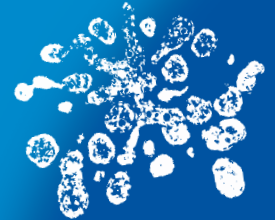


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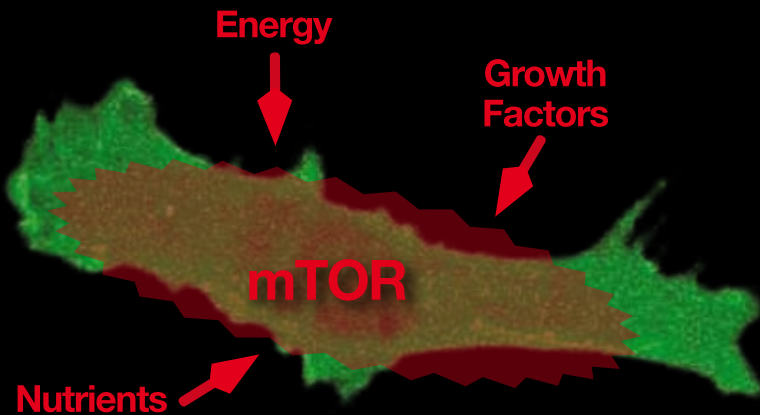
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Montse Sanchez-Cespedes

Dario R. Alessi

Tomi Mäkelä

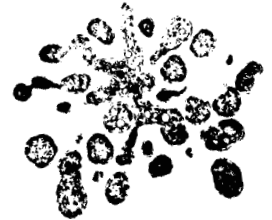


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## Signalling upstream of mTOR

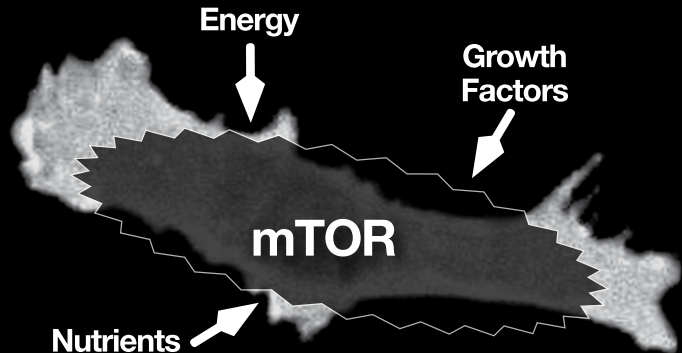
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Tomi Mäkelä, *University of Helsinki, Helsinki, Finland*

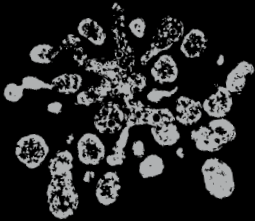




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# **Signalling upstream of mTOR**

Madrid | 3-5 November 2008

# PROGRAM





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## MONDAY, November 3<sup>rd</sup>

Welcome Address  
Montse Sanchez-Cespedes

**Session 1:**  
Cellular energetic  
metabolism

**Chair:** Susanne J. Baker

09:15|**11:00**

D. Grahame Hardie  
Benoît Viollet  
Richard F. Lamb

>> **Coffee break and poster session**

11:30|**13:20**

David M. Sabatini  
George Thomas  
Megan Cully  
Noelia Valbuena

>> **Lunch and poster session**

**Session 2:**  
LKB1 and cancer

**Chair:** Elisabeth P. Henske

14:30|**16:15**

Dario R. Alessi  
Tomi P. Mäkelä  
Kwok-Kin Wong

>> **Coffee break and poster session**

16:45|**18:15**

Reuben J. Shaw  
Montse Sanchez-Cespedes  
Paola Marignani

## TUESDAY, November 4<sup>th</sup>

**Session 3:**  
The PI3K/PTEEN input  
on mTOR

**Chair:** Kun-Liang Guan

09:30|**11:15**

Nissim Hay  
Stephen M. Cohen  
Susanne J. Baker

>> **Group Picture**

>> **Coffee break and poster session**

11:45|**13:35**

Roya Khosravi-Far  
Ana C. Carrera  
Arkaitz Carracedo  
Guillermo Velasco

>> **Lunch and poster session**

**Session 4:**  
Other inputs to mTOR  
regulation

**Chair:** Tomi P. Mäkelä

14:35|**16:05**

Tony Hunter  
Karen Cichowski  
Katharina Uhlenbrock

>> **Coffee break and poster session**

16:35|**18:05**

Jongkyeong Chung  
Brendan D. Manning  
Aaron Robitaille

## WEDNESDAY, November 5<sup>th</sup>

**Session 5:**  
Mechanisms of mTOR  
control, disease and  
cancer therapy

**Chair:** Richard F. Lamb

09:30|**11:15**

John Blenis  
Kun-Liang Guan  
Elizabeth P. Henske

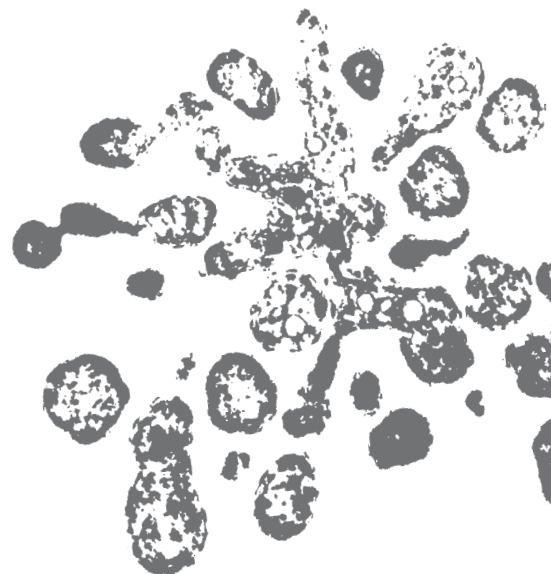
>> **Coffee break and poster session**

11:45|**12:45**

Roman Thomas  
Violeta Serra  
Hans-Guido Wendel

Closing remarks  
and Poster Prize

>> **Lunch**



**MONDAY, November 3<sup>rd</sup> <<**

Welcome Address 09:00|**09:10**  
Montse Sanchez-Cespedes  
**CNIO, Madrid, Spain**

**Session 1: Cellular energetic metabolism**  
**Chair: Susanne J. Baker**  
**St. Jude Children's Research Hospital, Memphis, USA**

D. Grahame Hardie 09:15|**09:50**  
**University of Dundee, Dundee, UK**  
*AMPK – a regulator of energy balance upstream of mTOR that can sense both AMP:ATP ratios and glycogen*

Benoît Viollet 09:50|**10:25**  
**Department of Endocrinology, Metabolism and Cancer, Institut Cochin Inserm U567, Paris, France**  
*Crosstalk between the AMPK and mTOR/S6K pathways in the regulation of cell growth and protein synthesis*

Richard F. Lamb 10:25|**11:00**  
**Institute of Cancer Research, London, UK**  
*Regulation of mTOR by nutrients*

**>> Coffee break and poster session** 11:00|**11:30**

David M. Sabatini 11:30|**12:05**  
**Whitehead Institute for Biomedical Research, Cambridge, USA**  
*Regulation of growth by the mTOR pathway and essential metabolic genes in cancer*

George Thomas 12:05|**12:40**  
**University of Cincinnati, Cincinnati, USA**  
*Nutrients, energy and mTOR/S6K1 signaling*

Short talk: Megan Cully 12:40|**13:00**  
**Signal Transduction Laboratory, Cancer Research UK, London, UK**  
*p38 positively regulates growth and Tor signalling*

Short talk: Noelia Valbuena 13:00|**13:20**  
**Institute of Molecular and Cellular Biology of Cancer (CSIC), University of Salamanca, Salamanca, Spain**  
*AMP-activated protein kinase (AMPK)-signalling upstream of TOR in Schizosaccharomyces pombe*

**>> Lunch and poster session** 13:20|**14:30**



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## Session 2: LKB1 and cancer

Chair: Elisabeth P. Henske

**Brigham and Women's Hospital, Harvard Medical School, Boston, USA**

- 14:30|**15:05** Dario R. Alessi  
**University of Dundee, Dundee, UK**  
*Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN deficient mice*
- 15:05|**15:40** Tomi P. Mäkelä  
**University of Helsinki, Helsinki, Finland**  
*Mesenchymal LKB1 is required for suppression of polyposis and TGF-beta signalling*
- 15:40|**16:15** Kwok-Kin Wong  
**Dana-Farber Cancer Institute, Boston, USA**  
*LKB1 and lung tumorigenesis*
- 16:15|**16:45** >> **Coffee break and poster session**
- 16:45|**17:20** Reuben J. Shaw  
**The Salk Institute, La Jolla, USA**  
*LKB1/AMPK pathway: connecting energy stress to cell growth*
- 17:20|**17:55** Montse Sanchez-Cespedes  
**CNIO, Madrid, Spain**  
*Genetic and molecular characterization of LKB1-mutant tumors*
- 17:55|**18:15** Short talk: Paola Marignani  
**Dalhousie University, Halifax, Canada**  
*Discovery of novel LKB1 signalling targets*

## >> TUESDAY, November 4<sup>th</sup>

### Session 3: The PI3K/PTEN input on mTOR

Chair: Kun-Liang Guan

**University of California at San Diego, San Diego, USA**

- 09:30|**10:05** Nissim Hay  
**University of Illinois at Chicago, Chicago, USA**  
*Interplay between Akt, FoxO and mTOR*
- 10:05|**10:40** Stephen M. Cohen  
**Temasek Life Sciences Laboratory, Singapore, Singapore**  
*Regulation of Akt activity: genetic studies for Drosophila*

Susanne J. Baker <b>St. Jude Children's Research Hospital, Memphis, USA</b> <i>PTEN/PI3K signaling in the nervous system</i>	10:40  <b>11:15</b>
<b>&gt;&gt; Group Picture. Coffee break and poster session</b>	11:15  <b>11:45</b>
Roya Khosravi-Far <b>Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, USA</b> <i>FoxO Family of Tumor Suppressors in Oncogene-Induced Evasion of Apoptosis</i>	11:45  <b>12:20</b>
Ana C. Carrera <b>CNB/CSIC, Madrid, Spain</b> <i>Specific functions of phosphoinositide 3-kinase p110alpha and beta in cell cycle</i>	12:20  <b>12:55</b>
Short talk: Arkaitz Carracedo <b>Harvard Medical School, Boston, USA</b> <i>Targeting mTOR-converging pathways for cancer therapy</i>	12:55  <b>13:15</b>
Short talk: Guillermo Velasco <b>Complutense University of Madrid, Madrid, Spain</b> <i>Cannabinoids activate an endoplasmic reticulum stress response that inhibits the Akt/mTORC1 axis and promotes autophagy-mediated cancer cell death</i>	13:15  <b>13:35</b>
<b>&gt;&gt; Lunch and poster session</b>	13:35  <b>14:35</b>
<b>Session 4: Other inputs to mTOR regulation</b> Chair: Tomi P. Mäkelä <b>University of Helsinki, Helsinki, Finland</b>	
Tony Hunter <b>The Salk Institute, La Jolla, USA</b> <i>Differential phosphorylation of mTOR in the mTORC1 and mTORC2 complexes</i>	14:35  <b>15:10</b>
Karen Cichowskia <b>Harvard Medical School, Boston, USA</b> <i>Exploring the role of the mTOR pathway in Ras-driven tumorigenesis</i>	15:10  <b>15:45</b>
Short talk: Katharina Uhlenbrock <b>Max Plank Institute for Molecular Physiology, Dortmund, Germany</b> <i>Modulators of Rheb signaling towards mTORC1 – reconsidering the roles of FKBP38 and TCT</i>	15:45  <b>16:05</b>
<b>&gt;&gt; Coffee break and poster session</b>	16:05  <b>16:35</b>



**10<sup>th</sup>**  
**ECRS**  
**Cancer**  
**Conferences**  
**2008**

- 16:35|**17:10** Jongkyeong Chung  
**Korea Advanced Institute of Science and Technology, Taejon, Republic of Korea**  
*Regulation of the TOR-dependent signaling in Drosophila*
- 17:10|**17:45** Brendan D. Manning  
**Harvard University, Boston, USA**  
*A complex relationship between the TSC1-TSC2 complex and the mTOR complexes*
- 17:45|**18:05** Short talk: Aaron Robitaille  
**University of Basel, Basel, Switzerland**  
*mTOR autophosphorylation at Ser-2481 is a molecular marker for mTORC2 activity*

**>> WEDNESDAY, November 5<sup>th</sup>**

**Session 5: Mechanisms of mTOR control, disease and cancer therapy**

**Chair:** Richard F. Lamb

**Institute of Cancer Research, London, UK**

- 09:30|**10:05** John Blenis  
**Harvard Medical School, Boston, USA**  
*mTOR signaling and cell growth control in normal and cancer cells*
- 10:05|**10:40** Kun-Liang Guan  
**University of California at San Diego, San Diego, USA**  
*Regulation and function of the TSC-mTOR pathway*
- 10:40|**11:15** Elizabeth P. Henske  
**Brigham and Women's Hospital, Harvard Medical School, Boston, USA**  
*Tuberous sclerosis and LAM: pathogenic mechanisms*
- 11:15|**11:45** **>> Coffee break and poster session**
- 11:45|**12:05** Short talk: Roman Thomas  
**Max Plank Institute for Neurological Research, Cologne, Germany**  
*A chemical genomics approach to dissect Pi3 kinase and mTOR dependency in non-small cell lung cancer*
- 12:05|**12:25** Short talk: Violeta Serra  
**Vall d'Hebron University Hospital, Barcelona, Spain**  
*NVP-BEZ-235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits growth of cancer cells with activating PI3K mutations*


Short talk: Hans-Guido Wendel 12:25|**12:45**  
**Memorial Sloan-Kettering Cancer Center, New York, USA**  
*Tumorigenic activity and therapeutic inhibition of Rheb GTPase*

Closing remarks and poster prize

Dario R. Alessi 12:45|**13:00**  
**University of Dundee, Dundee, UK**

**>> Lunch 13:00**

Nature Reviews Cancer sponsors a Prize to the best Poster that consists in an official certificate and ONE YEAR subscription to the journal

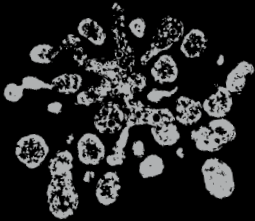
nature publishing group 

**TALKS:** 25-minute

**SHORT TALK:** 15-minute

**DISCUSSION:** 10-minute after each talk / 5-minute after each short talk

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# **Signalling upstream of mTOR**

Madrid | 3-5 November 2008

SPEAKER  
**ABSTRACTS**





# AMPK – a regulator of energy balance upstream of mTOR that can sense both AMP:ATP ratios and glycogen

**D. Grahame Hardie**

Division of Molecular  
Physiology, College  
of Life Sciences,  
University of Dundee,  
Dundee, Scotland,  
UK

The AMP-activated protein kinase (AMPK) acts as a sensor of cellular energy status. AMPK occurs as heterotrimers comprising catalytic alpha subunits and regulatory beta and gamma subunits, and genes encoding these are found in the genomes of essentially all eukaryotes. In budding yeast (*Saccharomyces cerevisiae*) the AMPK orthologue is the SNF1 complex, which is required for the response to glucose deprivation. The system may therefore have evolved as an ancient starvation response system. In *Caenorhabditis elegans*, the AMPK orthologue is required for the extension of lifespan in response to caloric restriction. The alpha subunits contain a kinase domain that is only active after phosphorylation at Thr-172 within the activation loop. In most mammalian cells, the upstream kinase that phosphorylates this site is the LKB1 complex. LKB1 was originally defined as a human tumor suppressor, because it is mutated in Peutz-Jeghers syndrome (an inherited susceptibility to cancer) and is deleted in many cases of non-small cell lung carcinoma. Although LKB1 also acts upstream of a small family of AMPK-related kinases, it seems likely that many of its tumor suppressor functions are mediated by AMPK itself.

The gamma subunits of AMPK contain two sites that reversibly bind AMP or ATP in a mutually exclusive manner. LKB1 appears to phosphorylate Thr-172 continually, but binding of AMP to the twin sites on the gamma subunit inhibits dephosphorylation of Thr-172, causing net conversion of the kinase to the active, phosphorylated form. In unstressed cells, these regulatory sites are normally occupied by ATP, so that the kinase is maintained in its dephosphorylated, inactive form. Thus, a rise in the cellular AMP:ATP ratio (a signal that the energy status of the cell is compromised) switches on the AMPK system via a very sensitive mechanism.

Metabolic stresses that either inhibit ATP production (e.g. ischemia, hypoxia, glucose deprivation) or accelerate ATP consumption (e.g. exercise in muscle) switch on the AMPK system. AMPK then phosphorylates numerous downstream targets that activate catabolic pathways generating ATP, while switching off ATP-consuming processes, including biosynthesis of lipids, carbohydrates and proteins, and cell growth and proliferation. A particularly important target by which AMPK limits cell growth is the mTOR complex 1 (TORC1). AMPK inhibits TORC1 via phosphorylation of both TSC2 and Raptor. The beta subunits of AMPK contain a conserved carbohydrate-binding module that causes binding of mammalian AMPK to glycogen. Recent work in our laboratory suggests that this domain can sense the state of cellular glycogen stores. If they are replete, AMPK triggers phosphorylation and inactivation of glycogen synthase, providing a feedback regulation on glycogen synthesis. Conversely, if glycogen stores become depleted, glycogen-bound AMPK is inhibited, causing glycogen synthase to be dephosphorylated to catalyze rapid re-synthesis of glycogen.

# Crosstalk between the AMPK and mTOR/S6K pathways in the regulation of cell growth and protein synthesis

**Benoît Viollet<sup>1,2</sup>**

All cells must coordinate diverse processes including cell division, cell migration and cell polarity with the energy status of the cell. The mammalian target of rapamycin (mTOR) assembles a signaling network essential for the regulation of cell growth and proliferation. Precise control of mTOR is achieved through multiple inputs, including growth factors, nutrients and cellular energy status. AMPK appears to represent a point of conversion of regulatory signals monitoring systemic and cellular energy status, affecting processes from ribosomal biogenesis to actin regulation. Activation of AMPK has been shown to inhibit protein synthesis through the repression of mTOR signalling pathway, leading to the inhibition of p70S6 kinase (S6K), an important inducer of skeletal muscle. A crosstalk between S6K and AMPK exists in skeletal muscle and may function as major determinants of the adaptive responses to nutrition and exercise. Suppression of nutrient-activated mTOR/S6K signaling is sufficient to trigger an energy stress response that is coordinated by AMPK. This AMPK-dependent metabolic program blunts the growth responses to nutrient availability and thereby plays a central role in the atrophic phenotype of S6K-deficient animals. Using two animal models in which the predominant catalytic and regulatory AMPK isoforms expressed in glycolytic skeletal muscle have been genetically deleted, we provided direct evidence linking AMPK activation to mTOR signaling inactivation. We determined that AICAR-induced inhibition of insulin-mediated mTOR signaling is intact in AMPKalpha2 KO and AMPKgamma3 KO mice. We also examined AMPK activity and its potential association with the regulation of skeletal muscle growth/overload-induced hypertrophy using AMPK-deficient animals. Our recent results pointed out an essential role for AMPK activation in skeletal muscle to limit the hypertrophic response.

