



The Pezcoller
Foundation

Journal



Summary

- Editorial June 2013
- 25th Pezcoller Symposium:
Abstracts of oral presentations
Abstracts of posters
- Call for 2014 International Award
For Cancer Research

June 2013

It's with great pleasure that I can report that the recipient of the 2013 Pezcoller Foundation-AACR International Award for Cancer Research is Peter K. Vogt, PhD, professor of Molecular Medicine of the Scripps Research Institute, La Jolla, CA.

The Selection Committee met in Philadelphia on November 30, 2012 and was chaired by René Bernards, PhD, the Netherland Cancer Institute, Amsterdam.

Members of the Committee were Maria Blasco, Ph. D., Spanish National Cancer Center, Madrid, E - Carlo M. Croce, M.D., Ohio State University, Columbus, OH - Riccardo Dalla-Favera, M.D., Columbia University Institute for Cancer Genetics, New York, NY - Michelle M. Le Beu, Ph. D. University of Chicago, Chicago, IL - Beverly S. Mitchell, M.D., Stanford Cancer Center, Stanford, CA - Marco A. Pierotti, Ph. D., Istituto Nazionale Tumori, Milan, I - Paul Workman, Ph. D., Institute of Cancer Research, Sutton, UK. Dr. Vogt was recommended as the recipient of the Award for his work with oncogenic retroviruses having yielded discovery of oncogenes of major importance in human cancer, among them *myc*, *jun* and *PI3K*. His studies of *PI3K* mutants in cancer show that this protein is unique, specific cancer target, perhaps the most promising therapeutic target currently available.

Peter Vogt was introduced at the 2013 AACR Annual Meeting in Washington D.C. where he delivered to a large audience the Pezcoller Lecture: "PI3K - from simplicity to complexity and back".

Afterwards the Award was given to Dr. Vogt on May 10 with a solemn ceremony in the prestigious hall of the Buonconsiglio Castle in Trento, Italy. In the same week he gave the "Korsmeyer Lecture" in Padova at the VIMM, Venetian Institute for Molecular Medicine to honor the memory of the late Stanley Korsmeyer who received the Pezcoller-AACR Award in 2004.

At the Award's ceremony in Trento, Dr. René Bernards introduced Dr. Peter Vogt "he has made very outstanding contributions to our understanding of how retroviruses cause cancer. He made several seminal discoveries over his 50-year career. His discovery include the identification of the genomic structure of Rous sarcoma virus. Using conditional mutants of the virus he was able to demonstrate that viral genes encode the cancer-causing effects of the virus. His collaborative work with Stehelin, Bishop and Varmus also led to the realization that DNA related to viral oncogenes is also present in mammalian genomes. Dr. Vogt's work has also resulted to the identification of *SRC*, *CJUN* and *PIK3CA* as normal cellular versions of viral oncogenes. Especially the identification of *PIK3CA* as a cancer-causing oncogene has led to the development of a novel class of cancer therapeutics that is currently in late stage of clinical development. At a recent example his work demonstrating that the *PI3K* p110 delta subunit is oncogenic when over-expressed has resulted in the development in a targeted drug, which is demonstrating considerable efficacy in clinical trials in leukemia and lymphoma." In conclusion Dr. Vogt's seminal discoveries have resulted in very fundamental insights in how cancer arises and has made possible new personalized cancer therapies, based on insights into the genetic defects of individual cancers. This issue of the Journal is dedicated to the 25th Pezcol-

ler Symposium entitled "Metabolism and Tumorigenesis" which will be held in Trento from June 20 to June 22, 2013. The meeting has been co-organized by William Kaelin, David Livingston, Massimo Loda and Karen Vousden with the support of Enrico Mihich.

The topic of the symposium will be cell metabolism and the focus on metabolic abnormalities often revealed in tumor cells. Certain metabolic abnormalities in tumors are grounded in the operations of mutant or dysfunctional genes, underscoring the value of these perturbations in the tumorigenesis process. This Symposium will explore key aspects of cancer cell metabolism with an emphasis on understanding the mechanisms that give rise to it, on defining how it serves the survival needs of tumors, on identifying abnormal tumor metabolic phenotypes and on assessing the potential clinical effects of interfering with these abnormalities.

Five are the sessions with the following titles: "The Krebs Cycle and Cancer Cells", "Metabolic Pathways in Cancer Cells", "Modeling Cancer Metabolism", "Imaging and New Technologies", "Therapeutic Opportunities." The invited participants are: **Kevin Brindle** Cambridge Research Institute, Cambridge, UK; **Anne Brunet** Stanford University School of Medicine, Stanford, CA; **Eyal Gottlieb** The Beatson Institute for Cancer Research, Glasgow, Scotland; **Grahame Hardie** University of Dundee, Dundee, Scotland; **Peter Jackson** Stanford University School of Medicine, Stanford, CA; **William Kaelin** Dana Farber Cancer Institute, Boston, MA; **David Livingston** Dana Farber Cancer Institute, Boston, MA; **Massimo Loda** Dana Farber Cancer Institute, Boston, MA; **Elizabeth Maher** UT Southwestern Medical Center, Dallas, TX; **Alberto Mantovani** Istituto Clinico Humanitas, Milan, Italy; **Steven McKnight** UT Southwestern Medical Center, Dallas, TX; **Gerry Mellino** University of Leicester, Leicester, UK; **Enrico Mihich** Dana Farber Cancer Institute, Boston, MA; **Ray Pagliarini** Novartis Institute for Biomedical Research, Cambridge, MA; **Eytan Rupp** Tel Aviv University, Jerusalem, Israel; **David Sabatini** Massachusetts Institute of Technology, Cambridge, MA; **Alan Saghetlian** Harvard University, Cambridge, MA; **Owen Sansom** The Beatson Institute for Cancer Research, Glasgow, Scotland; **Almut Schulze** London Research Institute, London, UK; **Reuben Shaw** Salk Institute for Biological Studies, La Jolla, CA; **Karen Vousden** The Beatson Institute for Cancer Research, Glasgow, Scotland; **Katherine Wellen** University of Pennsylvania, Philadelphia, PA; **Linda Hsieh-Wilson** California Institute of Technology, Pasadena, CA; **Richard Wooster** Glaxo Smith Kline, Collegeville, PA; **Katharine Yen** Agios Pharmaceuticals, Cambridge, MA.

The abstracts of this symposium are in the following pages.

Gios Bernardi M.D.
Editor and President Emeritus

Picture on front page: 2013 Pezcoller Foundation-AACR International Award for Cancer Research ceremony in Trento. From the left: Prof. Davide Bassi, President, Dr. Gios Bernardi President Emeritus, Prof. Peter K. Vogt, winner.

25th Pezcoller Symposium

Metabolism and Tumorigenesis

Trento, Italy, June 20-22, 2013

ABSTRACTS OF ORAL PRESENTATIONS

Magnetic resonance imaging of tumour metabolism

Kevin M. Brindle
Department of Biochemistry, University of Cambridge, UK

Patients with similar tumour types can have markedly different responses to the same therapy. The development of new treatments would benefit, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients, allowing rapid selection of the most effective treatment [1].

