AMPK in gonads, more than a cell energy sensor

Pascal Froment

AMPK: Biological Action and Therapeutic Perspectives

SEPTEMBER 28 - OCTOBER 3, 2014
LUCCA, ITALY

CO-ORGANIZERS:

David Carling, PhD
MRC CLINICAL SCIENCES CENTRE
LONDON, ENGLAND

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During these challenging financial times, it is imperative for the scientific community to work collaboratively to safeguard the exchange of knowledge and ideas that promote vital scientific progress. Our sincere appreciation goes to the many people and supporters who helped bring this conference to fruition and to ensuring its success.

We wish you all the best for an informative and rewarding experience!

Guy Fogleman, PhD
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PROGRAM AGENDA

AMPK: Biological Action and Therapeutic Perspectives

September 28 – October 3, 2014

Lucca, Italy

Co-Organizers:

David Carling
MRC Clinical Sciences Centre
London, England

Benoit Viollet
Institut Cochin
Paris, France

Sunday, **September 28, 2014**	
4:00PM – 9:00PM	Conference Registration
6:00PM – 7:00PM	FASEB Opening Reception
7:00PM – 8:30PM	Dinner
8:30PM – 8:45PM	Opening of Conference Welcome: Benoit Viollet and David Carling
Monday, **September 29, 2014**	
7:30AM – 9:00AM	Breakfast
Session 1: **9:00AM – 12:30PM**	**Advances in understanding the regulation of AMPK** Session Chair: David Carling, MRC Clinical Sciences Centre, London UK
9:00AM – 9:15AM	Welcome from FASEB
9:15AM – 9.45AM	Steve Gamblin, MRC NIMR *"Structural & Functional Studies on AMPK and its activation by drugs"*

9.45AM –10:15AM	Grahame Hardie, Dundee University *"Canonical and non-canonical regulation of AMPK"*
10:15AM – 10:35AM	Short Talk: Ravi Kurumbail, Pfizer *"Structural basis for activation of AMPK"*
10:35AM – 11:10AM	Group photo & FASEB Sponsored Coffee Break
11:10AM – 11.40AM	Bruce Kemp, St. Vincent's Medical Institute *"AMPK activation without phosphorylation; drugs and AMP unite"*
11:40AM – 12:00PM	Short Talk: Matt Sanders, AstraZeneca *"Activation of AMPK by small molecules"*
12:00PM – 12:30PM	Mark Rider, UCL Brussels *"New small-molecule AMPK activators in skeletal muscle"*
12:30PM – 2:00PM	Lunch
Session 2: **2:00PM – 5:20PM**	**AMPK regulation of Metabolism I** Session Chair: Kei Sakamoto, Nestle Institute of Health Sciences, Lausanne, CH
2:00PM - 2:30PM	Takashi Kadowaki, Tokyo University *"A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity"*
2:30PM – 3:00PM	Masahisa Jinushi, Hokkaido University *"Role of AMPK in macrophages in immune tolerance"*
3:00PM – 3:20PM	Short Talk: Isabel Lopez, University of Lausanne *"Participation of CDK4 in the regulation of mitochondrial metabolism and energy homeostasis"*
3:20PM – 3:50PM	Break
3:50PM – 4:10PM	Short Talk: Yvonne Oligschlager, Maastricht University *"Cycling of AMPK, another way of exercise to combat type 2 diabetes"*
4:10PM – 4:40PM	Miguel Lopez, University Santiago de Compostela *"The hypothalamic AMPK-BAT axis: a canonical regulator of energy balance"*
4:40PM – 5:00PM	Short Talk: Cornelia D. Cudrici NIH, Bethesda *"A New Target for Anti-inflammatory Effect of Methotrexate - AMP activated protein kinase"*

5:00PM – 5:20PM	Short Talk: Guy Rutter, Imperial College *"LKB1 and AMPK differentially regulate pancreatic beta cell identity"*
6:00PM – 7:30PM	Dinner
7:30PM -9:30PM	**Poster session 1**
Tuesday, **September 30, 2014**	
7:30AM – 9:00AM	Breakfast
Session 3 **9:00AM – 12:10PM**	**LKB1 and AMPK-related kinases** Session Chair: Bruce Kemp, St. Vincent's Institute of Medical Research, Melbourne, AUS
9:00AM – 9:30AM	Olga Goransson, Lund University *"Role and regulation of SIK2 in human adipose tissue"*
9:30AM –10:00AM	Kei Sakamoto, Nestle Institute *"The LKB1-SIK pathway in metabolic control"*
10:00AM – 10:20AM	Short Talk: Kristopher Clark, University of Dundee *"Targetting the salt-inducible kinase as a novel strategy to treat inflammation"*
10:20AM – 10:50AM	FASEB Sponsored Coffee Break
10:50AM – 11:20AM	Reuben Shaw, Salk Institute *"The LKB1-AMPK pathway: metabolic stress leads to metabolic rewiring"*
11:20AM – 11:50AM	Robert Screaton, University of Ottawa *"Sik2 (Sugar Inducible Kinase!) and functional compensation in the pancreatic beta cell"*
11:50AM – 12:10PM	Short Talk: Biplab Dasgupta, Cincinnati Children's Hospital *"The Tumor Suppressor LKB1 regulates Myelination through Mitochondrial Metabolism"*
12:30PM – 2:00PM	Lunch
Session 4: **2:00PM – 5:50PM**	**AMPK in heart and skeletal muscle** Session Chair: Jorgen Wojtaszewski, Copenhagen University, Denmark
2:00PM - 2:30PM	Luc Bertrand, UC Louvain, Brussels *"New metabolic targets of AMPK for treating cardiac pathologies"*

2:30PM – 3:00PM	Larry Young, Yale University Medical School *"Critical physiologic functions of the cardiac LKB1-AMPK pathway"*
3:00PM – 3:20PM	Short Talk: Sandrine Horman, UCL Brussels *"Reduced scar formation and contractility lead to exaggerated left ventricular dilatation after myocardial infarction in mice lacking AMPKalpha1"*
3:20PM – 3:50PM	Break
3:50PM – 4:10PM	Short Talk: Mark Evans, University of Edinburgh *"AMP-activated protein kinase is necessary for cardiorespiratory adjustments during hypoxia"*
4:10PM – 4:40PM	Arash Yavari, Oxford University *"PRKAG2 – insights into heart rate regulation"*
4:40PM - 5:10PM	Erik Richter, University of Copenhagen *"Role of AMPK in regulation of muscle metabolism during exercise"*
5:10PM – 5:30PM	Short Talk: Helen Heathcote, Glasgow University "A role for PKC in the regulation of VEGF-stimulated AMPK?"
5:30PM – 5:50PM	Short Talk: Randa Abdel Malik, Goethe-University, Frankfurt *"The AMPKα2 subunit in myeloid cells, but not in endothelial cells, regulates post-ischemic revascularization through modulation of the HIF-1α pathway"*
6:00PM – 7:30PM	Dinner
7:30PM – 9:00PM	**Meet the expert session**
Wednesday, **October 1, 2014** 7:30AM – 9:00AM	Breakfast
Session 5 **9:00AM – 12:10PM** 9:00AM - 9:30AM	**Mouse models to study AMPK signaling pathways** Session Chair: Benoit Viollet, Institut Cochin, Paris, France Pascal Froment, INRA, France *"AMPK in gonads, more than a cell energy sensor*"
9:30AM –10:00AM	Benoit Viollet, Institut Cochin, Paris, France *"Tissue-specific deletion of AMPK catalytic subunits: defining AMPK-dependent and -independent metabolic actions"*
10:00AM – 10:20AM	Short Talk: Angela Woods, MRC Clinical Sciences Centre *"A novel mouse model for studying activation of AMPK in vivo"*

10:20AM – 10:50AM	FASEB Sponsored Coffee Break
10:50AM – 11:20AM	Greg Steinberg, McMaster University *"AMPK regulation of lipid metabolism"*
11:20AM – 11:50AM	Jérôme Tamburini, Institut Cochin, Paris, France *"Targeting AMPK in acute myeloid leukemia"*
11:50AM – 12:10PM	Short Talk: Ian Salt, Glasgow University *"AMP-activated protein kinase suppresses inflammatory signalling and chemokine secretion in adipose tissue"*
12:30PM – 2:00PM	Lunch
Session 6: **2:00PM – 5:30PM**	**AMPK and Cancer** Session Chair: Grahame Hardie, University of Dundee, Dundee UK
2:00PM - 2:30PM	Rosa Senaris, University Santiago de Compostella *"Role of the fuel-sensing enzyme AMPK in astrocytic tumour cell proliferation"*
2:30PM – 3:00PM	Tracey Rouault, NIH *"Reduced AMPK activation and expression correlates with highly aggressive growth in hereditary renal cancer caused by FH deficiency"*
3:00PM – 3:20PM	Short Talk: Said Izreig, McGill University *"Repression of LKB1 by miR-17-92 is required for Myc-driven metabolism in B-cell lymphoma"*
3:20PM – 3:50PM	Break
3:50PM – 4:10PM	Short Talk: Annapoorni Rangarajan, Indian Institute of Science *"AMPK phosphorylates novel substrate PEA15 to facilitate anchorage-independent growth of mammary cells"*
4:10PM – 4:40PM	Russell Jones, McGill University *"Regulation of tumor metabolism by the LKB1-AMPK pathway"*
4:40PM – 5:10PM	Almut Schultze, Wuerzburg University *"Metabolic reprogramming in cancer supports cell growth and survival"*
5:10PM – 5:30PM	Short Talk: Theodoros Tsakiridis, McMaster University *"Translating tumour and radiation biology of AMPK into early phase oncology trials"*
6:00PM – 7:30PM	Dinner

7:30PM – 9:30PM	**Poster session 2**
Thursday, **October 2, 2014** 7:30AM – 9:00AM	Breakfast
Session 7 **9:00AM – 12:30PM**	**AMPK regulation of Metabolism II** Session Chair: Larry Young, Yale University School of Medicine, New Haven, USA
9:00AM - 9:30AM	Neil Ruderman, Boston University Medical School *"AMPK downregulation in humans: studies in insulin resistant and sensitive bariatric surgery patients"*
9:30AM –10:00AM	Barbara Kahn, Harvard Medical School *"Discovery of a Novel Class of Naturally-Occurring Lipids with Anti-Diabetic and Anti-inflammatory Effects"*
10:00AM – 10:20AM	Short Talk: Antonio Velazquez-Arellano, Universidad Nacional Autónoma de Mexico "*Insulin sensitivity is inversely related to cellular energy status, as revealed by biotin deprivation"*
10:20AM – 10:50AM	FASEB Sponsored Coffee Break
10:50AM – 11:10AM	Short Talk: Richard Roy, McGill University *"AMPK buffers adverse epigenetic change and consequent transgenerational reproductive defects following acute nutrient/energy stress"*
11:10AM – 11:40AM	Shengcai Lin, Xiamen University *"Regulation of AMPK activation and mTOR suppression by Axin"*
11:40AM – 12:00PM	Short Talk: Stephen Pinkosky, McMaster University "*The Coenzyme A Ester of ETC-1002 Mediates Direct β1-Selective Activation of AMP-Activated Protein Kinase and Provides Evidence for a Novel Nutrient-Sensing Mechanism"*
12:00PM – 12:30PM	Jorgen Wojtaszewski, Copenhagen University *"AMPK in muscle insulin sensitivity"*
12:30PM – 1:00PM	**Business Meeting**
12:30PM – 2:00PM	Lunch
1:00PM – 6:30PM	**Optional Organized Group Activity/Free Afternoon**
6:30PM – 8:00PM	Dinner

8:30PM-9:00PM	Announcement of Tabor Award Winner and Poster Awards
9:00PM - Late	**Party!**
Friday, **October 3, 2014**	
7:30AM – 9:00AM	Breakfast
Session 8: **9:00AM – 11:00AM**	**Emerging Roles for AMPK** Session Chair: Erik Richter, University of Copenhagen, Denmark
9:00AM – 9:30AM	Uwe Schlattner, INSERM U1055, Grenoble *"Analyzing AMPK conformational changes by fluorescence energy transfer: an AMPK FRET sensor"*
9:30AM – 9:50AM	Short Talk: Elton T. Young, University of Washington *"Phosphoproteomics Identifies Transcription-Coupled mRNA Decay as a Snf1-Regulated Pathway"*
9:50AM – 10:10AM	Short Talk: Rémi Mounier, Université Claude Bernard Lyon1 *"Self-renewal and metabolism of muscle stem cells are regulated by AMPKα1"*
10:10AM – 10:40AM	David Carling, MRC Clinical Sciences Centre, London *"Investigating the role of the AMPK cascade in prostate cancer"*
10:40AM – 11:00AM	Closing Comments Benoit Viollet and David Carling
11:00AM	Departures Boxed Lunches Available

END OF CONFERENCE

For additional information contact:
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SPEAKER ABSTRACTS

Monday AM, Session 1: Advances in understanding the regulation of AMPK

Structural & Functional Studies on AMPK and its activation by drugs

Steve Gamblin
MRC National Institute for Medical Research

AMP-activated protein kinase (AMPK) plays a major role in regulating cellular energy balance by sensing and responding to increases in AMP/ADP concentration relative to ATP. Binding of AMP causes allosteric activation of the enzyme and binding of either AMP or ADP promotes and maintains the phosphorylation of threonine 172 within the activation loop of the kinase. AMPK has attracted widespread interest as a potential therapeutic target for metabolic diseases including type 2 diabetes and, more recently, cancer. A number of direct AMPK activators have been reported as having beneficial effects in treating metabolic diseases, but there has been no structural basis for activator binding to AMPK. Here we present the crystal structure of human AMPK in complex with a small molecule activator that binds at a site between the kinase domain and the carbohydrate-binding module, stabilising the interaction between these two components. The nature of the activator-binding pocket suggests the involvement of an additional, as yet unidentified, metabolite in the physiological regulation of AMPK. Importantly, the structure offers new opportunities for the design of small molecule activators of AMPK for treatment of metabolic disorders.

Monday AM, Session 1: Advances in understanding the regulation of AMPK

Canonical and non-canonical regulation of AMPK

D. Grahame Hardie
University of Dundee

AMP-activated protein kinase (AMPK) occurs as heterotrimeric complexes comprising catalytic α subunits and regulatory β and γ subunits. Genes encoding these subunits are found in the genomes of essentially all eukaryotic species, suggesting that the development of AMPK was an early event during eukaryotic evolution. In the budding yeast *Saccharomyces cerevisiae*, genes encoding the AMPK ortholog are required for the switch from the rapid glycolysis and anabolism observed in proliferating cells grown in high glucose, to the mitochondrial oxidative catabolism and much slower growth observed in cells maintained in low levels of glucose. Since mitochondrial biogenesis remains an important output of the AMPK system in mammalian cells, these observations suggest that an ancestral function of the AMPK system was to monitor the output of endosymbiotic organelles (i.e. mitochondria and chloroplasts) and increase their production of ATP to match the demand created by cellular function.

The γ subunits of mammalian AMPKs contain three sites that bind AMP, ADP and ATP in competition. Replacement of ATP by AMP at one or more of these sites causes three effects that activate AMPK: (i) promotion of phosphorylation of Thr172 by the LKB1 complex (which can produce >100-fold activation in cell-free assays, although the changes in intact cells are much more modest); (ii) inhibition of Thr172 dephosphorylation by protein phosphatases (which we are currently attempting to identify); (iii) allosteric activation, which is substantial (>10-fold) and significant in intact cells. Of these effects, only the second is mimicked by binding of ADP. These represent the “canonical” mechanisms of activation of AMPK by energy stress, but we are also studying alternative “non-canonical” mechanisms for regulation of AMPK function. These include: (i) phosphorylation of Thr172 by the Ca^{2+}/calmodulin dependent protein kinase CaMKKβ; which in our hands is not enhanced by binding of AMP or ADP; (ii) regulation of AMPK by glycogen and α1->4-linked oligosaccharides; (iii) phosphorylation of Ser487 and other sites within the “ST loop” of AMPK-α1, by Akt and other kinases. We have shown that Ser487 phosphorylation inhibits Thr172 phosphorylation by both LKB1 and CaMKKβ, because the phosphorylated ST loop interacts with the small lobe of the kinase domain, physically occluding access of Thr172 to the upstream kinases.

AMPK is also activated by many drugs or natural products, some of which have been used to treat human disease for decades or even longer. These include the anti-diabetic drugs metformin and phenformin (both derived from the natural product galegine) and the anti-inflammatory agent salicylate, which is a natural product of plants and the major breakdown product of the synthetic salicylate derivative, aspirin (acetyl salicylate). Many novel synthetic AMPK activators have been identified, and we have recently found that the anti-diabetic drug canagliflozin (developed as an inhibitor of sodium-glucose cotransporter-2) activates AMPK at therapeutically relevant concentrations. The mechanisms by which these compounds activate AMPK will be discussed.

Monday AM, Session 1: Advances in understanding the regulation of AMPK

Crystal structure, biophysics and enzyme kinetics reveal the molecular switches turned on by synthetic ligands to activate AMPK

Ravi Kurumbail
Pfizer

In pursuit of novel therapy for the treatment of diabetes and other metabolic diseases, the pharmaceutical industry has been seeking direct activators of AMPK because of its central role in whole body energy homeostasis. In general, developing enzyme activators is a challenge which for AMPK gets further complicated by its heterotrimeric composition and the presence of multiple isoforms that differ between tissues and species. To provide molecular insights into activation, we have solved the crystal structure of mammalian α1β1γ1 isoform with a series of ligands related to A-769662, the most widely studied direct AMPK activator. Consistent with the previous report on α2β1γ1, A769662 and its analogs bind at a novel allosteric site between the carbohydrate binding module of the β subunit and the kinase domain of the α subunit. These observations have been further corroborated by biochemical and biophysical studies including NMR, SPR, HDX and site-directed mutagenesis. Moreover, kinetic studies reveal that A769662 exerts its effect by primarily lowering the K_m for the substrate peptide in contrast to AMP which increases the overall V_{max} of the kinase reaction. Thus natural and synthetic activators regulate the kinase activity of AMPK from opposite poles and by different molecular mechanisms

Monday AM, Session 1: Advances in understanding the regulation of AMPK

AMPK activation without phosphorylation; drugs and AMP unite

Bruce E. Kemp
St. Vincent's Institute of Medical Research and University of Melbourne

The AMP-activated protein kinase (AMPK) is a metabolic stress-sensing □□□heterotrimer responsible for energy homeostasis, making it a therapeutic target for metabolic diseases such as type 2 diabetes and obesity. AMPK signaling is triggered by phosphorylation on the AMPK □-subunit activation loop Thr172 by upstream kinases. Dephosphorylated, naive AMPK is thought to be catalytically inactive and insensitive to allosteric regulation by AMP and direct AMPK-activating drugs. Multiple activating drugs have been reported. Here we show that A-769662 alone can activate AMPK independently of □-Thr172 phosphorylation, provided □subunit -Ser108 is phosphorylated (Scott et al 2014). A-769662 has been shown to bind to the □-subunit CBM in association with the kinase small lobe (Xiao et al. 2013). A-769662 and AMP individually modestly stimulate the activity of dephosphorylated AMPK, together they stimulate activity >1,000-fold, bypassing the requirement for □-Ser108 phosphorylation. Consequently A-769662 and AMP together activate naive AMPK entirely allosterically and independently of upstream kinase signaling. The profound synergy between AMP and direct activating drugs has provided several new insights into drugs and metabolites that may influence AMPK activity.

Scott, J. W., Ling, N., Issa, S. M., Dite, T. A., O'Brien, M. T., Chen, Z. P., Galic, S., Langendorf, C. G., Steinberg, G. R., Kemp, B. E. & Oakhill, J. S. 2014. Small Molecule Drug A-769662 and AMP Synergistically Activate Naive AMPK Independent of Upstream Kinase Signaling. Chem Biol, 21, 619-27.

Xiao, B., Sanders, M. J., Carmena, D., Bright, N. J., Haire, L. F., Underwood, E., Patel, B. R., Heath, R. B., Walker, P. A., Hallen, S., Giordanetto, F., Martin, S. R., Carling, D. & Gamblin, S. J. 2013. Structural basis of AMPK regulation by small molecule activators. Nat Commun, 4, 3017.

Monday AM, Session 1: Advances in understanding the regulation of AMPK

Structural basis of AMPK regulation by small molecule activators

Matthew J Sanders
MRC National Institute for Medical Research

AMP-activated protein kinase (AMPK) has attracted wide-spread attention as a therapeutic target for treating aspects of the metabolic syndrome. The first direct AMPK activator, A-769662, was developed by Abbott Laboratories and was beneficial in animal models of the metabolic syndrome. We have been interested in understanding the regulation of AMPK by A-769662 and other direct activators. We have previously shown that A-769662 activates AMPK through a different mechanism to AMP/ADP; mutations that alter the nucleotide regulation do not affect A-769662 activation of AMPK. Furthermore, we discovered that truncating the β subunit of AMPK so that it lacked the carbohydrate-binding module (CBM) abolished activation by A-769662. In order to understand the regulation and binding of direct AMPK activators, we carried out a number of biophysical and structural studies. We have used circular dichroism, biolayer interference and hydrogen deuterium exchange mass spectrometry to monitor drug regulation/binding to AMPK. In addition, we have use X-ray crystallography to determine the structure of AMPK complexed with various activators. Taken together, these results demonstrate that the drug binding site is located between the kinase domain and the CBM.

Monday AM, Session 1: Advances in understanding the regulation of AMPK

New small-molecule AMPK activators in skeletal muscle

Mark H. Rider
de Duve Institute and Université catholique de Louvain

AMP-activated protein kinase (AMPK) is an attractive therapeutic drug target for treating metabolic disorders such as type 2 diabetes and cancer. We explored the possibility that inhibiting AMP-metabolizing enzymes might be a strategy for potentiating AMPK activation. Pre-incubation of rat epitrochlearis muscles with two pharmacological AMPD inhibitors termed compound 3 (cpd 3) and compound 4 (cpd 4) potentiated rises in AMP, AMP:ATP ratio, AMPK Thr172 and ACC Ser218 phosphorylation induced by electrical stimulation, but without affecting glucose transport. Mice harbouring a whole body AMPD1 deletion were generated and in incubated extensor digitorum longus and soleus muscles from Ampd1 KO mice, increases in AMP levels and AMP:ATP ratio by electrical stimulation were potentiated considerably compared with muscles from wild-type mice. However, enhancement of AMPK activity was significant only in soleus, suggesting control of AMPK by factors other than changes in adenine nucleotides. AMPK activation via inhibition of AMPD would not be a viable approach to treat metabolic disease, but the AMPD inhibitors that have been developed would be useful tools for enhancing AMPK activation in muscle and other cells and tissues during ATP-depletion.

Effects of a small-molecule direct AMPK activator (ex229 from patent application WO2010036613) have been studied in muscle compared with contraction and those of AICAR and A769662 treatment. In incubated rat epitrochlearis muscles, ex229 dose-dependently increased AMPK activity of α1-, α2-, β1- and β2-containing complexes with significant increases in AMPK activity seen at a concentration of 5 μM. At a concentration of 100 μM, AMPK activation was similar to that observed after contraction and importantly led to an ~2-fold increase in glucose uptake. In AMPK α1-/α2-subunit catalytic subunit double knockout myotubes incubated with ex229, the increases in glucose uptake and ACC phosphorylation seen in control cells were completely abolished, suggesting that the effects were AMPK-dependent. When muscle glycogen levels were reduced by ~50% after starvation, ex229-induced AMPK activation and glucose uptake were amplified in a wortmannin-independent manner and in L6 myotubes incubated with ex229, fatty acid oxidation was increased. In summary, ex229 efficiently activated skeletal muscle AMPK and elicited metabolic effects in muscle appropriate for treating type 2 diabetes by stimulating glucose uptake and increasing fatty acid oxidation.

Monday PM, Session 2: AMPK regulation of Metabolism I

A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity

Takashi Kadowaki
University of Tokyo

Adiponectin secreted from adipocytes binds to adiponectin receptors AdipoR1 and AdipoR2, and exerts antidiabetic effects via activation of AMPK and PPAR-α tathways, respectively. Levels of adiponectin in plasma are reduced in obesity, which causes insulin resistance and type 2 dibetes. Thus, orally active small molecules that bind to and activate AdipoR1 and AdipoR2 could ameliorate obesity-related diseases such as type 2 diabetes. Here we report the identification of orally active synthetic small-molecule AdipoR agonists. One of these compounds, AdipoR agonist (AdipoRon), bound to both AdipoR1 and AdipoR2 in vitro. AdipoRon showed very similar effects to adiponetin in muscle and liver, such as activation of AMPK and PPAR-α pathways, and ameliorated insulin resistance and glucose intolerance in mice fed a high-fat diet, which was completely obliterated in AdipoR1 and AdipoR2 double-knockout mice. Moreover, AdipoRon ameliorated diabetes of genetically obese rodent *db/db* mice, and ameliorated neointimal foundation induced by cuff-injury. Finally, AdipoRon prolonged the shortened lifespan of *db/db* mice on a high-fat diet. Thus, orally active AdopoR agonists such as AdipoRon are promising therapeutic approach for the treatment of obesity-related diseases such as type 2 diabetes and cardiovascular disease.

References

1) *Nature Medicine* 7:941-946, 2001, 2) *Nature Medicine* 8: 856-863, 2002, 3) *Nature* 423: 762-769, 2003, 4) *J.Clin.Invest.* 116: 1784-1792, 2006, 5) *Nature Medicine* 13: 332-339, 2007, 6) *Cell Metabolism* 6: 49-64, 2008, 7) *Nature (Article)*464: 1313-1319, 2010, 8) *Proc. Natl. Acad. Sci. USA* 108: 5753-5758, 2011, 9) *Cell Metabolism* 13: 123-124, 2011, 10) *Cell Metabolism* 13: 401-412, 2011, 11) *Cell* 148:624, 2012, 12) *Cell* 148:834, 2012, 13)*Cell Metabolism* 17:185-196,2013, 14) *Nature (Article)* 503: 493-499,2013

Monday PM, Session 2: AMPK regulation of Metabolism I

AMPK-mediated autophagy induction in macrophages mediates immune tolerance.