Department of  
Endocrinology,  
Metabolism and  
Cancer, *Institut  
Cochin, Université  
Paris Descartes,*  
CNRS (UMR 8104)  
and Inserm, U567,  
Paris, France



## Regulation of mTOR by nutrients

**Richard F. Lamb**, Virginia Mieulet and Lijun Yan

Cancer Research UK  
Centre of Cell and  
Molecular Biology,  
Institute of Cancer  
Research, Chester  
Beatty Laboratories,  
London, UK

The mTORC1 pathway is regulated by growth factors, hormones and nutrients such as glucose and amino acids. I will discuss: 1) how amino acids regulate the activity of an mTORC1 activator, MAP4K3, and 2) how amino acids signal to another signalling pathway-MEK-ERK.

Amino acids promote regulatory T-loop phosphorylation MAP4K3, at a site required for kinase activity and activation of mTORC1. This site is acutely regulated by amino acids via a PP2A phosphatase.

Innate immune responses to microorganisms are also highly influenced by host nutrient status, although the mechanisms involved are unknown. I will present data indicating that nutrient amino acids signal not only to mTORC1, but also the MAP kinase pathway in macrophages responding to the Toll-Like Receptor 4 (TLR4) ligand, lipopolysaccharide (LPS). We find that a MEK-family kinase regulates both pathways and is itself regulated by amino acid availability. These results suggest an explanation for the influence of host nutrient status on innate immune cell activation.

# Regulation of growth by the mTOR pathway and essential metabolic genes in cancer

**David M. Sabatini**

I will discuss two areas of interest to our lab, the regulation of the mTOR pathway and our efforts to identify essential metabolic genes in cancer cells. mTOR is the target of the immunosuppressive drug rapamycin and the central component of a nutrient- and hormone-sensitive signaling pathway that regulates cell growth and proliferation. We now appreciate that this pathway becomes deregulated in many human cancers. We have identified two distinct mTOR-containing proteins complexes, one of which regulates growth through S6K and another that regulates cell survival through Akt. These complexes, mTORC1 and mTORC2, define both rapamycin-sensitive and insensitive branches of the mTOR pathway. I will provide an overview of mTOR signaling as well as discuss the regulation of mTORC1 by insulin and nutrients.

Whitehead Institute  
for Biomedical  
Research, Nine  
Cambridge Center,  
Cambridge, USA



## Nutrients, energy and mTOR/S6K1 signaling

**George Thomas**

Department of  
Medical Oncology,  
Genome Research  
Institute, University of  
Cincinnati, Cincinnati,  
USA

The major pathogenic consequences of nutrient overload are insulin resistance and type 2 diabetes, which are displacing smoking as the major contributor to cardiovascular disease, the main cause of human morbidity and death. Importantly, recent studies show that nutrient overload is also a key risk factor for a number of cancers including colon, breast, and prostate. The major factors associated with nutrient overload-mediated dysmetabolic states are increased dietary fats and carbohydrates. However, increased protein consumption is also a major contributor, acting through elevated levels of circulating amino acids (AAs), particularly branched-chain AAs (BCAA). Earlier we set out to elucidate the molecular components and signaling pathways by which increased nutrients, particularly AA-mediated mTORC1 activation. These studies led us to a wortmannin sensitive, class 1 PI3K-independent input to mTORC1 signaling pathway, which we identified as hVps34. Ectopic expression of hVps34 drives S6K1 activation, whereas siRNA depletion of hVps34 blocks this response. Recent studies show that amino acids, particularly, leucine, leads to a rise in intracellular  $[Ca^{+2}]$  increasing the direct interaction of  $Ca^{+2}/CaM$  to an evolutionarily conserved motif in hVps34, which is required for lipid kinase activity and increased mTORC1 signaling. Unexpectedly, others have recently shown that the hVps34 response does not appear to be conserved in *Drosophila*, despite the conserved CaM binding site in the *Drosophila* orthologue of hVps34, dVps34. We have been studying the difference between *Drosophila* and mammalian cells, which have provided a novel insight into the role of nutrients in mediating mTORC1 activation.

# p38 positively regulates growth and Tor signalling

**Megan Cully<sup>1</sup>, Alice Genevet<sup>2</sup>, Nic Tapon<sup>2</sup>, Sally Leever<sup>3</sup>  
and Julian Downward<sup>1</sup>**

The Tor signalling pathway integrates signals measuring both the capacity and the desirability for growth. While many components of the Tor pathway have been described, there are also many unknown components, both upstream and downstream of Tor itself.

To identify novel components of the Tor signalling network, an RNAi screen was performed in *Drosophila* S2 cells. This screen identified the p38 pathway as a regulator of Tsc2-mediated changes in cell size. In both *Drosophila* and mammalian tissue culture models, inhibition of p38 signalling using RNAi prevents the phosphorylation of S6 and 4EBP1 in response to amino acids, and also decreases Tsc2 RNAi-mediated increases in cell size. Consistent with a model in which p38 activates Tor, the activation of p38 using H2O2 or anisomycin increases the phosphorylation of the downstream Tor targets S6/S6K and 4EBP1. Furthermore, long-term activation of the p38 pathway increases cell size. To investigate the role of p38 in the activation of Tor *in vivo*, we generated *Drosophila* mutants null for either p38b or the upstream kinase mkk3/licorne. Interestingly, mutants for either licorne or p38b are small. This decrease in organism size seems to be due at least in part to a cell autonomous decrease in cell size. Mutants for licorne or p38b are nutrition-sensitive; low-nutrient food accentuates the small organism phenotypes as well as the partial lethality of the p38b null allele. We are currently investigating potential mechanisms underlying these observations.

Our data suggest that p38 can activate Tor. This may be important during processes such as the activation of the immune system or during angiogenesis, where increased translation in response to stress would be key to mounting an appropriate response.

<sup>1</sup>. Signal Transduction Laboratory, Cancer Research UK London, London, UK  
<sup>2</sup>. Apoptosis and Proliferation Control Laboratory, Cancer Research UK London Research Institute, London, UK  
<sup>3</sup>. Growth Control Laboratory, Cancer Research UK London Research Institute, London, UK



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Short Talk

# AMP-activated protein kinase (AMPK): signalling upstream of TOR in *Schizosaccharomyces pombe*

Noelia Valbuena and Sergio Moreno

Laboratory, 10,  
Institute of Molecular  
and Cellular  
Biology of Cancer  
(CSIC), University  
of Salamanca,  
Salamanca, Spain

TOR (target of rapamycin) has a crucial role in the regulation of cell growth and cell size. Amino acids, growth factors and cellular energy levels regulate TOR activity. TOR is a serine/threonine kinase conserved from yeast to humans. In mammals, the TSC1-TSC2/Rheb/TOR pathway regulates cellular growth by activation of translation, transcription and ribosome biogenesis. Fission yeast is a good model to study this pathway because different to *Saccharomyces cerevisiae* all the elements of this pathway (TSC1/2, Rheb and TOR) are conserved.

The AMP-activated protein kinase (AMPK) is a heterotrimeric protein complex that works as a sensor of the cellular energy status, conserved in all eukaryotes. AMPK responds to changes in intracellular AMP levels during metabolic stress caused by exercise, hypoxia or lack of cell nutrients or glucose. Several studies have identified AMPK as a negative regulator of TSC2 and this appears to be responsible for limiting protein synthesis and cell growth, and protecting against apoptosis, during cellular stresses such as glucose starvation.

We have studied the role of Ssp2, the catalytic subunit of the AMPK complex in *S. pombe*, and its connection with TOR. Previously, we and others have shown that fission yeast Tor2 is central to controlling the switch between cell growth and cell differentiation in response to nutrient availability. We have found that the *ssp2* mutant shows the opposite phenotype to Tor2 inactivation. In *ssp2* mutant, cell differentiation is diminished, and the cells show a slower cell cycle arrest in G1 (a prerequisite for cell differentiation in *S. pombe*). On the other hand, translation, transcription and ribosome biogenesis are not repressed under nutrient depletion. We will propose a model for AMPK function in nutrient depletion and its relation with Tor2 in *S. pombe*.

# Important role of the LKB1-AMPK pathway in suppressing tumourigenesis in PTEN deficient mice

Xu Huang<sup>1</sup>, Stephan Wullschlegler<sup>1</sup>, Natalia Shpiro<sup>1</sup>, Victoria A. McGuire<sup>2</sup>, Kei Sakamoto<sup>1</sup>, Yvonne L. Woods<sup>3</sup>, Wendy McBurnie<sup>3</sup>, Stewart Fleming<sup>3</sup> and **Dario R. Alessi**<sup>1</sup>

The LKB1 tumour suppressor phosphorylates and activates AMPK when cellular energy levels are low, thereby suppressing growth through multiple pathways, including inhibiting the mTORC1 kinase that is activated in the majority of human cancers. Blood glucose lowering type-2 diabetes drugs also induce LKB1 to activate AMPK, indicating that these compounds could be used to suppress growth of tumour cells. In this study we investigated the importance of the LKB1-AMPK pathway in regulating tumourigenesis in mice resulting from deficiency of the PTEN tumour suppressor, which drives cell growth through over-activation of the Akt and mTOR kinases. In my talk I will provide evidence showing that inhibition of AMPK resulting from a hypomorphic mutation that decreases LKB1 expression does not lead to tumourigenesis on its own, but markedly accelerated tumour development in PTEN<sup>+/-</sup> mice. In contrast, activating the AMPK pathway by administration of PTEN<sup>+/-</sup> mice metformin, phenformin or A-769662, significantly delayed tumour onset. I will also provide evidence that LKB1 is required for activators of AMPK to inhibit mTORC1 signalling as well as cell growth in PTEN deficient cells. I will discuss how these results highlight in an animal model relevant to understanding human cancer, the vital role that the LKB1-AMPK pathway plays in suppressing tumourigenesis resulting from loss of PTEN tumour suppressor. They also suggest that pharmacological inhibition of LKB1 and/or AMPK would be undesirable, at least for the treatment of cancers in which the mTORC1-pathway is activated. Most importantly our data demonstrate the potential of AMPK activators such as clinically approved metformin as anti-cancer agents, which will suppress tumour development by triggering a signalling pathway that potently inhibits cell growth.

<sup>1</sup>. Department of Developmental Neurobiology, MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee, UK  
<sup>2</sup>. College of Life Sciences, University of Dundee, Dundee, UK  
<sup>3</sup>. Department of Molecular Pathology, University of Dundee, Ninewells Hospital, Dundee, UK



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# Mesenchymal LKB1 is required for suppression of polyposis and TGF-beta signalling

**Tomi Mäkelä**

Department of Cell  
Biology, Genome-  
Scale Biology  
Program and Institute  
of Biomedicine,  
*Biomedicum Helsinki*,  
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Germline mutations of LKB1 cause Peutz-Jeghers syndrome (PJS) manifesting with gastrointestinal polyps that contain in addition to epithelia a prominent stromal component. To explore the possible role of the non-epithelial component in the PJS gastrointestinal tumorigenesis, we inactivated *Lkb1* specifically in SM22 expressing mesenchymal cells by use of conditional *Lkb1* allele and SM-CreERT2 allele. Interestingly we find that loss of *Lkb1* limited to mesenchyme resulted in premature lethality due to gastrointestinal polyposis. To investigate the possible molecular mechanisms involved in polyposis we studied the activity of the mTOR pathway, but found no evidence for alterations in the mesenchymal cells with immunohistochemical analysis. Furthermore, analysis of gastrointestinal tracts of AMPK $\alpha$ 1<sup>-/-</sup> and AMPK $\alpha$ 2<sup>-/-</sup> mice did not reveal signs of polyposis suggesting that this pathway is not critical for polyp development. Analysis of tumor stroma indicated a change in the ratio of myofibroblasts and smooth muscle cells suggesting altered myogenic differentiation. Interestingly, *Lkb1* was found to affect TGF $\beta$  signaling as *Lkb1* deletions in primary mouse embryo fibroblasts (MEFs) attenuated TGF $\beta$ -dependent transcription and myofibroblast differentiation. Moreover, *Lkb1* deficient MEFs demonstrated reduction in active TGF $\beta$ , and *Lkb1* recombined tumor mesenchyme and the epithelium adjacent to it demonstrated compromised TGF $\beta$  signaling. Thus defective TGF $\beta$  signaling from mesenchyme to epithelium was identified as a possible mechanism for increased epithelial proliferation in these tumors. Independently, *Lkb1* deletion in endothelial cells resulted also in defective TGF $\beta$ -mediated signaling with adjacent cells, demonstrating the TGF $\beta$  modulating role of *Lkb1* also in other tissues. These data demonstrate that *Lkb1* signaling defects in mesenchymal cells drive polyp formation identifying an indirect mechanism of tumor suppression.

# LKB1 and lung tumorigenesis

**Kwok-Kin Wong**

Germline mutation in serine/threonine kinase 11 (STK11, also called LKB1) results in Peutz-Jeghers syndrome, characterized by intestinal hamartomas and increased incidence of epithelial cancers. Although uncommon in most sporadic cancers, inactivating somatic mutations of LKB1 have been reported in primary human lung adenocarcinomas and derivative cell lines. Here we used a somatically activatable mutant Kras-driven model of mouse lung cancer to compare the role of Lkb1 to other tumour suppressors in lung cancer. Although Kras mutation cooperated with loss of p53 or Ink4a/Arf (also known as Cdkn2a) in this system, the strongest cooperation was seen with homozygous inactivation of Lkb1. Lkb1-deficient tumours demonstrated shorter latency, an expanded histological spectrum (adeno-, squamous and large-cell carcinoma) and more frequent metastasis compared to tumours lacking p53 or Ink4a/Arf. Pulmonary tumorigenesis was also accelerated by hemizygous inactivation of Lkb1. Consistent with these findings, inactivation of LKB1 was found in 34% and 19% of 144 analysed human lung adenocarcinomas and squamous cell carcinomas, respectively. Expression profiling in human lung cancer cell lines and mouse lung tumours identified a variety of metastasis-promoting genes, such as NEDD9, VEGFC and CD24, as targets of LKB1 repression in lung cancer. These studies establish LKB1 as a critical barrier to pulmonary tumorigenesis, controlling initiation, differentiation and metastasis.

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# The LKB1/AMPK pathway: connecting energy stress to cell growth

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The serine/threonine kinase LKB1 is a tumor suppressor gene mutated in the familial cancer condition Peutz-Jeghers syndrome (PJS), as well as in 30-40% of sporadic non small cell lung cancer (NSCLC). We and others previously discovered that one of the critical substrates of LKB1 is the AMP-activated protein kinase (AMPK). AMPK is a highly conserved sensor of cellular energy status found in all eukaryotic cells that is activated under conditions of low intracellular ATP following stresses such as nutrient deprivation or hypoxia. Using a proteomic and bioinformatics approach, we recently identified the mTOR-binding subunit raptor as a critical substrate of AMPK mediate its effects on growth control. AMPK directly phosphorylates the mTOR binding partner raptor on two serine residues that are conserved throughout eukaryotes, and this phosphorylation induces 14-3-3 binding to raptor. We demonstrate that the phosphorylation of raptor by AMPK is required for the inhibition of mTORC1 and for cell cycle arrest following energy stress. These findings uncover a novel conserved effector of AMPK that mediates its role as a metabolic checkpoint coordinating cell growth with energy status. As this represents the first discovery of phosphorylation event directly inhibiting the mTORC1 complex, these findings suggest a possible therapeutic window for treatment of tumors bearing loss of TSC or hyperactivation of mTOR by other genetic lesions with AMPK agonists. In addition, the AMPK-raptor connection reinforces the idea that LKB1-deficient tumors such as those arising in PJS patients and sporadic NSCLC may be uniquely sensitive to rapamycin analogs, which we have recently explored in preclinical trials in LKB1 heterozygous mice. We have also found that hamartomas in LKB1<sup>+/-</sup> mice exhibit functional increases in glucose metabolism as visualized by FDG-PET, which is suppressed by rapamycin treatment. HIF-1a and HIF-1a target genes including GLUT1 and Hexokinase2 are upregulated in LKB1-dependent tumors providing a molecular explanation for the increased FDG-PET and rapamycin sensitivity. These and related therapeutic possibilities will be discussed. The connection between LKB1, AMPK, and mTOR signaling further illustrates molecular connections underlying the development of both cancer and metabolic syndrome.

# Genetic and molecular characterization of LKB1 - mutant tumors

**Montse Sanchez-Cespedes**

Germ-line mutations at LKB1, also called STK11, are the cause of the Peutz-Jeghers syndrome (PJS), which is characterized by an increased risk of several types of cancer. Moreover, LKB1 is somatically inactivated in some tumors of sporadic origin, specially in lung cancer. LKB1 encodes a serine-threonine kinase implicated in several cell functions such as cell polarity and signal transduction. LKB1, in a complex with STRAD and MO25, phosphorylates and activates the AMP-activated protein kinase (AMPK), an energy sensor that mediates the inhibition of the mammalian target of rapamycin (mTOR) through the activation of TSC2 (tuberin). Expression profiles of lung adenocarcinomas according to their LKB1 mutation status identified LKB1-specific variations in gene expression including de-regulation of transcripts involved in signal transduction, cytoskeleton, transcription factors and metabolism of AMP (AMPD3 and APRT). Endogenous levels of AMPK activity in LKB1-mutant lung cancer cells and in lung primary tumors were low. According to the role of AMPK in regulation of TSC2/mTOR activity, LKB1-mutant cells were unable to inactivate mTOR in a situation of energetic stress. This ability was restored after reintroduction of wild type LKB1. Inactivation of LKB1 in cancer cells frequently coexisted with alterations at other indirect modulators of mTOR such as PIK3CA and KRAS. This proves that multiple pathways that control mTOR activity are simultaneously altered in cancer and thus have independent roles in its development. In addition, it may help explain why neither LKB1 status nor alterations in other genes such as KRAS, ERBB2 or EGFR predict sensitivity to the mTOR inhibitor rapamycin.

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## Discovery of novel LKB1 signalling targets

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The gene responsible for the autosomal dominant disorder Peutz-Jeghers Syndrome is located on chromosome 19p13.3 and encodes a tumour suppressor kinase LKB1<sup>1,2</sup>. In the last decade, the identification of LKB1-mediated signalling pathways initiated with the discovery of the first binding partner of LKB1, the chromatin remodeling ATPase-helicase Brg1<sup>3</sup>, upstream kinases that phosphorylate LKB1<sup>4</sup>, activating adaptor proteins  $\alpha$ pseudokinase STE20-related adaptor (STRAD) and scaffolding mouse protein 25 (MO25)<sup>5-7</sup>, the involvement of LKB1 in AMPK-mediated cell energy metabolism<sup>8</sup>, cell polarity<sup>9-11</sup> and our most recent discovery that LKB1 catalytic deficient mutants possess oncogenic properties<sup>12</sup>. With each newly identified LKB1 protein interaction, the potential to uncover LKB1 molecular signalling networks improves, revealing how LKB1 is involved in mediating normal cellular events as well as aberrant signalling events that leads to the development and progression of disease. To identify emergent properties of LKB1, we developed a gel-free mass spectrometry strategy to isolate LKB1 binding partners. In this strategy we immunoprecipitated endogenous LKB1 from human embryonic kidney cell lysate, followed by proteolytic digestion directly from the immunoprecipitants. Peptide mixtures were analyzed by ESI-MS/MS. With this approach we identified a LKB1 proteome comprised of novel proteins found in complex with LKB1, one of which we refer to as A2 protein. We confirmed that the binding of A2 protein to LKB1 is independent of LKB1 catalytic activity since A2 protein binds to several different LKB1 catalytic deficient mutants. Furthermore using both transmission electron and immunofluorescent microscopy we confirmed the localization of A2 protein at the endoplasmic reticulum through an N-terminus endoplasmic reticulum targeting sequence. The function of LKB1-A2 protein interaction is currently under investigation. Funded by the Canadian Institutes for Health Research (grant #MOP-67039).

