

Analysis of Molecular Profiling Data to Understand Mechanisms of Disease, Targets & Biomarkers

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Introduction

IPA® is a web-based software application that enables life science researchers to analyze, integrate, and understand data derived from many different platforms including gene expression, microRNA, and SNP microarrays; metabolomics and proteomics experiments; siRNA screens, and small-scale experiments that generate gene and chemical lists.

IPA is an all-in-one solution that includes the following:

- Search and Explore Capabilities
- Data Analysis Tools
 - Basic Analysis
 - Toxicology Analysis
 - Biomarker Analysis
 - Metabolomics Analysis
- Communication and Collaboration Tools

Here we present a case study in which ovarian cancer gene expression profiles associated with copy-number-alterations (CNAs) were analyzed in IPA. In this study IPA provided a bridge between molecular profiles of cancer and cancer-specific processes, phenotypes & pathways. Insights from IPA generated testable hypotheses and helped narrow in on key genes and relationships to focus validation experiments on.

Materials and methods

Researchers can upload and analyze in IPA gene or chemical datasets that use any of the over 20 types of identifiers outlined here.

In this case ~500 genes with significant CNA-associated gene expression changes in ovarian tumors were uploaded & analyzed in IPA. CNAs in ovarian tumors were assessed with Affymetrix 500K SNP Chips. Gene expression patterns of these tumors were profiled with Affymetrix U133A and B chips.

Original datasets are available here: [High-resolution analysis of copy number alterations and associated expression changes in ovarian tumors](#). BMC Med Genomics 2009 May 6;2(1):21. Haverty PM, Hon LS, Kaminker JS, Chant J, Zhang Z.

Log Ratio	p-value	z-score	ID	Notes	Symbol	Entrez Gene Name	Location	Type(s)	Drug(s)
+5.308	1.77E-10	2.02E-09	206246_s_at		HMPK1	nitrogen-activated	Cytoplasm	kinase	
+5.115	3.32E-16	1.18E-14	201839_s_at		EPCAM	epithelial cell adhesion	Plasma Membrane	cell adhesion	hormonal agent
+4.712	1.11E-15	3.03E-14	209692_at		EYAA2	eyes absent homolog	Nucleus	phosphatase	
+4.319	4.89E-13	8.60E-12	212923_at		CCNE1	cyclin E1	Nucleus	transcription regulator	
+4.003	4.42E-12	7.47E-11	227201_s_at		CCNE1	cyclin E1	Nucleus	transcription regulator	
+3.732	3.00E-15	6.93E-14	221245_s_at		FDS5	frizzled homolog 5	Plasma Membrane	protein-coupled receptor	
+3.603	1.31E-12	2.14E-11	224913_s_at		TIPSD5	translocase of inner	Cytoplasm	phosphatase	

Figure 1. Ovarian Cancer Gene Expression Profile uploaded, mapped, and annotated in IPA just prior to running a Core Analysis.

Analysis Workflow in IPA:

- Run an IPA Core Analysis to link genotypes (molecular profiling data) to phenotypes and molecular events (Cellular and Disease Processes, Signaling and Metabolic Pathways, Molecular Networks).
- Use My Pathway tools to integrate additional therapeutic & clinical context, experimental data, and prioritize genes of interest for validation studies.

Results

IPA Maps Gene Expression Changes to Cancer-Relevant Cellular Processes and Disease Phenotypes

Molecular and Cellular Functions

Name	p-value
Cellular Movement	4.24E-05 - 4.90E-02
Amino Acid Metabolism	6.98E-05 - 4.90E-02
Post-Translational Modification	6.98E-05 - 4.71E-02
Small Molecule Biochemistry	6.98E-05 - 4.90E-02
Cell Death	1.33E-04 - 4.90E-02

Diseases and Disorders

Name	p-value
Immunology Disease	6.13E-04 - 3.43E-02
Inflammatory Response	6.13E-04 - 4.58E-02
Cancer	1.13E-03 - 4.90E-02
Hematological Disease	1.13E-03 - 3.43E-02
Reproductive System Disease	1.91E-03 - 4.22E-02

Key findings: apoptosis of tumor cells (2.79E-02), ovarian carcinoma (3.30E-02).