Masahisa Jinushi
Hokkaido University

Emerging evidences have been unveiled the critical role of macrophages in the regulation of immune suppression and tolerance, greatly influencing the pathogenesis of chronic innhection and cancer. In particular, phagocytosis of apoptotic cells by macrophages serves as one of the hallmark for maintening tissue homeostasis and controlling immunological sttus in local microenvironments.However, it remains largely unknown about the molecular mechanisms by which macrophage-mediated phagocytosis of tumor cells regulates tumor immunosurveillance and modulates therapeutic responses of anticancer agents such as chemotherapy and immunotherapy. In this study, we identified T cell immunoglobulin- and mucin domain-containing molecule-4 (TIM-4) as a indespensable mediator to repress antitumor adaptive immune responses through recognition and engulfment of chemotherapy-medited dying tumor cells. TIM-4 was found to be highly expressed on tumor-associated macrophages (TAMs), and danger-associated molecular patterns (DAMPs), such as HMGB-1, S100A8, ATP, released from chemotherapy-damaged tumor cells have an ability in inducing TIM-4 expression on TAMs recruited from bone marrow-derived precursors. Interestingly, TIM-4-positive populations were mostly identical to the $F4/80^{high}CD11b^{low}CD206(+)$ M2-like subsets of TAMs, arising the possibilityl that there is phenotypic and functional similarities between TIM-4-positive and M2 subsets of TAMs . Upon engulfment of apoptotic tumor cells, TIM-4 was internalized into cytosol and directly interacted with AMPKα1, leading the phosphorylation of AMPKα1 and autophagy initiator ULK1. The TIM-4-AMPKα1 interaction resulted in the activation of autophagic processes which are critical for over-degrading ingested tumors and their associated antigens, leading to reduced antigen presentation and impaired tumor-specific CTL responses. Consistently, blockade of the TIM-4-AMPKα1-autophagy pathway in macrophages augmented the antitumor effect of chemotherapeutics by enhancing tumor-specific CTL responses. Our finding provides insight into the immune tolerance mediated by phagocytosis of dying cells, and pharmaceutical targeting of the TIM-4-AMPKα1 interaction constitutes as a unique strategy for augmenting antitumor immunity and improving cancer chemotherapy.

Monday PM, Session 2: AMPK regulation of Metabolism I

Participation of CDK4 in the regulation of mitochondrial metabolism and energy homeostasis

I.C. Lopez-Mejia
Université de Lausanne

Specific cellular functions, such as proliferation, survival, growth, and senescence, are triggered by specific stimuli and require a specific adaptive metabolic response. Cyclins, cyclin-dependent kinases (CDKs), the transcription factor E2F1, and pRB are major regulators of cell growth and proliferation and as such, they could participate to the onset of adapted metabolic responses. Much has been studied about the participation of cyclin-dependent kinase 4 (CDK4) in cell growth and proliferation, whereas little attention has been paid to its implication in metabolic regulation. Our recent results point to the participation of CDK4 in key steps of mitochondrial function and energy homeostasis.

When fatty acid oxidation (FAO) and glycolysis were measured with the seahorse analyzer in MEFs from *Cdk4*$^{+/+}$, *Cdk4*$^{-/-}$ and *Cdk4*$^{R24C/R24C}$ (carrying an allele encoding for a CDK4 protein that is resistant to the inhibition by the Ink4 family members) embryos, we observed that the deletion of Cdk4 led to an increase in FAO and a decrease in anaerobic glycolysis. On the other hand, the hyperactive Cdk4 mutant drove a significantly decreased FAO and an extremely high glycolytic rate.

Interestingly, the increased oxidative activity in *Cdk4*$^{-/-}$ MEFs was correlated to an increased AMPK activity, suggesting that CDK4 may be a repressor of AMPK. We demonstrated that CDK4 interacts with AMPK, and phosphorylates several AMPK subunits including the AMPK a2 subunit.

Interestingly, AMPK a2$^{-/-}$ MEFs exhibit the same metabolic phenotype as *Cdk4*$^{R24C/R24C}$ MEFs, that is to say increased glycolysis and decreased FAO. Finally, the AMPK activator A769662 fails to further activate FAO in *Cdk4*$^{-/-}$ MEFs.

Overall, our results suggest that CDK4 participates in energy homeostasis through the modulation of AMPK activity via direct phosphorylation.

Monday PM, Session 2: AMPK regulation of Metabolism I

Cycling of AMPK, another way of exercise to combat Type 2 Diabetes?

Yvonne Oligschläger
Maastricht University

Background: AMP-activated protein kinase (AMPK; αβγ) is a key metabolic enzyme involved in maintaining energy homeostasis at different subcellular localizations. Generally, in response to cellular stress, active AMPK increases myocellular insulin-independent glucose import. AMPK is also known to phosphorylate and inhibit glycogen synthase at glycogen. AMPK-glycogen-binding is mediated by the carbohydrate-binding module (CBM) within the β-subunit, but only a subfraction of AMPK is bound to glycogen.
Results: In cell-free assays, inactive AMPK binds to a model sugar, whereas active AMPK does not. AMPK remains attached to the sugar, if upstream kinases are added after initial binding has occurred. This implies that auto-phosphorylation rather than activation is responsible for the loss of sugar binding affinity. X-ray structural data shows that β-threonine 148 (T148) is centrally located within the CBM. As revealed by mutagenesis studies, the phospho-mimicking mutant (T148D) is no longer capable of sugar-binding. This finding has been confirmed in cardiac/muscle cells using GFP-fused CBM-T148D. We also found that CBM-wild-type detects glycogen in cells. Moreover, cellular T148 phosphorylation increases upon AMPK activation, as determined by a phospho-specific T148 antibody.
Conclusions: These data suggest T148 (auto)phosphorylation as a potential mechanism responsible for loss of AMPK-glycogen-binding. Future studies will provide further insight into the impact of full-length β-T148 phosphorylation on localization-dependent function of AMPK. We anticipate that shifting AMPK away from glycogen can have beneficial effects on cardiac/muscle metabolism, e.g. with respect to glycogen storage in diabetic muscle.Manipulating substrate utilization in diabetic muscle by targeting the subcellular localization of AMPK

Monday PM, Session 2: AMPK regulation of Metabolism I

The hypothalamic AMPK-BAT axis: a canonical regulator of energy balance

Miguel López
University of Santiago de Compostela

The AMP-activated protein kinase (AMPK) is the downstream constituent of a kinase cascade that acts as a sensor of cellular energy levels. Current data unequivocally indicate that hypothalamic AMPK plays a key role in the control of the whole body energy balance, by integrating peripheral signals, such as hormones and metabolites, with central signals, such as neuropeptides, and eliciting changes in feeding and energy expenditure, by controlling brown adipose tissue (BAT) thermogenesis. Understanding this key molecule and especially its functions at central level may provide new therapeutic targets for the treatment of metabolic alterations and obesity.

Monday PM, Session 2: AMPK regulation of Metabolism I

A New Target for Anti-inflammatory Effect of Methotrexate - AMP activated protein kinase

Cornelia D. Cudrici
NIH

Background: Methotrexate (MTX) remains a first line therapy for treatment for rheumatoid arthritis and other rheumatic diseases. Although MTX has been used for a many years, the molecular mechanisms of its anti-inflammatory action are not completely understood. Through inhibiting AICAR transformylase, MTX increases the levels of AICAR (aminoimidazole-4-carboxamide ribonucleotide). AICAR is thought to mediate its anti-inflammatory effects through elevating the concentration of adenosine, which can act through G-protein coupled receptors to reduce inflammatory responses. However, AICAR, can mimic AMP to activate AMP-activated protein kinase (AMPK), an important cellular energy sensor that is responsible for maintaining systemic and cellular energy balance. AMPK plays a pivotal role in metabolism, growth, inflammation and immunity. We hypothesize that AMPK activation may mediates a major portion of the anti-inflammatory effects of MTX, and that this may account for some of the the efficacy of MTX in rheumatic diseases.
Methods: Human monocytes derived macrophages (MDM) and murine immortalized bone marrow-derived macrophages (iBMDM) were treated with MTX, AICAR and also with A769662 (a small-molecule which activates AMPK independently of AMP) and AMPK phosphorylation and total AMPK was detected by Western blotting .We have also used compound C, a selective ATP-competitive inhibitor of AMPK in order to determine whether MTX exhibits anti-inflammatory through AMPK. We treated MDM and iBMDM with LPS for six hours following treatment with various concentrations of MTX, AICAR and Compound C and detected production of pro-inflammatory cytokines in vitro.
Results: MTX induced AMPK phosphorylation in a time dependent manner, with effects comparable to the synthetic AMPK activator A 769662 and AICAR .We observed that methotrexate is able to induce AMPK activation and reduce production of pro-inflammatory cytokines (Il-6 and TNF-α) in MDM and iBMDM in response to LPS. Compound C is able to partially everse the effects of MTX on LPS-induced cytokine production
Conclusions: Our findings raise the possibility that some anti-inflammatory effects of MTX are mediated by AMPK. These results suggest that AMPK may be a target for the action of current ‘antimetabolite’ anti-inflammatory agents and a target for the development of new anti-inflammatory drugs.

Monday PM, Session 2: AMPK regulation of Metabolism I

LKB1 and AMPK differentially regulate pancreatic beta cell identity

Guy A. Rutter
Imperial College London

Differentiation of pancreatic beta cells is essential for the normal regulation of insulin secretion and requires the selective repression of genes which may otherwise impair hormone release. Using massive parallel sequencing we show here that highly beta cell-restricted deletion of the tumor suppressor Liver Kinase B1 (LKB1/STK11) strikingly increases the expression of subsets of hepatic (Alb, Iyd, Elovl2) and neuronal (Nptx2, Dlgap2, Cartpt, Pdyn) genes, enhancing glutamate-signaling. These changes are partially recapitulated by the loss of AMP-activated protein kinase (AMPK), which also up-regulates beta cell “disallowed” genes (Slc16a1, Ldha, Mgst1, Pdgfra). Correspondingly, targeted promoters are enriched for neuronal (Zfp206) and hypoxia-regulated (HIF1□) transcription factors. Thus, LKB1 and AMPK, through only partly overlapping mechanisms, maintain beta cell identity by suppressing alternate pathways leading to neuronal, hepatic and other characteristics. Modulation of LKB1 or AMPK signalling may thus provide a means to control the loss of beta cell identity in Type 2 diabetes.

Tuesday AM, Session 3: LKB1 and AMPK-related kinases

Role and regulation of SIK2 in human adipose tissue

Olga Göransson
Lund University

Salt-inducible kinase 2 (SIK2) is an AMPK-related kinase with prominent expression in adipose tissue. We have shown that SIK2 is regulated at many levels by catecholamines, which are highly relevant to adipocyte function. Others have shown that SIK2 is up-regulated in adipose tissue from obese diabetic (db/db) mice. Molecular targets and biological functions of SIK2 in adipose tissue however remain largely unknown, and there are to date no studies of SIK2 in humans or its potential role in the development of obesity and type 2 diabetes.
To address these issues we monitored effects of SIK2 silencing, over-expression and pharmacological inhibition in both rodent and human adipocytes. Moreover, we studied acute and long-term regulation of SIK2 in human adipocytes, as well as its expression in adipose tissue from lean vs obese and/or insulin resistant individuals.
Our data reveal that SIK2 mRNA and protein is markedly down-regulated in subcutaneous adipose tissue from obese and insulin-resistant individuals – in contrast to what was observed in db/db mice. Moreover, SIK2 expression is inhibited by TNF-□ providing a potential mechanism for the reduced expression of SIK2 in obesity. Modulation of SIK2 activity and/or expression demonstrated that CRTC2, CRTC3 and HDAC4 are regulated by SIK2, and that SIK2 is required for GLUT4 expression and glucose uptake in both rodent and human adipocytes. Our results suggest that SIK2 may play a protective role in the development of obesity and/or type 2 diabetes, and warrants further studies of SIK2 regulation and function in adipocytes.

Tuesday AM, Session 3: LKB1 and AMPK-related kinases

The LKB1-SIK pathway in metabolic control

Kei Sakamoto
Nestlé Institute of Health Sciences SA

LKB1 is a master kinase that regulates cellular metabolism and growth through AMPK and 12 other closely-related kinases. Liver-specific ablation of LKB1 causes increased glucose production in hepatocytes in vitro and hyperglycaemia in fasting mice in vivo. We investigated the role that salt-inducible kinases (SIK1, 2 and 3), members of the AMPK-related kinase family, play in gluconeogenic in the liver and other metabolic processes.

Tuesday AM, Session 3: LKB1 and AMPK-related kinases

Targeting the Salt-Inducible Kinases as a novel strategy to treat inflammation

Kristopher Clark
University of Dundee

Macrophages are plastic cells that acquire a pro-inflammatory, classically-activated phenotype to initiate the inflammatory process but switch to an anti-inflammatory, regulatory phenotype to promote the resolution of inflammation. A potential therapeutic strategy for the treatment of chronic inflammatory and autoimmune diseases would therefore be to administer drugs that could induce the formation of regulatory macrophages at sites of inflammation. To this end, we recently identified the Salt-Inducible Kinases (SIKs) as key molecular switches in the programming of regulatory macrophages. Treatment of macrophages with small-molecule inhibitors of the SIKs elevates the production of the anti-inflammatory cytokines IL-10 and IL-1ra while suppressing the production of the pro-inflammatory cytokines IL-6, IL-12 and TNFα. Moreover, this macrophage population also expresses higher levels of markers of regulatory macrophages including LIGHT, SPHK1 and Arginase 1. At the meeting, I will present evidence that the clinically approved anti-cancer drugs Bosutinib and Dasatinib promote the formation of regulatory macrophages at sites of inflammation by inhibiting the SIKs. Notably, the effects of Bosutinib and Dasatinib on macrophage polarization are lost when macrophages are engineered to express a drug-resistant mutant of SIK2. Our findings suggest that both Bosutinib and Dasatinib could be repurposed for the treatment of chronic inflammatory diseases such as rheumatoid arthritis and psoriasis. Importantly, these drugs have few side effects. In contrast to expectations, cancer patients suffering from Type II diabetes show a marked improvement in their glucose handling often leading to a complete cessation of insulin injections after initiating their treatment with Dasatinib. We therefore propose that selective SIK inhibitors will be well tolerated in human patients and will be efficacious in the treatment of chronic inflammatory and autoimmune diseases without overtly destabilizing glucose and fat metabolism.

Tuesday AM, Session 3: LKB1 and AMPK-related kinases

the LKB1-AMPK pathway: metabolic stress leads to metabolic rewiring

Reuben J. Shaw
The Salk Institute for Biological Studies

AMPK is a highly conserved sensor of cellular energy status found in all eukaryotic cells that maintains cellular metabolic homeostasis by reprogramming growth, metabolism, and autophagy in the face of cellular stresses. AMPK is activated by direct binding of AMP and ADP to its regulatory subunits, which enhances it phosphorylation by its upstream kinase, LKB1 which is a tumor suppressor gene frequently inactivated in sporadic human lung cancer and cervical cancer. In addition to this direct connection to cancer, AMPK is activated by a number of diabetes therapeutics and we have studied the role of LKB1 and AMPK in cancer and diabetes in genetically engineered mouse models. Thus LKB1 is a unique energy-state sensitive regulator of growth and metabolic reprogramming via its effects on AMPK. Over the past decade, our laboratory has performed a multi-tiered screen to identify highly conserved direct substrates of AMPK that may mediate its effects on metabolism and growth control. These studies have led to the identification of components of the mTOR signaling pathway (raptor, TSC2), the autophagy pathway (ULK1), and transcriptional regulators of metabolism (Srebp1, HDAC4/5/7) all as direct substrates of AMPK. New methodologies has also led us to additional novel targets shared between AMPK and its related family member or exclusive to AMPK, which further elaborate the processes and themes of metabolic reprogramming in response to energy stress or other hormonal inputs. Based on our molecular understanding, we have pursued a number of strategies to pursue metabolic vulnerabilities of tumors bearing alterations in this pathway, as well as testing potential avenues for treatment of metabolic diseases.

Tuesday AM, Session 3: LKB1 and AMPK-related kinases

Sik2 (Sugar Inducible Kinase!) and Functional Compensation in the Pancreatic Beta Cell

Robert Screaton
Children's Hospital of Eastern Ontario Research Institute

Energy sensing by the AMP-activated protein kinase (AMPK) is of fundamental importance in cell biology. In the pancreatic beta cell, AMPK is a central regulator of insulin secretion. The capacity of the beta cell to increase insulin output is a critical compensatory mechanism in prediabetes, yet its molecular underpinnings are unclear. We have delineated a complex consisting of the AMPK-related kinase SIK2, the CDK5 activator CDK5R1/p35, and the E3 ligase PJA2 essential for beta cell functional compensation. Following glucose stimulation, SIK2 phosphorylates p35 at Ser91, to trigger its ubiquitination via PJA2 and promote insulin secretion. Furthermore SIK2 accumulates in beta cells in models of metabolic syndrome to permit compensatory secretion; in contrast, beta cell knockout of SIK2 leads to accumulation of p35 and impaired secretion. This work demonstrates that the SIK2-PJA2-p35 complex is essential for glucose homeostasis and provides a link between p35-CDK5 and the AMPK family in excitable cells.

Tuesday AM, Session 3: LKB1 and AMPK-related kinases

The Tumor Suppressor LKB1 regulates Myelination through Mitochondrial Metabolism

Biplab Dasgupta
Cincinnati Children's Hospital Medical Center

Myelination of peripheral axons by Schwann cells (SC) is essential for proper transmission of action potentials. A prerequisite to myelination is SC differentiation, and compelling recent evidence indicates that metabolic reprograming from a glycolytic to oxidative metabolism occurs during cellular differentiation. It is unknown if this reprograming is required for SC differentiation. Little is also known about genes that regulate this metabolic transition. We have discovered that metabolic reprograming is required during SC differentiation and the tumor suppressor Lkb1 regulates this critical step. Lkb1 deletion in SCs causes hypomyelination, muscle atrophy, hindlimb dysfunction and peripheral neuropathy. Lkb1 null SCs failed to optimally activate mitochondrial oxidative metabolism during differentiation. Mitochondrial deficits were reflected by diminished oxygen consumption and production of the TCA cycle substrate citrate, which is a precursor to cellular lipids. Consequently, myelin lipids were reduced in Lkb1 mutant sciatic nerves. Citrate synthase (CS) activity was reduced in Lkb1 mutant SCs and overexpression of CS or exogenous citrate rescued differentiation/myelination defects of Lkb1 mutant SCs. We propose that Lkb1-mediated metabolic shift through regulation of CS activity during SC differentiation increases mitochondrial metabolism and lipogenesis, necessary for normal myelination.

Tuesday PM, Session 4: AMPK in heart and skeletal muscle

New metabolic targets of AMPK for treating cardiac pathologies

Luc Bertrand
Université catholique de Louvain

AMPK was firstly described to be a cellular fuel gauge that senses energy deprivation like during myocardial ischemia. In line with this aspect, we and others previously demonstrated that AMPK activation during such stress is protective for the heart. Beside this protective action during myocardial ischemia, the role of AMPK extends to the regulation of other cardiac parameters including insulin sensibility and myocardial hypertrophy. Indeed, AMPK activation is able to increase cardiomyocyte response to insulin. This increase is characterized by an elevation of the insulin-mediated PKB/Akt and AS160 phosphorylation and an overstimulation of glucose uptake. Possible molecular mechanisms able to explain this insulin-sensitizing action of AMPK will be presented. On the other hand, it has been previously shown that AMPK activation by relatively unspecific AMPK activators like metformin or resveratrol was able to block cardiac hypertrophy development. We recently used the more specific AMPK activator A-769662 to verify this paradigm. As expected, A-769662 is a potent hypertrophy inhibitor. Our results also reveal the existence of new AMPK downstream targets involved in this anti-hypertrophic action of AMPK. These new targets will be detailed during the presentation.

Tuesday PM, Session 4: AMPK in heart and skeletal muscle

LKB1-AMPK regulation of the growth and the response to injury in the heart

Lawrence H. Young
Yale University School of Medicine

The LKB1-AMPK pathway plays a critical role in the cardiac stress response to ischemia and pressure overload. This talk will discuss AMPK regulation of mitochondrial function in the ischemic heart, where AMPK maintains mitochondrial oxidative capacity and prevents oxidative stress during reperfusion that leads to JNK pathway activation and permeability transition pore opening. We will also present data on the modulation of AMPK pathway activation by D-dopachrome tautomerase a novel ligand of the CD74 receptor.

Tuesday PM, Session 4: AMPK in heart and skeletal muscle

Reduced scar maturation and contractility lead to exaggerated left ventricular dilation after myocardial infarction in mice lacking AMPKalpha1

Sandrine Horman
Université catholique de Louvain

Objective: Cardiac fibroblasts (CF) are crucial in left ventricular (LV) remodelling after myocardial infarction (MI). They predominantly express the alpha1 catalytic subunit of AMP-activated protein kinase (AMPKalpha1), while AMPKalpha2 is the major catalytic isoform in cardiomyocytes. AMPKalpha2 is known to protect the heart by preserving the energy charge of cardiac myocytes during injury, but whether AMPKalpha1 interferes with maladaptative heart responses remains unexplored. In this study, we aim at further substantiating the role of this AMPK isoform in the pathogenesis of post-MI LV remodelling and more particularly in the regulation of fibrotic properties of CF.

Methods: AMPKalpha1 knockout (KO) and wild type (WT) mice were subjected to permanent ligation of the left anterior descending coronary artery to mimic MI. Cardiac fibrosis was monitored using QRT-PCR analysis, histology and immunohistofluorescent staining. LV function and remodelling was assessed by echocardiography.

Results: In the absence of AMPKalpha1, the CF proliferative response was increased in infarcted myocardia. It resulted in elevated levels of fibrotic factors but did not lead to excessive matrix deposition or degradation in KO infarcts. While CF proliferation was increased, expression of the myodifferentiation marker alpha-smooth muscle actin was decreased. This faulty maturation of myofibroblasts might derive from down-regulation of the transforming growth factor-beta1/p38 mitogen-activated protein kinase pathway in KO infarcts. Although infarct size was similar in KO and WT hearts subjected to MI, these changes resulted in defective scar collagen maturation. This was associated with an exacerbated adverse remodelling as indicated by increased LV diastolic dimension 30 days after MI.

Conclusion: Our data genetically demonstrate the centrality of AMPKalpha1 in post-MI scar formation and highlight the specificity of this catalytic isoform in cardiac fibroblast/myofibroblast biology.

Tuesday PM, Session 4: AMPK in heart and skeletal muscle

AMP-activated protein kinase is necessary for cardiorespiratory adjustments during hypoxia

Mark Evans
University of Edinburgh

The AMP-activated protein kinase (AMPK) has been proposed to underpin appropriate cardiorespiratory adjustments during hypoxia (Evans et al., 2006), and has been identified as an effector of hypoxic pulmonary vasoconstriction (Evans et al., 2005) and a modulator of carotid body excitation during hypoxia (Wyatt et al., 2007; Ross et al., 2011). Our further investigations suggest that AMPK may adjust cell function by regulating a variety of ion channels that have been variously categorized as “oxygen-sensing”, including the large conductance Ca^{2+}- and voltage-activated potassium channel (BK_{Ca}), Kv2.1 (Evans et al., 2005; Ross et al., 2011; Ikematsu et al., 2011) and most recently Kv1.5 (unpublished). Recombinant, active AMPK heterotrimers and AMPK activators appropriately regulate macroscopic currents carried by identified hypoxia-sensitive channels of pulmonary arterial myocytes, carotid body type I cells and hippocampal neurons. Consistent with these findings AMPK directly phosphorylates and appropriately regulates the recombinant counterpart of many hypoxia-sensitive channels stably expressed in HEK 293 cells. Moreover, our developing studies on wild type and transgenic mice further strengthen the proposal (Evans, 2006) that AMPK is key to hypoxic pulmonary vasoconstriction and ventilatory adjustments during hypoxia. Therefore AMPK regulates O_2 and thus energy (ATP) supply at the cellular and whole body level.

Evans et al. (2005). J. Biol Chem., 280, 41505-41511.
Evans (2006). J. Physiol., 574, 113-123.
Ikematsu et al. (2011). PNAS, 108,18132-7
Ross et al. (2011). J. Biol. Chem., 286, 11929-11936.
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Tuesday PM, Session 4: AMPK in heart and skeletal muscle

PRKAG2 – insights into heart rate regulation

Arash Yavari
University of Oxford

Background
Mutations in the γ2 subunit of AMP-activated protein kinase (AMPK) – a conserved serine threonine kinase regarded as the cell's energy sensor – cause a cardiomyopathy characterised by left ventricular hypertrophy, bradycardia and conduction disease, ventricular pre-excitation and myocardial glycogen storage. Existing overexpressing transgenic models suggest glycogen excess to be the cause for pre-excitation, but do not explain the prominent sinus bradycardia occurring in patients. Whether γ2 might play a role in the physiological modulation of heart rate is also unknown.

Methodology
We used gene-targeting to generate a faithful disease model by inserting the R302Q mutation into the equivalent position (R299Q) of the orthologous murine γ2 gene (*Prkag2*). To explore the physiological role of γ2 we generated a global γ2 knock-out (KO).

Results
The R299Q γ2 mutation induces basal activation of γ2-containing AMPK complexes. Cardiac phenotyping of R299Q γ2 knock-in mice revealed no evidence of cardiomyopathy (MRI and invasive haemodynamics) and no pre-excitation, but did identify intrinsic sinus bradycardia. This concurred with downregulation of *Hcn* expression (encoding f-channels which conduct the sinus node pacemaker 'funny' current, I_f). Isolated sinoatrial node (SAN) myocytes from R299Q γ2 knock-in mice exhibited reduced automaticity and I_f density, with no direct effect of mutant γ2 on f-channels. Immunoblotting for γ2 revealed a relative enrichment of this γ subunit isoform in murine sinus node. To investigate whether γ2 might play a physiological role in modulating heart rate, global γ2 KO mice were generated. These mice displayed increased intrinsic heart rate, isolated sinus cell rate, I_f and *Hcn* expression. Chronic endurance exercise using a voluntary computerised running wheel protocol induced activation of cardiac AMPK and an intrinsic bradycardia together with reduced isolated SAN cell rate and I_f in wild type Cre+ control mice but failed to do so in γ2 KO mice despite equivalent distances run.

Conclusions
Mutant R299Q γ2 alters SAN automaticity and pacemaking via an effect on *Hcn* expression and I_f. The sinus bradycardia and chronotropic incompetence in patients with *PRKAG2* mutations likely reflects in part an f-channelopathy. The contrasting findings from the γ2 KO coupled with the finding that γ2 is an important mediator of athletic bradycardia argue for a novel physiological role for γ2 in the regulation of mammalian intrinsic heart rate. Thus γ2 AMPK is a regulator of whole-organ energy homeostasis via modulation of sinus rate and myocardial oxygen and hence energy consumption.

Tuesday PM, Session 4: AMPK in heart and skeletal muscle

Role of AMPK in regulation of muscle metabolism during exercise

Erik A. Richter
University of Copenhagen

During muscle contraction/exercise the metabolic rate in muscle increases many times. An important fuel for contracting muscle is glucose extracted from the blood. It has been a matter of controversy whether AMPK is regulating muscle glucose uptake during exercise or whether Ca^{++} released from the sarcoplasmic reticulum apart from initiating muscle contraction also increases muscle glucose uptake. Our experiments aimed at investigating the relative role of AMPK and Ca^{++} in increasing glucose uptake during muscle contractions have shown that AMPK, and not Ca^{++}, is directly involved in increasing muscle glucose uptake during contractions. However, AMPK is not fully accountable for increasing muscle glucose uptake. It is well known that stretch of muscle also increases muscle glucose uptake. Our experiments indicate that stretch-activated glucose uptake in muscle is independent of AMPK. Together, metabolic activation of AMPK and stretch of muscle fibers can account for contraction-induced muscle glucose uptake.

Tuesday PM, Session 4: AMPK in heart and skeletal muscle

A role for PKC in the regulation of VEGF-stimulated AMPK?

