COMPLEXITY OF MOLECULAR SIGNALING NETWORKS FOR VARIOUS TYPES OF CANCER AND NEUROLOGICAL DISEASES CORRELATES WITH PATIENT SURVIVABILITY

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Objective

- Objective: Correlating molecular network statistics and bioinformatics data with clinical and patient statistics.

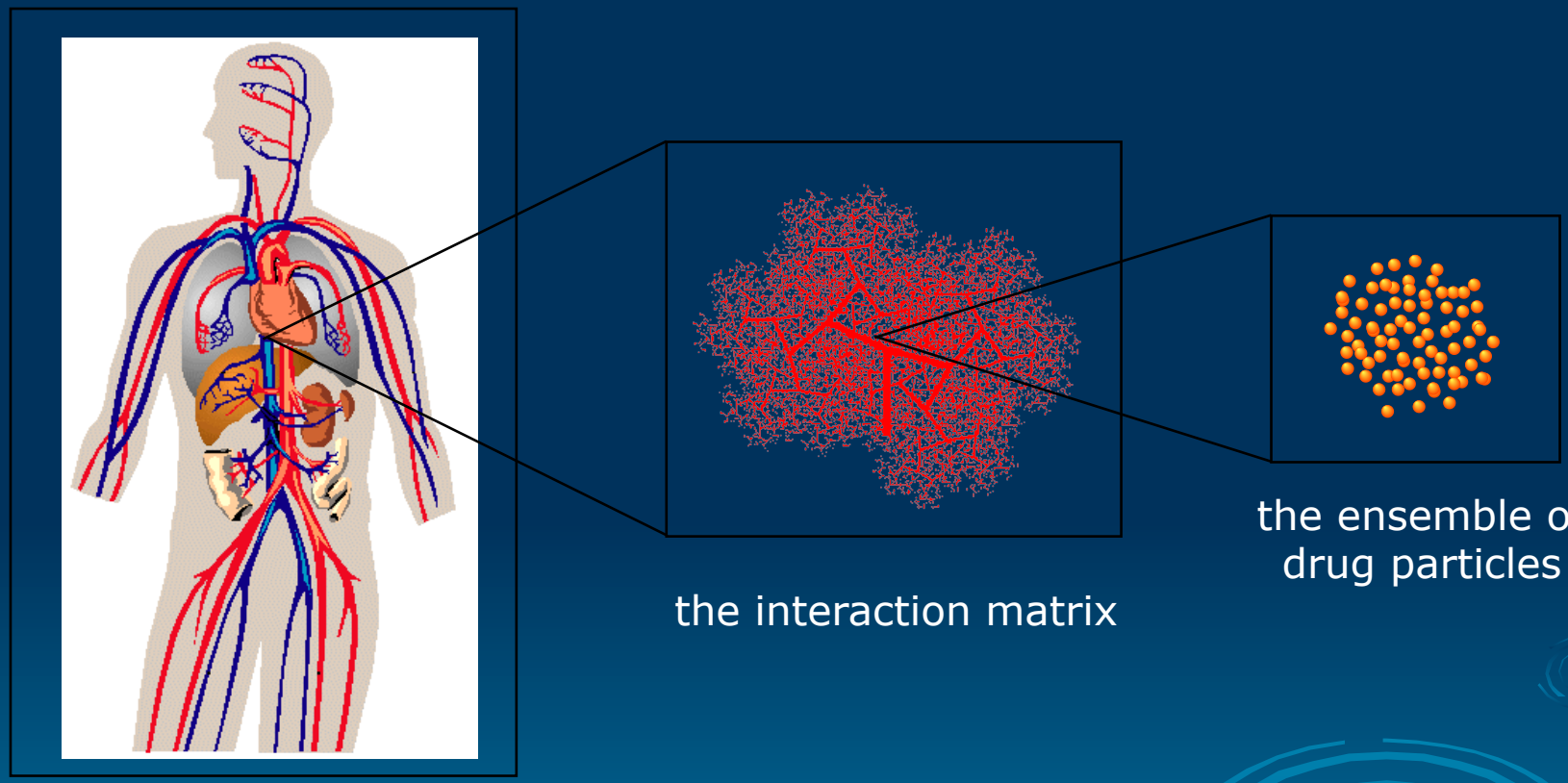
- Signaling Databases
- Patient Databases
- Mappings

D. Breitkreutz, L. Hlatky, E. A. Rietman, and J. A. Tuszynski, Molecular Signaling Network Complexity Is Correlated with Cancer Patient Survivability, **Proceedings of the National Academy of Sciences of the USA,** Jun 5;109(23):9209-12. Epub 2012 (2012)

Introduction

- Overall Goal: To look at cancer and chemotherapy in a different way and to ultimately improve treatment.
- Focus: Investigate a quantitative measure of the robustness of cancer signaling pathways.
- Cell biology normally focuses on individual components and processes.
- Network biology focuses on the interaction of all components of a biological system.
- Chemotherapy traditionally focuses on single targets.
- Cellular processes are more complex than this.
- We want to find a way to evaluate the effectiveness of chemotherapeutics on a network level.

The Pharmacokinetic System



the medium

the interaction matrix

the ensemble of drug particles

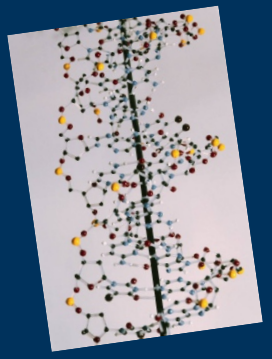
What is Systems Biology?

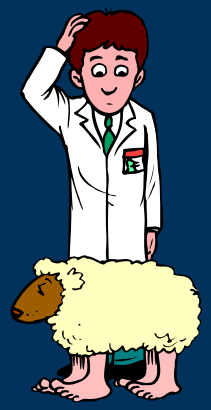
- Systems Biology - *The study of the mechanisms underlying complex biological processes as integrated systems of many interacting components.*

- *Systems biology involves:*
- *(1) collection of large sets of experimental data*
- *(2) proposal of mathematical models that might account for at least*
- *some significant aspects of this data set,*
- *(3) accurate computer solution of the mathematical*
- *equations to obtain numerical predictions, and*
- *(4) assessment of the quality of the model by comparing*
- *numerical simulations with the experimental data.*

Molecular Biology vs. Systems Biology

- In molecular biology, gene structure and function is studied at the molecular level.
- In systems biology, specific interactions of components in the biological system are studied – cells, tissues, organs, and ecological webs.

