What is so special with Systems Biology?
What it delivers?
Examples from cancer studies

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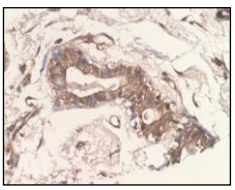
Systems Biology course, KI
13.06.2008

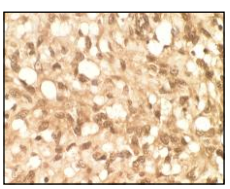
Overview

- Cancer is a systemic disease

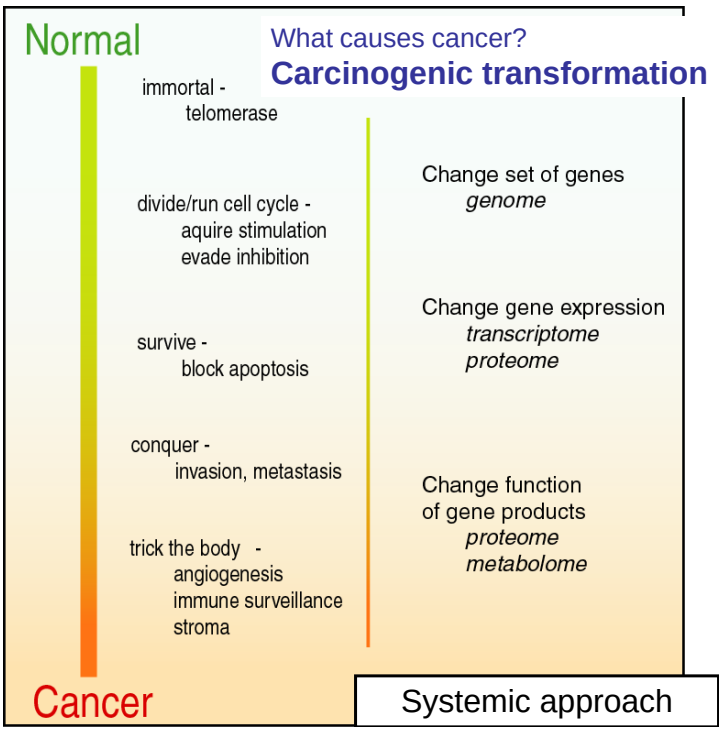
-Systemic analysis tools
- Data presentation
- Clustering
- Analysis of dependencies
- Modeling
- Interrogation

-Examples of systemic analysis
- Carcinogenesis studies
- Clinical applications



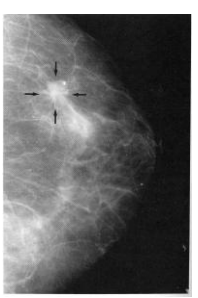


Human breast
Upper: non-cancerous
Lower: inf. duct.carcinoma



Breast cancer

Europe
detected cases - 350.000/year*
deaths - 130.000/year*



*** Find novel mechanisms of tumorigenesis**

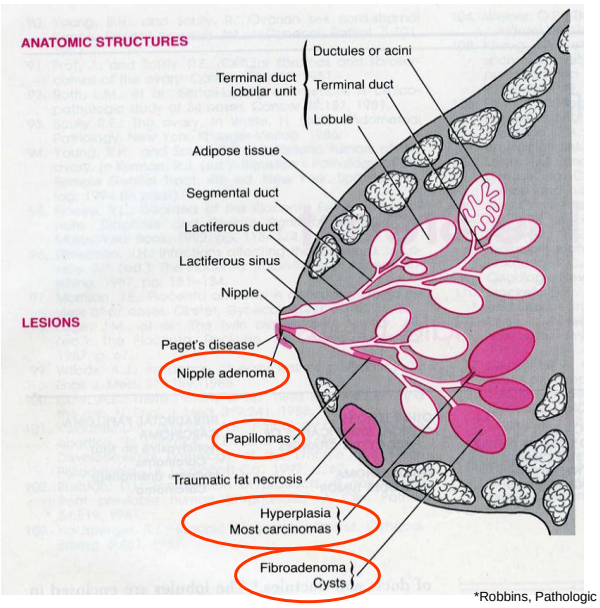
*** Find novel anti-cancer drug targets**

*** Development of "smart" drugs**

*** Find markers of breast cancer**

*estimates for 2000; Tyczynski et al., ENCR CFS, 2, 2002

Human breast and lesions

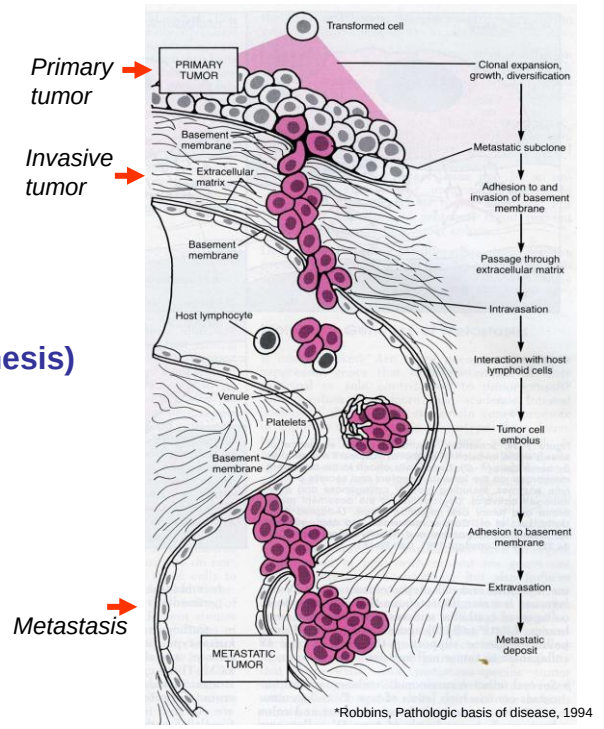


*Robbins, Pathologic basis of disease, 1994

Histopathological type of invasive breast carcinoma	Frequency
Invasive ductal carcinoma, not otherwise specified	50–80%
Invasive lobular carcinoma	5–15%
Mixed type, lobular and ductal features	4–5%
Tubular/invasive cribriform carcinoma	1–6%
Mucinous carcinoma	<5%
Medullary carcinoma	1–7%
Invasive papillary carcinoma	<1–2%
Invasive micropapillary carcinoma	<3%
Metaplastic carcinoma	<5%
Adenoid cystic carcinoma	0.1%
Invasive aprocrine carcinoma	0.3–4%
Neuroendocrine carcinoma	2–5%
Secretory carcinoma	0.01–0.15%
Lipid-rich carcinoma	<1–6%
Acinic-cell carcinoma	7 cases
Glycogen-rich, clear-cell carcinoma	1–3%
Sebaceaous carcinoma	4 cases

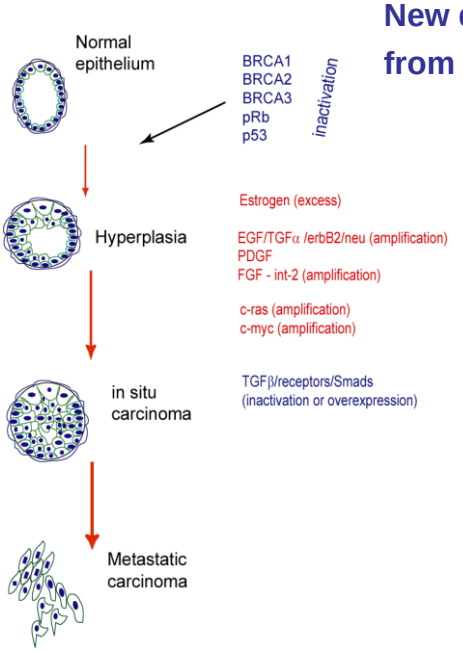
*Weigelt et al., Nature Rev. Cancer, 2005

Breast cancer treatments (stages of tumorigenesis)

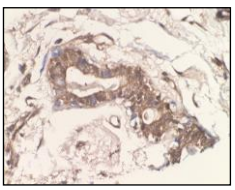


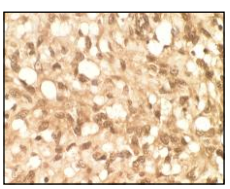
*Robbins, Pathologic basis of disease, 1994

New drugs and markers come from in-depth and omics studies



Markers		Drugs
BRCA1	CA125	Tamoxifen
BRCA2	CA12-3	Raloxifen
ER/PgR	BR 27.29	Fulverstrant
p53	cSHMT	Herceptin
uPA	Tbx3	Trastuzumab
PAI-1	utrophin	ZD1839
ErbB2/neu		*examples
CEA		
Detection		**+ Treatments**
Mammography		Surgery Radiotherapy Chemotherapy





Human breast
Upper: non-cancerous
Lower: inf. duct.carcinoma

Normal

What causes cancer?

Carcinogenic transformation

immortal -
telomerase

divide/run cell cycle -
aquire stimulation
evade inhibition

survive -
block apoptosis

conquer -
invasion, metastasis

trick the body -
angiogenesis
immune surveillance
stroma

Change set of genes
genome

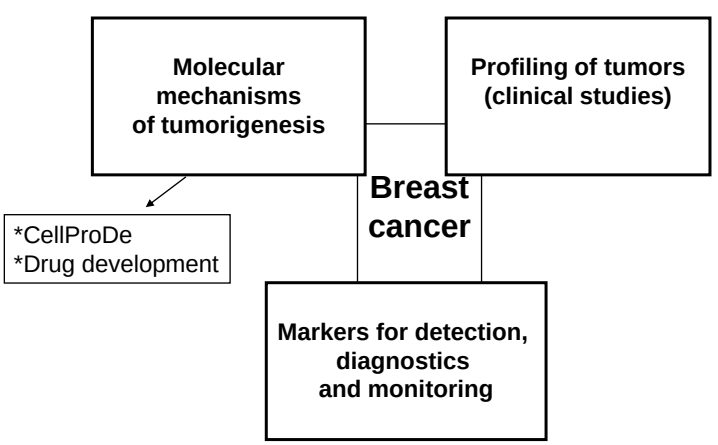
Change gene expression
transcriptome
proteome

Change function
of gene products
proteome
metabolome

Cancer

Systemic approach

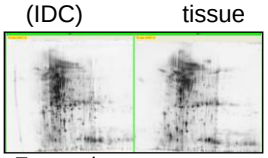
Deliverables of Proteomics



Ways to study molecular mechanisms of breast cancer

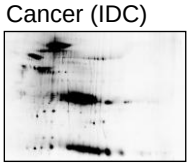
Clinical samples
tissues, tumors, blood/plasma

Cancer (IDC) ”normal” tissue



Expression

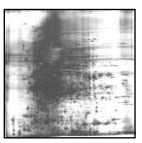
Cancer (IDC)



Phosphoproteins

Zakharchenko et al, 2007, Lomnytska et al., 2007

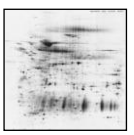
Human primary cells
Immortalization-specific proteins have been identified
Jia et al., 2007



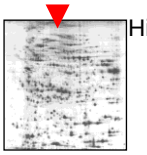
Animal models
Xenografts, Proteomics of cells and tumors formed in animals

Sjöblom, Yakymovych et al.,2004, Cunha et al., 2008

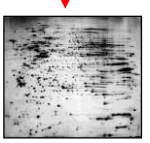
Cultured cells
Expression and PTMs



Normal (184A, HMEC)



High proliferation dysplasia; (MCF10A)



Tumorigenic (MCF7)

Metastatic

Woksepp, Bhaskaran, Jia, Lin, Yakmovych, Zakharchenko, Stasyk, Dubrovska,

What proteomics generates?