Figure 2. Output of Functional Analysis Component of Core Analysis. One of the main outputs of an IPA Core Analysis is the Functional Analysis – which directly links genes from the dataset (here, with CNA-driven up or down regulated transcripts) to Cellular, Organismal, and Disease Phenotypes. IPA identifies a strong association between differentially expressed genes in the ovarian cancer profiles and cancer-related phenotypes such as apoptosis & cell movement. Findings from the Ingenuity® Knowledge Base also rapidly identify genes from the data set that have been associated with ovarian cancer in previous studies, helping validate the researcher's experimental approach.

IPA Identifies Key Signaling Pathways Perturbed in Ovarian Cancer Molecular Profiles

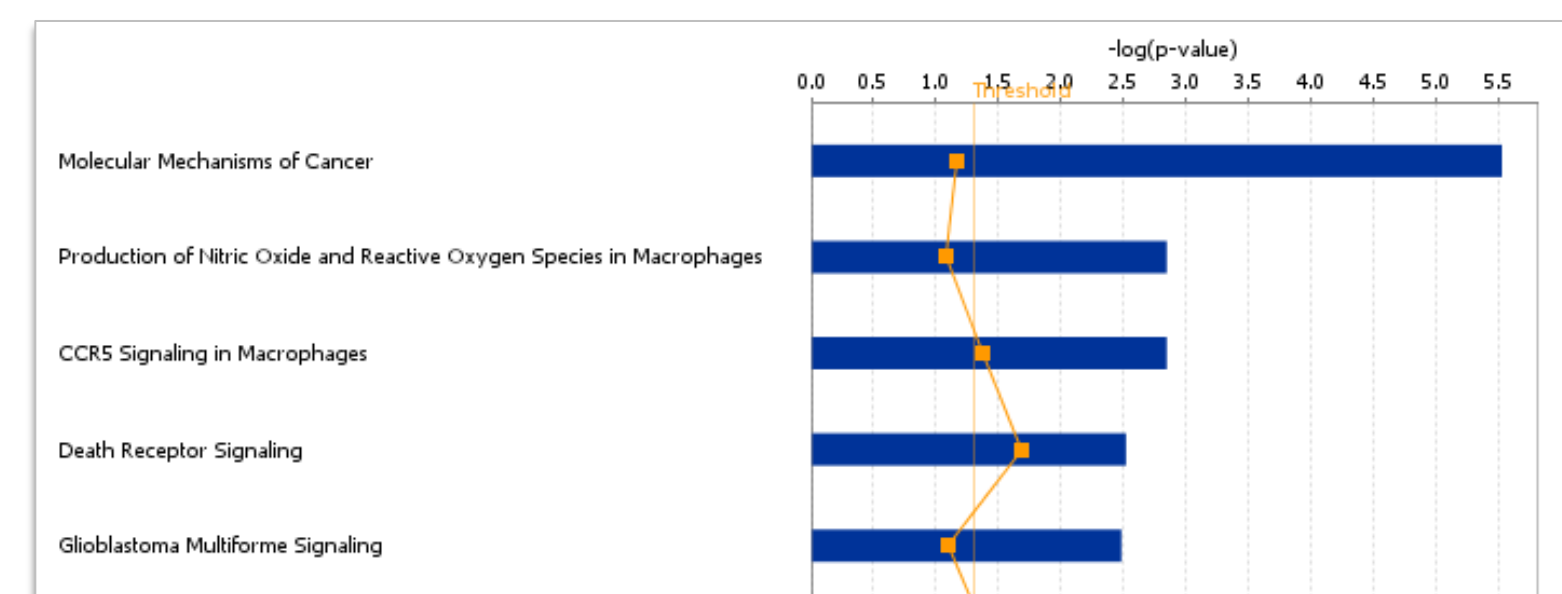
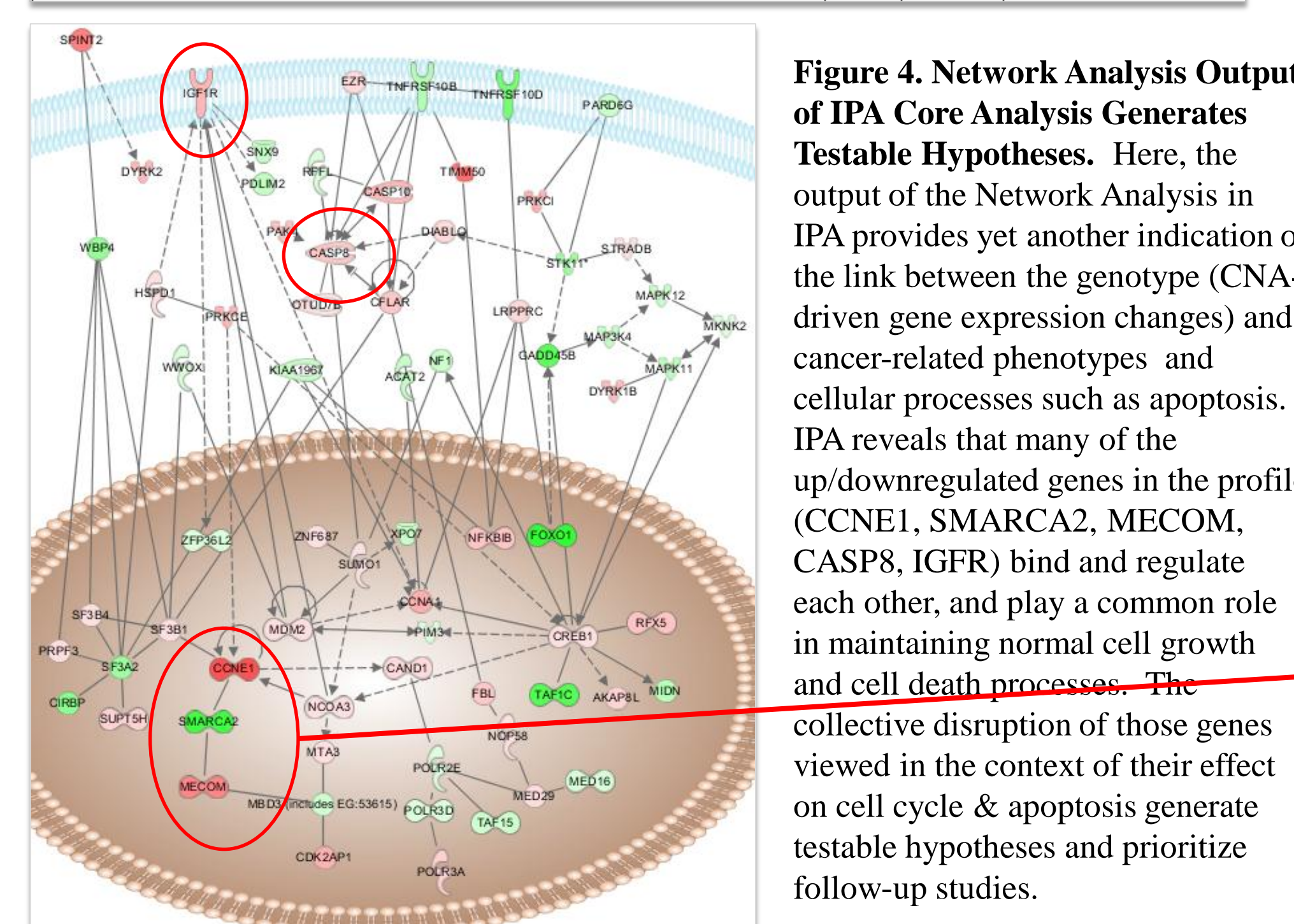