1. Hemminki, A., et al. (1997). *Nat Genet.* **15**, 87-90
2. Jenne, D.E., et al. (1998). *Nat Genet.* **18**, 38-43
3. Marignani, P.A., et al. (2001). *J Biol Chem.* **276**, 32415-32418
4. Sapkota, G.P., et al. (2002). *Biochem J.* **362**, 481-490
5. Baas, A.F., et al. (2003). *Embo J.* **22**, 3062-3072
6. Boudeau, J., et al. (2003). *Embo J.* **22**, 5102-5114
7. Marignani, P., et al. (2007). *Cancer Biology Therapy.* **6**, 1627-1631
8. Hardie, D.G. (2005). *Curr Opin Cell Biol.* **17**, 167-173
9. Baas, A.F., et al. (2004). *Trends Cell Biol.* **14**, 312-319
10. Shelly, M., et al. (2007). *Cell.* **129**, 565-577
11. Barnes, A., et al. (2007). *Cell.* **129**, 549-563
12. Scott, K.D., et al. (2007). *Cancer Res.* **67**, 5622-5627

# Interplay between Akt, FoxO, and mTOR

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In the first part of this presentation, new regulatory circuits that underlie interplay between Akt, FoxO, and mTOR will be described. We found that activated FoxO1 inhibits mTORC1 by TSC-dependent and TSC-independent mechanisms. At the same time FoxO1 increased mTORC2 activity that consequently activates Akt. The exact mechanisms by which FoxO regulates mTORC1 and mTORC2 activities will be described. We propose that in certain conditions of growth factors or nutrient limitations when FoxO activity is elevated, it transiently inhibits mTORC1, thereby decreasing protein synthesis—a major consumer of cellular energy, while transiently activating Akt, which increases cellular energy metabolism. Thus, FoxO proteins may mediate Akt-mTOR regulatory circuits to maintain cellular energy homeostasis.

In the second part of this presentation, the ability of hyperactive mTORC1 to inhibit apoptosis will be described. TSC-deficient cells are sensitized to apoptosis in the presence of growth factors due to the inhibition of Akt activity by the negative feedback mechanism induced by the hyperactive mTORC1. Unexpectedly, however, TSC-deficient cells are markedly resistant to growth factor withdrawal induced apoptosis. We show that the hyperactive mTORC1 in TSC2-deficient cells inhibits apoptosis induced by growth factor withdrawal. The hyperactive mTORC1 in Tsc2<sup>-/-</sup> cells promotes resistance to growth factor withdrawal induced apoptosis, in part by elevating the expression of HIF1 $\alpha$ , which in turn increases GLUT1 and HKII expression. The elevation of HIF1 $\alpha$  - Tsc2<sup>-/-</sup> cells exclusively in the absence of serum, is due to both, increased HIF1 $\alpha$  mRNA levels and sustained mRNA translation -. In addition, the sustained inhibition of GSK3 $\beta$ , and Mcl-1 expression in the absence of growth factors, contribute to the resistance of Tsc2<sup>-/-</sup> cells to apoptosis. However, inhibition of mTORC1 activity by either rapamycin or the knockdown of Raptor cannot sensitize Tsc2<sup>-/-</sup> cells to growth factor withdrawal induced apoptosis because of the elevated Akt activity as a consequence of mTORC1 inhibition.

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## Regulation of Akt activity: genetic studies for *Drosophila*

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Animals use the Insulin/TOR signaling pathway to mediate their response to fluctuation in nutrient availability. Energy and amino acids are monitored at the single-cell level via the TOR branch of the pathway and systemically via insulin signaling to regulate cellular growth and metabolism. Using a combination of genetics, expression profiling and chromatin immunoprecipitation, we have examined nutritional control of gene expression and identified a network of convergent regulation of protein biosynthesis by the FOXO and TOR branches of the nutrient-sensing pathway. TOR exists in two forms, the rapamycin-sensitive TOR complex 1, mediates amino acid and energy-level signals by controlling translation. TOR exists in a second complex, which is rapamycin insensitive and which had recently been reported to regulate AKT activity. Mutants that lack an essential component of TORC2 have led to surprising new insights into the mechanism of AKT regulation by dual phosphorylation. TORC2-mediated phosphorylation of AKT is needed, but only for very high-level activation of the pathway, as occurs in *pten* mutants. AKT without phosphorylation of this site is sufficient to support nearly normal development (Hietakangas & Cohen 2007). Hyperactivation of AKT is a common cause of cancer (e.g. in PTEN mutant tumors), suggesting that components of TORC2 may be a source of useful targets for anticancer drugs.

## PTEN/PI3K signaling in the nervous system

Nader Chalhoub, Lionel Chow, Raelene Endersby, Xiaoyan Zhu and **Suzanne J. Baker**

PTEN is the central negative regulator of PI3K signaling. Loss of PTEN function has profound consequences in the nervous system, where inactivating somatic mutations occur frequently in glioblastomas, and inherited mutations are associated with a number of neurological abnormalities in humans including macrocephaly, ataxia, seizures, Lhermitte-Duclos disease, mental retardation and autism. We selectively inactivated Pten in different cell types and at different developmental stages and showed key roles in tumor suppression, normal development, and homeostatic maintenance in the nervous system *in vivo*. The functional impact of Pten deletion varies depending on the specific context. We will present studies to evaluate the contribution of mTor, as well as other upstream effectors in the PI3K signaling pathway, including p110 $\alpha$ , Pdk1 and Akt, to these Pten-deficient phenotypes using pharmacological and genetic approaches.

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# FoxO Family of Tumor Suppressors in Oncogene-Induced Evasion of Apoptosis

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Forkhead family of transcription factors, including FoxO subgroup, plays key roles in a variety of cellular functions including cellular survival and cell death. We and others have demonstrated that the FoxO proteins are one of the key factors in mediating oncogene-induced evasion of apoptosis in leukemia. The BCR-ABL kinase plays an essential role in the pathogenesis of chronic myeloid leukemia (CML) and some cases of acute lymphocytic leukemia (ALL). Even though the ABL kinase inhibitor, imatinib mesylate, has shown great promise in the treatment of CML, the persistence of residual disease and the occurrence of resistance have prompted investigations into the molecular effectors of BCR-ABL. Here we show that BCR-ABL stimulates the proteasome-dependent inhibition of members of Forkhead family of tumor suppressor genes (including FoxO1 and FoxO3a) in cell culture, in an in vivo mouse model for leukemia and in BCR-ABL-positive CML and ALL patients. Consequently, inhibition of the proteasome pathway, using bortezomib, causes regression of CML-like disease and prevents BCR-ABL-induced suppression of FoxO3a and its pro-apoptotic targets TRAIL and BIM. Notably, we show that bortezomib treatment of a BCR-ABL-positive ALL patient result in a durable cytogenetic and molecular remission, which is accompanied by an increase in the expression of FoxO1 and FoxO3a. We further demonstrate the contribution of FoxO3a function towards bortezomib sensitivity in imatinib-resistant BCR-ABL T315I cells. Our data delineate the involvement of FoxO3a, in BCR-ABL-induced evasion of apoptosis and suggests FoxO proteins as candidate therapeutics in the treatment of BCR-ABL-induced leukemia and potentially other types of cancer.

# Specific functions of phosphoinositide 3-kinase p110 $\alpha$ and beta in cell cycle

Miriam Marqués, Amit Kumar, Isabel Cortés, Carmen Hernández  
and **Ana C. Carrera**

Phosphoinositide 3-kinase (PI3K) is an early signaling molecule that regulates cell growth and cell cycle entry. PI3K is activated immediately after growth factor receptor stimulation (at G0/G1 transition) and again in late G1. The two ubiquitous PI3K isoforms (p110 $\alpha$  and  $\beta$ ) are essential during embryonic development and are thought to control cell division. Nonetheless, it is presently unknown which one is activated during cell cycle and whether or not they both control S phase entry. We found that p110 $\alpha$  was activated first in G0/G1, followed by a minor p110 $\beta$  activity peak. In late G1, p110 $\alpha$  activation preceded that of  $\beta$ , which showed maximum activity at this time. p110 $\beta$  activation required Ras-activity, whereas p110 $\alpha$  was first activated by Tyrosine kinases and then further induced by active-Ras. Interference with p110 $\alpha$  and  $\beta$  activity diminished activation of downstream effectors with different kinetics, with a selective action of p110 $\alpha$  at blocking early G1 events. We show that inhibition of either p110 $\alpha$  or p110 $\beta$  reduced cell cycle entry. These results reveal that PI3K $\alpha$  and  $\beta$  present distinct activation requirements and kinetics in G1 phase, with a selective action of PI3K $\alpha$  at G0/G1 phase transition. Nevertheless, both PI3K $\alpha$  and  $\beta$  regulate S phase entry.

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Short Talk

## Targeting mTOR-converging pathways for cancer therapy

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The PI3K-mTOR pathway includes some of the most mutated, amplified and lost genes in human cancers. Hence, efficient targeting of this pathway has become a main goal in cancer research. Indeed, the deconstruction of the PI3K signaling has uncovered new attractive therapeutic approaches to halt cancer. From PTEN to mTOR, we have recently proposed novel approaches to regulate this pathway. On the one hand, nuclear localization of PTEN –regulated by monoubiquitination- is critical for its tumor suppressive activity. Therefore, targeting regulators of this pathway, such as PTEN E3-ligases and deubiquitinases could ultimately lead to the development of clinical tools which increase PTEN anticancer activity. Importantly, PTEN is lost and mutated in many cancers leading to a decrease in the dose of the tumor suppressor. However, complete loss of PTEN activates a p53-dependent failsafe senescence response. Hence, the understanding of the mechanistic insights of PTEN-loss induced senescence, opens new avenues for prosenescence therapy for cancer through the targeting of the PTEN pathway. On the other hand, at the bottom of the PI3K pathway resides a complex in charge of protein translation -mTORC1, which is a source of feedback loops regulating upstream pathways. Therefore, the identification of novel feedback-regulated pathways, together with the utilization of genetic models to predict the effectiveness of mTOR-targeting compounds, are critical for the development of the most appropriate single and combinatorial anticancer approaches.

# Cannabinoids activate an endoplasmic reticulum stress response that inhibits the Akt/mTORC1 axis and promotes autophagy-mediated cancer cell death

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The hemp plant *Cannabis sativa* produces approximately 70 unique compounds known as cannabinoids, of which delta(9)-tetrahydrocannabinol (THC) is the most important owing to its high potency and abundance in cannabis. One of the most exciting areas of research in the cannabinoid field is the study of the potential application of cannabinoids as antitumoural agents. Thus, cannabinoid administration has been shown to curb the growth of several types of tumour xenografts in rats and mice. Based on this preclinical evidence, a pilot clinical trial has been recently run to investigate the antitumoural action of THC on recurrent gliomas. Recent findings have shown that the pro-apoptotic and tumour growth-inhibiting activity of cannabinoids relies on the up-regulation of the transcriptional co-activator p8 and its downstream target the pseudo-kinase Tribbles homologue 3 (TRB3). However, the mechanisms that promote the activation of this signalling route as well as the targets downstream of TRB3 that mediate its tumour cell-killing action remain elusive. Our data unravel the mechanism underlying this action by showing that THC, via ceramide accumulation and eIF2 $\alpha$  phosphorylation, activates an endoplasmic reticulum stress response that promotes autophagy via TRB3-dependent inhibition of the Akt/mTORC1 axis. We also show that autophagy is upstream of apoptosis in cannabinoid-induced cancer cell death and that activation of this pathway is necessary for the anti-tumoural action of cannabinoids in vivo. These findings define a new route for promoting the autophagy-mediated death of tumour cells that may have important therapeutic implications.

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# Differential phosphorylation of mTOR in the mTORC1 and mTORC2 complexes

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The mTOR pathway is exquisitely regulated through feedback mechanisms in mammalian cells. The mTORC1 complex, a regulator of both growth factor and nutrient signaling via S6K, is modulated by a negative feedback loop in which S6K, activated downstream of mTORC1, phosphorylates IRS1/2, uncoupling them from activated insulin/IGF-1 RTKs, and also inducing their degradation, leading to reduced PI-3K signaling to Akt and decreased mTORC1 activity. Activated mTOR also negatively regulates NF- $\kappa$ B induction by TNF $\alpha$  and DNA damage through crosstalk to the ERK and PI-3K pathways. In another potential negative feedback loop high Akt activity reduces activating phosphorylation of Akt at S473 by mTORC2, a complex that regulates the actin cytoskeleton and several AGC family kinases, such as Akt, through hydrophobic motif phosphorylation. In studies of how mTORC1 and mTORC2 might be regulated by differential phosphorylation, we have found that mTOR is phosphorylated differentially when associated with mTORC1 and mTORC2, when isolated from 293 and U2OS cells stimulated with insulin/IGF-1; mTORC1 contains mTOR phosphorylated predominantly on S2448, whereas mTORC2 contains mTOR phosphorylated predominantly on S2481. Phosphorylation of both sites requires intact mTOR complexes. S2481 may be a mTORC2-dependent autophosphorylation site, perhaps dependent on mTORC2 dimerization, whereas S2448 is a known S6K site, and could be phosphorylated as another feedback response. Prolonged rapamycin treatment, which induces mTORC2 disassembly, reduces mTOR pS2481 levels. Using S2481 phosphorylation as a marker for mTORC2 sensitivity to rapamycin, we found mTORC2 to be rapamycin sensitive in several cancer cell lines in which mTORC2 activity was reported to be rapamycin insensitive, based on unchanged Akt S473 phosphorylation level (e.g. MDA-MB-468 cells). Based on Rictor/mSin1 depletion, it appears that residual mTORC2 activity in these cells is primarily responsible for rapamycin-resistant S473 phosphorylation, although there may also be a contribution from DNA-PK, which, like mTOR, is a PIKK.

# Exploring the role of the mTOR pathway in Ras-driven tumorigenesis

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The mTOR pathway is activated as a consequence of genetic alterations in several human oncogenes and tumor suppressors. As such mTOR inhibitors are being evaluated as potential therapies in a number of human tumor types with varying success. However, the key to unlocking the therapeutic potential of these agents may lie in 1) identifying sensitive tumor-types, 2) establishing the specific combination of alterations that confer sensitivity, 3) elucidating mechanisms of resistance and 4) rationally developing combination therapies. We have been focusing our efforts on Ras-driven tumors. First, we have been investigating malignancies associated with the familial cancer syndrome neurofibromatosis type 1 (NF1), which is caused by loss of function mutations in a RasGAP gene. Accordingly, aberrant Ras signaling underlies disease pathogenesis. We have found that the mTOR pathway is hyper-activated in aggressive nervous system tumors from NF1 patients and that tumor cell lines are exquisitely sensitive to mTOR inhibitors. Moreover, the mTOR inhibitor rapamycin potently suppressed the development of these sarcomas in a genetically engineered mouse tumor model. These data suggest that mTOR inhibitors may represent a potential therapy for these aggressive malignancies that are often refractory to standard therapies. Interestingly, we have found that NF1 is mutated in other sporadic tumor-types, which are similarly sensitive to mTOR inhibition. Thus NF1 appears to be one genetic alteration that is important for conferring sensitivity to these agents. We have begun to extend these studies to understanding how a genetically-distinct subset of tumors that harbor K-Ras mutations may respond to mTOR inhibitors.

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## Modulators of Rheb signalling towards mTORC1 – reconsidering the roles of FKBP38 and TCTP

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The Ras-like small G-protein Rheb is a key upstream activator of the mTOR/S6K pathway of translational control. Despite the intensive research carried out on the mechanisms of activation, regulation and on additional components contributing to Rheb/mTOR signalling many missing links in the cascade remain to be identified.

Evidence for mTOR being a direct Rheb target is not convincing, arguing for a mediator of activation. Recently, the FK506-binding protein FKBP38 has been proposed to act as a bona fide Rheb effector which, by binding to Rheb-GTP, gets released from an inhibitory interaction with mTOR. However, our data document that Rheb does not interact directly with FKBP38 suggesting that Rheb regulates mTOR activity independently of FKBP38. Nevertheless, we confirm a role for FKBP38 as a negative regulator of mTOR signalling, by inhibiting S6K(T389) phosphorylation by means of its enzymatic peptidyl prolyl cis-trans isomerase activity, providing an alternative view on the mechanism of mTOR regulation.

Ras-like G-Proteins are positively regulated by guanine nucleotide exchange factors (GEFs). Recently, genetic studies implicated translationally controlled tumor protein (TCTP) in the PI3K/TOR pathway in *Drosophila*. Since the structural characterisation of TCTP had shown similarity to Mss4, a GEF for the Rab family, TCTP was postulated to function as a GEF for Rheb. This hypothesis has been supported by biochemical experiments for both *Drosophila* and mammalian systems. However, we demonstrate by the use of purified mammalian proteins in biochemical assays as well as by employing cell-based studies, that TCTP is very unlikely to be a GEF for Rheb.

# Regulation of the TOR-dependent signaling in *Drosophila*

**Jongkyeong Chung**

Previous studies have demonstrated that the mTOR signaling pathway is highly conserved in *Drosophila*. Using the well established genetic and biochemical tools in *Drosophila*, we are characterizing novel upstream regulators of TOR. With our loss-of-function mutants for LKB1, AMPK and several AMPK-related kinases, we investigated whether their in vivo functions are related to TOR and S6K. As demonstrated in mammalian systems, AMPK negatively regulates S6K activity in *Drosophila*, and we also found that AMPK-related kinases showed functional redundancy in regulation of S6K activity. We have also been studying other regulators for TOR and RAPTOR in *Drosophila*, such as their specific binding proteins and E3 ligases, to understand how TOR can receive various extracellular signals and transfer them to downstream targets in the cell.

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# A complex relationship between the TSC1-TSC2 complex and the mTOR complexes

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Akt/PKB both regulates and is regulated by the TSC1-TSC2 complex. Upon its activation downstream of growth factors and PI3K, Akt directly phosphorylates TSC2 on multiple sites. While the molecular mechanism is not well understood, these phosphorylation events relieve the inhibitory effects of the TSC1-TSC2 complex on Rheb and mTOR complex 1 (mTORC1), thereby activating mTORC1 in response to growth factors. Through negative feedback mechanisms, mTORC1 activity inhibits growth factor stimulation of PI3K. This is particularly evident in cells and tumors lacking the TSC1-TSC2 complex, where Akt signalling is severely attenuated due to constitutive activation of mTORC1. We have recently uncovered an additional level of complexity in the relationship between Akt, the TSC1-TSC2 complex, and mTOR. The growth factor-stimulated kinase activity of mTOR complex 2 (mTORC2), which normally enhances Akt signalling by phosphorylating its hydrophobic motif (S473), was found to be defective in a variety of cell lines lacking the TSC1-TSC2 complex. We have several lines of evidence demonstrating that this effect on mTORC2 can be separated from the inhibitory effects of the TSC1-TSC2 complex on Rheb and mTORC1. Furthermore, cells lacking either the TSC1-TSC2 complex or mTORC2 display similar defects in regulatory phosphorylation events on both Akt and PKC $\alpha$ . Surprisingly, the TSC1-TSC2 complex was found to physically associate with mTORC2, but not mTORC1, further suggesting distinct mechanisms of regulation. I will provide an update on our ongoing studies to define the role of Akt-mediated phosphorylation of TSC2 for mTORC1 regulation and the mechanism by which the TSC1-TSC2 complex regulates mTORC2. These represent fundamentally important questions toward our understanding of the molecular wiring of this pathway underlying a large number of human tumor syndromes and cancers.

