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**Molecular Imaging as a tool for bench-to-bedside research and therapy**

The ability to assay cancer biologic features is key to advances in both the basic biomedical science and clinical patient care. Advances in our ability to assay molecular processes including gene expression, protein expression, and molecular and cellular biochemistry have fueled recent advances in our understanding of many diseases and our ability to treat them. Most assays require sampling of cells or tissue to perform the measurements. Thus the cell culture system, animal model, or patient must be perturbed in order to perform the assay. This requirement makes serial assays over time more difficult and leaves open the possibility that the assay itself may change the state of the system being measured. The ability to measure biologic processes without perturbing them would be highly desirable and would offer complementary information to that obtained by most traditional assay methods. Advances in both technology and cancer science have led to the ability to perform non-invasive molecular assay. One recent advance, is the ability to image regional biochemistry and molecular biology, termed molecular imaging [1,2]. Molecular imaging applicable to the entire range of biological systems from cell culture to humans, is molecular imaging. Molecular imaging differs from standard anatomic imaging in that the focus is on measuring regional biology rather than anatomic structure. Quantitative analysis is an important feature of this type of imaging, for example, the ability to measure regional tumor receptor expression. As such, molecular imaging can be considered an *in vivo* assay technique, capable of measuring regional tumor biology without perturbing it. This makes molecular imaging a unique tool for probing disease biology that is complementary to traditional assay methods, and a potentially very powerful tool for translational science.

This talk will review methods for functional and molecular imaging, emphasizing radionuclide methods, especially PET, and with a focus on translational studies in humans. Molecular imaging of cancer will be used as an example to show how molecular imaging can (1) provide prognostic information based upon *in vivo* measures of cancer differentiation and aggressiveness, (2) measure the regional expression of possible therapeutic targets, (3) provide early assessment of response to therapy, and (4) provide insights into the *in vivo* biology of cancer and its response to treatment. The talk will emphasize imaging methods relevant to LAM and related diseases, including receptor expression, metabolism, and perfusion/angiogenesis. The goal is to provide an introduction to current molecular imaging methods, and their early application to humans, to help better direct the use of these new techniques for LAM research,

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## Bruce Kristal, PhD

Brigham and Women's Hospital/Harvard School of Public Health

### Validated Biomarkers of Caloric Restriction in rats: Markers of Disease Risk in Humans?

**Objectives:** Caloric restriction (CR) robustly increases longevity/reduces morbidity in mammals, eg., breast cancer risk is generally decreased >90% in CR rodents. CR-mediated effects are usually dominant to other risk factors and are directly analogous to human data linking obesity with poor health outcomes, including cancer. We tested if biomarkers of diet in rats predicts future disease in humans.

**Methods:** Metabolomics measurements in sera/plasma were conducted w/HPLC/Coularray detectors (N=600 rats, ~1700 humans). Classification/ predictive power were tested, optimized, and validated using a series of megavariable data analysis approaches in sequential blinded cohorts.

**Results:** Models distinguish diet groups with >95% accuracy. Diets varying in extent/duration of CR were used to develop models for intermediate caloric intakes. Markers were adapted for human study, analytically validated at instrumentation (N=30; 100% blinded split accuracy) and sample collection levels (N=34; most stable under worst case conditions), then biologically validated (N=70 in triplicate, metabolites/profiles had intraclass correlation coefficients from -0.65-0.85). Initial study reveal differences on this marker profile associated with energy intake and risk of future breast cancer in 210 case/control pairs nested within the Nurses' Health Study.

**Conclusion:** Metabolomics profiles offer a potential biochemical approach to validate nutritive status and contribute to prediction of disease.

## Jane Yu, PhD

Brigham and Women's Hospital and Harvard Medical School

### Evidence from expression profiling, proteomics, metabolomics and FDG-PET of an estrogen-dependent metabolic switch in tuberin-null cells

We previously demonstrated that estrogen promotes the survival of tuberin-deficient ELT3 cells both in vitro and in vivo, and that estrogen treatment of mice bearing ELT3 cells xenograft tumors promotes lung metastasis. This estrogen-induced cell survival and metastasis was inhibited in vivo by the MEK inhibitor CI-1040 (Yu et al. PNAS 2009).