Figure 3. Output of Pathway Analysis Component of Core Analysis. A second major component of an IPA Core Analysis is the identification of key Signaling, Metabolic & Disease Pathways associated with the CNA-related gene expression changes. This analysis reinforces the insights gained from the Functional Analysis by revealing strong associations to related pathways such as Molecular Mechanisms of Cancer and Death Receptor Signaling.

Network Analysis Supports Connection Between Copy Number-Driven Gene Expression Changes and Cancer Relevant Processes: Apoptosis, Cell Growth

Molecules in Network	Score	Focus Molecule	Top Functions
106	70	Cell Death, Cellular Growth and Proliferation, Amino Acid Metabolism	



Initial Conclusions

- IPA analysis of CNA-driven expression changes in ovarian cancer tumors:
- Validated their experimental approach by revealing existing knowledge linking genes in the profile to ovarian cancer
 - Identified strong links between up/down regulated genes and apoptosis
 - Identified biological processes, pathways and genes that may be potential drivers of carcinogenesis based on their association with CNA-driven expression changes

Next Step: Prioritize genes for follow up studies by identifying assays available to measure their transcript/protein levels in a clinical setting.

Integration of Therapeutic Context Prioritizes Genes of Interest from Apoptosis Network

Overlay: Biomarkers

Application	Disease	# Molecules	Molecule(s)
Diagnosis	prostatic carcinoma	1	MEC1
Diagnosis	breast cancer	1	CASP8
Diagnosis	pancreatic cancer	1	STK11
Diagnosis	sarcoma	1	IGFBP1
Efficacy	breast cancer	2	IGFBP1, NCOA3
Efficacy	head and neck cancer	1	IGFBP1
Efficacy	solid tumor	1	CASP8
Efficacy	non-hodgkin lymphoma	1	MEC1
Efficacy	colorectal cancer	1	IGFBP1
Prognosis	gastrointestinal stroma...	1	EZR
Prognosis	Burkitt's lymphoma	1	CFLAR
Prognosis	melanoma	1	MEC1
Unspecified Application	Huntington's disease	1	CFLAR

Secondary Outcome Measures:
Antitumor activity [Designated as safety issue: No]
In vivo molecular and biological effects of CDDO as measured by changes in biomarkers of apoptosis and cell cycle arrest (e.g., caspase 3, caspase 9, and caspase 9 cleavage products, p21, and poly-ADP-ribose polymerase [PARP] cleavage products) [Designated as safety issue: No]

Figure 5. Pathway Overlay Features Integrate Additional Context. The Overlay features in IPA prioritize genes of interest in the Apoptosis Network by identifying genes that may also be significantly deregulated in other cancer datasets, and by layering in knowledge about drug targets and exploratory clinical biomarkers. For example, Findings in IPA reveal that an ELISA exists to measure levels of activated, phosphorylated IGF1R in clinical samples and that CASP8 is being used as a secondary outcome marker (impact on apoptosis and cell cycle arrest) for CDDO anti-tumor therapy.

IPA My Pathway Report
MECCOM Neighborhood Report

Report Date: 2010-05-19
IPA Version: 3.0 (Release Date: 2010-02-10)
Dataset Version: 2009 (Release Date: 2010-05-14)

Top Functions: Cell Death, Cellular Growth and Proliferation, Amino Acid Metabolism

Drug Summary: Summary of drug targeting molecules in My Pathway

Drug Name	Class	Target	Brand Name	Drug Pathway
irinotecan	antitumor	topoisomerase II	irinotecan	multiple myeloma/leukemia/colorectal cancer/pancreatic cancer
irinotecan hydrochloride	antitumor	topoisomerase II	irinotecan	multiple myeloma/leukemia/colorectal cancer/pancreatic cancer
irinotecan hydrochloride	antitumor	topoisomerase II	irinotecan	multiple myeloma/leukemia/colorectal cancer/pancreatic cancer
irinotecan hydrochloride	antitumor	topoisomerase II	irinotecan	multiple myeloma/leukemia/colorectal cancer/pancreatic cancer

Figure 6. Interactive Reports Provide Deep Knowledge of Targets, Pathways. Multiple lines of evidence from the Ingenuity Knowledge Base highlight MECOM and its interactions with other ovarian cancer genes as an area for further investigation. MECOM is upregulated in the CNA dataset, is upregulated in other ovarian cancer studies (Findings from the Ingenuity Knowledge Base), is a target of miRNA upregulated in ovarian cancer (see dataset from Dahiya et al), is in a complex with HDAC (a target of CML therapeutic intervention), and binds SMARCA2 (downregulated in the CNA dataset, a target of miRNA downregulated in OC, and binds ovarian cancer markers).

