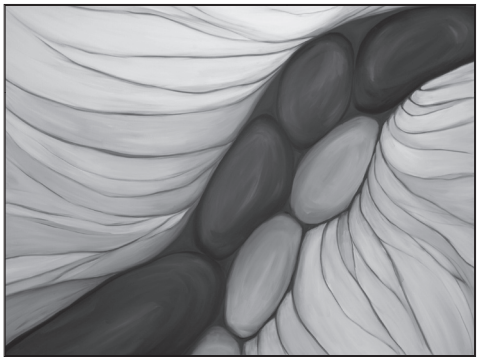
Given the important role of transcription factors in gene expression, the target gene identification of known transcription factors has been an important, but difficult issue. With the increasing size of available genomic databases, the development of computational tools to identify such novel targets from these databases poses a significant challenge, and has important implications in the study of human diseases such as cancer and cardiovascular diseases.

Methods

A new methodology is developed based on the suffix tree data structure to identify novel targets of transcription factors. The method is applied to hypoxia-inducible factor 1 (HIF-1), one of the most important transcription factors. The basic approach is based on algorithms for multiple-pattern multiple-sequence matching using suffix trees. A leave-k-out cross-validation method is used to verify the performance of the methodology.

Results

We used 15 known HIF-1 target gene sequences as a training set to search the gene database and found 258 potentially HIF-1 targets, including all 25 known HIF-1 targets. To further investigate whether the identified potential novel HIF-1 targets are functional, we selected COX-2, one of the 233 potentially novel HIF-1 targets for analyzing its biological function in the wet laboratory. We demonstrated that COX-2 is a biologically relevant HIF-1 target. In the follow-up literature search, an additional 17 putative genes in the final output were confirmed to be upregulated by HIF-1 or hypoxia. These results demonstrate that the methodology is an effective computational approach for identifying novel HIF-1 targets. Using HIF-1 as a model system, we show that our new methodology may have a general application to search for novel targets of other transcription factors in the future.

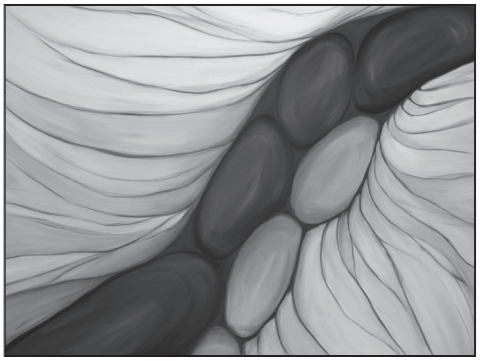


Poster #8: Rheb Inhibits C-Raf Kinase Activity and Growth Factor-Induced B-Raf/C-Raf Heterodimerization in a Rapamycin-Insensitive Manner

Magdalena Karbowniczek and Elizabeth Petri Henske

Fox Chase Cancer Center, Philadelphia, PA

Tuberous Sclerosis Complex (TSC) is a tumor suppressor gene syndrome whose manifestations can include seizures, mental retardation, autism, and tumors in the brain, retina, kidney, heart, and skin. The products of the TSC1 and TSC2 genes, hamartin and tuberin respectively, heterodimerize and inhibit the mammalian target of rapamycin (mTOR). Angiomyolipomas, which occur in the majority of sporadic lymphangioleiomyomatosis (LAM) and tuberous sclerosis complex (TSC) patients, are benign tumors composed of abnormal vessels, immature smooth muscle cells, and fat cells. Genetic studies on sporadic LAM-associated angiomyolipomas demonstrated that all three components (smooth muscle, vessels, and fat) are derived from a common progenitor cell (Karbowniczek et al., *Am J Pathol* 2003), indicating the ability of TSC2-deficient cells to differentiate into multiple lineages. These findings suggest that tuberin and hamartin play a role in regulation of cell differentiation, and led us to focus on Raf and p42/p44 MAPK signaling downstream of the TSC1/TSC2 complex. In prior work (Karbowniczek et al., *J Biol Chem* 2004), we found that siRNA downregulation of tuberin (TSC2) decreases B-Raf kinase activity, and that Rheb, the target of the GTPase activating domain of tuberin (TSC2), interacts with and inhibits B-Raf kinase in a Rapamycin-resistant manner. Here, we report that Rheb inhibits the phosphorylation of B-Raf kinase at S445, a site previously thought to be constitutively phosphorylated. The activity of S445D and S445A B-Raf mutants was inhibited by Rheb, suggesting that Rheb's inhibition of B-Raf activity and Rheb's inhibition of B-Raf phosphorylation are independent activities. Rheb did not inhibit the activity of V600E or T598E/S601D B-Raf mutants. We also found that Rheb inhibits growth-factor-induced C-Raf kinase activity and Serine 338 phosphorylation. Rheb did not inhibit the constitutively active C-Raf-DDED mutant (S338D/Y341D/T491E/S494D). In addition to Rheb's inhibition of B-Raf and C-Raf phosphorylation and activity, Rheb inhibited C-Raf/B-Raf heterodimerization. C-Raf/B-Raf heterodimerization is known to impact the activity of both C-Raf and B-Raf. Therefore, the decreased heterodimerization may be the mechanism through which Rheb regulates the activity of C-Raf. Rheb's inhibition of B-Raf/C-Raf heterodimerization, as well as Rheb's inhibition of B-Raf and C-Raf phosphorylation and activity, are Rapamycin insensitive, indicating that they not mediated by mTOR complex 1. This has potential clinical relevance because Rapamycin is currently in clinical trials for patients with TSC and/or LAM. In summary, we report here for the first time that the activity and S338/S445 phosphorylation of C-Raf and B-Raf kinases are regulated by Rheb. Rheb inhibits C-Raf and B-Raf activity and inhibits C-Raf/B-Raf dimerization. Regulation of Raf kinase activity by Rheb may play a central role in the coordination of cellular differentiation signals and cell fate decisions, and may thereby participate in the pathogenesis of LAM.



Poster #9: TOR Complex 2 and the Akt/TSC/Rheb Pathway Negatively Regulate Nutrient Capture via Phagocytosis

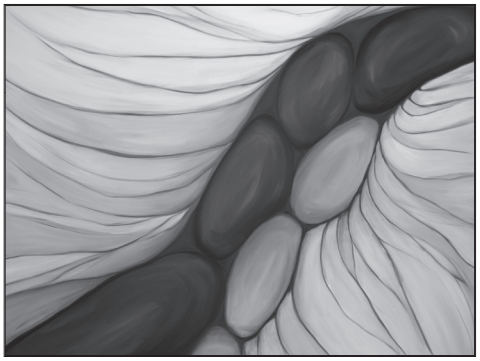
Daniel Rosel, Taruna Khurana, Amit Majithia, Xiuli Huang, Ramantha Bhandari, and Alan R. Kimmel

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

The TOR kinase functions within (at least) two distinct complexes, TORC1 and TORC2, that integrate signals arising from nutrients, cellular energy state, and other stimuli to regulate growth and cytoskeletal response. TORC1 is comprised of TOR, raptor, and LST8 (mammalian GßL) and is required for cellular growth. TOR/raptor interactions and TORC1 function are disrupted by rapamycin. In contrast, TORC2 is dispensable for growth, but regulates actin polarization in yeast, mammalian cells, and *Dictyostelium*. The components of TORC2 are not as well defined across species as those of TORC1. TORC2 consists of TOR and LST8, but unlike TORC1, it does not contain raptor and does not exhibit sensitivity to inactivation by rapamycin. TORC2 of *Dictyostelium*, yeast, and mammals also contain a Pia (yeast AVO3, mammalian Rictor) subunit, and TORC2 of *Dictyostelium* and yeast possesses RIP3 (yeast AVO1).

TORC1 is subject to antagonistic controls that coordinate growth parameters and intracellular energy status. These signals become integrated at the Tuberous Sclerosis Complex (TSC), a Rheb-GAP (GTPase activating protein), which functions in a negative pathway for TORC1 regulation. The downstream target of TSC is the ras-related, small G protein Rheb. Rheb-GTP serves as an activator of TORC1 and, in turn, TSC is negatively or positively influenced through respective phosphorylations by Akt or the AMP-dependent protein kinase (AMPK). Growth factors that activate the PI3K/Akt pathway inhibit TSC and activate TORC1, whereas depletion of intracellular energy stores activates AMPK and TSC, suppressing TORC1 function and cellular growth. The pathways for growth regulation are more complex and are still only partially understood; furthermore, upstream regulators of TORC2 have yet to be elaborated. Here, using *Dictyostelium* as a model, we have focused on the role of TORC2 as a potential co-regulator of growth with TORC1 and suggest a functional interrelationship between TORC1 and TORC2.

