



SYNOPSIS BY TECHNOLOGY

Genomics/Microarray, Proteomics, RNAi, ChIP-chip

GENOMICS/MICROARRAY

A network-based analysis of systemic inflammation in humans. Nature. 2005 Aug 31 as doi: 10.1038/nature03985. Steve E. Calvano, Wenzhong Xiao, Daniel R. Richards, Ramon M. Felciano, Henry V. Baker, Raymond J. Cho, Richard O. Chen, Bernard H. Brownstein, J. Perren Cobb, S. Kevin Tschoeke, Carol Miller-Graziano, Lyle L. Moldawer, Michael N. Mindrinos, Ronald W. Davis, Ronald G. Tompkins, Stephen F. Lowry and Inflamm and Host Response to Injury Large Scale Collab. Res. Program.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16136080&itool=iconabstr&query hl=1

Members of the collaborative research program called Inflammation and Host Response to Injury are seeking to identify the biological processes that underlie systemic inflammation, the uncontrolled, body-wide inflammation associated with severe trauma and burns. In a novel study approach, healthy human volunteers were injected with endotoxin, triggering the innate immune response that leads to acute inflammation. This inflammatory response is self-limiting and resolves within 24 hours. The team evaluated gene expression in whole blood leukocytes taken from subjects before endotoxin exposure and at several time points afterwards. They found that endotoxin induced changes in the expression of 3,714 genes.

To more clearly understand the temporal nature of the gene expression changes, the team created a model inflammatory cell that included 292 genes that participate in innate immunity and inflammation, and their direct molecular pathway interactions as detailed in the Ingenuity Pathways Knowledge Database. The time course expression data revealed how these genes are modulated during the immunoinflammatory response to endotoxin challenge and its resolution. Among their findings:

- P Expression of many proinflammatory cytokines and chemokines (for example, *TNFSF2* (*TNF*), *IL1A*, *IL1B*, and *CSC1* (*GRO α*)) reach peak levels at 2-4 hr after exposure to endotoxin.
- P Expression of numerous transcription factors that activate and curb the innate immune response is increased at 4-6 hr after exposure to endotoxin.
- P Increases in the mRNA of secreted and membrane-associated proteins (*IL1RAP*, *IL1R2*, *IL10*, and *TNFRSF1A*) occurs 4-6 hrs after exposure to endotoxin.

Using the Ingenuity Pathways Analysis application, the team constructed a gene network of the global biological processes involved in the response to endotoxin challenge. Within this computationally generated global network, they identified nine

sub-networks or *functional modules* involved in mitochondrial energy production, protein synthesis, protein degradation, the COP9 signalosome, and the proteasome. Their work documents how endotoxin affects these highly interconnected network modules. Notably, modules involved in mitochondrial energy production, protein synthesis, and protein degradation were suppressed, implicating their involvement in innate immune system tolerance

Supplemental information to the paper includes more details about the network analysis methodology and demonstrates how to use the Ingenuity Pathways Analysis application to elucidate coordinated pathway function from microarray time-series datasets.

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Microarray analysis reveals genetic pathways modulated by tipifarnib in acute myeloid leukemia. BMC Cancer 2004, 4:56. Mitch Raponi, Tober T Belly, Judith E. Karp, Jeffrey E. Lancet, David Atkins and Yixin Wang.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15329151&itool=iconpmc&query_hl=12

Dysregulated signaling pathways are important contributors to cell proliferation in various types of cancer. The biological activity of numerous proteins in signal transduction pathways requires farnesylation, a posttranslational modification that involves the addition of a farnesyl moiety. Farnesyl transferase inhibitors (FTIs) are an new class of drugs that inhibit tumor growth, presumably by diminishing uncontrolled cell signaling that leads to cancerous cell proliferation.

The FTI tipifarnib is currently in clinical trials for treatment of various cancers and shows promising potential for some blood cancers. To help identify pharmacologic biomarkers of tipifarnib activity and the gene pathways involved in the drug's mechanism of action, scientists conducted differential gene expression analysis in acute myeloid leukemia cells treated with tipifarnib and used the Ingenuity Pathways Analysis application to analyze the genes affected by tipifarnib.

Network analysis identified five highly significant networks associated with the cell cycle, proliferation, chemotaxis, and immunity. The networks include genes that are known to be directly or indirectly affected by FTIs. Moreover, Neighborhood Explorer revealed other genes not identified by microarray analysis that may be regulated by tipifarnib. Thus Ingenuity Pathways Analysis enabled a larger framework for identifying potential tipifarnib gene targets. The genes revealed by the network analysis may be useful for determining candidates for tipifarnib therapy.

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Gene Expression Profiling of the Left Ventricles in a Rat Model of Intrinsic Aerobic Running Capacity. Physiol Genomics. 2005 Jul 20. Soon Jin Lee, Justin A. Ways, John C. Barbato, David Essig, Krista Pettee, Sarah J. DeRaedt, Siming Yang, David A. Weaver, Lauren G Koch, and George T Cicila
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16033863&itool=iconabstr&query_hl=1

In a study of cardiorespiratory function and aerobic running capacity (ARC), scientists used global genomic approaches to identify differentially expressed cardiac genes that may serve as cardiovascular risk factors. They employed a rat model

comprising two strains: DA (a high ARC strain) and COP (a low ARC strain). Genetic linkage demonstrated that genes associated with aerobic capacity were heritable and the chromosomal locations were identified.

