



## Publication Synopses

Discovery, Lead Identification, Pre-Clinical, Clinical

### DISCOVERY

#### **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](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](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 Torre<sup>1</sup>, Wing H. Wong<sup>2</sup> 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](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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**Glucocorticoid Receptor-Dependent Gene Regulatory Networks.** PLoS Genetics. August 2005. Volume 1(2):e16. Phillip Phuc Le, Joshua R. Friedman, Jonathan Schug, John E. Brestelli<sup>1</sup>, 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=40](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16110340&query hl=40)

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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**Egr-2 and Egr-3 are negative regulators of T cell activation.** Nature Immunology. 6:472 – 480, (2005) Published online 17 April 2005. Meredith Safford, Samuel Collins, Michael A. Lutz, Amy Allen, Ching-Tai Huang, Jeanne Kowalski, Amanda Blackford, Maureen R. Horton, Charles Drake, Ronald H. Schwartz and Jonathan D. Powell  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15834410&query\\_hl=2](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15834410&query_hl=2)

T cell anergy is defined as a nonresponsiveness to activation in the absence of costimulatory molecules. The mechanism by which specific molecules induce T cell anergy remains unclear. Egr2 and Egr3 are differentially expressed during both T and B cell anergy. However, the precise functions of Egr2 and Egr3 have not been addressed.

To identify the genes and pathways that promote T cell anergy, five conditions were used to stimulate T cells, and T cell anergy was assessed. Expression profiles from stimulated T cells were analyzed by the Affymetrix7 GeneChip7 U74A, B and C arrays. The functional relationships of the candidate genes were explored using the Ingenuity Pathways Analysis application.

One hundred fifty-six unique genes were identified in stimulated T cells and the majority of these encoded transcription factors. As displayed in Supplementary Figure 1 of this paper, three potential networks were recognized by the Ingenuity Pathways Analysis application and the Ingenuity Pathways Knowledge Base. Transcription factor Egr2 is prominent in one of the networks.

The expression of Egr2 and Egr3 was further analyzed from microarray data. Both Egr2 and Egr3 expression was upregulated by TCR activation and inhibited by CsA.

CsA corresponded to inhibition of IL2 production— that is, anergy induction. Real time PCR, immunoblotting, *in vivo* animal model testing, and transgenic model testing validated these results. This study demonstrates that the transcription factors Egr2 and Egr3 induce T cell anergy by upregulating a set of T cell inhibitory genes or other mediators.

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**Temporal and spatial transcriptional programs in murine kidney**

**development.** *Physiol Genomics*. 2005 Jul 5; [Epub ahead of print]. G. Challen, B. Gardiner, G. Caruana, X. Kostoulas, G. Martinez, M. Crowe, D. F Taylor, J. Bertram, M. Little, and S. M Grimmond

[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15998744&query\\_hl=9](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15998744&query_hl=9)

Development of the mouse kidney, including induction of the ureteric bud (UB) and metanephric mesenchyme (MM), is still only partially understood. Global expression profiling is required to measure both temporal and spatial changes during development. However, both the number of developmental stages and number of genes identified in these studies is limited.

In this study, researchers performed expression profiling throughout all stages of kidney development using several experimental strategies. Using oligonucleotide arrays, the temporal profiles of the MM were monitored at 24-hour intervals from 10.5dpc through the neonatal period. Early metanephric development was further studied by directly comparing RNA from 10.5 vs. 11.5 vs. 13.5dpc kidneys. Spatial profiling was performed by comparing MM (10.5dpc) to adjacent intermediate mesenchyme.

The data were analyzed by applying B statistics at a threshold of 0.0, a Welch ANOVA test ( $p < 0.005$ ), and a Benjamini/Hochberg correction. The Ingenuity Pathway Analysis application and the Ingenuity Pathways Knowledge Base were used for pathway analysis and network classification. It was shown that temporal and spatial profiles have high concordance with the published rat array data using GeneSpring gene tree analysis. The results were validated by *in situ* hybridization using mouse tissues.

There were 3600 genes identified in the temporal experiment by statistical analysis. All were imported into the Ingenuity Pathway Analysis software. A total of 813 upregulated genes were identified and classified into six top scored networks using the Ingenuity Pathways Knowledge Base. Pathway analysis characterized the genes in kidney development: cell cycle, DNA replication and RNA post transcriptional genes in early development, and biosynthetic and immune response genes in later development. Ingenuity Pathways Analysis identified several signaling pathways not identified by other methods. Genes involved in these pathways include Wnt, Tgf $\beta$ , IGF, VEGF, and insulin signaling during early kidney development, Erk Map signaling in early nephrogenesis, and PPAR and other metabolism genes in later kidney development.