In their ecological niche, *Dictyostelium* utilize bacteria as a nutrient source during growth. We now show that loss of TORC2 components Lst8, Pia, or Rip3 promotes nutrient uptake via phagocytosis, an actin-mediated process. This contrasts with the positive role of TORC1 for growth and of TORC2 for cytoskeletal response. Furthermore, our data suggest that TORC2 may be subject to similar control by the TSC/Rheb pathway as is TORC1. Inactivating mutations of Akt and Rheb promote phagocytosis, while TSC2 deficiency or expression of the Rheb-GTP variant suppresses phagocytosis. Further, expression of Rheb-GTP is unable to inhibit phaocytosis in either lst8- or rip3-nulls, suggesting that Rheb acts in a dependent pathway upstream of TORC2. Thus, growth signals that activate TORC1 may also activate TORC2 to suppress phagocytosis and nutrient capture. We suggest that the balanced and contrasting activities of TORC1 and TORC2 are essential to coordinate and optimize growth rates with energy supply. Nonetheless, TORC1 does not play a direct role in phagocytosis. Neither Raptor-depletion nor short-term (<2 hr) rapamycin treatment alters phagocytois. However, long-term rapamycin (>4 hr) treatment will promote phagocytosis in WT, but not in raptor-depleted, cells. Consequently, the TORC2 complex may be subject to destabilization and inhibition by rapamycin, albeit indirectly and temporally delayed. We propose that TORC1 and TORC2 are not static complexes, but are in equilibrium, and that the rapamycin-induced destabilization of TOR/raptor interactions within TORC1 shifts the equilibrium from TORC2, in effect recruiting additional TORC1 for growth and promoting phagocytosis by reducing levels of the TORC2 inhibitory complex.



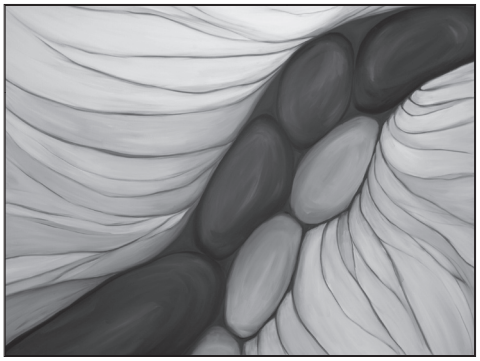
Poster #10: Elevated Fatty Acids Decrease Basal Protein Synthesis by mTOR-Mediated Mechanism, but Not the Anabolic Effect of Leucine in Skeletal Muscle

Charles Lang

Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA

Elevations in free fatty acids (FFAs) impair glucose uptake in skeletal muscle. However, there is no information pertaining to the effect of elevated circulating lipids on either basal protein synthesis or the anabolic effects of leucine and insulin-like growth factor (IGF)-I. In chronically catheterized conscious rats, the short-term elevation of plasma FFAs by the 5 h infusion of heparin + intralipid decreased muscle protein synthesis by approximately 25 percent under basal conditions. Lipid infusion was associated with a redistribution of eukaryotic initiation factor (eIF)-4E from the active eIF4E·eIF4G complex to the inactive eIF4E·4E-BP1 complex. This shift was associated with a decreased phosphorylation of eIF4G but not 4E-BP1. Lipid infusion did not significantly alter either the total amount or phosphorylation state of mTOR, TSC2, S6K1, or the ribosomal protein S6 under basal conditions. In control rats, oral leucine increased muscle protein synthesis. This anabolic response was not impaired by lipid infusion, and no defects in signal transduction pathways regulating translation initiation were detected. In separate rats receiving a bolus injection of IGF-I, lipid infusion attenuated the normal redistribution of eIF4E from the active to inactive complex, and largely prevented the increased phosphorylation of 4E-BP1, eIF4G, S6K1, and S6. This IGF-I resistance was associated with enhanced Ser307 phosphorylation of insulin receptor substate (IRS)-1. These data indicate that short-term elevation of plasma FFAs impairs basal protein synthesis in muscle by altering eIF4E availability, and this defect may be related to impaired phosphorylation of eIF4G, not 4E-BP1. Moreover, hyperlipidemia leads to a relative selective impairment in IGF-I action but does not produce a leucine resistance in skeletal muscle.

This research was supported by GM38032.

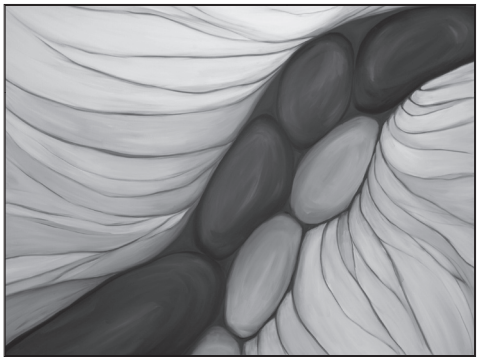


Poster #11: Integration of Nutrient Sensing with the Proliferative Response via the Molecular Scaffold Kinase Suppressor of Ras (KSR)

Diane L. Costanzo[1], Paul Pfluger[2], Jeffery L. Stock[4], Aimee Schreiner[1], Tina Treece[1], Ming-Hoi Wu[1], Andrey S. Shaw[3], John McNeish[4], Matthias H. Tschöp[2], and Robert E. Lewis[1]

[1]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE; [2]Obesity Research Center, Department of Psychiatry, University of Cincinnati, Cincinnati, OH; [3]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO; [4]Genetic Technologies, Pfizer Global Research and Development, Groton, CT

The Raf/MEK/ERK kinase cascade is a potent regulator of multiple cell fates. Recent data demonstrate that the molecular scaffold Kinase Suppressor of Ras 1 (KSR1) plays a critical role in regulating the intensity and duration of signaling through this signaling cassette, directing its output toward either proliferative or adipogenic programs. A related family member, KSR2, may have both distinct and overlapping functions. Previous observations have demonstrated that KSR1-/- mice are essentially normal. A closer examination revealed that KSR1-/- mice have hypertrophic adipocytes, though no increase in overall fat pad mass. As adipocyte size has been correlated with altered glucose homeostasis, we tested glucose tolerance following KSR1 disruption in DBA1/LacJ and C57BL6/J strains. Glucose tolerance was normal in KSR1-/- mice on the DBA1/LacJ background, despite adipocyte hypertrophy. However, deletion of KSR1 in C57BL6/J mice caused profound glucose intolerance. Comparable levels of glucose intolerance were observed in wild type C57BL6 mice only after 12 weeks of feeding on a high fat diet. Interestingly, glucose intolerance was not exacerbated in KSR1-/- mice by a high fat diet. Disruption of KSR2 has profound effects on glucose homeostasis that are demonstrable even in the DBA1/LacJ background. Analysis of mouse embryo fibroblasts from knockout mice reveals alterations in basal glucose uptake and nutrient-dependent cell proliferation that suggests the metabolic effects of KSR1 and KSR2 may be cell autonomous. Coupled with the potent effect of KSR1 in regulating Raf and MEK activation, these observations suggest a previously unidentified, but physiologically important, interdependence between the Raf/MEK/ERK signaling cassette and mechanisms regulating cellular metabolism.

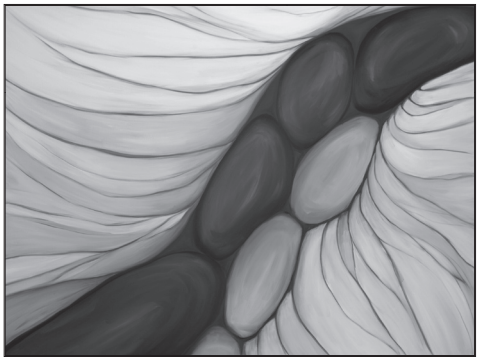


Poster #12: A Potential Role for the Redox Enzyme Proline Oxidase in mTOR-AMPK Signaling Pathway

Jui Pandhare[1], Sandra K. Cooper[2], and James M. Phang[1]

[1]Laboratory of Comparative Carcinogenesis, Center for Cancer Research, NCI-Frederick, Frederick, MD; [2]Basic Research Program, SAIC-Frederick, Inc., Frederick, MD

Proline oxidase (POX) is a mitochondrial inner membrane redox enzyme catalyzing the conversion of proline to pyrroline-5-carboxylate (P5C). The cycling of proline and P5C between the mitochondria and cytoplasm forms a redox shuttle that can transfer reducing potential for generation of ATP or donate electrons for ROS generation. Thus, proline degradation may bioenergetically support a number of important cellular functions, including the coupling of cellular oxidative metabolism to apoptosis. In another context, the mammalian target of rapamycin (mTOR) is a Ser/Thr protein kinase that integrates signals from growth factors, nutrients, stress, and cellular energy levels to control cell growth. The mTOR-specific inhibitor, rapamycin, was found to stimulate POX promoter activity and upregulate expression of POX with concomitant increase in POX catalytic activity in RKO cells, indicating that POX may be playing a functional role in the mTOR pathway. Rapamycin treatment slowed the growth rate and within 6 h resulted in an initial decline of intracellular ATP levels. However, accompanying the increase in POX expression by 12 h, the ATP levels started to rise. In addition, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), the activator of AMP-activated protein kinase (AMPK), also stimulated POX expression. AMPK is a cellular energy sensor that blocks anabolic pathways (i.e., protein synthesis) and increases the production of ATP. Both mTOR inhibition and AMPK activation are sensitive to cellular energy levels and affect protein synthesis through the inhibition of p70 S6k activity. Because POX expression is stimulated by both rapamycin and AICAR, we hypothesize that POX may be regulated to generate ATP for maintaining cells in a vegetative state when protein synthesis is halted due to the inhibition of mTOR and/or decreased energy levels. Thus, POX may be an alternative energy source for survival as well as for programmed cell death.