We have been developing methods for detecting the early responses of tumours to therapy, including magnetic resonance (MR) imaging of tumour cell metabolism using hyperpolarized ¹³C-labelled cellular metabolites. Nuclear spin hyperpolarization can increase sensitivity in the MR experiment by >10,000x. This has allowed us to image the location of labelled cell substrates and, more importantly, their metabolic conversion into other metabolites. These substrates include pyruvate [2], lactate [3], glutamine [4], glutamate [5], fumarate [6], bicarbonate [7] and ascorbate [8]. We have shown that exchange of hyperpolarized ¹³C label between lactate and pyruvate can be imaged in animal models of lymphoma and glioma and that this flux is decreased post-treatment [2,9]. We showed that hyperpolarized [1,4-¹³C]fumarate can be used to detect tumour

cell necrosis post treatment in lymphoma [6] and that both the polarized pyruvate and fumarate experiments can detect early evidence of treatment response in a breast tumour model [10] and also early responses to anti-vascular [11] and anti-angiogenic drugs [12]. Fumarate can also be used to detect necrosis in other tissues, such as the kidney [13]. We have shown that tissue pH can be imaged from the ratio of the signal intensities of hyperpolarized H¹³CO₃⁻ and ¹³CO₂ following intravenous injection of hyperpolarized H¹³CO₃⁻ [7] and that tumour redox state can be determined by monitoring the oxidation and reduction of [1-¹³C] ascorbate and [1-¹³C]dehydroascorbate respectively [8]. Related to this, we have shown that cytoplasmic lipid droplets, which give an intense ¹H MR signal that can be detected in vivo, are a reflection of intramitochondrial oxidative stress [14]. More recently we have shown that we can monitor tumour glycolysis by measuring the conversion of hyperpolarized [U-²H, U-¹³C]glucose to lactate. Labelled lactate production was higher in the tumour than in surrounding normal tissue and was markedly decreased at 24 h after treatment with a chemotherapeutic drug.

1. Brindle, K. *Nat Rev Cancer* 8, 94-107 (2008).
2. Day, S.E., et al. *Nat Med* 13, 1382-1387 (2007).
3. Kennedy, B.W.C., et al. *J. Am. Chem. Soc.* 134, 4969-4977 (2012).

4. Gallagher, F., et al. *Magn. Reson. Med.* 60, 253-257 (2008).
5. Gallagher, F., et al. *Magn Reson Med* 66, 18-23 (2011).
6. Gallagher, F.A., et al. *Proc Natl Acad Sci U S A* 106, 19801-19806 (2009).
7. Gallagher, F., et al. *Nature* 453, 940-943 (2008).
8. Bohndiek, S.E., et al. *J Am Chem Soc* 133, 11795-11801 (2011).
9. Day, S.E., et al. *Magn Reson Med* 65, 557-563 (2011).
10. Witney, T.H., et al. *Brit. J. Cancer* 103, 1400-1406 (2010).
11. Bohndiek, S.E., et al. *Mol Cancer Ther* 9, 3278-3288 (2010).
12. Bohndiek, S.E., et al. *Cancer Research* 72, 854-864 (2012).
13. Clatworthy, M.R., et al. *Proc. Natl. Acad. Sci. U. S. A.* 109, 13374-13379 (2012).
14. Boren, J., et al. *Cell Death and Differentiation* 19, 1561-1570 (2012).

The Plasticity of Aging

Anne Brunet
Stanford University School of Medicine,
Stanford, CA

Aging, long thought to be solely the byproduct of “wear and tear”, is in fact a highly plastic process regulated by a combination of genetic and environmental factors. My lab is interested in discovering genes that regulate lifespan and in exploring how the products of these genes integrate environmental stimuli that promote longevity, such as dietary restriction. The pathway connecting the insulin signaling pathway to FoxO transcription factors is well known to play a pivotal role in aging from worms to mammals. One part of my lab is focused on understanding how the insulin-FoxO pathway acts to regulate gene expression programs and cellular responses that are important for longevity in mammals. We are particularly interested in the role of longevity genes and pathways, including the insulin-FoxO pathway, in aging neural stem cells. My lab also uses unbiased approaches in the nematode *C. elegans* and in mammalian cells to identify novel pathways that control organismal longevity,

particularly in response to dietary restriction. Finally, we are developing the extremely short-lived African killifish *N. furzeri* as a new vertebrate model for aging studies to explore the genetic architecture of longevity in vertebrates.

AMP-activated protein kinase: friend or foe in cancer?

D. Grahame Hardie
Division of Cell Signalling & Immunology,
College of Life Sciences, University of Dundee,
Dundee, Scotland, UK

The AMP-activated protein kinase is a highly conserved sensor of cellular energy status that is conserved in essentially all eukaryotes. AMPK exists universally as complexes containing catalytic α subunits and regulatory β and γ subunits, and is activated by phosphorylation at a conserved threonine residue (Thr-172) within the α subunit kinase domain. Displacement of ATP by AMP and/or ADP at multiple binding sites on the γ subunit (which occurs when ADP:ATP and AMP:ATP ratios increase as a result of cellular energy stress) causes conformational changes in the complex that enhance net Thr-172 phosphorylation (by promoting phosphorylation and inhibiting dephosphorylation), leading to a large increase in kinase activity. Binding of AMP (but not ADP) also causes further allosteric activation.

The hunt for the upstream kinase that phosphorylated Thr-172 led to the identification of complexes containing LKB1, which had previously identified as a tumor suppressor by human and mouse genetics. This raised the possibility that AMPK might mediate some, if not all, of the tumor suppressor functions of LKB1. At least three considerations support this idea: (i) AMPK activation can trigger cell cycle arrest; (ii) AMPK activation inhibits the mTORC1 pathway and other anabolic pathways required for cell growth; (iii) in the longer term, AMPK opposes the reliance on glycolysis seen in most tumor cells, while promoting the more energy-efficient oxidative metabolism observed in most quiescent cells. We have obtained evidence using a genetically engineered mouse model that the presence of AMPK

delays the onset of tumors, or renders them less aggressive; results obtained using this model will be presented. Consistent with the idea that the LKB1-AMPK pathway is a tumor suppressor, it is down-regulated in many tumors, and the mechanisms by which this arises will also be discussed.

The LKB1-AMPK pathway therefore appears to protect against the development of cancer. Paradoxically, however, it may be easier to treat cancers where the pathway has been lost, because if still present it enhances the survival of tumor cells treated with certain classes of cytotoxic agents. Therefore while AMPK is a “friend” in terms of preventing initiation of cancer, it can be a “foe” when it comes to treatment of cancer.

The von Hippel-Lindau Tumor Suppressor Protein: Insights into Oxygen Sensing and Cancer Metabolism

William G. Kaelin Jr., M.D.

Howard Hughes Medical Institute, Dana-Farber Cancer Institute and Brigham and Women's Hospital, Harvard Medical School, Boston, MA

The pVHL tumor suppressor protein is the substrate recognition subunit of an ubiquitin ligase complex that targets the alpha subunit of the HIF transcription factor for polyubiquitylation and proteasomal degradation. The binding of pVHL to HIF α requires that HIF α be hydroxylated on one (or both) of two conserved prolyl residues by members of the EglN (also called PHD) family of prolyl hydroxylases. EglN1 (PHD2) is the primary HIF α prolyl hydroxylase under normal conditions, with EglN2 (PHD1) and EglN3 (PHD3) playing compensatory roles under certain conditions. EglN2 and EglN3 also appear to have HIF-independent activities linked to control of cell proliferation and apoptosis, respectively. The EglNs are 2-oxoglutarate-dependent dioxygenases that require 2-oxoglutarate (also called α -ketoglutarate), oxygen, and iron in order to function. Other 2-oxoglutarate-dependent dioxygenases include the JmjC histone demethylases and the TET DNA hydroxylases. We recently showed that KDM5A (also called JARID1A

or RBP2) is an H3K4 demethylase and is a potential therapeutic target in murine cancers linked to *RB1* or *MEN1* inactivation. Some cancers are linked to inactivating mutations affecting SDH and FH, leading to the accumulation of succinate and fumarate, respectively, or to gain of function IDH mutations, leading to the accumulation of R-2-hydroxyglutarate. Succinate, fumarate, and R-2HG can alter (usually inhibit) various 2-oxoglutarate-dependent dioxygenases. IDH1 and IDH2 mutations have been observed in several different tumors including gliomas and leukemias. We recently discovered that R-2HG, in contrast to the alternative enantiomer S-2HG, potentiates, rather than inhibits, EglN activity and linked this to the ability of mutant IDH1 to transform human astrocytes. Moreover, we found that R-2HG, but not S-2HG, is sufficient to promote leukemic transformation and that its effects are reversible. In this setting inactivation of EglN, as occurs with S-HG, appears to be antithetical to transformation. R-2HG promotes leukemic transformation by inhibiting targets such as TET2, while sparing EglN activity.