# mTOR autophosphorylation at Ser-2481 is a molecular marker for mTORC2 activity

Aaron M. Robitaille and Michael N. Hall

The target of rapamycin (TOR) is a central controller of cell growth, structurally and functionally conserved from yeast to human. TOR is an atypical Ser/Thr kinase that forms two functionally distinct multiprotein complexes, TORC1 and TORC2. Nutrients and growth factors activate mammalian TOR (mTOR) complex 1 (mTORC1). Growth factors such as insulin activate mTOR complex 2 (mTORC2). The best-characterized substrates of mTOR are S6K and 4E-BP1 (mTORC1) and Akt/PKB (mTORC2). The molecular mechanism of growth factor mediated mTORC2 activation is poorly understood. We show that insulin stimulates the autophosphorylation of mTOR at Ser-2481 specifically in mTORC2. Raptor and Rictor are essential components of mTORC1 and mTORC2, respectively. Knockout of Rictor, but not of Raptor, abolished mTOR autophosphorylation at Ser-2481. Furthermore, prolonged treatment with rapamycin inhibited Ser-2481 phosphorylation in cancerous and immortalized cell lines. Thus mTORC2 autophosphorylation at Ser-2481 can be used as a biomarker for mTORC2 activity. We then investigated if Phosphoinositide 3-kinase (PI3K) is involved in mTORC2 activation. Wortmannin and LY294002, two specific inhibitors of PI3K, inhibit insulin-stimulated mTOR Ser-2481 and Akt/PKB Ser-473 phosphorylation *in vivo*. These and additional results on the regulation of mTORC2 will be presented.

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## mTOR signaling and cell growth control in normal and cancer cells

**John Blenis, Andrew Choo, Jamie Dempsey, Greg Hoffman, Marina Holz, Sang Gyun Kim, Sarah Mahoney, Xiaoju “Max” Ma and Yonghao Yu**

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The mammalian target of rapamycin (mTOR), in association with raptor and Lst8, comprises the mTOR Complex 1 (mTORC1). mTORC1 signaling is inhibited by the TSC1/2 tumor suppressor complex. The TSC complex integrates information from a variety of extracellular and metabolic cues which regulate signaling downstream of PI3-kinase, Ras, Wnt, nutrients, stress induction and cellular energy status. The consequence of receiving so many inputs is that mTORC1 signaling is carefully monitored and regulated in the cell. Unfortunately, gain-of-function mutations in Ras, PI3-kinase, Raf and various receptor and cytoplasmic tyrosine kinases, or loss-of-function mutations in tumor suppressors like PTEN, TSC1/2, NF1, LKB1 and others result in constitutive activation of mTORC1 signaling. Our goal has been to understand how this pathway is regulated and how when improperly activated it contributes to the tumor cell phenotype. We are currently focused on improving our understanding of how mTORC1 signaling contributes to the control of selective and general protein synthesis, and how this is contributing to cellular growth control. We have also completed RNAi-based screens, phospho-proteomic screens and S6K1 tandem affinity purifications, which we hope will aid in further defining this complex signaling system.

# Regulation and function of the TSC-mTOR pathway

**Kun-Liang Guan**

The mammalian target of rapamycin (mTOR) is a central cell growth regulator. TOR forms two distinct functional complexes, TORC1 and TORC2. The TSC1 and TSC2 tumor suppressor genes are key upstream regulators of TORC1. TSC2 functions as a GTPase activating protein, GAP, to inhibit the Rheb small GTPase, which is a potent director activator of TORC1. In TSC mutant cells, TORC1 is highly activated and the uncontrolled TORC1 activity likely contributes to the pathogenesis of TSC and related diseases. As a central growth controller, TORC1 activity has to be regulated by a wide range of extra and intra cellular signals, such as growth factors, energy levels, amino acids, and hypoxia. Studies from our lab laboratory have demonstrated that the AMP activated protein kinase, AMPK, plays a key role in TORC1 inhibition in response to energy starvation. We have also shown that the Rag GTPases mediates the amino acid signals to TORC1 activation. Our studies reveal a potential mechanism how cell growth can be coordinated with cellular nutrient status.

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## Tuberous sclerosis and LAM: pathogenic mechanisms

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The primary focus of my laboratory is the pathogenesis of tuberous sclerosis complex (TSC) and the related disorder, lymphangioleiomyomatosis (LAM). TSC is a multisystem disorder in which the manifestations can include tumors in the brain, heart, kidney, and skin, as well as mental retardation and autism. My laboratory has a particular interest in the renal manifestations of TSC (cysts, angiomyolipomas, and carcinomas) and in the pulmonary manifestation, LAM. About 30% of women with TSC develop LAM, which is an unusual lung disease in which benign-appearing smooth muscle cells proliferate extensively in the lungs, leading to cystic, emphysema-like lung destruction and lung failure. LAM pathogenesis appears to involve one of the most unusual pathogenic mechanisms in human disease: the metastasis of histologically benign cells.

The protein products of the TSC1 and TSC2 genes (hamartin and tuberin, respectively) physically interact to inhibit the activity of the kinase, TORC1. This mTOR inhibition is achieved the small GTPase Rheb which is regulated by the GAP (GTPase activating protein) region of TSC2. A great deal of attention has been focused on the role of TORC1, which is inhibited by Rapamycin, in the pathogenesis of TSC and LAM. A key area of uncertainty is whether Rheb has TORC1-independent targets that are disease-relevant.

Currently the laboratory is examining 1) the mechanisms through which estrogen may promote the metastasis of TSC2-null cells, thereby promoting LAM pathogenesis, 2) the role of the TSC pathway in neural development, using a *Drosophila* model, and 3) the connections between the TSC proteins and the Birt-Hogg-Dube (BHD) protein. BHD (like TSC) is associated with renal cell carcinoma and cystic lung disease. In *Schizosaccharomyces pombe* we have found that the BHD homolog functions in the TOR signaling pathway, but in opposition to the TSC proteins.

# A chemical genomics approach to dissect Pi3 kinase and mTOR dependency in non-small cell lung cancer

Stefanie Fischer and **Roman Thomas**

We have developed a chemical genomics approach that involves high-throughput cell-based screening of large collections of cancer cell lines that are heavily annotated in gene copy number, expression and mutation spaces. We employed a diverse library of isoform-selective and broad-spectrum Pi3 kinase and mTOR inhibitors to identify tumours with particular Pi3 kinase and mTOR dependency resulting in massive apoptosis when challenged with appropriate perturbagens. Computational approaches identified genetic correlates of Pi3 kinase and mTOR dependency, thus yielding potential predictors for clinical patient stratification. In summary, we demonstrate an approach for chemical and genetic dissection of oncogene dependency as a rich source for genotype-phenotype correlations enabling to dissect Pi3 kinase and mTOR dependency in non-small cell lung cancer.

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## NVP-BEZ-235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits growth of cancer cells with activating PI3K mutations

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PI3K pathway deregulation is a common event in human cancer, either by the presence of hot spot activating mutations of the PI3K isoform (PIK3CA) or through inactivation of the tumor suppressor PTEN. $\alpha$ p110 Both phenomena result in increased pathway activity and have been proven to be oncogenic both *in vitro* and *in vivo*. Furthermore, sustained PI3K pathway activation contributes to therapeutic resistance to the anti-HER2 antibody trastuzumab. Selective targeting of PIK3CA is, therefore, an attractive strategy for cancer treatment. We investigated the effects of NVP-BEZ235, a dual PI3K / mTOR inhibitor, in several cancer cell lines bearing the hot spot PIK3CA mutations E545K and H1047R. To this end, we determined its antiproliferative activity in cellular and *in vivo* settings. The compound was found to specifically block downstream effectors of PI3K and mTOR, such as p-Akt and p-S6, and reduced the proliferation capacity of the tested tumor cells, including trastuzumab resistant cells. Endogenous FKHL1 transcription factor nuclear re-localization was used as a read out of decreased Akt activity. Consistent with the effect of PI3K inhibition on proliferation, progression through the G0-G1 phase of the cell cycle was found to be significantly retarded under NVP-BEZ-235 treatment.

The preceding results were further confirmed and extended by analyzing the effect of NVP-BEZ-235 on the growth of xenografts derived from BT474 cells (HER2 amplified) stably transfected with the potent H1047R activating PIK3CA mutation. In this xenograft model, NVP-BEZ-235 induced tumor regression at tolerated doses. *Ex-vivo* analyses of tumor and skin tissues showed significant reduction in the levels of phosphorylated Akt and S6 proteins.

Our findings demonstrate that the concomitant, dual inhibition of PI3K and mTOR by NVP-BEZ-235 potently inhibits proliferation and tumor growth in cancer cells with mutated PIK3CA. Future clinical studies will evaluate the effects of this compound in tumors with deregulated PI3K / mTOR pathways.

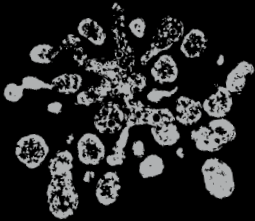
# Tumourigenic activity and therapeutic inhibition of Rheb GTPase

Konstantinos J. Mavrakis, Hong Zhu, Ricardo L. A. Silva, Wayne Tam, Jerry Pelletier  
and **Hans-Guido Wendel**

The AKT-mTOR pathway harbours several known and putative oncogenes and tumour suppressors. In a phenotypic screen for lymphomagenesis we tested candidate genes acting up- and downstream of mTOR in vivo. We find that Rheb, a proximal activator of mTORC1, can produce rapid development of aggressive and drug resistant lymphomas. Rheb causes mTORC1-dependent effects on apoptosis, senescence and treatment responses that resemble those of Akt. Moreover, Rheb activity towards mTORC1 requires farnesylation and is readily blocked by a pharmacological inhibitor of farnesyltransferase (FTI). In Pten deficient tumor cells inhibition of Rheb by FTI is responsible for the drug's anti-tumour effects, such that a farnesylation independent mutant of Rheb renders these tumours resistant to FTI therapy. Notably, RHEB is highly expressed in some human lymphomas, resulting in mTORC1 activation and increased sensitivity to rapamycin and FTI. Downstream of mTOR, we examined translation initiation factors that have been implicated in transformation in vitro. Of these, only eIF4E was able to enhance lymphomagenesis in vivo. In summary, the Rheb GTPase is an oncogenic activity upstream of mTORC1 and eIF4E and a direct therapeutic target of farnesyltransferase inhibitors in cancer.

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# **Signalling upstream of mTOR**

Madrid | 3-5 November 2008

POSTER  
**SESSION**





# 1. Akt regulates L-Type calcium channel activity by modulating Cav $\alpha$ 1 protein stability

**Daniele Catalucci**<sup>1,2</sup>, Deng-Hong Zhang<sup>1</sup>, Jaime DeSantiago<sup>3</sup>, Franck Aimond<sup>4</sup>, Guillaume Barbara<sup>5</sup>, Jean Chemin<sup>5</sup>, Eckard Picht<sup>3</sup>, Francesca Rusconi<sup>2</sup>, Nancy Dalton<sup>1</sup>, Dario R. Alessi<sup>6</sup>, Kirk L. Peterson<sup>1</sup>, Sylvain Richard<sup>4</sup>, Donald M. Bers<sup>3</sup>, Joan Heller Brown<sup>7</sup> and Gianluigi Condorelli<sup>1,2</sup>

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The insulin, growth factor-1 (IGF-1)/phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays a crucial role in a broad range of biological processes involved in the modulation of local responses as well as processes implicated in metabolism, cell proliferation, transcription, translation, apoptosis, and growth. In the heart, the IGF-1/PI3K/Akt pathway is involved in the regulation of contractile function and impairment of this signaling pathway is considered an important determinant of cardiac function and failure. The Akt (also called PKB) family of serine/threonine kinases consists of 3 isoforms (Akt-1, -2, and -3) that are activated by IGF-1 and insulin through PI3K and eventually phosphoinositide-dependent kinase-1 (PDK1). Activation or inhibition of Akt in cardiomyocytes as well as in neuronal cells has been shown to increase or reduce Ca<sup>2+</sup> current (ICa,L), respectively, suggesting a pivotal role of Akt in regulating L-type Ca<sup>2+</sup> channel complex (LTCC) function and cell Ca<sup>2+</sup> handling. The importance and ubiquity of Ca<sup>2+</sup> as an intracellular signaling molecule suggests that altered channel function could give rise to widespread cellular and organ defects. Indeed, a variety of cardiovascular diseases have been related to alterations in the density or function of the LTCC. However, the molecular basis for dysregulation of LTCC function and the possible involvement of Akt in ICa,L regulation remains unresolved. Here, taking advantage of a mouse line with tamoxifen-inducible and cardiac-specific deletion of PDK1, the upstream activator of all three Akt isoforms, we identify a novel post-translational mechanism by which Akt modulates LTCC protein density and function under physiological conditions, highlighting the pivotal role of this kinase in cardiac function. In cardiomyocytes, the LTCC is composed of different subunits: the pore-forming subunit Cav $\alpha$ 1, and the accessory  $\beta$ , and  $\alpha$ 2 $\delta$  subunits. Interestingly, we demonstrated that the Cav $\alpha$ 1 contains highly conserved PEST sequences (signals for rapid protein degradation) that direct rapid protein degradation and in-frame deletion of these PEST sequences resulted in increased Cav $\alpha$ 1 protein levels. Interestingly, our findings show that Akt associate with and phosphorylate Cav $\beta$ 2, the LTCC chaperone for Cav $\alpha$ 1, and phosphorylation of the Akt consensus site in Cav $\beta$ 2 antagonized Cav $\alpha$ 1 protein degradation. This Akt-dependent phosphorylation of Cav $\beta$ 2, antagonizes Cav $\alpha$ 1 protein degradation by prevention of Cav $\alpha$ 1-PEST sequences recognition, leading to increased LTCC density, and consequent modulation of Ca<sup>2+</sup> channel function. This novel mechanism by which Akt modulates LTCC stability could profoundly influence cardiac myocyte Ca<sup>2+</sup> entry, Ca<sup>2+</sup> handling, and contractility.

## 2. What can we learn from animal models that can be useful in human thyroid pathology?

**Alexandra Faustino<sup>1</sup>, Ana Sofia Rocha<sup>1,2</sup>, Helena Pópulo<sup>1</sup> and Ana Paula Soares<sup>1,3</sup>**

A genetically engineered mouse strain with a selective deletion of the Pten gene in thyroid follicular cells (Pten<sup>L/L</sup>;TPO-Cre mice) has been previously created and described<sup>1</sup>. This animal model reveals a constitutive activation of the PI3K/AKT pathway and leads to the development of diffuse colloid goiter in all the animals and follicular adenoma in 70% of the females<sup>1</sup>. Mammalian Target of Rapamycin (mTOR) was later identified as being the key effector of this chronic PI3K activation and its inhibition with RAD001 was proved to be efficient in restoring the normal proliferation index in the mouse model<sup>2</sup>.

In the current work we aimed to determine whether the mTOR pathway is also activated in human thyroid benign proliferative diseases. One hundred and thirty three thyroid tissues including 79 goiters, 32 thyroid adenomas and 21 adjacent normal tissues were analysed by immunohistochemistry for mTOR upstream (pAKT Ser473, pAKT Thr308) and downstream (pS6 Ser235/236, p4E-BP1 Thr37/46) molecules.

Increased expression of AKT in both phosphorylated states (residues Thr308 and Ser473) and of unphosphorylated and phosphorylated mTOR (Ser2448) was observed in both types of thyroid lesions when compared with adjacent normal tissues. In thyroid adenomas an over-expression of p4E-BP1, downstream molecule of mTORC1, was also observed. Furthermore when we compare the expression levels between the two types of benign lesions, over-expression of pAKT Ser473, pAKT Thr308, pS6 Ser235/236 and of p4E-BP1 Thr37/46 was observed in adenomas.

In conclusion, the mTOR pathway seems to be in part over-activated in thyroid nodular hyperplasia and benign tumours. Targeting of mTOR could be a valuable strategy to develop an efficient treatment of thyroid proliferative and neoplastic disorders.

1. Yeager N, Klein-Szanto A, Kimura S, Di Cristofano A. Pten Loss in the Mouse Thyroid Causes Goiter and Follicular Adenomas: Insights into Thyroid Function and Cowden Disease Pathogenesis. *Cancer Res.* 2007 Feb 1; **67**(3):959-66

2. Yeager N, Brewer C, Cai KQ, Xu XX, Di Cristofano A. Mammalian target of rapamycin is the key effector of phosphatidylinositol-3-OH-initiated proliferative signals in the thyroid follicular epithelium. *Cancer Res.* 2008 Jan 15; **68**(2):444-9

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### 3. Regulation of serum and glucocorticoid induced protein kinase-1 (SGK1) by mTOR complex-2 (mTORC2)

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SGK1 is a member of the AGC family of protein kinase and is activated by agonists including growth factors. SGK1 regulates diverse effects of extracellular agonists by phosphorylating regulatory proteins that control cellular process such as ion transport and growth. Like other AGC family kinases, activation of SGK1 is triggered by phosphorylation of a Thr residue within the T-loop of the kinase domain and a Ser residue lying within the C-terminal hydrophobic motif (Ser422 in SGK1). PDK1 phosphorylates the T-loop of SGK1. The identity of the hydrophobic motif kinase is unknown. Recent work has established that mammalian Target Of Rapamycin complex-1 (mTORC1) phosphorylates the hydrophobic motif of the S6K whilst mTOR Complex-2 (mTORC2) phosphorylates the hydrophobic motif of Akt. Here we demonstrate that SGK1 hydrophobic motif phosphorylation and activity is ablated in knockout fibroblasts lacking the mTORC2 subunits Rictor, Sin1 or mLST8. Furthermore, phosphorylation of the NDRG1, a physiological substrate of SGK1, was also abolished in Rictor, Sin1 or mLST8 deficient fibroblasts. mTORC2 immunoprecipitated from wild type, but not mLST8 or Rictor knockout cells, phosphorylated SGK1 at Ser422. However, immunoprecipitated mTORC1 failed to phosphorylate SGK1 at Ser422, under conditions which it phosphorylated the hydrophobic motif of S6K. Consistent with mTORC1 not regulating SGK1, rapamycin suppressed phosphorylation of S6K, without affecting SGK1 phosphorylation or activation. These findings indicate that mTORC2 plays a vital role in controlling the hydrophobic motif phosphorylation and activity of SGK1. It may explain why in previous studies phosphorylation of substrates such as FOXO that could be regulated by SGK, are reduced in mTORC2 deficient cells. Our data indicate that NDRG1 phosphorylation represents an excellent biomarker for mTORC2 activity.