To elucidate pro-survival events mediated by estrogen in tuberin-null cells, we compared Tsc2-null ELT3 cell xenograft tumors from estrogen or placebo-treated mice using expression profiling (Affimetrix rat expression array) and proteomic analysis (iTRAQ-8 plex).

Expression profiling of ELT3 cell xenograft tumors revealed that estrogen upregulates the expression of genes in lipid and amino acid metabolism pathways. Among these genes, the estrogen-induced expression of amino adipatetransferase (AADAT), which is involved in lysine degradation, has been confirmed by immunoblotting of tumor lysates.

Using iTRAQ proteomics from four estrogen and four placebo-treated ELT3 xenograft tumors, we identified 40 proteins that are significantly regulated by estrogen, including proteins involved in carbohydrate metabolism (creatine kinase), amino acid degradation (aspartate aminotransferase) and lipid metabolism (isocitrate dehydrogenase).

Metabolic profiling by LC/MS/MS of TSC2-null angiomyolipoma-derived cells from a LAM patient (621-101 cells) showed a temporal effect of rapamycin (10 nM) on the accumulation of pentose phosphate pathway intermediates and glycolytic metabolites. Cellular amino acid levels were reduced after rapamycin treatment.

Consistent with a metabolic switch occurring in LAM, we found, using immunohistochemical staining, that the tumor-associated pyruvate kinase isoform M2 is abundantly expressed in ELT3 cell xenograft tumors, lung metastatic lesions from estrogen-treated mice bearing ELT3 cell xenograft tumors, and in human LAM cell nodules. Finally, using FDG-PET, we found that xenograft ELT3 cell tumors in estrogen-treated mice exhibited higher levels of uptake compared with placebo-treated mice.

In conclusion, gene expression profiling indicated that estrogen enhances the expression of genes which products regulate glucose and amino acid metabolism. Proteomic analysis showed estrogen-regulated candidates involved in glycolysis, amino acid, and lipid metabolisms. Metabolomic screening revealed that cells lacking tuberin have a metabolic response to rapamycin treatment. FDG-PET imaging showed enhanced uptake in estrogen-treated xenograft tumors. Collectively, these data indicate that cellular metabolic alterations may contribute to the pathogenesis of LAM. Targeting metabolic regulators might have therapeutic benefit for women with LAM.

Michael J. Welsh, MD  
University of Iowa

## A Porcine Model of Cystic Fibrosis Exhibits Lung Manifestations of the Human Disease

It has been two decades since discovery of the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). However, we still lack answers to many questions about how the loss of CFTR function causes disease in the lung, pancreas, liver, intestine and other organs, and cystic fibrosis (CF) remains a lethal disease. An impediment to progress in understanding the pathogenesis of CF and to developing mechanism-based therapies and preventions has been lack of an animal model other than mice - mice with targeted alterations of their *CFTR* gene fail to develop many of the manifestations that are typical of CF. Because pigs share many anatomical, biochemical, and physiological characteristics with humans, we used homologous recombination in somatic cells and somatic cell nuclear transfer to develop pigs with either a targeted disruption or a  $\Delta F508$  mutation of their *CFTR* gene. These animals exhibited defective chloride transport and replicated aspects of the disease seen in newborn patients with CF. This porcine model may provide opportunities to address persistent questions about CF pathogenesis and accelerate discovery of therapeutics.

**William Hardie, MD**  
Cincinnati Children's Hospital Medical Center

**Inhibition of PI3K reverses transforming growth factor-alpha induced pulmonary fibrosis**

**Rationale:** TGF $\alpha$  is a ligand activating the epidermal growth factor receptor (EGFR). TGF $\alpha$  conditionally expressed under control of a lung epithelial-specific promoter in transgenic mice causes a severe and progressive pulmonary fibrosis associated with activation of the PI3K-Akt/mTOR pathway. Features of fibrosis in the TGF $\alpha$  model also seen in human fibrotic disease include progressive cachexia, restrictive changes on lung mechanics, secondary pulmonary hypertension, transformation of myofibroblasts, fibroblastic foci, and progression of disease in the absence of inflammation. The progressive fibrosis in the TGF $\alpha$  model allows for testing interventions to reverse established and progressive disease. This study sought to determine the role of the PI3K pathway in the maintenance of TGF $\alpha$ -mediated pulmonary fibrosis.