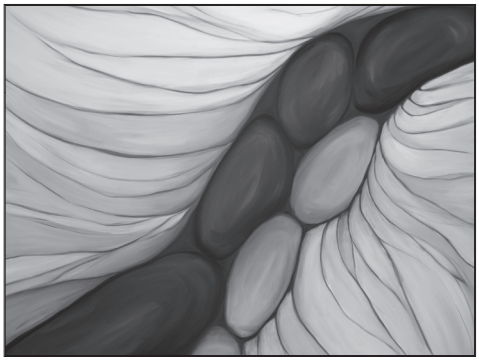


Poster #13: Chemopreventive and Other Effects of PDK1/AKT Pathway Inhibition in a Transgenic Mouse Model of Prostate Cancer

Aaron Sargeant

Chen Laboratory, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH

The PDK1/Akt pathway is an important regulator of multiple biological processes, including cell growth, survival, and glycogen metabolism. To evaluate the chemopreventive relevance of inhibiting this pathway in prostate cancer, transgenic mice (transgenic adenocarcinoma of the mouse prostate or TRAMP) that model lesion progression in man were treated with OSU03012, a novel molecularly targeted agent synthesized in our laboratory and currently undergoing preclinical evaluation in the Rapid Access to Intervention Development program at the NCI. Beginning at 10 weeks of age when clinically relevant prostatic intraepithelial neoplasia (PIN) lesions are well developed, TRAMP mice received OSU03012 once daily by oral gavage for 8 weeks. In addition to anti-cancer effects, general systemic consequences of drug administration were assessed by comprehensive clinical and histopathologic evaluation. Treatment significantly decreased the weight of all four prostate lobes as well as the severity of microscopic lesions in the dorsal and lateral lobes compared to vehicle-control mice. The incidences of carcinoma and metastasis were decreased, although not to a statistically significant level. The presence of drug and pathway inhibition in prostate tissue were confirmed by HPLC and western analyses, respectively. Body fat and weight of treated mice were decreased independent of food intake. This change and observed histopathologic lesions, including centriacinar hepatocellular atrophy and multifocal, multiphasic segmental skeletal myonecrosis, can be linked to sustained PDK1/Akt inhibition partly through downstream inactivation of glycogen synthase. We conclude that targeting of the PDK1/Akt pathway has chemopreventive relevance in prostate cancer and other *in vivo* effects mediated by alteration of bioenergetic signaling.

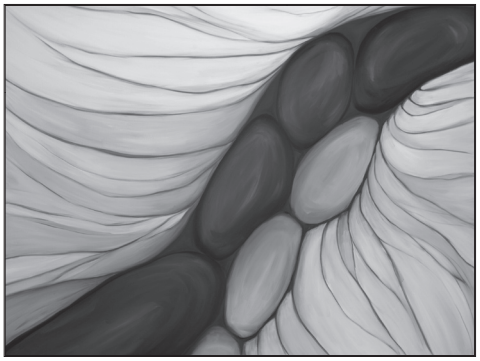


Poster #14: Temporal Dynamics of Tyrosine Phosphorylation in the Insulin Signaling Pathway

Katrin Schmelzle[1], Susan Kane[2], Scott Gridley[2], Gustav E. Lienhard[2], and Forest M. White[1]

[1]Biological Engineering Division, Massachusetts Institute of Technology, Cambridge, MA; [2]Biochemistry Department, Dartmouth Medical School, Hanover, NH

The insulin signaling network is crucial for the regulation of blood glucose levels and metabolic control. Dysregulation of this network may result in the development of type 2 diabetes or other metabolic diseases. Although the role of tyrosine phosphorylation in insulin signaling is well known, only a limited number of phosphorylation sites have been identified. To address this issue and investigate temporal response, we have conducted a quantitative, mass spectrometry-based analysis of insulin-induced tyrosine phosphorylation in 3T3-L1 adipocytes. In our approach, we have identified and quantified more than 100 tyrosine phosphorylation sites on several dozens of proteins, including insulin receptor, IRS1/2, and ERK1/2. The temporal profile of most sites shows an initial increase in phosphorylation upon insulin stimulation with fairly stable phosphorylation levels over time. A few sites exhibit other distinct profiles such as: (a) continuous increase in phosphorylation, or (b) an initial increase followed by a decrease in phosphorylation (e.g., proteins involved in the MAP kinase pathway). In addition to several previously known sites, many new sites have been identified, including sites on proteins involved in glucose uptake. In conclusion, our findings reveal an extensive picture of insulin-induced tyrosine phosphorylation and might ultimately contribute to the identification of potential new drug targets in diabetes and other metabolic diseases.



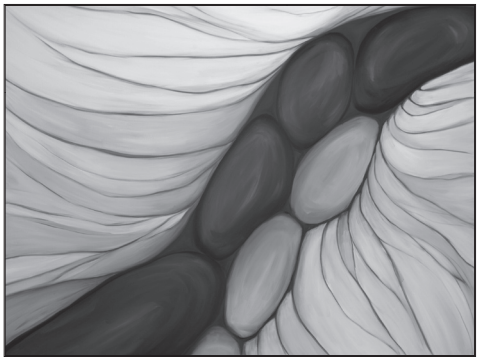
Poster #15: Enhanced Energy Expenditure, Insulin Sensitivity, and Resistance to Diet-Induced Obesity in Mice Lacking BCATm

***Pengxiang She[1], Sarah K. Bronson[1], Susan M. Hutson[2], and Christopher J. Lynch[1]**

[1]Department of Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA; [2]Nutrition Research Center, Wake Forest University Health Sciences, Winston-Salem, NC

Branched chain amino acids (BCAAs), especially leucine, are nutrient signals that regulate protein synthesis and cell growth pathways affected by mTOR and insulin secretion. BCAAs may also affect insulin signaling. To understand the role of leucine metabolism in these effects, we generated mice lacking (KO) the mitochondrial isoform of branched-chain aminotransferase (BCATm) using the Cre-LoxP system. BCATm catalyzes the first step of BCAA metabolism in non-neural tissues. The global BCATm KO and wild-type mice were maintained on a choice of complete and BCAA-free diets. Although KO mice selected far less of the BCAA-containing diet, their total plasma BCAAs were still elevated approximately 21-fold compared to wild-type mice. BCATm KO mice had an unusual metabolic phenotype. Male KOs exhibited a 10-15 percent lower body weight (associated mainly with decreased fat body mass), while caloric intake was comparable to wild type. Fasting plasma glucose and insulin were decreased by 24 and 67 percent, respectively. The KOs had lower blood glucose along with 51 and 55 percent decreases in areas under curve during GTTs and ITTs and lower fasting plasma leptin (-88%), adiponectin (-50%), resistin (-34%), FFA (-40%), beta-hydroxybutyrate (-50%), and fed plasma alanine (-44%). Rates of oxygen consumption were increased by 32 percent in the KO mice, suggesting increased energy expenditure. Plasma thyroxine (T4) and brown fat mRNA expression for thyroid hormone activating enzyme type 2 iodothyronine deiodinase (D2), UCP-1, PGC-1alpha, and beta-adrenergic receptor 3 were unaltered. Locomotive activity and rectal core temperature were also unaltered. On a 60 percent fat diet, male KO mice were resistant to diet-induced obesity. Enhanced insulin sensitivity and energy expenditure still existed when plasma BCAAs were normalized by dietary manipulation, suggesting that loss of BCAA catabolism rather than elevated BCAA is responsible for these metabolic changes. We are currently exploring the underlying mechanisms in these animals to evaluate the potential of BCATm as a target for diabetes/obesity therapies.

***Recipient of Travel Award**

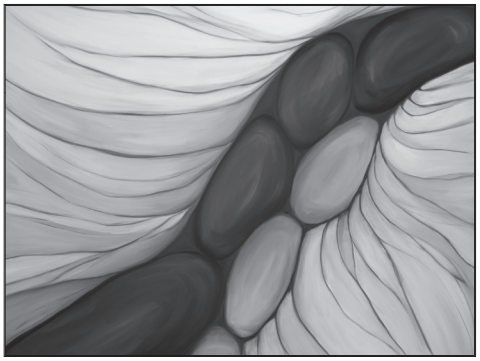


Poster #16: Metformin and AICAR Prevent Insulin Resistance Caused by Sustained Hyperglycemia in HepG2 Cell

Gabriela Suchankova[1], Mengwei Zang[2], Asish Saha[3], and Neil B. Ruderman[3]

[1]Diabetes and Metabolism Unit, Department of Medicine, Boston University School of Medicine, Boston, MA; [2]Vascular Biology Unit, Whitaker Cardiovascular Institute, Boston, MA; [3]Diabetes and Metabolism Unit, Department of Medicine, Boston University School of Medicine, Boston, MA

Glucose toxicity due to sustained hyperglycemia is one of the causes of insulin resistance (IR) in patients with type 2 diabetes and other metabolic disorders. Recently, we demonstrated that sustained hyperglycemia causes IR in cultured HepG2 cells. An associated change was a decrease in the activity of the fuel-sensing enzyme, AMP-activated protein kinase (AMPK); however, its relevance to the development of IR was not studied. To examine this question, HepG2 cells were incubated for 24 h with 5 or 25 mM glucose in the presence or absence of metformin (1-2 mM) or AICAR (0.2-1 mM), two agents known to activate AMPK in these cells. In keeping with earlier results, we found that incubation with 25 mM glucose for 24 h decreased phosphorylation of AMPK (Thr 172) and ACC (Ser 79), as well as insulin-induced phosphorylation of Akt (Ser 473) and its downstream target GSK-3 (Ser 21/9). The decreases in all of these parameters were prevented by metformin in a dose-dependent manner. Incubation with 25 mM glucose for 2 h caused qualitatively similar changes in p-AMPK and insulin signaling, both of which were prevented by metformin and AICAR. Finally, we examined the effect of resveratrol, a polyphenol that activates Sir2, a deacetylase thought to mediate the effects of caloric restriction on longevity. Incubation of these cells with 100 mM resveratrol for 1 h increased p-AMPK by twofold, as well as p-ACC in cells. Whether it affects the insulin signaling is presently under study. In conclusion, the results indicate that restoration of p-AMPK by metformin and AICAR prevent insulin resistance in HepG2 cells incubated in a high glucose media. Whether other AMPK activators have a similar effect and the mechanism by which they act in this setting remains to be determined.