Helen R. Heathcote
University of Glasgow

AMP-activated protein kinase (AMPK) has recently been demonstrated to be inhibited by phosphorylation of the α1 catalytic subunit at Ser485 (Ser491 in α2). Several Ser485/Ser491 kinases have been proposed, including PKA and Akt. We have previously demonstrated that vascular endothelial growth factor (VEGF), the key stimulus for endothelial cell proliferation and migration transiently activates AMPK in a Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK)-dependent manner in human endothelial cells (ECs). We further demonstrated that AMPK is required for VEGF-stimulated endothelial cell proliferation. Intriguingly, despite the requirement for AMPK in VEGF-stimulated endothelial cell proliferation, activation of AMPK alone suppressed proliferation in the absence of VEGF, consistent with many studies in non-endothelial cells. Our published data therefore indicate that VEGF-mediated regulation of AMPK may involve mechanism(s) other than phosphorylation and activation of AMPK at Thr172. We therefore examined whether VEGF regulated AMPK Ser485 phosphorylation in ECs by immunoblotting of lysates from ECs incubated in the presence or absence of VEGF and selective kinase inhibitors.

VEGF robustly stimulated AMPKα phosphorylation at both Thr172 and Ser485/491, yet the time course of phosphorylation at each site was different, with Thr172 phosphorylation preceding Ser485 phosphorylation and Ser485 phosphorylation maintained for ~20 minutes. VEGF has been demonstrated to activate multiple protein kinases including Akt, ERK and PKC. Incubation of ECs with the Akt inhibitor AKti (1μM, 60 mins) had no effect on VEGF-stimulated phosphorylation of AMPK Ser485. VEGF-mediated phosphorylation of AMPKα Ser485 was abrogated by the removal of extracellular calcium, but was not altered by the CaMKK inhibitor STO-609, at concentrations that inhibit Thr172 phosphorylation (10μM, 60 mins), indicating that VEGF does not cause autophosphorylation of Ser485, yet phosphorylates in a Ca^{2+}-dependent manner. In contrast, the PKC inhibitor GF109203X (1μM, 60mins) abolished VEGF-stimulated Ser485 phosphorylation ($n=3$, $p<0.01$), under conditions that did not alter VEGF-stimulated AMPK phosphorylation at Thr172. We therefore examined the role of PKC in VEGF-mediated Ser485 phosphorylation. The PKC activator phorbol myristate acetate (PMA) rapidly stimulated Ser485 phosphorylation. Furthermore, downregulation of PKC isoforms using targeted siRNA or chronic incubation with PMA (200 nM, 24 hours) attenuated VEGF-stimulated Ser485 phosphorylation.

These data suggest that VEGF-stimulated AMPK phosphorylation at Thr172 and Ser485 is mediated via post-VEGF receptor signalling pathways dependent on calcium, but independent of each other, with Thr172 phosphorylation mediated by CaMKK and Ser485 phosphorylation mediated by PKC. PKC-mediated AMPK phosphorylation at Ser485 represents a novel mechanism of AMPK regulation.

Tuesday PM, Session 4: AMPK in heart and skeletal muscle

The AMPKα2 subunit in myeloid cells, but not in endothelial cells, regulates post-ischemic revascularization through modulation of the HIF-1α pathway

Randa Abdel Malik
Goethe-University

Immune cells play a key role in regulating angiogenesis through the production and release of a wide range of angiogenic mediators. Given that the AMP-activated protein kinase (AMPK) is activated in response to hypoxia and other cellular stresses, we investigated the consequences of deleting the α2 subunit in myeloid cells on angiogenesis and vascular repair as well as neutrophil function.

Wild-type and AMPKα2$^{-/-}$ mice were subjected to femoral artery ligation. The recovery of blood flow was significantly impaired (80%) in AMPKα2$^{-/-}$ mice versus the wild-type littermates as measured by laser Doppler analysis. To determine the reason for the impaired revascularization in AMPKα2$^{-/-}$ mice, hindlimb ischemia was induced in mice lacking the AMPKα2 in endothelial (AMPKα2flox/flox x VE-Cadherin Cre, AMPKα2$^{\Delta EC}$) or myeloid cells (AMPKα2flox/flox x LysM Cre, AMPKα2$^{\Delta MC}$) specifically. Surprisingly, blood flow recovery was unaffected in AMPKα2$^{\Delta EC}$ mice. In contrast, reperfusion of the ischemic limb after surgery was significantly impaired (80%) in AMPKα2$^{\Delta MC}$ mice. This could be linked to reduced ischemic limb capillary densitiy (CD31 staining) and to a reduced collateral formation in the thigh muscle of AMPKα2$^{\Delta MC}$ mice (μ-CT angiography). In addition, myeloid cell infiltration into the ischemic muscle was altered in AMPKα2$^{\Delta MC}$ mice as determined by flow cytometry. At Day 1 post-surgery neutrophil number was increased compared to wild-type mice, however, 3 days after surgery AMPKα2$^{\Delta MC}$ mice showed significantly decreased myeloid cell infiltration into the ligated limb. Mechanistically, downregulation of the α2 subunit, but not the α1 subunit, in HEK293 cells significantly reduced HIF-1α protein expression after incubation in 1% hypoxia or stimulation with cobalt chloride. Gene expression of the angiogenic factors SDF-1α and MMP-9 were significantly decreased in neutrophils isolated from AMPKα2$^{\Delta MC}$ mice. On the other hand, inflammatory mRNA expression levels of TNF-α and IL-1β were increased.

These results indicate that the AMPKα2 plays a crucial role in regulating the inflammatory state and angiogenic potential of myeloid cells promoting vascular repair in vivo by modulating HIF-1α signalling.

Wednesday AM, Session 5: Mouse models to study AMPK signaling pathways

AMPK in gonads, more than a cell energy sensor

Pascal Froment
INRA

Oogenesis and spermatogenesis are complex and dynamic processes that are regulated by endocrine, paracrine and autocrine signals. These signals are exchanged between the oocyte and the somatic cells of the follicle or in male between male germ cells and Sertoli cells. The role of AMP-activated protein kinase (AMPK), an important regulator of cellular energy homeostasis was analyzed.

A deficiency in alpha1AMPK in male mice led to loss of fertility associated with hyperandrogenism of testicular origin. Strangely, no modification of the testis structure was observed : neither in testis size or cell composition nor in apoptosis or in proliferation . However, sperm a1AMPK-/ - show a lack of mobility in association with head structural anomalies, compared with male controls. Mitochondria in spermatozoa present also alteration and could be a cause of the low mobility. In addition, hyperandrogenia is linked to hyperactivity in Leydig cells. Gonadotropins secretion did not seems to be modified in a1AMPK-/ - mice.

In female mice, we have specifically excised a1AMPK in oocyte to assess the function of this kinase in female germ cells. A decrease about 20% in oocyte production was observed in ZP3-α1AMPK-/- female mice. As in male, increasing abnormal mitochondria and alterations in the mitochondria distribution were correlated with a decrease in the mitochondrial function as attested by a decrease in ATP production. When compared to wild-type oocytes, the expression of important junctional proteins such as Cx37 was reduced in mutant animals.

Both in male and female transgenic model, absence of α1AMPK induced a reduction in Histone Deacetylase activity which was associated with an increase in Histone H3 acetylation at the K9/K14 residue.

In conclusion, these results suggest that AMPK modifies germ cells competence through energy processes, cytoskeleton and germ cells/somatic cell communication.

Wednesday AM, Session 5: Mouse models to study AMPK signaling pathways

Tissue-specific deletion of AMPK catalytic subunits: defining AMPK-dependent and -independent metabolic actions

Benoit Viollet
INSERM U1016

To sustain metabolism, intracellular ATP concentration must be regulated within an appropriate range. This coordination is achieved through the function of the AMP-activated protein kinase (AMPK), a cellular “fuel gauge”. AMPK integrates multiple extra- and intracellular input signals directly or indirectly linked to limited energy availability to coordinate a wide array of compensatory, protective, and energy-sparing responses at the cellular, organ, and organism levels. By maintaining energy balance, both at the single-cell and the whole-body levels, activation of AMPK appears to be a unique challenge to prevent and treat the metabolic abnormalities associated with the metabolic syndrome. Generation of tissue-specific deletion of AMPK catalytic subunits have largely contributed to the elucidation of the role of the energy sensor AMPK during metabolic adaptation as well as its potential therapeutic use for metabolic disorders: by using liver-specific AMPK KO mice, we challenged the paradigm that hepatic AMPK is required for acute gluconeogenesis regulation by showing that AMPK is dispensable for metformin- and AICAR-mediated suppression of glucose production in primary hepatocytes and AICAR-induced increase in glucose disappearance in vivo. We also provided evidence for the crucial role of AMPK in the regulation of hepatic lipid metabolism following indirect and direct AMPK agonist administration. In addition, we demonstrated that AMPK acts as a sensor and safeguard of the hepatic adenylate energy pool during an acute challenge to the energy status of the liver. By using muscle-specific AMPK KO mice, we reinforced the central role of AMPK in regulating skeletal muscle exercise capacity and mitochondrial function by showing that loss of muscle AMPK impairs endurance exercise capacity most likely due to defects in the regulation of mitochondrial oxidative phosphorylation. Lastly, by using mice lacking AMPK in the myeloid lineage, we reported both a delay and an impairment of muscle regeneration. We established the predominant role for AMPKα1 in phagocytosis-induced macrophage skewing from pro- to anti-inflammatory phenotype at the time of resolution of inflammation. These results have important implications for therapeutics designed to target the pathogenesis of both metabolic and inflammatory diseases.

Wednesday AM, Session 5: Mouse models to study AMPK signaling pathways

A novel mouse model for studying the effects of AMPK activation in vivo

Angela Woods
MRC Clinical Sciences Centre

In order to investigate the effects of constitutive AMPK activation *in vivo*, a transgenic mouse model has been created by inducing the expression of an AMPKγ1 subunit, containing an activating mutation, using the CreLoxP system. This mutation has been shown to produce an AMPK complex in transfected cells 3-5 times more active than the wild type protein, and that is resistant to allosteric AMP activation and dephosphorylation.
We have generated mice expressing this activated form of AMPK in different tissues in order to determine the tissue specific roles AMPK plays *in vivo*.

Wednesday AM, Session 5: Mouse models to study AMPK signaling pathways

AMPK regulation of lipid metabolism

Gregory R. Steinberg
McMaster University

The AMP-activated protein kinase (AMPK) is acutely regulated by a number of hormones and nutrients to maintain cellular energy balance. Energy homeostasis is achieved through direct phosphorylation of more than 30 substrates that alter all aspects of cellular growth and metabolism. Importantly, AMPK is also switched on by exercise, metformin and salicylate. Acetyl-CoA carboxylase (ACC) was the first identified AMPK substrate (ACC1 at Ser79 and ACC2 at Ser212). ACC generates malonyl-CoA, a metabolic intermediate that is essential for fatty acid synthesis and an allosteric inhibitor of fatty acid oxidation. We have generated mice harboring a targeted knock-in (Ser-Ala) mutation in both ACC1 and ACC2 and have crossed these mice to generate ACC double knock-in (DKI) mice (Fullerton et al. Nat Med 2013). We find that on a control chow diet ACC DKI mice have elevated lipogenesis and lower fatty acid oxidation leading to fatty liver, glucose intolerance and insulin resistance, but not obesity. Remarkably, ACC DKI mice made obese by high-fat diet feeding, are refractory to the lipid-lowering and insulin-sensitizing effects of metformin due to an impaired ability to suppress liver de novo lipogenesis. These studies establish that inhibitory phosphorylation of ACC is essential not only for the control of lipid metabolism, but also insulin sensitivity. In this presentation we will discuss recent findings linking AMPK regulation of fatty acid metabolism and its potential importance for the treatment of non-alcoholic fatty liver disease, insulin sensitivity and cellular proliferation.

Wednesday AM, Session 5: Mouse models to study AMPK signaling pathways

Co-activation of AMPK and mTORC1 is synthetically lethal in acute myeloid leukemia

Jérôme Tamburini
INSERM U1016

Virtually all recurrent molecular alterations in acute myeloid leukemia (AML) functionally converge to cause signal transduction pathway dysregulation that drives cellular proliferation and survival. The mammalian target of rapamycin complex 1 (mTORC1) is a rapamycin-sensitive signaling node defined by the interaction between mTOR and raptor. Pharmacologic inhibition with rapamycin or second-generation mTOR kinase inhibitors has shown limited anti-AML activity, suggesting that addiction to this oncogene is not a recurrent event in AML. Here we report that sustained mTORC1 activity is nonetheless essential for the cytotoxicity induced by pharmacologic activation of AMP-activated protein kinase (AMPK) in AML.

Our studies employed a novel AMPK activator (GSK621). Using CRISPR and shRNA-mediated silencing of the AMPK [illegible] vity was necessary for the anti-leukemic response induced by this agent. GSK621-induced AMPK activation precipitated autophagy. Blocking autophagy via shRNA-mediated knockdown of ATG5 and ATG7 protected AML cells from GSK621, suggesting that autophagy promotes cell death in the context of active AMPK. GSK621 cytotoxicity was consistently observed across twenty different AML cell lines, primary AML patient samples and AML xenografts in vivo. GSK621-induced AMPK activation also impaired the self-renewal capacity of MLL-ENL- and FLT3-ITD-induced murine leukemias.

Strikingly, GSK621 did not induce cytotoxicity in normal CD34+ hematopoietic progenitor cells. We hypothesized that the differential sensitivity to GSK621 could be due to the difference in amplitude of mTORC1 activation in AML and normal CD34+ cells. In contrast to most reported cellular models in which AMPK inhibits mTORC1 both directly (through raptor phosphorylation) and indirectly (through TSC2 phosphorylation), sustained mTORC1 activity was seen following GSK621-induced AMPK activation in AML. Inhibition of mTORC1 abrogated AMPK-induced cytotoxicity in AML cells, including primary AML patient samples. This protective effect was mediated by mTORC1-dependent modulation of the ATF4/CHOP stress response pathway. The ultimate functional consequence was that, rather than diminishing GSK621-induced cytotoxicity, persistent mTORC1 activity was in fact synthetically lethal with AMPK activity in AML cells. This synthetic lethality could be recapitulated in normal CD34+ progenitors by constitutive activation of mTORC1 using a lentivirally-transduced myrAKT construct. It could also be enhanced in AML cells by mTORC1 overactivation induced by CRISPR-mediated deletion of *TSC2*.

Taken together, these data show that the magnitude of mTORC1 activity determines the degree of cytotoxicity triggered by AMPK activation. Our results therefore support AMPK activation as a promising therapeutic strategy in AML and other mTORC1-active malignancies which warrants further investigations in clinical trials.

Wednesday AM, Session 5: Mouse models to study AMPK signaling pathways

AMP-activated protein kinase suppresses inflammatory signalling and chemokine secretion in adipose tissue

Ian P. Salt
University of Glasgow

Background: Obesity is characterized by hypertrophic adipocytes and increased infiltration of macrophages which stimulate production of cytokines and chemokines, including tumour necrosis factor-α (TNFα), interleukin-1β (IL-1β) and interleukin-6 (IL-6), which have been proposed to play a key role in insulin resistance. AMP-activated protein kinase (AMPK) has been reported to have anti-inflammatory actions independent of its effects on carbohydrate and lipid metabolism, yet the role of AMPK in the regulation of adipose tissue inflammatory signalling is currently poorly characterised

Methods: 3T3-L1 adipocytes and RAW 264.7 macrophages were incubated in the presence or absence of the AMPK activators prior to incubation with pro-inflammatory stimuli. Secretion of chemokines into conditioned media was determined by multiplex bead immunoassay or ELISA. Lysates and cDNA were prepared from adipose tissue from *AMPKα1*$^{-/-}$ mice and *AMPKα1*$^{+/+}$ littermates. Phosphorylation and activity of signalling intermediates were determined by immunoblotting. Expression of classically activated, pro-inflammatory (M1) and alternatively-activated, anti-inflammatory (M2) macrophage markers was assessed by qRT-PCR.

Results: A769662 and infection with adenovirus expressing a constitutively active AMPK mutant suppressed IL-1β-stimulated NFκB nuclear translocation in 3T3-L1 adipocytes. Conversely, this was abrogated upon adenoviral expression of a dominant negative AMPKα1 mutant. AMPK activation also ameliorated TNFα/IL-1β-stimulated phosphorylation of IκB, IKK, JNK, ERK1/2 and p38 MAPKs in 3T3-L1 adipocytes. AMPK activation also attenuated IL-6-stimulated STAT3 phosphorylation. In 3T3-L1 adipocytes, AMPK activators abrogated cytokine-stimulated secretion of IL-6 and the chemokines IP-10 (CXCL10), KC (CXCL1) and MCP-1. Furthermore, AMPK activation reduced secretion of IL-5, MCP-1 and MIP-1α from LPS-stimulated RAW 264.7 macrophages. AMPK activation inhibited differentiation of pre-adipocytes into adipocytes that was associated with an early (12-48 hours) inhibition of STAT3 phosphorylation. Both visceral and subcutaneous adipose tissue from *AMPKα1*$^{-/-}$ mice exhibited increased basal phosphorylation of STAT3 and JNK, supporting a role for AMPK in the suppression of pro-inflammatory signalling pathways in vivo. Expression of adipose tissue M1 (*Tnf, Nos2*) and M2 (*Arg1, Chi313/Ym1, Retnla/Fizz1*) macrophage markers were not significantly different between genotypes, although there was a tendency toward reduced expression of the anti-inflammatory M2 macrophage markers *Arg1* and *Retnla/Fizz*1 in adipose from *AMPKα1*$^{-/-}$ mice.

Conclusion: AMPK activation in adipose tissue suppresses multiple proinflammatory signalling pathways and secretion of cytokines and chemokines. Although there were no significant differences in M1 and M2 marker gene expression, *AMPKα1*$^{-/-}$ mice exhibit a modest upregulation of basal proinflammatory signalling.

Wednesday PM, Session 6: AMPK and Cancer

Role of the fuel-sensing enzyme AMPK in astrocytic tumor cell proliferation

Rosa Señarís
Universidade de Santiago de Compostela

AMPK is an energy sensor that controls cell metabolism, and it has been related with apoptosis and cell cycle arrest. Although its role in metabolic homeostasis is well documented, its function in cancer is much less clear. In this study, we examined the role of AMPK in several models of mice astrocytoma, human glioblastomas and xenografts. AMPK was constitutively activated in mice astrocytes expressing oncogenic H-RasV12 and PTEN deletion in parallel with high cell division rates. In clinical specimens of human glioblastoma, elevated levels of activated AMPK appeared especially in areas of high proliferation surrounding the blood vessels. Genetic deletion of AMPK or attenuation of its activity in these mice cells and in human tumor cells was sufficient to reduce cell proliferation. The levels of pAMK were always related to the levels of phosphorylated Rb at Ser804, which might indicate an AMPK mediated phosphorylation of Rb. Moreover, we demonstrated a role of the high levels of pAMK of these tumors in lipid tumor cell metabolism, an essential function to maintain the high proliferation levels. Together, our findings indicate that the initiation and progression of astrocytic tumours relies upon AMPK-dependent control of the cell cycle and metabolism, thereby identifying AMPK as a candidate therapeutic target for these and other type of tumors with high levels of AMPK .

Wednesday PM, Session 6: AMPK and Cancer

Reduced AMPK activation and expression correlates with highly aggressive growth in hereditary renal cancer caused by fumarate hydrates deficiency

Tracey A. Rouault
NIH

Hereditary leiomyomatosis and renal cancer (HLRCC) is an inherited syndrome caused by loss of fumarate hydratase (FH). In general, patients have a germline mutation in one allele, and cancer develops in renal cells that lose a second copy of the allele through mutation or deletion. Uterine leiomyomas are not malignant, but renal cancers are extremely aggressive. Cell lines from renal cancers of HLRCC patients were compared to cell lines from cancers caused by mutations in VHL and SDHB. FH deficient cell lines manifested reversal of the TCA cycle, aerobic glycolysis, and decreased expression and phosphorylation of AMPK. We hypothesize that reduced AMPK activity promotes lipid synthesis and anabolic reactions that enhance growth and metastasis. Other less aggressive renal cancers did not manifest significant changes in AMPK expression or activation.

Reference:
Tong, W. H., Sourbier, C., Kovtunovych, G., Jeong, S. Y., Vira, M., Ghosh, M., Romero, V. V., Sougrat, R., Vaulont, S., Viollet, B., Kim, Y. S., Lee, S., Trepel, J., Srinivasan, R., Bratslavsky, G., Yang, Y., Linehan, W. M., and Rouault, T. A. (2011). The glycolytic shift in fumarate-hydratase-deficient kidney cancer lowers AMPK levels, increases anabolic propensities and lowers cellular iron levels. Cancer Cell *20*, 315-327.

Wednesday PM, Session 6: AMPK and Cancer

Repression of LKB1 by miR-17-92 is required for Myc-driven metabolism in B-cell lymphoma

Said Izreig
McGill University

Cancer is a disease characterized by uncontrolled cell proliferation. Oncogene activation often engages pathologic metabolic programs to meet the demands of malignant growth. Increased expression or activity of the proto-oncogenic transcription factor Myc is observed in many human cancers. Myc acts to orchestrate metabolic reprogramming in tumor cells through the coordinated expression of metabolic pathway genes, including enzymes that regulate glycolytic and tricarboxylic acid (TCA) cycle metabolism. Among the transcriptional targets of Myc are microRNAs (miRNAs), which are small, non-coding RNAs that negatively regulate the translation of target mRNAs. Here we demonstrate that the Myc-activated miR-17-92 miRNA cluster is required for Myc-dependent changes in lymphoma metabolism. Deletion of the miR-17-92 in Myc-dependent B cell lymphomas (Δ/Δcells) resulted in a significant reduction in tumor metabolism, marked by decreases in glycolysis, glutamine metabolism, and mTORC1 activity. We have identified miR-17 and -20 as the key components of the miR-17-92 cluster that regulate tumor cell metabolism. We demonstrate that the tumor suppressor LKB1 is a direct target of miR-17/20, and that miR-17/20 negatively regulates LKB1 protein expression in cells. Re-expression of miR-17/20 or silencing of LKB1 expression via shRNA in Δ/Δcells rescued the metabolic defects of these tumor cells and restored their tumorigenic potential in vivo. Finally, we demonstrate that the mitochondrial complex I inhibitor phenformin, whose cytotoxicity is partly dependent on LKB1 expression, induces cell death in human B-lymphoma cell lines with high Myc or miR-17-92 expression. Taken together, these observations highlight a novel regulatory circuit whereby Myc enhances pro-proliferative metabolism in lymphoma through miRNA-dependent suppression of LKB1 signaling. Our data provide preclinical rationale for the use of phenformin in treating Myc-dependent lymphoma where LKB1 expression is repressed.

Wednesday PM, Session 6: AMPK and Cancer

AMPK phosphorylates novel substrate PEA15 to facilitate anchorage-independent growth of mammary cells

Annapoorni Rangarajan
Indian Institute of Science

Matrix-detachment triggers a form of apoptosis, termed as ‘anoikis’, in epithelial cells. In contrast, acquisition of anoikis-resistance is a hallmark of solid tumors. Interestingly, recent studies have uncovered that even within the normal epithelia, there exists a very small population of cells that can survive matrix-detachment and generate three-dimensional structures when cultured in suspension in vitro. Such floating spheroids generated by normal human mammary epithelial cells (HMECs) have been termed as “mammospheres” that are enriched in stem/progenitor cells. Therefore, understanding the molecular mechanisms that enable normal HMECs to withstand anoikis and generate mammospheres is likely to provide important insights into normal mammary gland biology as well as breast cancer progression.

Here we show that mammosphere formation by normal HMECs is accompanied with increased activation of AMP-activated protein kinase (AMPK). Pharmacological inhibition or knockdown of AMPK abrogaded mammosphere formation, revealing that AMPK activation is required for mammosphere formation. Further, inhibition of AMPK led to increased apoptosis in suspension, suggesting that AMPK may contribute to anoikis-resistance during mammosphere formation.

Concomitant with AMPK activation, we detected increased Ser116 phosphorylation of the anti-apoptotic protein PED/PEA15, and its recruitment to the DISC complex. Knockdown of PEA15, or overexpression of the non-phosphorylatable S116A mutant of PEA15, increased apoptosis and abrogated mammosphere formation. Further, inhibition of AMPK prevented PEA15 phosphorylation in suspension, together suggesting that Ser116 phosphorylation of PEA15 downstream of AMPK activation may play a critical role in the inhibition of apoptosis and mammosphere formation. We further demonstrate that AMPK directly interacts with and phosphorylates PEA15 at Ser116 residue, thus identifying PEA15 as a novel AMPK substrate.

Since anchorage-independent growth property is a hallmark of solid tumors, we investigated the role of the AMPK-PEA15 axis in solid tumor growth. Breast cancer cells cultured as spheroids showed elevated phosphorylation of AMPK and PEA15. Like in normal HMECs, inhibition or knockdown of AMPK or overexpression of S116A mutant of PEA15 led to increased apoptosis, abrogaded sphere formation in vitro, and impaired tumor formation xenograft mouse models. Thus, our study identifies a novel AMPK-PEA15 signaling axis in the anchorage-independent growth of both normal and cancerous mammary epithelial cells, suggesting that breast cancer cells may employ mechanisms of anoikis-resistance already inherent within a subset of normal HMECs. Thus, targeting the AMPK-PEA15 axis might prevent breast cancer dissemination and metastasis.

Wednesday PM, Session 6: AMPK and Cancer

Regulation of tumor metabolism by the LKB1-AMPK pathway

Russell G. Jones
McGill University

The AMP-activated protein kinase (AMPK) is a metabolic sensor that helps maintain energy homeostasis in mammalian cells and tissues. AMPK is situated in the center of a signaling network involving established tumor suppressors (LKB1, TSC2, p53) and cell growth regulators (mTORC1, HIF-1α). However, the role of AMPK in cancer remains controversial as AMPK can exert pro- or anti-tumorigenic roles depending on context. We have recently demonstrated that AMPK can function as a tumor suppressor in Myc-dependent lymphoma, and that loss of LKB1-AMPK signaling promotes a metabolic shift to the Warburg effect that enhances tumor cell growth and proliferation. Here we will discuss a novel regulatory circuit that influences pro-growth metabolism in tumor cells through miRNA-dependent suppression of the LKB1-AMPK pathway. We will also present evidence supporting a role for AMPK in the regulation of mitochondrial OXPHOS and ROS production. The implication of these data for tumor cell growth, metabolism, and adaptation to metabolic stress will be discussed.

This work was supported by grants from the CIHR (MOP-93799) and Terry Fox Foundation (TFF-116128).