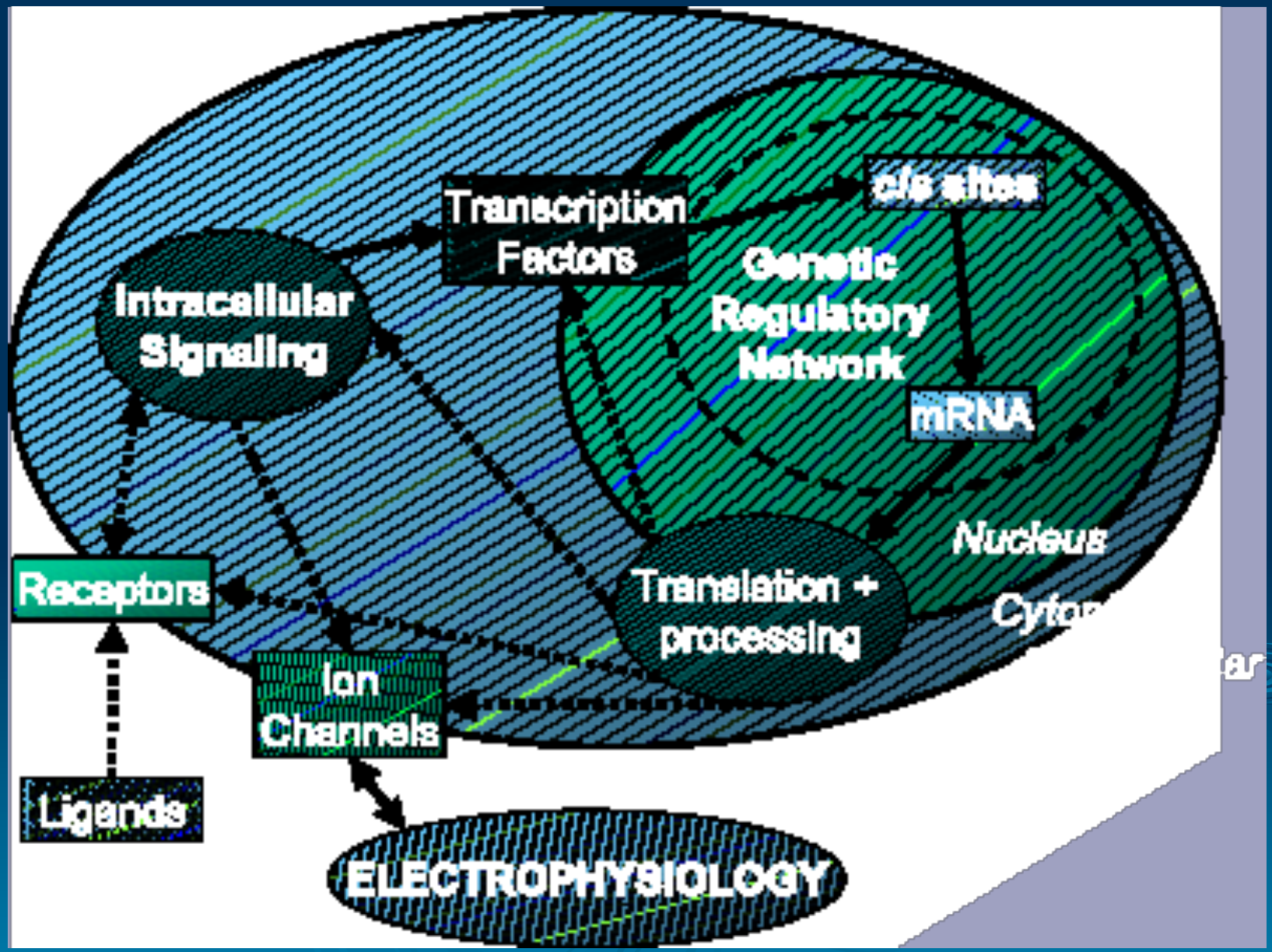


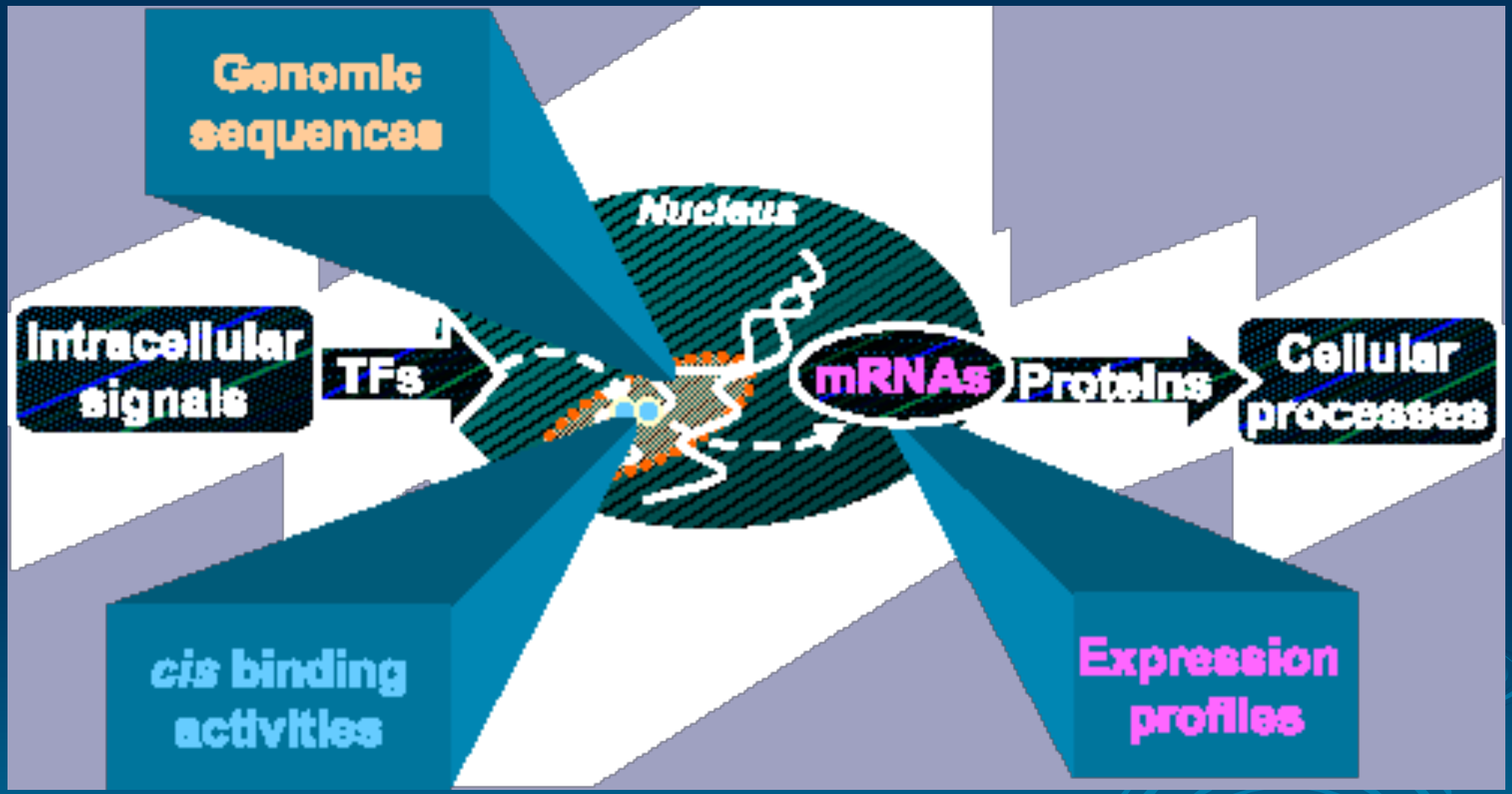


From Systems Biology to Computational Biology

Biological Systems are complex, thus, a combination of experimental and computational approaches are needed.

Linkages need to be made between molecular characteristics and systems biology results


Transcription Factors
Intracellular Signalling
Genetic Regulatory Network
mRNA
Nucleus
Translation + processing
Receptors
Ion Channels
Ligands
ELECTROPHYSIOLOGY


Genomic sequences
Nucleus
Intracellular signals
TFs
mRNAs
Proteins
Cellular processes
cis binding activities
Expression profiles

Biochemical Networks

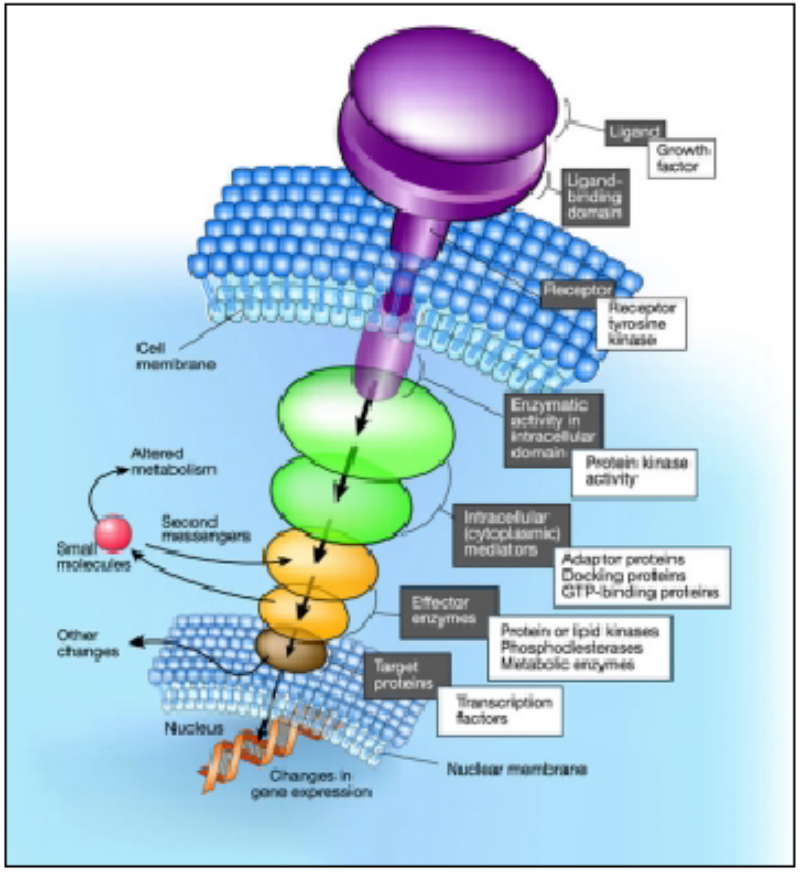
At the level of cells communication is signal transduction

Outside → Insulin

Membrane → Insulin - Receptor

Inside: cytosol → Intracellular signalling pathways: G-proteins, adaptors & kinases

Nucleus → Transcription factors and gene expression



Sources of biological data

- information stored in the genetic code (DNA)
- protein sequences
- 3D structures of biomolecules
- experimental results from various sources
- clinical data
- patient statistics
- scientific literature

SEER database

- Surveillance Epidemiology and End Results (SEER) at NCI
- Cancer patient statistics, among others