**Methods:** TGF $\alpha$  was expressed for 8 weeks. After 4 weeks of TGF $\alpha$  expression mice were treated daily with the PI3K inhibitor PX-866 (3 mg/kg po.) or vehicle and compared to controls. Changes in body weights from baseline, lung mechanics (using the Flexi-Vent system), and lung fibrosis, assessed by total lung collagen with the Sircol collagen assay and pentachrome staining of lung sections were determined.

**Results:** Mice treated with PX-866 after fibrosis was already established demonstrated significantly reduced lung fibrosis assessed by lung histology and total lung collagen as well as reduced changes in body weight and lung mechanics compared with vehicle treated mice. Body weights, lung collagen and mechanics remained altered in PX-866 treated mice compared with controls consistent with partial reversal of fibrosis.

**Summary:** Inhibition of the PI3K signaling pathway with PX-866 partially reversed TGF $\alpha$ /EGFR-induced pulmonary fibrosis. Our findings demonstrate the PI3K-Akt is a major, but not exclusive, effector pathway involved in the maintenance of EGFR-mediated pulmonary fibrosis. These findings support PI3K inhibition as a potential therapeutic option for progressive fibrotic disease, but also support investigation of other signaling pathways, such as the MAPK, which may also mediate fibrosis maintenance.

Mouse Group (n)	Body Weight (% change)	Resistance (cmH2O*s/ml)	Compliance (ml/cmH2O*kg)	Collagen (ug/ml)
Control (10)	+15 $\pm$ 2%	1.2 $\pm$ .1	1.4 $\pm$ .1	19 $\pm$ 1
TGF $\alpha$ vehicle (9)	-26 $\pm$ 6%*	5.7 $\pm$ .7*	0.4 $\pm$ .1*	71 $\pm$ 9*
TGF $\alpha$ PX-866 (9)	-1 $\pm$ 2%**	3.5 $\pm$ .6**	0.6 $\pm$ .1**	51 $\pm$ 4**

\*=p<0.05 compared to control by ANOVA

\*\*=p<0.05 compared to control and TGF $\alpha$  vehicle by ANOVA

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**Kazunori Tobino, MD**  
Juntendo University, School of Medicine

## **Imaging Studies of LAM with Computed Tomography**

“Where do LAM cells come from?”

We studied the chest CT images of 138 patients with LAM and also abdominal CT images of 76 patients among them. Although this is a cross-sectional study, the frequency of “lymphatic lesion” in each part of the body may answer to the question.

“How do LAM cells spread in the lung?”

The method of characterizing fractals, which is the concept of fractal geometry developed by Mandelbrot to describe quantitatively the variations in size and shape seen in natural objects, has been successfully applied to pulmonary physiology. For example, Mishima and colleagues analyzed the number and size of low attenuation areas (LAAs) on CTs in patients with chronic obstructive lung disease (COPD) and determined that LAAs on CT scans display fractal properties. LAAs on CT image are confirmed to correspond to emphysema in the lung parenchyma. Pulmonary cysts are also recognized as LAAs on CT, however, there is no report that assessed the usefulness of the methods of characterizing fractals of LAAs to analyze the pathophysiology in cystic lung diseases. Moreover, in most of previous reports, LAAs were quantified about number, size and extent in the lung, but not about their shape and distribution. Therefore, we characterized the pulmonary cysts on CT in patients with cystic lung diseases (i.e. LAM and Birt-Hogg-Dubé syndrome) by quantification of cysts including the shape, and also assessed the usefulness of the methods of characterizing fractals of LAAs to analyze the pathophysiology in cystic lung diseases, comparing with COPD. The results of the present study may answer to the second question.

**Jared Hagaman, MD**  
University of Cincinnati

**Screening for Lymphangiomyomatosis with High-Resolution CT in Young, Nonsmoking Women Presenting With Spontaneous Pneumothorax is Cost-Effective**

**Rationale:** Women with pulmonary lymphangiomyomatosis (LAM) who present with a sentinel spontaneous pneumothorax (SPTX) will experience an average of 2.5 additional pneumothoraces. The diagnosis of LAM is typically delayed until after the second pneumothorax. Our hypothesis is that targeted screening of a LAM enriched population of nonsmoking women between the ages of 25-54 who present with a sentinel pneumothorax with high resolution CT (HRCT) will facilitate early identification, definitive therapy and improved quality of life for patients with LAM.