## 4. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces autophagy through a TAK1-AMPK-mTOR signaling pathway in human epithelial cells

**Griselda Herrero-Martin<sup>1</sup>, Maria Hoyer-Hansen<sup>2</sup>, Celina Garcia-Garcia<sup>1</sup>, Claudia Fumarola<sup>3</sup>, Thomas Farkas<sup>2</sup>, Marja Jäätelä<sup>2</sup> and Abelardo-Lopez-Rivas<sup>1</sup>**

Mammalian target of rapamycin (mTOR) is an autophagy negative regulator that is also regulated by AMP-activated protein kinase (AMPK). We have demonstrated that tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) induces accumulation of autophagosomes in human breast epithelium cells MCF10A and human retinal pigment epithelial cells hTERT-RPE1. TRAIL-induced autophagy in MCF10A is mediated by the activation of AMPK that, in turn, inhibits mammalian target of rapamycin complex 1 (mTORC1). Interestingly, the TRAIL-induced AMPK activation and autophagy are dependent on transforming growth factor  $\beta$  activating kinase 1 (TAK1) but do not require the known AMPK activating kinases LKB1 or Ca<sup>2+</sup>/calmodulin-dependent kinase kinase  $\beta$  (CaMKK  $\beta$ ). Furthermore, TAK1 is also required for the AMPK activation induced by TRAIL in murine embryonic fibroblasts (MEFs) and for the TRAIL-induced AMPK activation and autophagy in RPE1 cells. In summary, these results suggest that TRAIL induces autophagosome formation through the TAK1-AMPK-mTOR signalling pathway in MCF10A cells.

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## 5. A functional siRNA screen for nutrient and growth factor regulation of mTORC1 signalling

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Several fundamental aspects of the signalling network responsible for mTORC1 activation remain poorly understood. Specifically, although the role of TSC2 in down regulating Rheb following growth factor activation is well established, neither the mechanisms responsible for up-regulating the pathway by stimulating Rheb GTP-binding, nor the mechanism by which Rheb stimulates mTORC1 kinase activity, have been well characterized. In addition, the mechanism by which mTORC1 kinase activity is regulated by cellular amino acid levels is poorly defined; the upstream amino acid sensor has not been identified nor is it apparent how amino acid depletion impinges on mTORC1 activity. In order to address these questions, we developed a high throughput, cell-based assay for measuring phosphorylation of rpS6 at Ser235/236 in mammalian tissue culture cells. This assay takes advantage of the 'In Cell Western' (ICW) technique using the Aeries infrared imaging system (Licor Biosciences). Using this approach, we have performed a large scale siRNA screen in human cancer cells to identify likely targets for pharmacological inhibition of the pathway. As both growth factor and amino acid inputs are required for full activation of mTORC1 and subsequent phosphorylation of rpS6, knockdown of components required for both the growth factor and amino acid branches of the mTORC1 signalling network lead to a reduction in the phospho-rpS6 signal and score as hits in the primary screen. Importantly, known kinases involved in growth factor-mediated inputs leading to rpS6-phosphorylation, including IRS2, IGF1R, PI3-kinase, PDK1, and S6K2, score as hits in the screen, validating our approach. In addition to these known pathway components, a number of uncharacterized genes scored as strong hits in our screen and we are currently characterizing their mechanism of action. Bioinformatics analysis of the siRNA data set identifies a number of conserved pathways that play previously unappreciated roles in regulation of mTORC1 activity which may serve as novel targets for therapeutic intervention for the treatment of cancer and metabolic diseases associated with deregulated mTORC1 signalling.

## 6. Evidence for an LKB1/AMPK/eNOS cascade regulated by HGF, S-adenosylmethionine and NO in hepatocyte proliferation

Mercedes Vázquez, Marta Varela-Rey, Nieves Embade, Nuria Martínez, David Fernández, Laura Gómez, Santiago Lamas, Shelly C. Luc, J.M. Mato and **M. Luz Martínez-Chantar**

S-Adenosylmethionine (SAME) is involved in numerous complex hepatic processes such as hepatocyte proliferation, death, inflammatory responses, and anti-oxidant defence. One of the most relevant actions of SAME is the inhibition of hepatocyte proliferation during liver regeneration. In hepatocytes, SAME regulates the levels of cytoplasmic HuR, an RNA-binding protein that increases the half-life of target mRNA such as cyclin D1 and A2, via inhibition of HGF-mediated AMP-activated protein kinase (AMPK) phosphorylation. Because AMPK is activated by the tumour suppressor kinase LKB1, and AMPK activates endothelial nitric oxide (NO) synthase (eNOS), and NO synthesis is of great importance for hepatocyte proliferation, we hypothesized that in hepatocytes HGF may induce the phosphorylation of LKB1, AMPK and eNOS through a process regulated by SAME, and that this cascade might be crucial for hepatocyte growth. Here we demonstrate that the proliferative response of hepatocytes involves eNOS phosphorylation via HGF-mediated LKB1 and AMPK phosphorylation, and that this process is regulated by SAME and NO. We also show that knockdown of LKB1, AMPK, or eNOS with specific iRNA inhibits HGF-mediated hepatocyte proliferation. Finally, we found that the LKB1/AMPK/eNOS cascade is activated during liver regeneration after partial hepatectomy and that this process is impaired in mice treated with SAME before hepatectomy, in knockout mice deficient in hepatic SAME, and in eNOS knockout mice. Conclusion: We have identified for the first time an LKB1/AMPK/eNOS cascade regulated by HGF, SAME and NO that functions as a critical determinant of hepatocyte proliferation during liver regeneration after partial hepatectomy.

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## 7. TGF - $\beta$ induces SIK1 to negatively regulate type I receptor kinase signalling

**Aristidis Moustakas**, Peter Lönn, Michael Vanlandewijck, Marcin Kowanetz, Katarzyna Kowanetz and Carl-Henrik Heldin

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Signal transduction by transforming growth factor  $\beta$  (TGF -  $\beta$ ) coordinates physiological responses in diverse cell types. TGF -  $\beta$  signals via type I and type II receptor serine/threonine kinases and intracellular Smad proteins that regulate transcription. The pathway is controlled by multiple mechanisms that safeguard its proper and timely action. We have identified an inducible gene target of TGF -  $\beta$  /Smad signalling, the kinase SIK1, which negatively regulates signalling together with Smad7. SIK1 and Smad7 form a complex and cooperate to downregulate the activated type I receptor, ALK5. We further show that both the kinase and UBA domain of SIK1 are required for proper ALK5 degradation, with ubiquitin functioning to enhance SIK1-mediated receptor degradation. We then investigated deeper this mechanism and demonstrate that the E3 ubiquitin ligase Smurf2 cooperates with SIK1 in downregulating the TGF -  $\beta$  type I receptor. Smurf2 does not directly bind to SIK1, but participates in the receptor complex via the proline/tyrosine-rich motif of Smad7. Furthermore, SIK1 phosphorylates Smad7 in distinct serine residues. Our model supports a central role of Smad7 as a mediator of the action of SIK1 on the TGF -  $\beta$  type I receptor, a mechanism that also engages the ubiquitin ligase Smurf2. Finally, loss of endogenous SIK1 results in enhanced gene responses of the fibrotic and cytostatic programs of TGF -  $\beta$ . We thus identify in SIK1 a negative regulator that controls TGF -  $\beta$  receptor turnover and physiological signalling.

## 8. CoA synthase interacts with PI3 kinase on mitochondria

Ivan Nemazanyy, Oksana Breus and Valeriy Filonenko

Coenzyme A (CoA) is an obligatory cofactor in all living organisms. Biosynthesis of CoA is a universal pathway in prokaryotes and eukaryotes, involving five enzymatic steps, which consume pantothenate (Vitamin B5), ATP, and cysteine. In the first step, pantothenic acid is phosphorylated by pantothenate kinase (PanK) to generate 4'-phosphopantothenic acid (4'-PPA). Then, 4'-phosphopantothenoylcysteine synthase converts the first intermediate into 4'-phosphopantothenoylcysteine, which is subsequently decarboxylated to 4'-phosphopantetheine (4'-PP) by phosphopantothenoylcysteine decarboxylase. The last two steps are facilitated by a bifunctional enzyme CoA synthase, which mediates the conversion of 4'-PP to dephospho-CoA and subsequently CoA.

Recently transient expression studies and confocal microscopy allowed us to demonstrate that full-length CoA synthase is associated with the mitochondria, whereas the removal of the N-terminal region relocates the enzyme to the cytosol. In addition, we showed that the N-terminal sequence of CoA synthase (amino acids 1–29) exhibits a hydrophobic profile and targets green fluorescent protein exclusively to mitochondria. Further analysis, involving subcellular fractionation and limited proteolysis, indicated that CoA synthase is localized on the mitochondrial outer membrane.

Also, we have demonstrated that CoA synthase associates specifically with ribosomal protein S6 kinase (S6K1). Ribosomal protein S6 kinase (S6K) as a downstream target of mTOR/PI3K signalling pathways is a key regulator of cell size and growth.

PI3Ks are enzymes with both protein and lipid kinase activities that have ubiquitous cellular functions. The PI3K signalling pathway and its downstream targets have been intensively studied for their role in cell proliferation, survival, cycle control, as well as other cellular functions.

Here we report for the first time that CoAsy can bind p85 subunit of PI3K in pull down and immunoprecipitation experiments. At the next step we have shown that PI3K can localize to mitochondria. Moreover, we were able to immunoprecipitate PI3 kinase using aCoAsy antibodies from mitochondrial fraction – which indicates that CoAsy interacts with PI3 kinase on mitochondria. Physiological relevance of this interaction should be further elucidated.

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## 9. Regulation of CoA synthase by posttranslational modifications

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CoA synthase is a bifunctional enzyme which mediates the final stages of CoA biosynthesis. In previous studies, we have reported molecular cloning, biochemical characterization, and subcellular localization of CoA synthase (CoASy). CoA and its derivatives - Acetyl-CoA and Acyl-CoA are important players in cellular metabolism and signal transduction.

Recently, we have demonstrated that CoA synthase associates specifically with ribosomal S6 kinase (S6K1). Ribosomal protein S6 kinase (S6K) as a downstream target of mTOR signalling pathways is a key regulator of cell size and growth. To further uncover a potential link between mTOR/S6K signalling pathway and energy metabolism we looked for posttranslational modifications of CoASy, like Ser/Thr phosphorylations and whether such phosphorylation is involved in modulation of CoASy activity.

Using bioinformatic approach we selected several potential phosphorylation sites on CoASy by Ser/Thr kinases including Akt, mTOR, PKC and PKA. At the next step, using recombinant CoASy as a substrate we have confirmed sites localization and *in vitro* CoASy phosphorylation by some of the listed kinases. Also, we generated phosphospecific antibodies to two of confirmed sites and proved the phosphorylation of CoASy *in vivo*. We are currently examining the role of identified CoASy posttranslational modifications and the progress on this project will be presented.

## 10. Akt activation synergizes with Tp53 loss in oral epithelium to produce a novel mouse model for head and neck squamous cell carcinoma

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TOP216 is a novel oxyphenisatin derivative with low nanomolar cytotoxic activity against a subset of human cancer cell lines with striking selectivity (>1000 fold) against naturally resistant cell lines. Here we demonstrate that incubation of TOP216-sensitive cell lines, but not naturally resistant cell lines such as MDA-MB-231 or MCF10A, leads to inhibition of cellular TORC1 and eIF2a activity, shutdown of protein synthesis, induction of caspase 3/7 activity, and cell death. Cell lines with induced resistance to TOP216 showed no cross-resistance to a panel of known chemotherapeutic drugs, suggesting that TOP216 affects cell viability through a novel mechanism. *In vivo*, TOP216 induced tumour stasis and regression (including cures) in mouse xenograft models of human breast, prostate, ovarian and pancreatic cancer, both when administrated intravenously and perorally. Large PC-3 tumours were shown to regress following a single peroral dose of TOP216. Currently we are investigating the mechanism of action of TOP216 using genomic, proteomic, and metabolomic techniques with the aim to progress this interesting class of anti-cancer compounds into clinical trials.

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## 11. *Bacillus licheniformis* inhibits TGF- $\beta$ -induced matrix metalloproteinase-2 and -9 synthesis via Akt/mTOR signalling in HCT 116 human colorectal cancer cells

Jeong-Kyu Park, Chul-Su Yang, Ki-Hye Kim, Hong-Hee Choi, Eun-Kyeong Jo and Hwa-Jung Kim

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The phosphoinositide 3-kinase (PI3K)/Akt/ mammalian target of rapamycin (mTOR) pathway controls many cellular processes that are important for the formation and progression of cancer, including apoptosis, translation, angiogenesis, and cell cycle progression. Thus, the inhibition of the PI3K/Akt/mTOR pathway is an attractive concept for cancer therapy. Various types of non-pathogenic bacteria have been shown to infiltrate, replicate and then preferentially accumulate in tumours. The basis for the potent tumor cell killing seen by some cancer-related bacteria, but not others like *Bacillus mesentericus* and *B. subtilis*. *B. licheniformis* has been commonly isolated from human or animal tumours.

In this study, we investigated the possibility that non-pathogenic *B. licheniformis* was evaluated the novel therapeutics for treating metastatic cancers. TGF -  $\beta$  modulates the expression of several matrix metalloproteinases (MMPs) through the PI3K/Akt/mTOR pathway. We found that TGF -  $\beta$  up-regulated MMP-2 and -9 synthesis via Akt/mTOR signalling in the HCT 116 human colorectal cancer cell line. TGF -  $\beta$  induced Akt and S6K1 phosphorylation in the HCT 116. The inhibition of mTOR by rapamycin blocked TGF -  $\beta$ -induced activation of S6K1, and MMP-2 and -9 synthesis. *B. licheniformis* led to Akt phosphorylation in the HCT 116 after 2 days with a peak on 4 days, but blocked S6K1 activation by TGF -  $\beta$  after 2 days, and MMP-2 and -9 synthesis by TGF -  $\beta$  after 2 days with a complete blocking on 3 days.

Together, *B. licheniformis* completely inhibits TGF -  $\beta$ -induced MMP-2 and -9 synthesis via Akt/mTOR signalling and reduces the metastatic potential of the HCT 116 human colorectal cancer cells.

## 12. Role of mTORC1 and mTORC2 in muscle cancer cachexia

**Michele Pelosi** and Antonio Musarò

Cancer cachexia is a debilitating syndrome, characterized by severe skeletal muscle degeneration and muscle wasting. This progressive loss of skeletal muscle mass affects about 50% of the cancer patients, and one-third of cancer mortalities result from cachexia rather than tumor burden.

Little is known about the molecular etiology of cancer cachexia, and hence few targets have been identified for a possible therapy.

A rapamycin-sensitive pathway has recently emerged among the positive regulators of physiological process of myogenic differentiation. Experiments show that mTOR functions can control the multistep myogenesis at more than one stage. Moreover, mTOR signaling has been involved in the control of IGF-1 induced hypertrophy in skeletal muscle.

We report here our investigation on mTORC1 and mTORC2 signaling in myogenic cells subjected to cachexia-inducing stimulus.

We used murine colon-26 adenocarcinoma (C-26) cells conditioned medium to induce cachexia in the C2C12 murine myogenic cell line. We observed that cachexia-inducing stimulus modulated the mTORC1 and mTORC2 signaling, as shown by the reduced phosphorylation of molecular targets, such as p70 S6 Kinase (Thr389), S6(Ser235/236) and phospho-AKT(Ser473). The altered muscle phenotype was also associated with reduced expression of the myogenic transcription factors, such as Mrf-4 and MEF-2C, and with reduced phosphorylation of FoxO3a and consequent increased nuclearization.

Our work suggests the possibility to modulate mTORC1/2 signalling as a possible therapy in muscle cancer cachexia. Moreover, we suggest the skeletal muscle among a possible target of the adverse effects of the mTOR-perturbing molecules, used as anticancer in humans.

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## 13. Regulation of autophagy by kinases and phosphatases

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### Apoptosis

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### Background:

Autophagy is an evolutionary conserved lysosomal pathway involved in the turnover of cellular macromolecules and organelles. Autophagy proceeds in a low basal level in most if not all cells, and it plays a crucial role in nutrient delivery, remodeling, differentiation, and removal of damaged proteins and organelles. In spite of its essential role in tissue homeostasis, the molecular mechanisms regulating autophagy in cancer is poorly understood. Kinases and phosphatases control the reversible process of phosphorylation and are misregulated in many types of cancer. Given that protein and lipid phosphorylation controls autophagy and the cells survival signaling, strategies for targeting kinases and phosphatases are in highest priority for improved therapeutic intervention.

### Results:

Using large scale RNAi, we report here the identification of human phosphatases that are essential for autophagy regulation and cancer cell survival; we also identified a new group of phosphatases that reverts the autophagy activation under the treatment of cytotoxic drug siramesine, a sigma-2 receptor ligand that kills cancer cells by destabilizing their lysosomes. Our sigma-2-ligand induced autophagy siRNA screening identified 16 phosphatases and regulatory subunits which reverted autophagy effect out from 307 genes (5% of total 307 genes included in to phosphatase library). Our carefully evaluated secondary screen data indicates that phosphatases that are transiently suppressed regulate signal cascades activating autophagy. Sequence analysis of siramesine effect reverting phosphatases show that majority of them interacts with signaling proteins, respectively. In addition, analysis of protein phosphatases found in siramesine induced autophagy screen show power of methodology, as 60% of autophagy reverting phosphatases are not yet studied as oncogenic signaling molecules.

### Conclusions:

We conclude that combining RNA interference with current functional autophagy model (MCF7-LC3 EGFP) breast cancer cell line and visual estimation of autophagy activation combined with profiling of mTOR activity with phosphorylation of P70S6K and 4E-PB1 is an effective way to identify genes functioning in an autophagy regulating signaling process.

## 14. Identification of phosphorylation sites on Rictor

**Caroline Treins** and Julian Downward

The mammalian target of rapamycin (mTOR) is a conserved Ser/Thr kinase that controls cell growth, proliferation and survival through two structurally and functionally distinct multi-protein complexes, mTORC1 and mTORC2. Rapamycin-sensitive mTORC1 consists of mTOR and two associated proteins, Raptor (Rapamycin sensitive companion of mTOR) and mLST8/GβL. This complex plays a major role in the regulation of cell growth in response to amino acids, energy sufficiency and growth factors. mTORC2 is a rapamycin-insensitive complex, which also contains mTOR and mLST8/GβL, and in addition contains Rictor (Rapamycin insensitive companion of mTOR), and Sin1 (SAPK-interacting protein 1). mTORC2 has been shown to phosphorylate Akt/PKB and PKC, and to be involved in the regulation of the actin cytoskeleton, but little is known regarding the molecular events involved in the regulation of its activity. It has been suggested that Rictor is prone to phosphorylation, although neither the significance of this phosphorylation nor the specific sites involved have been described. Nevertheless, these phosphorylation events may be involved in the regulation of mTORC2 activity and could lead to the identification of new components of the mTORC2 signaling pathway. In order to identify specific phosphorylation sites on Rictor, we performed mass spectrometry analysis on immunoprecipitated Rictor from 293 cells. We identified 17 phosphorylation sites within Rictor. Among them we focus our work on the characterization of one specific phosphorylation site by studying its regulation and its potential involvement in the regulation of mTORC2 activity.