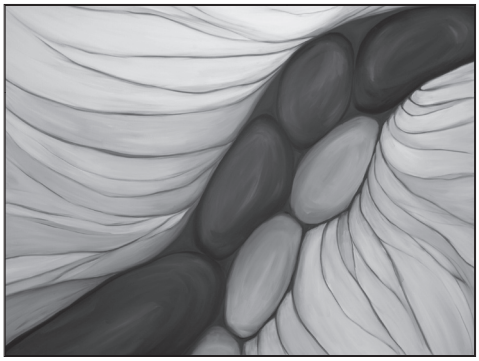


Poster #17: Activation of Signaling Components Leading to Translation Initiation in Skeletal Muscle of Neonatal Pigs Is Regulated by Feeding and Development

Agus Suryawan and Teresa A. Davis

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX

The neonatal period is characterized by rapid growth driven by high rates of skeletal muscle protein synthesis. This high rate of protein synthesis, which is induced by feeding, declines with development. In this study, we measured the activation of several growth factor- and nutrient-induced signaling components that positively or negatively regulate mRNA translation initiation in skeletal muscle of 7- and 26-day-old pigs. The activation of the inhibitors of protein synthesis (PTEN, PP2A, and TSC1/TSC2) increased with age ($P<0.05$). Interestingly, neither abundance nor activation of AMPK-alpha, a potent inhibitor of protein synthesis, changed with age. The activation of positive regulators of protein synthesis (mTOR, S6K1, and 4EBP-1) decreased with age ($P<0.05$). Furthermore, raptor abundance and raptor-mTOR complex were higher in 7-day-old compared to 26-day-old pigs ($P<0.05$), consistent with higher mTOR activation in younger pigs. Thus, the results suggest that the activation of both growth factor and nutrient signaling components leading to mRNA translation initiation changes with age in parallel with the changes in protein synthesis in skeletal muscle of neonatal pigs.

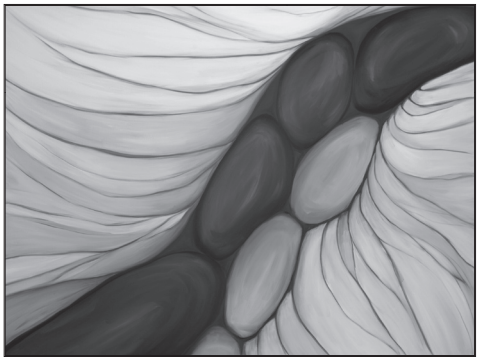


Poster #18: Activation of mTOR by Insulin Is Associated with Stimulation of 4EBP1 Binding to Dimeric mTOR Complex 1

Lifu Wang[1], Christopher J. Rhodes[2], and John C. Lawrence, Jr.[1]

[1]Department of Pharmacology, University of Virginia, Charlottesville, VA; [2]Pacific Northwest Research Institute, Department of Pharmacology, University of Washington, Seattle, WA

Insulin stimulates protein synthesis through phosphorylation of the eIF4E-binding protein, 4EBP1. This effect is rapamycin-sensitive and mediated by mTOR complex 1 (mTORC1), which contains mTOR, raptor, and mLst8. Here, we demonstrate that incubating 3T3-L1 adipocytes with insulin produces a stable increase in the kinase activity of mTORC1 measured after immunoprecipitating complexes with raptor antibodies. The increase in activity was associated with an increase in binding of 4EBP1 to raptor. Binding was assessed using two different approaches. In the first, binding was measured after incubating immunoprecipitated raptor with purified 4EBP1. In the second, binding was measured by determining the amount of raptor captured by an affinity resin to which 4EBP1 had been covalently bound. Disruption of mTOR-raptor interaction by either of the detergents Triton X-100 or NP40, abolished the effects of insulin in binding and activity. After size exclusion chromatography, most of TORC1 was recovered in a peak indicative of a Mr=840,000. Insulin stimulated both 4EBP1 kinase activity and binding activity of mTORC1 in this peak. Co-immunoprecipitations of epitope-tagged subunits provided evidence of mTOR-mTOR, raptor-raptor, and mTOR-raptor interactions in this peak. The results indicate that the major insulin-responsive form is dimeric mTORC1, a structure containing two heterotrimers of mTOR, raptor, and mLst8.

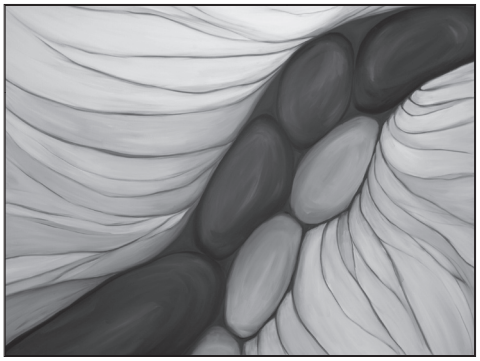


Poster #19: Regulation of Insulin Synthesis by mTOR in MIN6 Insulinoma Cells

Rebecca L. Brown[1], Poonam Saxena[1], Lorna Dickson[3], Margaret Milewski[2], Sushila Dalal[1], Gregory R. Vlacich[1], Christopher J. Rhodes[3], and Gene C. Webb[1]

[1]Department of Medicine and of [2]Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL; [3]Pacific Northwest Research Institute, Seattle, WA

Coordination of protein translation and secretion occupies a central role in the function of many eukaryotic cells. Although modulation of ATP levels derived from glucose metabolism has been demonstrated to couple glucose stimulation to insulin secretion through direct action on the ATP-sensitive K(+) channel, it is not clear which factor or factors couple glucose stimulation to specific increases in proinsulin synthesis. We provide evidence that ATP is also responsible for this coupling. Metabolic inhibitors 2-DG and FCCP, through distinct mechanisms, brought about dose-dependent modulation of cellular ATP levels that provided separable regulation of proinsulin synthesis and secretion. Regulation of synthesis was dependent on signaling through the mTOR pathway as defined by ATP dose-dependent activation of mTOR toward downstream effectors eIF4E-BP and p70S6K. The mTOR-specific inhibitor rapamycin uncoupled ATP levels from mTOR activation and proinsulin synthesis but not secretion. Expression of a kinase-dead allele of mTOR was also able to specifically inhibit proinsulin synthesis. The ATP-sensitive K(+) channel inhibitor diazoxide blocked activation of the insulin receptor in a dose-dependent manner but had no effect on proinsulin synthesis. Immunoneutralization of secreted insulin further demonstrated that insulin is not necessary for signaling through the mTOR pathway or for proinsulin synthesis.



Poster #20: Estradiol-Induced Activation of the Mammalian Target of Rapamycin Pathway Is Mediated by Phosphatidylinositol 3-Kinase/Akt and Tuberin (Also Presented as a Late Breaking News Topic)

***Jane Yu and Elizabeth Petri Henske**

Fox Chase Cancer Center, Philadelphia, PA

Tuberous Sclerosis Complex (TSC) results from loss of TSC1 or TSC2. The TSC1 and TSC2 gene products, hamartin and tuberin, associate as a functional complex to inhibit mTOR via Rheb. Lymphangioleiomyomatosis is a TSC-linked pulmonary smooth muscle cell proliferation that occurs almost exclusively in women, suggesting that estrogen is linked to the function of tuberin. However, the pathways through which estrogen signals to tuberin are not well understood. To investigate tuberin's role in regulating cytoplasmic estrogen signaling, we first determined whether estrogen activates mTOR activity by examining the phosphorylation of downstream targets of mTOR, p70 ribosomal S6 kinase (p70S6K) and S6, in MCF-7 cells. 17-beta estradiol rapidly increased the phosphorylation of p70S6K (3-fold) and S6 (6-fold) within 15 minutes. The estrogen-induced activation of S6K and S6 was blocked by Rapamycin, Wortmannin, and PD28095, suggesting that the PI3K/mTOR and MEK/MAPK pathways co-mediate estrogen signaling to mTOR. Because Rheb is known to activate mTOR and S6K, we next asked whether estrogen regulates Rheb activation. Estradiol increased the active GTP-fraction of endogenous Rheb by fourfold within 10 minutes in MCF-7 cells. Tuberin is a substrate of protein kinases Akt and RSK1, and both Akt and RSK1 can be activated by estrogen. Estradiol stimulated endogenous tuberin phosphorylation at T1462 (a site of phosphorylation by both Akt and RSK1) approximately fourfold. Expression of wild-type tuberin or tuberin carrying alanine mutations at the two Akt phosphorylation sites (S939A and T1462A) inhibited the estradiol-induced phosphorylation of S6. To investigate tuberin's role in regulating genomic estrogen signaling, we confirmed that expression of tuberin resulted in significant inhibition (fivefold) of ERE-luciferase activity compared with vector control, as has been shown previously by the Noonan laboratory. We also tested patient-derived TSC2 mutants: N1643K, P1675L, N1651S, and R611Q. The mutant forms of tuberin did not inhibit luciferase activity to the same extent as wild-type tuberin. These results suggest for the first time the potential disease-relevance of tuberin's transcriptional repression. In summary, we demonstrate for the first time that estradiol activates the mTOR/S6K/S6 pathway in a PI3K/Akt and MAPK/RSK1 dependent manner. Estradiol's activation of mTOR is inhibited by tuberin, and is associated with an increase in GTP-Rheb and phospho-tuberin. These data imply that tuberin integrates estrogen signals from multiple pathways to regulate mTOR activity. We also demonstrate that wild-type tuberin, but not mutant forms, inhibits ERE-luciferase activity. These signaling pathways may have relevance to both the pathogenesis and therapy of LAM.