type of data and informational value

LIST OF PROTEINS

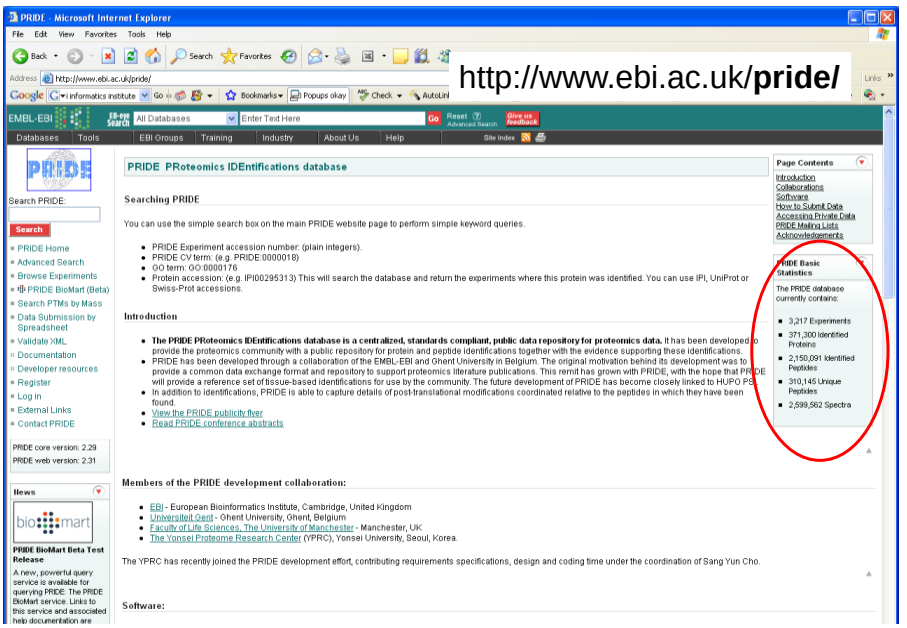
Comprehensive *vs* selected

All proteins
(e.g. total extraction)

Enrichment/purification

- by PTMs
- by PTMs chemistry
- by chemical modifications (peptide backbone, e.g. ICAT)
- by activity (ABPP)
- by purification (PTMs and complexes)

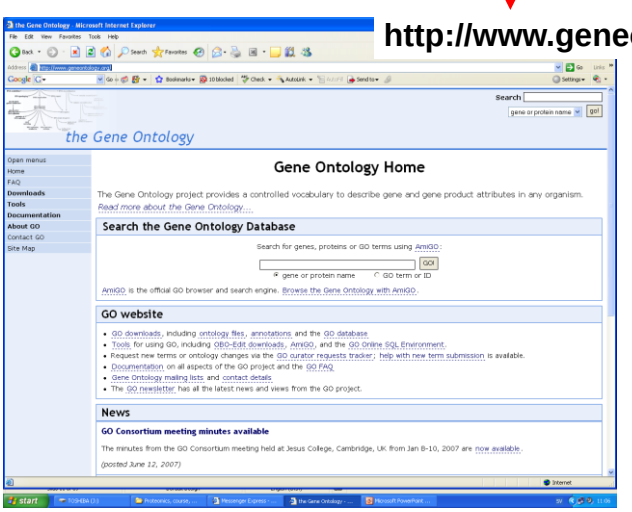
Deposition of information



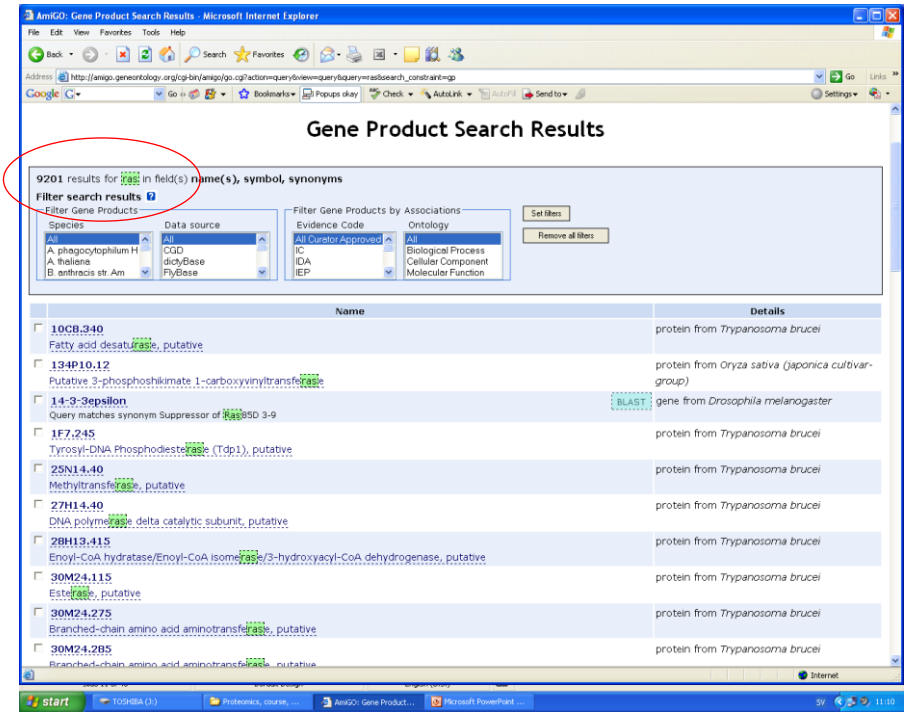
http://www.ebi.ac.uk/**pride/**

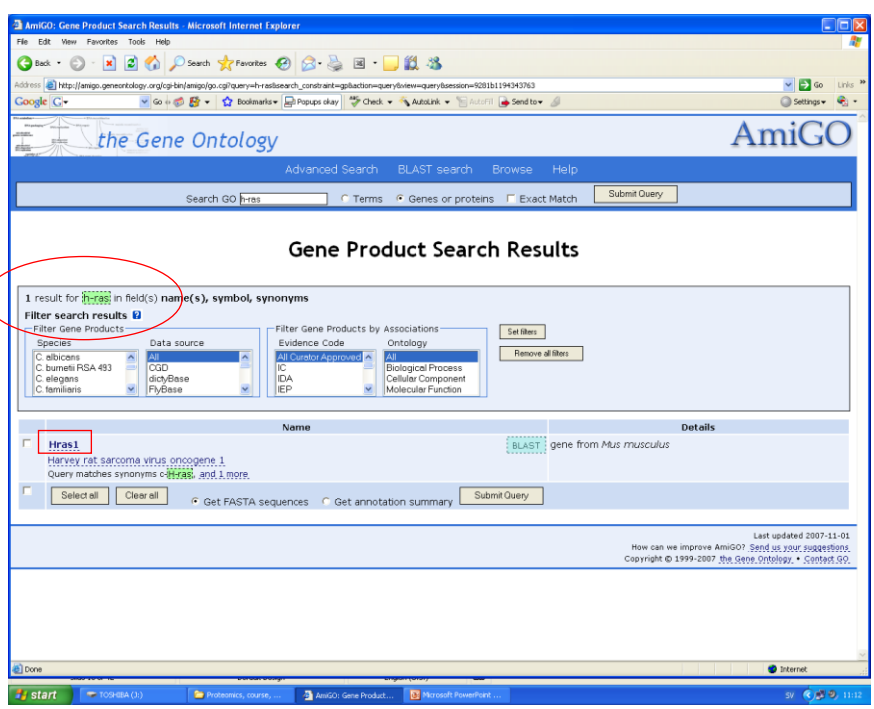
Clustering:

Functional ***/alphabet/***



http://www.geneontology.org/


AmiGO: Gene Product Search Results - Microsoft Internet Explorer
Gene Product Search Results
9201 results for ras in field(s) name(s), symbol, synonyms
Filter search results
Filter Gene Products
Species
Data source
Filter Gene Products by Associations
Evidence Code
Ontology
Set filters
Remove all filters
Name
Details
10C8.340
Fatty acid desaturase, putative
protein from Trypanosoma brucei
134P10.12
Putative 3-phosphoshikimate 1-carboxyvinyltransferase
protein from Oryza sativa (japonica cultivar-group)
14-3-3epsilon
Query matches synonym Suppressor of Ras85D 3-9
BLAST
gene from Drosophila melanogaster
1E7.245
Tyrosyl-DNA Phosphodiesterase (Tdp1), putative
protein from Trypanosoma brucei
25N14.40
Methyltransferase, putative
protein from Trypanosoma brucei
27H14.40
DNA polymerase delta catalytic subunit, putative
protein from Trypanosoma brucei
28H13.415
Enoyl-CoA hydratase/Enoyl-CoA isomerase/3-hydroxyacyl-CoA dehydrogenase, putative
protein from Trypanosoma brucei
30M24.115
Esterase, putative
protein from Trypanosoma brucei
30M24.275
Branched-chain amino acid aminotransferase, putative
protein from Trypanosoma brucei
30M24.285
protein from Trypanosoma brucei


AmiGO: Gene Product Search Results - Microsoft Internet Explorer
the Gene Ontology
AmiGO
Advanced Search
BLAST search
Browse
Help
Search GO h-ras
Terms
Genes or proteins
Exact Match
Submit Query
Gene Product Search Results
1 result for h-ras in field(s) name(s), symbol, synonyms
Filter search results
Filter Gene Products
Species
Data source
Filter Gene Products by Associations
Evidence Code
Ontology
Set filters
Remove all filters
Name
Details
Hras1
Harvey rat sarcoma virus oncogene 1
Query matches synonyms c-H-ras1, and 1 more.
BLAST
gene from Mus musculus
Select all
Clear all
Get FASTA sequences
Get annotation summary
Submit Query
Last updated 2007-11-01
How can we improve AmiGO? Send us your suggestions.
Copyright © 1999-2007 the Gene Ontology. • Contact GO

17-beta-hsd 1
hsd17b1
edh17b2
MGC2803
cpn10
hspe1
hsp10
krt10
hmg-14
hmg-17
hmgn1
anxa1
lpc1
lmna
lmn1
emd2
fpld
cmd1a
hgps
lgmd1b
krt10
krt10
tacstd1
trop1
m4s1
mic18
ly74
mnda

Proteins
Presented in the ”right” GO alphabet

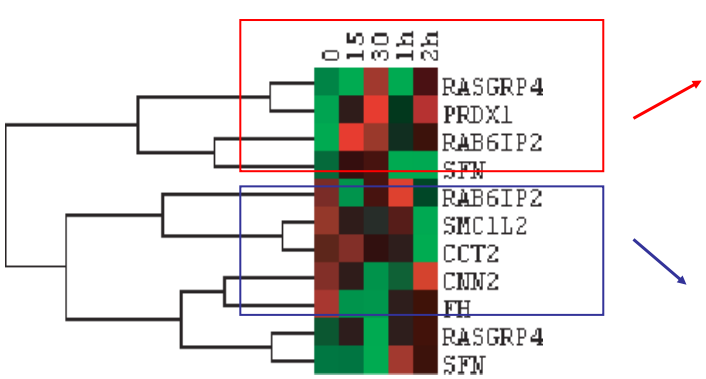
.... But it is based on genes.