**Methods:** We constructed a Markov state-transition model to assess the cost-effectiveness of screening. Rates of SPTX and prevalence of LAM in populations stratified by age, gender and smoking status were derived from the literature. Costs of testing and treatment were extracted from 2007 Medicare data. We compared a strategy utilizing HRCT screening followed by pleurodesis for patients with LAM, versus no HRCT screening.

**Results:** The prevalence of LAM in nonsmoking women between the ages of 25-54 with SPTX is estimated at 5% based on available literature. In our base case analysis, screening for LAM with HRCT is the most cost-effective strategy with a marginal cost-effectiveness ratio of \$32,980 per quality adjusted life year gained. Sensitivity analysis showed that HRCT screening remains cost-effective for groups in which the prevalence of LAM in the population subset screened is greater than 2.5%.

**Conclusion:** Screening for LAM with HRCT in non-smoking women age 25-54 that present with SPTX is cost-effective. Physicians are advised to screen for LAM with HRCT in this population.

**Masaki Hirose, PhD**

National Hospital Organization Kinki-Chuo Chest Medical Center

### **Serial measurement of serum vascular endothelial growth factor (VEGF)-D in patients with lymphangiomyomatosis**

**Background:** Lymphangiomyomatosis (LAM) is a progressive rare lung disease that exclusively affects women of childbearing age. Recent studies have revealed that measurement of serum VEGF-D is useful for the diagnosis of LAM. However, there are few reports about clinical evaluation of serial measurement of serum VEGF-D level. Thus, we evaluated the seventeen cases of serum VEGF-D level and clinical course. And we hypothesized that serum VEGF-D reflects pulmonary functions.

**Subjects and Methods:** Patients with LAM (n=17, sporadic LAM: 15, TSC-LAM: 2) who had been followed for 1.6-6.6 years (mean: 3.9 years) were studied. Serum VEGF-D levels were serially measured by ELISA using commercially available kit (R&D systems) and were evaluated with clinical measures including pulmonary function tests.

**Results & Discussion:** Serum VEGF-D level did not correlated with pulmonary functions ( $FEV_{1.0}/FVC$ , %VC, %FVC, %DLco, RV/TLC). The levels were affected by various kinds of clinical conditions, such as treatment with sirolimus, but not with gonadotropin-releasing hormone agonists. Although the number of patient was small, serum VEGF-D level tend to be high in patients with lymphatic abnormalities.

These results may suggest that serum VEGF-D level may reflect systemic abnormality or activities of LAM, rather than the local disease severity of the lung. We concluded that serial measurement of serum VEGF-D level is useful to follow-up LAM patients.

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Po-Shun Lee, MD  
Brigham and Women's Hospital

## TSC2 dependent up-regulation of COX2 and prostaglandin production in LAM

**Introduction:** We have previously found that COX2 and prostacyclin (PGI<sub>2</sub>) synthase are transcriptionally up-regulated in TSC2-null LAM-like cells. Since prostaglandins have been implicated in tumorigenesis, we study COX2 and prostaglandin regulation in cellular, and animal models of TSC/LAM and in clinical LAM samples.

**Methods:** Human and murine (MEF) TSC2-deficient cell lines and the corresponding TSC2-addback controls were used for this study. Pharmacologic inhibition of mTORC1 by rapamycin (20nM) was tested. Levels of protein expression were quantified by immunoblotting and prostaglandin levels were assayed in conditioned media by ELISA. Immunohistochemistry(IHC) were performed on pulmonary LAM tissues, and on renal tumors derived from *Tsc2*<sup>-/-</sup> mice.

**Results:** We observed rapamycin-resistant increase expressions of prostaglandin-endoperoxide synthase 2 (COX2) and prostacyclin (PGI<sub>2</sub>) synthase in the human TSC2-null cells compared to the respective TSC2+ controls. Conditioned media from TSC2-null cells demonstrated significantly higher levels of prostaglandins, especially prostacyclin, compared to TSC2+ addbacks. Rapamycin treatment failed to suppress levels of prostaglandin in the TSC2-null cells. Similar findings were found in *Tsc2*-null or TSC2 addback MEFs. Immunohistochemistry (IHC) of LAM tissues confirmed COX2 expressions within LAM nodules. Similarly, renal tumors from *Tsc2*<sup>-/-</sup> mice showed positive COX2 expression on IHC.