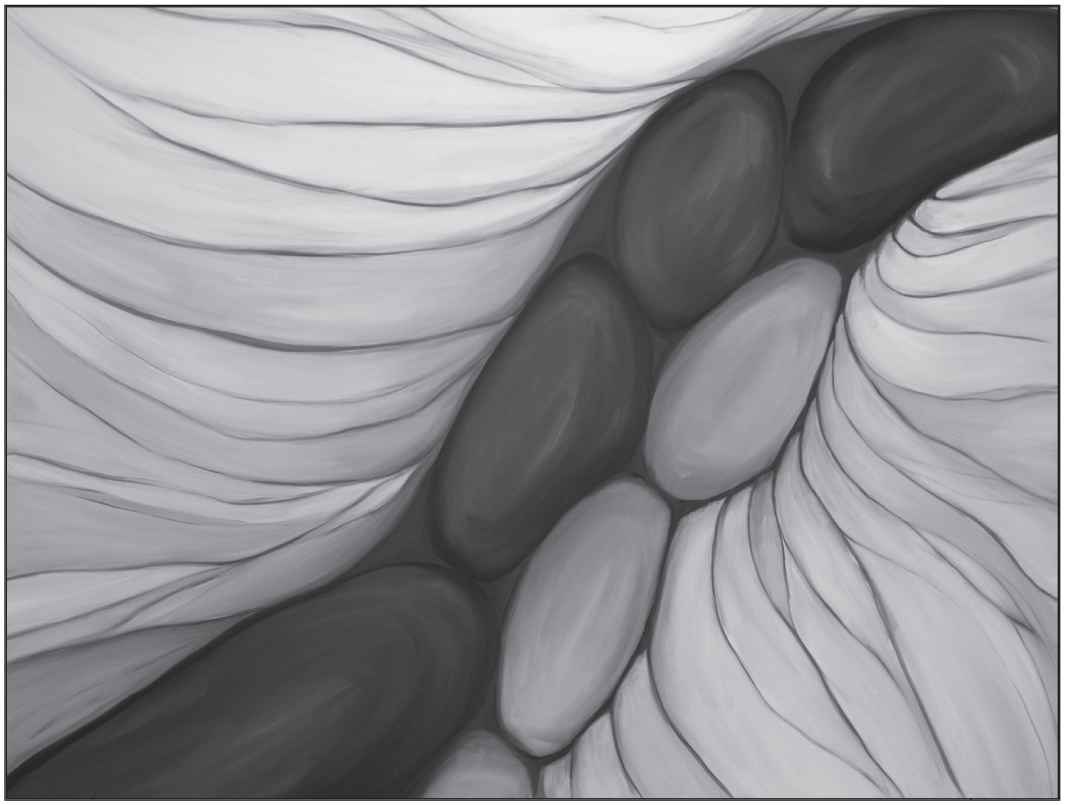
***Recipient of Travel Award**

Notes

Notes

Notes

Speakers List



Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Speakers List

Joseph Avruch, M.D., Ph.D.
Department of Molecular Biology/Medicine
Massachusetts General Hospital
Simches Research Building, Room 6408
185 Cambridge Street
Boston, MA 02114
Phone: (617) 726-6909
Email: avruch@molbio.mgh.harvard.edu

Joseph Bateman, Ph.D.
Wolfson Centre for Age-Related Diseases
King's College London
Guy's Campus
London, SE1 1UL
United Kingdom
Phone: 44-207-848-8144
Email: joseph_matthew.bateman@kcl.ac.uk

John Blenis, Ph.D.
Department of Cell Biology
Harvard Medical School
240 Longwood Avenue
Boston, MA 02115
Phone: (617) 432-4848
Email: jblenis@hms.harvard.edu

James Brugarolas, M.D., Ph.D.
Center for Developmental Biology
University of Texas Southwestern Medical Center
5323 Harry Hines Boulevard
Dallas, TX 75390-9133
Phone: (214) 648-4059
Email: james.brugarolas@utsouthwestern.edu

Sandra Dabora, M.D., Ph.D.
Hematology Division
Brigham and Women's Hospital and Harvard Medical School
CHRB, 6th Floor
One Blackfan Circle
Boston, MA 02115
Phone: (617) 355-9004
Email: sdabora@partners.org

Bruce Edgar, Ph.D.
Division of Basic Sciences
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue North, Room #B2-152
Seattle, WA 98109
Phone: (206) 667-4185
Email: bedgar@fhcrc.org

Leif Ellisen, M.D., Ph.D.
Division of Hematology and Medical Oncology
Harvard Medical School
Massachusetts General Hospital Cancer Center
55 Fruit Street, Room GRJ-904
Boston, MA 02114
Phone: (617) 726-4315
Email: ellisen@helix.mgh.harvard.edu

Charis Eng, M.D., Ph.D.
Department of Genetics
Cleveland Clinic Genomic Medicine Institute
9500 Euclid Avenue, NE5
Cleveland, OH 44146
Phone: (216) 444-3440
Email: engc@ccf.org

Stephen Groft, Ph.D.
Director
Office of Rare Diseases
National Institutes of Health
Room 1B19, MSC 2084
31 Center Drive
Bethesda, MD 20892
Phone: (301) 402-4336
Email: grofts2@mail.nih.gov

Nissim Hay, Ph.D.
Department Biochemistry and Molecular Genetics
University of Illinois at Chicago
900 S. Ashland Avenue
Chicago, IL 60607
Phone: (312) 355-1684
Email: nhay@uic.edu

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Speakers List

Tony Hunter, Ph.D.
Department of Molecular and Cell Biology
The Salk Institute
10010 N. Torrey Pines Road
La Jolla, CA 92037
Phone: (858) 453-4100
Email: hunter@salk.edu

John C. Lawrence, Ph.D.
Department of Pharmacology
University of Virginia
P.O. Box 800735
1300 Jefferson Park Avenue
Charlottesville, VA 22908-0735
Phone: (434) 924-1584
Email: jcl3p@virginia.edu

Zhijun Luo, M.D., Ph.D.
Department of Medicine
Boston University School of Medicine
650 Albany Street, Room 820
Boston, MA 02118
Phone: (617) 414-1033
Email: zluo@bu.edu

Brendan Manning, Ph.D.
Department of Genetics and Complex Diseases
Harvard University, School of Public Health
665 Huntington Avenue, Room SPH2-117
Boston, MA 02115
Phone: (617) 432-5614
Email: bmanning@hsph.harvard.edu

Duojia Pan, Ph.D.
Department of Molecular Biology and Genetics
Johns Hopkins University School of Medicine
615 PCTB
725 N. Wolfe Street
Baltimore, MD 21205
Phone: (410) 502-3179
Email: djpan@jhmi.edu

Griffin Rodgers, M.D.
Acting Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 9N-318
Bethesda, MD 20892-1822
Phone: (301) 402-2418
Email: gprod@helix.nih.gov

Reuben Shaw, Ph.D.
Department of Molecular and Cell Biology Laboratory
The Salk Institute
10010 N. Torrey Pines Road
La Jolla, CA 92037
Phone: (858) 453-4100
Email: shaw@salk.edu

Dinah Singer, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-8636
Email: ds13j@nih.gov

George Thomas, Ph.D.
Department of Genome Science
Genome Research Institute
University of Cincinnati
2180 E. Galbraith Road
Cincinnati, OH 45237
Phone: (513) 558-7100
Email: thomasg4@uc.edu