Protein Ontology is under construction

http://www.ebi.ac.uk/

Clustering:

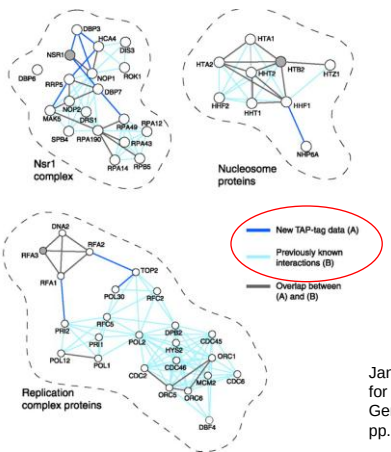
Time points



Clustering:

Structural Groups

organelles



Jansen et al. (2003) A Bayesian Networks Approach for Predicting Protein-Protein Interactions from Genomic Data. Science, Vol. 302. no. 5644, pp. 449 - 453

Clustering:

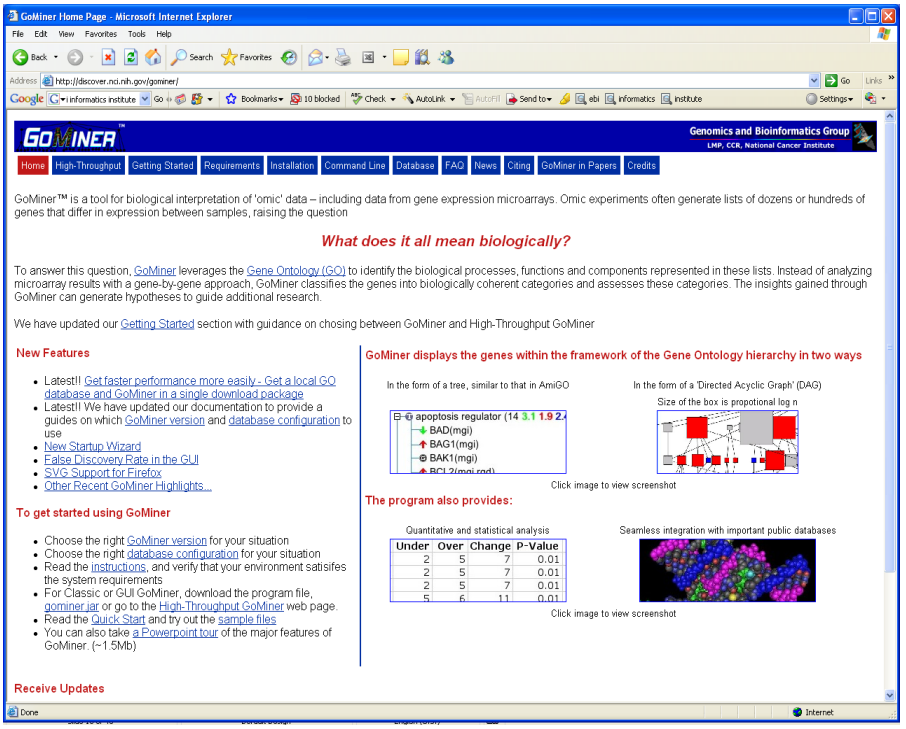
Functional

/sentences/

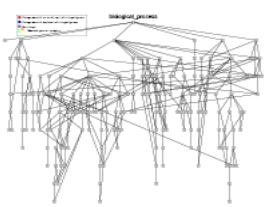
Example:

GoMiner

http://discover.nci.nih.gov/gominer/

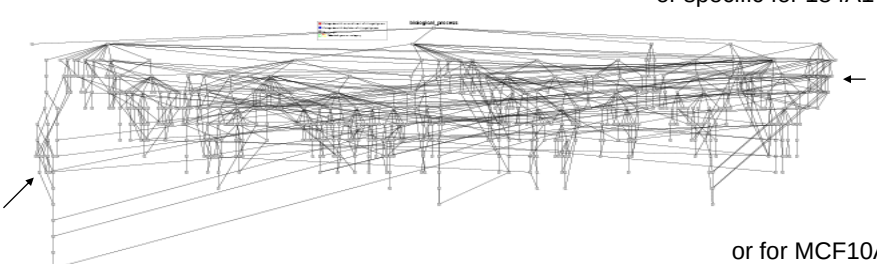


Clustering:

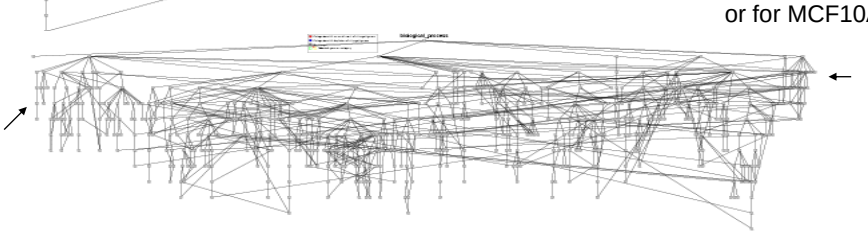


Overview of TGFβ targets common for both cell lines,

or specific for 184A1



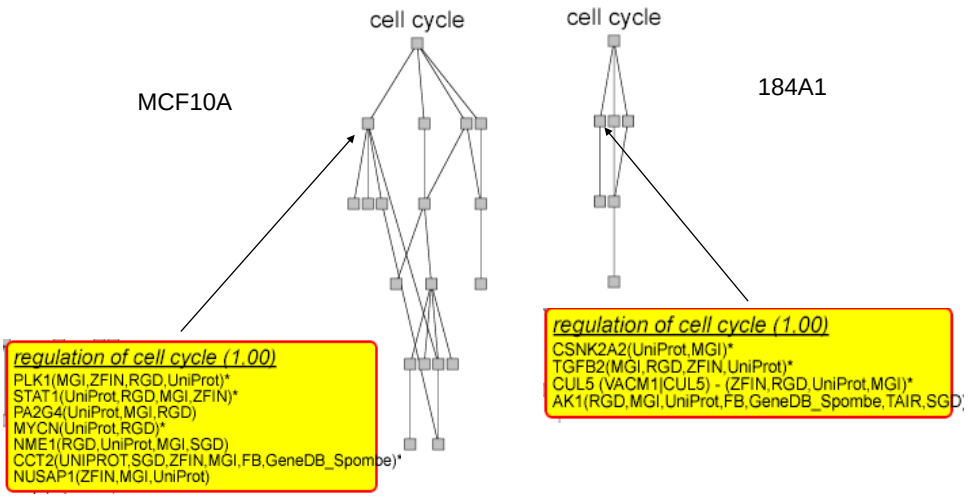
or for MCF10A



*DAGs represent funtional clusters of common and specific proteins

Clustering:

Different pattern of TGFβ-dependent regulation of the cell cycle



**Primary data were generated by Hanna Woksepp (184A1 cells) and Nimesh Bhaskaran (MCF10A cells)

- ”traditional” analysis
- large-scale analysis without analysis of dependencies

- systemic analysis

Systemic analysis is a study of properties which appear as a result of interaction of components

(”it takes two to tango”, or ”cancer is not a gene disease”)

Systemic Analysis

Functional analysis

”Bottom – up”

Results → Process

↓

Explanations

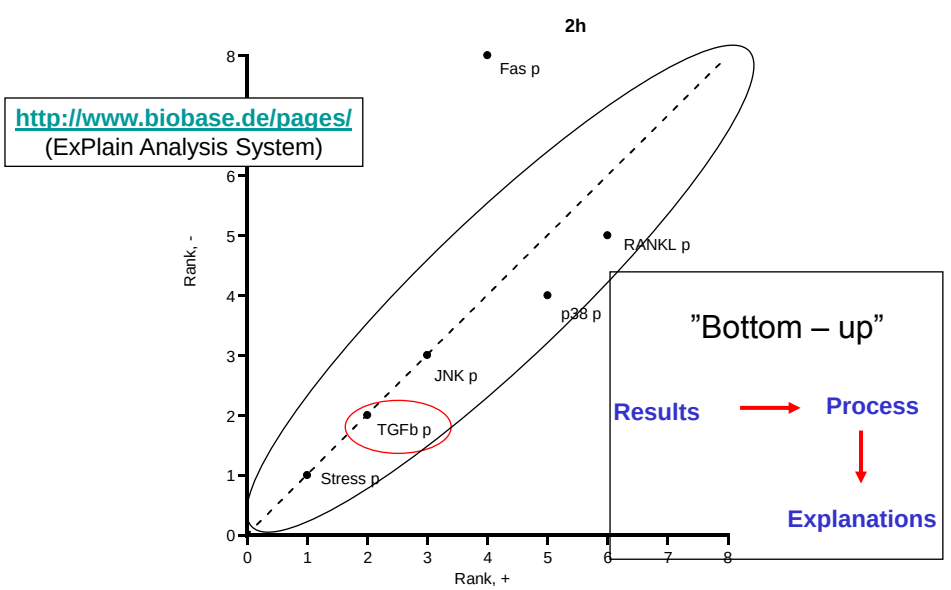
”Top – down”

Process → Results

↓

Explanations

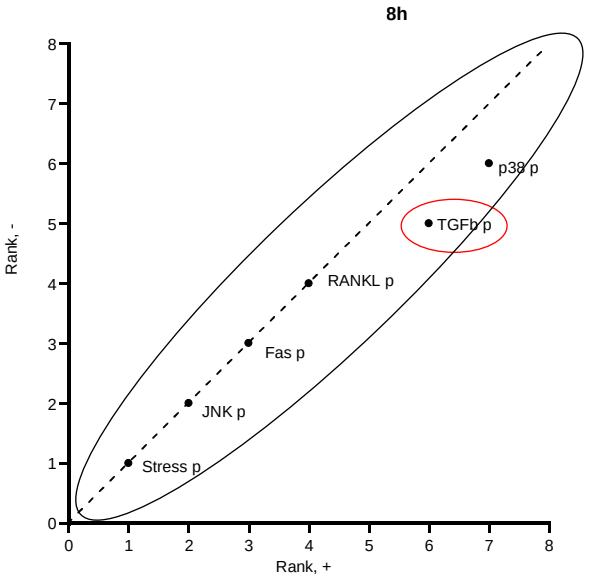
How much of TGFβ signalling is induced by TGFβ?



http://www.biobase.de/pages/
(ExPlain Analysis System)

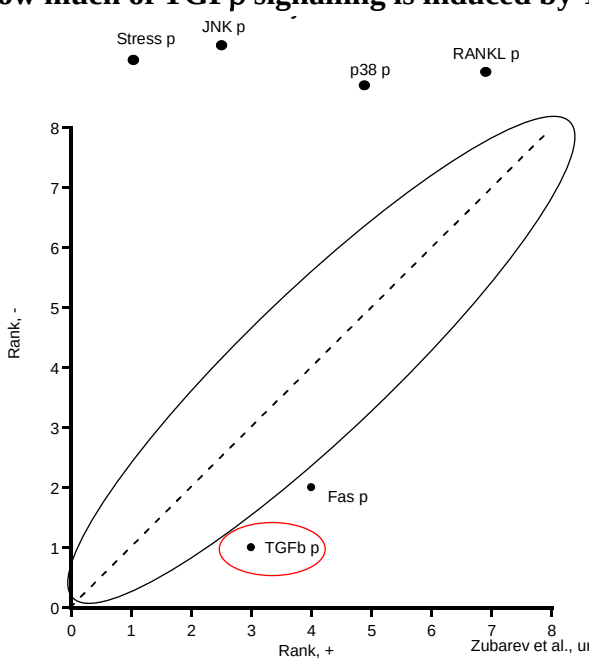
Zubarev et al., unpublished observation

How much of TGFβ signalling is induced by TGFβ?



Zubarev et al., unpublished observation

How much of TGFβ signalling is induced by TGFβ?