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## 15. TOP216, a novel small molecule mTOR pathway inhibitor with profound anti-cancer activity

Søren Jensby Nielsen, **Christina Trojel-Hansen**, Kamille Dumong Petersen, Mette Knak Christensen, Steven Butcher, Fredrik Björkling, Peter Buhl Jensen and Maxwell Sehested

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TOP216 is a novel oxyphenisatin derivative with low nanomolar cytotoxic activity against a subset of human cancer cell lines with striking selectivity (>1000 fold) against naturally resistant cell lines. Here we demonstrate that incubation of TOP216-sensitive cell lines, but not naturally resistant cell lines such as MDA-MB-231 or MCF10A, leads to inhibition of cellular TORC1 and eIF2a activity, shutdown of protein synthesis, induction of caspase 3/7 activity, and cell death. Cell lines with induced resistance to TOP216 showed no cross-resistance to a panel of known chemotherapeutic drugs, suggesting that TOP216 affects cell viability through a novel mechanism. *In vivo*, TOP216 induced tumour stasis and regression (including cures) in mouse xenograft models of human breast, prostate, ovarian and pancreatic cancer, both when administrated intravenously and perorally. Large PC-3 tumours were shown to regress following a single peroral dose of TOP216. Currently we are investigating the mechanism of action of TOP216 using genomic, proteomic, and metabolomic techniques with the aim to progress this interesting class of anti-cancer compounds into clinical trials.

## 16. Lkb1 is required for TGF $\beta$ - mediated myofibroblast differentiation

**Kari Vahtomeri<sup>1</sup>, Eeva Ventelä<sup>1</sup>, Kaisa Laajanen<sup>1</sup>, Pekka Katajisto<sup>1</sup>, Pierre-Jean Wipff<sup>2</sup>, Boris Hinz<sup>2</sup>, Tea Vallenius<sup>1</sup>, Marianne Tiainen<sup>1</sup> and Tomi P. Mäkelä<sup>1</sup>**

Inactivating mutations of the tumor suppressor kinase LKB1 underlie Peutz-Jeghers syndrome (PJS) characterized by gastrointestinal hamartomatous polyps with a prominent smooth muscle and stromal component. Recently it was noted that PJS-type polyps develop in mice in which Lkb1 deletion is restricted to SM22-expressing mesenchymal cells. Here we have investigated the stromal functions of Lkb1 possibly underlying tumor suppression. Ablation of Lkb1 in primary mouse embryo fibroblasts (MEFs) leads to attenuated Smad activation and TGF $\beta$ -dependent transcription. Also myofibroblast differentiation of Lkb1<sup>-/-</sup> MEFs is defective resulting in markedly decreased formation of  $\alpha$ -smooth muscle actin (SMA) positive stress fibers and reduced contractility. The myofibroblast differentiation defect was not associated with altered serum response factor (SRF) activity and was rescued by exogenous TGF $\beta$  indicating that inactivation of Lkb1 leads to defects in myofibroblast differentiation through attenuated TGF $\beta$  signaling. These results suggest that tumorigenesis by Lkb1-deficient SM22-positive cells involves defective myofibroblast/smooth muscle cell differentiation.

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## 17. Protein-protein interaction network of LKB1 and 14 substrate kinases

**Tea Vallenius, Jianmin Wu, Ana-Maria Osiceanu and Tomi P. Mäkelä**

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Inactivating germline mutations in the LKB1 tumor suppressor gene lead to hereditary Peutz-Jeghers syndrome associated with an increased risk of cancer. Moreover, sporadic inactivating mutations of LKB1 are characteristic for non-small cell lung adenocarcinomas.

LKB1 in a complex with STRAD and MO25 phosphorylates and activates 14 substrate kinases implying the requirement of LKB1 in cell polarity, energy metabolism and cell proliferation. In addition, our recent studies indicate the involvement of TGFbeta signaling in PJS polyps.

However, an important unsolved question remains: through which pathways does defects in LKB1 signaling cause tumorigenesis and what are the critical downstream targets? As one approach to address this, we have initiated to construct a protein-protein interaction (PPI) network downstream of LKB1 including both a collection of characterized PPIs, and novel PPIs identified through systematic screens.

To this end we have developed a web-based Protein Interaction Network Analysis platform (PINA) which integrates six manually curated PPI databases, ability to add your own novel PPIs and offers several visualization, graph theoretical and Gene Ontology tools for advanced PPI network level analysis for biologists. The features of PINA will be presented, as well as a construction of LKB1 and 14 substrate kinase network.

## 18. The role of the tumour suppressor LKB1 in vertebrate organogenesis and cancer

Yme van der Velden<sup>1</sup>, Maarten van Lohuizen<sup>1</sup>, Hans Clevers<sup>2</sup> and Anna-Pavlina Haramis<sup>1</sup>

Mutations in the serine-threonine kinase LKB1 lead to a gastrointestinal hamartomatous polyposis disorder with increased predisposition to malignancies of epithelial origin, in particular of the gastro-intestinal tract. LKB1 activates AMPK under low energy conditions and that leads to growth suppression through several pathways including inhibition of the mTOR pathway. Additionally, activation of LKB1 induces complete polarization of human intestinal epithelial cells. To gain insight into how LKB1 mediates its effects during development and cancer, we have identified two mutations in the single zebrafish LKB1 ortholog by TILLING. Both mutations are stop codons in the kinase domain and fortuitously the Y246 mutation has also been identified in Peutz-Jeghers patients and has been shown to abolish the catalytic activity of LKB1.

Lkb1 mutant zebrafish die at the larval stage owing to severe problems in maintaining intestinal architecture. Mutant larvae are indistinguishable from wt siblings the first 5 days of development. Soon after yolk absorption, we observe an abrupt collapse of the intestinal villi and flattening of the epithelium. Perhaps surprisingly, cell polarization of intestinal epithelial cells is not affected as localization and levels of actin and aPKC on the apical membrane are normal. Lkb1 mutant intestines at day 7 pf show dramatic reduction of ifabp staining indicating failure to maintain differentiation. This phenotype is not accompanied by increased apoptosis neither decreased proliferation.

Interestingly, lkb1 mutant intestines resemble those of starved wild-type larvae at around day 12 pf. Consistent with this finding we observe premature depletion of liver glycogen. We are currently investigating whether also at the molecular level the lkb1 mutants exhibit accelerated metabolism. We are also comparing transcriptional profiles of wt and lkb1 mutants with the aim to unravel the pathways regulated by LKB1 and a possible interconnection between maintenance of cell polarity, physiology and energy status as well as implications for cancer.

Preliminary analysis of adult aged lkb1/+fish, indicates that they are susceptible to bile duct adenocarcinomas, establishing that LKB1 acts as a tumor suppressor also in fish.

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2008

## 19. Smurf2 cooperates with SIK1 during TGF $\beta$ type I receptor downregulation

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Signal transduction pathways are controlled by multiple mechanisms that safeguard their proper and timely action. We have recently described a novel mechanism of negative regulation of Transforming Growth Factor $\beta$  (TGF- $\beta$ ) signaling via the serine/threonine kinase salt-inducible kinase (SIK) 1, one of the members of the AMP kinases phosphorylated by LKB1. SIK1 forms protein complexes with the TGF- $\beta$  type I receptor and with the inhibitory Smad7 and leads to type I receptor downregulation after signaling. The SIK1 gene is transcriptionally induced by TGF- $\beta$ /Smad signaling and thus SIK1, like Smad7, participates in a negative feedback regulatory mechanism. We have investigated the molecular mechanism of this effect and demonstrate that the E3 ubiquitin ligase Smurf2 cooperates with SIK1 in downregulating the TGF- $\beta$  type I receptor. Smurf2 does not directly bind to SIK1, but participates in the receptor complex via the proline/tyrosine-rich motif of Smad7. Furthermore, SIK1 phosphorylates Smad7 on distinct serine residues. Our model supports a central role of Smad7 as a mediator of the action of SIK1 on the TGF- $\beta$  type I receptor, a mechanism that also engages the ubiquitin ligase Smurf2.

## 20. Regulation of epidermal growth factor receptor turnover by the PTEN tumor suppressor

**Igor Vivanco, Daniel Rohle, Mathias Versele, Nicolaos Palaskas, Michael Evans, Teli Hsueh, Cameron Brennan, Sigrid Rajasekaran, Daniel Wolle, Raj A.K. Rajasekaran, Ivan Dikic, Hong Wu, Timothy Perera and Ingo K. Mellingshoff**

Temporal and spatial regulation of growth factor signals is critical for normal development. The ubiquitin ligase c-Cbl plays an important role in this process by promoting the degradation of activated growth factor receptors. Escape from Cbl-mediated downregulation mediates the unattenuated signalling of many mutant growth factor receptors in cancer. Here we show that inactivation of PTEN in transformed and non-transformed cells impairs Cbl-mediated ubiquitination and downregulation of EGFR. Instability of the Cbl complex in PTEN deficient cells is associated with enhanced dependence of EGFR on HSP90 function and results in a modest increase in EGFR activity. This increase in EGFR signal output is sufficient to rescue EGFR driven cells from cell death induction by EGFR kinase inhibitors and can be overcome by more complete EGFR inhibition. Overexpression of dominant negative c-Cbl phenocopies the effects of PTEN on EGFR kinase inhibitor resistance. Our results establish a new link between EGFR and the PI3K pathway and implicate defects in EGFR downregulation as a novel mechanism for EGFR kinase inhibitor resistance.

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## 21. The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase

**Mahvash Zakikhani**, Ryan J.O. Dowling, Nahum Sonenberg  
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Population studies provide evidence that obesity and insulin resistance are associated not only with elevated serum insulin levels and reduced serum adiponectin levels, but also with increased risk of aggressive prostate and colon cancer. We demonstrate here that adiponectin activates AMP activated protein kinase (AMPK) in colon (HT-29) and prostate (PC-3) cancer cells. These results are consistent with prior observations in myocytes, but we demonstrate that in epithelial cancer cells AMPK activation is associated with reduction in mTOR activation as estimated by Ser 2448 phosphorylation, reduction in p70S6 kinase as estimated by Thr389 and ribosomal protein S6 activation as estimated by Ser235/236 phosphorylation, reduction in protein translation as estimated by 35S methionine incorporation, and with growth inhibition. Adiponectin-induced growth inhibition is significantly attenuated when AMPK level is reduced using siRNA methods, indicating that AMPK is involved in mediating the antiproliferative action of this adipokine. Thus, adiponectin has the characteristics of a AMPK-dependent growth inhibitor that is deficient in obesity, and this may contribute to the adverse effects of obesity on neoplastic disease. Furthermore, metformin was observed to activate AMPK and to have growth inhibitory actions on colon (HT-29) and prostate (PC-3) cancer cells. Both (HT-29) and (PC-3) cells were protected against metformin – induced growth inhibition by siRNA targeting AMPK. Metformin also decreased mTOR activity as assessed by phosphorylation of two downstream targets, p70S6 kinase and ribosomal protein S6 and a general decrease in mRNA translation in HT-29 and PC-3 cancer cells, suggesting that this compound may be of particular value in attenuating the adverse effects of obesity on neoplasia.

## 22. mTOR signaling in heart diseases

**Denghong Zhang, Daniele Catalucci, Roberto Rizzi, Jianlin Zhang, Ju Chen  
and Gianluigi Condorelli**

The mammalian target of rapamycin (mTOR) plays a critical role in the regulation of cell growth and in responding to stress, energy states. Heart disease remains a leading cause of death globally and the No. 1 cause of death in the United States. Rapamycin inhibits cardiac hypertrophy through mTOR pathway. However, the mechanism of rapamycin inhibition is still to be determined. The early embryonic lethality was observed in mTOR knockout mice, to bypass this early embryonic lethality, we developed mutant mice with conditional mTOR allele, which can be deleted in cardiomyocytes in a tissue-specific and temporal manner. Use this tamoxifen-inducible cardiac specific mTOR knockout mouse model for a time course gene deletion analysis. Preliminary data show deletion of mTOR in adults cause dilated hearts and lower fractional shortening, finally cause heart failure and death. We hope to clarify the role of mTOR pathway in regulating many aspects of cardiac biology, from cardiomyocyte cell size to angiogenesis, apoptosis and fibrosis. These data will explain the mechanistic mTOR signaling of heart diseases and potentially impact functional studies of rapamycin during clinic trials.

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## 23. A biochemical approach to identify a Rheb-specific GEF

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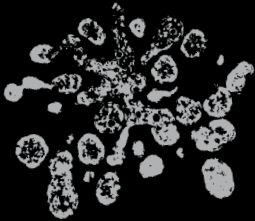
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The small Ras-like GTPase Rheb is an essential element in the signal transduction route leading to activation of mTOR, which functions as an integrator of growth factor signaling and nutrient status. Regulation of Rheb by the product of the tuberous sclerosis gene TSC2 is relatively well understood. For example, insulin signaling leads to the activation of phosphatidylinositol-3 kinase (PI-3K) and subsequently, PKB. This latter kinase phosphorylates and thereby inhibits TSC2. TSC2 acts as a GTPase activating protein (GAP) for Rheb. Blocking the action of TSC2 increases the fraction of GTP-bound Rheb, which may directly or indirectly activate mTOR.

GTP-loading of Rheb is most likely under the control of a guanine nucleotide exchange factor. Although TCTP was recently proposed to act as a Rheb-specific GEF, here we present several lines of evidence that this is highly unlikely. Purified TCTP does not show any GEF activity towards Rheb in real-time photospectrometric measurements. Furthermore, a direct interaction between both proteins could not be detected and knocking down TCTP does not affect phosphorylation of mTOR targets. In order to identify a Rheb-specific GEF, we employed GST-Rheb affinity columns using conditions that would favor an interaction between both proteins. Mass spectrometry of proteins specifically interacting with GST-Rheb identified two enzymes that function in the urea cycle/arginine biosynthetic pathway. Although we consider it unlikely that they function as a Rheb-GEF we decided to test the functional significance of this interaction. The initial results of this analysis will be presented.



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# **Signalling upstream of mTOR**

Madrid | 3-5 November 2008

# INVITED SPEAKERS **PORTFOLIO**

A compilation of short scientific biographies of organizers and speakers in accordance  
with the order of the program





## D. Grahame Hardie

Professor of Cellular Signalling  
Division of Molecular Physiology  
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Grahame Hardie studied Biochemistry at the University of Cambridge, and completed his PhD in Biological Sciences at Heriot-Watt University, Edinburgh in 1974. In 1977 he was appointed as a Lecturer in the Department of Biochemistry, University of Dundee, where he has remained ever since, being promoted to Professor of Cellular Signalling in 1994. He was elected a Fellow of the Royal Society of Edinburgh in 1998, the Academy of Medical Sciences in 2002 and the Royal Society in 2007, and received an Honorary degree from the Medical University of Bialystok, Poland in 2008. In 2008 he will receive the Rolf Luft award for research in endocrinology and metabolism. He is currently Head of the Division of Molecular Physiology at Dundee.

Grahame's main research achievement has been to define the AMP-activated protein kinase (AMPK) system and to elucidate its physiological function. He gave the kinase its name<sup>1</sup>, was the first to purify it to homogeneity<sup>2</sup>, identified the major upstream kinases<sup>3,4</sup>, and identified the key regulatory AMP/ATP-binding<sup>5</sup> and glycogen-binding<sup>6</sup> domains. AMPK is an upstream regulator of mTOR that is now recognized to be the target for metformin, the front line drug for treatment of Type 2 diabetes, and is also being developed as a target for drugs aimed at treatment of cancer.



<sup>1</sup> Hardie, D. G., Carling, D. & Sim, A. T. R. (1989). *Trends Biochem. Sci.* **14**, 20-23

<sup>2</sup> Davies, S. P. et al. (1994). *Eur. J. Biochem.* **223**, 351-357

<sup>3</sup> Hawley, S. A. et al. (2003). *J. Biol.* **2**, 28

<sup>4</sup> Hawley, S. A. et al. (2005). *Cell Metab.* **2**, 9-19

<sup>5</sup> Scott, J. W. et al. (2004). *J. Clin. Invest.* **113**, 274-284

<sup>6</sup> Hudson, E. R. et al. (2003). *Current Biol.* **13**, 861-866

## Benoît Viollet

Department of Endocrinology, Metabolism and Cancer  
Institut Cochin Inserm U567  
Paris, France

Benoît Viollet is currently research scientist in the Department of Endocrinology, Metabolism and Cancer at Institut Cochin, INSERM in Paris. He was born in Paris and was educated in France. He started his undergraduate studies in Strasbourg at the ESBS/University Louis Pasteur where he earned his B.Sc. in 1992. He obtained his PhD in Molecular and Cellular Biology in 1997 after a 4 year research in the laboratory of Prof. Axel Kahn at Inserm U129 in Paris. During this time, he investigated the molecular mechanisms involved in the regulation of gene transcription by glucose. After, he moved to the Pasteur Institute to work as a post-doctoral fellow in the laboratory of Prof. Moshe Yaniv. He addressed the function of Hepatocyte nuclear factor 1 (HNF1), a transcription factor involved in the regulation of a large set of hepatic genes. He joins Institut Cochin of the INSERM as a staff scientist in 2000, where is set up his own group dedicated to the study the pathophysiological regulation of genes implicated in energy metabolism and their possible implication in type 2 diabetes and related metabolic syndromes. His current interests involve functional characterisation of AMP-activated protein kinase (AMPK), a serine/threonine protein kinase that functions as an intracellular energy sensor by facilitating ATP production and suppressing unnecessary ATP use in energy-stressed cells. By generating and using animal models deficient for the catalytic subunits of AMPK, he has shown the role of AMPK in the adaptation of cellular metabolism in the liver, adipose tissue skeletal and cardiac muscles and tumor cells via the regulation of fatty acid oxidation, glucose uptake, glycolysis and hepatic glucose production.





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## Richard F. Lamb

Institute of Cancer Research  
Cancer Research UK Centre for Cell and Molecular Biology  
London, UK



Richard Lamb was born in Scotland and undertook his PhD in Glasgow at the Beatson Institute for Cancer Research. He completed postdoctoral work in the laboratories of Brad Ozanne in Glasgow and Alan Hall in London, and with the latter discovered a role of the TSC1 gene in Rho GTPase signalling and cytoskeletal regulation. In 2001 he began his laboratory at the Institute of Cancer Research in London. Following on from genetic studies in *Drosophila* implicating TSC in cell size regulation, in 2001 his laboratory discovered that mammalian TSC1-2 regulates the activities of S6K and 4E-BP1, two critical effectors of mTOR. Subsequent work with cells deficient in TSC2- in collaboration with George Thomas's lab.- established the principle of the use of rapamycin to selectively target cells with activated mTOR signaling, which has contributed to current clinical trials with this agent. In 2003 further work on signalling established molecular mechanisms underlying a negative feedback regulation that suppresses PI3K signalling in cells lacking TSC1-2 function-providing a possible explanation for the relative poor response of certain tumours to rapamycin analogues. Current research is focused on the control of cell signaling pathways by amino acid nutrients.