Inbred strains of DA and COP as well as bred F1(DAXCOP) were used in the experiment. RNA isolated from left ventricles after tests was applied to the Affymetrix7 Rat Genome U34 array set. Quantitative PCR and identification of chromosomal locations were performed to validate and confirm the signature genes. The Ingenuity Pathways Analysis application was applied to generate gene networks and identify the functional relationships of the candidate genes.

By comparing the high ARC strain (DA) to the low ARC strain (COP), 199 differentially expressed probe sets were identified. Nine probe sets were matched to known chromosomal locations based on their sequences and validated by quantitative real time PCR. Using the Ingenuity Pathways Analysis application, 50 differentially expressed genes were identified from 13 biological networks containing a total of 135 genes, as shown in supplemental Table 3 of the paper. Three large networks with 40 differentially expressed genes indicating potential functions are listed in Table 4 of the paper. Chromosomal locations of 85 genes from these networks were examined, but these genes were not among the 199 genes identified by the microarray analysis. Four genes recognized by the Ingenuity Pathways Analysis application were mapped to known ARC regions. These genes may be considered potential risk factors for cardiorespiratory disease.

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Identification of Transcriptional Networks during Liver Regeneration. J Biological Chemistry. 2005. 280(5):3715-3722. Peter White, John E. Brestelli, Klaus H. Kaestnert, and Linda E. Greenbaum.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15546871&itool=iconabstr&query hl=8

The regenerative ability of the liver in humans and animals is well known, however little is known about the mechanisms that regulate the proliferation of hepatocytes. The partial hepatectomy model in rodents is an extensively used experimental model of hepatic growth in which partial removal of the liver induces normally quiescent hepatocytes and non-parenchymal liver cells to reenter the cell cycle and restore the liver in about two weeks. Using this model, researchers at the University of Pennsylvania Medical School identified differentially expressed genes from mouse liver cDNA samples taken at 0, 2, 16, and 40 hr post-hepatectomy (time points that correspond to the priming phase, hepatocyte mid-G1 phase, and peak of the hepatocyte S phase in the partial hepatectomy model).

To help understand their role in regulatory and signaling networks that control hepatic proliferation *in vivo*, the differentially expressed genes were uploaded to the Ingenuity Pathways Analysis (IPA) application. Although Fos, JunB, JunD, and Myc were not detected by microarray analysis due to sensitivity limitations, Ingenuity Pathways Analysis included these genes as likely members in a network that regulates early growth responses at the 2 hr time point. Subsequent quantitative PCR analysis confirmed that these genes were upregulated. This underscores the power of IPA when microarray analysis is limited by sensitivity or a gene of interest is not represented on an array. The network analysis also revealed an association between Myc and DUSP6 (MPK3), an inhibitor of extracellular signal-regulated

kinase/mitogen-activated protein kinase signaling activity- a new finding for the partial hepatectomy model.

At the 16 hr time point, network analysis showed that the proteins encoded by the differentially expressed Myc targets are involved in cytokine signaling, matrix remodeling, and cell cycle progression. At the 40 hr time point, differentially expressed genes participate in regulatory networks involved in DNA replication, mitotic spindle assembly, and mitotic checkpoint control.

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Identification of candidate genes associated with salivary adenoid cystic carcinomas using combined comparative genomic hybridization and oligonucleotide microarray analyses.

The International Journal of Biochemistry & Cell Biology, September 1, 2005; 37(9): 1869-80. Atsushi Kasamatsu, Yosuke Endoa, Katsuhiko Uzawa, Dai Nakashima, Hirofumi Koike, Susumu Hashitani, Tsutomu Numata, Masahiro Urade, Hideki Tanzawa

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15908262&itool=iconabstr&query hl=6

Researchers studying adenoid cystic carcinoma (ACC) of the salivary gland identified genes for Ingenuity Pathways Analysis using a two-step strategy based on comparative genome hybridization and microarray analysis. They focused on up-regulated ACC-associated genes on ACC-associated loci with increased DNA copy number. Using the Ingenuity Pathways Analysis global functional analysis and network analysis features, the investigators discovered how a subset of these genes interact in a network of molecular functions related to cancer. Their study yields new and valuable information that contributes to our understanding of the molecular basis of ACC and may help identify possible targets for therapeutic intervention.

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Anti-angiogenic activity of the mutant Dutch A(beta) peptide on human brain microvascular endothelial cells.

Brain Res Mol Brain Res. 2005 May 20;136(1-2):212-30. Daniel Paris, Ghania Ait-Ghezala, Venkatarajan S. Mathura, Nikunj Patel, Amita Quadros, Vincent Laporte, Mike Mullan.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15893605&itool=iconabstr&query hl=3

Cerebral amyloid angiopathy, the deposition of (beta)-amyloid (A-beta) in the cerebrovasculature, characterizes a rare disorder called hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). A single point mutation of the (beta)-amyloid precursor protein leads to HCHWA-D, resulting in recurrent hemorrhagic stroke at middle age, vascular dementia, and fatal cerebral bleeding.

Wild-type A(beta) has been shown to be anti-angiogenic, and both structural and functional cerebrovascular abnormalities are associated with Alzheimer's disease. This study found Dutch A(beta) to be an even more potent inhibitor of angiogenesis. To better understand the molecular mechanisms that cause the anti-angiogenic activity of Dutch A(beta) researchers profiled genes that were differentially expressed in human brain microvascular endothelial cells exposed to an anti-angiogenic dose of Dutch A(beta). The genes affected by Dutch A(beta) were uploaded to the Ingenuity Pathways Analysis application and network analysis

revealed that exposure to Dutch A(beta) dysregulates networks involved in cellular migration, cellular proliferation, angiogenesis, atherosclerosis, and tumorigenesis.