Zubarev et al., unpublished observation

Conclusion:

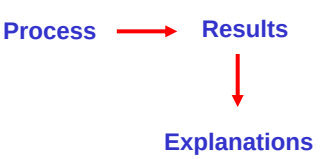
TGFβ regulates targets which are not considered to be TGFβ targets by majority of TGFβ scientists.

Shall TGFβ signalling change its shape?

systemic analysis

Functional analysis

”Top – down”



Example:
Ingenuity Pathway Analysis (http://www.ingenuity.com)

In application to TGFbeta-regulated proteins in human breast epithelial cells

Workflow

Cells,
Treated or not with TGFbeta

2D gels

Image analysis
(statistics)

Identification
(mass spectrometry)

17-beta-hsd 1
hsd17b1
edh17b2
MGC2803
cpn10
hspe1
hsp10
krt10
hmg-14
hmg-17
hmgn1
anxa1
lpc1
lmna
lmn1
emd2
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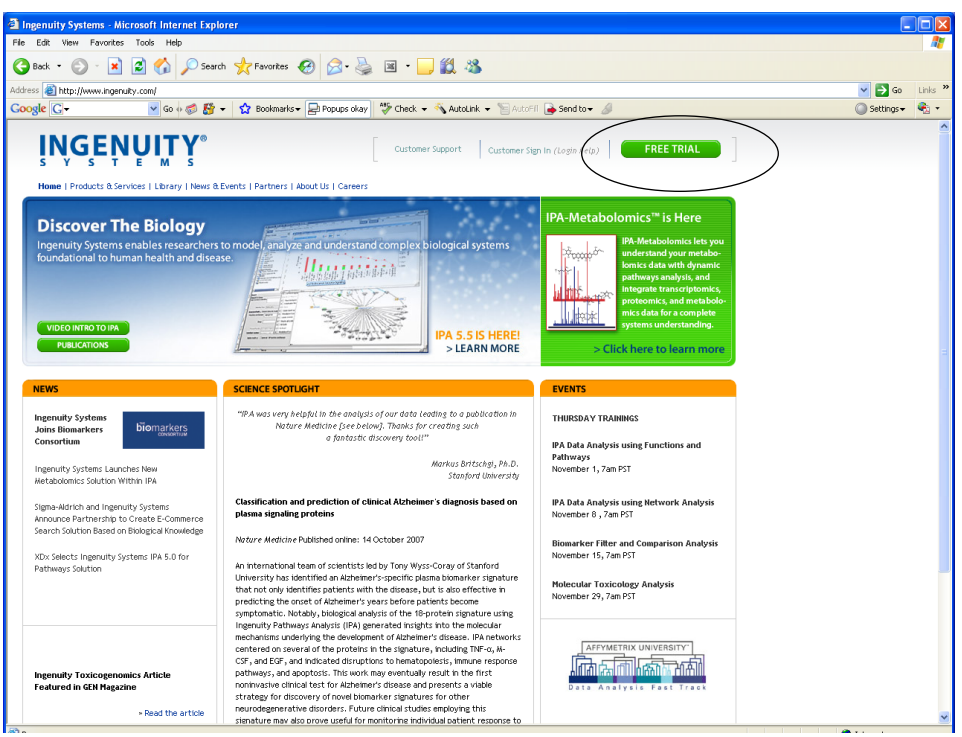
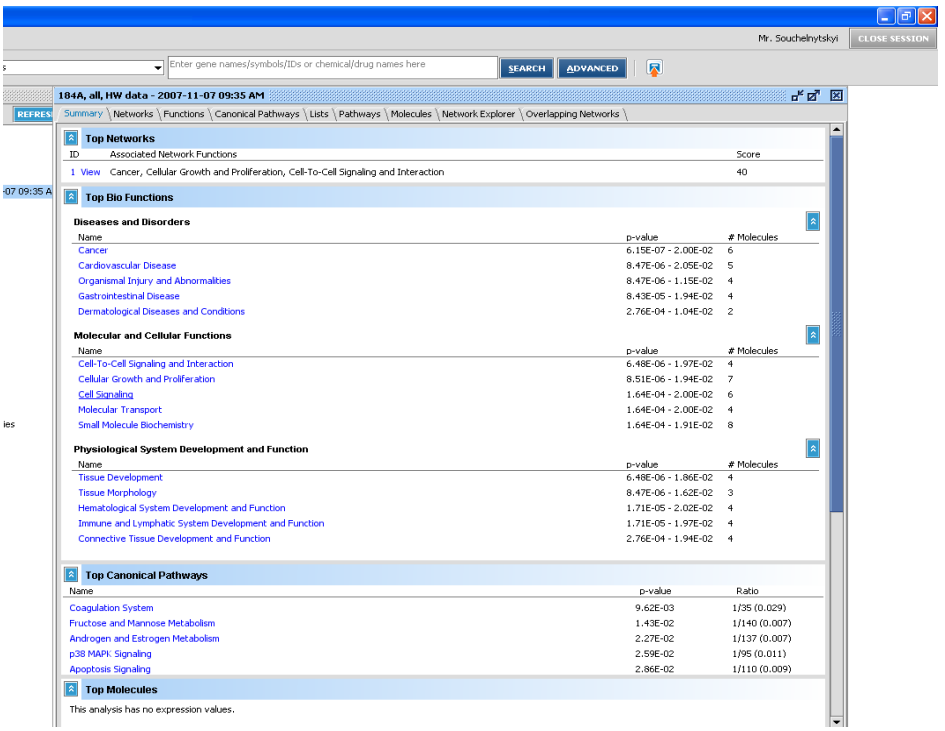
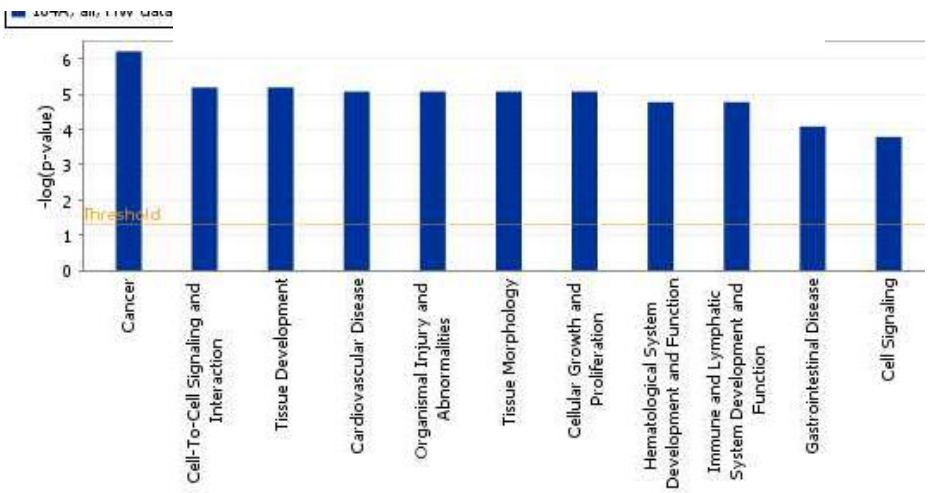
tacstd1
trop1
m4s1
mic18
ly74

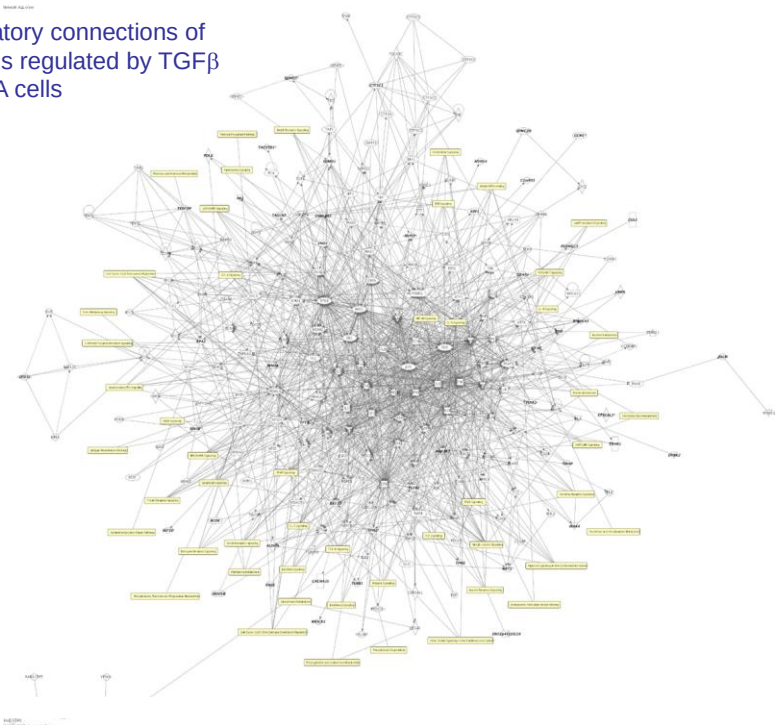




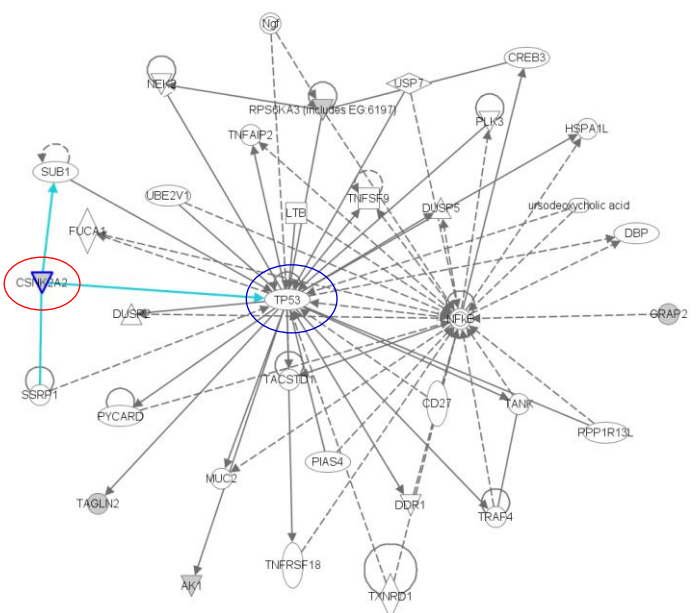
Functions affected by TGFbeta-regulated proteins