## David M. Sabatini

Member, Whitehead Institute  
Associate Professor Biology, MIT  
Investigator, Howard Hughes Medical Institute  
Senior Associate Member, Broad Institute  
Member, Koch Institute for Integrative Cancer Research at MIT

Whitehead Institute for Biomedical Research  
Nine Cambridge Center  
Cambridge, USA

David Sabatini is a Member of the Whitehead Institute for Biomedical Research, Senior Associate Member at The Broad Institute MIT, and Member of the Koch Institute for Integrative Cancer Research at MIT, as well as Associate Professor of Biology at the Massachusetts Institute of Technology. He was also recently named an Investigator of the Howard Hughes Medical Institute.

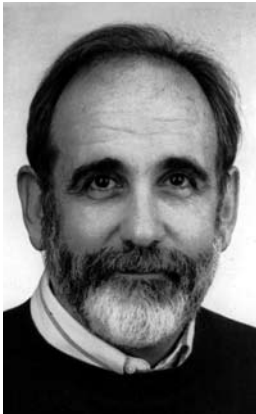
David and his lab at the Whitehead Institute study the basic mechanisms that regulate cell growth, the process whereby cells and organisms accumulate mass and increase in size. These pathways are often deranged in human diseases, such as diabetes and cancer. His current focus is on a cellular system called the Target of Rapamycin (TOR) pathway, a major regulator of growth in many eukaryotic species. In addition to his work on growth control, David is developing and applying new technologies that facilitate the analysis of gene function in mammalian cells. He has developed 'cell-based microarrays' that allow one to examine the cellular effects of perturbing the activity of thousands of genes in parallel. David is a founding member of The RNAi Consortium (TRC) of labs in the Boston area that is developing and using genome-scale RNA interference (RNAi) libraries targeting human and mouse genes.

David received his B.S. from Brown University magna cum laude and his M.D./Ph.D. from Johns Hopkins University in 1997 in the lab of Dr. Solomon H. Snyder in the Department of Neuroscience. Later in the same year, David was appointed a Whitehead Fellow at the Whitehead Institute for Biomedical Research. This was followed in 2002 by a dual appointment to Member at the Whitehead and Assistant Professor of Biology at the Massachusetts Institute of Technology. David has received a number of distinctions, including being named a W. M. Keck Foundation Distinguished Young Scholar, a Pew Scholar, a TR100 Innovator.



# George Thomas

Department of Medical Oncology  
Genome Research Institute  
University of Cincinnati  
Cincinnati, USA



George Thomas received his BA from UC San Diego in 1969 and his PhD from UC Santa Cruz in 1975. He then moved to the Friedrich Miescher Institute for Biomedical Research (FMI) in Basel, Switzerland in 1975 as a Fellow of the European Molecular Biology Organisation. In 1979 he became a Junior Group Leader at the FMI and in 1983 he was promoted to Senior Group Leader. In 1991 he was voted in as a member of EMBO and in 1995 was awarded the Max Cloëtta Prize for Medical Research. He has served on the Scientific Advisory Board of Novartis Oncology and The Genetics Company. In January of 2005 he joined the Genome Research Institute, University of Cincinnati, where he holds the John and Gladys Strauss Endowed Chair in Cancer Research. He is recognised for his groundbreaking studies in the purification and cloning of S6K1 and S6K2 and for his studies in elucidating the role of these kinases in cell growth and the identification of the upstream regulatory components, which control their activity, including the TSC1/TSC2 tumour suppressor complex. More recently his group has demonstrated how this pathway contributes to obesity and type 2 diabetes under conditions of nutrient overload.

## Dario Alessi

Deputy Director  
MRC Protein Phosphorylation Unit  
University of Dundee  
Dundee, UK

Dario Alessi was born in France, went to School in Brussels and studied for a BSc and PhD in Biochemistry at the University of Birmingham with Ian Trayer and David Trentham at the National Institute of Medical Research, Mill Hill, London. Between 1991-1996 Dario performed postdoctoral research in Professor Sir Philip Cohen's laboratory in the MRC Protein Phosphorylation Unit at the University of Dundee, where he became interested a class of enzyme termed kinases that are regulated by insulin and growth factors. In 1997 Dario was appointed as a principal investigator at the MRC Protein Phosphorylation Unit, where he has worked ever since on the molecular details of how insulin, growth factors kinases and other signalling molecules exert their physiological effects and on how to exploit these observations to develop novel treatments for disease. Dario Alessi has published over 140 peer reviewed research papers and been awarded the Colworth Medal (1999), The Eppendorf Young European Investigator Award (2000), Morgagni Young Investigator Prize (2002), Pfizer Academic Award for Europe (2002), Royal Society of Edinburgh Makdougall Brisbane Prize (2002), Philip Leverhulme Prize (2002), FEBS Anniversary Prize (2002), The RD Lawrence Lecture (2004), the EMBO Gold Medal (2005), the Royal Society Francis Crick medal (2006) and in 2008 was elected Fellow of the Royal Society.





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## Tomi P. Mäkelä

Professor of Biochemistry and Molecular Biology  
Dean of Research at the Faculty of Medicine  
Genome-Scale Biology Program and Institute of Biomedicine  
*Biomedicum Helsinki*, University of Helsinki  
Helsinki, Finland



Tomi Mäkelä received his Ph.D. degree in 1992 for the studies carried out in K. Alitalo's lab in University of Helsinki, Finland. He then spent a three-year postdoctoral period (1993-1996) in R. Weinberg's laboratory at the Whitehead Institute for Biomedical Research, during which time he identified cyclin H, the regulatory subunit of the CDK-activating kinase CAK and part of the basal transcription factor TFIIH. Following his postdoctoral period he has, with his laboratory located in Biomedicum, University of Helsinki, continued his interest in cell cycle regulation and CAK, and following the identification of the LKB1 tumor suppressor has studied its function especially with mouse models.

Currently he is Professor of Biochemistry and Molecular Biology and Dean of Research at the Faculty of Medicine. In addition he has directed the Helsinki Biomedical Graduate School and is the co-director of the Finnish Academy Center of Excellence in Translational Genome-Scale Biology.

## Kwok-Kin Wong

Instructor in Medicine, Harvard Medical School  
Department of Medical Oncology  
Dana-Farber Cancer Institute  
Boston, USA

Kwok-Kin Wong obtained his MD and PhD degrees from Columbia Presbyterian Medical Center in 1996. Following two years of internal medicine training at Massachusetts General Hospital in Boston, he received his medical oncology and postdoctoral training from 1998-2003 through the Hematology/Oncology Dana-Farber/Partners program. He joined the DFCI faculty as an independent investigator in 2003 and was promoted to Assistant Professor in 2006. Dr. Wong's ongoing research program seeks to identify and characterize novel genetic alterations driving lung cancer initiation and progression, as well as acquired resistance to targeted therapeutics. His research program has focused in the development of novel mouse models of lung cancer based on discrete genetic alterations that occur in human cancers. He uses these models not only as a means to better understand the pathogenesis of lung cancer, but also as preclinical model systems that enable the development and validation of novel targeted therapeutics for lung cancer prior to in-human testing. Dr. Wong is the recipient of the Miriam Berkman Spotnitz Award (1996, Columbia University), DFCI Lung Cancer Career Development Award (2003, DFCI) and the Kimmel Scholar Award (2004, Sidney Kimmel Foundation) and is funded by the National Institute of Health and Joan's Legacy Foundation.





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## Reuben J. Shaw

Hearst Endowment Assistant Professor  
Molecular and Cell Biology Laboratory  
The Salk Institute for Biological Studies  
La Jolla, USA



Reuben Shaw is an assistant professor in the Molecular and Cellular Biology Laboratory here at the Salk Institute. Dr. Shaw's lab studies signaling pathways that control the development of type 2 diabetes and cancer. Dr. Shaw grew up in upstate New York and did undergraduate studies in Biology at Cornell University. After that, Reuben did his PhD in the lab of Dr. Tyler Jacks at the Cancer Center at Massachusetts Institute of Technology (MIT). After his PhD, he performed postdoctoral research at Harvard Medical School in the lab of Dr. Lewis Cantley. At Harvard Med, Dr. Shaw discovered a very unexpected connection between cancer, cell growth, metabolism, and type 2 diabetes. Dr. Shaw now continues these studies in his lab at Salk.

## Montse Sanchez-Cespedes

Lung Cancer Group Leader  
Molecular Pathology Programme  
Spanish National Cancer Research Centre (CNIO)  
Madrid, Spain (until October 2008)

Current address:  
Genes and Cancer Group  
Epigenetics and Biology Program  
*Institut Catala Oncologia (ICO)*  
Barcelona, Spain

Montse Sanchez-Cespedes specialized in Genetics and Molecular Biology and graduated from the University of Barcelona. She carried out her Ph.D. work at the Molecular Biology of Cancer Laboratory of the "Germans Trias i Pujol" Hospital in Badalona. From 1997 to 2001 she was a Postdoctoral Fellow at the Johns Hopkins University School of Medicine (Baltimore-USA) working at the laboratory of David Sidransky. She focused on the identification of novel genetic and molecular alterations in cancer. More specifically, she looked for recurrent chromosomal abnormalities in lung tumours and genetically analysed candidate tumour suppressor genes in these regions. She joined the CNIO in October 2001 where she worked as a Junior Group Leader of the Lung Cancer Group from 2004 to 2008. At present, she leads the Genes and Cancer Group at the Epigenetics and Biology Program at the Catalan Institute of Oncology (ICO) in Barcelona, Spain. Her main interest is the identification of genes which are important in human cancer, and the understanding of the biological pathways to which these genes belong. During her postdoctoral years at Johns Hopkins she found that the LKB1 gene was frequently inactivated in lung cancer. Recently, the group has observed that BRG1, the ATPase component of the chromatin remodelling complex SWI/SNF, is also genetically inactivated in lung tumours. Thus, one of the major goals of her lab is to contribute to the unveiling of the biological role of LKB1 and BRG1 in cancer development. Her list of over 50 original publications and reviews in well recognised international journals is proof of her experience and in-depth knowledge in the field of cancer research.





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## Nissim Hay

Professor  
Department of Biochemistry and Molecular Genetics  
University of Illinois at Chicago  
Chicago, USA



Nissim Hay received his PhD from the Weizmann Institute in 1984, working on attenuation of transcription using SV40 as a model system. He then moved to the University of California San Francisco for a postdoctoral work with Dr. J. Michael Bishop. In 1990 he joined the faculty of the University of Chicago, and in 1998 he moved to the University of Illinois, at Chicago, where he is a Professor in the Department of Biochemistry and Molecular Genetics. Since 1997, after work in his laboratory showed that the serine/threonine kinase Akt is the major downstream effector of growth factor mediated cell survival, and that Akt is sufficient and required for the activation of mTOR by growth factors, research in his laboratory has been focused on the PI3K/Akt/mTOR signaling pathway. In particular, the research addresses the role of this pathway in apoptosis, and cell proliferation, its relevance to the genesis of cancer, and how these are coupled to the role of this pathway in energy metabolism, both at the cellular and organismal levels.

# Stephen Cohen

Executive Director  
Temasek Life Sciences Laboratory  
Singapore, Singapore

Dr. Stephen Cohen studied Zoology at University of Toronto. His PhD training was at Princeton University and he did post-doctoral work at MIT and later at the Max Planck Institute in Tübingen Germany, where he began to work on *Drosophila* developmental genetics. He was a Howard Hughes Medical Institute Assistant Investigator at Baylor College of Medicine in Houston Texas, before moving to the European Molecular Biology Laboratory in 1993. In 1996, he became Head of the Developmental Biology Unit at EMBL and was elected as a member of the European Molecular Biology Organisation. In 2007 he moved to Singapore to take a position as Executive Director of the Temasek Life Sciences Laboratory. In 2008 he was elected as a Fellow of the Royal Society of London. Dr. Cohen serves on the editorial boards of Science, Genes and Development, Developmental Cell and as an Editor of Developmental Biology. His research over the last decade has focused on morphogen gradients, growth control and metabolism and more recently on microRNAs.





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## Suzanne J. Baker

Associate Member  
Department of Developmental Neurobiology  
St. Jude Children's Research Hospital  
Memphis, USA



Suzanne Baker received her Ph.D. in Molecular Biology and Human Genetics from The Johns Hopkins University under the guidance of Dr. Bert Vogelstein. As a graduate student, she was the first to identify p53 point mutations in human cancer, providing genetic evidence of its role as a tumor suppressor. After postdoctoral training with Dr. Tom Curran at the Roche Institute of Molecular Biology, she joined the faculty in the Department of Developmental Neurobiology at St. Jude Children's Research Hospital in 1995.

The focus of her research is the regulation of normal and neoplastic growth in the brain. Her research employs conditional regulation of genes in mouse brain to evaluate function in a physiological setting. Her laboratory has shown multiple effects of PTEN inactivation in brain including enhanced tumorigenesis, aberrant regulation of cell size, proliferation and developmental migration defects depending on the cell-type and developmental stage in which the pathway is studied. These mouse models provide novel model systems in which to study context-dependent function of signaling pathways.

## Roya Khosravi-Far

Associate Professor of Pathology  
Harvard Medical School  
Beth Israel Deaconess Medical Center  
Boston, USA

Dr. Khosravi-Far received her Ph.D. in Genetics and Molecular Biology from the University of North Carolina at Chapel Hill, where she studied Ras-induced signal transduction pathways and malignant transformation in the laboratory of Dr. Channing Der. Dr. Khosravi-Far carried out her postdoctoral training in Cancer Biology and Immunology at the Massachusetts Institute of Technology with Dr. David Baltimore, where she studied signal transduction pathways that regulate programmed cell death. In 1999, Dr. Khosravi-Far joined Harvard Medical School and Beth Israel Deaconess Medical Center as an Assistant Professor of Pathology and a member of Dana Farber Cancer Institute's Cancer Biology and Hematology programs. In 2005, Dr. Khosravi-Far was promoted to Associate Professor of Pathology. She has also served as the Chair of the Biological and Biomedical Sciences Graduate Admissions at Harvard Medical School. Dr. Khosravi-Far is a council women of Women in Cancer Research/American Association in Cancer Research. She is an American Cancer Society Scholar and has served on various National Cancer Institute and Department of Defense Cancer Research Study Sections. Currently, she serves on the Editorial *Boards of Cancer Research, Cancer Biology and Therapy, Apoptosis and Journal of Biological Chemistry*. Dr. Khosravi-Far's research focuses on investigating oncogene-induced evasion of apoptosis and mechanisms involved in resistance of tumors to apoptosis. In particular, she is interested in the involvement of Forkhead family of transcription factors and TNF-Related Apoptosis Inducing Ligand in evasion of apoptosis.





## Ana C. Carrera

Research Professor in Biomedicine  
Director of Department of Immunology and Oncology  
*Centro Nacional de Biotecnología (CNB/CSIC)*  
Madrid, Spain

Ana Carrera did her PhD thesis work in the laboratory of Dr. Manuel Ortíz de Landázuri at the *Hospital de la Princesa* from 1985-88, and received the PhD in Chemistry from the *Universidad Autónoma de Madrid*. From 1989-1992, Carrera was a postdoctoral fellow in the group of Dr. Thomas Roberts at the Dana Farber Cancer Institute, Harvard Medical School. During that time, she worked on the structural-functional analysis of pp56Lck, a Src family Tyr kinase. In 1992, she returned to Spain as a research scientist in the laboratory of Dr. Carlos Martínez-Alonso at the *Centro de Biología Molecular*, where she formed a group to study the biological function of pp56Lck. She was tenured in 1996 at the *Centro Nacional de Biotecnología* (CNB, Spanish National Research Council), of which she was Vice Director (2004-2006). Since 1996, she has worked on the involvement of phosphoinositide 3-kinases in cancer and inflammation. In 2006 She become Profesor of the Spanish Scientific Research Council, as well as Director of the Department of Immunology and Oncology at the CNB. Dr. Carrera has received the following distinctions:

- 2003 EMBO member
- 2005 Member of the Spanish National Cancer Network
- 2005 Second Descartes prize for "Excellence in scientific collaborative research"
- 2005 Prize of the Spanish Association of lupus patients
- 2006 Research professor in biomedicine



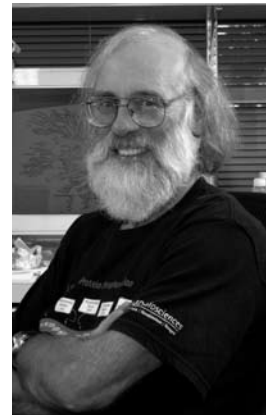
### Recent representative papers:

1. Miriam Marqués, Amit Kumar, Isabel Cortés, Ana Gonzalez-García, Carmen Hernández, M. Carmen Moreno-Ortiz, and Ana C. Carrera "3-kinases p110alpha and p110beta regulate cell cycle entry, exhibiting distinct activation kinetics in G1 phase". (2008). *Mol Cell Biol. Apr*; **28**:2803-14
2. Z. García, V. Silio, M. Marqués, I. Cortés, A. Kumar, C. Hernandez, A. I. Checa, A. Serrano and A. C. Carrera. "A PI3K activity-independent function of p85 regulatory subunit in control of mammalian cytokinesis". (2006). *EMBO J.* **25**(20), 4740-4751. Epub 2006 Oct 5
3. Z. García, A. Kumar, M. Marqués, I. Cortés and A.C. Carrera. "PI3K controls early and late events in mammalian cell division". (2006). *EMBO J.* **25**, 655-61
4. Barber, D. F., Bartolomé, A., Hernández, C., Flores, J., Redondo, C. Fernández-Arias, C., Camps, M., Rulke, T., Schwarz, M.K., Rodríguez, S., Martínez-A., C., Balomenos, D., Rommel, C. and Carrera, A. C. "PI3K inhibition blocks glomerulonephritis and extends lifespan in murine models of systemic lupus". (2005). *Nature Medicine.* **11**:933-935
5. Alvarez, B., Garrido, E., and Carrera, A. C. "Forkhead transcription factors contribute to execution of the mitotic program in mammals". (2001). *Nature.* **413**, 744 -747

## Tony Hunter

Professor and American Cancer Society Professor  
Molecular and Cell Biology Laboratory  
The Salk Institute  
La Jolla, USA

Tony Hunter is an American Cancer Society Research Professor, Director of the Molecular and Cell Biology Laboratory and Director of The Cancer Center at the Salk Institute for Biological Studies in La Jolla, California. He studies how cell growth and division is regulated, and how mutations in genes that regulate growth lead to cancer. His group has made significant contributions in the area of signal transduction, defining how protein phosphorylation generates signals that stimulate or rein in growth. In 1979, he discovered that phosphate could be attached to tyrosine residues in proteins. This seminal discovery opened the door to the study of tyrosine kinases and their role in signal transduction and cell growth and development, and the role that dysregulated tyrosine phosphorylation plays in cancer and other human diseases. This knowledge resulted in the development of a new class of cancer drug that block the activity of mutationally activated tyrosine kinases. His current efforts are aimed at elucidating how protein phosphorylation, ubiquitination, and SUMOylation events regulate cell proliferation and growth, and cell cycle checkpoint activation in response to DNA damage. His recent work has highlighted the importance of crosstalk and feedback loops in the PI-3 kinase-Akt-mTOR cell growth pathway, has elucidated mechanisms of activation of the ATM protein kinase in response to double strand DNA breaks, and has identified a role for the ERK MAP kinase pathway in the motility of early breast carcinoma cells. He has received many awards for his discovery of tyrosine kinases, and he is a Fellow of the Royal Society of London, an Associate Member of the European Molecular Biology Organization (EMBO), a Fellow of the American Academy of Arts and Sciences, a Member of the National Academy of Sciences, a Member of the Institute of Medicine, and a Member of the American Philosophical Society.