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mSin3A corepressor regulates diverse transcriptional networks governing normal and neoplastic growth and survival. Genes & Development 19:1581-1595, 2005

Jan-Hermen Dannenberg, Gregory David, Sheng Zhong, Jaco van der Torre¹, Wing H. Wong² and Ronald A. DePinho

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15998811&itool=iconabstr&query_hl=10

Sin3 is a component of an evolutionarily conserved multi-protein corepressor complex that mediates gene silencing in eukaryotes. The mammalian Sin3 corepressor complex (mSin3) associates with histone deacetylases (HDACs) and achieves transcriptional silencing through the chromatin-modifying activities of these enzymes. A wide range of sequence-specific DNA-binding transcription factors can recruit the mSin3/HDAC complex to the regulatory region of a target gene. The mSin3/HDAC complex activity is essential to cellular differentiation and development, proliferation, and apoptosis. Aberrant interactions between transcription factors and the mSin3/HDAC complex are associated with the pathogenesis of cancer as well as other diseases.

mSin3 includes two splice forms called mSin3A and mSin3B. Investigators applied the Ingenuity Pathways Analysis application to the mSin3A transcriptome to better define the roles of this corepressor component. Network analysis confirmed several known transcription factor nodes through which mSin3A modulates gene expression, including the Myc-Mad, E2F, and p53 transcriptional networks. Furthermore, the network analysis identified new mSin3A interactions, revealing unexpected connections between mSin3A and networks regulated by FOS, PPAR, STAT, and FALZ. PPAR is especially noteworthy because the mSin3A transcriptome includes many genes involved in mitochondrial respiration and metabolism, and the PPAR pathway, which is implicated in mitochondrial respiration and metabolism, appears to be regulated by histone deacetylation.

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Acute Myeloid Leukemia with Translocation t(8;16) Demonstrates Specific Cytomorphological, Cytogenetic, and Gene Expression Characteristics and Can Clearly Be Discriminated from Other AML with Balanced Translocations.

Blood (ASH Annual Meeting Abstracts) 2004 104: Abstract 2897. Torsten Haferlach, MD, Helmut Loeffler, MD, Alexander Kohlmann, Martin Dugas, MD, Wolfgang Hiddemann, MD, Wolfgang Kern, MD, Susanne Schnittger, PhD and Claudia Schoch, MD

http://meeting.bloodjournal.org/cgi/content/abstract/104/11/2897?maxtoshow=&HITS=&hits=&RESULTFORMAT=&fulltext=Ingenuity+Pathway&andorexactfulltext=and&searchid=1120238602924_11824&stored_search=&FIRSTINDEX=0&resourcetype=1

Chromosome translocation occurs in acute myeloid leukemia (AML). Fusion genes generated from chromosomal rearrangements have been classified into distinct biological subsets in AML. Although cytomorphology and gene expression patterns

have also been used to define subsets of AML, some cases of AML cannot be classified according to the FAB categories.

The authors suggest AML-t(8;16) is derived from a very early stem cell in the myeloid and monoblastic cell lineages. In this meeting presentation, they presented gene expression analysis from four cases of AML-t(8;16) using the Affymetrix7 GeneChip7 U133 A+B arrays. They compared expression of AML-t(8;16) with 46 AML FAB M1, 41 M4, 9 M5a and 16 M5b, along with presentation of karyotypes.

Hierarchical clustering analysis indicated that there are genes shared between FAB M4 and M5b, but not with the M1 group. Additional comparisons were made between experimental data and WHO classifications, however no consistent results were obtained. Using the Ingenuity Pathways Analysis application, the top 100 differentially expressed genes were further analyzed. Fifteen genes associated with AML-t(8;16) were involved in the CMYC-pathway. Eleven genes are upregulated (BCOR, COXB5, CDK10, FL11, HNRPA2B1, NSEP1, PDIP38, RAD50, SUPT5H, TLR2, and USP33) and four genes are down regulated (EGR, GATA2, NCOR3, and RPS20). The results suggest that AML-t(8;16) is a distinct subtype of AML with a specific gene expression profile.

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A Molecular Classification of Papillary Renal Cell Carcinoma. Cancer Research 2005, 65(13):5628. Ximing J. Yang, *et al.*
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15994935&itool=iconabstr&query hl=2

Gene expression profiling has revealed distinguishing molecular signatures for several types of kidney cancer, including papillary renal cell carcinoma (PRCC), the second most common type of kidney cancer. In this study, scientists identified two molecular subtypes of PRCC (class 1 and class 2 PRCC) based on gene expression signatures, cytogenetic profiles, and histological evidence.

To extend the characterization of these subtypes, the researchers analyzed the genes that are differentially expressed in class 1 and class 2 PRCC with the Ingenuity Pathways Analysis application. The most significant networks showed that G₁-S checkpoint genes are dysregulated in class 1 PRCC whereas G₂-S checkpoint genes are dysregulated in class 2 PRCC. The insights gained from the network analysis enabled further interesting perspectives on PRCC and the investigators observe that:

- P c-met is upregulated in class 1 tumors. Interestingly, during liver regeneration, the hepatocytes in conditional *met*-mutant mice show impaired exit from quiescence (transition from G₀-G₁) and diminished replication (entry and progression through S phase). The involvement of met signaling in G₁-S checkpoint dysregulation is a pertinent area of future research.
- P DNA TopII α is a diagnostic marker for class 2 PRCC. TopII inhibitors produce G₂ arrest and therefore may be therapeutic candidates for class 2 PRCC.