Wednesday PM, Session 6: AMPK and Cancer

Metabolic reprogramming in cancer supports cell growth and survival

Almut Schulze
Würzburg University

The hallmarks of cancer include uncontrolled proliferation, reduced cell death and the loss of tissue homeostasis. The loss of normal control of cell growth and proliferation is the consequence of aberrant regulation of cellular signaling pathways through the activation of oncogenes or loss of tumor suppressor function. Alterations in metabolic activity have emerged as one of the features of cancer cells and many oncogenic signaling pathways directly regulate the activity of metabolic processes. We have investigated the involvement of metabolic processes in the proliferation and survival of cancer cells. These studies demonstrated that cancer cells have to balance their bioenergetics requirements with anti-oxidants synthesis, pH regulation and the activation of stress response pathways. Disruption of this balance leads to loss of viability and may offer therapeutic opportunities.[2]

Wednesday PM, Session 6: AMPK and Cancer

Translating tumour and radiation biology of AMPK into early phase oncology trials

Theodoros Tsakiridis
McMaster University

In the past few years our group investigated the response of AMP-activated kinase (AMPK) to clinical doses of radiation therapy (RT). We found that AMPK is not only a metabolic but also a genomic stress sensor that is activated in epithelial tumour cells downstream of Ataxia Telengiectasia Mutated (ATM) and leads to induction of p53 and cyclin dependent kinase inhibitors such as p21cip1 and p27kip1. We observed that AMPK is involved in mediation of the G2-M cycle arrest and the cytotoxicity of RT. Knock down of AMPK in cancer cells and knockout models of AMPK show dramatic activation of the Akt-mTOR pathway. In tumours RT induced growth inhibition and chronic suppression of angiogenesis that was associated with sustained activation of ATM and AMPK and inhibition of Akt and mTOR axis. For that we utilized well-tolerated agents to target AMPK to inhibit tumour growth and enhance RT responses in lung and prostate cancer.
The anti-diabetic drug metformin (MET) is used by more than 120 million people worldwide for the treatment of diabetes and there are no reports of increased toxicity when combined with RT or chemotherapy. MET at low microM concentrations, achievable in the serum of diabetic patients, activated the AMPK pathway, inhibited growth and enhanced RT responses in cancer cells and tumours. The anti-proliferative and radio-sensitizing activity of MET was depended on AMPK and led to effective inhibition of the Akt-mTOR pathway. MET increased the pro-apoptotic and anti-angiogenic action of RT.
In retrospective analyses of clinical data from our institution we found that diabetic patients with locally advanced (LA) non-small cell lung cancer (NSCLC) treated with RT and chemotherapy have improved survival after chemo-RT treatment if they receive metformin for the treatment of diabetes. Based on our pre-clinical and retrospective clinical studies we proposed prospective clinical trials in lung cancer. Two phase II trials of MET in combination of chemo-RT now open to accrual to investigate this AMPK activator in LA-NSCLC. The Ontario Clinical Oncology Group (OCOG) ALMERA and NSABP-RTOG-GOG (NRG) LU001 studies investigate MET in combination with concurrent chemo-RT in LA-NSCLC. LU001 examines the pure chemo-radio-sensitizing action of MET using the drug only concurrently with chemo-RT while ALMERA examines the benefits of MET when used both concurrently with chemo-RT and continues for 1 year. Tumour bio-specimens collected in NRG-LU001 and OCOG-ALMERA are expected to help understand whether expression or mutation status of tumour (LKB1, K-Ras, p53, EGFR and Alk) and microenvironment (HIF1a and micro-vessel density) markers could serves as biomarkers of MET response in NSCLC.
In on-going pre-clinical studies we investigate the ability of other well-tolerated and widely-used anti-inflammatory and anti-cholesterol agents that activate AMPK, and their combination with MET, to modulate growth of lung and prostate cancer models. Results suggest that salicylate and statins modulate AMPK activity, inhibit tumour cell growth and enhance the effects of MET through inhibition of lipogenesis and suppression of the activity of the mTOR pathway. These concepts are being converted into clinical studies.
This work illustrates a promising paradigm of translating laboratory findings on the role of AMPK in tumour biology into early phase oncology trials.

Thursday AM, Session 7: AMPK regulation of Metabolism II

AMPK downregulation in humans: studies in insulin sensitive and resistant bariatric surgery patients

Neil B. Ruderman
Boston University Medical Center

Markedly obese individuals show an increased predilection to atherosclerotic cardiovascular disease, (ASCVD) type 2 diabetes and certain cancers. Available data suggests that morbidity and mortality from these abnormalities is diminished by bariatric surgery and in particular (at least for ASCVD) in patients with hyperinsulinemia and insulin resistance. We have previously shown that AMPK activity is diminished in multiple adipose tissue depots (SC, omental, epiploic) of insulin resistant compared to insulin sensitive bariatric surgery patients and confirmed this in a second study. The decrease in AMPK activity was associated with increases in oxidative stress in these depots and changes in gene expression (PGC1α, macrophages, T-cells and various inflammatory cyokines) which were typically more marked in visceral than in subcutaneous fat. Ongoing studies are examining these and various clinical parameters in the insulin sensitive and resistant patients following bariatric surgery and appropriate controls

Thursday AM, Session 7: AMPK regulation of Metabolism II

Discovery of a Novel Class of Naturally-Occurring Lipids with Anti-Diabetic and Anti-inflammatory Effects

Barbara B. Kahn
Beth Israel Deaconess Medical Center and Harvard Medical School

Increased adipose tissue (AT) lipogenesis is associated with enhanced insulin sensitivity. Mice overexpressing Glut4 in AT (AG4OX) have elevated AT lipogenesis and increased glucose tolerance in spite of obesity and elevated circulating fatty acids. To determine if the lipid profile contributes to improved glucose homeostasis in AG4OX, we performed lipidomic analysis of AT. This revealed a 16-18-fold increase in a novel class of lipids in AG4OX AT vs wildtype (WT) mice. Using a targeted MS approach, we identified 16 novel lipid family members with multiple isomers based on structural variations. Novel lipid levels are highest in brown adipose tissue followed by subcutaneous (SQ) and perigonadal (PG) white AT (WAT). Levels in liver, pancreas, kidney, muscle, heart and brain are substantially lower than AT. Levels of these lipids are acutely regulated by fasting. Most isomers are reduced 50-65% in serum and AT of insulin-resistant *vs* insulin-sensitive people. Nearly all isomers in humans correlate remarkably strongly with insulin sensitivity determined by clamp. Lipid isomers are also reduced in SQ WAT in mice fed HFD. Oral administration of these lipids lowers ambient glycemia and enhances glucose tolerance in aged chow-fed mice and mice with diet-induced obesity while stimulating GLP1 and insulin secretion. These lipids enhance glucose-stimulated insulin secretion 1.5-fold in human pancreatic islets and GLP1 secretion 3-fold in enteroendocrine cells. In cultured adipocytes, they enhance glucose uptake and insulin-stimulated GLUT4 translocation. These lipids also suppress inflammatory processes in dendritic cells. Biological effects of these lipids are mediated through lipid-responsive GPCRs and knockdown of specific GPCRs blocks the effects on glucose transport and GLUT4 translocation. In sum, we identified a novel lipid class that improves glucose-insulin homeostasis. Restoration of the low levels in insulin-resistant people may be effective to treat type-2 diabetes.

Thursday AM, Session 7: AMPK regulation of Metabolism II

Insulin sensitivity is inversely related to cellular energy status, as revealed by biotin deprivation

Antonio Velazquez-Arellano
Universidad Nacional Autónoma de Mexico

Several nutrients participate in respiratory metabolism and are essential for ATP production, one of them being biotin, cofactor of the anaplerotic pyruvate carboxylase. Adaptations to fluctuating nutritional-derived energy levels involve both AMPK at the cellular level and insulin at the systemic one. Here we show that biotin deprived rats are more tolerant to glucose as shown by both oral and intraperitoneal glucose tolerance tests, during which insulin plasma levels were significantly diminished in deficient rats compared to controls. Biotin-deficient rats had lower blood glucose concentrations during intraperitoneal insulin sensitivity tests than controls. Furthermore, more glucose was infused to maintain euglycemia in the biotin deficient rats during hyperinsulinemic euglycemic clamps studies. These results demonstrate augmented sensitivity to insulin in biotin-deprived rats. We had previously found severe liver ATP shortage and AMPK activation in biotin deprivation. We have now uncovered augmented AMPK activation in both biotin-deprived in vivo muscle and in cultured L6 muscle cells. In these cells, glucose uptake was increased by AMPK activation by AICAR, and diminished by its knockdown by specific siRNAs directed against its α1 and α2 catalytic subunits; all of these effects being largely independent of the activity of the insulin signaling pathway, that was inhibited with Wortmannin. Nevertheless, there is an increased activation of basal insulin signal transduction pathway in biotin-deficient L6 cells evidenced by augmented phosphorylation of its proteins IRβ, IRS and AKT. These effects are probably mediated by the AMPK-mTORC1-S6K1 pathway, since S6K1 is an inhibitor of insulin signaling at the IRS step. The enhanced insulin sensitivity in biotin deficiency likely has adaptive value for organisms due to the hormone promotion of uptake and utilization not only of glucose, but also of other nutrients such as branched-chain amino acids, whose deficiency has been reported to increase insulin tolerance.

Thursday AM, Session 7: AMPK regulation of Metabolism II

AMPK buffers adverse epigenetic change and consequent transgenerational reproductive defects following acute nutrient/energy stress

Richard Roy
McGill University

Chromatin modifications are often associated with changes in gene expression that can persist throughout the lifetime of the organism or even span multiple generations. Using the C. elegans model system we have found that the master metabolic regulator AMP-activated protein kinase (AMPK) is essential for metabolic adaptation and subsequent survival during periods of acute nutrient/energy stress. If however animals are recovered prior to expiration animals manifest numerous growth and developmental defects that culminate in a 70% rate of sterility among starved/recovered AMPK mutants; a phenotype never observed in non-starved AMPK mutants or starved wild type animals. Moreover, AMPK is also required to buffer modifications to the chromatin landscape to ensure that gene expression remains inactive in the primordial germ cells (PGC) during adverse physiological conditions. In its absence both H3K4me3 and H3K9me3 levels increase giving rise to bivalent marks in the PGCs that ultimately compromise the reproductive fitness of, not only the generation that experienced the stress, but also up to ten subsequent unstressed generations. AMPK phosphorylates the COMPASS complex histone methyltransferase SET-2, while monoubiquitylated H2B levels, a key indicator of transcriptional progression and a chromatin modification presumed critical for SET-2 recruitment, also increase in its absence. Our data suggest that AMPK impinges on two or more molecular mechanisms to ensure that transcription remains attenuated until an energy/nutrient-dependent contingency is satisfied, thereby coordinating the initiation of germ line stem cell division and development with environmental conditions.

Thursday AM, Session 7: AMPK regulation of Metabolism II

Regulation of AMPK activation and mTOR suppression by Axin

Sheng-Cai Lin
Xiamen University

AMPK (AMP-activated protein kinase) plays a central role in maintaining cellular energy homeostasis. In response to low energy status, AMPK is activated to enhance catabolic activities with concurrent inhibition of anabolic processes such as fatty acid synthesis. AMP activates AMPK through at least three mechanisms, which are: i) allosteric changes of AMPK, ii) phosphorylation at the conserved T172 residue by LKB1, and iii) prevention of dephosphorylation. We have recently discovered the mechanism by which AMP, as a low energy-charge signal, can autonomously initiate the assembly of an activating complex for AMPK in response to starvation. AMP binding causes a higher affinity of AMPK for the scaffold protein Axin that also binds to LKB1. Hence, we have uncovered the intracellular molecular link between increased AMP levels and LKB1 phosphorylation of AMPK. We also provided genetic evidence to show that Axin is required for robust AMPK activation inside the cell, and further suggest that LKB1 association with AMPK occurs on the scaffold Axin. Moreover, we have identified an additional factor that plays a crucial role in AMPK activation by anchoring the Axin-based complex onto lysosome, and will also present a molecular mechanism for the inverse relationship between AMPK and mTOR.

The Coenzyme A Ester of ETC-1002 Mediates Direct β1-Selective Activation of AMP-Activated Protein Kinase and Provides Evidence for a Novel Nutrient-Sensing Mechanism

Stephen L. Pinkosky
Esperion Therapeutics, Inc.

The AMP-activated protein kinase (AMPK) senses cellular energy status through competitive binding of AMP, ADP, and ATP by the γ regulatory subunit, and responds to energy deficit by mediating concomitant inhibition of long chain fatty acid (LCFA) synthesis and activation of β-oxidation. However, the discovery of a structurally diverse class of synthetic AMPK activators that bind the β subunit provides evidence for a distinct regulatory domain that modulates AMPK activity independent of changes in cellular energy status. The physiological relevance of this regulatory domain as well as its natural ligand remains undefined. Our investigations into the mechanism of action of ETC-1002, a novel phase IIB clinical stage small molecule, have provided insight into the physiological role of the β subunit by demonstrating that the coenzyme A (CoA) -activated form of ETC-1002 (ETC-1002-CoA) directly activates AMPK. In a cell free assay, ETC-1002-CoA activated recombinant human AMPKαβγ complexes in a β1-dependent manner and protected AMPKα Thr172 from PP2Cα –mediated dephosphorylation. To elucidate the natural ligand mimicked by ETC-1002-CoA, we screened a series of metabolic intermediates, coenzymes, and vitamins that share structural elements with CoA, and found that only LCFA-CoA esters activated AMPK. Saturated LCFA-CoAs (C12 to C16) were optimal and activated recombinant human AMPKα1β1γ1 complexes by 2- to 3.5-fold (EC50 = ~ 1.0 μM) while their respective free acids were inactive. This activation occurred at saturating concentrations of AMP (30 μM) suggesting that LCFA-CoAs bind and activate AMPK through a distinct mechanism. Similar to the allosteric β1-selective AMPK activator, A769622, palmitoyl-CoA activated both AMPK α1- and α2-containing complexes, was β1-selective, and prevented PP2Cα-mediated AMPKα Thr172 dephosphorylation (EC50 = 3.0 μM). In support of the physiological relevance of this mechanism, the treatment of primary human hepatocytes with palmitate increased AMPK Thr172 and ACC Ser79 phosphorylation and reduced the rate of de novo lipid synthesis. Consistent with β1 selectivity, palmitate-induced AMPKα Thr172 and ACC Ser79 phosphorylation were attenuated in embryonic fibroblasts from AMPKβ1 KO mice when compared to wild type cells. The therapeutic relevance of this mechanism was investigated by treating mice fed a high fat high cholesterol diet with ETC-1002, which is converted to ETC-1002-CoA in liver, but is not a substrate for esterification or β-oxidation. Mice treated with ETC-1002 demonstrated increased hepatic ACC Ser79 phosphorylation, and reduced body weight gain, hepatic triglycerides, fasting glucose and insulin when compared to vehicle treatment. These studies provide evidence for a novel nutrient-sensing mechanism by which LCFA-CoAs mediate β1-selective activation of AMPK, and provide a mechanistic rationale for the therapeutic benefit of the investigational drug, ETC-1002.

Thursday AM, Session 7: AMPK regulation of Metabolism II

AMPK in muscle insulin sensitivity

Jorgen Wojtaszewski

University of Copenhagen

Elucidating the importance of AMPK activation during exercise in regulation of muscle metabolism has been a major task ever since the observation that AMPK was potently activated by exercise and that pharmacological AMPK ended influenced various aspects of metabolism. These investigations have been challenged by pharmacological inhibitors of AMPK with seemingly limited bioactivity in mature muscle and by transgenic approaches which might have been biased by remnant AMPK activities. Recently, we have investigating the role of muscle AMPK by the use of muscle specific alpha 1 and alpha 2 AMPK double knockout mice. In my presentation, I will discuss our latest observations suggesting that AMPK may regulate fat oxidation during muscle contraction/exercise, and that AMPK has the potential to increase insulin action through regulation of the GTPase-activating protein; TBC1D4. This interplay between insulin signaling and AMPK leads to an important role of AMPK in post exercise glycogen synthesis and the phenomenon of muscle glycogen supercompensation.

Friday AM, Session 8: Emerging Roles for AMPK

Analyzing AMPK conformational changes by fluorescence energy transfer: an AMPK FRET sensor

Uwe Schlattner
INSERM U1055

AMPK is a multi-protein nanomachine that is activated by multiple, complex mechanisms, allowing fine tuning of AMPK activity in different situations of metabolic stress. Binding of adenine nucleotides to the gamma subunit plays a major role in either direct allosteric activation of AMPK or modulation of AMPK phosphorylation and dephosphorylation by upstream kinases and phosphatases. These activation mechanisms require crosstalk between AMPK subunits by a nucleotide-induced conformational switch. We have engineered an AMPK complex that allows a direct, real-time readout of the AMPK conformational state by fluorescence energy transfer (FRET). This molecular sensor confirms the exquisite sensitivity of AMPK to low micromolar concentrations of AMP, shows the exclusive ability of ATP, but not MgATP, to compete with AMP, and allows insight into the role of CBS domains for allosteric AMPK activation.

Friday AM, Session 8: Emerging Roles for AMPK

Phosphoproteomics Identifies Transcription-Coupled mRNA Decay as a Snf1-Regulated Pathway

Elton T. Young
University of Washington

Snf1, the Saccharomyces cerevisiae ortholog of adenosine monophosphate-activated protein kinase is activated by glucose starvation and other stresses to regulate a variety of cellular processes. To understand the mechanisms by which Snf1 controls these processes we identified 147 proteins that were phosphorylated in a Snf1-dependent manner using label-free quantitative mass spectrometry. They represented virtually all categories of cellular metabolism and physiology. Two different consensus phosphorylation sites were evident in the Snf1 targets suggesting that they represent both direct substrates of Snf1 and substrates of unidentified kinases. Protein kinases and protein phosphatases were two prominent categories as were proteins involved in mRNA metabolism and protein synthesis. Recent evidence indicates that Snf1 promotes mRNA stability in a promoter-specific manner. We found three genes, CCR4, DHH1 and XRN1 in the mRNA metabolism category of the Snf1 phosphoproteome that promoted rapid decay of Snf1 transcripts in the presence of glucose. Despite the reduced rate of decay in deletion mutants of XRN1 and CCR4, their deletion also inhibited accumulation of the same mRNAs. The apparent defect in mRNA synthesis could be overcome in a promoter-specific manner by activating transcription with a chimeric activator protein, suggesting a defect in initiation of transcription. Unexpectedly, deletion of XRN1 was synthetically lethal with deletion of REG1, an inhibitor of Snf1. The synthetic lethality was rescued by deleting SNF1 suggesting that Xrn1 is required to overcome inappropriate activation of Snf1. Mutating three Snf1-dependent phosphorylation sites in XRN1 inhibited derepression and glucose-induced mRNA decay providing evidence that Snf1 modulates the activity of Xrn1.

Friday AM, Session 8: Emerging Roles for AMPK

Self-renewal and metabolism of muscle stem cells are regulated by AMPKα1

Rémi Mounier
CNRS UMR 5534 and Université Claude bernard Lyon1

During skeletal muscle regeneration, muscle stem cells activate and recapitulate the myogenic program to repair the damaged myofibers. A subset of these cells will not enter into the myogenesis but will self-renew to return into quiescence. The control of this process is crucial to maintain skeletal muscle homeostasis and the molecular mechanism that control muscle stem cells self-renewal only start to be understood. Recent studies highlight the importance of the metabolism switch in the regulation of stem cell fate and particularly the exit from and the entry into the quiescent state. In this way, AMPK seems to be an excellent candidate in the control of stem cell fate choice. AMPK is a master metabolic regulator. Its activation mainly depends on the increase of the AMP/ATP ratio and it induces a regulation of the anabolic/catabolic balance. Moreover, AMPK has been shown to regulate cell cycle and cell growth. Although it is a well-studied molecule, its action on stem cell fate choice has never been investigated.
In this project, we decipher the role of AMPKa1 in muscle stem cells self-renewal by *ex vivo* (isolated fibers) and *in vitro* (muscle stem cell primary cultures by cell sorting) experiments. These reliable procedures permit us to pointing out that in absence of AMPKa1 muscle stem cell differentiate in a lower extend and self-renew much more (+300%). We also performed *in vivo* experiments, using specific conditional mouse strains in which AMPKa1 is specifically deleted in muscle stem cells ($AMPKa1^{fl/fl}$; $Pax7^{CREERT2/+}$). The model of cardiotoxin injury in the *Tibialis Anterior* couple with the measurement of specific readouts of skeletal muscle regeneration allow us to analyze the different states of this process. Thus, a strong decrease in myofiber size 14 and 28 days post injury is observed. Moreover, a significant increase in the total number of fibers *per* muscle (+40%) associated with an important increase in the total number of satellite cell *per* muscle (+30%) 28 days post injury is noticed. This phenotype is not present in mice specifically deleted for AMPKa1 in myofiber ($AMPKa1^{fl/fl}$; $HSA^{CRE/+}$). Metabolism of muscle stem cells is explored *in vitro* by analyses of mitochondrial respiration and by measurement of the activity of key enzymes of glycolysis. In this way, our results support the hypothesis that in absence of AMPKa1 muscle stem cells are not able to use mitochondria to synthesize energy. To pursue and thanks to a very efficient collaboration with Anne Brunet, research of specific targets/substrates of AMPKa1 involved in the metabolic switch of muscle stem cells during skeletal muscle regeneration will allow us to decipher the specific role of AMPKa1 in the self-renewal.
To conclude, our work permits to establish a new and crucial role of AMPKa1 in muscle stem cell fate choice by switching the metabolism during skeletal regeneration, linking for the very first time self-renewal and metabolism in this context.

Friday AM, Session 8: Emerging Roles for AMPK

Investigating the role of the AMPK cascade in prostate cancer

David Carling
MRC Clinical Sciences Centre

AMPK and CaMKKβ have both been implicated in prostate cancer. In one study, silencing of the gene for the β1 subunit of AMPK reduced prostate cancer cell growth *in vitro* (Ros *et al*. 2012). In separate studies, expression of CaMKKβ was found to be up-regulated in prostate cancer cells and was required for prostate cancr cell growth and migration (Frigo *et al*. 2010; Massie *et al*. 2011). We have used cell-based studies and *in vivo* mouse mouse models to explore the role of the AMPK cascade in prostate cancer progression. I will discuss our results and their implications for the role of the AMPK cascade in prostate cancer.

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POSTER ABSTRACTS

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Poster #1

Nutrients Downregulate AMPK by Multiple Mechanisms in Rat Skeletal Muscle

Kimberly A. Coughlan, Thomas W. Balon, Neil B. Ruderman and Asish K. Saha

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Previously we have found that excesses of glucose or branched chain amino acids, notably leucine, cause insulin resistance in incubated rat skeletal muscle within 60 min. We established that they do so by downregulating AMP-activated protein kinase (AMPK) activity, which in turn activates mTOR/p70S6K. How glucose and leucine decrease AMPK activity remains unknown.
To examine this question, studies were carried out in rat extensor digitorum longus (EDL) muscles incubated with glucose and leucine for up to 2 hours and in red gastrocnemius muscle of rats infused with glucose for 3-8 hours at a rate that produces hyperglycemia, hyperinsulinemia, insulin resistance and decreased AMPK activity.
Incubation of the EDL in a high glucose (25 mM) or a glucose (5.5 mM) + leucine (100 μM) medium significantly diminished AMPK activity by 50% within 30 min as assessed by both the SAMS peptide assay and Thr172 phosphorylation, with further decreases occurring up to at least 2 h. The initial decrease in AMPK activity was followed by inhibitory phosphorylation of the catalytic α-subunit of AMPK at Ser485/491 which was first observed at 1 hr, while a decrease in SIRT1 expression and related parameters and an increase in PP2A activity were not observed until 2 h. Changes in the same parameters were observed during the glucose infusion, however with somewhat different timing. In this model, AMPK activity was diminished after 3h, while PP2A was increased substantially later, following the same sequence of events as in incubated muscle. In contrast, changes in Ser485/491 phosphorylation and SIRT1 expression, and related parameters, although in the same direction as in incubated muscle, were observed at the same time as the decrease in AMPK activity. These findings suggest that different mediators might be responsible for the timing, albeit not by the occurrence of the initial and sustained decrease in AMPK activity in the two models. Whether the later occurring changes in these mediators of AMPK activity specifically contribute to the regulation of such events as gene expression and organelle function as well as sustaining changes in fuel metabolism remains to be determined.

Poster #2

SAD-A kinase (BRSK2) in pancreas

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SAD-A kinase, alias BRSK2 is a member of the AMPK-related family of protein kinases. SAD-A kinase is highly expressed in the brain, where it plays a role in neuronal development. SAD-A kinase is also expressed in the pancreas. In part due to the lack of high affinity antibodies suitable for immunohistochemistry conflicting data exist about the cellular localization of SAD-A kinase within the pancreas and a potential role of the kinase in insulin secretion by beta-cells. Here, the gene expression of SAD-A kinase was assessed in islets and whole pancreas analysis of human, rat and mouse origin. The expression of SAD-A kinase was found to be higher in whole pancreas than in islets. No significant change of SAD-A kinase expression was detected in islets from diabetic origin. In both, purified mouse beta-cells and MIN6 mouse insulinoma SAD-A kinase was expressed. The phosphorylation status of SAD-A kinase increased in response to glucose in Min6 cells. siRNA-mediated reduction of SAD-A kinase expression by approx. 50 % had no effect on insulin secretion stimulated by either high glucose or GLP-1 agonism. In conclusion, we found no evidence for a beta-cell-specific role of pancreatic SAD-A kinase.

Poster #3

Phosphorylation of CLIP-170 by AMPK plays a crucial role for the speed of microtubule polymerization and directional cell migration

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Many types of physiological stress following the increase of intracellular AMP levels lead to AMPK activation. AMPK has been reported to coordinate the control of glucose metabolism, lipid metabolism, transcriptional regulation, cell growth and cell polarity depending on the phosphorylation levels of many substrates of AMPK. The downstream targets of AMPK participating in these functions, however, have not been fully identified. We identified CLIP-170 (cytoplasmic linker protein of 170 KDa) as a novel AMPK substrate using unique two-step column chromatography and mass spectrometry. Here we show that AMPK directly phosphorylates CLIP-170 at Ser311 and phosphorylation of CLIP-170 by AMPK is required for regulation of cell polarity and migration. CLIP-170, one of the microtubule plus end proteins, directly binds freshly polymerized distal ends of growing microtubules and rapidly dissociates from the older microtubule lattice. Both inhibition and depletion of AMPK resulted in prolonged accumulation of nonphosphorylated CLIP-170 at microtubule plus ends and a reduction in the speed of tubulin polymerization. Forced expression of a nonphosphorylatable mutant form of CLIP-170 consistently resulted in the same phenotypes. The prolonged accumulation of CLIP-170 reflected the reduced speed of tubulin polymerization, suggesting that AMPK is required for regulation of microtubule dynamics. Furthermore, inhibition of AMPK perturbed the directional cell migration and motility regulated by cytoskeletal reorganization; we ascribe this phenomenon to impairment of efficient microtubule dynamics. Thus, AMPK regulates cell polarity and migration by regulating microtubule dynamics through phosphorylation of CLIP-170.