**Conclusions:** We report that LAM lesions express COX2, and that COX2 up-regulation is TSC2 dependent and rapamycin resistant. Our data suggest the prostaglandin dysregulation may have a role in LAM pathogenesis. Furthermore, because prostaglandins can be readily assayed biologic fluids such as urine, prostaglandin levels may represent a novel biomarker.

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Vera P. Krymskaya, PhD  
University of Pennsylvania

## TSC2, mTORC2 and Actin Cytoskeleton: Relevance to Lung Cyst Formation

Pulmonary lymphangiomyomatosis (LAM) manifests by neoplastic growth of atypical smooth muscle LAM cells and lung cyst formation. While significant progress has been made in understanding cellular and molecular mechanisms of LAM cell growth, little is known about pathobiology of lung cyst formation in LAM. Cell-cell contacts and cell adhesion maintain tissue integrity; disease-associated alterations of signal transduction pathways regulating these interactions promote disruption of cell-cell contact integrity and normal tissue architecture. Actin cytoskeleton and Rho GTPases are key modulators of cell architecture, cell-cell contacts integrity and cell adhesion. Previously we demonstrated that TSC1/TSC2 modulate actin cytoskeleton and cell adhesion through reciprocal activation of RhoA and Rac1. mTORC2 also regulates actin cytoskeleton through Rho GTPases. Our current study demonstrates a link between TSC2 and mTORC2 in regulating actin

Caroline A. Owen, MD, PhD  
Brigham and Women's Hospital

## Lung Pathologies in LAM: Lessons from COPD

**Introduction:** Sporadic LAM is a systemic disorder of unknown etiology occurring mainly in premenopausal women and associated with somatic mutations in the TSC2 gene. It is characterized by the proliferation of abnormal smooth muscle cells (LAM cells) in the lung interstitium and axial lymphatics of the thorax and abdomen. This LAM cell proliferation is associated with the development of widespread thin-walled lung cysts and obstruction of the lymphatics and conducting airways. LAM has several features in common with a more well studied disease: chronic obstructive pulmonary disease (COPD). LAM and COPD are both associated with: 1) cystic lung destruction and increased lung levels of proteinases; 2) lung inflammation; and 3) and lung remodeling and increased lung levels of growth factors.

**Lung Destruction:** In COPD, there is substantial evidence that proteinases (especially MMPs and serine and cysteine proteinases) promote the development of airspace enlargement (1). Proteinases expressed by activated inflammatory cells and lung structural cells degrade the lung extracellular matrix (ECM) leading to airspace enlargement. Proteinases and cigarette smoke also promote lung septal cell apoptosis leading to loss of the alveolar walls (1-3). LAM patients have widespread lung cysts and increased lung expression of various proteinases [MMP-1, -2, -9, and -14, urokinase type plasminogen activator (u-PA), and cathepsin K (4-6)]. Together, these proteinases can degrade all components of the lung ECM. In COPD lungs, proteinases have been co-localized with degraded elastic fibers (7). In LAM lungs, some proteinases are strongly co-localized with degraded ECM proteins in lung cysts or with disrupted epithelial cell basement membranes. This is consistent with proteinases destroying the alveolar walls in LAM. MMP-14 and u-PA which are expressed on cell surfaces activate latent proMMPs and may contribute to the increased rates of proMMP-2 activation that is associated with LAM cells (8). MMP-14- or uPA-mediated activation of proMMP2 on the surface of LAM cells may promote LAM cell migration as well as lung ECM destruction.

**Lung Inflammation:** COPD is characterized by chronic lung inflammation and increased lung levels of pro-inflammatory mediators. The latter increase inflammatory cell recruitment and proteinase expression by inflammatory cell and lung structural cells (1,9). Many LAM patients have inflammation in airways that are surrounded by LAM cells. LAM patients also have increased BAL levels of chemokines including CCL2, CXCL1, and CXCL5 (10). LAM cells produce these chemokines, express receptors for chemokines, and migrate in response to CCL2. Thus, lung inflammation in LAM may not only increase the lung burden of destructive inflammatory cell proteinases but also promote migration of LAM cells into the lung.