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Acute Myeloid Leukemia with a Complex Aberrant Karyotype is a Distinct Biological Entity Characterized by Genomic Imbalances and a Specific Gene

Expression Profile. Genes, Chromosomes & Cancer 43:227-238 (2005) Claudia Schoch, Wolfgang Kern, Alexander Kohlmann, Wolfgang Hiddemann, Susanne Schnittger, Torsten Haferlach

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15846790&itool=iconabstr&query hl=2

Using cytogenetic analyses and gene expression profiling, researchers delineated a more precise and consistent definition of acute myeloid leukemia (AML) with a complex aberrant karyotype, an AML subtype that has a very poor outcome. AML with complex aberrant karyotype has a molecular signature that distinguishes it from all other AML subtypes. This expression profile includes several upregulated genes involved in DNA repair and DNA-damage-induced checkpoint signaling (*RAD21*, *RAD1*, *RAD23B*, and others).

Evaluating the differentially expressed genes with the Ingenuity Pathways Analysis application generated a biological network that provides the first clues to the pathways that may be dysregulated in the pathogenesis of this disease.

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Genomic approaches for reconstructing gene networks. Pharmacogenomics. 2005 Apr;6(3):245-58. Norman H. Lee

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16013956&query hl=1

Learn more about the strategies and technologies scientists use to build gene networks, including microarrays and cluster analysis, bioinformatics methods, ChIP-chip, and RNAi. Norman Lee explains how these techniques are used, assesses their strengths and limitations, and describes discoveries they enabled.

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DBC2 Significantly Influences Cell-cycle, Apoptosis, Cytoskeleton and Membrane-trafficking Pathways. J Mol Biol. 2005 Feb 11;346(1):83-9. Epub 2004 Dec 8. Siripurapu V, Meth J, Kobayashi N, Hamaguchi

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15663929&itool=iconabstr&query hl=2

Researchers at the Cold Spring Harbor Laboratory employed a strategy that combined RNAi and Ingenuity Pathways Analysis to gain new insights into the function of the tumor suppressor DBC2 (deleted in breast cancer 2). To determine the genes affected by DBC2, the research team performed RNA profiling of HeLa cells transformed with DBC2. Knockdown of DBC2 using RNAi confirmed the affected genes.

Using the Ingenuity Pathways Analysis application, the investigators identified two high-scoring networks in which more than half of the genes are affected by DBC2. One network regulates cell growth through apoptosis and cell-cycle control (S phase, G1/S transition), and the other is associated with cytoskeleton, membrane trafficking, Golgi assembly, and fusion. Their work lays the foundation for the functional characterization of DBC2 in tumor suppression.

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RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. Dev Biol. 2005 Jul 1;283(1):40-57. Fanyi Zeng, Richard M. Schultz
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15975430&itool=iconabstr&query_hl=1

The mammalian oocyte provides a "maternal legacy" of proteins, transcripts, and other factors that initially govern embryonic development. Zygotic gene activation (ZGA) is crucial to the successful transition from maternal to embryonic control of development. Researchers identified the genes that are newly expressed in mouse preimplantation embryos during ZGA by analyzing the transcriptomes of 1-cell and 2-cell embryos treated with (alpha)-amanitin, a transcription inhibitor. Genes that were (alpha)-amanitin-sensitive as well as upregulated in the 2-cell embryo compared to both 1-cell embryos and eggs were evaluated with the Ingenuity Pathways Analysis (IPA) application.

IPA global functional analysis revealed that the top four high-level functions associated with the data include RNA post-transcriptional modification, protein synthesis, gene expression, and cell cycle. The network with the highest significance score includes *Myc* at the network's center. *Myc* is also a node in six other significant networks associated with diverse biological themes, suggesting *Myc* plays a central role in regulating the expression of numerous subsets of genes vital to embryogenesis.

In addition to ZGA, a chromatin-based transcriptionally repressive state develops at the 2-cell stage. Histone hyperacetylation has been shown to activate transcription in embryos. Interestingly, the second most significant network, associated with cell cycle and DNA replication, shows many interactions between histone deacetylase 1 (HDAC1) and other focus genes. The network also identified 11 genes that physically interact with HDAC1. These results are the basis for a hypothesis-driven approach to further research on the role of *Myc* in genome activation and HDAC1 in genome repression during mouse embryogenesis.

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Multiclass cancer classification and biomarker discovery using GA-based algorithms. Jane Jijun Liu, Gene Cutler, Wuxiong Li, Zheng Pan, Sihua Peng, Tim Hoey, Liangbiao Chen and Xuefeng Bruce Ling
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15814557&itool=iconabstr&query_hl=3

Tumor classification based on patterns of gene expression holds great potential for accurate diagnosis and discovery of biomarkers that are important to the development of targeted therapies. Looking to improve tumor prediction performance, investigators applied a novel computational strategy that combined the genetic algorithm (GA) and all paired support vector machine (SVM) methods to a subset of the NS160 data, a database of genes expression profiles of 9712 spotted cDNAs from 68 cancer cell lines. Their approach generated highly accurate and robust predictive gene sets.