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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](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](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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**Transcriptional Profiling Identifies Genes Differentially Regulated by the BCR/ABL Fusion Oncogene.** Blood (ASH Annual Meeting Abstracts), Nov 2004; 104: 1537. Alexander Kohlmann, Claudia Schoch, Susanne Schnittger, Sylvia Merk, Martin Dugas, Wolfgang Hiddemann, Wolfgang Kern and Torsten Haferlach.

[http://meeting.bloodjournal.org/cgi/content/abstract/104/11/1537?maxtoshow=&HITS=&hits=&RESULTFORMAT=&fulltext=Ingenuity+Pathway&andorexactfulltext=and&searchid=1120238602924\\_11824&stored\\_search=&FIRSTINDEX=0&resourcectype=1](http://meeting.bloodjournal.org/cgi/content/abstract/104/11/1537?maxtoshow=&HITS=&hits=&RESULTFORMAT=&fulltext=Ingenuity+Pathway&andorexactfulltext=and&searchid=1120238602924_11824&stored_search=&FIRSTINDEX=0&resourcectype=1)

Chronic myeloid leukemia (CML) is associated with chromosomal translocation t(9;22). A chromosomal aberration is also found in this location in 30% of adult ALL (acute lymphoblastic leukemia). In this meeting presentation, the authors aimed to identify signature genes that are associated with this oncogenic phenotype, distinct from other subtypes of leukemia.

Discovery and validation expression profiling experiments were performed using the Affymetrix7 GeneChip7 U133A (A+B) and U133 2.0 array sets, respectively. The samples used for the discovery set were enriched monoclonal cells obtained from 218 adult patients. The gene list generated from this set was then validated using a second set of specimens (validation set) collected from another 110 patients.

Using a nonsupervised and a supervised approach for profiling, differentially expressed genes can be separated into subtypes. The genes exhibiting the highest degree of upregulation or downregulation were also identified in both the discovery set and the validation set using the classification algorithm (SVM) analysis.

Further analysis of the differentially expressed genes with the Ingenuity Pathways Analysis software showed biological differences between CML and ALL at t(9;22) that were clearly demonstrated in their pathways and functional networks. The upregulated genes in CML networks were related to leukemia metabolism immune response, non-selective vesicle transport, and humoral defense. The transcriptional profile of granulated promyelocytes in CML was clearly different from non-granulated immature promyelocytes in ALL. Biological networks in ALL included genes involved in DNA metabolism/replication, cell cycle progression, and protein biosynthesis. No statistically significant signature genes were identified that were common to both subtypes with t(9;22), suggesting that different genes are involved in BCR/ABL-dependent leukemogenesis.

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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](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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**Purmorphamine Induces Osteogenesis by Activation of the Hedgehog Signaling Pathway.** Chemistry & Biology. September 2004, 11(9): 1229-1238. X. Wu, J. Walker, J. Zhang, S. Ding, P. Schultz  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15380183&query\\_hl=3](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15380183&query_hl=3)

Osteoporosis is caused by a disorder of the differentiation of mesenchymal precursor cells into osteoblasts. Mesenchymal stem cells (MSC) can differentiate into several cell lineages, including bone, cartilage, adipose, muscle, stroma, and tendon. It is known that a small molecule, purmorphamine, induces osteogenesis in multipotent mesenchymal progenitor cells. However, the mechanism by which purmorphamine modulates the complex process of osteoporosis remains obscure.

In this study, gene expression profiles were generated from mouse embryonic fibroblasts and mouse C3H10T1/2 cells treated with purmorphamine, BMP-4 (bone morphogenetic protein 4), and DMSO as a control. Treated cells were collected at 12 hr, 24 hr, 2 days, 4 days, or 6 days and analyzed with the Affymetrix7 GeneChip7 U74Av2 array. To identify biological pathways and networks, the research team applied the Ingenuity pathways Analysis application to the GeneChip array data. RT-PCR and a reporter assay were employed to confirm the signaling pathways.