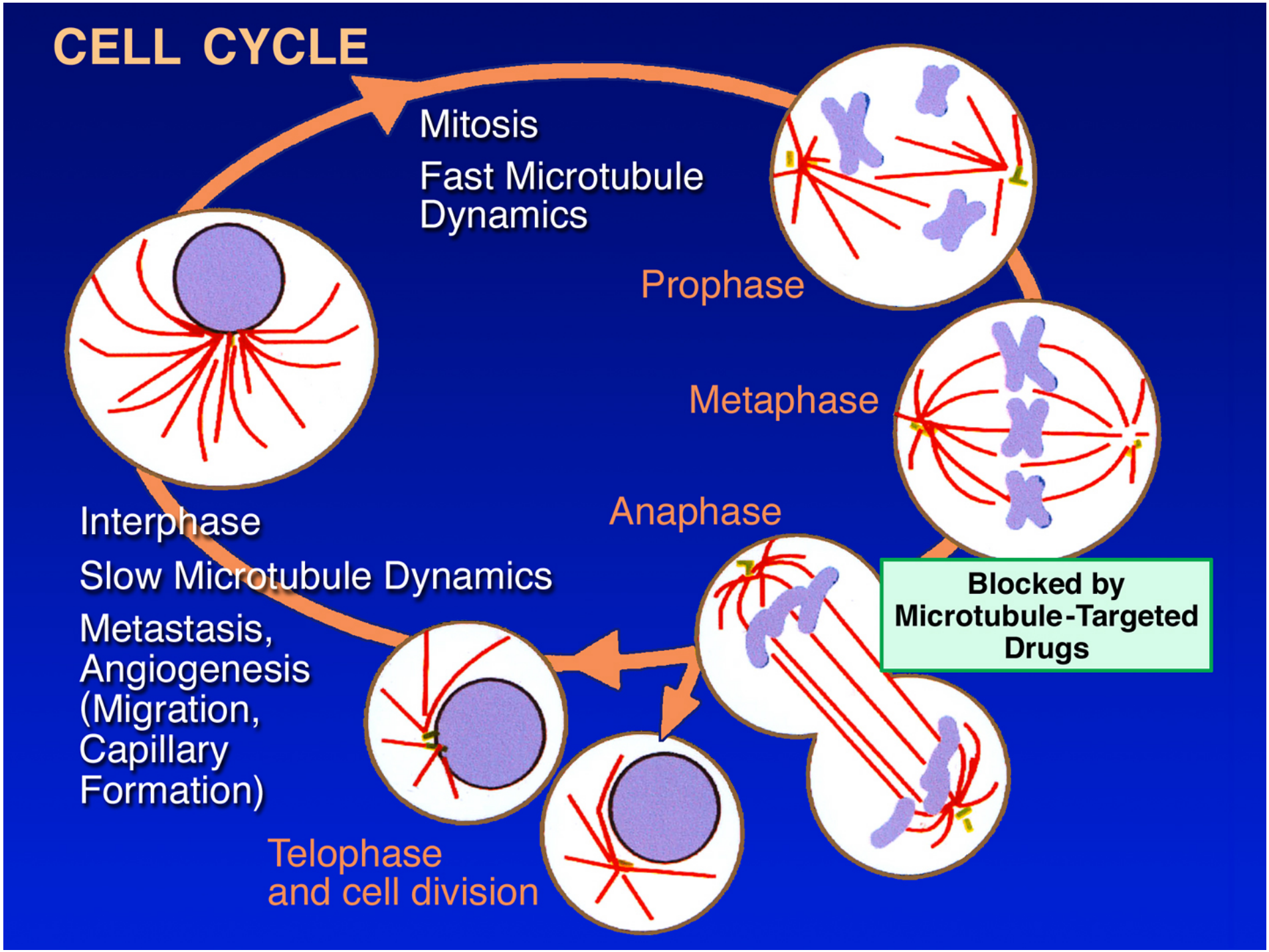
Chemotherapeutic agents' interactions with targets

Target : a molecule whose interaction with an anticancer agent will induce a cytotoxic effect

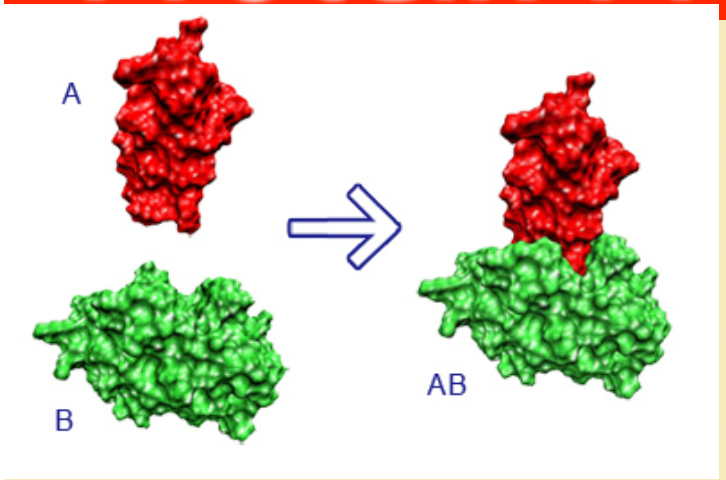
Targets are key molecules involved or required for cell mitosis and/or survival

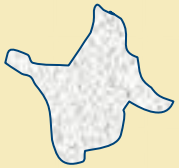
Conventional chemotherapy acts on dividing cells only, but does not distinguish normal and abnormal dividing cells

Targeted agents are designed to act on targets which are specific for tumor cells

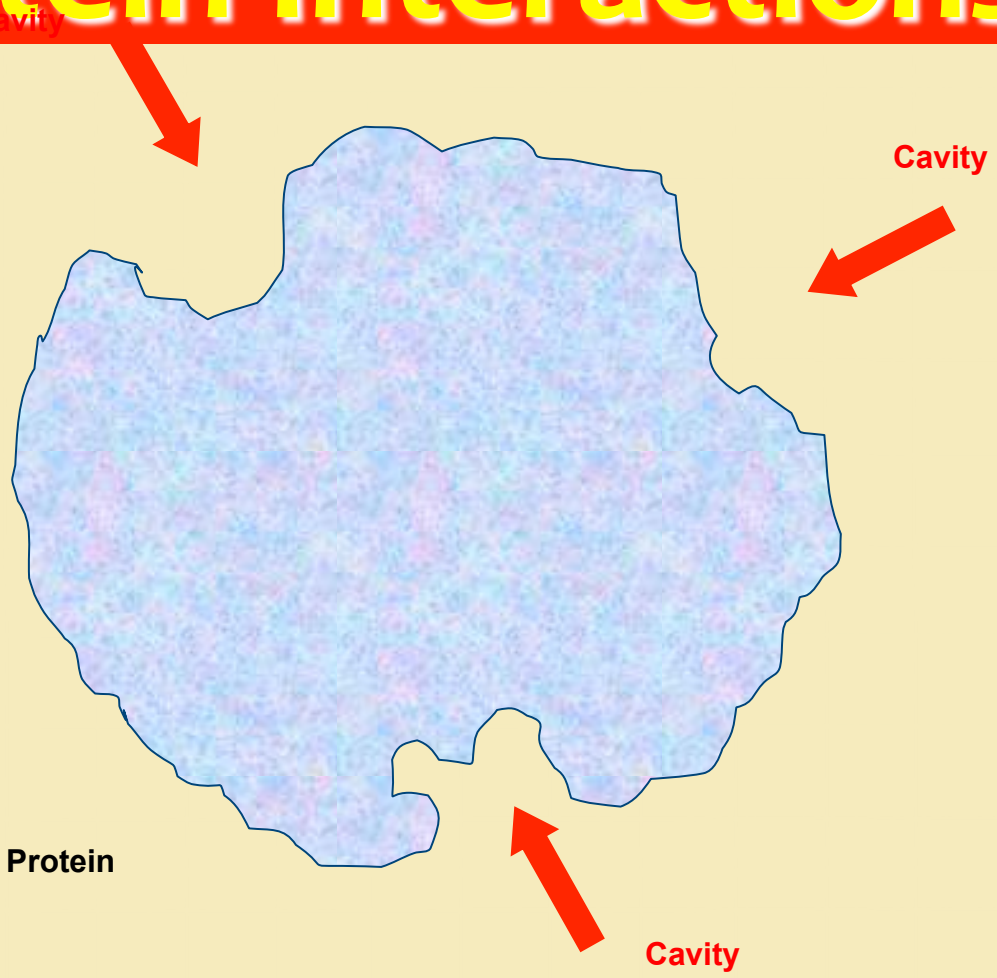

CELL CYCLE
Mitosis
Fast Microtubule Dynamics
Prophase
Metaphase
Anaphase
Blocked by Microtubule-Targeted Drugs
Telophase and cell division
Interphase
Slow Microtubule Dynamics
Metastasis, Angiogenesis (Migration, Capillary Formation)

Drug Binding: Inhibition of Protein-Protein Interactions





Drug / Ligand

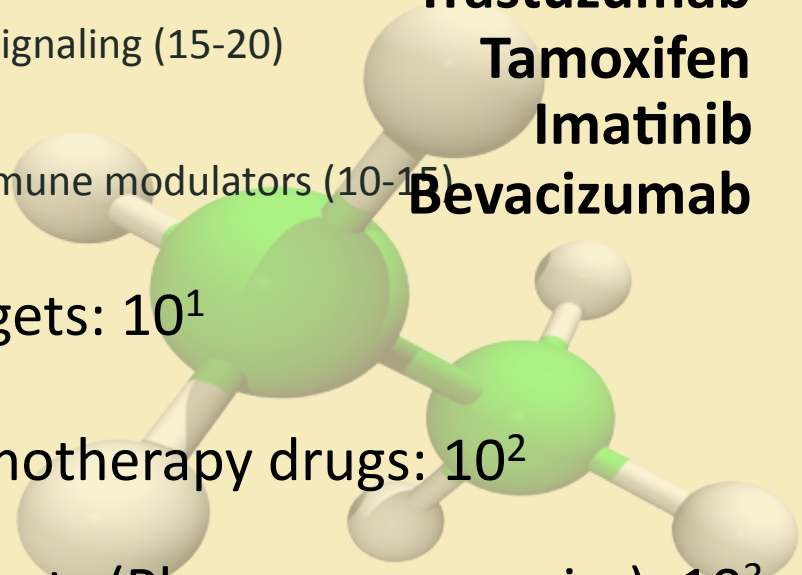


CHEMOTHERAPY DRUGS

Approximately 100 standard chemotherapeutic drugs:

1) Alkylating agents: Genotoxic (20-25)
2) Plant alkaloids: Inhibition of mitosis (10-15)
3) Antimetabolites: Inhibition of base synthesis (15-20)
4) Antibiotics: Derived from *Streptomyces* (10-15)
5) Targeted antibodies: Bind cell surface receptors (5-10)
6) Hormones: Inhibit or stimulate hormone signaling (15-20)
7) Directly targeted
8) Other indirect effects: Angiogenesis or immune modulators (10-15)