To help understand the biology underlying tumorigenesis, the GA/SVM-selected predictor genes were functionally characterized by the Ingenuity Pathways Analysis application. Fifty-six biological networks were generated and most of the pathways

associated with the networks are known to be involved in tumorigenesis. This functional analysis of predictor genes helped provide molecular insights fundamental to biomarker discovery.

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The Hepatic Transcriptome as a Window on Whole-Body Physiology and Pathophysiology. Toxicol Pathol. 2005;33(1):136-45. Kevin T. Morgan, Zaid Jayyosi, Moira A. Hower, Michael V. Pino, Timothy M. Connolly, Katja Kotlenga, Jieyi Lin, Min Wang, Hans-Ludwig Schmidts, Marc S. Bonnefoi, Timothy C. Elston, Gary A. Boorman

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15805065&itool=iconabstr&query hl=2

In this toxicogenomics study of the rat hepatic transcriptome, principal component analysis revealed that the duration of fasting before necropsy (6 hr or overnight) is associated with significant differences in patterns of gene expression. Genes with similar expression patterns from the 6 hr fasting group were functionally characterized using the Ingenuity Pathways Analysis application. The most significant network includes two distinct groups of genes: one involved in circadian rhythm and the other involved in lipid metabolism and energetics. These results indicate that circadian rhythm may be an important consideration in designing toxicogenomic studies.

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Impaired Revascularization in a Mouse Model of Type 2 Diabetes Associated With Dysregulation of a Complex Angiogenic-Regulatory Network.

Arteriosclerosis, Thrombosis, and Vascular Biology 2005;25:1603 Stephan Schiekofer; Gennaro Galasso; Kaori Sato; Benjamin J. Kraus; and Kenneth Walsh.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15920034&itool=iconabstr&query hl=3

To better understand how diabetes may affect angiogenesis, investigators set out to identify proteins potentially responsible for impaired neovascularization in $Lepr^{db/db}$ mice, a model of type 2 diabetes and obesity. The researchers identified differentially expressed transcripts of angiogenesis-related proteins from the hindlimb muscle of wild-type (WT) and $Lepr^{db/db}$ mice following ischemic surgery that produced vascular insufficiency.

Uploading the differentially expressed genes of WT mice (day 14 post-surgery compared to presurgery baseline) to the Ingenuity Pathways Analysis application generated a high scoring network comprising angiogenesis regulatory factors, proteasome subunits, translation regulatory factors, proteases, several matrix metalloproteinases (MMPs), and other proteins (for example, SPARC, myelin basic protein, elastin, and protein kinases). Thirty-four of the 35 nodes in the network were differentially regulated at day 14.

Interestingly, when the differentially expressed genes of $Lepr^{db/db}$ mice (day 14 post-surgery compared to presurgery baseline) were superimposed on the network derived from the WT genes, only ten of the 35 network nodes were differentially regulated. Further, genes associated with angiogenesis (MMPs, elastin, neuropilin-1, and VEGF-A) were upregulated in ischemic limbs of WT mice, but not $Lepr^{db/db}$ mice.

The study sheds light on a complex angiogenic-regulatory network and potential targets for therapeutic angiogenesis.

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Gene Expression Profiling of the PPAR-alpha Agonist Ciprofibrate in the Cynomolgus Monkey Liver.

ToxSci Advance Access published online on August 4, 2005 Toxicological Sciences, doi:10.1093/toxsci/kfi273. Neal F. Cariello, Elizabeth H. Romach, Heidi M. Colto, Hong Ni, Lawrence Yoon, J. Greg Falls, Warren Casey, Donald Creech, Steven P. Anderson, Gina R. Benavides, Debie J. Hoivik, Roger Brown, and Richard T. Miller

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16081524&itool=iconabstr&query hl=4

Fibrates are PPAR α (peroxisome proliferator-activated receptor- α) agonists that are used to treat high cholesterol. They not only lower plasma triglycerides, but also raise high-density lipoprotein levels. The response to fibrate exposure shows significant species-specific differences. In mice and rats, fibrates cause hepatic peroxisome proliferation and ultimately liver cancer; however, these effects are much less pronounced in humans and non-human primates. To help understand why this is so, researchers carried out hepatic transcriptional profiling of non-human primates following exposure to ciprofibrate.

Among the results, the study revealed that in non-human primates:

- P Genes relating to mitochondrial and peroxisomal β -oxidation are not as strongly upregulated as in rodents (2-fold increase compared to 10-fold increase)
- P Members of the *MYC*, *JUN*, and *NF κ B* gene families are downregulated. These are known to be upregulated in rodents.

Dysregulated genes were uploaded to the Ingenuity Pathways Analysis application for network analysis. A network of protein-protein interactions included *MYC* at the center node. The scientists propose that regulation of *MYC* is central to the response to ciprofibrate that includes reduced apoptosis and increased cell proliferation in the rodent liver, but not in the primate liver.

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Functional genomic characterization of delipidation elicited by *trans*-10, *cis*-12-conjugated linoleic acid (t10c12-CLA) in a polygenic obese line of mice.