Metabolic alterations in prostate cancer

*Massimo Loda, Giorgia Zadra, Carmen Priolo
Harvard Medical School, Dana Farber Cancer Institute, Boston, MA*

Cancer cells undergo profound metabolic changes as a result of either abnormal signaling pathways or mutations in genes encoding metabolic enzymes. A fundamental unanswered question is whether all oncogenic drivers harness a similar metabolic response in human tumors or whether each oncogenic event drives its own specific metabolic program. The clinical corollary of the latter is that specific metabolic enzymes/pathways could be therapeutically targeted based on the molecular phenotype of the tumor, individually or together with the putative driving oncogenes. Similarly, metabolic imaging modalities could be selected for groups of patients whose tumors harbor certain molecular lesions.

Metabolite profiling was performed on immortalized human prostate epithelial cells transformed by the MYC and AKT1 oncogenes, transgenic mice driven by the same oncogenes under the control of a prostate-specific promoter, and human prostate tumors characterized for the expression and activation of these proteins, respectively. An integrative analysis of the three datasets showed that AKT1 predominantly drives aerobic glycolysis while MYC overexpression is associated with dysregulation of lipid metabolism.

Increased de novo lipogenesis is known to be a hallmark of prostate cancer. Both lipogenic enzymes and AMPK, a major upstream negative regulator of lipogenesis, represent ideal therapeutic targets. We show that the suppression of de novo lipogenesis is the predominant mechanism responsible for AMPK-mediated prostate cancer growth inhibition over mTORC1. Intriguingly, PET with the lipid precursor 11C-acetate shows divergent results in prostate cancer xenografts following AMPK activation or fatty acid synthase inhibition in vivo.

Thus, prostate tumors exhibit metabolic fingerprints of their molecular phenotypes, which may have high impact on both diagnostics and targeted therapeutics.

Macrophage polarization and orchestration of metabolism

A. Mantovani

Istituto Clinico Humanitas IRCCS, University of Milan, Rozzano, Italy

Macrophages are key orchestrators of chronic inflammation. They respond to microenvironmental signals with polarized genetic and functional programmes. M1 macrophages which are classically activated by microbial products and interferon- γ are potent effector cells which kill microorganisms and tumours. In contrast, M2 cells, tune inflammation and adaptive immunity; promote cell proliferation by producing growth factors and products of the arginase pathway (ornithine and polyamines); scavenge debris by expressing scavenger receptors; promote angiogenesis, tissue remodeling and repair. M1 and M2 cells represent simplified extremes of a continuum

of functional states. Available information suggests that TAM are a prototypic M2 population. M2 polarization of phagocytes sets these cells in a tissue remodeling and repair mode and orchestrate the smouldering and polarized chronic inflammation associated to established neoplasia. Intrinsic metabolic features and orchestration of metabolism are key components of macrophage polarization and function. Recent studies have begun to address the central issue of the relationship between genetic events causing cancer and activation of protumour, smouldering, non resolving tumour-promoting inflammation. New vistas have emerged on molecules associated with M2 or M2-like polarization and its orchestration in cancer.

References

- Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol.* 2013; 229(2):176-85.
- Sica A and Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* 2012; 122, 787-795.
- Biswas SK, Mantovani A. Orchestration of metabolism by macrophages. *Cell Metab.* 2012; 15(4): 432-7
- Biswas S.K. and Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as paradigm. *Nat Immunol* 2010; 11, 889-896.

Keynote Address: Choking cancer via inhibition of a tumor-essential metabolic enzyme dispensable to normal tissues

Steven McKnight

UT Southwestern Medical Ctr, Dallas, TX

Mouse embryonic stem cells are unique in their dependence upon the catabolic conversion of threonine into glycine and acetyl-CoA. This specialized metabolic state is afforded by the copious expression of the threonine dehydrogenase (TDH) enzyme only in mouse ES cells. Potent and selective chemical inhibitors of the TDH enzyme kill mouse ES cells, yet have no detrimental effect on any cell or tissue types that do not express the TDH enzyme. These observations prompt the consideration as to whether any forms of

human cancer might selectively express any metabolic enzyme that might be of unique importance to tumors. Following conceptual advances offered by studies of prototrophic yeast grown under nutrient limiting conditions in a chemostat, we have gathered evidence that certain solid tumors utilize acetate as a critical carbon source. Such studies have led to the identification of an enzyme that converts acetate into acetyl-CoA that is dispensable to all tissues of adult mice, yet is of critical importance to a substantial fraction of hepatic tumors. Potent inhibitors of this enzyme have been discovered by a combination of high throughput screening and X-ray structure guided rational design. It is hoped that these chemical inhibitors of enzyme-mediated conversion of acetate into acetyl-CoA may someday qualify as non-toxic therapeutics for the treatment of acetate-dependent human tumors.

Characterizing IDH Mutations in Cancer

Raymond Pagliarini
Oncology Drug Discovery, Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA

Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) mutations result in proteins with a clear gain of enzymatic function: the overproduction of 2-hydroxyglutarate (2-HG), a small molecule “oncometabolite” with diverse effects on cellular function. Emerging evidence suggests that inhibition of IDH-dependent 2-HG production will be a viable strategy to treat patients bearing IDH mutations. However, the general lack of IDH-mutant models prevents a deeper understanding of how these mutations affect cancer biology. To address this gap, we generated a panel of isogenic IDH mutant and wild-type cells. Using this cell line panel, we have demonstrated clear IDH- and 2-HG-dependent signaling and metabolic phenotypes, and determined the reversibility of these phenotypes upon IDH inhibition. This work highlights the potential for direct IDH enzyme inhibition, as well as “synthetic sick” strategies, as options to target IDH mutant tumors.

J Biol Chem. 2012 Dec 7;287(50):42180-94. doi: 10.1074/jbc.M112.417832. Epub 2012 Oct 4.

Isocitrate dehydrogenase (IDH) mutations promote a reversible ZEB1/microRNA (miR)-200-dependent epithelial-mesenchymal transition (EMT).

Grassian AR, Lin F, Barrett R, Liu Y, Jiang W, Korpai M, Astley H, Gitterman D, Henley T, Howes R, Levell J, Korn JM, Pagliarini R.

Reconstruction of healthy and cancerous personalized human metabolic models and its utilization for studying the Warburg effect

Eytan Ruppin
Computer Science & Medicine, Tel-Aviv University, Israel

The emerging field of personalized medicine encompasses the use of marker-assisted diagnosis to improve health care. However, computational models describing cancer metabolism on an individual level have yet to be developed. In this talk I'll present a novel computational approach termed PRIME (Personalized Reconstruction of METabolic models), which addresses this challenge and generates individualized genome-scale metabolic models based on molecular and phenotypic data. We have applied PRIME to build personalized metabolic models for each of the healthy HapMap and NCI-60 cancer cell-lines. These over 250 individual metabolic models successfully predict a range of experimentally measured metabolically-related phenotypes including proliferation rates, gene essentiality, drug responses, metabolic biomarkers and known selective drug treatments in cancer. When applied to clinical data of over 700 individual samples, PRIME-derived models of breast cancer patients enhance the prediction of their prognosis beyond conventional markers.