The profiling results showed that only a very small fraction of genes were differentially expressed in response to the treatment. Cell cycle regulation and DNA synthesis-related genes were upregulated at early time points following treatment with purmorphamine, suggesting that purmorphamine stimulates cellular proliferation. Multiple osteogenesis genes were upregulated after two days of treatment with purmorphamine. The Ingenuity Pathways Analysis application was used to analyze biological pathways from 29 upregulated genes that responded to treatment with purmorphamine. The top scoring biological network was the Hedgehog signaling pathway, a finding validated by RT-PCR and a cellular reporter assay. The results suggest that purmorphamine modulates the Hedgehog signaling pathway and may have a therapeutic benefit.

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**A Comparison of Gene and Protein Expression in Primary Human Trabecular Meshwork Cells Cultured With Human Aqueous Humor or Fetal Bovine Serum.** Invest Ophthalmol Vis Sci 2005;46: E-Abstract 1345. K.G. Howell, A.M. Vrabel, A.A. Leontovich, M.C. Charlesworth, D.C. Muddiman, S. Raghavakaimal, D.H. Johnson and M.P. Fautsch  
[http://abstracts.iovs.org/cgi/content/abstract/46/5/1345?maxtoshow=&HITS=&hits=&RESULTFORMAT=&fulltext=Ingenuity+Pathways&andorexactfulltext=and&searchid=1120536105258\\_24833&stored\\_search=&FIRSTINDEX=0&resourcetype=1](http://abstracts.iovs.org/cgi/content/abstract/46/5/1345?maxtoshow=&HITS=&hits=&RESULTFORMAT=&fulltext=Ingenuity+Pathways&andorexactfulltext=and&searchid=1120536105258_24833&stored_search=&FIRSTINDEX=0&resourcetype=1)

Proteins in the aqueous humor are necessary to maintain trabecular meshwork (TM) cells in a normal homeostatic environment. Alteration in the type and amount of proteins in the aqueous humor may influence the genetic program and function of TM cells.

Investigators used Affymetrix7 GeneChip7 Human Genome U133 Plus 2.0 arrays and 2-D gel electrophoresis to study cultured human primary TM cells incubated with DMEM containing either 50% human aqueous humor or 10% FBS. The gene expression profiles were analyzed using GeneSpring software and the Ingenuity Pathway Analysis application. Significant numbers of genes and proteins are upregulated in primary human TM cell incubated in 50% human aqueous humor. The authors anticipate that future network analysis will enable the collection of gene regulation data from primary cultured TM cells, providing similar profiles to the *in vivo* state.

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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=Aabstract&list\\_uids=15975430&itool=iconabstr&query hl=1](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Aabstract&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](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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**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](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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**Analysis of gene expression profile in the glaucomatous human retina using DNA microarray.** Invest Ophthalmol Vis Sci., May 2004;45: E-Abstract 4393. Z.S. Boyd, G. Zhan, H.S. Pawar, D. Bozinov, J. Eudy, J.E. Richards, and R.V. Patil

[http://abstracts.iovs.org/cgi/content/abstract/45/5/4393?maxtoshow=&HITS=&hits=&RESULTFORMAT=&author1=Boyd%2C+S&fulltext=glaucomatous&andorexactfullt ext=and&searchid=1120591667676\\_19250&stored\\_search=&FIRSTINDEX=0&resour cetype=1](http://abstracts.iovs.org/cgi/content/abstract/45/5/4393?maxtoshow=&HITS=&hits=&RESULTFORMAT=&author1=Boyd%2C+S&fulltext=glaucomatous&andorexactfullt ext=and&searchid=1120591667676_19250&stored_search=&FIRSTINDEX=0&resour cetype=1)

Global gene expression profiling was performed using the Affymetrix7 GeneChip7 U133A array to identify gene expression associated with glaucoma. Four glaucomatous retinas and four normal samples were used. Candidate genes were categorized into therapeutically relevant networks using the Ingenuity Pathways Analysis application.

In glaucomatous retina, 14 genes were upregulated and 202 genes were downregulated relative to normal samples. The Ingenuity Pathways Analysis application classified the downregulated genes into seven networks: transport, cell death/apoptosis, gene transcription, cell signaling, response to stress, DNA repair, and immune response.

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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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**DNA-Microarray Analysis of Brain Cancer: Molecular Classification for Therapy.** Nature Reviews Neuroscience 5, 782-792 (2004). Paul S. Mischel, Timothy F. Cloughesy & Stanley F. Nelson.  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15378038&query hl=4](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15378038&query hl=4)

Primary brain tumors are highly lethal because they are usually refractory to available therapies. Paul Mischel and his colleagues provide a survey of approaches that use DNA microarray analysis to identify molecular diagnostic biomarkers and predict new therapies for brain cancers. DNA microarray analysis enables analysis of gene expression at the transcriptional level genome-wide and identification of differentially expressed genes among different patient groups, including those with high and low survival rates. Using tools like Ingenuity Pathways Analysis to further evaluate microarray data can reveal pathway and functional network information that will increase our understanding of cancer-associated genes and their proteins.