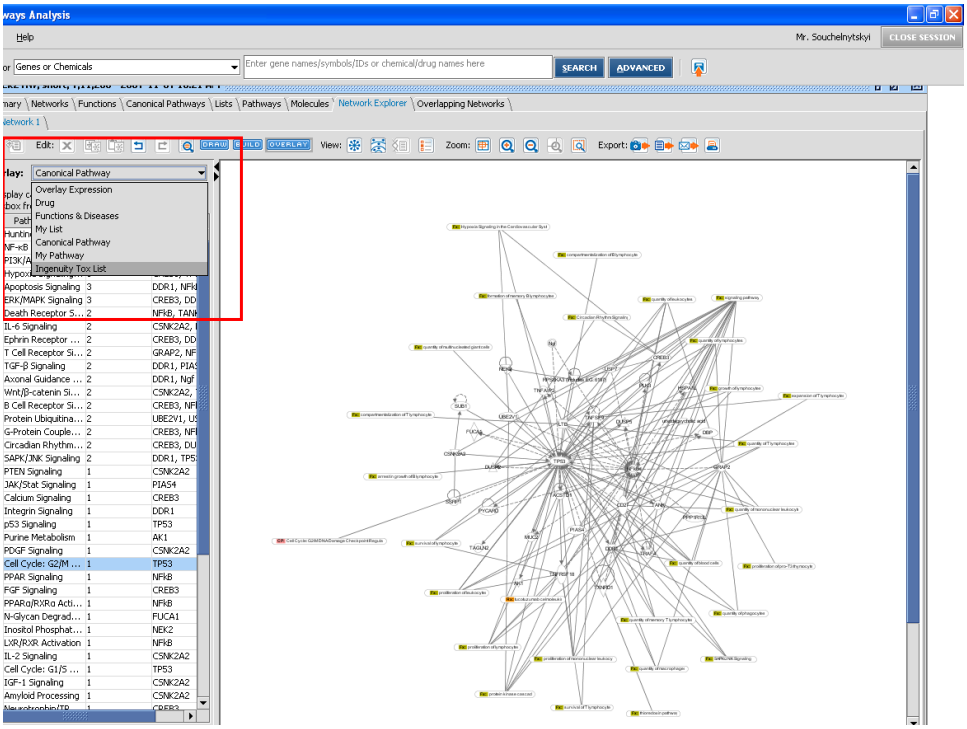


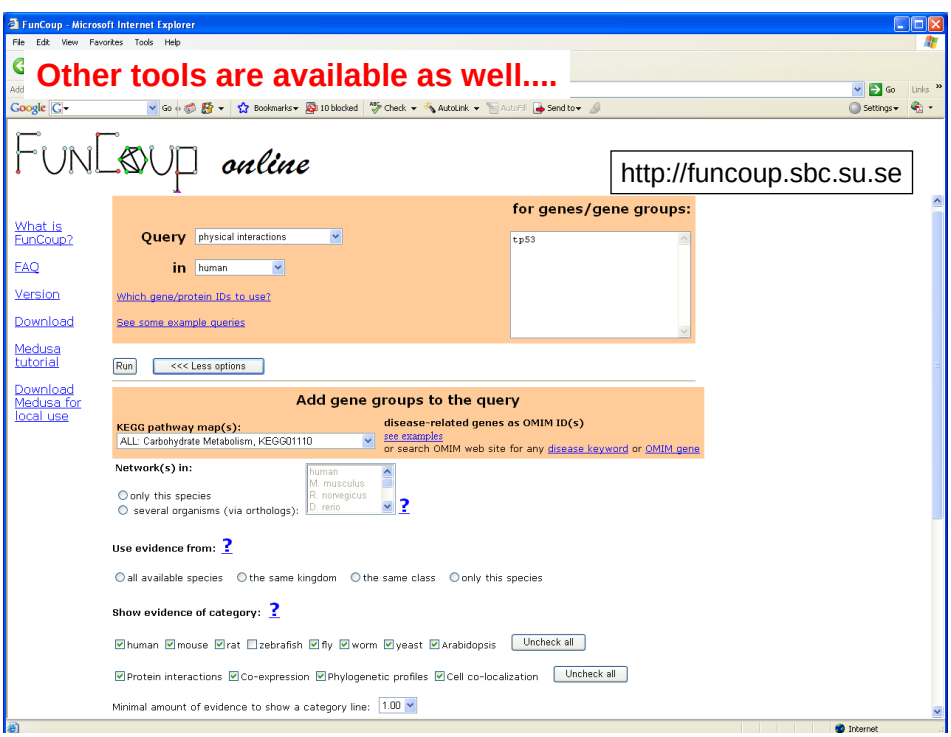
Regulatory connections of proteins regulated by TGFβ in 184A cells



CK2-dependent sub-network




Help
Mr. Souchelnytskyi
CLOSE SESSION
Genes or Chemicals
Enter gene names/symbols/IDs or chemical/drug names here
SEARCH
ADVANCED
Networks
Functions
Canonical Pathways
Lists
Pathways
Molecules
Network Explorer
Overlapping Networks
Edit:
View:
Zoom:
Export:
Canonical Pathway
Overlay Expression
Drug
Functions & Diseases
My List
Canonical Pathway
My Pathway
Ingenuity Tox List
Apoptosis Signaling 3 DDR1, NFkB
ERK/MAPK Signaling 3 CREB3, DD
Death Receptor S... 2 NFkB, TANK
IL-6 Signaling 2 CSNK2A2,
Ephrin Receptor ... 2 CREB3, DD
T Cell Receptor Si... 2 GRAP2, NF
TGF-β Signaling 2 DDR1, PIAS
Axonal Guidance ... 2 DDR1, Ngf
Wnt/β-catenin Si... 2 CSNK2A2,
B Cell Receptor Si... 2 CREB3, NFk
Protein Ubiquitina... 2 UBE2V1, US
G-Protein Couple... 2 CREB3, NFk
Circadian Rhythm... 2 CREB3, DU
SAPK/JNK Signaling 2 DDR1, TP5
PTEN Signaling 1 CSNK2A2
JAK/Stat Signaling 1 PIAS4
Calcium Signaling 1 CREB3
Integrin Signaling 1 DDR1
p53 Signaling 1 TP53
Purine Metabolism 1 AK1
PDGF Signaling 1 CSNK2A2
Cell Cycle: G2/M ... 1 TP53
PPAR Signaling 1 NFkB
FGF Signaling 1 CREB3
PPARg/RXRa Acti... 1 NFkB
N-Glycan Degrad... 1 FUCA1
Inositol Phosphat... 1 NEK2
LXR/RXR Activation 1 NFkB
IL-2 Signaling 1 CSNK2A2
Cell Cycle: G1/S ... 1 TP53
IGF-1 Signaling 1 CSNK2A2
Amyloid Processing 1 CSNK2A2


FunCoup - Microsoft Internet Explorer
Other tools are available as well....
FUNCOUP online
http://funcoup.sbc.su.se
What is FunCoup?
FAQ
Version
Download
Medusa tutorial
Download Medusa for local use
Query
physical interactions
in
human
Which gene/protein IDs to use?
See some example queries
for genes/gene groups:
tp53
Run
<<< Less options
Add gene groups to the query
KEGG pathway map(s):
ALL: Carbohydrate Metabolism, KEGG01110
disease-related genes as OMIM ID(s)
see examples
or search OMIM web site for any disease keyword or OMIM gene
Network(s) in:
only this species
several organisms (via orthologs):
human
M. musculus
R. norvegicus
D. rerio
Use evidence from:
all available species
the same kingdom
the same class
only this species
Show evidence of category:
human
mouse
rat
zebrafish
fly
worm
yeast
Arabidopsis
Uncheck all
Protein interactions
Co-expression
Phylogenetic profiles
Cell co-localization
Uncheck all
Minimal amount of evidence to show a category line:
1.00

Simulation

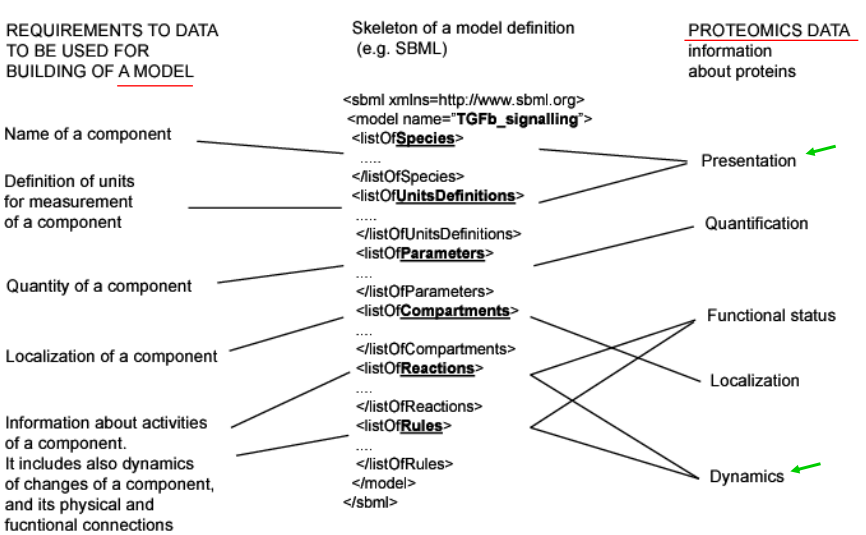
Acquisition of data
Presentation of data
Architecture of dependencies
Description of dependencies
Evaluation of results

Systems Biology or large-scale modeling

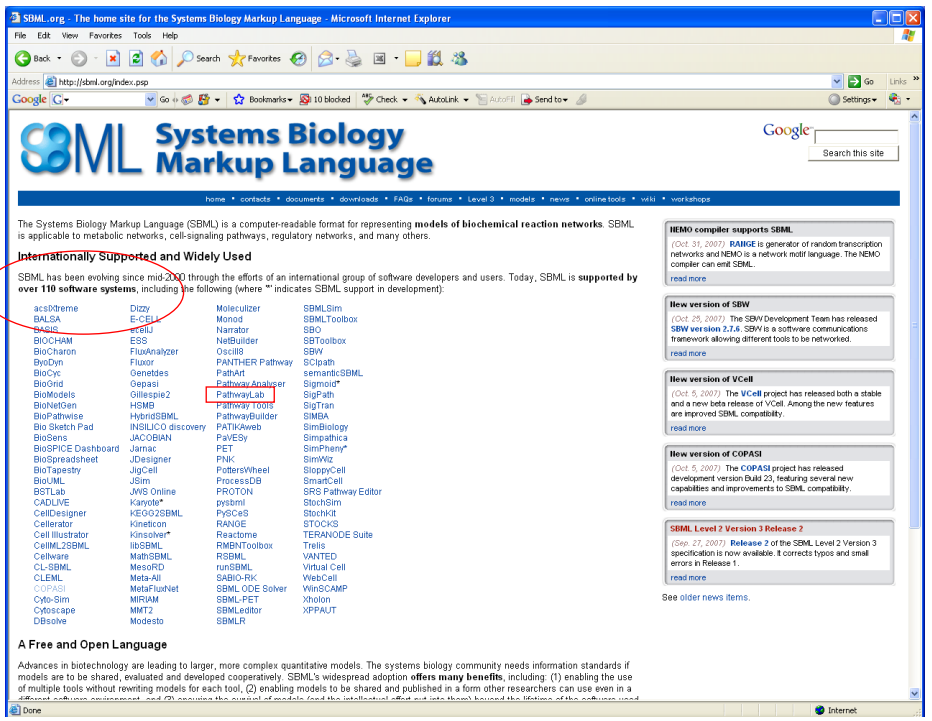
Study of systemic properties of biological systems, i.e. properties which appear as a result of interactions

(Souchelnytskyi, 2005)

Large-scale analysis of proteomics data



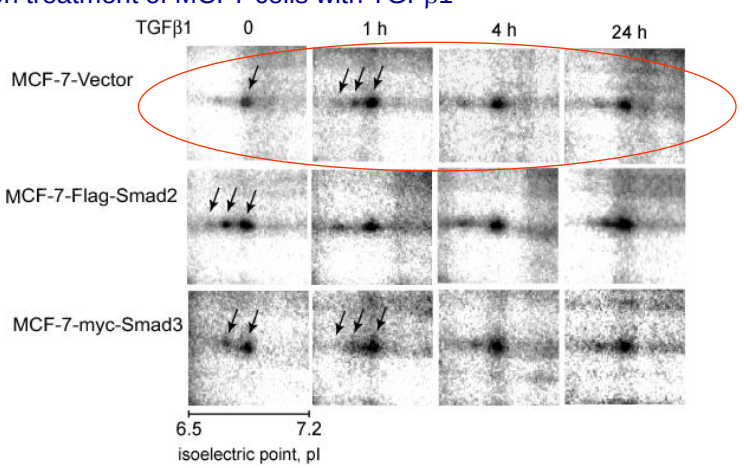
Souchelnytskyi, Proteomics, 2005



Example 1

Simulation

Primary proteomics data: transcription factor TFII-I is phosphorylated upon treatment of MCF7 cells with TGFβ1