**Lung Remodeling:** Many patients with COPD have small airway fibrosis and rodents chronically exposed to cigarette smoke also develop this lesion (11). In rodents, smoke stimulates the production of growth factors including TGF- $\beta$  which stimulate airway fibroblasts to deposit ECM proteins. LAM patients also have increased levels of TGF- $\beta$  in lung epithelial cells and in areas where there is LAM cell proliferation. Increased lung expression of TGF- $\beta$  is associated with increased expression of fibronectin which is a TGF- $\beta$  responsive gene (12). LAM cells also express high levels of other growth factors including insulin-like growth factors (IGFs) and IGF receptors (4). Proteinases that are increased in COPD and LAM activate latent TGF- $\beta$  and latent IGFs and the active forms of these growth factors may stimulate LAM cell proliferation as well as abnormal ECM deposition.

**Conclusions:** Much is known about the pathogenesis of airspace enlargement in COPD which is similar to the cystic lung destruction occurring in LAM patients. Thus, knowledge of COPD pathogenesis may provide insights into the causes of cystic lung destruction in LAM. Moreover, if ongoing clinical trials show that novel therapies limit lung destruction in COPD patients, it is possible these therapies will also be effective in limiting cystic lung disease in LAM patients.

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Eric S. White, MD

University of Michigan Medical School

## PTEN and the Fibroblast in Pulmonary Fibrosis: A Rationale for Studies in LAM

Idiopathic pulmonary fibrosis (IPF) is a progressive and ultimately fatal scarring disease of the lungs with no effective therapy. The tumor suppressor phosphatase and tensin homologue (PTEN) is a critical regulator of cell behaviors by providing a physiologic brake on the PI3K signaling pathway. As such, loss or mutation of PTEN (as occurs in many malignancies) results in unopposed Akt activity which allows for cellular survival, proliferation, and migration/invasion. PTEN also plays a major role in the behavior of non-malignant cells. Our lab has defined a role for PTEN in suppressing migration, proliferation, and myofibroblast differentiation of lung fibroblasts. We have also found that PTEN activity and expression is downregulated in lung fibroblasts from patients with IPF, which promotes their abnormal phenotypic behavior.

In LAM, immature smooth muscle cells (so-called "LAM cells") appear to be hyperproliferative and migratory. Much like IPF lung fibroblasts, LAM cells are not monoclonal expansions of a single cell. Perhaps most importantly, LAM cells derived from patients with tuberous sclerosis (as well as some patients with sporadic LAM) demonstrate mutations in the *TSC1* and/or *TSC2* genes. These genes encode proteins (hamartin and tuberlin, respectively) that are inhibited by the activity of Akt. Because PTEN is the major physiologic regulator of Akt activity, and because PTEN expression and activity is abnormally low in IPF lung fibroblasts, we have postulated that dysregulated PTEN expression or activity may be operative in patients with LAM. This talk will describe the role of PTEN in fibroblast phenotype and provide a rationale for the study of PTEN in LAM.

**Danielle Morse, MD**  
Brigham and Women's Hospital

### What Could We Learn About LAM From Other Chronic Lung Diseases?

Lymphangiomyomatosis (LAM) is characterized by proliferation, migration, and differentiation of smooth muscle (SM)-like LAM cells, leading to cystic destruction of the lung parenchyma. Similarly, idiopathic pulmonary fibrosis (IPF) is characterized by proliferation, migration and differentiation of fibroblasts towards a smooth-muscle like (myofibroblast) phenotype, contributing to destruction of the lung parenchyma, fibrosis and end-stage honeycomb lung. Although IPF has no known uniform genetic basis, features of the pathogenesis could inform how we understand the destruction of normal lung architecture in LAM. We will briefly review current thinking about the pathogenesis of fibrotic lung disease, and introduce a number of new findings from our laboratory and others that touch upon the role of metabolic pathways, autophagy and the stress response in IPF and emphysema, with an emphasis on potential overlap with LAM pathogenesis.