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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](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-6B-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-6B-IRF TFBS pair. Microarray analysis confirmed that nine of the genes are regulated by IFN or NF-6B. 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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**LEAD IDENTIFICATION**

**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](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.



## **PRE-CLINICAL**

**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](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.



**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](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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**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](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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**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](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](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 Lep<sup>db/db</sup> 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<sup>db/db</sup>* 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<sup>db/db</sup>* 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<sup>db/db</sup>* 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](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 $\alpha$  (peroxisome proliferator-activated receptor- $\alpha$ ) 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  $\beta$ -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 $\kappa$ 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](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- $\alpha$  (peroxisome proliferator-activated receptor- $\alpha$ ) 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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**Profiling Rnr KO and rd7 Mice to Identify Direct Targets of RNR (Nr2e3).**

Invest Ophthalmol Vis Sci., May 2005;46: E-Abstract 3068. A.L. Webber, P. Hodor, T. Zhang, D. Holder, and K. Petrukhin

[http://abstracts.iovs.org/cgi/content/abstract/46/5/3068?maxtoshow=&HITS=&hits=&RESULTFORMAT=1&andorexacttitle=and&andorexacttitleabs=and&fulltext=Ingenuity+Pathways&andorexactfulltext=phrase&searchid=1120249726909\\_14126&store\\_d\\_search=&FIRSTINDEX=0&sortspec=relevance&fdate=1/1/2005&resourcetype=1](http://abstracts.iovs.org/cgi/content/abstract/46/5/3068?maxtoshow=&HITS=&hits=&RESULTFORMAT=1&andorexacttitle=and&andorexacttitleabs=and&fulltext=Ingenuity+Pathways&andorexactfulltext=phrase&searchid=1120249726909_14126&store_d_search=&FIRSTINDEX=0&sortspec=relevance&fdate=1/1/2005&resourcetype=1)

An orphan nuclear receptor, Retina-specific nuclear receptor (RNR), was considered as a therapeutic target for retina degeneration. To provide evidence and support for the RNR as a therapeutic molecule, this work attempted to identify direct targets of RNR using gene expression profiling. The authors presented this study in the 2005 annual meeting of the Association for Research in Vision and Ophthalmology (ARVO).

Samples from both human patients with mutations in RNR and Rnr knockout (KO) mice were used for expression profiling with Agilent 60-mer oligonucleotide microarrays. The Ingenuity Pathways Analysis application was used to identify biological pathways and functional networks.

Molecular profiling revealed 422 genes that were differentially expressed and defined as potential RNR-dependent genes. The potential target genes were classified by Ingenuity Pathways Analysis into the following categories: lipid metabolism, fatty acid metabolism, cell-to-cell signaling, molecular transport, small molecular biochemistry, cell death, immunological diseases, and inflammatory diseases. Transcriptional regulation at the promoter region was analyzed in a subset of

differentially expressed genes generated from networks by evaluating transcription factor binding sites. Enrichment of promoters for the hexamer pairs of four genes, DR1, DR4, ER3 and IR3, was confirmed. These genes contain known nuclear binding sites and hexamer configurations.

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## CLINICAL

**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](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 (GROa)*) 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.



**Aquaporin-4 is increased in the sclerotic hippocampus in human temporal lobe epilepsy.** *Acta Neuropathol (Berl)*. 2004 Dec;108(6):493-502. Tih Shih Lee, Tore Eid, Shrikant Mane, Jung H. Kim, Dennis D. Spencer, Ole Petter Ottersen, and Nihal C. de Lanerolle  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15517312&query\\_hl=5](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15517312&query_hl=5)

Mesial temporal lobe epilepsy (MTLE) is a disorder characterized by chronic seizures. The hippocampus of patients with MTLE is typically fibrotic and shrunken by patterned loss of neurons and astroglial proliferation. The molecular basis of hippocampal sclerosis is still obscure.

The authors investigated whether aquaporin-4 (AQP4), a water channel transporter protein in the brain, plays a role in hippocampal sclerosis. AQP4 couples water transport to K<sup>+</sup> clearance. This study characterized the spatial distribution of AQP4, its expression profiles, and the regulation of genes in its functional pathways.