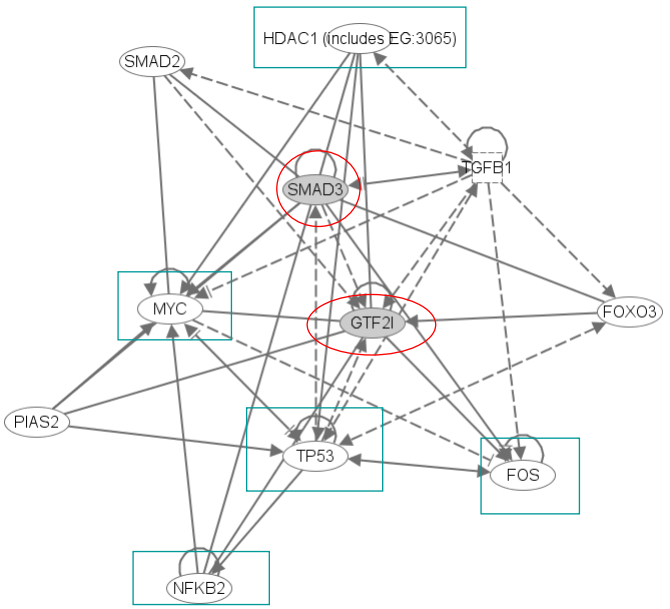


*Areas of images of 2D gels showing TFII-I-containing spot

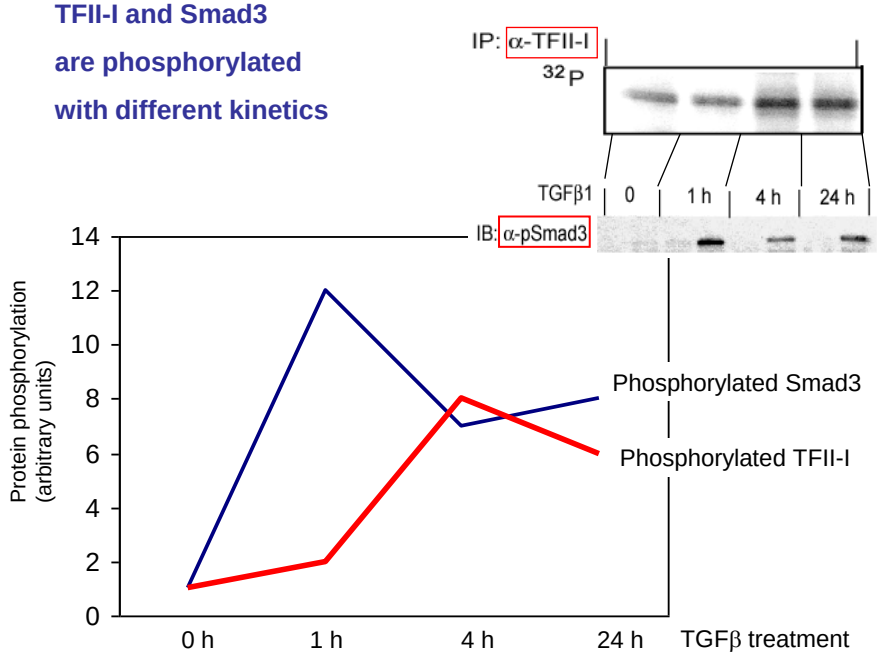
TFII-I interacts with c-myc, NFkB, ATF6, STAT1, HDAC3

Stasyk, Dubrovska, et al, 2005

TFII-I (gtf2i) and Smad3-centered network

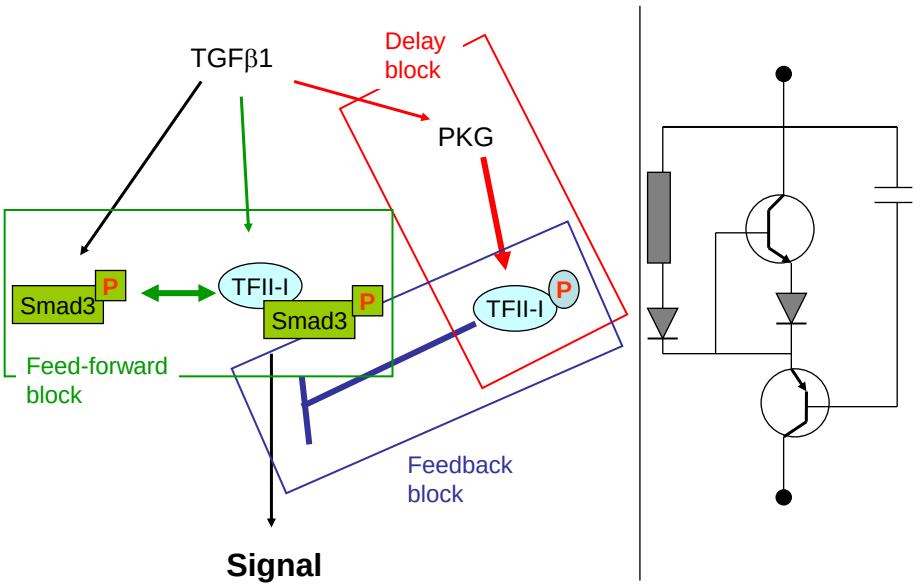


TFII-I and Smad3 are phosphorylated with different kinetics



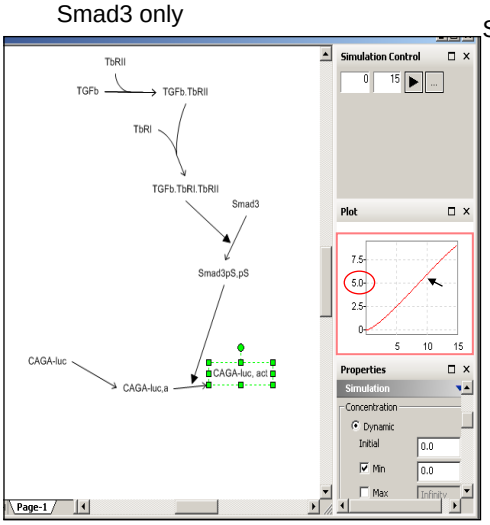
TGFβ-dependent phosphorylation of TFII-I as a negative feed-back mechanism

Feedback, feed-forward and time delay blocks

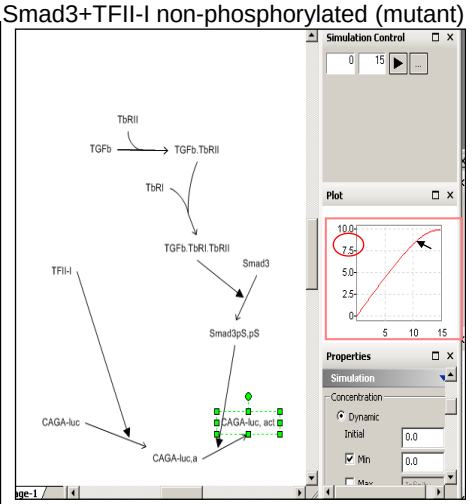


Modeling of signalling to transcriptional regulation by Smad3 and TFII-I

Smad3 only

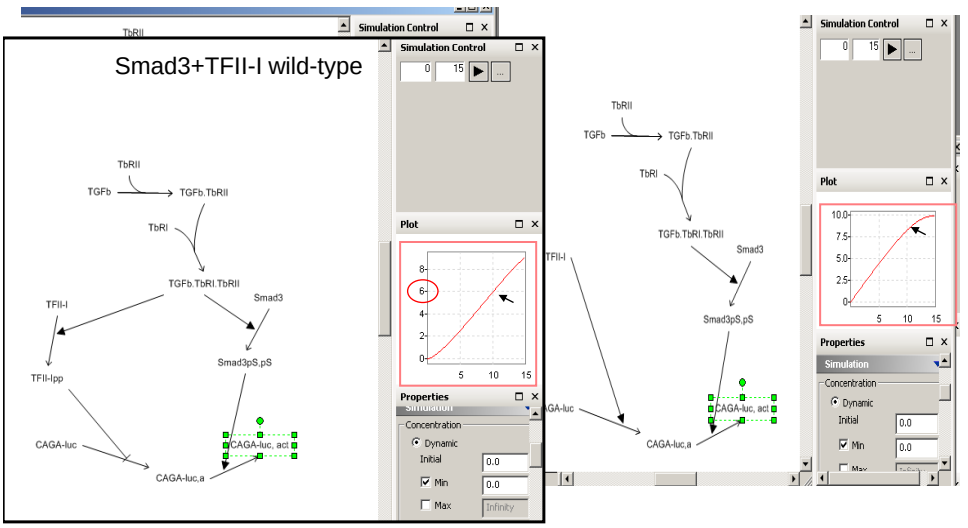


Smad3+TFII-I non-phosphorylated (mutant)

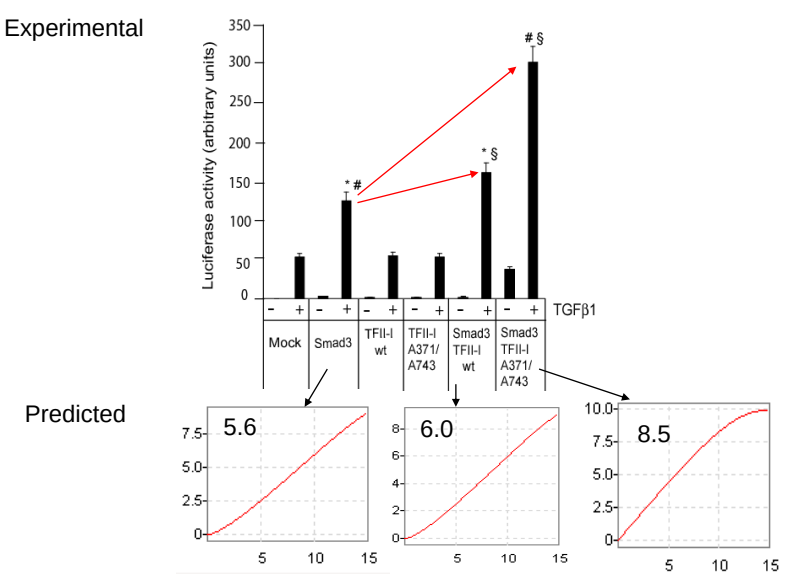


***PathwayLab**

Modeling of signalling
to transcriptional regulation by Smad3 and TFII-I



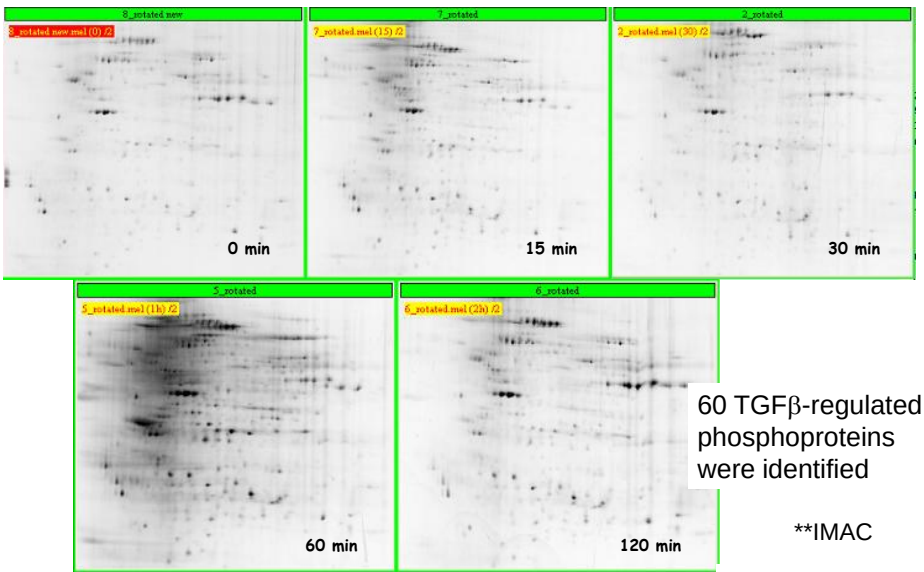
Modeling of signalling
to transcriptional regulation by Smad3 and TFII-I