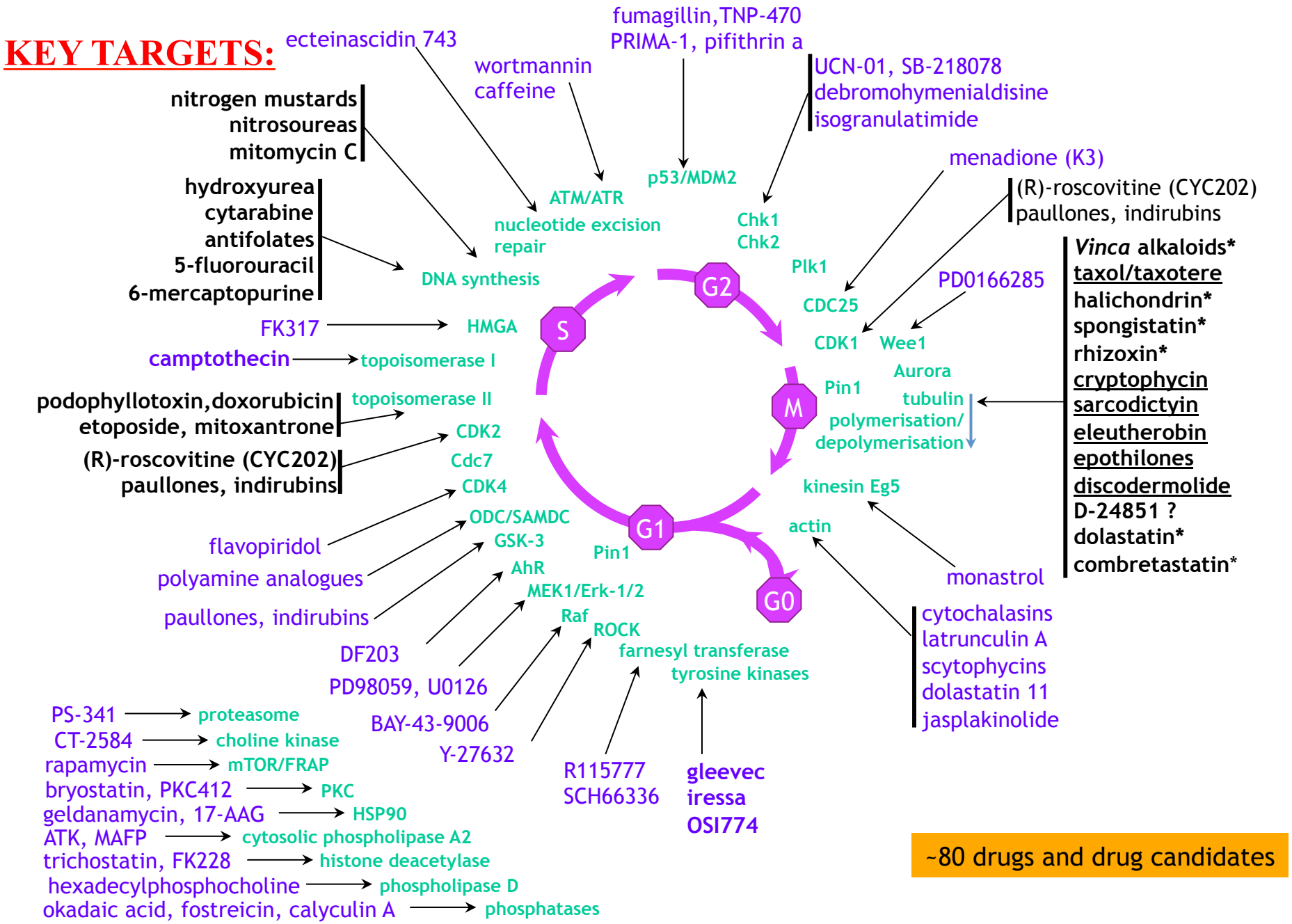
Cisplatin
Paclitaxel
Methotrexate
Doxorubicin
Trastuzumab
Tamoxifen
Imatinib
Bevacizumab



Number of current chemotherapy targets: 10^1

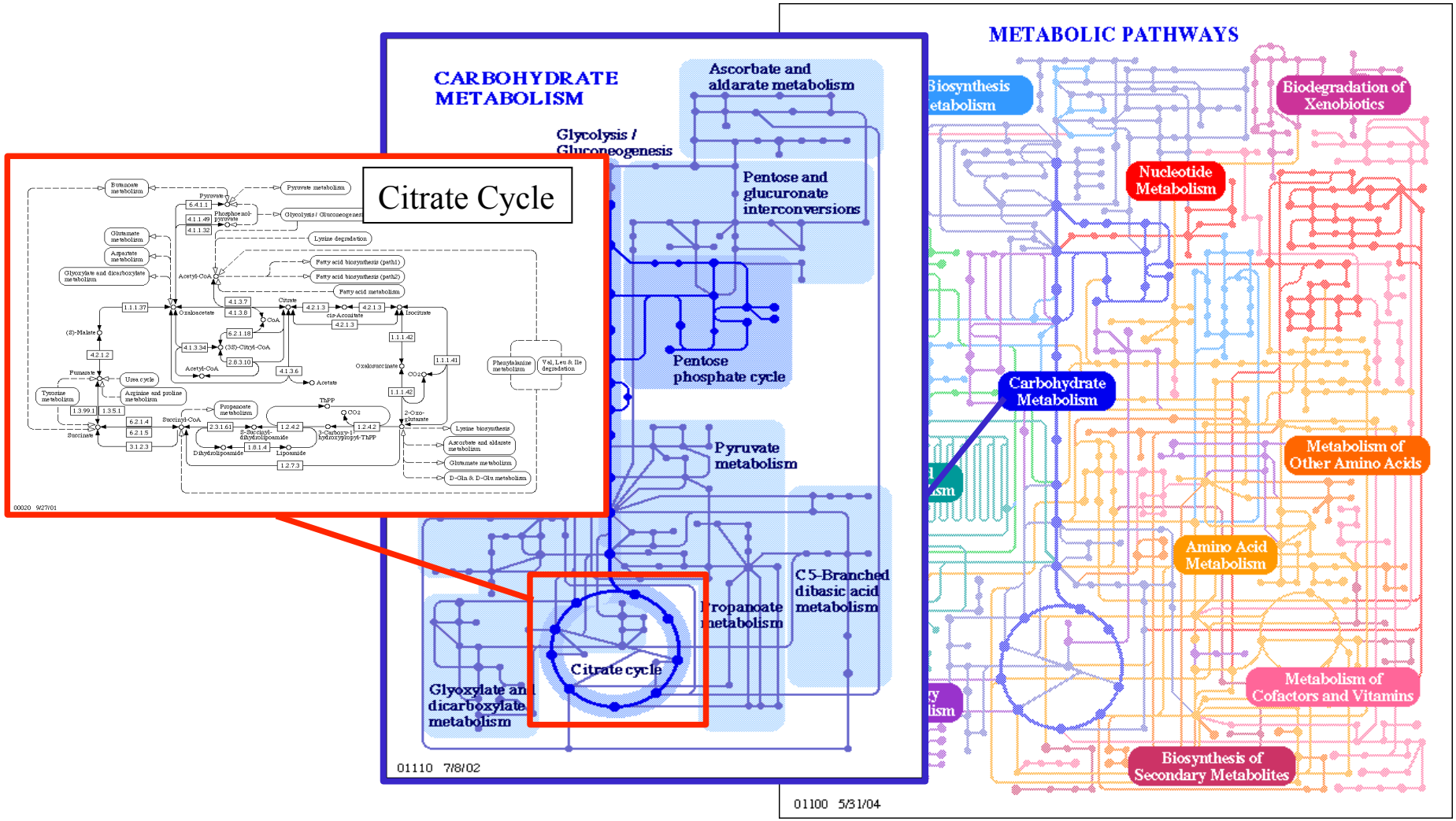
Number of chemotherapy drugs: 10^2

Potential Targets (Pharmacogenomics): 10^3



Source: Cell cycle laboratory, L. Meijer, Roscoff, France

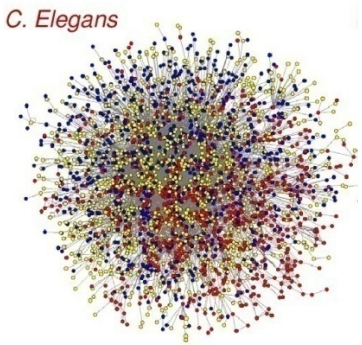
Biological pathways are complex networks

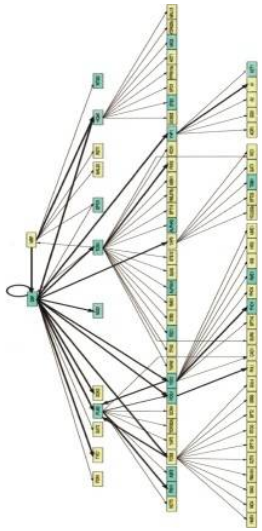


Biological networks: Gathering the knowledge

Physical interactions

Y2H, TAP, Co-IP, Protein chips, ChIP-chip
- Physical interactions (protein-protein / protein-DNA)
- Complexes



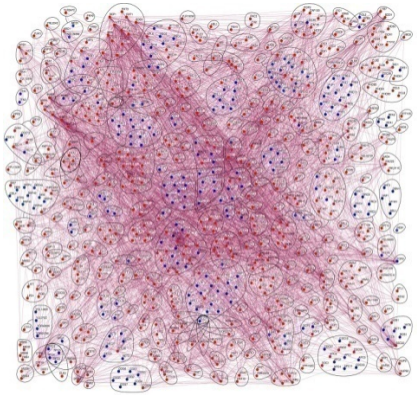


Protein interaction networks (Y2H)