Physiol. Genomics 21: 351-361, 2005. Ralph L. House, Joseph P. Cassady, Eugene J. Eisen, Thomas E. Eling, Jennifer B. Collins, Sherry F. Grissom and Jack Odle.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15888570&itool=iconabstr&query hl=20

The incidence of obesity has reached epidemic proportions nationally as well as internationally and the cost of medical treatment for obesity is substantial. As a result, the delipidative effects of conjugated linoleic acid (CLA) is a focus of research attention. The delipidative effects of CLA were found in the ICR line of mice which can lose 60% of their body weight in 4-5 weeks in response to CLA. The t10c12-CLA strain is very sensitive to the delipidative activity of CLA and was used in this study.

t10c12-CLA mice and control mice were the dietary treatment groups for a 14-day trial. Total RNA was isolated from the epididymal adipose tissue and subjected to

microarray expression analysis. Several interesting genes were identified using the Ingenuity Pathways Analysis application, including PPAR- γ (peroxisome proliferator-activated receptor- γ) and Cav-1 (fatty acid transport and casp-3 apoptosis pathway). This is the first study using genomic technologies to profile gene expression during CLA-induced degradation of body fat. Significantly, one of the identified genes, Cav-1, is involved in lipid metabolism and apoptosis.

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Functional gene expression analysis of clonal plasma cells identifies a unique molecular profile for light chain amyloidosis. Blood, 15 January 2005, Vol. 105, No. 2, pp. 794-803. Roshini S. Abraham, Karla V. Ballman, Angela Dispenzieri, Diane E. Grill, Michelle K. Manske, Tammy L. Price-Troska, Natalia Gonzalez Paz, Morie A. Gertz, and Rafael Fonseca
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15388584&itool=iconabstr&query_hl=4

Researchers at the Mayo Clinic demonstrated that the molecular signature of light chain amyloidosis (AL) is distinct from multiple myeloma (MM) and identified a subset of 12 genes that correctly classified AL and MM patients with 92% accuracy. To investigate the biological networks dysregulated in AL, they applied the Ingenuity Pathways Analysis (IPA) application to genes that were differentially expressed in AL and MM.

In light of their IPA network analysis, the investigators discuss two interesting hypotheses:

- P Interactions between *cyclinD1*, *CDK4*, and *Rb* may be involved in the rearrangement of the Ig light chain locus.
- P Dysregulated pathways involving the degradation, clearance, and intracellular folding of proteins may contribute to all amyloid diseases, regardless of the amyloid protein type.

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Genome-Wide Monocytic mRNA Expression in Polytrauma Patients for Identification of Clinical Outcome. SHOCK, 24:11-19, 2005. Peter Biberthaler, Viktoria Bogner, Henry V. Baker, M. Cecilia Lopez, Peter Neth, Karl-Georg Kanz, Wolf Mutschler, Marianne Jochum, and Lyle I. Moldawer.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15988315&itool=iconabstr&query_hl=14

Severe blunt trauma injuries can lead to systemic inflammatory response syndrome (SIRS), an inflammatory state of the entire body. SIRS has a complex pathophysiology involving both the innate and adaptive immune responses. It is important to understand SIRS as it is linked to the development of posttraumatic multiple organ failure. Toward this end, researchers analyzed the gene expression patterns of peripheral blood monocytes of trauma patients at several time points during the first 72 hrs after injury and documented a gene expression pattern associated with patients who succumbed to their injuries.

The investigators used the Ingenuity Pathways Analysis application to examine the genes associated with adverse clinical outcome and identified a pathway centered around c-JUN that includes several factors known to participate in SIRS. c-JUN is involved in monocytic differentiation and is activated by TNF α , an important mediator

of SIRS. These preliminary findings are essential to developing tailored therapies for trauma patients.

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Analysis of ARD1 Function in Hypoxia Response Using Retroviral RNA Interference. J. Biol. Chem., Vol. 280, Issue 18, 17749-17757, May 6, 2005. Tim S. Fisher, Shelley Des Etages, Lisa Hayes, Kim Crimin, and Baiyong Li. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15755738&itool=iconabstr&query_hl=37

HIF is a hypoxia-induced factor. The ARD1 gene encodes a HIF-1a acetylase. The enzymatic activity of ARD1 has been demonstrated in bacteria or lower eukaryotes, but not in mammals.

Using RNAi and microarray analysis, investigators sought to identify the function of ARD1 in the response to hypoxia that occurs in mammalian cells. Quantitative real time PCR and western blotting were used to validate the genomic results at the transcript and protein levels. The Ingenuity Pathways Analysis and Ingenuity Pathways Knowledge Base were applied to identify functional interactions and biological pathways. The study found that ARD1 is involved in both cell proliferation and energy metabolism.

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Genome-wide prediction and analysis of function-specific transcription factor binding sites. In Silico Biology 4, 0033. 2004. Fan Long, Hong Liu, Chang Hahn, Pavel Sumazin, Michael Q. Zhang and Asher Zilberstein <http://www.bioinfo.de/isb/2004/04/0033/>

Investigators devised a strategy to predict individual and pairs of transcription factor binding sites (TFBSs) and used the Ingenuity Pathways Analysis (IPA) application to confirm the biological role of the identified TFBSs. The study analyzed the NF- κ B-IRF TFBS pair in ENSEMBL annotated human genes. Genes that contained this TFBS pair were uploaded to IPA. The most significant network included 17 genes that contained the NF- κ B-IRF TFBS pair. Microarray analysis confirmed that nine of the genes are regulated by IFN or NF- κ B. Other data from the literature indicates that the remaining eight genes have direct or indirect interactions with the nine confirmed genes. The network analysis points out the limited sensitivity of microarray methods and shows how *in silico* TFBS identification can contribute to building gene networks.

