

Signal Transduction

Multipathway Model Enables Prediction of Kinase Inhibitor Cross-Talk Effects on Migration of Her2-Overexpressing Mammary Epithelial Cells

Neil Kumar, Raffi Afeyan, Hyung-Do Kim, and Douglas A. Lauffenburger

Departments of Chemical Engineering and Biological Engineering, and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts

Molecular Pharmacology 73:1668 (2008)

Kumar et al. used a high-throughput immunocytochemistry assay (the **ICW**) to quantify phosphorylation in wounded monolayers of HMEC cells expressing either low or high levels of HER2. The **ICW assay** provided a quantitative, multipathway modeling approach which contributed to the comprehending kinase inhibitor efficacy in the face of off-target and pathway cross-talk effects.

<http://molpharm.aspetjournals.org/cgi/reprint/73/6/1668>

Kinetics of Wnt-Driven b-Catenin Stabilization Revealed by Quantitative and Temporal Imaging

Rami N. Hannoush

Department of Protein Engineering, Genentech Inc., South San Francisco, California, USA

PLoS ONE 3(10):e3498 (2008)

Hannoush uses the ICW assays for rapid quantitative analysis of cellular b-catenin protein levels. The Wnt-stimulated accumulation of b-catenin to monitor the kinetics of post-translational stabilization of b-catenin. The method revealed an unprecedented level of detail about the rate of accumulation of intracellular b-catenin. He mentioned that the **ICW assay** may serve as basis for future kinetic modeling of the pathway, for assessing the cellular effects of inhibitors of GSK3b on b-catenin accumulation, or for screening compounds for Wnt pathway inhibition.

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0003498>

Akt and ERK1/2 pathways are components of the vasopressin signaling network in rat native IMCD

Trairak Pisitkun, Vinitha Jacob, Stephen M. Schleicher, Chung-Lin Chou, Ming-Jiun Yu, and Mark A. Knepper

Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

Am J Physiol Renal Physiol 295: F1030-F1043 (2008)

Using Odyssey Western blotting, Pistikun et al demonstrated that the V2 receptor-selective vasopressin analog dDAVP affects states of activation of both the Akt and ERK1/2 MAP kinase pathways in native rat renal IMCD cells. Changes in both pathways were relatively small as assessed by whole cell immunoblotting, but were extraordinarily consistent in the many experiments carried out in this study. They confirmed the presence of the ERK1/2 MAP kinase pathway components in IMCDs by immunofluorescence staining on tissue sections.

<http://ajprenal.physiology.org/cgi/content/abstract/295/4/F1030?maxtoshow=&HITS=&hits=&RESULTFORMAT=1&author1=Knepper%2Cma&andorexactitle=and&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&fdat e=1/1/2005&resourcetype=HWCIT>

Apoptosis

Deletion of the Ubiquitin Ligase CHIP Leads to the Accumulation, But Not the Aggregation, of Both Endogenous Phospho- and Caspase-3-Cleaved Tau Species

Chad A. Dickey, Mei Yue, Wen-Lang Lin, Dennis W. Dickson, Judith H. Dunmore, Wing C. Lee, Cynthia Zehr, Gemma West, Songsong Cao, Amber M. K. Clark, Guy A. Caldwell, Kim A. Caldwell, Christopher Eckman, Cam Patterson, Michael Hutton, and Leonard Petrucelli

Mayo Clinic College of Medicine, Jacksonville, Florida, University of Alabama, Tuscaloosa, Alabama, and University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

The Journal of Neuroscience, 26, 6985 (2006)

Dickey et al. establish that the microtubule-associated protein, tau, was accumulating because of impaired degradation resulting from deletion of a tau ubiquitin ligase called CHIP rather than simply enhanced kinase or decreased phosphatase activities. Using quantitative Western blotting on Odyssey to assess tau levels, they were able to validate that the levels of total tau protein in the CHIP^{-/-} mice were twice that in wild-type littermates.

<http://www.jneurosci.org/cgi/reprint/26/26/6985>

Enhanced Sensitivity to Cytochrome c-Induced Apoptosis Mediated by PHAPI in Breast Cancer Cells

Zachary T. Schafer, Amanda B. Parrish, Kevin M. Wright, Seth S. Margolis, Jeffrey R. Marks, Mohanish Deshmukh, and Sally Kornbluth

Departments of Pharmacology and Cancer Biology and Surgery, Duke University Medical Center; Institute for Genome Sciences and Policy, Duke University, Durham, North Carolina; and Neuroscience Center, University of North Carolina, Chapel Hill, North Carolina

Cancer Res 66, 2210 (2006)

Schafer et al found that breast cancer cells display an unusual sensitivity to cytochrome c-induced apoptosis when compared with their normal counterparts. This sensitivity, not observed in other cancers, resulted from enhanced recruitment of caspase-9 to the Apaf-1 caspase recruitment domain. Augmented caspase activation was mediated by PHAPI, which is overexpressed in breast cancers. Odyssey was used to determine the significance of PHAPI overexpression for the hypersensitivity of breast cancer cells to cytochrome c and then the degree of PHAPI overexpression in a set of patient samples.