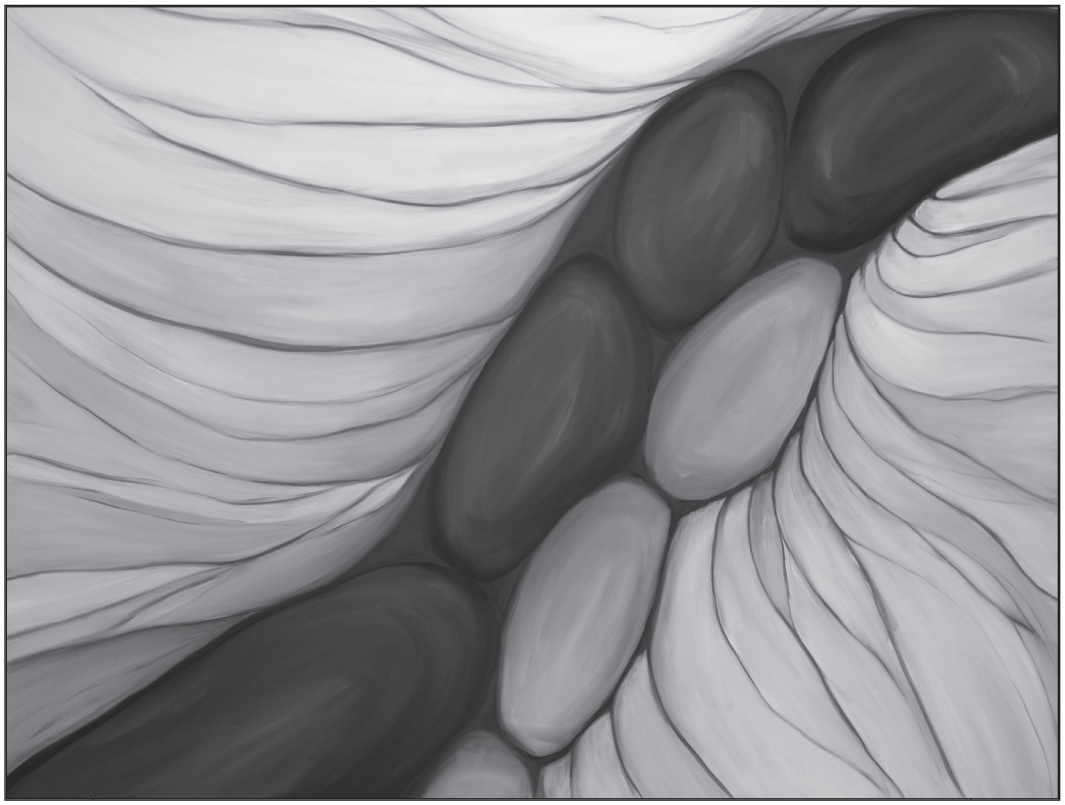
Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Speakers List

Craig Thompson, M.D.
Department of Cancer Biology
Abramson Family Cancer Research Institute
University of Pennsylvania School of Medicine
451 BRB II/III
421 Curie Boulevard
Philadelphia, PA 19104-6160
Phone: (215) 746-5515
Email: craig@mail.med.upenn.edu

Jane Yu, Ph.D.
Department of Human Genetics
Fox Chase Cancer Center
333 Cottman Avenue, Room P-3045
Philadelphia, PA 19111
Phone: (215) 728-2955
Email: jane.yu@fccc.edu

Participants List



Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Kristin Abraham, Ph.D.
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 795
6707 Democracy Bouleavard
Bethesda, MD 20892-5460
Phone: (301) 451-8048
Email: abrahamk@extra.niddk.nih.gov

Richard Anderson, M.D., Ph.D.
Genetics and Developmental Biology
National Institute of General Medical Sciences
National Institutes of Health
45 Center Drive
Bethesda, MD 20892
Phone: (301) 594-0943
Email: andersor@mail.nih.gov

Aristotelis Astrinidis, Ph.D.
Department of Medical Oncology
Fox Chase Cancer Center
333 Cottman Avenue, Room P3045
Philadelphia, PA 19111
Phone: (215) 728-2955
Email: aristotelis.astrinidis@fccc.edu

Masaya Baba, M.D., Ph.D.
Urologic Oncology Branch
National Cancer Institute
National Institutes of Health
1050 Boyles Street
Frederick, MD 21702
Phone: (301) 846-7351
Email: babam@ncifcrf.gov

Jonathan Backer, M.D.
Department of Molecular Pharmacology
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10801
Phone: (718) 430-2153
Email: backer@aecom.yu.edu

Marc Ballas
Medical Oncology Branch
National Cancer Institute
National Institutes of Health
5801 Nicholson Lane, Suite 1524
Bethesda, MD 20892
Phone: (917) 669-0718
Email: ballasm@mail.nih.gov

Ernesto Bernal-Mizrachi, M.D.
Department of Medicine
Washington University School of Medicine
Box 8127
660 S. Euclid Avenue, U12
St. Louis, MO 63110
Phone: (314) 362-7693
Email: ebernal@wustl.edu

Ramanath Bhandari
Laboratory of Cellular and Developmental Biology
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
50 South Drive
Bethesda, MD 20892
Phone: (301) 594-1361
Email: bhandarir@niddk.nih.gov

Benoit Bilanges, Ph.D.
Cancer Research Institute
University of California, San Francisco
2340 Sutter Street, Room N361
San Francisco, CA 94115
Phone: (415) 502-1865
Email: bbilanges@cc.ucsf.edu

Donald Blair, Ph.D.
Cancer Cell Biology Branch
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 5032
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-7028
Email: blaird@mail.nih.gov

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Val Bliskovsky, Ph.D.
Biosciences Business IG
National Cancer Institute
National Institutes of Health
37 Convent Drive, Room 3140
Bethesda, MD 20892
Phone: (301) 435-7249
Email: bliskovv@mail.nih.gov

Yves Boisclair, Ph.D.
Department of Animal Science
Cornell University
259 Morrison Hall
Ithaca, NY 14853
Phone: (607) 254-4704
Email: yrb1@cornell.edu

Maria Buse, M.D.
Department of Medicine, Biochemistry, and Molecular Biology
Medical University of South Carolina
96 Jonathan Lucas Street, CSB, R823
Charleston, SC 29425
Phone: (843) 792-3618
Email: busemg@musc.edu

Jaime Caro, M.D.
Department of Medicine
Thomas Jefferson University
1015 Walnut Street
Philadelphia, PA 19107
Phone: (215) 955-7775
Email: jaime.caro@jefferson.edu

Ariel Castro, Ph.D.
Department of Surgery
University of California, San Francisco
Mail Box 0875
2340 Sutter Street, Room N226
San Francisco, CA 94115
Phone: (415) 476-2392
Email: castroa@surgery.ucsf.edu

Timothy Clair
Laboratory of Pathology
National Cancer Institute
National Institutes of Health
Building 10, Room 2A33
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-1843
Email: timclair@helix.nih.gov

John Cole, Ph.D.
Cancer Etiology Branch
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Suite 5000
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-1718
Email: jc121b@nih.gov

Joe Crawford
Department of Research
Phoenix
P.O. Box 92926
Washington, DC 20090-2926
Phone: (301) 568-6098
Email: alawhite2000@yahoo.com

Thomas Darling, M.D., Ph.D.
Department of Dermatology
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814
Phone: (301) 295-3528
Email: tdarling@usuhs.mil

Teresa Davis, Ph.D.
Department of Pediatrics
USDA/ARS Children's Nutrition Research Center
Baylor College of Medicine
1100 Bates Street, Suite 9066
Houston, TX 77030-2600
Phone: (713) 798-7169
Email: tdavis@bcm.edu

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Phillip Dennis, M.D., Ph.D.
Medical Oncology Branch
Center for Cancer Reseach
National Cancer Institute
National Institutes of Health
National Naval Medical Center
Building 8, Room 5101
8901 Wisconsin Avenue
Bethesda, MD 20889
Phone: (301) 496-0929
Email: dennisp@mail.nih.gov

Min Du, Ph.D.
Department of Animal Science
University of Wyoming
1000 E. University Avenue
Laramie, WY 82071
Phone: (307) 766-3429
Email: mindu@uwyo.edu

Jane Fountain, Ph.D.
Neural Environment Cluster
National Institute of Neurological Disorders and Stroke
National Institutes of Health
Neuroscience Center
6001 Executive Boulevard, Room 2110
Bethesda, MD 20892
Phone: (301) 496-1431
Email: fountai@ninds.nih.mail

Oksana Gavrilova, Ph.D.
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 8, Room B2A-11
8 Center Drive
Bethesda, MD 20892
Phone: (301) 435-5370
Email: oksanag@intra.nidd.nih.gov

Laurie Goodyear, Ph.D.
Department of Metabolism
Joslin Diabetes Center
One Joslin Place
Boston, MA 02215
Phone: (617) 732-2474
Email: laurie.goodyear@joslin.harvard.edu

Jeff Green, M.D.
Laboratory of Cell Regulation and Carcinogenesis
National Cancer Institute
National Institutes of Health
41 Medlars Drive
Bethesda, MD 20892
Phone: (301) 435-5193
Email: jegreen@nih.gov

Donna Griebel, M.D.
Gastrointestinal and Other Cancers Research Group
Division of Cancer Prevention
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 2141
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (310) 594-5443
Email: griebeld@mail.nih.gov

Sushma Gurumurthy
Cancer Center
Massachusetts General Hospital
Simches Research Building, Room 4100
185 Cambridge Street
Boston, MA 02114
Phone: (617) 643-3157
Email: sgurumurthy@partners.org

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Carol Haft, Ph.D.
Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 793
6707 Democracy Boulevard
Bethesda, MD 20892
Phone: (301) 594-7689
Email: haftc@mail.nih.gov

Tina Han
Department of Nutrition and Food Science
University of Maryland
0112 Skinner Building
College Park, MD 20742
Phone: (301) 405-8775
Email: chan2@umd.edu

Wei Han, Ph.D.
Medical Oncology Branch
National Institutes of Health
Building 8, Room 5101
8901 Wisconsin Avnue
Bethesda, MD 20889
Phone: (301) 402-7698
Email: hanw@mail.nih.gov

Thurl Harris, Ph.D.
Department of Pharmacology
University of Virginia
1300 Jefferson Park Avenue
Jordan Hall, Room 5025
Charlottesville, VA 22908
Phone: (434) 924-1582
Email: teh3c@virginia.edu