Example 2

TGFβ1-dependent effect on phosphoproteome

MCF10A human breast epithelial cells

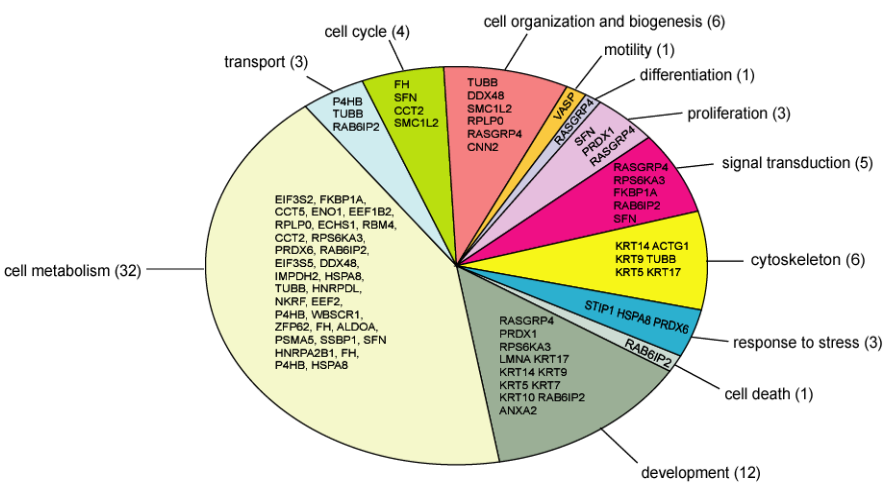


60 TGFβ-regulated phosphoproteins were identified

**IMAC

*Dubrovska et al., 2008

Functional clusters affected by TGFβ
(phosphoproteins)

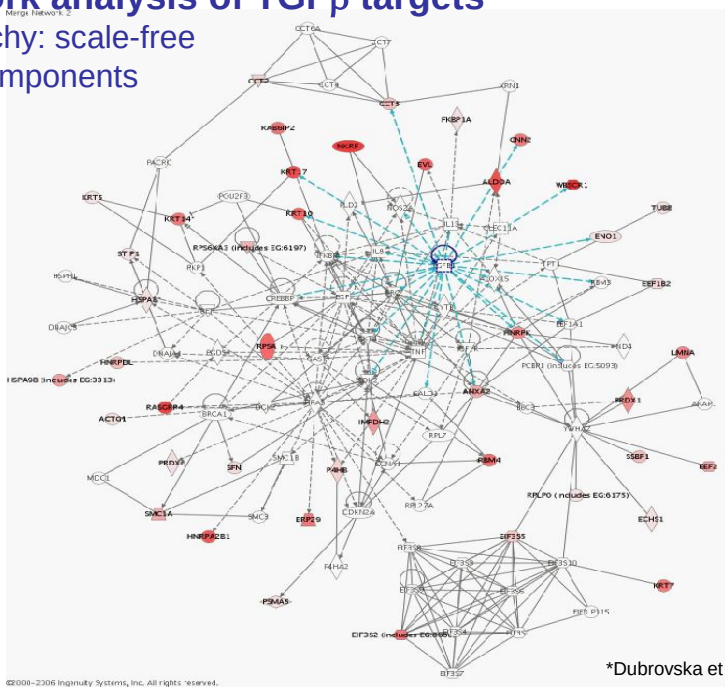


*Dubrovska et al., 2008

Network analysis of TGFβ targets

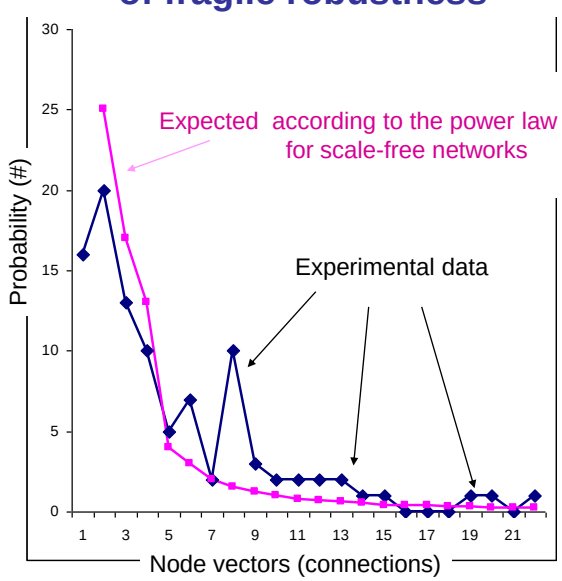
*hierarchy: scale-free

*key components



*Dubrovska et al., 2008

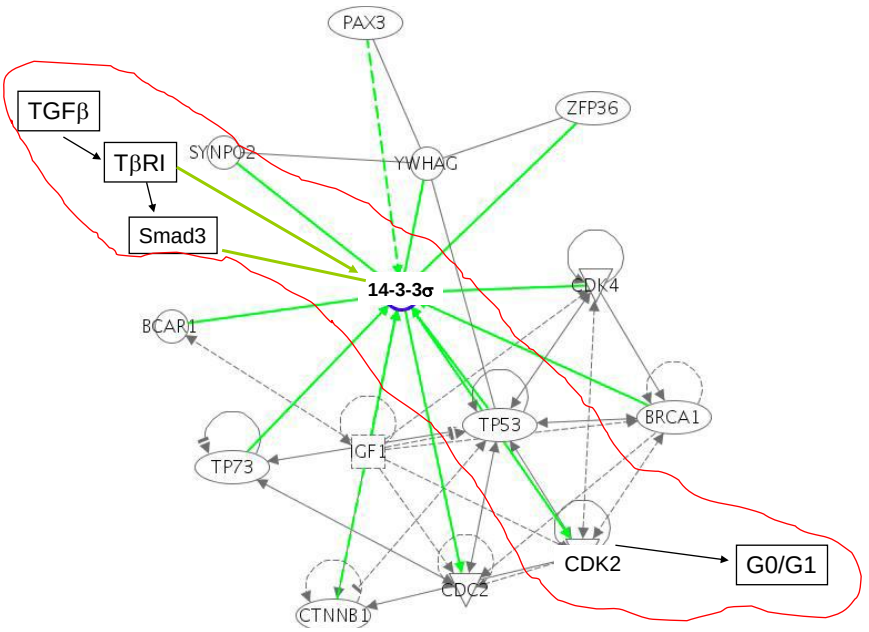
Deviating high-density nodes indicate points of fragile robustness



TGFβ (ligand)
EGF
H-Ras
TNF
AKT
Src
NF-kB
14-3-3σ

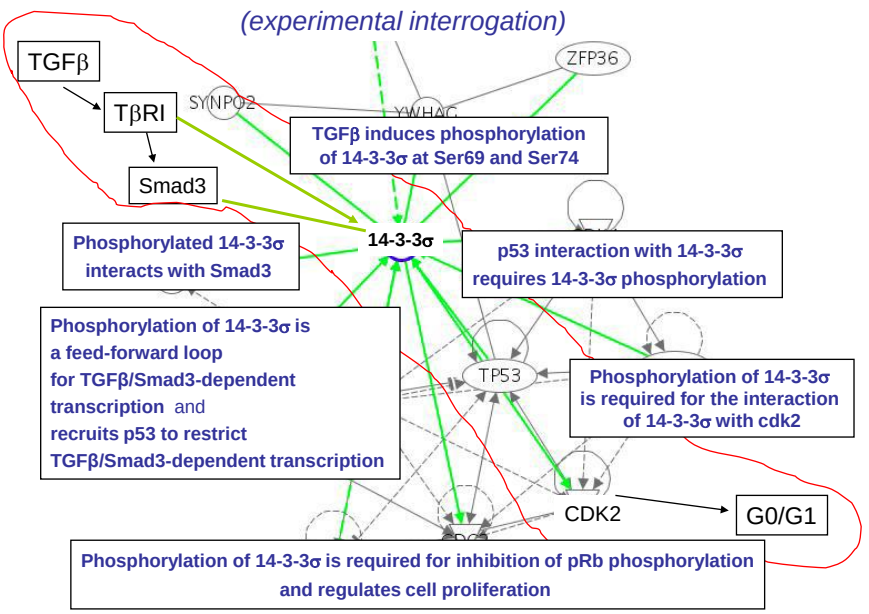
*examples

14-3-3σ –centered sub-network

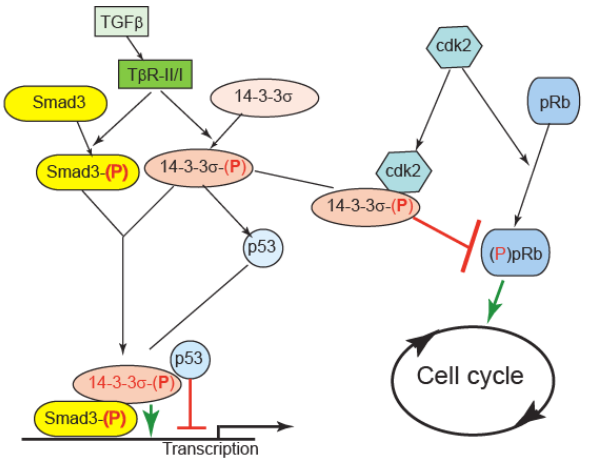


14-3-3σ –centered sub-network

(experimental interrogation)