<http://cancerres.aacrjournals.org/cgi/reprint/66/4/2210>

Age-dependent cell death and the role of ATP in hydrogen peroxide-induced apoptosis and necrosis

Noriyuki Miyoshi, Hammou Oubrahim, P. Boon Chock, and Earl R. Stadtman

Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

PNAS 103 1727 (2006)

Miyoshi used quantitative Western blotting on Odyssey to examine the effect of both cell age and hydrogen peroxide treatment on the phosphorylation of c-jun. They proposed that the decline in c-jun phosphorylation observed with cells from old donors that were not treated with hydrogen peroxide might compromise apoptotic signaling in these cells.

Quantifying a human cytokine array on Odyssey they also show here that cell aging is associated with significant increases in the hydrogen peroxide-induced leakage of various cytokines that are known to be implicated in the initiation of several age-related inflammatory disorders and diseases. Therefore, these results suggest leakage of cytokine proteins from the intracellular space of necrotic cells will affect neighboring live cells.

<http://www.pnas.org/content/103/6/1727.full.pdf+html>

Cancer

Quantitative analyses reveal the importance of regulated Hdmx degradation for P53 activation

Yunyuan V. Wang, Mark Wade, EeTsin Wong, Yao-Cheng Li, Luo Wei Rodewald, and Geoffrey M. Wahl

Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, CA; and Institute of Molecular and Cell Biology, Singapore

PNAS, Jul 2007; 104: 12365 - 12370

Wang et al. performed a Western blotting on Odyssey to quantify P53, Hdm2, and Hdmx levels in human normal and tumor cell lines to investigate the molecular basis of P53 regulation in normal and cancer cells after DNA damage. The data showed that the nuclear abundance of Hdm2 and Hdmx relative to P53 limits P53 activity in cells growing in culture. This study provides the first quantitative analysis of changes in the stoichiometry of endogenous P53, Hdm2, and Hdmx in response to P53-activating agents. The data also provided a basis for developing more refined mathematical models of P53 pathway regulation based on known kinetics of P53 transcriptional activation as a function of P53, Hdm2, and Hdmx subcellular concentration.

<http://www.pnas.org/content/104/30/12365.full.pdf+html>

A Novel Platform for Accelerated Pharmacodynamic Profiling for Lead Optimization of Anticancer Drug Candidates

Jeffrey Szwaya, Charles Bruseo, Enkeleda Nakuci, Denise McSweeney, Xiaoqin Xiang, David Senator, Dennis France, and Chang-Rung Chen

Department of Molecular Oncology, ArQule, Inc., Woburn, MA; Oncology Disease Area, Novartis Institutes for BioMedical Research, Cambridge, MA

J Biomol Screen, Mar 2007; 12: 159 - 166.

The authors characterize hundreds of HTS hits by quantitative Western blotting using a combination of a flatbed bufferless SDS-PAGE system, a dry ultra-rapid electroblotting apparatus, and the Odyssey. This platform significantly reduced the cycle time for hit evaluation and resulted in higher quality data that improved lead optimization. The annotation of inhibitors of 2 attractive oncology targets, BRAF kinase and Hsp90, are described.

<http://jbx.sagepub.com/cgi/content/abstract/12/2/159?maxtoshow=&HITS=&hits=&RESULTFORMAT=1&author1=szwaya%2C+j&andorexacttitle=and&fulltext=odyssey&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&fdate=1/1/2005&resourcetype=HWCIT>

The Molecular Scaffold Kinase Suppressor of Ras 1 Is a Modifier of RasV12-Induced and Replicative Senescence

Robert L. Kortum, Heidi J. Johnson, Diane L. Costanzo, Deanna J. Volle, Gina L. Razidlo, Angela M. Fusello, Andrey S. Shaw, and Robert E. Lewis

Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, Nebraska and Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri

J. Biol. Chem., 13 March 2009; 284(11): 6705-6715

The Lewis Lab used the Odyssey and the ICW assay to quantitate Erk activation in the study of Kinase Suppressor of Ras 1 (KSR1) which is a molecular scaffold for the Raf/MEK/ERK cascade.

<http://www.jbc.org/cgi/content/abstract/284/11/6705?maxtoshow=&HITS=&hits=&RESULTFORMAT=1&author1=lewis%2Cr&andorexacttitle=and&fulltext=odyssey&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&fdate=1/1/2005&resourcetype=HWCIT>