Elizabeth Henske, M.D.
Department of Medical Oncology
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19111
Phone: (215) 728-2428
Email: elizabeth.henske@fccc.edu

Christine Hollander
National Cancer Institute
National Institutes of Health
Building NNMC8, Room 5101
8901 Wisconsin Avenue
Bethesda, MD 20892
Phone: (301) 594-7069
Email: ch96b@nih.gov

Chien-Hui Hong
Department of Dermatology
Uniformed Services University of the Health Sciences
5801 Nicholson Lane, Room 611
North Bethesda, MD 20852
Phone: (301) 295-3820
Email: chong@usuhs.mil

Guo-Fu Hu, Ph.D.
Department of Pathology
Harvard Medical School
77 Avenue Louis Pasteur
Boston, MA 02115
Phone: (617) 432-6582
Email: guofu_hu@hms.harvard.edu

Jianxin Hu, Ph.D.
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 8C-209
10 Center Drive
Bethesda, MD 20892
Phone: (301) 496-9212
Email: jianxinh@intra.niddk.nih.gov

Jing Huang, Ph.D.
Department of Molecular and Medical Pharmacology
University of California, Los Angeles
23-231 CHS
650 Charles E. Young Drive South
Los Angeles, CA 90095
Phone: (310) 825-4329
Email: jinghuang@mednet.ucla.edu

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Bing-Hua Jiang, Ph.D.
Mary Babb Randolph Cancer Center
West Virginia University
1018 Health Sciences South
Morgantown, WV 26506
Phone: (304) 293-5949
Email: bhjiang@hsc.wvu.edu

Yue Jiang, M.S.
Lane Department of Computer Science and Electrical Engineering
West Virginia University
P.O. Box 6109
Morgantown, WV 26506
Phone: (304) 293-0405
Email: yue@csee.wvu.edu

Konstantin Kandror, Ph.D.
Department of Biochemistry
Boston University School of Medicine
715 Albany Street, Room K124
Boston, MA 02118
Phone: (617) 638-5049
Email: kkandror@bu.edu

Magdalena Karbowniczek, M.D., Ph.D.
Department of Medical Oncology
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19111
Phone: (215) 728-2955
Email: mmk_karbowniczek@fccc.edu

Taruna Khurana
Laboratory of Cellular and Developmental Biology
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
50 South Drive, Room 3345
Bethesda, MD 20892
Phone: (301) 594-1219
Email: tarunak@mail.nih.gov

Do-Hyung Kim, Ph.D.
Department of Biochemistry, Molecular Biology, and Biophysics
University of Minnesota
6-155 Jackson Hall
321 Church Street, S.E.
Minneapolis, MN 55455
Phone: (612) 626-3418
Email: dhkim@umn.edu

Eunjung Kim, Ph.D.
University of Michigan
Life Sciences Institute
210 Washtenaw Avenue, Room 6115
Ann Arbor, MI 48109
Phone: (734) 763-3273
Email: kimeunj@umich.edu

Alan Kimmel, Ph.D.
Laboratory of Cellular and Developmental Biology
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 50, Room 3351
50 South Drive
Bethesda, MD 20892
Phone: (301) 496-3016
Email: ark1@helix.nih.gov

Levy Kopelovich, Ph.D.
Division of Cancer Prevention
National Cancer Institute
National Institutes of Health
Executive Plaza North
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 594-0467
Email: kopelovich@nih.gov

Vera Krymskayam, Ph.D.
Department of Medicine
University of Pennsylvania
421 Curie Boulevard
Philadelphia, PA 19104
Phone: (215) 573-9861
Email: krymskay@mail.med.upenn.edu

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Charles Lang
Department of Cell Molecular Physiology
Penn State College of Medicine
500 University Drive
Hershey, PA 17033
Phone: (717) 531-5538
Email: clang@psu.edu

Sean Lee, Ph.D.
Genetics of Development and Disease Branch
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 10, Room 9N313
10 Center Drive
Bethesda, MD 20892
Phone: (301) 496-9739
Email: seanl@intra.niddk.nih.gov

Seung Yeon Lee
Department of Dermatology
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814
Phone: (301) 295-3820
Email: slee@usuhs.mil

Yong Lee
Department of Surgery
University of Pittsburgh
5117 Centre Avenue
Pittsburgh, PA 15213
Phone: (412) 623-3268
Email: leeyj@upmc.edu

Robert Lewis, Ph.D.
Eppley Institute
University of Nebraska Medical Center
987696 Nebraska Medical Center
Omaha, NE 68198-7696
Phone: (402) 559-8290
Email: rlewis@unmc.edu

Xin-Hua Liao
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 50, Room 3349
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 451-8256
Email: liaox@niddk.nih.gov

Huei-Min Lin
National Cancer Institute
National Institutes of Health
LCRC, Building 41, Room B1112
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 451-4961
Email: hueiminl@mail.nih.gov

Barbara Linder, M.D., Ph.D.
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 699
6707 Democracy Boulevard
Bethesda, MD 20892
Phone: (301) 594-0021
Email: linderb@mail.nih.gov

Marston Linenhan
Urologic Oncology Branch
National Cancer Institute
National Institutes of Health
Building 10, Room 1-5940
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-6353
Email: linehanm@mail.nih.gov

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Ying Liu
Department of Dermatology
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814
Phone: (301) 295-3820
Email: yiliu@usuhs.mil

Jaminelli Liwanag
Department of Signal Transduction
National Cancer Institute
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 451-3309
Email: liwanagj@mail.nih.gov

Philip Lucas, Ph.D.
Experimental Transplantation and
Immunology Branch
National Institutes of Health
Building 10, Room 3-3288
10 Center Drive
Bethesda, MD 20892
Phone: (301) 435-3542
Email: pjlucas@nih.gov

Christopher Lynch
Department of Cellular and Molecular Physiology
The Pennsylvania State University
College of Medicine
500 University Drive (MC H166)
Hershey, PA 17033
Phone: (717) 531-5170
Email: clynch@psu.edu

Carol MacLeod, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Suite 5066
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 594-8936
Email: macleodc@mail.nih.gov

Judy Mietz, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 5028
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-9326
Email: mietzj@mail.nih.gov

Beverly Mock, Ph.D.
Laboratory of Genetics
National Cancer Institute
National Institutes of Health
Building 37, Room 3146
37 Convent Drive, MSC 4258
Bethesda, MD 20892-4258
Phone: (301) 496-2360
Email: bev@helix.nih.gov

Richard Moran, Ph.D.
Massey Cancer Center
Virginia Commonwealth University School
of Medicine
Sanger Hall, Room 6-044
1101 E. Marshall Street
Richmond, VA 23298
Phone: (804) 828-9645
Email: rmoran@mail2.vcu.edu

Joel Moss, M.D., Ph.D.
Pulmonary Critical Care Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Building 10, Room 6D05
10 Center Drive, MSC 1590
Bethesda, MD 20892
Phone: (301) 496-1597
Email: mossj@nhlbi.nih.gov

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Len Neckers, Ph.D.
Urologic Oncology Branch
Center for Cancer Research
National Cancer Institute
National Institutes of Health
Building 10, Room 1-5940
10 Center Drive
Bethesda, MD 20892
Phone: (301) 496-5899
Email: len@helix.nih.gov

Mike Nickerson, M.S.
Department of Biomarkers and Pharmacogenomics
Transgenomic
11 Firstfield Road, Suite E
Gaithersburg, MD 20878
Phone: (240) 631-2001
Email: mnickerson@transgenomic.com

Sean Oldhamm, Ph.D.
Cancer Research Center
The Burnham Institute
10901 N. Torrey Pines Road
La Jolla, CA 92037
Phone: (858) 713-9934
Email: soldham@burnham.org

Ji-Hye Paik, Ph.D.
Department of Medical Oncology
Dana-Farber Cancer Institute
44 Binney Street, M416
Boston, MA 02115
Phone: (617) 632-6097
Email: jihye_paik@dfci.harvard.edu

Jui Pandhare, Ph.D.
Laboratory of Comparative Carcinogenesis
National Chemical Laboratory
Division of Biochemical Sciences
National Cancer Institute
National Institutes of Health
P.O. Box B, Building 538, Room 144
Frederick, MD 21702-1201
Phone: (301) 846-7352
Email: pandharej@ncifcrf.gov

Mary Ellen Perry, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 5034
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-7028
Email: perryma@mai.nih.gov

Chanika Phornphutkul, M.D.
Department of Pediatrics
Brown University/Rhode Island Hospital
593 Eddy Street, MPS 201
Providence, RI 02903
Phone: (401) 444-5504
Email: chanika_phornphutkul@brown.edu

Lawrence Quilliam, Ph.D.
Department of Biochemistrry and Molecular Biology
Indiana University School of Medicine
635 Barnhill Drive, Room 4075
Indianapolis, IN 46202-5122
Phone: (317) 274-8550
Email: lquillia@iupui.edu

Alexandra Racanelli
Department of Pharmacology/Toxicology
Virginia Commonwealth University School of Medicine
Sanger Hall 6-044
1101 E. Marshall Street
Richmond, VA 23298
Phone: (804) 828-9645
Email: racanelliac@vcu.edu

Mark Raffeld, M.D.
Laboratory of Pathology
National Cancer Institute
National Institutes of Health
Building 10, Room 2N110
10 Center Drive
Bethesda, MD 20892
Phone: (301) 496-1569
Email: mraff@box-m.nih.gov