Hippocampal specimens obtained from patients were processed for analysis by immunohistochemistry, quantitative real time PCR, and the Affymetrix7 GeneChip7 U133A array. Data generated from both light and electron microscopy combined with immunohistochemistry showed that the AQP4 protein was localized in the CA1 area of the mesial temporal lobe. In addition, AQP4 was localized to the membranes of astrocytic end-feet, next to endothelial cells. Increased AQP4 expression was detected by quantitative real time PCR as well as microarray analysis. As one of the reporter sequences on the U133A array, expression of AQP4 was also detected in the CA1 region of the hippocampus.

The Ingenuity Pathways Analysis software was used to investigate the molecular pathways and gene networks associated with AQP4 function in the hippocampal specimens from patients. As shown in the network in Figure 4 of this paper, the expression of six genes increased and five genes decreased. The elevation of AQP4 expression is correlated with increased GFAP (astrocyte marker glial fibrillary acidic protein) expression. Conversely, increased AQP4 is associated with decreased expression of dystrophin, a protein implicated in the anchoring of AQP4 to perivascular endfeet. Ingenuity Pathways Analysis also revealed that along with increased AQP4 expression in the sclerotic region of the hippocampus, expression levels of several genes related to dystrophin-associated protein complex were also altered. This complex anchors AQP4 protein to cellular membranes and regulates its function in the brain.



**New insights into MLL gene rearranged acute leukemias using gene expression profiling: shared pathways, lineage commitment, and partner genes.**

Leukemia (2005) 19, 953–964. A Kohlmann, C. Schoch, M. Dugas, S Schnittger, W. Hiddemann, W. Kern, and T. Haferlach  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15815718&query\\_hl=13](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15815718&query_hl=13)

In acute leukemia, the myeloid/lymphoid or mixed-lineage leukemia (MLL) gene, located at chromosomal band 11q23, is a recurrent target of chromosome translocation. The MLL fusion proteins play roles in signal transduction or transcriptional regulation during the oncogenic process. MLL gene rearrangements occur in both subtypes of leukemia, ALL (acute lymphoblastic leukemia) and AML (acute myeloid leukemia).

This study investigated global expression patterns and common target genes of ALL and AML leukemia subtypes, the MLL chimeric fusion genes, and how these fusion proteins affect the cellular properties of both myeloid and lymphoblastic lineages.

A series of 363 adult acute leukemia specimens were analyzed using the Affymetrix7 GeneChip7 U133 array sets. Although expression profiling indicated distinct expression signatures from two subtypes of leukemia, it could not clearly demonstrate an association between the differentially expressed genes and six partner genes that are known to interact with the MLL gene.

The Ingenuity Pathways Analysis application helped identify molecular pathways and biological networks associated with leukemia. A biological network (Figure 3 in this paper) distinguished the t(11q23)/MLL from other acute leukemia subtypes. A group of up- or down-regulated common target genes associated with t(11q23)/MLL leukemia was specified. A network also identified genes expressed differently in ALL with t(11q23)/MLL compared to AML with t(11q23)/MLL, as displayed in Figure 5 in the paper. ALL and AML subtypes were segregated according to the lineages, lymphoblastic or myeloid, respectively. Signature genes were identified in ALL and AML subtypes from the pathways. *PAX5*, an early B-cell lineage commitment factor, restricts developmental progression of lymphoid progenitors to the B-cell pathway. An essential B-cell regulator, *EBF*, functions in early B cell lymphopoiesis. Novel functional networks helped to better understand these two acute leukemia subtypes, including the identification of therapeutic targets.

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**AML M3 and AML M3 variant each have a distinct gene expression signature but also share patterns different from other genetically defined AML subtypes.**

Genes, Chromosomes, and Cancer, 43(2): 113-127. Torsten Haferlach, Alexander Kohlmann, Susanne Schnittger, Martin Dugas, Wolfgang Hiddemann, Wolfgang Kern, Claudia Schoch  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15751046&query\\_hl=2](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15751046&query_hl=2)

Acute promyelocytic leukemia (APL), a subtype of acute myeloid leukemia (AML), can be divided into two distinct subtypes: AML M3 and AML M3v. AML M3 has abnormal promyelocytes with heavy granulation and bundles of Auer rods while AML M3v shows non- or hypogranular cytoplasm and a bilobed nucleus. Patients with M3 and M3v subtypes often suffer severe bleeding that causes early death. Identification

of differentially expressed signature genes in these two subtypes will help to understand the morphological features associated with development, along with clinical differences in the diseases they cause.