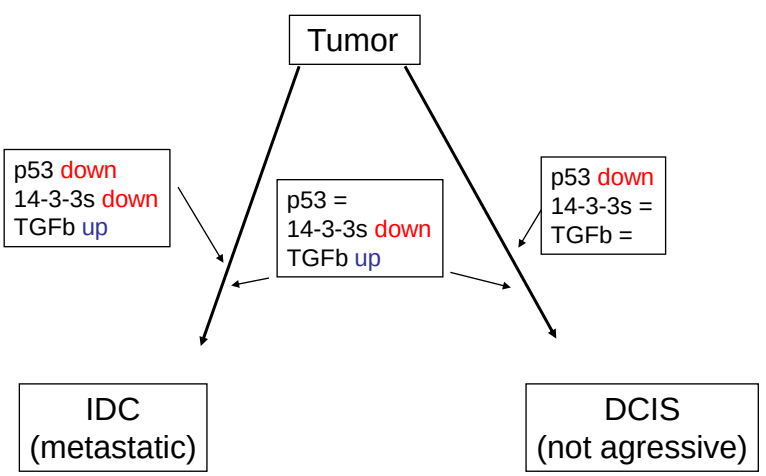


Cooperation between two tumor suppressors



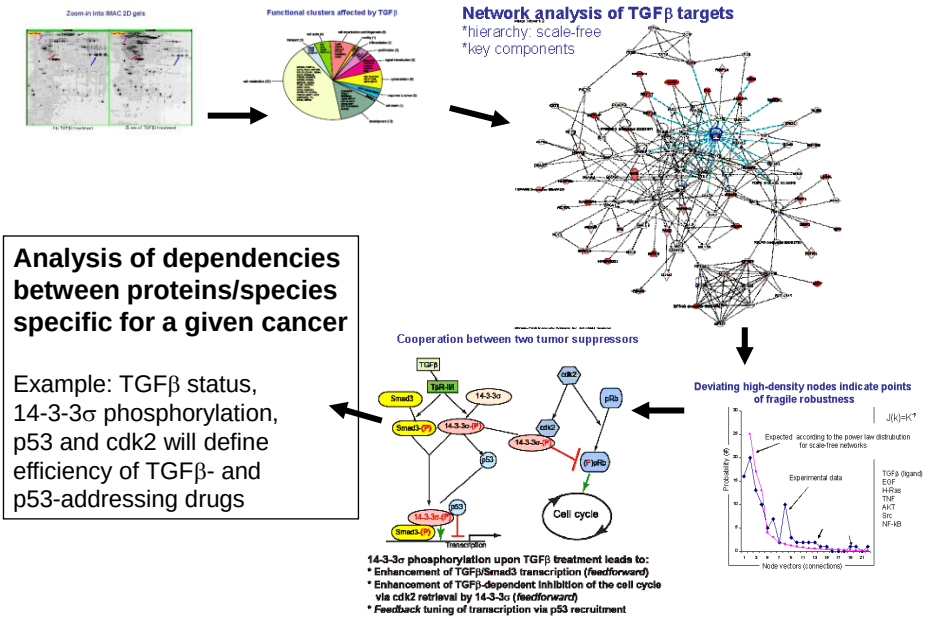
14-3-3σ phosphorylation upon TGFβ treatment leads to:
*** Enhancement of TGFβ/Smad3 transcription (*feedforward*)**
*** Enhancement of TGFβ-dependent inhibition of the cell cycle via cdk2 retrieval by 14-3-3σ (*feedforward*)**
*** *Feedback* tuning of transcription via p53 recruitment**

Status of network components may define probability of agressive tumor development



*examples; other components to be considered are Smad3, Smad2, Smad4, cdk2, pRb, CAGA- and E2F-read-outs.

From 2D gels, via systemic analysis to clinics



Does systemic approach is important for treatment of cancer?

It is in use now: combinations of various schemes of chemotherapy, radiotherapy, hormone-therapy, surgery.

But most often, use of combinatorial treatment is **empiric**

Example: FOAM scheme

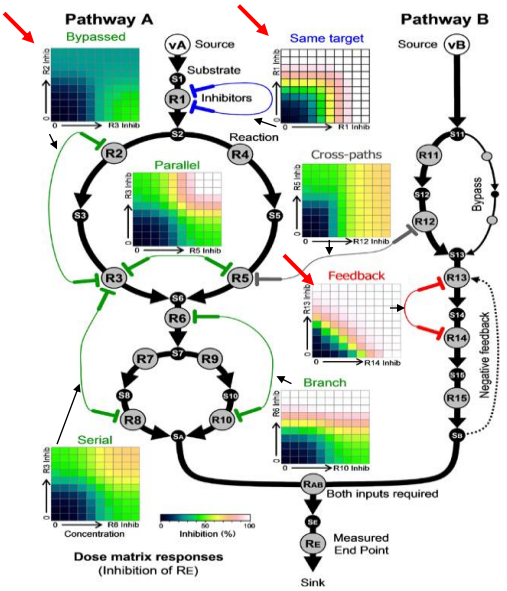
MMC – day 1

Adriamycin – days 1 and 29

5FU – days 1, 8, 29, 36

Vincristine – days 1, 8, 29, 36

Combinatorial treatment as the tool to improve clinical benefit



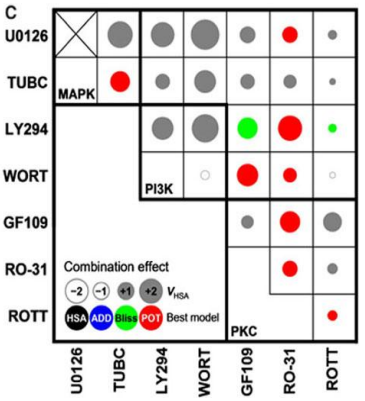


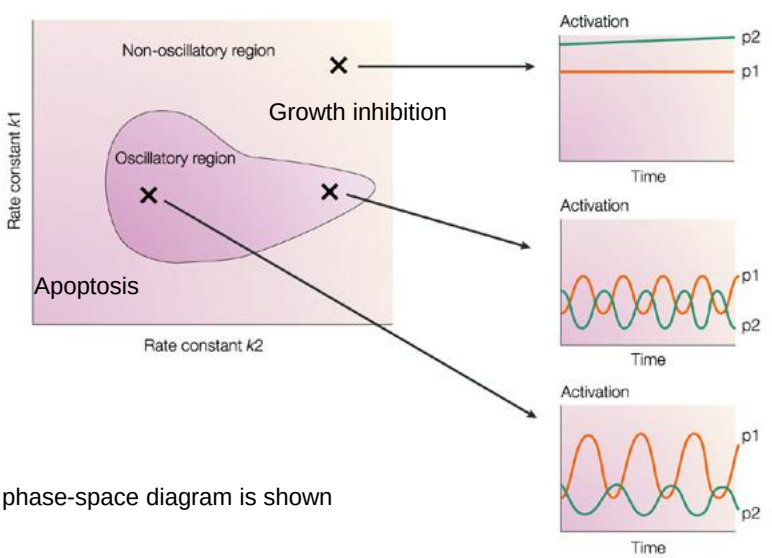
Table II Drugs targeting EGFR signalling used in the HCT116 proliferation experiment

Drug name	Abbrev.	CAS number	Conc. Range (μM)	Target kinase
U-0126	U0126	109511-58-2	0.10–26	MAPK/MEK[a]
5-iodotubercidin	TUBC	24386-93-4	0.04–10	MAPK/ERK[b]
LY 294002	LY294	154447-36-6	0.13–33	PI3-K[c]
Wortmannin	WORT	19545-26-7	0.09–23	PI3-K[d]
GF 109203X	GF109	133052-90-1	0.08–21	PKC[e]
Ro 31-8220	RO-31	138489-18-6	0.04–9.8	PKC[f]
Rottlerin	ROTT	82-08-6	0.08–19	PKC[g]

[a]Favata *et al* (1998).
[b]Fox *et al* (1998).

(Lehar et al., Mol. Sys. Biol., 2007)

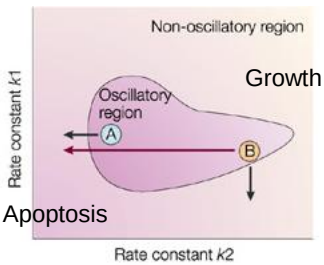
Search for efficient drugs and their combinations



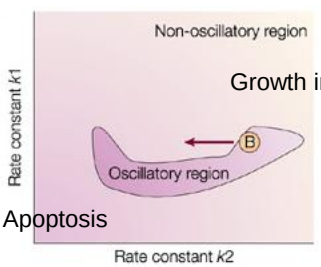
A phase-space diagram is shown

Kitano, Nature Rev. 2004

Search for efficient drugs and their combinations

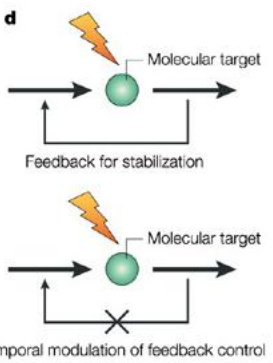


Apoptosis



Apoptosis

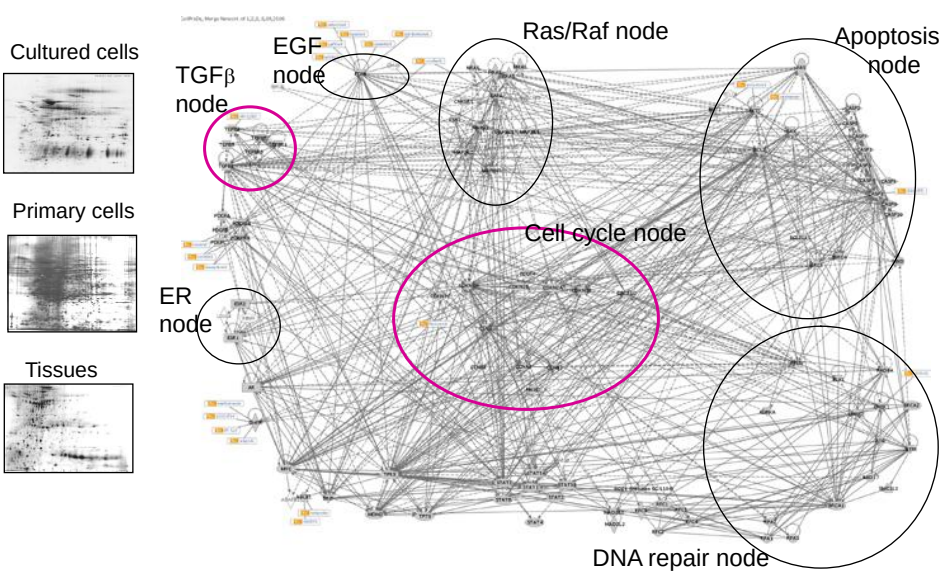
Shifting cell responsiveness
to cell death or to growth inhibition



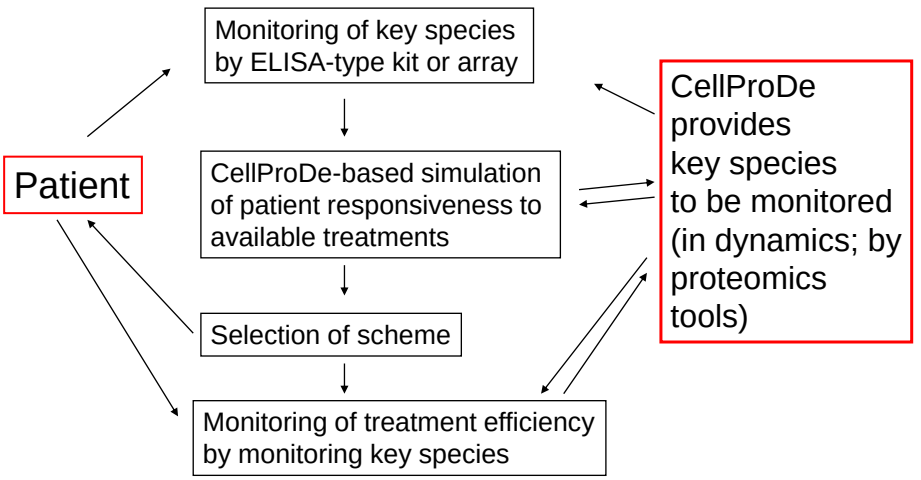
Kitano, Nature Rev. 2004

Cell Proliferation or Death (CellProDe) for individualized treatment and drug discovery

"Big" network with indication of some drugs which affect species of the network



CellProDe for individualized treatment



Summary

- Cancer is a systemic disease, and it requires a systemic approach

-Systemic analysis tools are under development, with significant progress reported for data presentation, clustering, analysis of dependencies, modeling and interrogation

-Examples of systemic analysis have shown that we can analyze systemic properties of relatively simple systems. The challenge is to model complex multi-component systems, e.g. tumor growth, and to find ways to destabilize them, e.g. eliminate cancer.