Integration of Multiple Lines of Evidence Generates Testable Hypotheses, Focuses Validation Studies

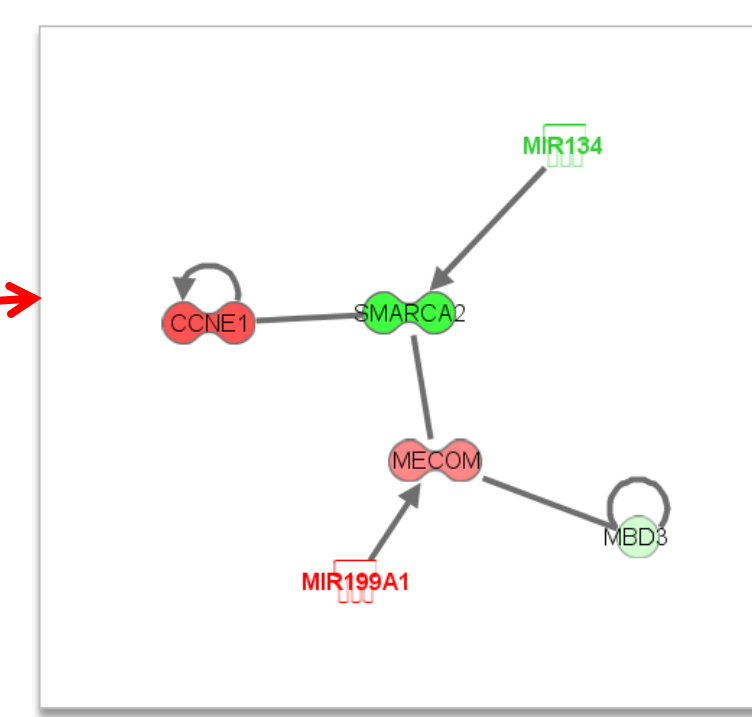


Figure 7. My Pathways features narrow in on testable hypotheses. The relationships between CCNE1, SMARCA2, MECOM (EV11), and MBD3 play an important role in cell proliferation and growth arrest checkpoints. (Findings from Ingenuity Knowledge Base). Deregulation of transcript levels (see original CNA dataset) and miRNA in ovarian tumors (see Dahiya et al dataset) suggest an important area for validation studies: validation of mRNA & protein levels in ovarian tumors, and their role as drivers of carcinogenic processes via targeted siRNA knockdowns or small molecule inhibitors.

Conclusions

IPA analysis of Copy Number Alteration (CNA)-driven gene expression changes in ovarian tumors provided valuable insight into genes, processes and pathways that are potential drivers of carcinogenesis. The time from data generation to mechanistic insight was rapidly accelerated by examining these molecular profiles within the dynamic biological context of IPA, and substantiated by the high quality, context-rich Findings, Phenotypes, Molecular Interactions, and Pathways in the Ingenuity Knowledge Base.

- IPA Core Analysis of CNA-specific gene expression changes identified key processes and pathways that may be driven by these gene alterations including:
 - Apoptosis networks
 - Molecular Mechanisms of Cancer and Death Receptor Signaling pathways
- IPA provided an initial pool of candidate genes that may be useful as markers of carcinogenesis in ovarian tissue, and identified existing clinical biomarker assays for those candidates.
- Examination of those candidate genes in the context of multiple lines of evidence (using the Overlay feature) narrowed in on subset of genes and hypotheses for validation studies
 - Overlaying additional mRNA, miRNA datasets point to the gene MECOM and its interacting partners as an area for further study.
- The hypotheses generated by this analysis now exist as interactive models in IPA that are directly supported by evidence from the Ingenuity Knowledge Base. In this way IPA can be used as a central resource for sharing insights with colleagues, and iteration/revision of models as new insights become available either through validation studies or Findings from the peer-reviewed literature.

Literature cited

High-resolution analysis of copy number alterations and associated expression changes in ovarian tumors. BMC Med Genomics 2009 May 6;2(1):21. Haverty PM, Hon LS, Kaminker JS, Chant J, Zhang Z.

MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS ONE 2008 Jun 18;3(6):e2436. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih IeM, Zhang Y, Wood W 3rd, Becker KG, Morin PJ.

For further information

The online IPA Learning Center is a great resource for IPA basic and advanced training courses, as well as participating in live scientific webinars. In addition, the Ingenuity website www.ingenuity.com provides a wealth of information on how IPA has been adopted broadly in the life sciences community. Find out how IPA has been used in your area of research by browsing our Science Spotlight Archive, or by searching our online library, where IPA has been referenced in approximately 5,000 scientific journals (through August 2011).

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