A global expression profile of bone marrow aspirates from 35 APL patients (19 AML M3 and 16 AML M3v) was generated using the Affymetrix7 GeneChip7 U133 array sets. Clustering analysis of expression patterns revealed that the APL samples were grouped distinctly from other AML subtypes and karyotype. In addition, specific gene expression signatures correlated with coagulation in APL compared to other AML subtypes. Thirteen genes were identified that differed in expression patterns between M3 and M3v subtypes. The findings were validated by real time PCR.

Ingenuity Pathways Analysis software and the Ingenuity Knowledge Base were applied for further analysis and confirmation of biological pathways and functional networks. Figure 2 in this paper shows a biological network distinguishing APL from other AML. A network also confirmed a known finding in which genes relevant to MHC-II antigen presentation are expressed less in APL. Another significant biological network, displayed in Figure 6 revealed that genes involved in clotting/coagulation were highly expressed in APL but not in other AML subtypes.

Other genes involved in maturation, granulation and nuclear configuration were also identified from functional networks. The DEFA gene was highly expressed in primary granules (APL M3) while the TCN2 gene was overexpressed in secondary granules (APL M3v). Importantly, this was the first example of discrimination between APL subtypes at the transcriptome level.

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### **Identification of Novel Candidate Oncogenes and Tumor Suppressors in Malignant Pleural Mesothelioma Using Large-Scale Transcriptional Profiling.**

American Journal of Pathology. 2005; 166:1827-1840. Gavin J. Gordon, Graham N. Rockwell, Roderick V. Jensen, James G. Rheinwald, Jonathan N. Glickman, Joshua P. Aronson, Brian J. Pottorf, Matthew D. Nitz, William G. Richards, David J. Sugarbaker, and Raphael Bueno  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15920167&query\\_hl=22](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15920167&query_hl=22)

Malignant pleural mesothelioma (MPM) is a highly lethal and poorly understood cancer. With limited treatment options, patients usually survive less than two years following diagnosis. Although MPM has been classified into three histological subtypes, there is no correlation between survival and response to treatment or histological classification.

In this study, 40 MPM tumor specimens, nine normal lung tissue samples and five cell lines were evaluated using the Affymetrix7 GeneChip7 U133A array. After applying clustering methods, two possible subclasses of MPM (C1 or C2) were grouped, representing distinct expression patterns. A total of 113 genes were differentially expressed in the C1 and C2 subclasses. Histological markers of 54 genes showed different expression levels between MPM histological classes. The study identified 328 upregulated genes and 311 downregulated genes as MPM tumor markers. Quantitative RT PCR, western blotting, and MPM tissue arrays validated expression of subset markers. The results showed that transcription and translation are coupled by NME2 (transcription factor) and CR1 (CREB binding protein).

The Ingenuity Pathways Analysis application was applied to discover novel pathogenic pathways during MPM progression. Analysis of the 328-upregulated genes in MPM tumor vs. normal tissues identified twelve networks. Seven networks that shared at least one gene were combined and are displayed in Figure 7 of this paper. Five genes were shared by at least two pathways. Two of them, SP1 and SPARC, have not been previously described in MPM studies. SP1, a transcription factor, regulates many cancer-related genes. SPARC is a matrix-associated protein. Pathway analysis showed that SPARC interacts with VTN and may function in angiogenesis in the context of MPM tumorigenesis. The functional pathways and networks generated by Ingenuity Pathways Analysis assisted in understanding MPM pathogenesis and tumorigenesis.

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**Profiling the Evolution of Human Metastatic Bladder Cancer.** Cancer Res. 2004 Nov 1;64(21):7813-21. Brian E. Nicholson, Henry F. Frierson, Mark R. Conaway, Javed M. Seraj, Michael A. Harding, Garret M. Hampton and Dan Theodorescu. [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Aabstract&list\\_uids=15520187&query\\_hl=2](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Aabstract&list_uids=15520187&query_hl=2)

Aggressive bladder carcinoma frequently results in metastatic lung cancer and is associated with a high death rate. There is limited understanding of this disease at the molecular level due to the poor availability of human metastatic tumor tissue and a lack of suitable animal models. To address this need, investigators developed progressively more metastatic human bladder cancer cell lines. They also developed an *in vivo* bladder-cancer lung metastasis model to identify gene expression changes that correlated with the degree of pulmonary metastasis.