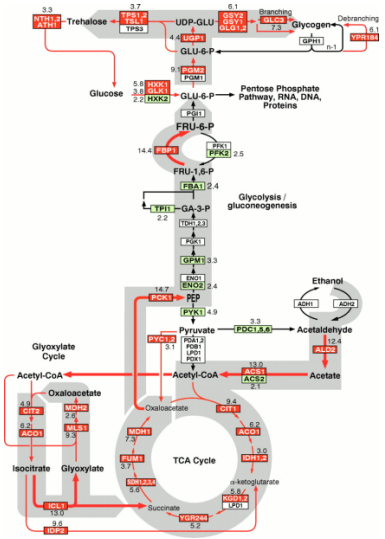
Regulatory networks (ChIP-chip)

Functional interactions

Knowledgebases, Computational predictions
- Functional interactions
- Pathways and functional ‘modules’

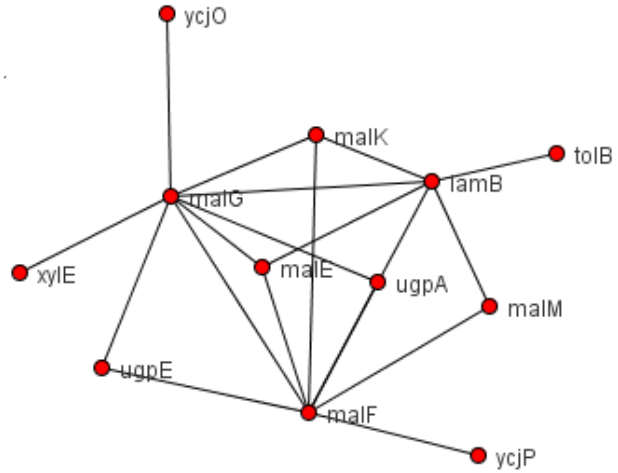


Genetic interaction Networks (SGA)



Metabolic networks (KEGG)

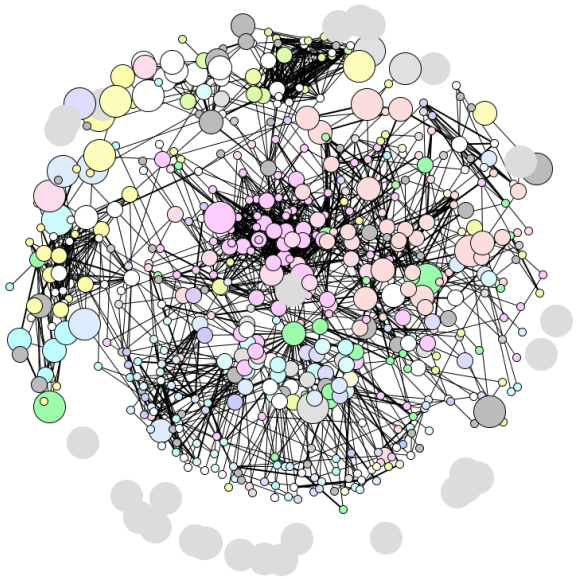
Networks may be analyzed using graph theory



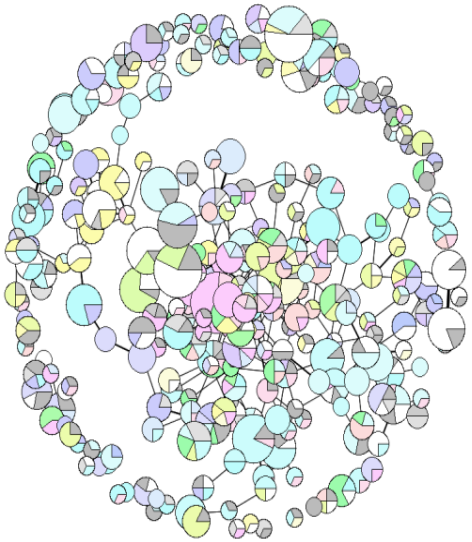
Nodes represent e.g. proteins, genes or substrates

Links between nodes represent interactions e.g. physical, genetic, biochemical

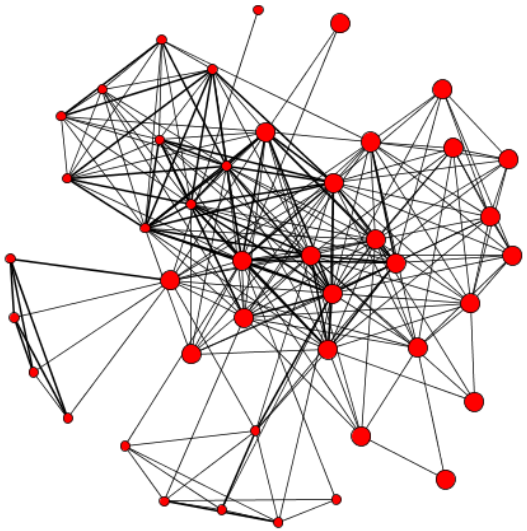
Analyses can be performed at different levels



Global



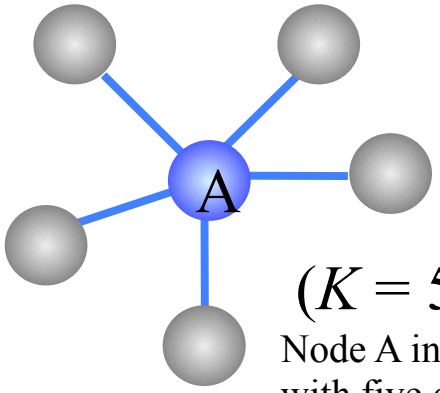
Complexes / cliques / pathways



Local

Topological properties of networks

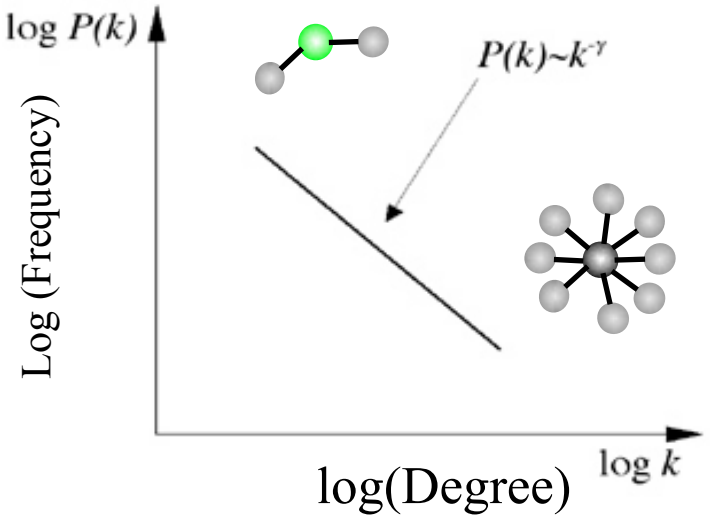
Global analyses of these properties over an entire network provide insights into its organization



($K = 5$)

Node A interacts with five other nodes

Many networks display small world / *scale free* behaviour (many nodes with few connections; few nodes with many connections)



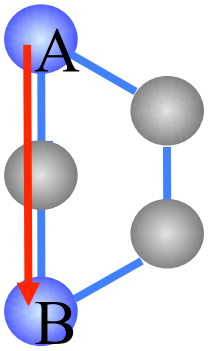
One of the more commonly used is

Node Degree

Scale free networks are thought to be more resistant to disruption

Topological properties of networks

Shortest path length

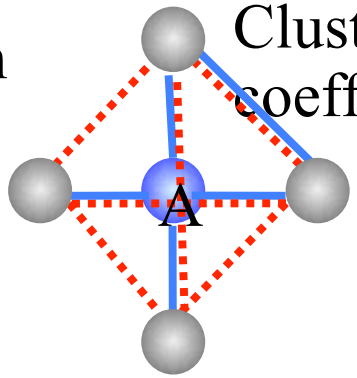


$(L = 2)$

The shortest path between A and B is via 2 links

Mean path length offers a measure of a networks overall navigability

Cluster coefficient

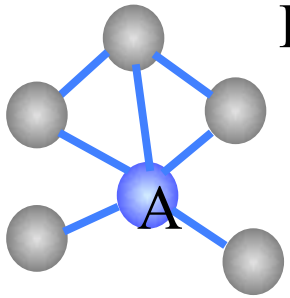


$(C = 1/6)$

Of the six possible connections between the neighbours of A, only one is actually made

Average cluster coefficient characterizes the overall tendency of the network to form clusters

Betweenness



$(B = 13/15)$

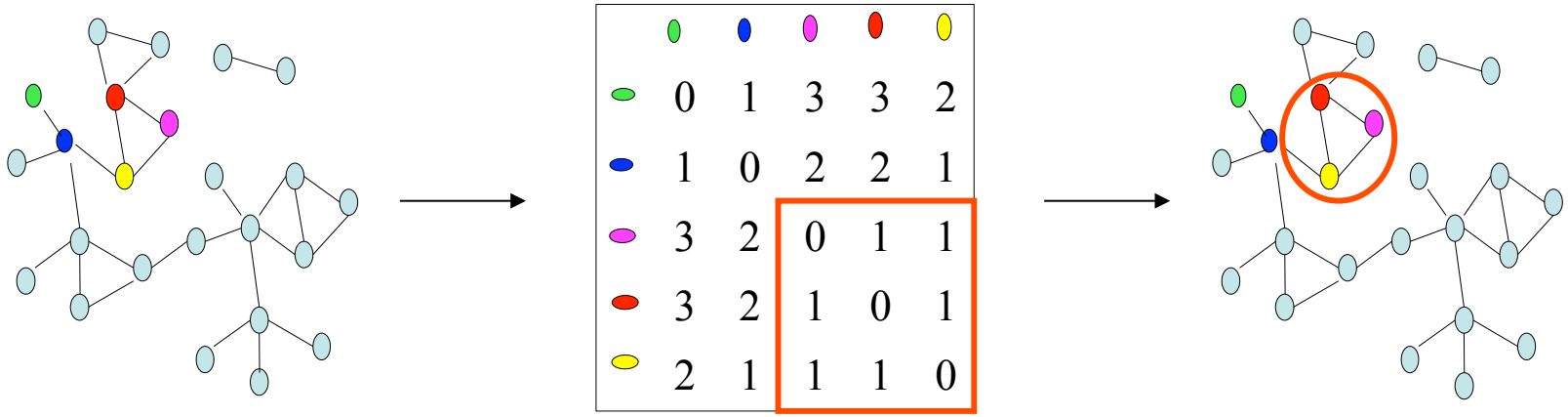
13 out 15 shortest paths in the network go through node A