Poster #4

Rosemary extract increases glucose uptake in skeletal muscle cells via AMPK activation

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Skeletal muscle (SKM) is important in glucose homeostasis and it is quantitatively the most important insulin target tissue. Impaired insulin action in this tissue leads to insulin resistance and type 2 diabetes mellitus. In recent years, activation of the energy sensor, 5' AMP-activated kinase (AMPK), has been viewed as a targeted approach to counteract insulin resistance. Chemicals found in plant extracts such as polyphenols have attracted attention for their potential use for treating insulin resistance. Recent in-vitro and in-vivo studies indicate that rosemary extract (RE) has anti-diabetic properties, although its effects on muscle and exact mechanisms involved are not known. In the present study, we examined the effects of RE and the mechanism of regulation of glucose uptake in SKM cells. RE stimulated glucose uptake in L6 myotubes in a dose- and time-dependent manner. Maximum stimulation was seen with 5 µg/ml of RE for 4 h (184±5.07% of control, $p<0.001$), a response comparable to maximum insulin (207±5.26% of control, $p<0.001$) and metformin (216±8.77% of control, $p<0.001$) stimulation. Furthermore, carnosic acid (CA) and rosmarinic acid (RA), major polyphenols found in RE, increased glucose uptake indicating that these compounds may be responsible for the RE effects. RE along with its major polyphenol constituents CA, and RA did not affect Akt phosphorylation while significantly increased AMPK phosphorylation. Furthermore, the RE-stimulated glucose uptake was significantly reduced by the AMPK inhibitor, compound C while remained unchanged by the PI3K inhibitor, wortmannin. RE, CA and RA did not affect GLUT1 or GLUT4 glucose transporter translocation in contrast to a significant translocation of both transporters seen with insulin or metformin treatment. Our study is the first to show a direct effect of RE on SKM glucose uptake by a mechanism that involves AMPK activation. Our findings are very important and suggest a potential use of rosemary extract to regulate glucose homeostasis and counteract insulin resistance.

Poster #5

High glucose concentration blocks anti-proliferative effects of metformin in MDA-MB-231 breast cancer cells

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Metformin, the most widely prescribed oral anti-diabetic drug, appears to protect against some types of cancer, including the breast cancer. While its anti-cancer action in diabetic patients could stem from improvements in glucose homeostasis, studies indicate that it may target cancer cells directly. Although exact molecular mechanism remains equivocal, evidence suggests that mitochondria are probably the major site of metformin action. By inhibiting mitochondrial ATP synthesis metformin evokes energy stress and thereby activates the AMP-activated protein kinase (AMPK), which could explain some of its anti-proliferative effects. However, AMPK activation may decrease or increase cancer cell survival under different conditions, suggesting metformin anti-cancer action may not exclusively depend on AMPK activation.

Here, we explored whether alterations in glucose availability modulates anti-proliferative effects of metformin and AICAR, a direct AMPK activator. Proliferating MDA-MB-231 breast cancer cells were grown in the presence of normal (1 g/L) or high (4.5 g/L) glucose concentration. As assessed by MTS assay and direct cell counting by haemocytometer, proliferation rate remained similar between normal and high glucose conditions, suggesting glucose is not a limiting factor for the MDA-MB-231 cell growth under standard cell culture conditions. AICAR suppressed proliferation to a similar extent in medium with normal or high glucose, indicating increased glucose availability could not overcome anti-proliferative effects of AMPK activation. Conversely, metformin failed to inhibit MDA-MB-231 cell proliferation under high glucose conditions, while proliferation was markedly suppressed by metformin in the presence of 1 g/L glucose.

Our results show that anti-proliferative effects of metformin are highly dependent upon glucose availability, while glucose concentration does not alter AICAR action. Taken together, these data indirectly suggest that glucose supply modulates effectiveness of metformin as an AMPK activator in MDA-MB-231 breast cancer cells. Alternatively, metformin may exert anti-proliferative effects in an AMPK-independent manner through an unknown glucose-sensitive mechanism. Finally, we demonstrate that media composition can have a major impact on metformin action and needs to be considered to avoid erroneous data interpretation.

Poster #6

AICAR stimulation metabolome-widely mimics electrical contraction in isolated rat epitrochlearis muscle

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Background - Physical exercise has potent therapeutic and preventive effects against metabolic disorders. A number of studies have suggested pivotal roles of 5'-AMP-activated protein kinase (AMPK) in regulating carbohydrate and lipid metabolism in contracting skeletal muscles. However, several recent studies using genetically manipulated animal models revealed that AMPK is not necessarily involved in the metabolic regulation in contracting skeletal muscles and suggested the significance of AMPK-independent pathways.

Purpose – The aim of the present study is to elucidate significance of AMPK-dependent and -independent signal pathways in contracting skeletal muscles in terms of metabolic regulation.

Methods - We conducted a capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS)-based metabolomic analysis that compared the metabolic effects of 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside (AICAR) stimulation with the electrical contraction ex vivo in isolated rat epitrochlearis muscles, in which both α1- and α2-isoforms of AMPK and glucose uptake were equally activated.

Results & Discussion - The CE-TOFMS analysis detected 184 peaks and successfully annotated 132 small molecules. AICAR stimulation exhibited high similarity to the electrical contraction in overall metabolites. Principal component analysis (PCA) demonstrated that the major principal component (explaining 63.8%) characterized common effects whereas the minor principal component distinguished the difference. PCA and a factor analysis suggested a substantial change in redox status as a result of AMPK activation. We also found a decrease in reduced glutathione levels in both AICAR-stimulated and contracting muscles. The muscle contraction-evoked influences related to the metabolism of amino acids, in particular, aspartate, alanine, or lysine, are supposed to be independent of AMPK activation.

Conclusion - Here we demonstrate that AICAR stimulation closely mimics the metabolomic changes in the contracting skeletal muscles. Our results substantiate the significance of AMPK activation in contracting skeletal muscles in terms of metabolome-wide regulation.

Poster #7

Partial hepatic resistance to IL-6-induced inflammation develops in type 2 diabetic mice, while the anti-inflammatory effect of AMPK is maintained

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Interleukin-6 (IL-6) induces hepatic inflammation and insulin resistance, and therapeutic strategies to counteract the IL-6 action in liver are of high interest. In this study, we demonstrate that acute treatment with AMP-activated protein kinase (AMPK) agonists AICAR and metformin efficiently repressed IL-6-induced hepatic proinflammatory gene expression and activation of STAT3 in a mouse model of diet-induced type 2 diabetes, bringing it back to basal non-stimulated level. Surprisingly, the inflammatory response in liver induced by IL-6 administration in vivo was markedly blunted in the mice fed a high-fat diet, compared to lean chow-fed controls, while this difference was not replicated in vitro in primary hepatocytes derived from these two groups of mice. In summary, our work reveals that partial hepatic IL-6 resistance develops in the mouse model of type 2 diabetes, while the anti-inflammatory action of AMPK is maintained. Systemic factors, rather than differences in intracellular IL-6 receptor signaling, are likely mediating the relative impairment in IL-6 effect.Key words: IL-6, AMPK, Inflammation, Liver, Type 2 diabetes

Poster #8

Regulation of mitochondrial ROS through an AMPK-dependent metabolic circuit

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Reactive oxygen species (ROS) are continuously produced by mitochondria and eliminated via antioxidant systems. Proper maintenance of this redox balance supports proper cellular function and can facilitate adaptation to metabolic stress. The AMP-activated protein kinase (AMPK), a central regulator of cellular metabolism, can be activated by ROS and has previously been linked to redox balance through control of ACC-dependent NADPH homeostasis. Here we show that AMPK functions in a feedback loop to limit mitochondrial ROS through regulation of mitochondrial metabolism. We demonstrate that the AMPK allosteric activator A-769662 promotes suppression of State III (ADP-stimulated) respiration in cells, leading to reduced mitochondrial oxidative phosphorylation (OXPHOS) and ROS production. Conversely, loss of AMPKa in mouse embryonic fibroblasts (MEFs) promotes increased production of mitochondrial ROS, leading to HIF-1a stabilization and a metabolic shift to the Warburg effect. In this poster we will discuss the possible mechanisms of AMPK-dependent control of OXPHOS and their implication for AMPK-dependent metabolic adaptation in cells.

This work was supported by the Canadian Institutes for Health Research Doctoral Award (to B.F). Canadian Institutes for Health Research MOP-93799 (to R.G.J.).

Poster #9

Skeletal muscle AMPK is required for maintaining FNDC5 expression

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Irisin is a recently characterized myokine that is produced in muscle tissue in response to exercise and cold exposure. Irisin is the secreted protein product of fibronectin type III domain-containing protein 5 (FNDC5) whose expression is controlled by the transcriptional co-activator, peroxisome proliferator-activated receptor gamma, co-activator 1 alpha (PGC1a). The AMP-activated protein kinase (AMPK) is essential for controlling numerous metabolic responses related to exercise and is an important regulator of PGC1a activity; therefore, we explored the role of AMPK in the regulation of irisin/FNDC5 using AMPK Beta1/Beta2 double muscle-null mice (DMKO) which lack skeletal muscle AMPK activity. Micro-array screening initially identified a reduction in FNDC5 expression and subsequent analysis confirmed that FNDC5 expression is dramatically reduced in resting muscles of AMPK DMKO mice compared to wildtype littermates. Acute or chronic activation of AMPK with the pharmacological AMPK activator AICAR did not increase PGC1a or FNDC5 expression in the tibialis anterior muscle. In contrast, four hours after treadmill running serum levels of FNDC5/irisin were increased in wildtype but not in AMPK DMKO mice exercised at the same relative intensity. Since AMPK DMKO mice are exercise intolerant, we developed an *in situ* contraction protocol that normalized muscle workloads. In wildtype mice, activating phosphorylation of AMPK was elevated immediately post contraction and was abolished in muscle from DMKO mice. In contrast, PGC1a was increased in both wildtype and AMPK DMKO mice 3 hours post contraction but FNDC5 expression was not altered. These data indicate that skeletal muscle AMPK is required for the maintenance of FNDC5 expression.

Poster #10

Activation of AMP- activated protein kinase is associated with mislocalization of TDP-43 in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) patients develop muscle weakness and impaired voluntary movement, which are caused by the degeneration of motor neurons. TAR-DNA binding protein-43 (TDP-43)-positive inclusions in the brain and the spinal cord are a hallmark of ALS. The signature protein of ALS, TDP-43, is a nuclear protein consisting of 414 amino acids. Accumulating evidence demonstrates that TDP-43 functions in a wide variety of important cellular pathways, including gene regulation, pre-mRNA processing, microRNA expression, and neuronal activity. In addition to the formation of TDP-43 inclusions, mislocalization of TDP-43 from the nucleus to the cytoplasm has been recognized as an early event of ALS that may cause detrimental effects. Because cytoplasmic TDP-43 inclusions are typically accompanied by nuclear clearance of TDP-43, ALS pathogenesis may, at least partially, be attributed to the loss of the nuclear functions of TDP-43. In this study, we demonstrate that cytoplasmic mislocalization of TDP-43 was accompanied by increased activation of AMP-activated protein kinase (AMPK) in motor neurons of ALS patients. The activation of AMPK in a motor neuron cell line (NSC34) or mouse spinal cords induced the mislocalization of TDP-43, recapitulating this characteristic of ALS. Collectively, AMPK may be a potential drug target for this devastating disease.

Poster #11

Control of Cell Motility through LKB1-MARK-dependent CLASP1/2 phosphorylation

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In motile cells, the establishment and maintenance of cell polarity controls cellular migration through the regulation of cytoskeletal components that are essential for membrane extension and cell adhesion at the leading edge of the cell. However, the molecular mechanisms that mediate these processes are not well characterized.

The liver kinase B1 (LKB1) tumor suppressor is a serine-threonine master kinase which plays critical roles coordinating cell growth, metabolic reprogramming and the establishment and maintenance of cell polarity. LKB1 directly regulates the phosphorylation and activity of a family of related protein kinases, including AMPK, MARK, SIK, NUAK and SAD kinases. The MARK kinases have been implicated in regulating cell polarity in many cell types; however, it is not known which of their downstream substrates mediate this function and how they may impact fundamental cell processes such as cell motility. To address these questions, we have conducted a search for novel substrates of the MARK kinases. Here we show that the microtubule-associated proteins CLASP1/2 are direct targets of the MARK1-4 family of AMPK-related kinases. We identify the critical sites regulated by MARKs on CLASP1/2 and further demonstrate that phosphorylation at these sites regulates microtubule dynamics in an LKB1- and MARK-dependent manner.

MARK expression in cells induced microtubule deacetylation and destabilization, which was mirrored by expression of a MARK phosphomimetic mutant of CLASP1/2. In contrast, expression of CLASPs that cannot be phosphorylated by the MARKs retained stabilized microtubules, suggesting that CLASPS are critical mediators of MARK-directed microtubule instability. Moreover, MARK phosphorylation of CLASP1/2 is required for the proper control of cell migration, as expression of CLASPS that cannot be phosphorylated by MARKs exhibited aberrantly accelerated motility in scratch-wound assays and 3D matrigel invasion assays. In contrast, MARK phosphomimetic CLASP1/2 mutants displayed dramatically decreased cell motility.

Together, our data reveal CLASP1/2 as novel phosphorylation targets of the MARK kinases and furthermore implicate LKB1 and MARKs as critical regulators of cell migration through CLASP-dependent microtubule dynamics. These results may provide insight into how LKB1 directs establishment of polarity during cell migration and help explain why cell motility can become aberrantly deregulated in LKB1-deficient settings.

Poster #12

Generation of a novel mouse model to modulate AMPK activity in vivo

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The AMP-activated protein kinase (AMPK) is a highly conserved master regulator of metabolism, which in response to metabolic stress restores cellular energy homeostasis by inhibiting energy-consuming anabolic processes and promoting energy-consuming catabolic processes. Beyond maintaining cellular energy levels, it is now clear that AMPK is a highly integrated node that plays an important role in numerous aspects of normal physiology and disease, such as food intake regulation, energy expenditure, the response to stresses like exercise and caloric restriction, aging, and diseases such as diabetes and cancer. However, the specific contribution of AMPK activity, or the lack thereof, to those physiological processes remains in many cases unclear, partly due to the paucity of *in vivo* models for the study of AMPK. To better assess physiological roles of AMPK, we have engineered a novel switchable mouse model platform in which activated alleles of AMPK can be induced, acutely and reversibly *in vivo*. This model consists in targeting a variety of AMPK mutants into a doxycycline-inducible cassette in the *ColA1* locus using a Flp/FRT recombinase-mediated targeting system. We have so far generated transgenic mice that inducibly express a constitutively active allele of AMPKα1 and are currently assessing the extent of AMPK activation in these mice. In addition, we have also targeted other AMPK alleles that we have tested for their ability to augment or inhibit AMPK activity and are in the process of generating those mice. In all, this platform represents a valuable tool to investigate the effects of AMPK activation and inhibition in specific physiological contexts *in vivo* and will allow us not only to uncover novel aspects of AMPK biology, but also to explore the potential therapeutic opportunities of such modulation of AMPK activity.

Poster #13

New sensitive and specific tools in the quantification of AMPK and related pathways targets; a focus on metabolic signaling in the liver

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Maintaining a balance between energy demand and supply is of critical importance for sustained cellular integrity and metabolic flexibility. Apart from intracellular changes in adenine nucleotide levels, a plethora of other mechanisms are thought to activate AMPK, while very little is so far known about how the phosphorylation status of the serine subunits may alter the overall activity of this kinase and transcriptionally affect its downstream targets. Crosstalk with PGC1a and SIRT1 is essential but sensitive quantification of changes either at the transcriptional or the translational level remains challenging. In the work presented, a new protein quantification technique based on gel electrophoresis in nano-capillaries is used to quantify the phosphorylation of AMPK at Thr-172 and of AMPKa1 at Ser-485 and compared to total AMPKa, AMPKa1 and AMPKa2 protein levels in a hepatic cellular model (HepG2, human hepatocarcinoma cell line). Quercetin, an acclaimed activator of AMPK is used to probe small changes under various nutritional related stresses and results obtained under different culture conditions are compared to the effects of AICAR. Transcriptional changes in SIRT1 and PGC1a are quantified by digital PCR and prove complementary in the understanding of the timeframe of evolution of events. Our results highlight the significance of these new technologies in the confident quantification of subtle changes. The knowledge obtained for the proper set up of various antibodies and titration of samples is also discussed.

Poster #14

AMPK inhibition of NKCC1 mediates hydrogen peroxide suppression of intestinal epithelial ion transport

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Reactive oxygen species cause epithelial dysfunction in inflammatory bowel disease (IBD) including disruption of homeostatic electrolyte transport processes in the gut. We have previously shown that hydrogen peroxide (H_2O_2) inhibits intestinal epithelial Ca^{2+}-dependent Cl^- transport by a mechanism involving inhibition of the basolateral Na-K-2Cl cotransporter, NKCC1, which governs Cl^- entry into intestinal epithelial cells (IEC) and thus plays the key role in maintaining the driving force for Cl^- secretion. Since electrolyte transport consumes large amounts of cellular energy, we investigated whether H_2O_2 inhibition of carbachol (CCh)-stimulated Ca^{2+}-dependent Cl^- secretion involves the cellular energy sensor, adenosine monophosphate-activated protein kinase (AMPK). Furthermore, we investigated whether AMPK affects NKCC1 function in H_2O_2 treated epithelial cells and isolated mouse colon. Methods: Protein phosphorylation was measured by Western blot, and ion transport was measured in Ussing chambers, or by measuring $^{86}Rb^+$ influx as a marker for K^+ transport.

Results: H_2O_2 (500 μM) increased AMPK-α (Thr^{172}) phosphorylation in T84 and HT-29 IEC lines with peak phosphorylation occurring from 5-15 minutes ($p<0.05$-0.01; n=7). The AMPK activator, AICAR, administered for 30 min, also significantly increased AMPK-α phosphorylation ($p<0.05$; n=4). Pre-treatment of polarized T84 monolayers with the AMPK inhibitor, Compound C (50 μM), blocked H_2O_2-mediated inhibition of CCh-stimulated Cl- secretion (50 μM Compound C; $p<0.05$; n=4), thus indicating that AMPK is also involved in H_2O_2 inhibition of Ca^{2+}-dependent Cl^- secretion. *Ex vivo* studies with proximal colonic mucosa mounted in Ussing chambers showed that the inhibitory effect of H_2O_2 on CCh-stimulated electrolyte transport was reduced in AMPK-α2 KO mice. To understand the mechanism of inhibition, we examined whether AMPK mediated H_2O_2 inhibition of NKCC1 transport of $^{86}Rb^+$. H_2O_2 by itself had no effect on baseline NKCC1 activity (bumetanide-sensitive $^{86}Rb^+$ influx), whereas H_2O_2 pretreatment inhibited CCh-stimulated $^{86}Rb^+$ influx (56% □ 8%, $p<0.001$, n=8). In the presence of Compound C (50 μM), the inhibitory effect of H_2O_2 on CCh-stimulated NKCC1 activity was eliminated indicating that AMPK inhibits NKCC1 function. Co-immunopreciptiation experiments suggested that AMPK (α-subunit Thr^{172}) may regulate NKCC1 through a physical association. Indeed, we observed that treatment of T84 IEC monolayers with H_2O_2 (500 μM) induced a rapid increase in NKCC1 ($Thr^{212,217}$) phosphorylation that peaked at 15 min as detected by Western blotting and subsequent densitometric analysis ($p<0.05$; n=4). The AMPK activator, AICAR, also significantly increased NKCC1 phosphorylation ($p<0.05$; n=4). H_2O_2-stimulated phosphorylation of NKCC1 was significantly reduced by pre-incubation with Compound C ($p<0.01$; n=4). Conclusion: H_2O_2 inhibits electrolyte transport through AMPK inhibition of NKCC1 activity potentially by a phosphorylation-dependent physical interaction. These data indicate a role for AMPK in suppression of epithelial transport in the setting of acute oxidant exposure.

Supported by the Crohn's and Colitis Foundation of America and NIH.

Poster #15

Anti-obesity effect of AMPK a2-subunit deletion in mice during high-fat feeding

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Background and aims: Previous results have demonstrated both acceleration (Villena *et al*, Diabetes, 2004) and reduction (Jelenik *at al*, Diabetes, 2010) in body weight gain in mice fed high-fat diet in response to whole-body deletion of AMPK α2-subunit. We hypothesized that the differences between studies could be related to the composition of high-fat diet used in different experiments. The aims of the present study were to (i) characterize the effect of the AMPK α2-subunit deletion in the context of dietary obesity induced by either by corn-oil based (cHF) or lard-based (HSD) diet, and (ii) to assess possible role of genetic background of the mice.

Procedures: Adult male mice with a whole-body deletion of the α2 catalytic subunit of AMPK (Viollet *et al*, J Clin Invest, 2003) on either obesity-prone C57BL/6J (B6/J) or obesity-resistant A/J background (obtained by appropriate backcrossing for at least 10 generations), and their wild-type littermates, were fed either cHF or HSD (with 60 % energy as fat in both diets) for 10 weeks.

Results: Both cHF and HSD induced body weight gain with a stronger effect in B6/J mice, and the induction was also stronger with HSD as compared with cHF. In B6/J mice, body weight gain correlated with adiposity and hepatic accumulation of lipids. In response to AMPK α2-subunit deletion, reduction of body weight gain was observed in the case of cHF diet, while body weight gain in mice fed HSD was not affected. In the A/J mice, with a relatively small induction of dietary obesity, the stronger effect of HSD on body weight was observed again, but body weight was not affected by AMPK α2-subunit deletion. As compared with B6/J mice, accumulation of hepatic lipids was relatively low. Only in A/J mice, AMPK α2-subunit deletion resulted in a higher accumulation of lipids in skeletal muscle (*m. quadriceps*), independent on the type of the diet, and the weight of the muscle was lower as compared with the wild-type littermates.

Conclusion: Only in the B6/J mice fed corn-oil based high-fat diet but not lard-based high-fat diet, the paradoxical anti-obesity effect of whole-body deletion of AMPK α2-subunit could be detected. Comparison between mice of different genetic backgrounds suggests association of the obesity-prone phenotype with hepatic lipid accumulation, but the anti-obesity effect of AMPK α2-subunit deletion could not be explained by the interference with hepatic lipid metabolism.

Grant: Supported by the Ministry of Health of the Czech Republic (NT13763-4)

Poster #16

Knockdown of hypothalamic AMPK in mice leads to weight loss

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AMP-activated protein kinase (AMPK) is a key enzyme in the regulation of energy metabolism, ultimately affecting food intake and body weight regulation. It has numerous roles in multiple tissues, however, functions of hypothalamic AMPK in food intake regulation remains to be elucidated. Hypothalamic AMPK integrates diverse hormonal and nutritional signals to regulate food intake and energy metabolism. Recent evidence suggests that altered AMPK activity in hypothalamus can lead changes in food intake and body weight regulation. In order to investigate the roles of hypothalamic AMPK in food intake and body weight regulation, genetic methods using lentiviruses which carry scrambled shRNA and AMPK-targeted (α1 and α2 subunits) short hairpin (sh) RNA was applied in neuropeptide Y (NPY) and agouti-related peptide (AgRP) co-expressing hypothalamic neurons. These neurons showed significantly decreased expression levels of AMPK (71.49%) and NPY (36.11%) and AgRP (22.40%). Knockdown of hypothalamic AMPKα1 and α2 in mice by intracerebroventrical injection with the lentiviruses led to significant body weight loss after 8 weeks of injection compared to scrambled-shRNA control mice (32.14%). Body mass (lean mass, fat mass, and total body weight) and metabolic profiles (food intake, physical activity, and energy expenditure) were further analyzed in scrambled- or AMPK-shRNA injected mice fed with a normal chow diet (ND) or a high fat diet (HFD). Body weights of the ND group and HFD group were reduced (19.69% and 27.27%), respectively. Interestingly, AMPK-shRNA injected mice in HFD group showed a significant decrease in both lean mass (35.65%) and fat mass (21.23%) compared to scrambled-shRNA control, although AMPK-shRNA injected mice in ND group showed only mild reduction in lean mass (4.85%) and fat mass (12.35%) compared to scrambled-shRNA control. Moreover, AMPK-shRNA injected mice in HFD group showed a significant increase in physical activity (36.54%) but food intake was not different from scrambled shRNA control. AMPK-shRNA injected mice in ND group showed only mild increased physical activity (16.21%) and reduced food intake (14.28%) without significance. Taken together, these results suggest that hypothalamic AMPK is in part involved in the regulation of accumulation of fat mass, thereby regulating body weight.

Poster #17

X-ray crystallographic studies of AMPK: Insights into AMPK function through interactions with small molecule allosteric modulators.

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The AMP-activated protein kinase (AMPK) is a highly conserved αβγ heterotrimer that acts as a master metabolic regulator responsible for cellular and whole body energy homeostasis. AMPK is regulated by ATP/AMP ratio in the cell. In response to a fall in intracellular ATP, AMPK activates energy-producing pathways and inhibits energy consuming pathways. This regulation is achieved through phosphorylation of enzymes involved in virtually every branch of cellular metabolism, including fat synthesis, protein synthesis and carbohydrate metabolism as well as phosphorylating transcription factors for long term regulatory effects

On a whole body level, AMPK is regulated by a diverse range of hormones including leptin, ghrelin and adiponectin; where it has a role in appetite and cell growth, as well as glucose, lipid and protein metabolism. It is for this whole body response that has made AMPK of pharmacological interest, given the growing epidemic of metabolic disorders including obesity, type 2 diabetes, cancer and cardiovascular disease. Activation of AMPK in vivo elicits a number of metabolic responses that would be expected to counteract the physical abnormalities associated with obesity and type 2 diabetes.

We have a number of small molecule activators and inhibitors known to act on AMPK independently of AMP. These small molecules are being used in crystallization experiments with previously solved full length and truncated AMPK constructs (Xiao et al, 2013; Chen et al, 2012; Littler et al, 2010; Polekhina et al, 2005).

We aim to use the structural information to improve our knowledge of AMPK enzyme activity and allosteric regulation.

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Poster #18

Different postnatal maturation of AMPK in skeletal muscle in obesity-prone and obesity-resistant mice

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Background and aims: Little is known about changes in AMPK subunits expression and AMPK activity in skeletal muscle during the early postnatal development, between birth and weaning. The aims of the present study were to (i) characterize the activity of AMPKα1 and AMPKα2 isoforms and expression of the genes encoding its catalytic subunits in murine skeletal muscle in mice during the first month of life, and (ii) assess possible roles of both gender and genetic background of the mice.
Materials and methods: Male (M) and female (F) pups of the obesity-prone C57BL/6 (B/6) mice and obesity resistant A/J mice were born and maintained at 30 °C, mothers were fed Chow diet, and mice were weaned at 28 days (D) of age. Gastrocnemius muscle was collected by freeze-clamping and AMPK activity was determined using AMARA peptide substrate. Gene expression was assessed by real-time quantitative RT-PCR. Evaluation of data was performed by ANOVA.