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**Akiko Hata, PhD**  
Tufts University School of Medicine

### MicroRNA Regulation of Smooth Muscle Phenotypes

MicroRNAs (miRNAs) are small non-coding RNAs that participate in the spatiotemporal regulation of mRNA and protein synthesis. Aberrant miRNA expression leads to developmental abnormalities and diseases, such as cardiovascular disorders and cancer; however, the stimuli and processes regulating miRNA biogenesis are largely unknown. The transforming growth factor  $\beta$  (TGF $\beta$ ) superfamily of growth factors, including bone morphogenetic proteins (BMPs) and TGF $\beta$ s, orchestrates fundamental biological processes in development and in the homeostasis of adult tissues, including the vasculature. We show that induction of a contractile phenotype in vascular smooth muscle cells (VSMCs) by TGF $\beta$  and BMPs is mediated by miR-21. Surprisingly, TGF $\beta$ /BMP signaling promotes a rapid increase in expression of mature miR-21 through a post-transcriptional step, promoting the processing of primary transcripts of miR-21 (pri-miR-21) into precursor miR-21 (pre-miR-21) by the Drosha complex. Smad proteins, the signal transducers of the TGF $\beta$  family of growth factors, are required for ligand-induced upregulation of pre-miR-21 and mature miR-21. We will discuss a potential mechanism of regulation of miRNA biogenesis by the BMP/TGF $\beta$  signaling pathway, which in turn modulates the phenotype of VSMCs.

**Akiko Hata, PhD**  
Tufts Medical Center

### Regulation of Vascular Smooth Muscle Cell Phenotype by microRNAs

The transforming growth factor  $\beta$  (TGF $\beta$ ) super-family of growth factors, including bone morphogenetic proteins (BMPs) and TGF $\beta$ s, orchestrates fundamental biological processes in development and in the homeostasis of adult tissues, including the vasculature. We show that induction of a contractile phenotype in vascular smooth muscle cells (VSMCs) by TGF $\beta$  and BMPs is mediated by miR-21. Surprisingly, TGF $\beta$ /BMP signaling promotes a rapid increase in expression of mature miR-21 through a post-transcriptional step, promoting the processing of primary transcripts of miR-21 (pri-miR-21) into precursor miR-21 (pre-miR-21) by the Drosha complex. Smad proteins, the signal transducers of the TGF $\beta$  family of growth factors, are required for ligand-induced upregulation of pre-miR-21 and mature miR-21. We will discuss a potential mechanism of regulation of miRNA biogenesis by the BMP/TGF $\beta$  signaling pathway.

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**Franco Bonetti, MD**  
Verona University

### LAM, PEComas, and Cathepsin K

Lymphangiomyomatosis (LAM) is part of a family of lesions composed by perivascular epithelioid cells (PEC), together with angiomyolipoma, sugar tumor, and PEComas. The pathogenesis of LAM is determined by mutations affecting tuberous sclerosis complex (TSC) genes, with eventual deregulation of the Rheb/mTOR/p70S6K pathway, and the potential therapeutic activity of mTOR inhibitors is currently under investigation. To better understand the molecular mechanisms involved in the pathogenesis of LAM, we investigated the expression of cathepsin-k (a papain-like cysteine protease with high matrix-degrading activity). The rationale of this choice was based on the recent demonstration that mTOR inhibitors can regulate major functional activities of osteoclasts, including the expression of cathepsin-k. The immunohistochemical study included 12 cases of LAM. Twelve angiomyolipomas and several lung diseases (sarcoidosis, organizing pneumonia, usual interstitial pneumonia, emphysema) were investigated as controls.

In all LAM cases, strong cathepsin-k immunoreactivity was demonstrated, restricted to lymphangiomyomatosis cells. Similar expression levels were observed in renal angiomyolipomas. These observations extend the knowledge regarding the immunophenotypic profile of LAM cells, and provide a useful new marker for diagnosis in difficult cases (eg, in small transbronchial biopsies). The strong expression of such a potent papain-like cysteine protease in LAM cells can significantly contribute to the progressive remodelling of lung parenchyma observed in this deadly disease, with eventual formation of lung cysts. It is possible to speculate that mTOR inhibitors may exert part of their action by limiting the destructive remodelling of lung structure.