Nodes with high betweenness are 'central' to the network

Exploiting topological properties to detect cliques

Common patterns of local interactions may be exploited to define cliques

One method uses shortest path lengths to create a matrix of local connectivity's



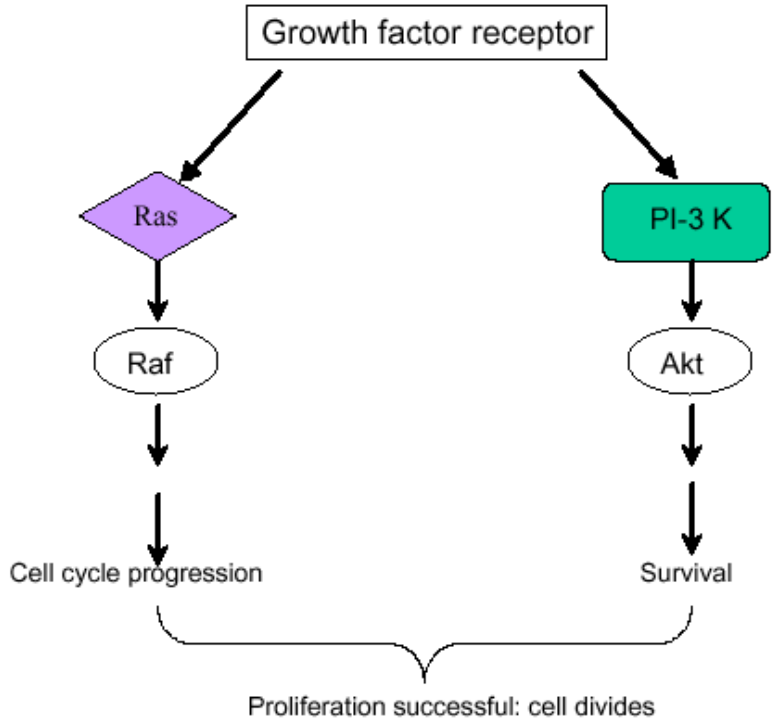
Other methods include use of clustering coefficients, or specialized graph clustering algorithms

What do cliques represent ? (complexes, pathways, ‘functional’ modules)

Who are the main players connecting cliques ?

Concurrency in Biochemical Networks

Biochemical networks are also concurrent communicating systems. Pathways consist of sequences of interactions which sometimes affect other parallel pathways. As an example, consider two pathways involved in cell division. The Ras- Raf pathway which triggers the cell division and the PI- 3K- Akt pathway which keeps the cell alive are both triggered by the same growth factor. The sequences of interactions in both pathways run concurrently with some interaction i. e. Akt inhibits Raf.



Cancer Cell Network

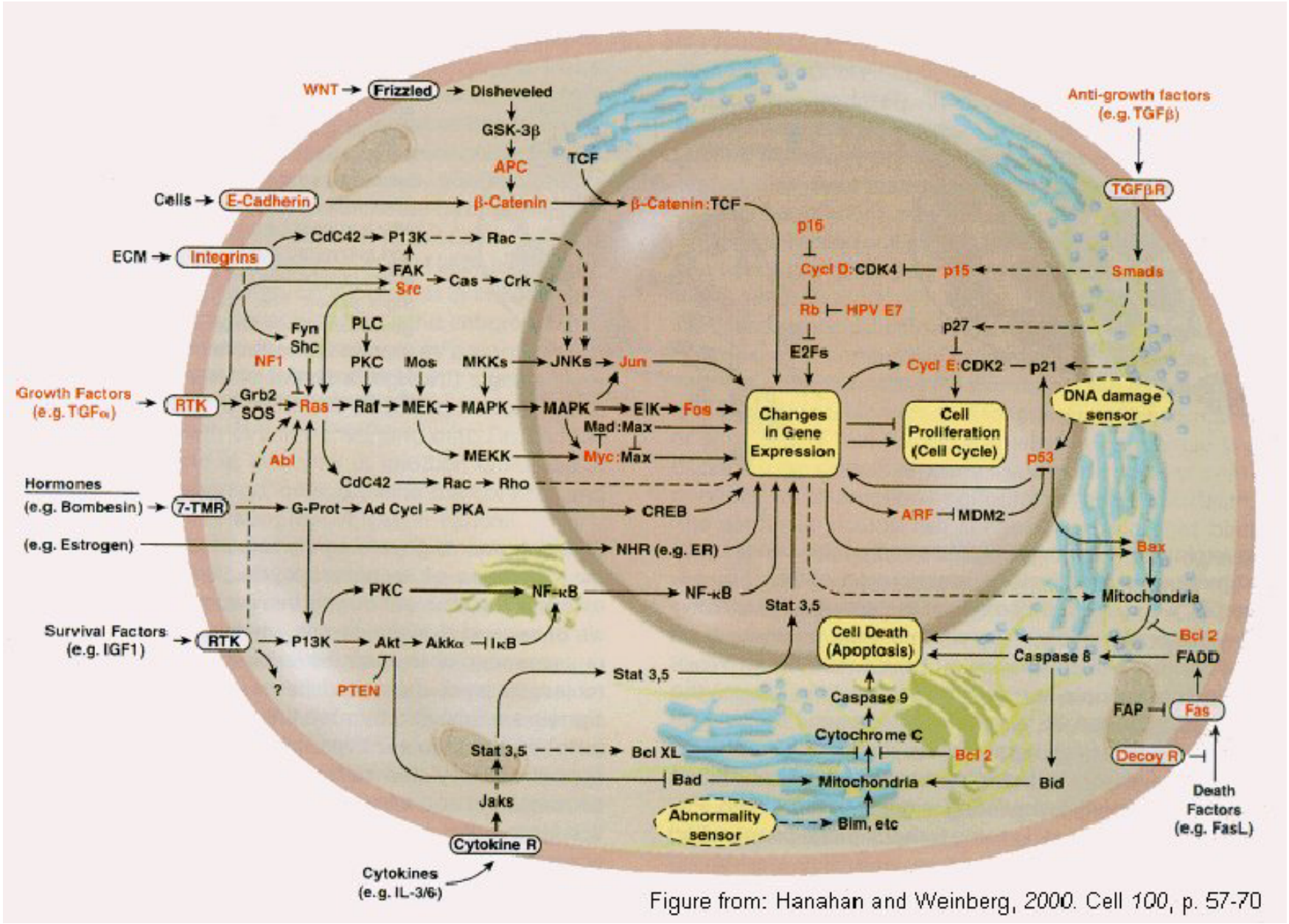


Figure from: Hanahan and Weinberg, *2000*. Cell *100*, p. 57-70

Information available

- Data base of drugs approved and investigational, their mode of action, targets, applications in cancer
- Biochemical pathways (identify where drugs inhibit them)
- List of “druggable targets”
- Methods of modeling networks and pathways

Robustness of Biological Networks

- We want to know how resilient these pathways are to chemotherapy.
- How does the inhibition of an interaction effect function of the entire network?
- Robustness is the measure of how well networks function under random perturbation.
- Network robustness can be quantified as entropy.