Three cell lines with increasingly metastatic features were developed and characterized by *in vitro* cell-based assays and *in vivo* survival and colonization assays. Expression profiles of metastatic progression phenotypes from four cell lines were evaluated using Affymetrix7 GeneChip7 arrays. Quantitative RT-PCR and western blotting were used to validate the expression of a subset of candidate genes.

One hundred twenty-one genes showed increased expression while expression of 43 genes was decreased. The majority of significant genes participated in cell-extracellular matrix interactions. Such interactions are known to be important determinants of metastasis. The Ingenuity Pathways Analysis software was applied to further identify biological pathways, protein-protein interaction networks, and their functional relationships in the metastasis model.

The two top scoring pathways generated by Ingenuity Pathways Analysis revealed functionally strong associations of genes involved in cell invasion and migration. Pathway 1 included the coassociation of fibronectin, tPA, uPA, MMP-14, TIMP, and IL8. Pathway 2 included integrins A3, A6, and B4, as well as amphiregulin and melanoma cell adhesion molecule. The pathway with the next highest score identified genes involved in the suppression of apoptosis, including BCL2\_L1, IER3, and BIK. It is known that suppression of apoptosis is an important feature of metastatic progression.

This study helped identify potentially important molecular and cellular alterations involved in the progression of bladder cancer from superficial lesions to pulmonary metastases.

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**Can gene expression profiling predict survival for patients with squamous cell carcinoma of the lung?**

Molecular Cancer. Dec. 2004, 3:35. Zhifu Sun, Ping Yang, Marie-Christine Aubry, Farhad Kosari, Chiaki Endo, Julian Molina and George Vasmatazis

[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Aabstract&list\\_uids=15579197&query\\_hl=32](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Aabstract&list_uids=15579197&query_hl=32)

Lung cancer causes more death worldwide than any other kind of cancer. Prognostic biomarkers are required to help classify the clinical outcomes of lung cancer patients. The authors attempted to identify reliable prognostic biomarkers from expression profiling of lung cancer patients' tissues by using DNA microarrays.

Tissues were collected from three groups of patients (15 total) with squamous cell lung cancer. Five were classified as aggressive, five as non-aggressive, and five were used as test controls. Three strategies for expression data analysis were applied, including unsupervised clustering, linear discrimination, and R package. The expression profiles showed overall similar patterns from aggressive and non-aggressive lung cancers. This indicates that only a small group of genes may be differentially expressed between these two clinically different groups.

Twenty-four networks were generated by applying the Ingenuity Pathways Analysis application to a subset of 126 overlapping genes selected by statistical analysis. Using the Ingenuity Pathways Knowledge Base, seven of the top networks were strongly associated with patient survival, as listed in Table 2 of this paper. Further analysis using the RAB6A network predicted all samples correctly. The prognostic biomarkers can be further investigated using gene network analysis.

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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](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=Aabstract&list\\_uids=15994935&itool=iconabstr&query hl=2](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Aabstract&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 G<sub>1</sub>-S checkpoint genes are dysregulated in class 1 PRCC whereas G<sub>2</sub>-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 G<sub>0</sub>-G<sub>1</sub>) and diminished replication (entry and progression through S phase). The involvement of met signaling in G<sub>1</sub>-S checkpoint dysregulation is a pertinent area of future research.
- P DNA TopII $\alpha$  is a diagnostic marker for class 2 PRCC. TopII inhibitors produce G<sub>2</sub> 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](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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**Profiling Rnr KO and rd7 Mice to Identify Direct Targets of RNR (Nr2e3).**

Invest Ophthalmol Vis Sci., May 2005;46: E-Abstract 3068. A.L. Webber, P. Hodor, T. Zhang, D. Holder, and K. Petrukhin

[http://abstracts.iovs.org/cgi/content/abstract/46/5/3068?maxtoshow=&HITS=&hits=&RESULTFORMAT=1&andorexacttitle=and&andorexacttitleabs=and&fulltext=Ingenuity+Pathways&andorexactfulltext=phrase&searchid=1120249726909\\_14126&store\\_d\\_search=&FIRSTINDEX=0&sortspec=relevance&fdate=1/1/2005&resourceype=1](http://abstracts.iovs.org/cgi/content/abstract/46/5/3068?maxtoshow=&HITS=&hits=&RESULTFORMAT=1&andorexacttitle=and&andorexacttitleabs=and&fulltext=Ingenuity+Pathways&andorexactfulltext=phrase&searchid=1120249726909_14126&store_d_search=&FIRSTINDEX=0&sortspec=relevance&fdate=1/1/2005&resourceype=1)

An orphan nuclear receptor, Retina-specific nuclear receptor (RNR), was considered as a therapeutic target for retina degeneration. To provide evidence and support for the RNR as a therapeutic molecule, this work attempted to identify direct targets of RNR using gene expression profiling. The authors presented this study in the 2005 annual meeting of the Association for Research in Vision and Ophthalmology (ARVO).