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## Karen Cichowski

Assistant Professor  
Harvard Medical School and Brigham and Women's Hospital  
Ludwig Center, Dana-Farber Harvard Cancer Center  
Boston, USA

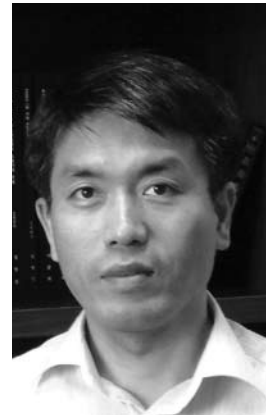


Dr. Karen Cichowski is an Assistant Professor at Harvard Medical School in the Genetics Division at Brigham and Women's Hospital. She is also a Ludwig Center Investigator in the Dana Farber Harvard Cancer Center. Dr. Cichowski performed her thesis work in the signal transduction field at the University of Pennsylvania and received her post-doctoral training at MIT with Dr. Tyler Jacks. Her laboratory combines biochemical, cellular and mouse modeling approaches to elucidate how deregulated Ras signaling contributes to tumorigenesis. One of her major efforts is in studying the familial cancer syndrome NF1. Her laboratory has shown that NF1 critically regulates the mTOR pathway and that mTOR inhibitors represent a potential therapy for this disease and sporadic tumors which harbor NF1 mutations. The ultimate goal of her work is to utilize biochemical and molecular insight gained from basic scientific studies to rationally design more effective therapies for NF1-deficient tumors and other Ras-driven cancers.

## Jongkyeong (JK) Chung

Professor  
Department of Biological Sciences and NCRI Center for Cell Growth Regulation  
Korea Advanced Institute of Science and Technology (KAIST)  
Taejeon, Korea

Jongkyeong (JK) Chung is Professor in the Department of Biological Sciences at KAIST and also a member of KAIST Institute for the Biocentury (KIB). He received his B.S. and M.S. degrees from Seoul National University in Korea and earned a Ph.D. degree in cell biology from Harvard University (John Blenis' Lab) in 1993. During his graduate studies, he firstly demonstrated S6 kinase as a target of PI3 kinase and rapamycin. After his postdoctoral experience at Dana-Farber Cancer Institute and Harvard Medical School, he joined the faculty of KAIST as an Assistant Professor in 1996. Since then, he has been using both mammalian cell system and *Drosophila* genetics to continually understand the regulatory mechanisms of cell growth and proliferation. He is also interested in understanding the pathological mechanism of Parkinson's disease. Recently, he has made original contributions to each field; AMPK was found to be a novel signal linking energy status and cell structures. He also established a novel link between the two genetic factors of Parkinson's disease, named as the PINK1-Parkin pathway.





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## Brendan D. Manning

Assistant Professor  
Department of Genetics and Complex Diseases  
School of Public Health, Harvard University  
Boston, USA



Dr. Brendan Manning completed his doctoral training at Yale University in 2000 and joined the laboratory of Dr. Lewis Cantley at Harvard Medical School as a postdoctoral fellow. During his time in the Cantley lab, he identified TSC2 as a novel target of the protein kinase Akt and found that the TSC1-TSC2 complex lies at the heart of a signaling network critical for cell growth control through the mammalian target of rapamycin (mTOR). In 2004, Dr. Manning joined the faculty of the Department of Genetics and Complex Diseases at the Harvard School of Public Health. His laboratory uses a combination of biochemistry, cell biology, and mouse genetics to study signaling pathways underlying tumor syndromes, cancer, and metabolic diseases, such as obesity and diabetes. A major research focus of the Manning laboratory is in understanding the regulation and pathophysiological functions of the Akt-TSC-mTOR pathway

## John Blenis

Professor  
Department of Cell Biology  
Harvard Medical School  
Boston, USA

John Blenis is a Professor of Cell Biology at Harvard Medical School. His research has focused on the cell biology and biochemistry of cancer cells from his undergraduate days at U.C. Berkeley, his graduate studies at Michigan State University, during his postdoctoral fellowship in Ray Erikson's laboratory at Harvard University and throughout his faculty career.

The major objectives of his research are to define and characterize the molecular basis of cellular signaling with a current focus on PI3-kinase, Ras and mTOR signal transduction. Current efforts are designed to understand how these pathways are regulated and then contribute to the normal and disease-related regulation of protein synthesis and cell growth, cell survival and cell death, migration and invasion, and cell cycle progression.

John Blenis is the recipient of several awards including the Junior Faculty Research Award from the American Cancer Society, Established Investigator Award from the American Heart Association, John A. Boezi Memorial Alumnus Award from Michigan State University, and the NIH/NCI MERIT award.





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## Kun-Liang Guan

Professor

Department of Pharmacology and UCSD Moores Cancer Center

University of California

San Diego, USA



Kun-Liang Guan, B.S. (1982) Hangzhou University, China; Ph.D. (1989) Purdue University; Assistant professor to professor (1992-2007), University of Michigan; Halvor Christensen Professor (2003-2007), University of Michigan; Professor (2007-present), University of California San Diego. National Science Foundation study section (1994-1999); National Institutes of Health study section (1999-2003); Editorial board member (1997-2002), Journal of Biological Chemistry; The Arnold Kent Balls Award (1989), Purdue University; MacArthur Fellow (1998), the John D. & Catherine T. MacArthur Foundation; Young Investigator Award, Society of Chinese Bioscientist Association; Scherling-Plaugh Award (1999), American Society of Biochemistry and Molecular Biology. Procter & Gamble Lecture (199), Purdue University; Susan Swerling Lecture (2004), Dana-Farber Cancer Institute; Faculty Recognition Award (2004), University of Michigan; Distinguished Alumni Award (2006), Purdue University. Dr. Guan's research interesting is in signal transduction and cancer cell biology.

## Elizabeth (Lisa) Petri Henske

Director of the LAM Center at the Brigham and Women's Hospital  
Harvard Medical School  
Boston, USA

Elizabeth (Lisa) Petri Henske, MD is Director of the Brigham and Women's Hospital LAM Center, in Boston MA. She joined the faculty at the Brigham and Women's Hospital, Dana-Farber Cancer Institute, and Harvard Medical School in September 2008. Previously she was a tenured Senior Member at Fox Chase Cancer Center in Philadelphia PA.

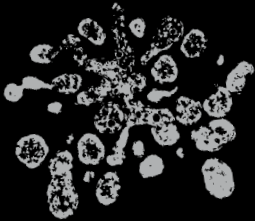
Dr. Henske's research focuses on lymphangioleiomyomatosis (LAM) and tuberous sclerosis complex (TSC). TSC is a tumor suppressor gene disorder that leads to benign tumors in multiple organs, including the brain, heart, and kidney, as well as seizures, mental retardation, and autism. LAM, the pulmonary manifestation of TSC, is notable because it affects almost exclusively women and results from the metastasis of histologically benign cells. The Henske laboratory uses a broad range of approaches to study TSC and LAM, including genetic analyses of human tumor specimens, cell-based and biochemical assays, and mouse, *Drosophila*, and yeast models.

Dr. Henske graduated summa cum laude from Yale University, attended Harvard Medical School, and was trained in Internal Medicine and Hematology-Oncology at the Massachusetts General Hospital. She has received the Scientific Advancement Award from The LAM Foundation (2000), the Manuel Gomez Award from the Tuberous Sclerosis Alliance for "extraordinary scientific and humanitarian efforts to find a cure for Tuberous Sclerosis" (2005), and the Medtronic Prize from the Society for Women's Health Research (2007) for "an outstanding scientist whose work has led or will lead directly to the improvement of women's health."

Dr. Henske is a member of the American Society for Clinical Investigation. She is past Chair of the NIH Cellular and Molecular Biology of the Kidney Cellular and currently Chairs the DOD Neurofibromatosis Research Program Integration Panel. She is an elected member of the Tuberous Sclerosis Alliance (TSA) Board of Directors, where she chairs the International Professional Advisory Board.



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# **Signalling upstream of mTOR**

Madrid | 3-5 November 2008

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Ana C. Carrera

**acarrera@cnb.uam.es**

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## >> February 2008

### **Development and cancer – CNIO Cancer Conference**

04/02/2008 - 06/02/2008

**ORGANIZERS:** Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

**REGISTRATION:** [www.cnio.es/cc](http://www.cnio.es/cc)

### **Trasladando la proteómica al laboratorio clínico**

12/02/2008

**ORGANIZERS:** Sociedad Española de Bioquímica Clínica y Patología Molecular, Grupo de Marcadores Tumorales

**REGISTRATION:** [www.seqc.es](http://www.seqc.es)

### **II Jornada traslacional en oncología pediátrica: del diagnóstico molecular a la clínica**

22/02/2008

**ORGANIZERS:** Juan Cruz Cigudosa, Anna González-Neira, Javier Benítez, Instituto Roche

**REGISTRATION:** [www.institutoroche.es](http://www.institutoroche.es)

## >> March 2008

### **CNIO-ONCOTRAIN Meeting: “New battlefields in human cancer - Attacking in many fronts”**

10/03/2008 - 11/03/2008

**ORGANIZERS:** ONCOTRAIN Program

**REGISTRATION:** [www.cnio.es/meetings](http://www.cnio.es/meetings)

## >> April 2008

### **Jornada Científica COLOMICS: “Avances en cáncer de colón”**

14/04/2008

**ORGANIZERS:** Felix Bonilla, Ignacio Casal, Antonio Garcia de Herreros y Alberto Muñoz

**REGISTRATION:** [www.cnio.es/meetings](http://www.cnio.es/meetings)

### **CASP Meeting**

21/04/2008 - 22/04/2008

**ORGANIZERS:** Alfonso Krzysztof Fidelis, Andriy Kryshchovych, John Moul, Burkhard Rost, Anna Tramontano, Michael Tress and Alfonso Valencia

**REGISTRATION:** [www.cnio.es/meetings](http://www.cnio.es/meetings)

## **ENFIN – DREAM Conference: assessment of computational methods in systems biology**

28/04/2008 - 29/04/2008

**ORGANIZERS:** Alfonso Valencia, Pascal Kahlem; Co-Organizer: Ana Rojas Mendoza

**REGISTRATION:** [www.cnio.es/meetings](http://www.cnio.es/meetings)

## **>> May 2008**

### **Marie Curie Gard: interplay among genetics, epigenetics and non-coding RNAs**

04/05/2008 - 07/05/2008

**ORGANIZERS:** Manel Esteller and George Calin

**REGISTRATION:** [www.mc-gard.eu](http://www.mc-gard.eu)

## **>> June 2008**

### **3<sup>rd</sup> ESO-CNIO Familial Cancer Conference**

05/06/2008 - 06/06/2008

**ORGANIZERS:** Javier Benitez, Ros Eeles and Hans Vasen

**REGISTRATION:** [www.cnio.es/meetings](http://www.cnio.es/meetings)

### **Structure and mechanisms of essential complexes for cell survival - CNIO Cancer Conference**

23/06/2008 - 25/06/2008

**ORGANIZERS:** Niko Grigorieff, Eva Nogales and Jose María Valpuesta

**REGISTRATION:** [www.cnio.es/cc](http://www.cnio.es/cc)

### **EPIGENOME Meeting**

26/06/2008 – 29/06/2008

**ORGANIZERS:** Oskar Fernández-Capetillo, Ana Losada, María A. Blasco and the Epigenome network

## **>> October 2008**

### **4<sup>th</sup> CNIO-Leica advanced live cell microscopy workshop**

09/10/2008-10/10/2008

**ORGANIZERS:** María Montoya and Leica Microsystems

**REGISTRATION:** [www.leica-microsistemas.com/cnio](http://www.leica-microsistemas.com/cnio)



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### **CNIO Special Workshop “Stress signaling and cancer”**

13/10/2008 – 15/10/2008

ORGANIZERS: Erwin Wagner, Nic Jones and Ángel Nebreda

REGISTRATION: [www.cnio.es/meetings](http://www.cnio.es/meetings)

### **ESO-CNIO European oncology course in native language: “*Tumores Neuroendocrinos. Mecanismos moleculares y aplicaciones clínicas. Actualización del diagnóstico y el tratamiento*”**

16/10/2008 – 17/10/2008

ORGANIZERS: Ramón Salazar, José Ángel Díaz and Mercedes Robledo

REGISTRATION: [www.cnio.es/es/cursos/cursos.asp](http://www.cnio.es/es/cursos/cursos.asp)

### **Recombinat Antibodies: new developments for future challenges**

20/10/2008 - 22/10/2008

ORGANIZERS: Luis Álvarez-Vallina, Luis Ángel Fernández, Jorge L. Martínez-Torrecuadrada and Serge Muyldermans

REGISTRATION: [www.cnio.es/meetings](http://www.cnio.es/meetings)

### **OncoCycle Programme: “*Ciclo Celular y Cáncer*”**

27/10/2008 - 28/10/2008

ORGANIZERS: Mariano Barbacid, Marcos Malumbres, Jesús M. Paramio and Miguel Vidal

REGISTRATION: [www.cnio.es/meetings](http://www.cnio.es/meetings)

## **>> November 2008**

### **Signalling Upstream of mTOR - CNIO Cancer Conference**

03/11/2008 - 05/11/2008

ORGANIZERS: R. Alessi, Tomi P. Mäkelä and Montserrat Sanchez-Céspedes

REGISTRATION: [www.cnio.es/cc](http://www.cnio.es/cc)

## **>> February 2009**

### **Stem cells and cancer - CNIO Cancer Conference**

23/02/2008-25/02/2008

ORGANIZERS: Eduard Batlle, María A. Blasco, Elaine Fuchs and Mirna Perez-Moreno

REGISTRATION: [www.cnio.es/cc](http://www.cnio.es/cc)

## >> June 2009

### **ESO Meeting - New therapies in cancer - Epigenetic drugs and beyond**

11/06/2009 – 12/06/2009

**ORGANIZERS:** Miguel Ángel Piris, Manel Esteller and Silvia Marsoni

## >> July 2009

### **Cancer-om-atics: Multilevel interpretation of cancer genome data - CNIO Cancer Conference**

06/07/2009 – 08/07/2009

**ORGANIZERS:** Søren Brunak, Nuria Malats, Chris Sander and Alfonso Valencia

## >> November 2009

### **The energy of cancer - CNIO Cancer Conference**

02/11/2009 – 04/11/2009

**ORGANIZERS:** Manuel Serrano, Toren Finkel, David Sabatini, David Sinclair



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## >> 2008

### **Development and cancer**

04/02/2008 - 06/02/2008

**ORGANIZERS:** Konrad Basler, Ginés Morata, Eduardo Moreno and Miguel Torres

### **Structure and mechanisms of essential complexes for cell survival**

23/06/2008 - 25/06/2008

**ORGANIZERS:** Niko Grigorieff, Eva Nogales and Jose María Valpuesta

### **Signalling Upstream of mTOR**

03/11/2008 - 05/11/2008

**ORGANIZERS:** Dario R. Alessi, Tomi P. Mäkelä and Montserrat Sanchez-Cespedes

## >> 2007

### **Molecular mechanisms in lymphoid neoplasm**

**ORGANIZERS:** Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe, Miguel Angel Piris

19/02/2007 - 21/02/2007

### **Myc and the transcriptional control of proliferation and oncogenesis**

**ORGANIZERS:** Robert N. Eisenman, Martin Eilers, Javier León

11/06/2007 - 13/06/2007

### **Links between cancer, replication stress and genomic integrity**

**ORGANIZERS:** Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez, André Nussenzweig

05/11/2007 - 07/11/2007

## >> 2006

### **Telomeres and Telomerase - CNIO / José Steiner Cancer Conference**

**ORGANIZERS:** María A. Blasco, Jerry Shay

13/11/2006 - 15/11/2006

### **Medicinal chemistry in oncology**

**ORGANIZERS:** Fernando Albericio, James R. Bischoff, Carlos García-Echeverría, Andrew Mortlock

02/10/2006 - 04/10/2006

### **Inflammation and cancer**

**ORGANIZERS:** Curtis Harris, Raymond DuBois, Jorge Moscat, Manuel Serrano

22/05/2006 - 24/05/2006

## **PTEN and the AKT route**

**ORGANIZERS:** Ana Carrera, Pier Paolo Pandolfi, Peter Vogt  
08/05/2006 - 10/05/2006

## **>> 2005**

### **Cancer and aging**

**ORGANIZERS:** María A. Blasco, Kathy Collins, Jan Hoeijmakers, Manuel Serrano  
07/11/2005 - 09/11/2005

### **MAP kinases and cancer**

**ORGANIZERS:** Philip Cohen, Dundee, Roger Davis, Worcester, Chris Marshall, Ángel Nebreda  
30/05/2005 - 01/06/2005

### **Animal tumour models and functional genomics**

**ORGANIZERS:** Allan Balmain, Mariano Barbacid, Anton Berns, Tyler Jacks  
07/03/2005 - 09/03/2005

## **>> 2004**

### **Cadherins, catenins and cancer**

**ORGANIZERS:** Amparo Cano, Hans Clevers, José Palacios, Franz Van Roy  
29/11/2004 - 01/12/2004

### **Structural biology of cancer targets**

**ORGANIZERS:** Ernest Laue, Guillermo Montoya, Alfred Wittinghofer  
27/09/2004 - 29/09/2004

## **>> 2003**

### **Apoptosis and cancer**

**ORGANIZERS:** Gabriel Nuñez, Marisol Soengas, Scott Lowe  
01/12/2003 - 03/12/2003

### **Small GTPases in human carcinogenesis**

**ORGANIZERS:** Juan Carlos Lacal, Channing Der, Shuh Narumiya  
16/06/2003 - 18/06/2003

### **Targeted search for anticancer drugs**

**ORGANIZERS:** Amancio Carnero, David H. Beach  
17/03/2003 - 19/03/2003



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>> **2002**

### **Mechanisms of invasion and metastasis**

**ORGANIZERS:** Joan Massagué, Richard Hynes

18/11/2002 - 20/11/2002

### **The cell cycle and cancer**

**ORGANIZERS:** Marcos Malumbres, Charles Sherr, Jiri Bartek

30/09/2002 - 02/10/2002

### **Cancer epigenetics: DNA methylation and chromatin**

**ORGANIZERS:** Manel Esteller, Stephen B. Baylin

29/05/2002 - 31/05/2002



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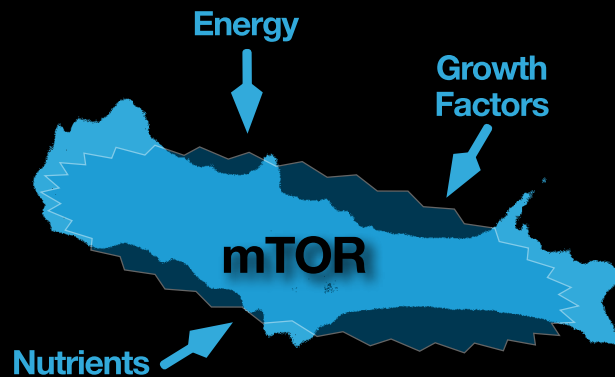
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