We then harness the NCI-60 models to perform a genome-scale investigation of the Warburg effect, showing how the cells' metabolic rewiring enforces them to secrete lactate in order to maintain their growth. We further show that the ratio between

the production of ATP in the Glycolysis and its production in OXPHOS, a defining index of the “Warburgness” of cells, is strongly associated with several central cancer-related features, including drug response and the expression of metabolic oncogenes. Interestingly, this ATP production ratio is highly positively correlated with the cells’ motility, emphasizing the role of the Warburg effect in supporting the more aggressive metastatic stages of tumor development. Finally, we predict gene knock-outs that can reverse the ATP production ratio without killing the cells. These targets are expected to inhibit cell migration while attenuating the selection of more aggressive tumor cells.
[Joint work with Keren Yizhak, Yedael Waldman and Gideon Stein]

Regulation of growth by the mTOR pathway

David M. Sabatini, M.D., Ph.D.
Member, Whitehead Institute
Professor Biology, MIT
Investigator, Howard Hughes Medical Institute
Senior Associate Member, Broad Institute
Member, Koch Institute for Integrative Cancer Research at MIT

mTOR is the target of the immunosuppressive drug rapamycin and the central component of a nutrient- and hormone-sensitive signaling pathway that regulates cell growth and proliferation. We now appreciate that this pathway becomes deregulated in many human cancers and has an important role in the control of metabolism and aging. We have identified two distinct mTOR-containing proteins complexes, one of which regulates growth through S6K and another that regulates cell survival through Akt. These complexes, mTORC1 and mTORC2, define both rapamycin-sensitive and insensitive branches of the mTOR pathway. I will discuss new results from our lab on the regulation and functions of the mTORC1 and mTORC2 pathways.

Profiling Natural Small Molecules

Alan Saghatelian
Associate Professor, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA

Profiling methods rely on the ability to detect molecules from complex biological milieu. Though profiling methods are common for nucleic acids and proteins, other classes of natural molecules, including small molecules have been more difficult to implement. Given the importance of metabolites in health and disease the development of new strategies to interrogate this group of molecules is of paramount importance. Therefore, we have developed and applied methods for metabolite profiling to discover novel pathways related to cancerous proteins.

Vinayavekhin N & Saghatelian A (2011) Discovery of a protein-metabolite interaction between unsaturated fatty acids and the nuclear receptor Nur77 using a metabolomics approach. *J Am Chem Soc* 133(43):17168-17171.

Kim YG, Lone AM, Nolte WM, & Saghatelian A (2012) Peptidomics approach to elucidate the proteolytic regulation of bioactive peptides. *Proc Natl Acad Sci U S A* 109(22):8523-8527.

Translational elongation is limiting for the initiation of tumourigenesis following Apc loss.

Owen Sansom
Beatson Institute of Cancer Research, Glasgow, UK

The Adenomatous Polyposis Coli (APC) gene is mutated in approximately 80% of colorectal cancers. Its major function is to negatively regulate the Wnt signalling pathway and loss of Apc leads to the nuclear accumulation of β -catenin and the expression of 100's TCF/LEF target genes. Within the intestinal epithelium loss of the Apc tumour suppressor leads to a crypt progenitor cell phenotype where intestinal enterocytes fail to differentiate, hyperproliferate and are no longer able to migrate. We have previously shown that the c-Myc transcription factor is a

key target following Apc loss, with co-deletion of c-Myc rescuing the phenotypes of Apc loss. To examine the mechanisms by which Myc is essential for the phenotypes of Apc loss we examined the expression of number of candidate pathways downstream of MYC and found mTOR and the phosphorylation of 4EEBP1 and S6 kinase to be Myc dependent. Inhibition of mTORC1 function either genetically via raptor deletion or pharmacologically via rapamycin suppressed the proliferation of Apc deficient cells. Moreover rapamycin treatment arrested proliferation and drove differentiation of established adenomas. Mechanistically this was via suppression of signalling downstream of S6 Kinase, specifically the phosphorylation of EF2 kinase. Genetic loss of EF2 kinase stopped the ability of rapamycin to inhibit proliferation of Apc deficient cells. Furthermore in vitro analysis showed that APC deficient cells had enhanced elongation rates which were suppressed by inhibition of mTORC1.

Thus the marked transcriptional response following Apc loss requires a similar increase in translation which is driven through increased translation elongation downstream of S6 Kinase. If mTOR is inhibited this causes a growth arrest and a switch in cellular fate. Removal of rapamycin allows translation then to proceed and the regrowth of tumours suggesting that when tumours outgrow their nutrient supply a reversible growth arrest occurs. Further mutation of KRAS then allows mTORC1 independent activation of S6 kinase in a MYC dependent manner making cancer cells intrinsically resistant to mTORC1 inhibition. Taken together these data suggest targeting mTORC1 activity via rapalogs make be beneficial in early stage cancer driven by Apc deficiency lacking KRAS mutation or in patients that carry germline mutation of APC.

Metabolic reprogramming in cancer supports cell growth and survival

Almut Schulze
CRUK London Research Institute, London, UK

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.

This work was funded by Cancer Research UK.

References:

Griffiths, B, Lewis, C.A., Bensaad, K., Ros, S., Zhang, Q., Ferber, E.C., Konisti, S., Peck, B., Miess, H., East. P., Wakelam, M., Harris, A.L., and Schulze, A. (2013) Inhibition of SREBP induces ER-stress by altering cellular lipid composition and blocks cancer cell growth. *Cancer & Metabolism*. 1:3
Ros S, Santos CR, Moço S, Baenke F, Kelly G, Howell M, Zamboni N and Schulze A. (2012) Functional screen identifies 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB4) as an important regulator of prostate cancer cell survival. *Cancer Discovery* 2:328-343

The LKB1 tumor suppressor pathway: decoding metabolic links and therapeutic targeting

Reuben J. Shaw
Molecular and Cell Biology Laboratory, Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, CA

The serine/threonine kinase LKB1 is a tumor suppressor gene mutated in the familial cancer condition Peutz-Jeghers syndrome (PJS), as well as in 30% of sporadic non-small cell lung cancer (NSCLC). One of the critical substrates

of LKB1 is the AMP-activated protein kinase (AMPK). AMPK is a highly conserved sensor of cellular energy status found in all eukaryotic cells that restores metabolic homeostasis following stress. Thus LKB1 is a unique energy-state sensitive regulator of growth and metabolic reprogramming via its effects on AMPK. Our laboratory has performed a three-pronged screen to identify novel 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. Collectively, these studies uncovered novel conserved effectors of LKB1 and AMPK that mediate their role as a metabolic checkpoint coordinating cell growth with energy status. The connection of AMPK to tumor suppression has also led many to examine whether compounds that normally serve to activate AMPK, may in fact exhibit anti-cancer activities. Notably, the most widely used type 2 diabetes therapeutic in the US and worldwide, metformin, is a mitochondrial OXPHOS inhibitor which activates AMPK. We have therefore examined the potential anti-cancer effects of metformin and its more potent analog phenformin, in the context of genetically engineered mouse models of lung cancer. Whereas cells containing an intact LKB1-AMPK pathway respond to this metabolic stress by undergoing growth arrest, cells lacking LKB1 are unable to sense the damage and continue to divide ultimately undergoing apoptosis. Thus we examined whether this class of compounds may show selective therapeutic efficacy in preclinical trials in Kras-dependent lung cancer mouse models with loss of LKB1 as compared to those bearing loss of p53.