Samples from both human patients with mutations in RNR and Rnr knockout (KO) mice were used for expression profiling with Agilent 60-mer oligonucleotide microarrays. The Ingenuity Pathways Analysis application was used to identify biological pathways and functional networks.

Molecular profiling revealed 422 genes that were differentially expressed and defined as potential RNR-dependent genes. The potential target genes were classified by Ingenuity Pathways Analysis into the following categories: lipid metabolism, fatty acid metabolism, cell-to-cell signaling, molecular transport, small molecular biochemistry, cell death, immunological diseases, and inflammatory diseases. Transcriptional regulation at the promoter region was analyzed in a subset of differentially expressed genes generated from networks by evaluating transcription factor binding sites. Enrichment of promoters for the hexamer pairs of four genes, DR1, DR4, ER3 and IR3, was confirmed. These genes contain known nuclear binding sites and hexamer configurations.

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**Identification of genes and molecular pathways involved in the progression of premalignant oral epithelia.** Mol Cancer Ther., 4:865-875 2005. Abhijit G.

Banerjee, Indraneel Bhattacharyya and Jamboor K. Vishwanatha.  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15956244&query\\_hl=3](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15956244&query_hl=3)

Early diagnosis of oral premalignancy is key to increasing the survival rate for oral cancer. This study focused on the identification of biomarkers from oral biopsy specimens and archival tissues. A subset of candidate genes identified by microarray analysis was validated by quantitative real time PCR, immunohistochemistry, and bioassays.

The Ingenuity Pathways Analysis application was used to identify malignant progression-related pathways and functional networks in oral precancer. An important enzyme in the arachidonic acid metabolic pathway, lipocalin-type prostaglandin D2 synthase, is downregulated in premalignant stages. Two invasion-related biomarkers, psoriasin and versican, are upregulated in oral premalignant and malignant archival tissues.

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**Molecular Classification of Tamoxifen-Resistant Breast Carcinomas by Gene Expression Profiling.** Journal of Clinical Oncology, Vol 23, No 4 (February 1), 2005: pp. 732-740. Maurice P.H.M. Jansen, John A. Foekens, Iris L. van Staveren, Maaïke M. Dirkzwager-Kiel, Kirsten Ritstier, Maxime P. Look, Marion E. Meijer-van Gelder, Anieta M. Sieuwerts, Henk Portengen, Lambert C.J. Dorssers, Jan G.M. Klijn, Els M.J.J. Berns.  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list\\_uids=15681518&itool=iconabstr&query\\_hl=24](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15681518&itool=iconabstr&query_hl=24)

Tamoxifen has become a standard therapy for treating all stages of estrogen receptor (ER) positive breast cancer. One of the major challenges in treatment with tamoxifen is resistance to this antiestrogen, since half of the patients with ER-positive breast cancer do not respond to tamoxifen. Biomarkers are urgently needed to classify breast cancer patients prior to treatment into tamoxifen-sensitive or -insensitive groups.

Expression profiling was performed on 112 ER-positive breast cancer patients using spotted oligonucleotide arrays. Genes that were differentially expressed in tamoxifen-sensitive patients compared to tamoxifen-resistant patients were validated by quantitative real time PCR. Forty-four signature genes were identified and validated through analysis of a training set and a test set. The expression of eight signature genes was measured and confirmed the correlation between microarray and quantitative real time PCR. Functional annotation classified the signature genes into the following groups: estrogen action, apoptosis, extracellular matrix formation, and immune response. Seventeen signature genes were regulated by or associated with estrogen receptor actions.

The Ingenuity Pathways Analysis application was applied to further assess the association between this subset of signature genes and tamoxifen responsiveness. Seven genes were associated with apoptosis, including IL4R, LDHA, MAP2K4, NPM1, SIAH2, CASP2, and TXN2. Two anti-apoptosis genes, API5 and BNIP3, were also recognized. The signature genes identified by this study may help to predict antiestrogen therapy resistance in ER-positive patients.

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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](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](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 $\alpha$ , an important mediator of SIRS. These preliminary findings are essential to developing tailored therapies for trauma patients.

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