Results: At 10D, the activity of AMPKα1 was significantly higher in comparison with the AMPKα2 activity in all tested groups (A/J F ~1.9-fold; A/J M ~3.3-fold; B/6 F ~2.6-fold; B/6 M ~3.7-fold). Between 10D and 28D, the AMPKα1 activity decreased in mice of both strains except for A/J F (A/J M ~2-fold; B/6 M ~2.6-fold; B/6 F ~3.7-fold). In A/J mice at 28D, activity of AMPKα2 was higher than that of AMPKα1 (A/J F ~1.4-fold; A/J M ~1.6-fold). Total activity of AMPK (α1+α2) in B/6 mice decreased significantly between 10D and 28D (B/6 F ~1.9-fold; B6 M ~ 1.5-fold) but it stayed constant in A/J mice. Expression of AMPKα1 gene was constant in both A/J and B/6 mice between 10D and 28D. Expression of AMPKα2 gene increased between 5D and 28D in both strains (A/J F 5D 3.10±0.96 vs. 28D 12.04±0.80 AU; A/J M 5D 3.54±0.47 vs. 28D 16.00±3.21 AU; B6 F 5D 2.24±0.28 vs. 28D 12.85±1.10 AU; B6 M 5D 2.00±0.08 vs. 28D 9.66±0.80 AU).

Conclusion: During early postnatal development, i.e., the period of the switch from high- to low-fat intake, strain-specific changes in AMPK activity in murine skeletal muscle were observed. While in the obesity-resistant A/J mice the activity stayed constant, it declined in the obesity-prone B/6 mice. The developmental change in AMPK activity reflected the activity of AMPKα1 isoform, but not the expression of the genes for either of the catalytic subunits of the enzyme. Changes in AMPK activity in skeletal muscle during early postnatal development may affect propensity to obesity in adulthood, depending on the genetic background of the mice.

Grant: Czech Science Foundation (MITOCENTRUM - grant 14-36804G)

Poster #19

Increased Cytoplasmic NAD(P)+/NAD(P)H Ratio by NQO1 Activator Ameliorates Vascular complication.

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Recently, obesity become popular medical and social problems with potentially devastating consequences because it clusters with type 2 diabetes, hypertension and hyperlipidemia in the metabolic syndrome, which is an important risk factor for cardiovascular disease incidence and mortality. So far, caloric restriction and increasing energy expenditure through exercise are only effective methods to solve these problems. AMP-activated protein kinase (AMPK) functions as a fuel sensor in the cell and is activated when cellular energy is depleted. In previous report, we presented that alpha-lipoic acid (Alpha-LA) decreased hypothalamic AMPK activity and causes profound weight loss in rodents by reducing food intake and enhancing energy expenditure. Recently, we found that pharmacologically-induced cytoplasmic NAD(P)+/NAD(P)H ratio might stimulate the rates of glycolysis, fatty acid oxidation through the increase mitochondrial oxidative phosphorylation and adaptive mitochondrial biogenesis. Furthermore, this might be a useful therapeutic approach for patients with metabolic syndrome. When cytoplasmic NAD(P)H:quinone oxidoreductase 1 (NQO1) is activated by exogenous compounds, the cytoplasmic NAD(P)+/NAD(P)H equilibrium is shifted towards oxidized NAD(P)+. Under these conditions, the high NAD(P)+/NAD(P)H ratio stimulates mitochondrial oxidative phosphorylation and glycolysis and activates sirtuins. Here we show that the mechanism by which NQO1-mediated oxidation of NAD(P)H leads to enhanced mitochondrial fatty acid oxidation involves activation of AMP-activated protein kinase (AMPK). Furthermore, NQO1-mediated oxidation of NAD(P)H ameliorates most of phenotypes of metabolic syndrome, including obesity, glucose intolerance, dyslipidemia, and fatty liver disease in ob/ob mice and in mice on a high-calorie diet with diet-induced obesity. Neointimal formation, the leading cause of restenosis, is caused by proliferation of vascular smooth muscle cells (VSMCs). In this study, we found that Bl, one of NQO1 activators which regulates NAD(P)/NAD(P)H redox potential reduces neointimal formation after balloon injury in vivo. Bl prevents VSMC's proliferation caused by G1 cell cycle arrest via an AMPK dependent mechanism. These data suggest that cellular NAD/NADH level followed by treatment of Bl in NQO1-expressing cells displays beneficial effects in the treatment of metabolic syndromes including atherosclerosis at least in part via upregulation of AMPK. Therefore these studies will provide the regulation of NAD+/NADH redox potential may be novel therapeutic target for the treatment of metabolic syndromes.

Poster #20

The Role of AMPK in Prostate Cancer

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AMP-activated kinase (AMPK) constitutes a hub for cellular metabolic control and therefore represents a promising therapeutic target for prostate cancer (PCa), downregulating anabolic pathways and growth in conditions of cellular stress. PCa is characterised by increases in lipogenesis and activation of the PTEN/PI3K/AKT/mTOR pathway; whether AMPK activation is sufficient to block PCa cell growth remains to be determined. There is growing controversy as to whether activating AMPK is always detrimental to cancer cells, with some studies associating AMPK with survival advantage. We have recently determined the mode of action of a potent and specific small molecule activator of AMPK. In order to resolve current controversies in the literature we have used a combination of in vitro and in vivo studies to validate AMPK as a therapeutic target.

Poster #21

In vivo screen for AMPK substrates and phosphorylation sites reveals an inhibitory role for this energy-sensing kinase in cell invasion

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AMP-activated protein kinase (AMPK) is a central energy gauge that helps prevent aging and age-related diseases, including cancer. Whether AMPK's role in cancer is via its known metabolic substrates or other substrates is unknown. Using an in vivo chemical genetics screen, we identify over 100 potential novel AMPK substrates, including 19 new high confidence substrates and their phosphorylated residues. This methodology identifies for the first time large-scale AMPK phosphorylation motifs in vivo. Interestingly, AMPK high confidence substrates are strongly enriched for proteins involved in cellular motility, adhesion, and invasion. We find that the AMPK phosphorylation site on the RHOA guanine nucleotide exchange factor NET1A prevents extracellular matrix degradation and inhibits cell invasion. Our results reveal the extent to which the energy-sensing AMPK inhibits cell invasion, which has important implications for the prevention of metastasis and pharmacological targeting of AMPK.

Poster #22

Activation of AMPK Regulates Hippocampal Neuronal pH by Recruiting Na+/H+ Exchanger NHE5 to the Cell Surface

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Strict regulation of intra- and extracellular pH is an important determinant of nervous system function as many voltage-, ligand- and H^+-gated cationic channels are exquisitely sensitive to transient fluctuations in pH elicited by neural activity and by pathophysiologic events such as hypoxia-ischemia and seizures. Multiple Na^+/H^+ exchangers (NHEs) are implicated in maintenance of neural pH homeostasis. However, aside from the ubiquitous NHE1 isoform, their relative contributions are poorly understood. NHE5 is of particular interest as it is preferentially expressed in brain relative to other tissues. In hippocampal neurons, NHE5 regulates steady-state cytoplasmic pH, but intriguingly the bulk of the transporter is stored in intracellular vesicles. Here, we show that NHE5 is a direct target for phosphorylation by the AMP-activated protein kinase (AMPK), a key sensor and regulator of cellular energy homeostasis in response to metabolic stresses. In NHE5-transfected non-neuronal cells, activation of AMPK by the AMP mimetic AICAR or by antimycin A, which blocks aerobic respiration and causes acidification, increased cell surface accumulation and activity of NHE5 and elevated intracellular pH. These effects were effectively blocked by the AMPK antagonist compound C, the NHE inhibitor HOE694, and by mutation of a predicted AMPK recognition motif in the NHE5 C-terminus. This regulatory pathway was also functional in primary hippocampal neurons, where AMPK activation of NHE5 protected the cells from sustained antimycin A-induced acidification. These data reveal a unique role for AMPK and NHE5 in regulating the pH homeostasis of hippocampal neurons during metabolic stress.

Poster #23

[6]-Gingerol exerts glucose regulation role through AMPK pathway

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[6]-Gingerol, a pungent ingredient of ginger (Zingiber officianle Roscoe, Zingiberaceae) has been used to control diabetes and dyslipidaemia. Its metabolic role is poorly understood. In this study, [6]-Gingerol increased AMP-activated protein kinase (AMPK) phosphorylation in mouse C2C12 skeletal muscle cells. [6]-Gingerol also stimulated glucose uptake in AMPK dependently. Both inhibition of AMPKa2 and knock-down of AMPKa2 blocked [6]-Gingerol-induced glucose uptake. [6]-Gingerol increased the PP2A phosphorylation. The interaction between AMPKa2 and PP2A was confirmed by co-immunoprecipitation. In addition, this interaction was increased by AMPK activator, suggesting the involvement of PP2A in [6]-Gingerol-mediated AMPK phosphorylation. [6]-Gingerol increased the phosphorylation of Rab GTPase-activating protein AS160. AMPK inhibitor blocked [6]-Gingerol-induced AS160 phosphorylation, implying that AS160 played a role on AMPKa2 downstream. Moreover, [6]-Gingerol increased the expression of Rab5 mRNA and protein. Knock down of AMPKa2 blocked [6]-Gingerol-induced increased Rab5 expression, suggesting the involvement of AMPK in Rab5 expression. [6]-Gingerol increased the expression of GLUT4 mRNA and protein levels. A colorimetric absorbance assay showed that [6]-Gingerol stimulated translocation of GLUT4 to the plasma membrane, and that this effect was suppressed in cells pre-treated with Compound C. Furthermore, insulin-mediated glucose uptake was potentiated by co-treatment with [6]-gingerol. This potentiation was not appeared in the present of Compound C, suggesting that AMPK activation is involved in [6]-Gingerol-mediated insulin sensitizing effect. Our results suggest that [6]-Gingerol has an important role in glucose metabolism via the AMPKa2-mediated AS160-Rab5 pathway and the other way is through potentiation of insulin-mediated glucose regulation.

Poster #24

AMP-activated protein kinase (AMPK) is a regulator of autophagic cell death in hippocampal neural stem (HCN) cells following insulin withdrawal.

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AMP-activated protein kinase (AMPK) is a central regulator of cellular processes and metabolism. Besides its role in maintaining cellular energy homeostasis, AMPK also plays a critical role in determining the degrees of cell polarity, growth, autophagy, and cell death. Our previous study has shown that the adult hippocampal neural stem (HCN) cells undergo autophagic cell death (ACD) in response to insulin withdrawal in vitro. The role of AMPK in regard to ACD has not been determined in HCN cells. Here, we report that pharmacological inhibition of AMPK activity by Compound C or overexpression of a dominant-negative AMPK reduced ACD. Furthermore, knockdown of AMPK by siRNA reduced ACD. On the other hand, activation of AMPK by AICAR or overexpression of a constitutive-activate form of AMPK increased ACD. Activation of AMPK enhanced the rate of autophagolysosome turnover, which reflects the autophagy activity. Collectively, our result suggests a novel role of AMPK in the HCN cells as a regulator of ACD upon insulin withdrawal. Understanding the mechanisms governing AMPK-mediated ACD can provide novel strategies for utilization of endogenous neural stem cells.

Poster #25

Novel Small Compounds Targeting Diabetes

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Diabetes is a common chronic disease, with a staggering 1 in 12 people affected. As type 2 diabetes (T2D) accounts for more than 90 % of cases, there is an urgent need to develop new and effective therapies to modulate the symptoms of this disorder. T2D arises when the level of insulin produced fails to adequately stimulate cellular glucose uptake through the mobilisation of glucose transporter 4 (GLUT4), to the plasma membrane. Muscle contraction and certain pharmacological agents can also stimulate glucose transport independently of insulin action through adenosine-5'-monophosphate activated protein kinase (AMPK). Therefore, targeting these pathways to augment glucose transport may be a promising approach for the treatment of T2D. A series of novel compounds have been found to restore normal glucose and insulin sensitivity when given to mice previously rendered diabetic with a high fat diet. Current results suggest that these compounds activate the AMPK pathway as a result of a transient inhibition of the mitochondrial respiratory chain complex 1. The activation of AMPK leads to the phosphorylation of ACC, which stimulates fatty acid oxidation, and of AS160, which allows GLUT4 to translocate to the membrane, leading to an increase in glucose uptake. However, AS160 is activated in two cycles, the first of which does not seem to be related to AMPK. Since the compounds have been found to cause a strong initial increase in phosphorylation of another regulator of AS160, AKT, the first phase is therefore believed to be influenced by this protein. As these compounds appear to instigate glucose uptake in a superior manner to that of metformin, the leading anti-diabetic drug, the cellular effects of the compounds were compared to those produced by metformin. Metformin increases the phosphorylation of AMPK and ACC, yet appears to decrease the phosphorylation of AKT over time and does not cause a cyclical phosphorylation of AS160. As metformin does not increase the phosphorylation of AKT, this may explain the enhanced stimulatory effect of these compounds on glucose uptake.

Poster #26

A novel gamma-2 mutation resulting in hyper-activation of AMPK.

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Several mutations in human gamma-2 have been previously shown to be associated with hypertrophic cardiomyopathy and ventricular pre-excitation (premature excitation of the large chambers of the heart). These mutations include R302Q, H383R, T400N, N488I, Y487H, E506K, R531G, R531Q, R384T and S548P. Most are inherited in a dominant manner and have a relatively late onset of symptoms. A small number are de novo mutations with early onset, and these tend to be lethal during the neonatal period. We report the characterisation of a novel mutation in the gamma 2 subunit (K475E), which was found in an infant with hypertrophic cardiomyopathy that was diagnosed in utero by ultrasound. The patient has mild developmental delay with failure to thrive, and, unusually, is still alive at the age of 5. The mutation affects a residue (K475) equivalent to K242 in rat gamma-1, which in crystal structures lies very close to the phosphate group of ATP bound in site 1. This might be expected to lead to a gain in function due to loss of ATP binding and consequent decreased sensitivity to Thr172 dephosphorylation. Expression of the mutant protein in an inducible cell system has shown that AMPK complexes containing the mutant gamma 2 subunit are constitutively activated and shows increased levels of Thr172 phosphorylation. However, similar to other gamma-2 mutations, the mutant displays no allosteric activation by AMP in cell-free assays, or any activation or increased Thr172 phosphorylation in response to increased AMP levels in intact cells. The K475E mutation is a charge switch, replacing a positively charged side chain in the ATP-binding site of gamma-2 by a negatively charged side chain, which might be expected to inhibit binding of the negatively charged gamma-phosphate. However, the introduction of the negative charge might also mimic the presence of AMP in the binding site, resulting in the observed constitutive activation. This is supported by the fact that a K475A mutant, like K475E, is not responsive to AMP levels, but shows no increase in the basal activity.

Poster #27

AMPK inhibits ALOX15 induction in IL-4-polarized primary human macrophages

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AMP-activated protein kinase (AMPK) is reported to inhibit inflammation in cells of the immune system and promote an anti-inflammatory M2 polarization of macrophages. However, little is known about the impact of AMPK in macrophages polarized to classical M2 phenotype by IL-4/IL-13. In this study we show that AMPK activation selectively affects human macrophage response to IL-4 by inhibiting the expression of arachidonate 15-lipoxygenase (ALOX15). AMPK activation did not influence STAT6 activation, the major regulator of IL-4-induced gene expression. Inhibition of ALOX15 expression resulted in diminished levels of arachidonic acid lipoxygenase metabolites, 12-HETE and 15-HETE, in IL-4-polarized macrophages. This, in turn, may affect the responses of IL-4-polarized macrophages to subsequent inflammatory stimuli. Thus, AMPK alters arachidonic acid metabolism in IL-4-polarized macrophages, with implications for pro- vs. anti-inflammatory phenotype changes.

Poster #28

The SGLT2 inhibitor Canagliflozin activates AMPK

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In mammals, glucose is absorbed in the small intestine and the proximal tubules of the kidney by the sodium/glucose co-transporters SGLT1 and SGLT2, respectively; any glucose not reabsorbed by SGLT2 in the kidney is excreted in the urine. The first SGLT1/SGLT2 inhibitor identified was phlorizin, a glucose derivative isolated from the bark of apple trees, first described in 1835. Orally administered phlorizin is hydrolysed in the small intestine to the aglycone, phloretin. By inhibiting SGLT2 and hence promoting glucose excretion, phlorizin has beneficial effects on hyperglycaemia in animal models of diabetes, although its simultaneous inhibition of SGLT1 can cause severe diarrhoea and dehydration. More recently, the selective SGLT2 inhibitors canagliflozin and dapagliflozin, which are related in structure to phlorizin, have been approved for treatment of Type 2 diabetes in the USA and Europe. In clinical trials, canagliflozin increased urinary glucose excretion from 10g to 115g per day, whilst reducing plasma glucose by 25%. Canagliflozin also increases insulin sensitivity and reduces body weight, as well as causing reduced synthesis and increased oxidation of fatty acids.

The effects of canagliflozin on insulin sensitivity and fat metabolism prompted us to look at effects of these drugs on AMPK (interestingly, it was also reported in 1959 that phlorizin inhibited oxygen consumption by isolated mitochondria). We found that canagliflozin activated AMPK in intact cells at concentrations similar to those in plasma of patients. AMPK was also activated quite potently by phloretin, but dapagliflozin and phlorizin were less effective. Canagliflozin and phloretin both activated AMPK in cells expressing wild type gamma-2 but not the AMP-insensitive R531G mutant, suggesting that it acts by increasing cellular AMP levels. Consistent with this, canagliflozin and phloretin increased ADP:ATP ratios, and canagliflozin inhibited oxygen uptake, in intact cells. Canagliflozin had no effect on AMPK activity in cell free assays, nor did it affect allosteric activation by AMP or A-769662, indicating that it does not bind directly to AMPK. Taken together, these data indicate that canagliflozin and phloretin (like metformin) activate AMPK via a mechanism involving inhibition of the respiratory chain. We also utilised WT and AMPK-null MEFS to show that canagliflozin increased fatty acid synthesis in intact cells in an AMPK-dependent manner. This study raises the possibility that some of the beneficial effects of canagliflozin, such as increased insulin sensitivity, may be independent of SGLT2 inhibition and may be mediated instead via activation of AMPK.

Poster #29

Naturally occurring mutations in the γ-3 subunit of AMPK α2β2γ3 repress antagonism by ATP

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AMP-activated protein kinase (AMPK) is a protein kinase that serves as a central regulator of cellular and whole body energy metabolism. Recent studies have identified naturally occurring gain of function mutations in the AMPK regulatory γ-3 subunit (Arg225Trp and Arg225Gln). Individuals with these mutations exhibit an altered metabolic phenotype with increased glucose uptake and mitochondrial content in skeletal muscle, decreased intramuscular triglyceride and a resistance to muscular fatigue. In an effort to better understand the structural and biochemical underpinnings that drive these functional properties, we have recombinantly expressed the wild type AMPK α2β2γ3 and AMPK α2β2γ3 R225W and R225Q mutants and have profiled their biochemical activities in presence of different nucleotides. Our data shows that at high ATP concentrations, the γ3 mutants are activated by AMP significantly more than the wildtype protein. Arginine 225 is located in the CBS1 module of the γ3 subunit and forms an ion-pair with the gamma phosphate of ATP at site 1 as well as with the alpha phosphate of AMP at site 3. Based on our observations, we propose that relief of the antagonistic effect arising from high cellular ATP concentration is responsible for the gain of function phenotype observed for these γ3 mutants.

Poster #30

Dissociation between AMPK dysregulation and fatty liver development

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AMP-activated protein kinase (AMPK) is a key regulator of energy metabolism. AMPK plays an important role in the regulation of lipid metabolism by inhibiting fatty acid and cholesterol synthesis and by stimulating lipid oxidation. Several studies have shown an association between the intracellular accumulation of lipids and the loss of AMPK activity in the liver. These observations suggest that AMPK dysregulation may be a factor involved in the pathogenesis of hepatic steatosis. To investigate this hypothesis, we generated a new model of knockout mice lacking both alpha 1 and alpha 2 catalytic subunits of AMPK complex specifically in the liver. We analyzed the consequences of hepatic AMPK deletion on lipid metabolism in different nutritional conditions. Loss of AMPK activity in the liver did not alter hepatic triglyceride and cholesterol content, neither lipogenesis gene expression after a 24-hours fast or after a refeeding with a high carbohydrate diet. In addition, hepatic fatty acid oxidation assessed by the plasma ketone bodies levels was not modified in liver AMPK-deficient mice compared to control mice after a 24-hours fast. Surprisingly, lack of AMPK in the liver did not exacerbate fatty liver, hyperglycemia or glucose intolerance in mice fed a high-fat diet for 5 months.
These results demonstrate that the loss of hepatic AMPK activity is not a triggering or an aggraving factor in the development of fatty liver.

Poster #31

Endocannabinoid-AMPK signaling axis: a novel mechanism to counter DCM

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Endogenous cannabinoids (endocannabinoids) are lipid mediators that act as ligands for the cannabinoid receptors Cb1r and Cb2r; the two main endocannabinoids being arachidonoyl ethanolamide (AEA, anandamide) and 2-arachidonoylglycerol (2-AG). Although our understanding of the endocannabinoid signaling pathway has progressed, its role in cardiac metabolism is currently understudied. Insulin plays a key role in regulating substrate (glucose and fatty acids) utilization in cardiomyocytes. On the other hand, inflammation can impede insulin signaling and contribute to insulin resistance. In cardiomyocytes, this phenomena precipitates to the disorder termed as Diabetic Cardiomyopathy (DCM). AMP-activated protein kinase (AMPK) is the key cellular energy regulator and acts as a protective response to energy stress during metabolic deregulations, including altered substrate utilization in cardiomyocytes. In this study we have demonstrated that 2-AG activates AMPK signaling pathway in a CAMKK-dependent manner in cardiomyocytes. Inflammation induces impairment of insulin signaling leading to the suppression of glucose uptake, alterations in cell signaling and changes in gene expression in cardiomyocytes. However, rapid activation of AMPK via endocannabinoid restores substrate utilization and concomitant alterations in gene expression in the insulin-resistant condition. Furthermore, pharmacological inhibition of either CAMKK or AMPK dramatically abolishes the ameliorative effect of endocannabinoid in cardiomyocytes, validating the signaling axis involved in this process. Overall, the current study aims to utilize the endocannabinoid-mediated activation of AMPK signaling pathway as a novel therapeutic option against inflammation-induced DCM.

Poster #32

Activation of AMPK by metformin ameliorates the neuropathological symptoms in a mouse model of Lafora disease.

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Lafora disease (LD, OMIM 254780) is a rare fatal neurodegenerative disorder that usually occurs during childhood with generalized tonic-clonic seizures, myoclonus, absences, drop attacks or visual seizures. Unfortunately, at present, available treatments are only palliatives and no curative drugs are available yet. The hallmark of the disease is the accumulation of insoluble polyglucosan inclusions, called Lafora bodies (LBs), within the neurons but also in heart, muscle and liver cells. Mouse models lacking functional EPM2A or EPM2B genes (the two major loci related to the disease) recapitulate the Lafora disease phenotype: they accumulate polyglucosan inclusions, show signs of neurodegeneration and have a dysregulation of protein clearance and endoplasmic reticulum stress response. In this study, we have subjected a mouse model of LD (Epm2b-/-) to different pharmacological interventions aimed to alleviate endoplasmic reticulum stress. We have used two chemical chaperones, trehalose and 4-phenylbutyric acid. In addition, we have used metformin, an activator of AMP-activated protein kinase (AMPK), as it has a recognized neuroprotective role in other neurodegenerative diseases. Here, we show that treatment with 4-phenylbutyric acid or metformin decreases the accumulation of Lafora bodies and polyubiquitin protein aggregates in the brain of treated animals. 4-Phenylbutyric acid and metformin also diminish neurodegeneration (measured in terms of neuronal loss and reactive gliosis) and ameliorate neuropsychological tests of Epm2b-/- mice. As these compounds have good safety records and are already approved for clinical uses on different neurological pathologies, we think that the translation of our results to the clinical practice could be straightforward.

Poster #33

AMPK preserves endothelial tight junctions in coronary microcirculation during sepsis and prevents the onset of myocardial wall oedema

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Induced capillary leakage contributes to organ dysfunction during sepsis. However, the contribution of cardiac oedema to sepsis-induced left ventricular (LV) dysfunction and signalling pathways controlling the sepsis-induced myocardial oedema remains unclear. AMPK controls cytoskeleton organization in endothelial cells (ECs) and exerts anti-inflammatory effects. Our hypothesis was that it could influence vascular permeability and inflammation, thereby counteracting cardiac wall oedema during sepsis and preserving heart function.

Sepsis was triggered using a sub-lethal injection of lipopolysaccharide (LPS, 10 $mg.kg^{-1}$) in both WT and $\alpha_1AMPK^{-/-}$ mice. Echocardiography and MRI imaging revealed an increase in LV mass corresponding to exagerated LV wall oedema, 24 h after LPS injection in $\alpha_1AMPK^{-/-}$ compared to WT. This was associated with declined in LV end-diastolic volume. Despite more pronounced wall oedema, no difference in systolic function could be detected between WT and $\alpha_1AMPK^{-/-}$. Using dye extravasation, we demonstrated that $\alpha_1AMPK^{-/-}$ animals exhibited a dramatic increased in the LPS-induced vascular hyperpermeability in the heart. Mechanistically, this cardiac phenotype observed in LPS-treated $\alpha_1AMPK^{-/-}$ could not be attributed to major changes in the systemic inflammatory response. It was due to an increased disruption of interendothelial tight junctions as demonstrated by distortion of the ZO-1 linear pattern *in vivo*. AMPK activation by AICAr counteracted LPS-induced hyperpermeability in WT mice *in vivo* as well as in ECs in culture. AICAr preserved ZO-1 linear border pattern even in presence of LPS. AMPK knockdown using siRNA prevent AICAr to exert its action.

Our results demonstrate, for the first time the involvement of a signalling pathway in the control of LV wall oedema during sepsis. AMPK exerts a protective action through the preservation of interendothelial tight junctions. Interestingly, exaggerated LV wall oedema was not coupled with aggravated systolic dysfunction. However, it could contribute to diastolic dysfunction in septic patients.

Poster #34

INHIBITION OF JAK-STAT PATHWAY ACTIVATION BY AMP-ACTIVATED PROTEIN KINASE (AMPK) VIA DIRECT PHOSPHORYLATION OF JAK KINASES

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Chronic inflammation of conduit vessels and the microcirculation is a key feature of obesity and type 2 diabetes. Importantly, ablation of pro-inflammatory signalling has been shown to improve vascular function. AMP-activated protein kinase (AMPK) is a serine/threonine kinase proposed to be a therapeutic target for treating type 2 diabetes due to its role in regulating energy balance [1]. We therefore investigated whether AMPK was able to regulate signalling by IL-6 and other JAK-STAT-mobilising cytokines in vascular endothelial cells (ECs). Three distinct AMPK activators (rosiglitazone, AICAR, A769662) reduced sIL-6Ralpha/IL-6 trans-signalling complex-stimulated phosphorylation of STAT3, STAT1 and ERK1/2. Furthermore, AMPK's effects were attenuated by siRNA-mediated knockdown of AMPKalpha1 subunits. A769662 reduced sIL-6Ralpha/IL-6-mediated induction of STAT3 target genes SOCS-3 (49±10% reduction, n=3, $p<0.05$) and C/EBPdelta (55±12%, n=3, $p<0.05$) and similarly attenuated the ability of sIL-6Ralpha/IL-6 to promote monocyte chemotaxis. Interestingly, while several pathways known to act downstream of AMPK (eNOS, mTOR, SIRT1, multiple PTPs) could also significantly inhibit IL-6-signalling, the effect of AMPK occurred independently of these.