The role of the p53 pathway in metabolic adaptation and survival

Oliver Maddocks, Celia Berkers, Eric Cheung and Karen Vousden
The Beatson Institute for Cancer Research, Glasgow, UK

The p53 protein is an important tumor suppressor that functions in a number of ways

to prevent cancer development, including an ability to promote cell survival and modulate metabolism. p53-deficient cells that cannot properly mount such protective responses may be more vulnerable to certain types of metabolic stress, a characteristic that could be harnessed for therapy. We have recently found that p53 expression can help cells survive serine starvation. Serine starvation induces de novo serine synthesis by up-regulating the expression of enzymes in the serine synthesis pathway, causing the diversion of glycolytic intermediates and disruption of glycolysis. Interestingly, p53 is not necessary for the activation of the serine synthesis pathway, but seems to be required to allow cells to undergo this metabolic adaptation.

Several p53-induced proteins have been shown to play a role in limiting ROS and modulating metabolism, activities that could contribute to tumor suppression by helping cells to prevent or repair stress and damage. However, the inappropriate or deregulated expression of some of these p53-target genes may also support cancer progression. In this context, we have also been investigating the activities of TIGAR, a p53-inducible protein that functions to protect cells from cell death. TIGAR can act as a fructose-2,6-bisphosphatase, driving the pentose phosphate pathway (PPP), promoting NADPH production to restore reduced glutathione and protecting the cell from ROS-associated apoptosis and autophagy. We have recently found that TIGAR also functions under conditions of hypoxia to limit mitochondrial ROS through mechanisms that are independent of its fructose-2,6-bisphosphatase activity. In addition to limiting oxidative stress, TIGAR is also predicted to function to regulate other metabolic pathways, and we are presently investigating these activities *in vitro and in vivo*.

Metabolic reprogramming: links to the epigenome

Kathryn E. Wellen
Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA

Cancer cells are characterized by major alterations in both cellular metabolism and

epigenetic profiles. Current understanding of links between metabolism and chromatin in the context of cancer is currently very limited. We have previously demonstrated that acetylation of histones is sensitive to glucose availability through the enzyme ATP-citrate lyase (ACL), which produces acetyl-CoA from citrate. While this is likely to impact gene expression and other chromatin-dependent processes, the molecular mechanisms and functional significance of metabolic regulation of lysine acetylation are poorly understood. In this presentation, I will focus on current efforts to elucidate how metabolic signals are conveyed to chromatin. I will also discuss recent findings demonstrating a novel link between ACL and another chromatin-modifying enzyme, DNA methyltransferase 1, during adipocyte differentiation.

Linking O-GlcNAc Signaling to Cancer Metabolism and Tumor Growth

Linda C. Hsieh-Wilson
Division of Chemistry and Chemical Engineering, California Institute of Technology and the Howard Hughes Medical Institute, Pasadena, CA 91125

Cancer cells must satisfy the metabolic demands of rapid cell growth within a continually changing microenvironment. The dynamic post-translational modification of proteins by O-linked β -N-acetylglucosamine (O-GlcNAcylation) serves as a nutrient sensor to couple metabolic status to the regulation of cellular signaling pathways. O-GlcNAc transferase (OGT) catalyzes the transfer of N-acetylglucosamine from uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) to serine or threonine residues of many intracellular proteins, including signaling proteins important for insulin resistance, oncogenes and tumor suppressors, and transcriptional co-activators that control gluconeogenesis. Here we show that O-GlcNAcylation plays a key role in the regulation of glucose metabolism in cancer cells. Glycosylation was dynamically induced on phosphofructokinase 1 (PFK1) at Ser529 under hypoxic conditions in multiple cancer cell lines and human

tumor tissues, but not in rapidly proliferating normal T lymphocyte and fibroblast cells. O-GlcNAcylation inhibited PFK1 activity and redirected glucose flux through the pentose phosphate pathway, thereby conferring a selective growth advantage to cancer cells. Blocking glycosylation of PFK1 at Ser529 reduced cancer cell proliferation *in vitro* and impaired tumor formation *in vivo*. Ongoing metabolite profiling analyses reveal that OGT overexpression, which leads to increased intracellular levels of O-GlcNAc, alters the steady-state pools of nucleotides and unsaturated fatty acids; a preliminary assessment of these alterations in the metabolome and their significance will be discussed in the context of PFK activity. Altogether, these studies reveal a previously uncharacterized mechanism for the regulation of metabolic pathways in cancer and possible targets for therapeutic intervention.

Publications

Rexach, J. E., Rogers, C. J., Yu, S.-H., Tao, J., Sun, Y.-E., Hsieh-Wilson, L. C. "Quantification of O-Glycosylation Stoichiometry and Dynamics using Resolvable Mass Tags," *Nature Chem. Biol.* 9, 645-651 (2010).
Yi, W., Clark, P. M., Mason, D. E., Keenan, M. C., Hill, C., Goddard, W. A., III, Peters, E. C., Driggers, E. M., Hsieh-Wilson, L. C. "Phosphofructokinase 1 Glycosylation Regulates Cell Growth and Metabolism," *Science* 337, 975 (2012).

IDH Mutations and Tumorigenicity

F. Wang^{1ψ}, J. Travins^{1ψ}, B. DeLaBarre^{1ψ}, V. Penard-Lacronique^{2,3,4 ψ}, S. Schalm^{1ψ}, E. Hansen¹, K. Straley¹, A. Kernytsky¹, W. Liu¹, C. Gliser¹, H. Yang¹, S. Gross¹, E. Artin¹, V. Saada³, E. Mylonas^{2,3,4}, C. Quivoron^{2,3,4}, J. Popovici-Muller¹, J.O. Saunders^{1#5}, F.G. Salituro^{1,6}, S. Yan⁷, S. Murray¹, W. Wei⁸, Y. Gao⁹, L. Dang¹, M. Dorsch¹, S. Agresta¹, D.P. Schenkein¹, S.A. Biller¹, S.M. Su¹, S. de Botton^{2,3,4}, K.E. Yen^{1*}

Mutations in the isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) genes are present in ~20% of acute myeloid leukemia, and cause

a neomorphic enzyme activity that results in the production of 2-hydroxyglutarate (2HG). Mutational and epigenetic profiling of a large patient cohort of acute myeloid leukemia (AML) has revealed that IDH1/2-mutant AMLs display global DNA hypermethylation and impaired hematopoietic differentiation.

To further investigate the intrinsic effect of 2HG on hematopoietic proliferation and differentiation, we transfected an erythroleukemia cell line (TF-1) with either IDH1 or IDH2 mutant alleles. These cells overexpress the mutant enzyme, have high levels of 2HG, and exhibit GM-CSF independent growth. Consistent with clinical observations, overexpression of the IDH mutant proteins led to hypermethylation of both histones and DNA. These results suggest that mutations in IDH1/2 could lead to epigenetic rewiring of cells that could facilitate the gain of function phenotype. We are currently studying the global and specific effects of IDH1/2 mutant overexpression to gain a broader

understanding of the biological consequence of the IDH1/2 gain of function mutations

We have also generated mutation selective molecules that are capable of inhibiting IDHm enzymes. Upon compound treatment in vitro, we are able to reverse hypermethylation of both histones and DNA and induce cellular differentiation in IDHm cell lines and primary human IDHm AML patient samples(1, 2). These data suggest that an inhibitor of IDH1/2 mutations could correct the altered gene expression patterns seen in IDH1/2 mutant AML tumors leading to a profound effect on hematopoietic differentiation, proliferation and tumor growth.

1. J. A. Losman et al., (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* 339, 1621 (Mar 29, 2013).
2. F. Wang et al., Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science* 340, 622 (May 3, 2013).