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PROTEOMICS

Combined Proteomics and Pathways Analysis of Collecting Duct Reveals a Protein Regulatory Network Activated in Vasopressin Escape. Published on August 3, 2005 as doi: 10.1681/ASN.2005030322 JASN Express (J Amer Soc Nephrol 16:??) Ewout J. Hoorn, Jason D. Hoffert, and Mark A. Knepper http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16079266&itool=iconabstr&query_hl=2

Certain clinical conditions such as congestive heart failure or inappropriately elevated levels of circulating vasopressin can lead to free-water retention and progressive hyponatremia, a serious and potentially fatal electrolyte disorder. The extent of

hyponatremia is limited by vasopressin escape, a physiologic phenomenon that leads to increased water excretion independent of circulating vasopressin levels.

Working with a rat model, scientists at the National Institutes of Health sought to discover proteins from the inner medullary collecting duct that regulate vasopressin escape and the signaling pathways that are involved. Since vasopressin escape is characterized by reduced expression of the water channel aquaporin-2 (AQP2), the researchers focused on the proteins that co-regulate with AQP2, using differential gel electrophoresis (DIGE) to monitor their abundance and MALDI-TOF mass spectrometry to identify them.

The proteins found by DIGE were uploaded to the Ingenuity Pathways Analysis (IPA) application and generated a network of 33 proteins, dubbed the "vasopressin escape cluster". It included proteins that were not detected by DIGE, but whose involvement in vasopressin escape was verified by semiquantitative immunoblotting. Thus IPA enabled the discovery of low-abundance proteins, such as transcription factors, not found by DIGE.

The proteins in the vasopressin escape cluster that were identified by IPA, but not DIGE represent "hypotheses" in that their involvement in vasopressin escape can be tested by semiquantitative immunoblotting to confirm changes in their abundance. The results of this exciting approach implicate several transcription factors (c-myc, c-fos, c-jun, p53), the transcriptional co-factor SRC-1, and regulatory proteins (c-src, RACK1) in vasopressin escape.

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A combined proteome and microarray investigation of inorganic phosphate-induced pre-osteoblast cells. Molecular & Cellular Proteomics. Mol Cell Proteomics. 2005 Jun 14; [Epub ahead of print]. Kelly A. Conrads, Ming Yi, Kerri A. Simpson, David A. Lucas, Corinne E. Camalier, Li-Rong Yu, Timothy D. Veenstra, Robert M. Stephens, Thomas P. Conrads, and George R. Beck http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15958391&itool=iconfft&query_hl=1

The level of inorganic phosphate is elevated during the differentiation of osteoblasts. Inorganic phosphate serves as a signaling molecule, altering signal transduction pathways, gene expression, and cellular function during osteoblast differentiation that leads to bone mineralization. How these processes are temporally and spatially coordinated during osteoblast differentiation is poorly understood. Such coordination requires complicated communication between the osteoblast and the extracellular environment.

In this study, the authors used proteomics and microarray approaches to determine the effects of elevated phosphate on the osteoblast. Using quantitative proteomics, over 2500 proteins in control and inorganic phosphate-stimulated MC3T3-E1 pre-osteoblast cells were measured. The methods included cleavable isotope-coded affinity tagged reagents and mass spectrometry. Transcription profiles were generated using a NCI 22,000 oligo array. Correlation coefficients were computed using Pearson correlation and R-package. BioCarta collection and Gene Ontology were used for individual pathway analysis. A weak correlation between microarray analysis and the quantitative proteomic data was observed through the statistical

comparison. However, a strong correlation was shown for a subset of osteoblast relevant genes/proteins. This subset of proteins was posttranscriptionally regulated by elevated inorganic phosphate. It included Fra-1, a member of the activator (AP-1) family of transcription factors.

The Ingenuity Pathways Analysis application was used for functional analysis and pathway network identification. It revealed that expression of proteins related to cell cycle changed significantly in cells exposed to inorganic phosphate. A number of proteins, representing the highly functional relationships identified by Ingenuity Pathways Analysis, showed an increase in cell metabolism and proliferation. Ingenuity Pathways Analysis software also identified a number of significant changes in osteoblast-related processes. Importantly, a number of cell cycle related proteins increased post-phosphate stimulation in one network recognized by Ingenuity Pathways Analysis. The biological functions of this group of proteins were validated by immunoassays (flow cytometry) and a cell-based proliferation assay.

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Phosphotyrosine Signaling Networks in Epidermal Growth Factor Receptor Overexpressing Squamous Carcinoma Cells. Mol Cell Proteomics. 2005 Apr;4(4):356-76. April Thelemann, Filippo Petti, Graeme Griffin, Ken Iwata, Tony Hunt, Tina Settinar, David Fenyo, Neil Gibson, and John D. Haley.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15657067&itool=iconabstr&query hl=1

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptor tyrosine kinases that coordinate a host of extracellular signals and regulate diverse signaling pathways. Overexpression of EGFR upregulates cell cycle and anti-apoptosis pathways and leads to dysregulated cell cycle checkpoints. Overexpression or mutation of EGFR is linked to many types of cancer and inhibitors of EGFR activity show promising antiproliferative effects.