Graph Theory and Entropy

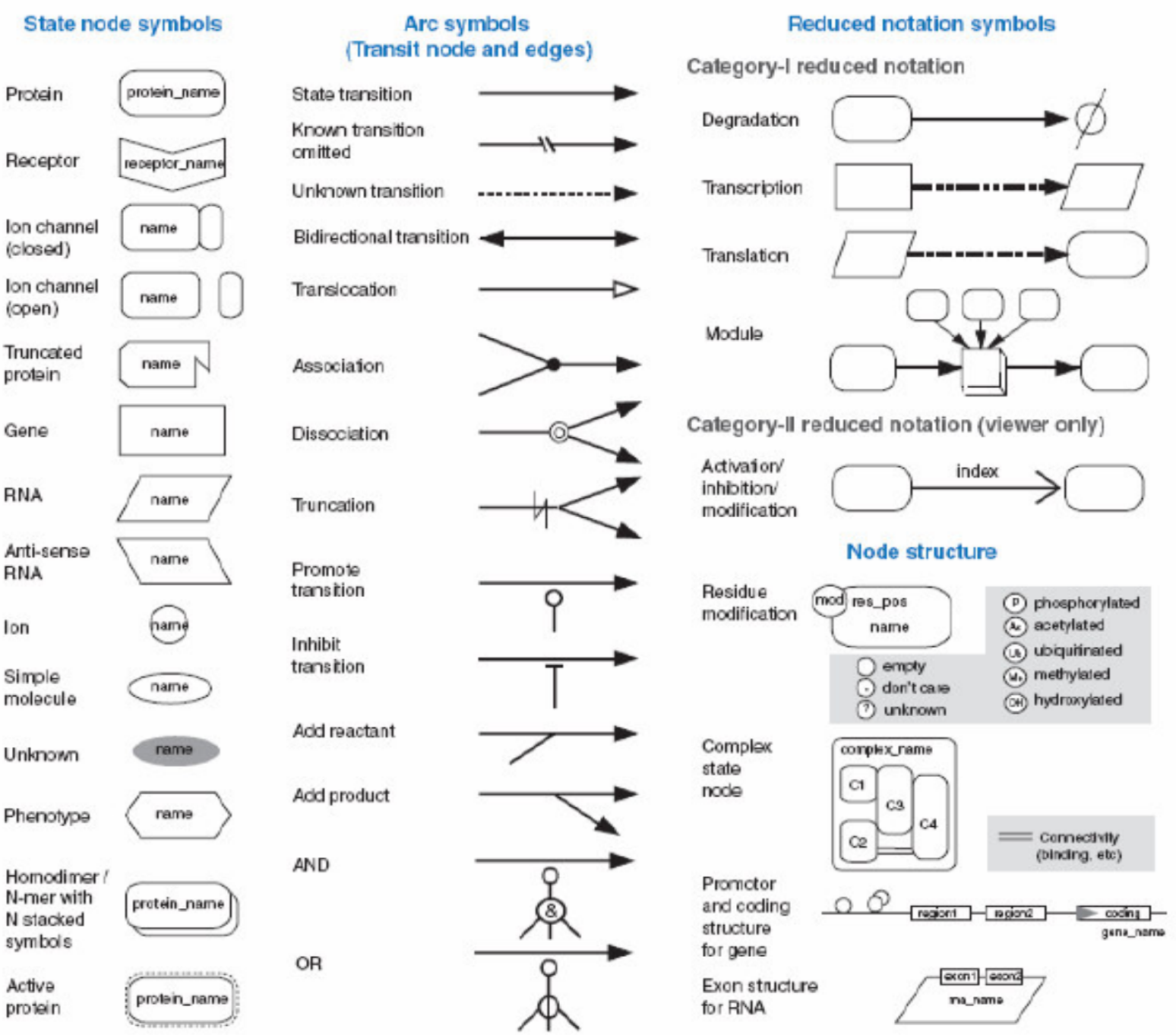
- A graph is a collection of nodes and edges.
- In this case nodes represent proteins and genes while edges represent interactions between them.
- The degree of a node is a count of how many edges lead to or from it.
- Pathways were converted into graphs using R and KEGGgraph.
- The entropy of these graphs is then given by $H = -\Sigma\, p(k)*\ln(p(k))$, where $p(k)$ is the probability that a node has degree k.

KEGG Pathway Networks

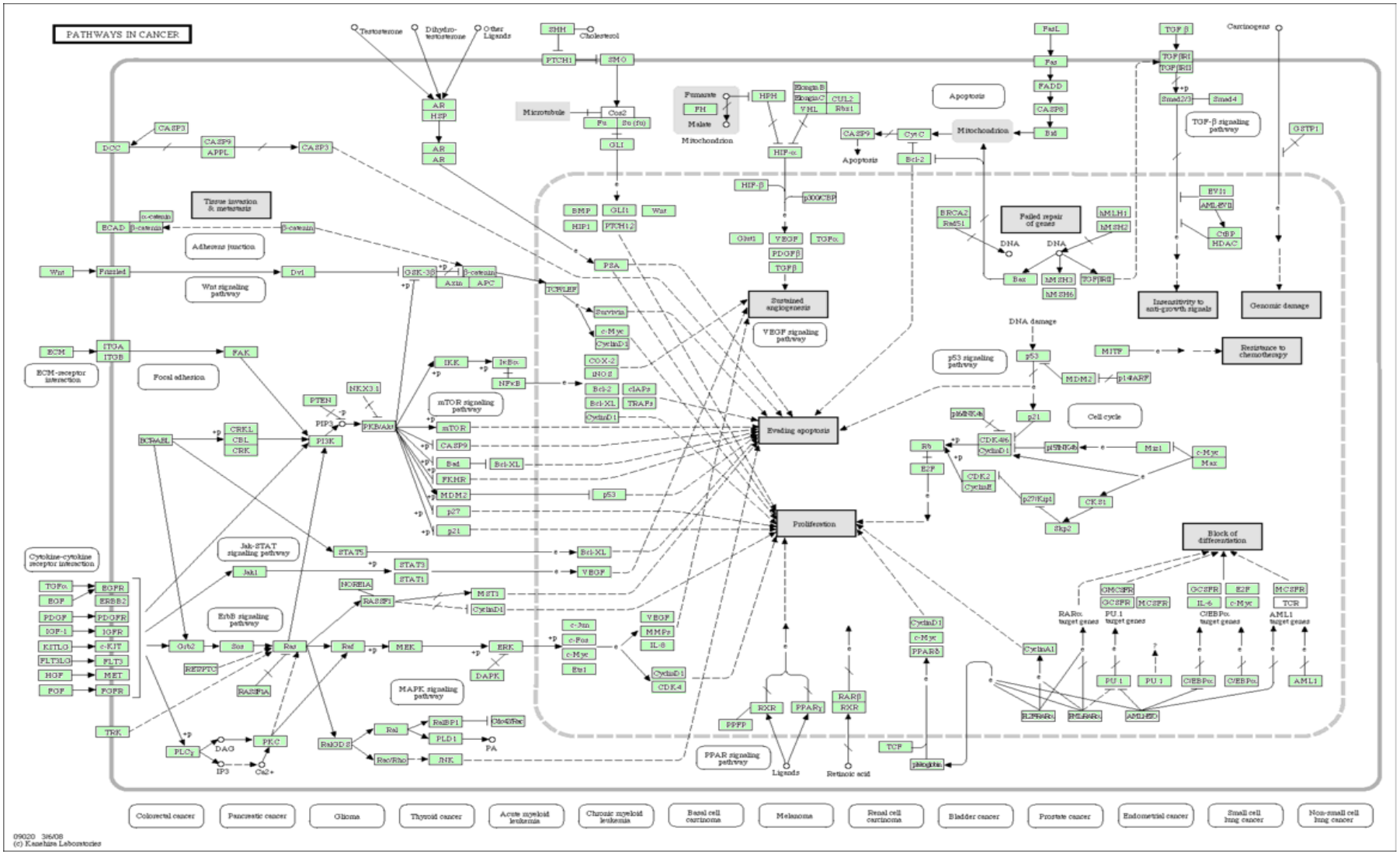
- Kyoto Encyclopedia of Genes and Genomes (KEGG)
- www.genome.jp/kegg
- A highly comprehensive pathway network for some cancers derived from extensive literature, textbooks, other database and expert knowledge.
- An R program, KEGGgraph, extracts the protein-protein interaction network for the relevant pathway, producing an adjacency list.

Analysis of Pathways

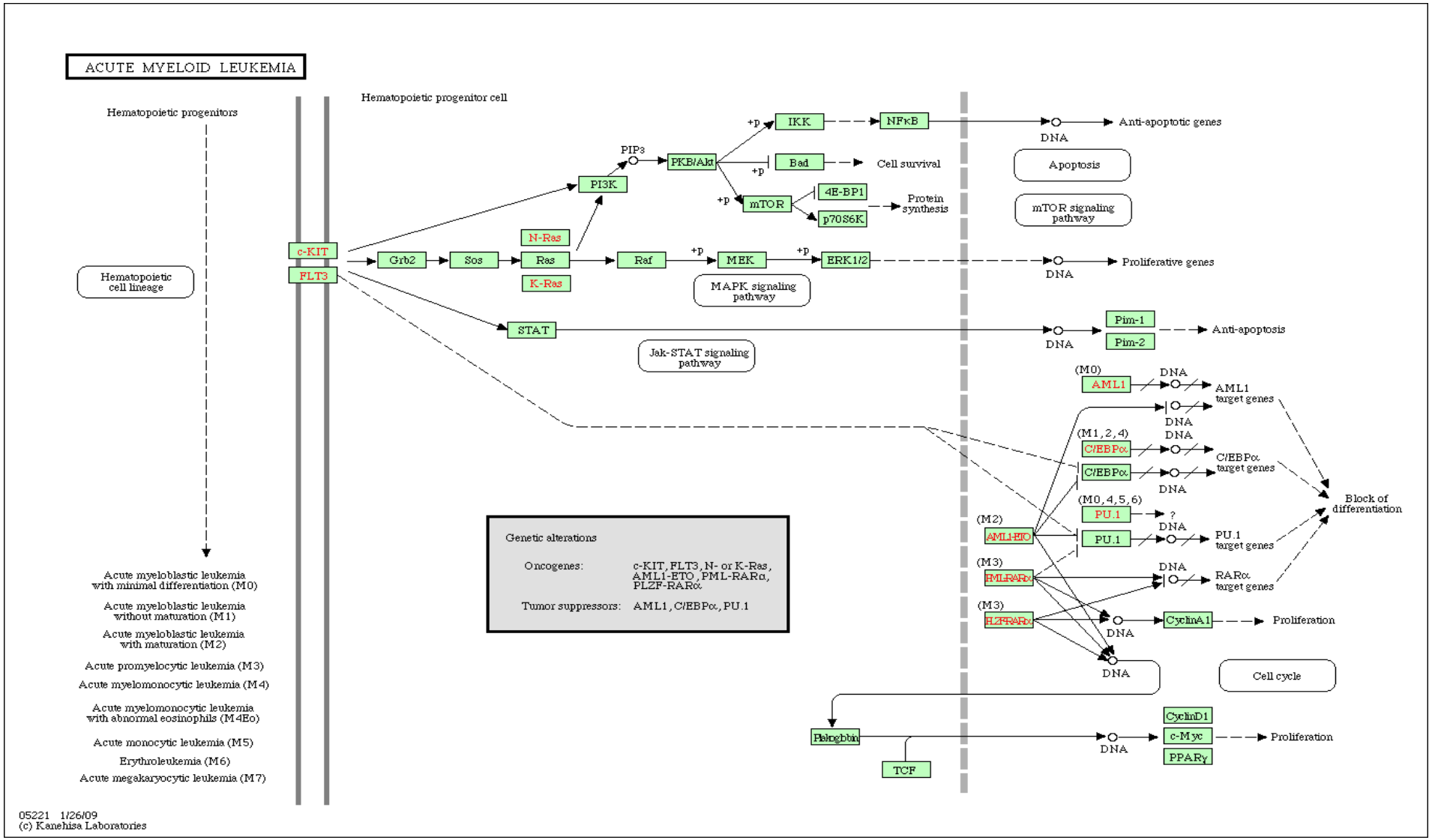
- The next step was to calculate the entropy of each pathway.
- This was done using both R and Excel.
- After that, to draw useful information from these entropies.
- We hypothesized that there should be a correlation between entropy and lethality.
- The most lethal cancers should be the most robust.