ABSTRACTS OF POSTERS

The extracellular form of NAMPT contributes to creating a proinflammatory environment in chronic lymphocytic leukemia

Valentina Audrito^{1,2}, Sara Serra^{1,2}, Tiziana Vaisitti^{1,2}, Nadia Raffaelli³, Menico Rizzi⁴ and Silvia Deaglio^{1,2}

¹Immunogenetics Unit, Dept. of Medical Sciences, University of Torino, Italy; ²Human Genetics Foundation (HuGeF), Torino, Italy; ³Dept. of Molecular Pathology and Innovative Therapies, Section of Biochemistry, Università Politecnica delle Marche, Ancona, Italy; ⁴Dept. of Chemical, Food, Pharmaceutical and Pharmacological Sciences, "Amedeo Avogadro" University of Eastern Piedmont, Novara, Italy.

Tumor transformation is accompanied by an altered metabolic state, characterized by a higher NAD turnover rate with crucial changes in both energy and signal transduction. Increasing evidence indicate that NAD plays important roles as a co-factor in redox reactions, but also as a signaling molecule in the regulation of calcium homeostasis and inflammation. Nicotinamide phosphoribosyltransferase (NAMPT), is the rate-limiting enzyme involved in NAD biosynthesis and extracellularly an essential cytokine/adipokine-like factor generating proinflammatory conditions in different tumor models as well as in acute and chronic inflammatory-metabolic diseases. In this work we investigated the functional significance of NAMPT in chronic lymphocytic leukemia (CLL), a lymphoproliferative disorder, strongly dependent on a growth supportive environment. Results showed that i) NAMPT mRNA, as well as its plasma levels, were significantly higher in CLL patients compared to healthy donors. Moreover, ii) activation of purified CLL lymphocytes was followed by eNAMPT secretion, indicating that it is the leukemic component that actively releases eNAMPT. Treatment of leukemic PBMCs, but not B

purified CLL lymphocytes, for 5 days with recombinant NAMPT resulted in increased numbers of adherent CD11b⁺ cells, displaying intracellular vacuoles and granules, consistent with macrophage differentiation. These cells secreted significant amounts of proinflammatory cytokines, including IL6, IL8 and CCL3. Furthermore, long-term exposure to eNAMPT enhanced the formation and the phagocytosis ability of nurse-like cells (NCLs), a myeloid population and a crucial component of the CLL microenvironment. In these cells, exogenous NAMPT triggered rapid phosphorylation of Erk1/2, STAT3 and NF- κ B activation.

These functions appear independent of the enzymatic activity of eNAMPT, as inferred by the inability of an enzymatically deficient NAMPT mutant to trigger IL6/STAT3 activation. In line with this conclusion, enzymatic NAMPT product nicotinamide mononucleotide (NMN) was ineffective in the system, while FK866, a selective NAMPT enzyme inhibitor, did not block eNAMPT-dependent functions.

Overall, these results reveals enzyme-independent functions of eNAMPT, critical in the induction of pro-inflammatory and pro-survival pathways in CLL microenvironment.

The glycolytic phenotype of cancer cells modulates resistance to anti-angiogenic therapy

Matteo Curtarello¹, Elisabetta Zulato¹, Giorgia Nardo¹, Silvia Valtorta², Giovanni Esposito¹, Elisabetta Rossi³, Anna Pastò¹, Giulia Guzzo⁴, Aichi Msaki¹, Alberto Amadori^{1,3}, Andrea Rasola⁴, Rosa Maria Moresco² and Stefano Indraccolo¹

¹ Istituto Oncologico Veneto - IRCCS, Padova, Italy

² Nuclear Medicine Department, San Raffaele Scientific Institute; Fondazione Tecnomed, University of Milan Bicocca; IBFM-CNR, Milan, Italy

³ Department of Surgery, Oncology and

Gastroenterological sciences, University of Padova, Padova, Italy

⁴ Department of Biology "A. Vallisneri", University of Padova, Padova, Italy

Anti-angiogenic therapy is increasingly used to treat cancer patients but therapeutic responses are often short-term due to intrinsic or extrinsic resistance. We previously showed that anti-VEGF therapy causes metabolic perturbations in tumors, including marked reduction in glucose and ATP levels. Moreover, highly glycolytic tumors developed broad necrotic areas following VEGF neutralization. Here, we aimed to investigate therapeutic effects of anti-VEGF treatment on experimental tumors with different glycolytic phenotypes as well as the possible modulation of metabolic features of tumor cells by anti-VEGF therapy. We found that poorly glycolytic tumors regressed following protracted anti-VEGF therapy, whereas highly glycolytic tumors became rapidly resistant. Resistance was associated with increased numbers of CD44⁺/CD117⁺ cancer stem cells following anti-VEGF therapy. Moreover, FAZA and FLT PET imaging showed that poorly glycolytic tumors chronically treated with anti-VEGF therapy - albeit did not progress - increased hypoxic and highly proliferative tumor areas. We also observed that protracted anti-VEGF therapy selects for highly glycolytic cells and this metabolic switch is stable and precedes tumor relapse. Interruption of anti-VEGF therapy counteracted this phenomenon and reduced frequency of tumor relapse. These results support the hypothesis that the highly glycolytic phenotype of tumor cells - either primary or secondary - confers resistance to VEGF blockade. Moreover, anti-angiogenic therapy appears to select stable metabolic features of tumor cells.

Glucose utilisation via glycogen phosphorylase sustains proliferation and prevents premature senescence in cancer cells

Elena Favaro¹, Karim Bensaad¹, Mei G. Chong², Daniel A. Tennant², David J.P. Ferguson³, Cameron Snell³, Graham Steers³, Helen Turley³, Ji-Liang Li¹, Ulrich L. Günther²,

Francesca M. Buffa¹, Alan McIntyre¹, Adrian L. Harris¹

¹Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford

²School of Cancer Sciences, University of Birmingham, Birmingham

³Nuffield Department of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford

Metabolic reprogramming in cancer cells provides energy and important metabolites required to sustain tumour proliferation. Hypoxia in tumours represents a hostile environment that can encourage these transformations and other adaptive responses that contribute to poor prognosis and resistance to radiation and chemotherapy. We report here that glycogen metabolism undergoes a characteristic, and pronounced upregulation in response to hypoxia in both tumour xenografts, and in cancer cell lines. More specifically, hypoxia stimulates glycogen accumulation and its subsequent utilisation, as well as the concurrent upregulation of several glycogen metabolising enzymes such as glycogen synthase (GYS1) and glycogen phosphorylase (PYGL). Interestingly, PYGL depletion prevented glycogen utilization, and led to glycogen accumulation in hypoxic cells. Furthermore, PYGL-depleted cells also exhibited increased intracellular levels of reactive oxygen species (ROS), and a reduction in proliferation due to increased p53-dependent induction of senescence. Moreover, depletion of PYGL was associated with markedly impaired tumorigenesis *in vivo*. Metabolic analyses indicated that glycogen degradation by PYGL is important for the optimal functioning of the pentose phosphate pathway in hypoxic cells. Taken together with our findings, a number of observations suggest that PYGL could represent a novel target for cancer therapy. Firstly, PYGL is one of the genes defining a hypoxic signature with prognostic significance in head & neck and breast cancer. Secondly, our meta-analysis of gene expression profiling studies revealed that PYGL is upregulated in several cancer types, as compared to normal tissues. Finally, patients with Hers' disease (that lack PYGL) are largely asymptomatic, suggesting that PYGL inhibitors are unlikely to have severe side-effects *per se*. We conclude

that glycogen metabolism is a key metabolic pathway induced by hypoxia that represents a targetable mechanism of metabolic adaptation in tumours.