A769662 also blocked STAT3 phosphorylation in response to interferon alpha (IFNalpha) as well as STAT1 phosphorylation following ectopic expression of constitutively active Val617Phe JAK2, suggesting that AMPK was exerting its inhibitory effects at one or more JAK isoforms. To test this hypothesis, we used overlapping peptide arrays for in vitro kinase assays to show that purified AMPK could directly phosphorylate specific Ser residues within the regulatory SH2 domain of JAK1 and JAK2 but not the related kinases JAK3 and Tyk2. Within the JAK1 25mer- peptide, 5 Ser residues were identified as potential phosphoacceptor sites, in vitro kinase assay of Ser/Ala-mutated versions of the JAK1 peptide found that AMPK phosphorylates Ser515 and Ser518. This was confirmed when wild type but not S515A/S518A double mutant, JAK1 precipitates with GST-14-3-3 protein in extracts from A769662 treated JAK1 null U4C cells transiently expressing either wild type or S515A/S518A double mutant JAK1.

Thus, AMPK is a functionally important suppressor of JAK-STAT activation by multiple cytokines, and may mediate its effects through specific phosphorylation and inhibition of JAK1 and JAK2. Ongoing work is identifying the biochemical mechanisms by which AMPK inhibits JAK activity, and assessing the significance of this modification for the anti-inflammatory and immunosuppressive actions of AMPK-activating drugs such as metformin and salicylate.

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Poster #35

AMPK activation prevents TGF- β1-Induced Epithelial-Mesenchymal Transition and myofibroblast activation

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TGF- β is profibrotic and contributes to tubulointerstitial fibrosis and progressive chronic kidney disease (CKD). We investigated the mechanism by which TGF-β exerts its profibrotic effects and specifically the role of AMPK in human and murine kidney tubular epithelial cells, interstitial fibroblasts and in vivo in a TGF- β1 transgenic mouse model. In cultured human and murine proximal tubular epithelial cells (PTECs), TGF-β1 treatment causes a decrease in AMPK phosphorylation on its activating site (Thr172) together with increased fibronectin and α-SMA expression and decrease in E-cadherin. TGF-beta exerts similar changes in interstitial fibroblasts. Activation of AMPK with AICAR or metformin or transfection of the cells with constitutively active AMPK markedly attenuated the phenotypic changes induced by TGF-β1. Conversely, inhibition of AMPK with ARA or siRNA-mediated knockdown of AMPK mimicked the effect of TGF- β and enhanced basal as well as TGF-β1-induced phenotypic changes. In the kidney cortex of TGF-β transgenic mice, there was a significant decrease in AMPK phosphorylation associated with increase in mesenchymal markers and decrease in E-cadherin compared to control littermates. Collectively, the data indicate that TGF-β exerts its profibrotic action in vitro and in vivo via inactivation of AMPK. AMPK activation prevents tubulointerstitial injury induced by TGF-beta. Activators of AMPK provide potential therapeutic strategy to prevent kidney fibrosis and progressive kidney disease.

Poster #36

Development And Characterization of Small Molecule AMPK Activators For Metabolic Diseases and Their Complications

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AMPK is an energy sensing kinase that is a critical regulator of whole body energy metabolism. Upon activation, AMPK alters energy utilization by stimulating ATP-producing pathways and inhibiting ATP-consuming pathways via phosphorylation of regulatory proteins in the acute phase and inducing longer-term adaptations through modulation of gene expression. Due to its central role in energy regulation, pharmacologic activation of AMPK is expected to be beneficial in treating metabolic disorders and in improving exercise endurance, as well as in treating patients with inflammatory diseases and cancers. We reported the identification of small molecule AMPK activators that potently stimulated AMPK T172 phosphorylation both *in vitro* and *in vivo*, and improved glucose tolerance in mouse models of diabetes. Mechanistic studies demonstrated that AMPK activation by the compounds was due to inhibition of mitochondrial complex I. Metabolomics analysis of treated mice indicated an up-regulation of fatty acid oxidation and ketogenesis in liver, muscle, and adipose tissues, which is indicative of increased mitochondrial oxidative phosphorylation. Furthermore, our compound ameliorated the development of progressive exercise intolerance in aged, diet-induced obese mice. These benefits were accompanied by the normalization of perfusion in resting and exercising muscle, and enhanced mitochondrial function. Global metabolite profiling of tissues and plasma from mice with diet-induced obesity chronically treated with the compound revealed tissue-selective effects, i.e. up-regulation of skeletal muscle glycolysis and lipolysis, whereas metformin treatment resulted in stronger reductions in glucose and lipid metabolites in the liver. These studies suggest that this class of small molecule AMPK activators has some degree of functional selectivity towards skeletal muscle, making them particularly suitable for the treatment of metabolic diseases and their complications such as intermittent claudication associated with peripheral artery disease.

Poster #37

Dual Action Drug That Activates or Inhibits AMPK Depending on the Beta Subunit Isoform

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The AMP-activated protein kinase (AMPK) exists as a heterotrimeric complex with tissue-specific subunit isoform expression, which functions as a critical regulator of cellular and whole-body energy metabolism. Depending on the tissue, activation or inhibition of AMPK activity can mitigate against metabolic defects associated with Type-2 diabetes and obesity, therefore AMPK is considered a promising target for new drugs aimed at treating these diseases. We have discovered a dihydroxyquinoline drug (MT47-100) that has the unusual property of being an activator of AMPK-beta1 but an inhibitor of AMPK-beta2 heterotrimers. The inhibitory activity towards AMPK-beta2 depends on the carbohydrate-binding module (CBM) and maps to a cluster of residues (Ile81, Phe91 and Ile92) present in the beta2-CBM but not beta1. Substitution of the corresponding residues into the beta1-CBM makes MT47-100 an inhibitor of AMPK-beta1 complexes. These findings raise the possibility of exploiting differences in the beta subunits to design activators and inhibitors of AMPK for therapeutic applications.

Poster #38

Impaired AMPK-ACC signalling inhibits ghrelin-stimulated increases in appetite

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Within the hypothalamus, activation of AMPK in response to peripheral hormones that signal negative energy balance promotes food intake by increasing the expression of orexigenic neuropeptides. The underlying molecular interactions are not fully understood, but recent studies have suggested a number of potential mechanisms, including regulation of hypothalamic lipid metabolism, interactions with the mammalian target of rapamycin or the deacetylase sirtuin-1 as possible mediators of AMPK's effect on whole-body energy balance.

The acute effects of AMPK on lipid metabolism are mediated by phosphorylation of acetyl-CoA carboxylase (ACC) 1 at Ser79 and ACC2 at Ser212, thereby inhibiting fatty acid synthesis and promoting fatty acid oxidation. To specifically investigate the physiological impact of this regulation, we generated mice with alanine knock-in mutations in both ACC1 (Ser79Ala) and ACC2 (Ser212Ala) (ACC double knock-in, ACC DKI). Compared with wild type mice, ACC DKI mice showed reduced body weight from 15 weeks of age concomitant with decreased subcutaneous fat pad weights and adipocyte size. Furthermore, ACC DKI mice had reduced appetite after an overnight fast and lower accumulated food intake over a 72h period. Importantly, we demonstrate that feeding in response to the orexigenic hormone ghrelin is attenuated and ghrelin-induced expression of the orexigenic neuropeptides NPY and AgRP is inhibited in ACC DKI mice. These results indicate that AMPK regulation of ACC is an important physiological mechanism in the control of feeding behaviour and body weight regulation.

Poster #39

Salicylate impairs lipolysis through the AMPK β1 subunit

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White adipose tissue is an important regulator of whole body energy metabolism by storing excess energy as triglyceride and mobilizing free fatty acids during times of energetic demand. Thus, a fine balance between storage and mobilization is required to ensure proper energy homeostasis. This study focussed on further understanding the role of AMPK, a highly conserved metabolic sensor, in adipose tissue. Our group in collaboration with others has recently shown that the ancient drug salicylate activates AMPK to promote fat utilization and lowers plasma free fatty acids (FFA) in vivo. We sought to understand the mechanism by which salicylate lowers plasma fatty acids. Treatment of 3T3-L1 adipocytes with AICAR and salicylate led to the activation of AMPK as detected by the phosphorylation of acetyl-CoA carboxylase (ACC) and hormone sensitive lipase (HSL S565). In addition, treatment of 3T3-L1 adipocytes with isoproterenol, a β-AR agonist, stimulated lipolysis, an effect which was reduced by both AICAR and salicylate pre-treatment. To determine the specificity of AICAR and salicylate suppression of lipolysis, we performed similar experiment in isolated adipocytes from AMPK β1 deficient mice (β1-KO). AICAR lowered isoproterenol stimulated lipolysis equally in wildtype and β1-KO adipocytes; however, salicylate only lowered lipolysis in adipocytes from wildtype mice. Overall, these results suggest that salicylate suppresses lipolysis through the AMPK β1 subunit. Further work is required to understand the in vivo role of salicylate in AMPK adipose tissue deficient mice.

Poster #40

Loss of Skeletal Muscle AMPK Blocks Autophagy Promoting Fasting-Induced Hypoglycemia and Aging-Induced Myopathy.

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Skeletal muscle autophagy is induced during fasting and is important for maintaining muscle and mitochondrial health. AMPK is known to regulate autophagy, but its physiologic role in regulating fasting-induced muscle wasting and aging-induced muscle dysfunction has not been evaluated. Here we find that fasting AMPK-MKO mice results in hypoglycemia and hyperketosis, an effect which is not due to dysregulated increases in fatty acid oxidation. Instead, fasting in AMPK-MKO mice fails to induce muscle proteolysis of the essential gluconeogenic amino acid alanine, phosphorylation of ULK1 Ser555 and aggregation of RFP-LC3 puncta indicative of impaired autophagy. Consistent with impaired autophagy aged AMPK-MKO mice (18 months old) had significantly more muscle fibrosis and centrally located nuclei than WT littermates. Aged AMPK-MKO mice also exhibited mitochondrial myopathy characterized by a greater number of giant mitochondria, mitochondrial DNA mutations and accumulation of the mitophagy protein Parkin; consistent with an inability to remove dysfunctional mitochondria in the absence of skeletal muscle AMPK. These findings establish an essential requirement for skeletal muscle AMPK-mediated autophagy in preserving blood glucose levels during prolonged fasting as well as maintaining muscle integrity and mitochondrial function during aging.

Poster #41

Synergistic effects of clinical doses of metformin and salicylate on AMPK activity, liver de novo lipogenesis and insulin sensitivity in mice and humans

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While metformin is the mainstay therapy for type 2 diabetes (T2D), many patients also take salicylate based drugs such as Aspirin for their cardioprotective effects. Salsalate (two salicylate molecules) is currently in clinical trials for the treatment of T2D and cardiovascular disease (CVD). Both metformin and salicylate (the in vivo active metabolite of aspirin and salsalate) increase AMP-activated protein kinase (AMPK) activity; however, their mechanisms of action are distinct, with metformin altering the adenylate charge of the cell, while salicylate interacts directly with the AMPK β1 subunit Ser-108. Activation of AMPK by both metformin and salicylate leads to phosphorylation and inhibition of acetyl-CoA carboxylase (ACC), the first rate limiting step in the control of fatty acid synthesis and an important determinant of non-alcoholic fatty liver disease (NAFLD). Given their distinct abilities to activate AMPK, we tested the hypothesis that there may be synergistic effects at low clinical doses of salicylate and metformin on AMPK activity and fatty acid synthesis. We find that in a purified cell-free assay, AMP enhances salicylate-induced activation of an AMPK $\alpha 1\beta 1\gamma 1$ heterotrimer by over 60-fold. In mouse primary hepatocytes, treatment with clinically relevant concentrations of metformin and salicylate have no effect on ACC phosphorylation and fatty acid synthesis; however, when combined they increase ACC phosphorylation and inhibit fatty acid synthesis. These findings are also observed in primary human hepatocytes. Chronic treatment of obese mice with clinically achievable doses of metformin and salsalate in combination reduces liver fat and improves glucose tolerance and liver insulin sensitivity. Importantly, metformin and aspirin also have additive effects on reducing pro-insulin (a marker of glycemic control/insulin sensitivity) in over 8000 people with dysglycemia. These data demonstrate the synergistic effects of salicylate and metformin on AMPK activity, liver fatty acid synthesis and insulin sensitivity indicating that combination therapy may be efficacious for NAFLD and T2D treatment.

Scott, J. W., Ling, N., Issa, S. M., Dite, T. A., O'Brien, M. T., Chen, Z. P., Galic, S., Langendorf, C. G., Steinberg, G. R., Kemp, B. E. & Oakhill, J. S. 2014. Small Molecule Drug A-769662 and AMP Synergistically Activate Naive AMPK Independent of Upstream Kinase Signaling. *Chem Biol,* 21**,** 619-27.

Poster #42

Activation of Ampk improves macrophage cholesterol homeostasis

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Atherosclerosis stems from imbalances in lipid metabolism and leads to maladaptive inflammatory responses. The AMP-activated protein kinase (Ampk) is a highly conserved serine/threonine kinase that regulates many aspects of lipid and energy metabolism, although its specific role in controlling macrophage cholesterol homeostasis remains unclear. We sought to address this question by testing the effects of Ampk-specific activators in primary bone marrow-derived macrophages from Ampk β1-deficient ($\beta1^{-/-}$) mice. Macrophages from Ampk $\beta1^{-/-}$ mice had enhanced lipogenic potential and diminished cholesterol efflux, although cholesterol uptake was unaffected. Specific activation of Ampk β1 via salicylate (the unacetylated form of aspirin) or A-769662 (a small molecule activator), decreased the synthesis of both fatty acids and sterols in WT but not Ampk $\beta1^{-/-}$ macrophages. In lipid-laden macrophages, salicylate decreased cholesterol uptake and increased cholesterol efflux to HDL and apoA-I, effects that occurred in an Ampk β1-dependent manner. Increased cholesterol efflux was also associated with increased gene expression of the ATP binding cassette transporters, Abcg1 and Abca1. Moreover, in vivo reverse cholesterol transport was significantly suppressed in mice that received Ampk $\beta1^{-/-}$ macrophages compared to WT control. Our data highlight the therapeutic potential of targeting macrophage Ampk with new or existing drugs for the restoration of cholesterol homeostasis during the early stages of atherosclerosis.

Poster #43

AMPK mediates stress-induced potency factor decrease in stem cells that induces differentiation

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At low exposures of many stressors (i.e. hyperosmotic sorbitol, genotoxic benzopyrene, or hypoxic O2<2%), AMPK adjusts metabolic mechanisms to sustain stem cells and maintain proliferation. But at higher stress exposures, stem cell accumulation is diminished and AMPK mediates potency factor loss necessary for differentiation. In placental trophoblast stem cells (mTSCs) AMPK mediates stress-induced decrease in Inhibitor of Differentiation (Id)2 and Caudal related homeobox (Cdx)2; transcription factors that maintain potency. In totipotent cells of the two-cell stage embryo AMPK mediates stress-induced decrease in Id2, Cdx2, and Oct4. In pluripotent embryonic stem cells (mESCs) AMPK mediates stress-induced decrease in Nanog and Oct4, transcription factors that maintain potency. The peak AMPK response to hyperosmotic stress in mESCs, mTSCs, and embryos is rapid (i.e. 15 minutes) and return to baseline activity is rapid (i.e. 60-90 minutes). But, stress-induced increase of Hand1, which is inhibited by Id2, requires stress-activated protein kinase/jun kinase (SAPK/JNK). Hand1 transcription factor is required for differentiation of mTSCs to produce the rodent equivalent of “Early Pregnancy Test” hormone (e.g placental lactogen-1/PL1) that is important in maintaining the maternal supply of nutrition to the embryo. The kinetics of SAPK activation is slower and much more long-lived, remaining at peak for 4hr and active through 10hr of stress. Thus AMPK mediates rapid potency loss and SAPK mediates longer activation of Hand1, both events are required to maintain the long-lived differentiated state of the stem cell. Interestingly, during AMPK- and proteasome-dependent Id2 protein loss, AMPK contributes to preservation of Id2 mRNA. When stress is removed Id2 protein is regained and cell proliferation returns to near normal rates. Our current hypothesis is that AMPK mediates a reversible differentiation of mTSCs to produce sufficient hormone for embryo survival, but that cells revert to stemness when stress is removed.

Poster #44

AMP-activated kinase (AMPK) and its role in inflammatory nociception

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The activation of adenosine monophosphate (AMP)-activated kinase (AMPK) has shown beneficial effects such as improvement of hyperglycemic states in diabetes as well as reduction of obesity and inflammatory processes. Furthermore, recent data indicated an additional important role of AMPK in the regulation of acute and inflammatory pain. We could show that activation of AMPK significantly alleviates mechanical hyperalgesia in zymosan-induced paw inflammation and the tonic nociceptive response in the formalin assay in mice. Since these findings suggest an impact of AMPK in major inflammatory signaling pathways, in continues studies we focused on signal transduction cascades underlying the AMPK-mediated antinociceptive effects.

Surprisingly, after finding significant inhibition of inflammatory nociception after AMPK activation by AICAR, we found no effect on the expression and activation of the typical proinflammatory gene COX-2 in the zymosan induced paw inflammation model as assessed by RT-PCR analysis and PGE2 ELISA. In addition, COX-2, iNOS or IL1b were also not regulated in the formalin test. However; treatment of mice with AICAR significantly decreased the formalin-induced expression of the 2 pain-relevant transcription factors c-fos and egr1 in the spinal cord which might influence the regulation of further pain genes.

In further experiments we investigated the effect of AICAR on thermal hyperalgesia in the zymosan model. In contrast to a significant inhibition of mechanical hyperalgesia, thermal hyperalgesia was not influenced by AICAR-induced AMPK activation and also not by a knock-out of AMPKa2. These results fit well with the observation that Ca2+ influx in primary DRG neurons of AMPKa2 wild type and knock-out mice showed no difference after stimulation of heat sensitive TRPV1 channels with capsaicin.

In summary, our results indicate that activation of AMPK decreases inflammatory mechanical but not thermal hyperalgesia at least partially by regulation of c-fos and egr1 dependent genes. Therefore, specific AMPK activators might constitute novel tools for the therapeutic treatment of different pain types.

Poster #45

Novel kinase recruiting mechanism was revealed by the structural study of RIPoptosome core

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Receptor interaction protein kinase 1 (RIP1) is a molecular cell-fate switch. RIP1, together with FADD and caspase-8, forms the RIPoptosome that activates apoptosis. RIP1 also associates with RIP3 to form the necrosome that triggers necroptosis. The RIPoptosome assembles via DD:DD interactions between RIP1 and FADD and DED:DED interactions between FADD and caspase-8. In this study, we analyzed the overall structure of the RIP1 DD:FADD DD complex, the core of the RIPoptosome. The results show that RIP1 DD and FADD DD form a stable complex in vitro similar to the previously described Fas DD:FADD DD complex, suggesting that the RIPoptosome and DISC complex share a common assembly mechanism. Both complexes adopt a helical conformation that requires the Type I, II and III interactions of the death domains.

Poster #46

Activation of skeletal muscle AMP-activated protein kinase γ1 improves muscle metabolism and insulin sensitivity

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The AMP-activated protein kinase (AMPK) is a major sensor of cellular energy homeostasis and stimulates ATP-generating processes such as lipid oxidation and glycolysis in peripheral tissues upon activation. The heterotrimeric enzyme consists of a catalytic α-subunit, a β-subunit that is important for enzyme activity and a non-catalytic γ-subunit that binds AMP and activates the AMPK complex. We generated a cre-inducible transgenic mouse model that expresses a mutant γ1-subunit (AMPKγ1H151R) in skeletal muscle resulting in chronic AMPK activation. The expression of the AMPKγ3 isoform, the predominant γ isoform in glycolytic skeletal muscle, was reduced by 50% in the extensor digitorum longus muscle of animals carrying the γ1H151R mutation, while the expression of several AMPK targets and associated enzymes (including acetyl-CoA carboxylase and hexokinase II) was increased. The mutation induces a 7-fold increase in glycogen content in gastrocnemius muscle in the absence of altered glycogen synthase protein content. While glucose tolerance was not affected by the expression of γ1H151R, the plasma insulin level under chow conditions was reduced, both at the basal state and 15 minutes after an intraperitoneal glucose injection, indicating increased insulin sensitivity in female mice. Furthermore, increased food intake without changes in body weight, body composition and organ weight were observed in γ1H151R-expressing mice. This absence of weight change may be due to increased overall energy expenditure and a tendency towards increased physical activity. Overall, skeletal muscle AMPK activation by the γ1H151R mutation plays an important role in skeletal muscle metabolism and affects whole-body energy homeostasis.

Poster #47

Investigating the Upstream Mechanisms leading to AMPK Activation under Detachment

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Anoikis-resistance is a prerequisite to metastasis. AMP activated protein kinase (AMPK) pathway has been implicated in anoikis-resistance and metastasis in different cancers. However, the molecular mechanisms that activate AMPK in the context of matrix-deprivation remain unexplored. We observed that AMPK pathway was activated in a rapid and sustained manner under matrix-deprived conditions. AMPK activation was found to be reversible and was inhibited on cell reattachment. Hence, we investigated the upstream mechanisms that may lead to the activation of AMPK upon matrix-deprivation.

Since detachment leads to loss of integrin-matrix cross-linking, we investigated the involvement of integrin signaling in regulating AMPK activation. We found that under detachment, FAK phosphorylation at Y397 and Y925 were reduced. Hence we hypothesized that integrin signalling negatively modulates AMPK. To test this, we took multiple approaches. (a) Under attached conditions, we inhibited FAK using a pharmacological inhibitor, PZ-0117. This did not alter pAMPK levels. (b) We modulated Src, a key downstream kinase, by transfecting constitutively active v-Src. We found that there was no change in the levels of active pAMPK under detached conditions. (c) We inhibited integrins at receptor level using RGD peptide. However, we did not detect a corresponding change in pAMPK levels. These results indicated that AMPK activation might be independently regulated under detachment and may not be the direct consequence of loss of integrin signalling.

Since ATP depletion is known to activate AMPK, we determined the ATP levels in detached conditions. We found that ATP levels did not change under detached conditions up to an hour, but the levels subsequently reduced by 24 hours. This indicated that energy stress might account for sustained AMPK activation, but the initial rapid activation might be independent of cellular energy status.

We then investigated the role of upstream kinases in the rapid activation of AMPK on detachment. The two well-known upstream kinases of AMPK are LKB1 and CaMKKβ. We found no difference in the expression levels of LKB1, a constitutively active kinase. When we performed knock down experiments of LKB1 in HEK 293T cells, we did find a reduction in the levels of AMPK activation. This indicated suggested that LKB1 might be partially responsible for AMPK activation under detachment. Interestingly, LKB1 deficient cell lines like HeLa S3, G361 and A549 were also able to activate AMPK under detachment to a similar extent as LKB1 containing cell lines like BT 474, MCF7 and HEK 293T. This suggested a role for CaMKKβ in AMPK activation under detachment. Consistent with this, inhibition of CaMKKβ with a specific inhibitor, STO 609, or its knockdown in G361, significantly reduced AMPK activation under detachment. Thus CaMKKβ could be a major player in AMPK activation on matrix-deprivation.

Poster #48

Role of AMPK in PTEN Lymphoma Model

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AMP-activated protein kinase (AMPK) is a highly conserved Ser/Thr protein kinase complex that senses cellular energy status by monitoring the cellular AMP/ATP ratio. Like most kinases, it is activated by phosphorylation of a residue within the activation loop of the kinase domain in the α subunit (Thr172 in rat AMPK alpha-2) and once activated, it regulates metabolic pathways by direct phosphorylation of enzymes, transcription factors and co-activators, thus switching off anabolic pathways that consume ATP and switching on catabolic pathways that generate ATP. The principal upstream kinase that phosphorylates Thr172 is the tumour suppressor LKB1. Germline inactivating mutations of LKB1 are responsible for Peutz-Jeghers syndrome, a hereditary predisposition to cancer. Somatic mutations of LKB1 are also found in a significant fraction of human cancers, including non-small lung cancers (c. 30%) and cervical cancers (c. 20%). These findings suggest that the LKB1-AMPK pathway might act as a tumour suppressor, and provided a link between the metabolic actions of AMPK and cancer. However, to date the role of AMPK in tumorigenesis and tumor metabolism has remained unclear. To address this question, we are investigating the effects of T cell-specific loss of the alpha-1 isoform of AMPK in a lymphoma model where PTEN is conditionally deleted from T cells using Cre recombinase expressed from the T cell-specific Lck promoter. An advantage of this system is that alpha-2 is not expressed in T cells, even when alpha-1 is knocked out.

In our initial studies, we have shown that loss of AMPK as well as PTEN does not appear to affect the timing of onset of lymphomas. However, the AMPK knockout tumours are larger, perhaps because they appear to have a defect in migration from the thymus. Thus, lymphomas with AMPK knockout are almost always restricted to the thymus, whereas those resulting from PTEN knockout alone are often also found in lymph nodes and spleen. Another advantage of this model is that we know that the lymphomas originate from the thymus, and we can sample the organ at the “pre-tumor” stage (4 weeks of age), by which time the genes have been deleted but no lymphomas have yet become evident. We are currently analyzing changes in the activity, phosphorylation or expression of proteins associated with cell proliferation and survival, such as AKT, mTORC1, S6K1, Myc and HIF-1α, in the pre-tumour and the tumour cells. In the longer term, we plan to study changes in metabolism caused by AMPK knockout in the pre-tumor cells.

Poster #49

Oxidative stress activates AMPK by increasing AMP and ADP

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AMP-activated protein kinase (AMPK) is activated >100-fold by phosphorylation of Thr172 within its kinase domain by upstream kinases, with the principal upstream kinase being the tumour suppressor LKB1. Binding of AMP to the ☐ subunit, an complementary mechanisms: (i) allosteric activation; (ii) promotion of Thr172 phosphorylation by LKB1; and (iii) inhibition of Thr172 dephosphorylation. Cellular stresses that inhibit ATP production, or that accelerate its consumption, activate AMPK by causing increases in cellular ADP:ATP and AMP:ATP ratios. Once activated, AMPK acts to restore energy homeostasis by switching on catabolic pathways and switching off anabolic pathways. Oxidative stress, often triggered experimentally by adding hydrogen peroxide to the cell medium, has been shown to activate AMPK and has also been implicated in a wide variety of physiological and pathological processes, including aging, diabetes, neurological degeneration and autoimmune disorders. In 2010, two mechanisms of activation of AMPK by oxidative stress were proposed. Our laboratory showed that phosphorylation of Thr172 and AMPK activity increased when HEK-293 cells expressing wild type AMPK-gamma-2 (WT cells) were treated with hydrogen peroxide, while this did not occur in cells expressing an AMP/ADP-insensitive R531G mutant of gamma-2 (RG cells). This suggested that activation was via the classical pathway, mediated by increases in AMP. However Zmijewski et al. proposed a more direct mechanism of activation independent of changes in cellular nucleotides, occurring through the oxidation of two redox-sensitive cysteine residues within the alpha subunit of AMPK. We have re-investigated this question using the method adopted by Zmijewski et al, which involves incubating HEK-293 cells with glucose oxidase. This generates a constant low level of hydrogen peroxide from glucose in the medium, and may be a better model of physiological levels of oxidative stress than direct addition of high concentrations of hydrogen peroxide, which we show to be extremely rapidly metabolized by cellular enzymes. Under these conditions, AMPK was still phosphorylated at Thr172 and activated mainly through an increase in cellular AMP:ATP and ADP:ATP ratios. As evidence for this, we showed that incubation with glucose oxidase caused large increases in these ratios, and also that AMPK activation was greatly reduced in RG cells compared with WT cells. However, a small residual activation did remain in RG cells, which may me mediated by the mechanism proposed by Zmijewski and coworkers. Using a novel assay, we also provide evidence that the increases in Thr172 phosphorylation observed after the treatment with hydrogen peroxide, are mediated in part by inhibition of Thr172 dephosphorylation.