To dissect EGFR signaling, scientists used affinity selection, LC-MS/MS, and MALDI methods to survey the proteins, EGFR peptides, and phosphopeptides from the model cell line HN5 following hyperstimulation of EGFR activity by exposure to EGF or inhibition by exposure to erlotinib, an EGFR kinase inhibitor. The researchers used the Ingenuity Pathways Analysis (IPA) application to determine the relations between the isolated proteins and complexes. Based on the information generated by IPA, along with data from the literature, they modeled the proteins involved in proximal EGFR signaling, cell adhesion, and cell-cell contact, and the effects of exposure to epidermal growth factor or erlotinib.

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Proteomic research: potential opportunities for clinical and physiological investigators. Am J Physiol Endocrinol Metab 286: E863-E874, 2004. K. Sreekumaran Nair, Abdul Jaleel, Yan W. Asmann, Kevin R. Short, and Sreekumar Raghavakaimal
<http://ajpendo.physiology.org/cgi/reprint/286/6/E863>

Proteins are functional molecules that are often modified posttranslationally and modulated by metabolic factors. Protein detection can be challenging due to variability in turnover rates and tissue specificity. Proteomics helps overcome these difficulties.

Integrating metabolic labeling, measurement of synthesis rates, mass spectrometry, and bioinformatics enable protein profiling. The Ingenuity Pathways Analysis application, can be used to define protein function and their signaling pathways. Proteomic profiling provides information for clinical research that will greatly benefit prognosis, diagnosis, and therapeutics. However, integrating the massive amounts of data generated from these studies remains a significant challenge.

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RNAi

Genomic approaches for reconstructing gene networks. Pharmacogenomics. 2005 Apr;6(3):245-58. Norman H. Lee
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16013956&query_hl=1

Learn more about the strategies and technologies scientists use to build gene networks, including microarrays and cluster analysis, bioinformatics methods, ChIP-chip, and RNAi. Norman Lee explains how these techniques are used, assesses their strengths and limitations, and describes discoveries they enabled.

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DBC2 Significantly Influences Cell-cycle, Apoptosis, Cytoskeleton and Membrane-trafficking Pathways. J Mol Biol. 2005 Feb 11;346(1):83-9. Epub 2004 Dec 8. Siripurapu V, Meth J, Kobayashi N, Hamaguchi
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15663929&itool=iconabstr&query_hl=2

Researchers at the Cold Spring Harbor Laboratory employed a strategy that combined RNAi and Ingenuity Pathways Analysis to gain new insights into the function of the tumor suppressor DBC2 (deleted in breast cancer 2). To determine the genes affected by DBC2, the research team performed RNA profiling of HeLa cells transformed with DBC2. Knockdown of DBC2 using RNAi confirmed the affected genes.

Using the Ingenuity Pathways Analysis application, the investigators identified two high-scoring networks in which more than half of the genes are affected by DBC2. One network regulates cell growth through apoptosis and cell-cycle control (S phase, G1/S transition), and the other is associated with cytoskeleton, membrane trafficking, Golgi assembly, and fusion. Their work lays the foundation for the functional characterization of DBC2 in tumor suppression.

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ChIP-chip

Glucocorticoid Receptor-Dependent Gene Regulatory Networks. PLoS Genetics. August 2005. Volume 1(2):e16. Phillip Phuc Le, Joshua R. Friedman, Jonathan Schug, John E. Brestelli1, J. Brandon Parker, Irina M. Bochkis, Klaus H. Kaestner
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16110340&query_hl=44

Glucocorticoids are essential steroid hormones that affect multiple organ systems. The synthetic analogs of glucocorticoids are broadly prescribed for their immunosuppressive and anti-inflammatory effects, but they often cause unwanted side effects. Glucocorticoids and their receptor (GR) are involved in complex transcriptional regulation and multiple signaling pathways. The mechanism of the action of the ligand-bound GR remains unclear.

Investigators combined two high-throughput technologies to identify direct targets of the glucocorticoid receptor. The genes that interact with GR and their different expression patterns will provide a global network of the glucocorticoid signaling pathways. Hopefully this information will help improve glucocorticoid therapy.

Mouse liver lobes were collected from animals treated with synthetic glucocorticoid and from control groups. The samples were used in two parallel experiments. Expression profiling was performed with Agilent DNA oligonucleotide arrays. Location analysis, called ChIP-on-chip (chromatin immunoprecipitation followed by DNA array analysis), was also performed using an antiserum raised against GR. The immunoprecipitated DNA was then applied to the Mouse PromoterChip BCBC-3.0 promoter microarray. Quantitative real time PCR was used to validate the enriched genetic loci.

Expression analysis revealed that 445 genes were differentially expressed in the treatment versus control group. One hundred and eighty-two genes were able to bind to the GR promoter according to ChIP-on-chip analysis. Combining the results of the two analyses, 53 genes were classified as "intersecting"—that is, differentially expressed and bound to GR. Functional networks were generated from the 53 genes using the Ingenuity Pathways Analysis application. Figure 6 in this paper shows the transcriptional regulatory network for GR. The network includes genes that were previously known to be targets of GR. Many of the other genes are candidate GR targets, including some novel GR target genes that may play critical roles in the glucocorticoid response.

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Genomic approaches for reconstructing gene networks. Pharmacogenomics. 2005 Apr;6(3):245-58. Norman H. Lee
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Learn more about the strategies and technologies scientists use to build gene networks, including microarrays and cluster analysis, bioinformatics methods, ChIP-chip, and RNAi. Norman Lee explains how these techniques are used, assesses their strengths and limitations, and describes discoveries they enabled.

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