Enhanced Expression of AK4 Supports Metabolic Requirements for Maintaining EMT Phenotype by activating HIF1- α in Lung Cancer

*Yi-Hua Jan and Michael Hsiao
Genomics Research Center, Academia Sinica,
Taipei, Taiwan*

The metabolic alterations in cancer progression remain largely unknown. Therefore, identifying genes that are critical for metabolic reprogramming in cancer invasion and metastasis may provide novel targets for cancer diagnosis and therapy. Previously, we have identified adenylate kinase 4 (AK4) as a marker for poor clinical outcome that promotes metastasis of lung cancer. However, the molecular mechanism of AK4-induced phenotype in cancer was not completely understood. By microarray analysis, we found downstream targets of HIF1- α were differentially regulated upon AK4 overexpression in CL1-0 lung cancer cells. Furthermore, AK4 overexpression induces glycolysis and results in accelerated glucose consumption, ATP utilization and lactate production. Overexpression of AK4 also increases the levels of reactive oxygen species, which exaggerates HIF1- α protein expression under hypoxia and concurrently induces epithelial-to-mesenchymal transition (EMT) in a HIF1- α -dependent manner. IHC analysis showed an inverse correlation between AK4 and E-cadherin and a positive correlation between AK4 and nuclear HIF1- α in lung cancer patients. Moreover, patients with AK4 high/E-cadherin low showed worse outcome compared to patients with AK4 low/E-cadherin high. Our study indicates that enhanced expression of AK4 in is a critical factor for triggering aerobic glycolysis and induced HIF1- α -mediated EMT in lung cancer.

Key words: AK4, HIF1- α , EMT, lung cancer invasion and metastasis

Overexpression of mitochondrial F₀F₁ATP synthase subunit epsilon promotes colon cancer metastasis through modulation of AMPK-AKT-HIF1 α signaling axis

*Tsung-Ching Lai, Yi-Hua Jan, and Michael Hsiao
Genomics Research Center, Academia Sinica,
Taipei, Taiwan*

Metastasis remains the major cause of death for colon cancer. To identify differentially expressed genes that were associated with metastatic colon cancer, we analyzed public available microarray datasets which contain normal colon tissues, polyps, primary colon tumors, liver metastatic tumors and lung metastatic tumors. We found *ATP5E* which encodes for mitochondrial F₀F₁ ATP synthase subunit epsilon was overexpressed in tumor compared normal while the other genes encode for ATP synthase subunit were repressed. Moreover, *ATP5E* levels positively correlated with colon cancer progression from poly, primary tumor, liver metastasis, to lung metastasis. We then validated the expression of ATP5E protein expression in colon cancer tissue and found ATP5E was overexpressed in colon cancer and correlated with poor prognosis. Knockdown of ATP5E expression in colon cancer cell lines inhibited invasion and migration. Furthermore, inhibition of ATP5E also reduced distal metastasis of colon cancer in vivo. Next, we found ATP5E could induce colon cancer cells to undergo epithelial-to-mesenchymal transition (EMT) and its expression is predominately associated with low E-cadherin expression in patients with metastatic colon cancer. Moreover, microarray data showed elevated *ATP5E* in metastatic colon cancer significantly associated with AMPK-AKT-HIF1- α signaling axis and knockdown of ATP5E could abolish HIF1- α protein expression under hypoxia through AMPK-AKT signaling. Taken together, our study indicates elevated ATP5E expression in colon cancer is a marker for poor prognosis that promotes colon cancer metastasis by modulating AMPK-AKT-HIF1- α signaling.

Key words: ATP synthase subunit, ATP5E, HIF1- α , colon cancer, metastasis

Lrp-1 Mediates Invasive Activity of Serpin³

Santina Quarta¹, Cristian Turato²,
Alessandra Biasiolo¹, Mariagrazia Ruvoletto¹,
Angelo Gatta¹, Patrizia Pontisso¹
¹Department of Medicine-DIMED, University of
Padua, ² I.O.V. (IRCCS), Padua, Italy

Background. The low-density lipoprotein receptor-related protein-1 is an ubiquitously expressed endocytotic receptor involved in the clearance of several serpins and serpin-protease complexes. However, for the clearance of the ov-serpin SERPINB3, no information is available yet. This serpin induces deregulation of adhesion processes and increases the cellular invasive potential by switching on the epithelial to mesenchymal transition program. Aim of the present study was to investigate whether SERPINB3 binds LRP-1 and whether the invasion effect induced by SERPINB3 is mediated by LRP-1.

Methods: HepG2 cells transfected with SERPINB3 were analyzed with immunofluorescence for co-localization with LRP-1. The Epithelial Mesenchymal Transition (EMT) induced by the incubation of HepG2 control cells with exogenous SERPINB3, after addition of a specific anti-LRP-1 antibody and of the inhibitor RAP, was followed by real-time monitoring instrument (xCELLigence) and by immunofluorescence analysis of EMT proteins.

Results: Our results show that SERPINB3 overexpressed in HepG2-transfected cells co-localizes with LRP-1. The real-time monitoring of the invasion capacity induced by SERPINB3 documented that, blocking LRP-1 with increasing amounts of anti-LRP-1 antibody and/or with the inhibitor RAP molecule, decreased the cellular invasive potential in a dose dependent manner. This effect was confirmed by the profile of epithelial to mesenchymal transition proteins, including vimentin, E-cadherin and beta-catenin, that reversed their invasive pattern after blocking LRP-1.

Conclusions: These results demonstrate that LRP-1 binds SERPINB3 and that it mediates cell invasiveness induced by this serpin.

Genome-wide analysis of p53-dependent transcriptional programs in tumor suppression

Claudia Tonelli,¹ Marco J. Morelli,² Arianna Sabò,² Andrea Piontini,¹ Mattia Pelizzola,² Stefano Campaner,² and Bruno Amati^{1,2}
¹Department of Experimental Oncology, European Institute of Oncology (IEO)
²Center for Genomic Science of IIT@SEMM, Fondazione Istituto Italiano di Tecnologia (IIT)

p53 is a transcription factor central to the regulation of cell fate in response to genotoxic stresses caused either by DNA damaging agents or oncogene activation. After a cell receives a stress stimulus, p53 activates a complex transcriptional network leading to cell cycle arrest, DNA repair, apoptosis or senescence. In concordance with its tumor suppressive function, p53 activity is frequently impaired in human cancers.

Although extensively studied, the detailed genetic programs through which p53 directs stress-specific biological responses remain to be clarified.

Using whole genome mapping of p53 and gene expression profiling, we are investigating the transcriptional circuitry employed by p53 in suppressing cancer development *in vivo*, in a mouse model of Myc-induced lymphoma.

Restoring p53 function in tumor cells is an attractive strategy for treating cancers and it has been shown to elicit tumor regression. We are testing whether the tumor suppressive response induced by p53 in the pre-tumoral phase of lymphoma development, differs from the one induced by pharmacological reactivation of p53 in tumors or by acute DNA damage.

Altogether, these data will shed light on key tumor suppressor mechanisms, whose reactivation remains a central goal in tumor therapy.

To our knowledge this will be the first characterization of the p53 signaling in fresh tissue samples, a more physiologically-relevant context.

Genome-wide investigation of FK866-induced effects in Jurkat cells

Chiara Zucal¹, Vito D'Agostino¹, Toma Tebaldi¹, Santina Bruzzone², Alessandro Quattrone¹, Alessio Nencioni³, Alessandro Provenzanj¹

¹ Centre for Integrative Biology, CIBIO, University of Trento, Trento, Italy

² Department of Experimental Medicine, Section of Biochemistry, University of Genoa, Genoa, Italy

³ Department of Internal Medicine, University of Genoa, Genoa, Italy

Acute lymphoblastic leukemia is a relatively uncommon form of cancer (17.3 per million in United States, of which T-ALL comprises about 25%) that usually affects infants, children and young adults. In T cell leukemia cell lines, apoptosis occurs when endogenous NAD synthesis is limited using FK866. This is a specific inhibitor of nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme in the regulation of NAD biosynthesis from the natural precursor nicotinamide, that on lymphocyte activation becomes up-regulated to compensate for the increased metabolic demands.