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Elizabeth Read-Connole, Ph.D.
Cancer Etiology Branch
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 5016
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-6085
Email: bconnole@mail.nih.gov

B. Tibor Roberts, Ph.D.
Office of Scientific Program and Policy Analysis
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building 31, Room 9A16
31 Center Drive
Bethesda, MD 20892
Phone: (301) 496-6623
Email: brucer@niddk.nih.gov

Richard Robinson
National Cancer Institute
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-6366
Email: rcrobin@helix.nih.gov

Nan Roche, Ph.D.
Laboratory of Cell Regulation
National Cancer Institute
National Institutes of Health
41 Library Drive
Bethesda, MD 20892
Phone: (301) 496-8346
Email: roche@helix.nih.gov

Aaron Sargeant, D.V.M.
Department of Medicinal Chemistry
The Ohio State University
510 W. 12th Avenue
Columbus, OH 43210
Phone: (614) 292-6796
Email: sargeant.9@osu.edu

Neeraja Sathyamoorthy, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 435-1878
Email: ns61r@nih.gov

Katrin Schmelzle
Department of Biological Engineering
Massachusetts Institute of Technology
Building 56-754
77 Massachusetts Avenue
Cambridge, MA 02139
Phone: (617) 324-0403
Email: kschmelzle@mit.edu

Laura Schmidt, Ph.D.
Urologic Oncology Branch
National Cancer Institute
National Institutes of Health
P.O. Box B, Building 560, Room 12-69
Frederick, MD 21702-1201
Phone: (301) 846-5856
Email: schmidtl@ncifcrf.gov

Pamela Schwartzberg, M.D., Ph.D.
National Human Genome Research Institute
National Institutes of Health
49 Convent Drive
Bethesda, MD 20892
Phone: (301) 435-1906
Email: pams@mail.nih.gov

Hasnaa Shafik, M.D., Ph.D.
Division of Extramural Activities
National Cancer Institute
National Institutes of Health
6116 Executive Boulevard, Room 8135
Bethesda, MD 20892
Phone: (301) 451-4757
Email: shafikh@mail.nih.gov

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Adam Shaywitz
Department of Endocrinology
Beth Israel Deaconness Medical Center
330 Brookline Avenue
Boston, MA 02215
Phone: (617) 667-4016
Email: ashaywit@bidmc.harvard.edu

Pengxiang She
Department of Cellular and Molecular Physiology
The Pennsylvania State University College of Medicine
MC H166
500 University Drive
Hershey, PA 17033
Phone: (717) 531-5344
Email: pzs11@psu.edu

Barbara Spalholz, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 5030
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-7028
Email: bs62d@nih.gov

David Stokoe, Ph.D.
Cancer Research Institute
University of California, San Francisco
2340 Sutter Street
San Francisco, CA 94115
Phone: (415) 502-2598
Email: dstokoe@cc.ucsf.edu

Jennifer Strasburger
Cancer Cell Biology Branch
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 5027
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-7028
Email: strasbuj@mail.nih.gov

Gabriela Suchankova, M.D., Ph.D.
Department of Medicine/Diabetes
Boston University School of Medicine
650 Albany Street, Room 825
Boston, MA 02118
Phone: (617) 638-7152
Email: gsuchan@bu.edu

Agus Suryawan, Ph.D.
Department of Pediatrics
USDA/ARS Children's Nutrition Research Center
Baylor College of Medicine
1100 Bates Street
Houston, TX 77030
Phone: (713) 798-7148
Email: suryawan@bcm.edu

Yien Che Tsai, Ph.D.
Laboratory of Protein Dynamics and Signaling
National Cancer Institute
National Institutes of Health
1050 Boyles Street
Frederick, MD 21702
Phone: (301) 846-5824
Email: yctsai@ncifcrf.gov

Takatoshi Ueki
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
50 South Drive, Room 3349
Bethesda, MD 20892
Phone: (301) 594-0537
Email: uekit@niddk.nih.gov

Francisca Vazquez, Ph.D.
Department of Cell Biology
Johns Hopkins University
725 N. Wolfe Street, WBSB116
Baltimore, MD 21231
Phone: (410) 955-4699
Email: fvazquez@jhmi.edu

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Cathy Vocke, Ph.D.
Urologic Oncology Branch
National Cancer Institute
National Institutes of Health
Building 10, CRC, Room 1-5888
10 Center Drive, MSC 1107
Bethesda, MD 20892-1107
Phone: (301) 402-1963
Email: vockec@mail.nih.gov

Todd Waldman, Ph.D., M.D.
Lombardi Cancer Center
Georgetown University
NRB E304
3970 Reservoir Road, N.W.
Washington, DC 20057
Phone: (202) 687-1340
Email: waldmant@georgetown.edu

Xiaolin Wan, Ph.D.
Pediatric Oncology Branch
Center for Cancer Research
National Cancer Institute
National Institutes of Health
Building 10, Room 1W-3816
10 Center Drive
Bethesda, MD 20892
Phone: (301) 451-7013
Email: xiaolinw@mail.nih.gov

Ji-An Wang
Department of Dermatology
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814
Phone: (301) 295-3820
Email: jwang@usuhs.mil

Lifu Wang, Ph.D.
Department of Pharmacology
University of Virginia
1300 JPA
Charlottesville, VA 22903
Phone: (434) 924-1582
Email: lw6j@virginia.edu

Noel Warfel, M.S.
National Cancer Institute
National Institutes of Health
8901 Wisconsin Avenue
Bethesda, MD 20889
Phone: (301) 435-5868
Email: warfeln@mail.nih.gov

Michelle Warren, M.S.
Urologic Oncology Branch
National Cancer Institute
National Institutes of Health
P.O. Box B, Building 560, Room 12-25
Frederick, MD 21702-1201
Phone: (301) 846-7324
Email: mwarren@ncifcrf.gov

Gene Webb, Ph.D.
Department of Medicine/Endocrinology
University of Chicago
Room AB420, MC 1027
5841 S. Maryland Avenue
Chicago, IL 60637
Phone: (773) 702-2332
Email: genewebb@midway.uchicago.edu

Allan Weissman, M.D.
Center for Cancer Research
National Cancer Institute
National Institutes of Health
P.O. Box B, Building 560, Room 22-103
Frederick, MD 21702-1201
Phone: (301) 846-7540
Email: amw@nih.gov

Vicky Whittemore, Ph.D.
Department of Research
Tuberous Sclerosis Alliance
801 Roeder Road, Suite 750
Silver Spring, MD 20910
Phone: (301) 562-9890
Email: vwhittemore@tsalliance.org

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Betsy Wilder, Ph.D.
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Two Democracy Plaza, Room 623
6707 Democracy Boulevard, MSC 5458
Bethesda, MD 20892-5458
Phone: (301) 594-1409
Email: betsywilder@nih.gov

Girma Woldemichael, Ph.D.
Molecular Targets Development Program
Center for Cancer Research
National Cancer Institute
National Institutes of Health
P.O. Box B, Building 560, Room 22-301
Frederick, MD 21702-1201
Phone: (301) 693-3435
Email: girma@ncifcrf.gov

Virginia Wray, Ph.D.
Division of Extramural Activities
National Cancer Institute
National Institutes of Health
6116 Executive Boulevard, Room 8125
Bethesda, MD 20892
Phone: (301) 496-9236
Email: vw8z@nih.gov

Julie Wu
National Human Genome Research Institute
National Institutes of Health
49 Convent Drive, Room 4A39
Bethesda, MD 20892
Phone: (301) 435-1907
Email: wujulie@mail.nih.gov

Keying Wu
Rhode Island Hospital
593 Eddy Street, MPS-219
Providence, RI 02903-4923
Phone: (401) 444-3921
Email: wu591211@yahoo.com

Gang Xi
Department of Medicine
University of North Carolina
2525 Booker Creek Road, Room13A
Chapel Hill, NC 27514
Phone: (919) 966-1142
Email: xigg2005@med.unc.edu

Xiaoping Yang, Ph.D.
School of Pharmacy
University of Wyoming
1000 E. University Avenue
Laramie, WY 82071
Phone: (307) 766-6147
Email: xpyang@uwyo.edu

Rihab Yassin, Ph.D.
Division of Cancer Biology
National Cancer Institute
National Institutes of Health
Executive Plaza North
6130 Executive Boulevard
Bethesda, MD 20892
Phone: (301) 496-7028
Email: ry38k@nih.gov

Yanlin Yu
Laboratory of Cell Regulation and Carcinogenesis
National Cancer Institute
National Institutes of Health
Building 37, Room 5002
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 402-4073
Email: yuy@mail.nih.gov

Allan Zhao, Ph.D.
Department of Cell Biology and Physiology
University of Pittsburgh
3500 Terrace Street, S-326, BSTWR
Pittsburgh, PA 15261
Phone: (412) 648-8148
Email: azhao@pitt.edu

Nutrient Sensing, Insulin Signaling, and Hamartoma Syndromes

Participants List

Pan Zheng, M.D., Ph.D.
Department of Pathology
Ohio State University
129 Hamilton Hall
1645 Neil Avenue
Columbus, OH 43210
Phone: (614) 292-2003
Email: pan.zheng@osumc.edu

Notes

Notes

Notes

Notes

Notes

Notes

Department of Health and Human Services
National Institutes of Health
National Institute of Diabetes and Digestive and Kidney Diseases
National Cancer Institute
NIH Office of Rare Diseases