Poster #50

Global Cellular Effects of AMPK Activation

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AMP-activated protein kinase (AMPK) has been identified as a potential drug target in both metabolic diseases and cancer, but the global effects of AMPK activation on cellular protein phosphorylation, gene expression and metabolism have not yet been fully characterised. We aim to define the changes in these processes effected by AMPK activation, to create a comprehensive view of the mechanisms by which this master regulator controls cellular energy balance, growth and proliferation.

AMPK is thought to be required for cells to switch from a state of rapid proliferation (which is associated with aerobic glycolysis, also known as the Warburg effect) to a quiescent state (where the more energy-efficient oxidative metabolism is used). This is relevant to the treatment both of tumours and of disorders of the immune system. For example, rapidly proliferating antigen-stimulated T cells must revert to a quiescent state to become memory cells, and this change is defective in the absence of AMPK (1).

We are using cytotoxic T lymphocytes as a model for rapidly proliferating cells that utilise aerobic glycolysis. We are investigating the effect of activating AMPK, using AMPK knockout cells as controls, on three global sets of changes:

a) The phosphoproteome, using the technique of stable isotope labeling in cell culture (SILAC). We have already identified several potential new direct targets for AMPK, and have preliminary evidence indicating that AMPK may modulate growth-related signaling pathways through the activation of downstream phosphatases, as well as kinases.

b) Expression of total mRNAs, and mRNAs that are being translated (i.e. associated with polysomes), using RNAseq analysis. AMPK appears to particularly inhibit translation of oncogenic mRNAs such as c-myc, while facilitating translation of metabolic mRNAs (2). It has also been reported that TORC1, which is down-regulated by AMPK, may specifically enhance the translation of mRNAs related to cell growth, such as hypoxia-inducible factor 1α (3).

c) Carbohydrate and lipid metabolism, using flux balance analysis (4). This approach should enable us to construct an overview of the influence of AMPK on the entire metabolic network of the cell, and to predict metabolic targets that contribute to its cytostatic effects.

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Poster #51

Functional effects of a pathogenic mutation in CRBN on the regulation of protein synthesis via the AMPK-mTOR cascade.

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Initially identified as a protein implicated in human mental deficit, cereblon (CRBN) was recently recognized as a negative regulator of adenosine monophosphate–activated protein kinase (AMPK) in vivo and in vitro. Here, we present results showing that CRBN can effectively regulate new protein synthesis through the mammalian target of rapamycin (mTOR) signaling pathway, a downstream target of AMPK. Whereas deficiency of Crbn repressed protein translation via activation of the AMPK-mTOR cascade in Crbn-knockout mice, ectopic expression of the wild-type CRBN increased protein synthesis by repressing endogenous AMPK. Unlike the wild-type CRBN, a mutant CRBN found in human patients, which lacks the last 24 amino acids, failed to rescue mTOR-dependent repression of protein synthesis in Crbn-deficient mouse fibroblasts. These results provide the first evidence that Crbn can activate the protein synthesis machinery through the mTOR signaling pathway by inhibiting AMPK. In light of the fact that protein synthesis regulated by mTOR is essential for various forms of synaptic plasticity that underlie the cognitive functions of the brain, the results of this study suggest a plausible mechanism for CRBN involvement in higher brain function in humans, and they may help explain how a specific mutation in CRBN can affect the cognitive ability of patients.

Poster #52

Manipulating substrate utilization in diabetic muscle by targeting the subcellular localization of AMPK

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The energy-sensor AMP-activated protein kinase (AMPK) cycles between a glycogen-bound and a free state. The muscle-specific regulatory AMPKβ2 subunit carries a high affinity carbohydrate-binding module (CBM). Upon energy stress, such as exercise, AMPK localization at glycogen allows for rapid inhibition of glycogen synthesis, whereas cytosolic AMPK is capable of stimulating insulin-independent glucose uptake. By inhibition of glycogen-binding of muscular AMPK, we hypothesize to promote glycogen synthesis and glucose import, both of which are desirable processes for type 2 diabetes patients.
Based on X-ray structures of AMPK-β1 and -β2 CBMs, we performed a structure-based virtual ligand screen. From a commercial 800,000 ligand database, we identified the 1000 best scoring compounds for each isoform. Only 20% of overlap was found between those 2 subsets of compounds. After biochemical testing of 870 candidates, more than 50 compounds appeared to interfere with AMPK-CBM binding to a model sugar. A selection of compounds is currently being further tested in a cellular-based assay. Based on our preliminary data we expect that isoform-specific targeting of AMPK-CBM is feasible.

Poster #53

Targeting cN-1A and cN-II soluble 5'-nucleotidases using knockout mice as a strategy for AMPK activation to combat diabetes

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The stimulation of glucose uptake in skeletal muscle by AMP-activated protein kinase (AMPK) activation underlies the beneficial effect of exercise for type 2 diabetic patients. Intracellular AMP concentrations rise during metabolic stress and AMP is either hydrolysed to adenosine by soluble cytosolic 5'-nucleotidase-1A or deaminated by AMP deaminase to IMP. IMP is then hydrolysed by cytosolic 5'-nucleotidase-II to inosine. We have explored whether pharmaceutical inhibition of AMP-metabolizing enzmes could be a strategy for potentiating AMPK activation in muscle and other tissues for treating type 2 diabetes.

Whole body Nt5c1a KO and Nt5c2 KO mice have been generated and are being studied. Interestingly, both male and female Nt5c2 KO mice show improved blood glucose clearance compared with wild-type animals following intraperitoneal glucose injection. The results of experiments on EDL and soleus muscles from wild-type and both Nt5c1a KO and Nt5c2 KO mice subjected to electrical stimulation for measurements of intracellular nucleotide levels, AMPK activity, ACC phosphorylation and glucose uptake will be presented.

Poster #54

Dynamics of AMPK β-Ser108 phosphorylation, a critical determinant for drug sensitivity

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AMP-activated protein kinase (AMPK) is a therapeutic target for metabolic diseases such as type 2 diabetes[1]. An increasing number of direct AMPK activating drugs such as the thienopyridone derivative A-769662 have been identified[2], but little progress has been made in elucidating their mechanism of action. A-769662 preferentially activates AMPK complexes containing the β1-isoform[3], and we have recently demonstrated a requirement for phosphorylation of Ser108 within the β-subunit carbohydrate binding module (CBM)[4]. We investigated mechanisms regulating Ser108 phosphorylation with the aim of increasing potency of AMPK direct activators.
We show that AMPK sensitivity to A-769662 is conferred exclusively by β-Ser108 phosphorylation and is independent of activation loop (α-Thr172) phosphorylation. In vitro experiments demonstrate that β-Ser108 is a cis-autophosphorylation site following AMPK activation by CaMKKβ. In COS7 mammalian cells β-pSer108 is dephosphorylated at a significantly slower rate compared to α-pThr172, producing AMPK complexes that are allosterically sensitive to drug alone. We also present findings relating to α-pThr172-independent mechanisms of β-Ser108 phosphorylation. Our study highlights the potential benefits of combinatorial therapies involving AMPK-targetting drugs and currently available AMP-elevating drugs such as metformin.

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Poster #55

Roles of LKB1 in hormone-secreting cells

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Introduction

Liver kinase B1 (LKB1), a tumour suppressor inactivated in Peutz-Jeghers syndrome (PJS), exerts its biological effects by directly phosphorylating AMPK (Sutherland CM et al, 2003). LKB1 has previously been shown to play a role in insulin secretion by selective deletion in pancreatic beta cells using the transgenes RIP2 Cre and Pdx1-CreER (Sun G et al, 2004; Granot Z et al, 2009). This leads to improved insulin content and secretion as well as increased beta cell size and altered cell polarity.

Whereas pancreatic alpha cells secrete glucagon, intestinal enteroendorine L-cells secrete the incretin hormone GLP-1 whose main role is in glucose-dependent insulin secretion. Here, we examine the effects of LKB1 deletion on the development and function of pancreatic alpha and intestinal L-cells.

Results

Beta cells using LKB1f/f Ins1Cre

Given that previous studies may have been confounded by recombination in extrapancreatic tissues, in the present study LKB1 was inactivated highly specifically in beta cell using a novel Ins1Cre driver line. Though insulin secretion was not affected in these mice, relative beta cell mass, assessed by optical proection tomography, was significantly increased ($P<0.05$) and there was an increase in beta/alpha cell ratio. Moreover, RNASeq analysis suggested that LKB1 regulates expression of genes involved in glutamate signalling. Correspondingly, signalling by the glutamate receptor agonist kainite to intracellular calcium changes and insulin secretion were significantly enhanced in betaLKB1 KO mice ($P<0.05$).

Alpha cells using LKB1f/f iGluCre

LKB1 was deleted in alpha cells using an iGluCre transgene under the control of the proglucagon promoter. Immunohisotchemistry revealed that the pancreatic alpha/beta cell ratio was significantly increased in these mice ($P<0.005$). However, intraperitoneal and oral glucose tolerance tests were normal. Likewise, when fasted overnight, there was little change in glucagon levels.

L-cells using LKB1f/f iGluCre

Using the same transgene as above, LKB1 was deleted in L-cells of the gut. This led to a slight increase (not significant) in circulating GLP-1 levels. Dramatically, mice with this transgene showed weight loss from 18 weeks and premature death at 20 weeks. On further analysis, large tumours were found in the duodenum.

Conclusions/Future work

We show here that LKB1 plays a significant role in multiple hormone-secreting cell types. Thus, loss of LKB1 leads to enhanced growth in all three cell types examined, most dramatically leading to polyp formation after deletion in intestinal L-cells. Further studies will explore in detail the molecular mechanisms, and downstream kinases involved, for example by using the same Cre drivers to delete AMPK in alpha and L-cells.

Poster #56

LKB1 and NUAK1 kinases regulate terminal axon branching in cortical neurons through control of presynaptic mitochondria capture and function.

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The molecular mechanisms underlying axon branching and circuit formation in the developing brain are still poorly understood. Impairment of these processes can lead to socially-devastating neurodevelopmental defects such as autism spectrum disorders or mental retardation. The polarity kinase LKB1/STK11 is required for the polarization of cortical neurons and in particular axon formation, both *in vitro* and *in vivo* (Barnes et al., *Cell* 2007). Using a CRE-based inactivation of the *Lkb1* gene after axon specification, we uncovered a previously unknown function for LKB1 in controlling the outgrowth and terminal branching of the axon of cortical neurons (Courchet, Lewis et al., *Cell* 2013). We also identified that this novel function of LKB1 involves the phosphorylation and activation of NUAK1/ARK5, a poorly characterized downstream target of the AMPK-related kinase family. In parallel, we discovered that the LKB1-NUAK1 kinases promote the capture of mitochondria specifically at nascent presynaptic sites. Finally, we performed a series of rescue experiments using the axon specific mitochondria anchor protein Syntaphilin as a molecular tool to control mitochondria trafficking, demonstrating that direct control of mitochondrial immobilization in axons mediates the function of the LKB1-NUAK1 kinase pathway during axon branching. To determine what function of presynaptic mitochondria underlie their function during axon branching, we have developed novel approaches to probe dynamics of ATP homeostasis in cortical neurons and their axons. Our results indicate that LKB1 or NUAK1 deficiency impairs the production of ATP by presynaptic mitochondria which play a critical role during axon morphogenesis.

This work was supported by a grant from the National Institute of Aging (NIA-NIH 5R01AG031524 to FP).

Poster #57

A Novel AMPK Activator that Improves Exercise Capacity and Skeletal Muscle Mitochondrial Function and Insulin Sensitivity in Obese Mice

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With the exception of exercise, commonly used medications for the treatment of type 2 diabetes such as metformin have little effect on improving skeletal muscle insulin sensitivity; therefore, there is a need for new therapies acting on this important tissue to maximize improvements in glycemic control. R419 is a recently characterized complex-I inhibitor that acutely activates AMPK and exerts metabolic effects on cultured myotubes and in muscle of db/db mice [1]. Whether this compound improves exercise capacity and insulin sensitivity via skeletal muscle AMPK signaling is unknown. To examine the effects of R419 on these parameters, wildtype (AMPK-WT) and AMPK β1β2 muscle-specific null (AMPK-MKO) mice were made obese through high-fat diet feeding for 6 weeks before being treated with or without R419 for the remaining 6 weeks of the HFD intervention. We find that like chronic exercise training, R419 treatment dramatically improves treadmill running capacity and skeletal muscle mitochondrial biogenesis in AMPK-WT mice. Remarkably, these effects were completely absent in AMPK-MKO mice indicating that skeletal muscle AMPK signaling is required to elicit increases in exercise capacity and mitochondrial biogenesis in response to R419 treatment. AMPK-WT and AMPK-MKO mice developed similar degrees of obesity, glucose intolerance and insulin resistance when fed the HFD. Treatment with R419 dramatically enhanced carbohydrate oxidation, glucose tolerance and skeletal muscle insulin sensitivity, without altering body mass; effects observed in both AMPK-WT and AMPK-MKO mice. There was no effect of R419 on liver insulin sensitivity. These data indicate that R419 increases exercise capacity and mitochondrial function via a skeletal muscle AMPK dependent pathway. Overall, R419 administration may be a viable new treatment for improving exercise capacity and skeletal muscle insulin sensitivity in obesity.

1: Jenkins Y, Sun TQ, Markovtsov V, Foretz M, Li W, Nguyen H, Li Y, Pan A, Uy G, Gross L, Baltgalvis K, Yung SL, Gururaja T, Kinoshita T, Owyang A, Smith IJ, McCaughey K, White K, Godinez G, Alcantara R, Choy C, Ren H, Basile R, Sweeny DJ, Xu X, Issakani SD, Carroll DC, Goff DA, Shaw SJ, Singh R, Boros LG, Laplante MA, Marcotte B, Kohen R, Viollet B, Marette A, Payan DG, Kinsella TM, Hitoshi Y. AMPK activation through mitochondrial regulation results in increased substrate oxidation and improved metabolic parameters in models of diabetes. PLoS One. 2013 Dec 5;8(12):e81870. doi: 10.1371/journal.pone.0081870. eCollection 2013

Poster #58

Hedgehog/AMPK-mediated control of polyamine metabolism supports neuronal and medulloblastoma cell growth

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Developmental Hedgehog (Hh) signaling controls proliferation of cerebellar granule cell precursors (GCPs) and its aberrant activation is a leading cause of medulloblastoma, the most frequent brain malignancy of the childhood. In this work we demonstrate that Hedgehog promotes biosynthesis of polyamines, small polycations involved in cell proliferation, by engaging a Hedgehog/AMPK axis leading to ODC translation. Upon activation of the Hh transducer Smo, AMPK phosphorylates the RNA binding protein CNBP. Phosphorylated CNBP increases its association with Suppressor of Fused (Sufu), followed by CNBP stabilization, ODC translation and polyamine biosynthesis. Notably, elevated CNBP, ODC and polyamines are hallmarks of Hedgehog-dependent medulloblastoma and genetic or pharmacological inhibition of this axis efficiently blocks the growth of medulloblastoma cells. Together, these data illustrate an unexpected AMPK-mediated mechanism of metabolic control by a morphogenic pathway with relevant implications in development and cancer.

Poster #59

Dual control of AMPK-induced vasodilation in resistance vessels by SERCA and BKCa channels

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AMPK stimulation is suggested to exert positive effects for the treatment of the metabolic syndrome, which is not only characterized by a metabolic, but also by a vascular component. Accordingly, a reduction of microvascular tone by AMPK e.g. in skeletal muscle, may improve insulin resistance. However, the effect of AMPK and the underlying mechanisms at the level of microvascular smooth muscle (VSM) are not yet fully elucidated. We hypothesized that AMPK could regulate VSM cytosolic free calcium $[Ca^{2+}]_i$, thereby modulating vascular tone. The aim of this study was therefore to analyze the functional consequences of AMPK activation with respect to tone and VSM $[Ca^{2+}]_i$ in microvessels. In isobaric preparations of preconstricted (norepinephrine) hamster skeletal muscle resistance arteries, pharmacological AMPK activation by two structurally unrelated compounds (A769662 and PT1) caused a dose-related vasodilation up to 91% of maximal diameter. The latter was accompanied by a decrease of $[Ca^{2+}]_i$ (Fura-2, up to 88 % of the calcium decrease induced by calcium entry blockers). These effects occurred independently of the endothelium. Whole cell patch clamp studies in freshly isolated VSM revealed activation of BK_{Ca} currents in the presence of AMPK stimulators. Accordingly, we found AMPK-mediated hyperpolarization up to 26 mV in VSM of isolated arteries. Surprisingly, the BK_{Ca} channel blocker iberiotoxin showed only minor effects on AMPK-induced vasodilation and calcium decrease in isolated vessels. In contrast, the selective SERCA inhibitor thapsigargin significantly reduced the $[Ca^{2+}]_i$ decrease by 42 % and the vasodilation by 61 %. A combined application of SERCA and BK_{Ca} channel inhibitors abolished the AMPK-induced vasomotor effects.

We conclude that AMPK directly modulates microvascular VSM tone by both, inducing BK_{Ca} channel-mediated hyperpolarization and calcium sequestration *via* SERCA activation. The SERCA driven calcium decrease seems to be the more crucial mechanism, since SERCA could fully compensate for BK_{Ca} channel inhibition. Thus, AMPK could be a potential target in the treatment of the metabolic syndrome by influencing microvascular tone.

Poster #60

Constructing and characterizing an AMPK FRET-sensor reporting conformational changes within the AMPK heterotrimer

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The AMP-activated protein kinase (AMPK), an evolutionary conserved, large heterotrimeric complex, is a central signaling hub and master regulator of energy metabolism and beyond, and implicated in various pathologies. The kinase is activated in situations of metabolic stress by multiple, complex mechanisms. These include activating phosphorylation at the α-subunit (by upstream kinases), inactivating dephosphorylation (by upstream phosphatases), as well as regulation via binding of AMP- and ADP to the γ-subunit and of an unknown regulator at the α/β-subunit interface. Activator binding increases the AMPK phosphorylation level and (in case of AMP) directly allosterically activates the kinase. These mechanisms require close communication between the AMPK subunits, involving a conformational switch upon activator binding as we proposed earlier. To further elucidate this mechanism, we have engineered an AMPK heterotrimer that allows a direct, real-time readout of the AMPK conformational state by fluorescence energy transfer (FRET). A bottleneck in engineering such multi-protein complexes is the exponential increase in work-load if several heterologous genes need to be altered, engineered and combined. We used the ACEMBL technology which harnesses site-specific and homologous recombination techniques in tandem to facilitate rapid, iterative revision of multi-protein complex expression. The resulting genetically encoded fluorescent biosensor can report conformational changes in the AMPK heterotrimer that are induced by adenine nucleotide binding within physiologically relevant concentration ranges. Since the monitored FRET signal correlates with the AMPK activation state, the sensor allows insight into the AMPK activation mechanism and may also be a tool of choice for AMPK-targeted drug screening.

Poster #61

Endothelial AMPK-alpha2 regulates glucose tolerance in mice

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Background: Insulin-induced vasodilatation of arterioles and subsequent increase in muscle microvascular perfusion is thought to be a requisite response to food intake, which is impaired in the metabolic syndrome. We hypothesized that endothelial AMPK regulates insulin-induced vasodilatation and subsequent whole body glucose uptake.
Methods: In our Tie2CreERT2 AMPKα2loxp/loxp endothelium-specific inducible knockout model (AMPK α2i-KO) binding of tamoxifen's active metabolite to the estrogen receptor of bacteriophage derived CreERT2-protein induces nuclear translocation and excision of AMPKα2 DNA. We investigated insulin-induced capillary recruitment (contrast enhanced ultrasonography), peripheral insulin sensitivity (hyperinsulinemic euglycemic clamp), blood pressure (telemetry) and glucose tolerance (oral glucose tolerance test).
Results: In AMPKα2-/- tissuewide KO mice, peripheral insulin sensitivity and insulin-induced capillary recruitment was reduced. After administrating tamoxifen to the AMPKα2i-KO mice null gene was clearly detectable in lung endothelial cells. Both caloric intake and body weight were similar to control mice. Strikingly, at 15 minutes after oral glucose load blood glucose was 36% higher in AMPKα2iEC mice compared to control (15.66 mmol/l, SD2.2 versus 11.52 mmol/l, SD1.3, $p<0.01$, n=3-7). Blood pressure was not altered significantly (n=2 preliminary results). Whereas microvascular filling velocity decreased significantly in control mice, it did not in AMPKα2iEC mice. Peripheral insulin sensitivity and microvascular blood volumes were unaltered, suggesting AMPKα2EC does not regulate peripheral insulin sensitivity.
Conclusions: In summary our results suggest that endothelial AMPK plays a role in the development of glucose intolerance while not altering peripheral insulin sensitivity. In the near future experiments will be performed in AMPKα1iEC and AMPKα1α2iEC mice.

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GENERAL INFORMATION

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CONTACT INFORMATION	Renaissance Tuscany Il Ciocco Resort & Spa Via Giovanni Pascoli, Castelvecchio Pascoli Barga Lucca 55051 Italy +39 0583 7691
ATM	An ATM is located under the archway near the shops by the reception area…opposite the mini market.
BAR	Located in the hotel lobby.
BUSES	There are 3 buses scheduled to leave The Renaissance Tuscany Il Ciocco Resort & Spa on Friday. The buses will leave at 4:30AM, 8:30AM, and 12:00PM. **RESERVATIONS ARE REQUIRED**. If you have not made your reservation yet, please talk with the FASEB Registration Desk by Wednesday morning. The ride to Pisa is approximately 90 minutes. Unfortunately, buses **CAN NOT** stop at the Pisa Railway Station.
CHECK-OUT	Check out is on Friday morning at 12:00 PM. Please remember to pay the hotel for any incidental expenses you may have incurred during your stay.
COFFEE BREAKS	Coffee service will be set up in the foyer behind the lecture hall.
MEALS	Served in La Veranda. Please check your program agenda for service times. Remember to wear your name badge at all times while in La Veranda.
EXCURSIONS	Day trips to local sites in Italy are coordinated through the hotel's front desk.
GUESTS & CHILDREN	Guests and children older than 12 years of age must be registered and paid as a guest to enjoy meals with attendees. Guests and children are not permitted to attend general sessions and poster sessions.
FAX	The front desk can send a fax for you for a small charge.

FASEB OFFICE HOURS	The FASEB Registration Desk/Office is located in the Usignolo Room (next to the Salone Pascoli) and will be open: Sunday: 4:00 PM – 9:00 PM Monday: 7:30 AM – 12:00 PM & 6:00 PM – 7:00 PM Tuesday: 7:30 AM – 12:00 PM & 6:00 PM – 7:00 PM Wednesday: 7:30 AM – 12:00 PM & 6:00 PM – 7:00 PM Thursday: 7:30 AM – 12:00 PM & 6:00 PM – 7:00 PM Friday: 7:30 AM – 12:00 PM
EMERGENCIES	Please dial 118
CLOSEST MEDICAL FACILITY	Hospital “Santa Croce” Via dell’Ospedale, 3 Castelnuovo di Garfagnana Lucca – Italy +39 0583 6691
GROUP PHOTO	The group photo will be taken during the morning coffee break on Monday. Photos will be emailed to all conference participants within 2 weeks of the conclusion of the conference.
INTERNET ACCESS	Computer facilities for attendees are located in room "Castagno" upstairs from the auditorium. There are two PCs connected to the Internet and two printers. There are also 12 Ethernet ports available for those who bring their own laptops. Please pay 10c (euro) per printed page. An honesty box is provided. Wireless access is available in the hotel lobby, auditorium, and “Castagno” computer room. You have to pay the hotel directly for this service. The cost is free for 30 minutes, € 7,00 for one hour and € 11,00 for 24 hours.
MOBILE PHONES	Please turn off mobile phones or put them on silent mode during general sessions. If you must take the call, please remove yourself from the room so that you do not disrupt the other participants.
MESSAGES & MESSAGE BOARD	A message board is displayed in the lobby of the Salone Pascoli. If a message is received, it will be posted on the board. Sessions will not be interrupted unless it is an emergency. You may also receive messages in hotel. Please remember to periodically check the board.

MONEY EXCHANGE	Please see the front desk.
NAME BADGES	Please wear your name badge at all times during sessions, poster sessions and meals.
PARKING	Complimentary parking is available.
POSTERS AND POSTER SESSIONS	Poster sessions will take place in Salone Pascoli. If you submitted an abstract you may present it at one of the scheduled poster sessions. The poster boards are numbered. Check the program materials for the time and day your poster has been scheduled.
RESTROOMS	The nearest restrooms to the session room are through the main door and up one flight of stairs.
SCIENTIFIC SESSIONS	All lectures take place in the Salone Pascoli. Please wear you name badge at all times. Spouses and guests are strictly NOT permitted to attend any session.
SHOPS	There are a few shops near the main lobby area of the hotel.
SPEAKERS & SESSION CHAIRS	Please arrive at the Salone Pascoli at least 30 minutes before your session begins to set-up your laptop for your talk. Someone will be available to assist you if necessary. Remember to pick up an Expense Estimate Worksheet at the FASEB Registration Desk. Completed forms should be turned in by Tuesday morning. The information provided will help the organizers distribute the funds should reimbursement be available.
SPORTING ACTIVITIES	Tennis courts are up the hill from the main gate (2.5km). Please check with the front desk for information. There is a weight room on the 5th floor (before the stairs to the lecture hall complex). The hours of operation are posted by the main elevator on the ground floor. Horseback riding and mountain bikes can be arranged through the front desk. All sports activities are chargeable.

TAXIS	The front desk can call a taxi for you. Approximate fares are: Barga-Gallicano € 26,00 Lucca € 84,00 Pisa € 137,00
TRAINS	The nearest station to Il Ciocco is Barga-Gallincano. The hotel is only 5KM away. To find train times, please visit www.trenitalia.it.
VALUABLES	FASEB and The Renaissance Tuscany Il Ciocco Resort & Spa are not responsible for the damage or loss of personal valuables. If there is a safe in your room, please lock them up or arrange for a safety deposit box at the front desk.