By using Jurkat cells as a model of acute T-cell leukemia, we evaluated the effect of FK866 by AnnexinV/7AAD FACS analysis revealing a decrease in the number of cycling cells after treatment. The calculated IC50 at 48 hours was 5.5 nM. A significant, dose-dependent reduction of NAD-levels in treated samples confirmed the efficacy and specificity of the drug.

To determine gene-expression changes caused by FK866-induced NAD depletion in leukemic T cells, we performed microarray analysis of RNA samples after sucrose gradient fractionation of Jurkat lysates (sub-polysomes, polysomes, total). Among the identified DEGs we analyzed polysomal/subpolysomal distribution in treated or untreated cells to characterize the multi-level gene-expression regulation effects of the drug. Up-regulated DEGs in the polysomal RNA from treated samples code for proteins involved in chromatin modification, nucleotide and RNA bioprocessing and metabolism.

The identified differentially expressed genes,

in the early phase of cell response to FK866, and the genes accounting for a specific post-transcriptional regulation may help the identification of pathways involved in pharmacoresistance.

Prognostic Role of Amp-Activated Protein Kinase (Ampk) Expression in Metastatic Colon Rectal Carcinoma Treated with Anti-Vegf Therapy

Zulato E., Bergamo F., De Paoli A., Griguolo G., Esposto G., De Salvo G., Lonardi S., Zagonel V., Indraccolo S.
Istituto Oncologico Veneto-IRCSS, Padova, Italy

Vascular targeting has been increasingly used as therapeutic approach for cancer patients. However, validated biomarkers to predict the optimal biological dose of the anti-angiogenic agents and aiding selection of those patients who are most likely to benefit from the anti-angiogenic treatment are currently not available.

We previously showed that anti-VEGF therapy causes metabolic perturbations in tumors, including marked reduction in glucose and ATP levels. Moreover, we observed that LKB1-AMPK is activated by anti-angiogenic therapy in tumor xenografts, influencing the type of pathological tumor response to VEGF neutralization.

Here, we investigate the role of AMPK *status* as prognostic and/or predictive marker of responses to anti-angiogenic therapy in metastatic colon rectal carcinoma (mCRC) patients treated with FOLFIRI-Bevacizumab. We retrospectively analyzed pAMPK and pACC expression in a cohort of 48 mCRC tumor samples, using immunohistochemistry (IHC) staining. Protein levels of pAMPK and pACC were compared using Spearman's correlation test both in primary tumors and in metastasis. IHC scores of the two phospho-proteins significantly correlated both in primary tumors ($p=0.0001$) and metastasis ($p=0.003$). Moreover, a trend towards correlation of pACC levels between matched primary tumors and metastasis was observed ($p=0.06$). To investigate the

possible prognostic/predictive value of these in situ markers, IHC scores of pAMPK and pACC were re-coded in 2 classes (scores ≤ 5 versus > 5) and correlated with clinical features of patients. Multivariate analysis disclosed that patients with low pAMPK or pACC levels (score ≤ 5) had shorter overall survival compared with patients with high pAMPK or pACC levels (HR= 3.07, $p=0.016$ and HR=4.83, $p=0.0008$, respectively). A borderline ($p=0.06$) effect of pACC levels on progression-free survival was also observed.

Our data indicate heterogeneous levels of AMPK activation in mCRC and point towards a prognostic impact of pAMPK expression for mCRC patients treated with FOLFIRI-Bevacizumab.

Harnessing Reactive Oxygen Species and Cell Death pathways for the treatment of Pediatric T-ALL

Micol Silic-Benussi², Ilaria Cavallari¹,
Francesca Rende¹, Sonia Minuzzo¹,
Mariangela Manicone¹, Varun K. Sharma¹,
Eugenia Sharova¹, Giuseppe Basso³, Stefano
Indraccolo² and Vincenzo Ciminale^{1,2}
¹Department of Oncology, University of
Padova, Italy.

²Istituto Oncologico Veneto-IRCCS, Padova, Italy.

³Dipartimento di Salute della Donna e del Bambino, University of Padova, Italy

Acute Lymphoblastic Leukemia (ALL) is the most common haematological disease in pediatric patients. 15% of newly diagnosed ALL are of T-cell subtype of which approximately 20% are refractory to the current prednisone-based therapies. The present study describes new therapeutic strategies aimed at breaking prednisone-resistance of refractory T-ALL by manipulation of reactive oxygen species (ROS) homeostasis.

The study employed an animal model based on the growth of primary T-ALL cells in SCID-NOD mice. T-ALL xenografts stabilized from 13 patients were tested. Several drugs were tested both in vitro and in vivo in the mouse xenograft model.

In experiments performed thus far, effective, long-term control of prednisone-resistant T-ALL in vivo was obtained through a "multimodal" strategy that combined one or more drugs that increase mitochondrial ROS production, decrease the activity of ROS-scavenging pathways and inhibit survival pathways engaged by ROS.

Individual T-ALL xenografts were effectively treated by different drug combinations, suggesting that prednisone resistance could involve different cellular pathways in different patients.

Call for 2014 Pezcoller Foundation-AACR International Award for Cancer Research

The prestigious Pezcoller Foundation-AACR International Award for Cancer Research was established in 1997 to annually recognize a scientist:

- who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research;
- who continues to be active in cancer research and has a record of recent, noteworthy publications;
- whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.

The Award is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the Award when their investigations are closely related in subject matter and have resulted in work that is worthy of the Award. In the rare event that there are dual winners of the Award, the cash award will be shared equally between them, and the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.

Candidates for the Award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the President of the AACR and the Council of the Pezcoller Foundation. The Committee will consider all nominations as they have been submitted; the Committee may not combine submitted nominations, add a new candidate to a submitted nomination, or otherwise make alterations to the submitted nominations. After careful deliberations by the Committee, its recommendations will be forwarded to the Executive Committee of the AACR and the Council of the Pezcoller Foundation for final consideration and determination.

Selection of the Award winner will be made on the basis of the candidate's scientific accomplishments. No regard will be given to race, gender, nationality, or religious or political view.

The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Previously the Pezcoller Foundation gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science, in collaboration with the ESO-European School of Oncology.

The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now have over 33,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 97 other countries around the world.

The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. Because of the commitment of the Pezcoller Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of the Award. This will strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting (April 5-9, 2014) in San Diego, CA and the memorial Korsmeyer lecture at the VIMM in Padua and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy (May 9, 2014). The award consists of a prize of € 75.000 and a commemorative plaque.

**Nomination Deadline:
September 12, 2013**

Questions about the nomination process:
Monique P. Eversley, M.S., Senior Coordinator,
Scientific Achievement Awards
American Association for Cancer Research,
17th Floor, 615 Chestnut Street,
Philadelphia, PA 19106-4404
Tel. +1 (215) 446-6126;
Email: awards@aacr.org
www.aacr.org/ScientificAwards



**The Pezcoller
Foundation**

Journal

Six-monthly review of the
Pezcoller Foundation
Via Dordi 8 - 38122 Trento - Italy
Tel. (39) 0461 980250
Fax (39) 0461 980350
e-mail: pezcoller@pezcoller.it
www.pezcoller.it

Proprietario/editore:
Fondazione Prof. Alessio Pezcoller - Trento
n. 36 - Registro delle Persone Giuridiche
presso il Commissario del Governo
della Provincia di Trento
Redazione: Via Dordi 8 - 38122 Trento
Direttore Responsabile: Gios Bernardi

"The Pezcoller Foundation Journal"
year 23, n. 40, Semestrale giugno 2013
Poste Italiane spa
Spedizione in abbonamento postale
D.L. 353/2003 (conv. In L. 27/02/2004 n. 46)
Art. 1, comma 2, CNS Trento
taxe percue / tassa riscossa