State node symbols
Protein
protein_name
Receptor
receptor_name
Ion channel (closed)
name
Ion channel (open)
name
Truncated protein
name
Gene
name
RNA
name
Anti-sense RNA
name
Ion
name
Simple molecule
name
Unknown
name
Phenotype
name
Homodimer / N-mer with N stacked symbols
protein_name
Active protein
protein_name
Arc symbols (Transit node and edges)
State transition
Known transition omitted
Unknown transition
Bidirectional transition
Translocation
Association
Dissociation
Truncation
Promote transition
Inhibit transition
Add reactant
Add product
AND
&
OR
Reduced notation symbols
Category-I reduced notation
Degradation
Transcription
Translation
Module
Category-II reduced notation (viewer only)
Activation/ inhibition/ modification
index
Node structure
Residue modification
mod
res_pos
name
empty
don't care
unknown
phosphorylated
acetylated
ubiquitinated
methylated
hydroxylated
Complex state node
complex_name
C1
C2
C3
C4
Connectivity (binding, etc)
Promotor and coding structure for gene
region1
region2
coding
gene_name
Exon structure for RNA
exon1
exon2
rna_name

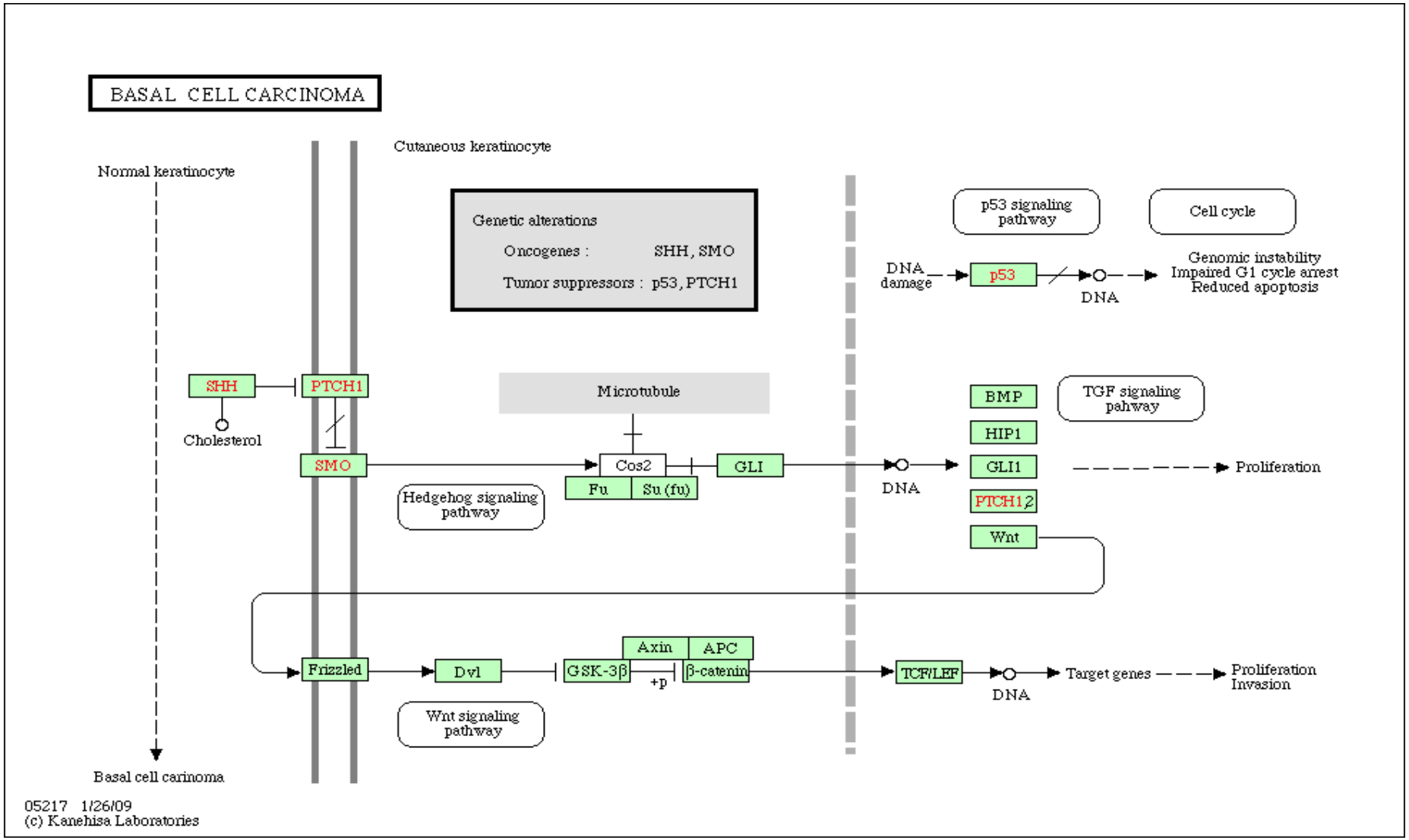
General cancer pathway



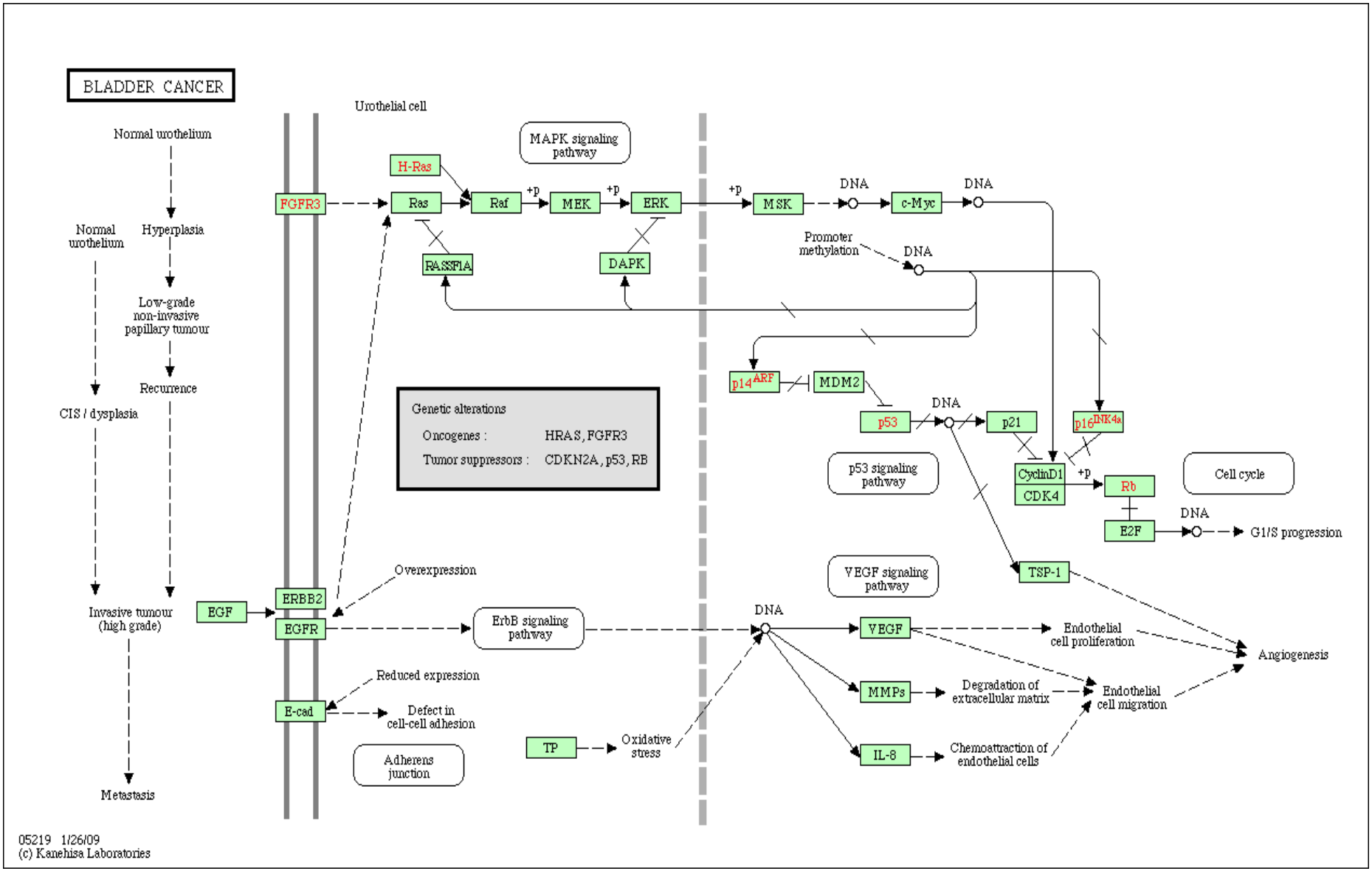
Acute myeloid leukemia



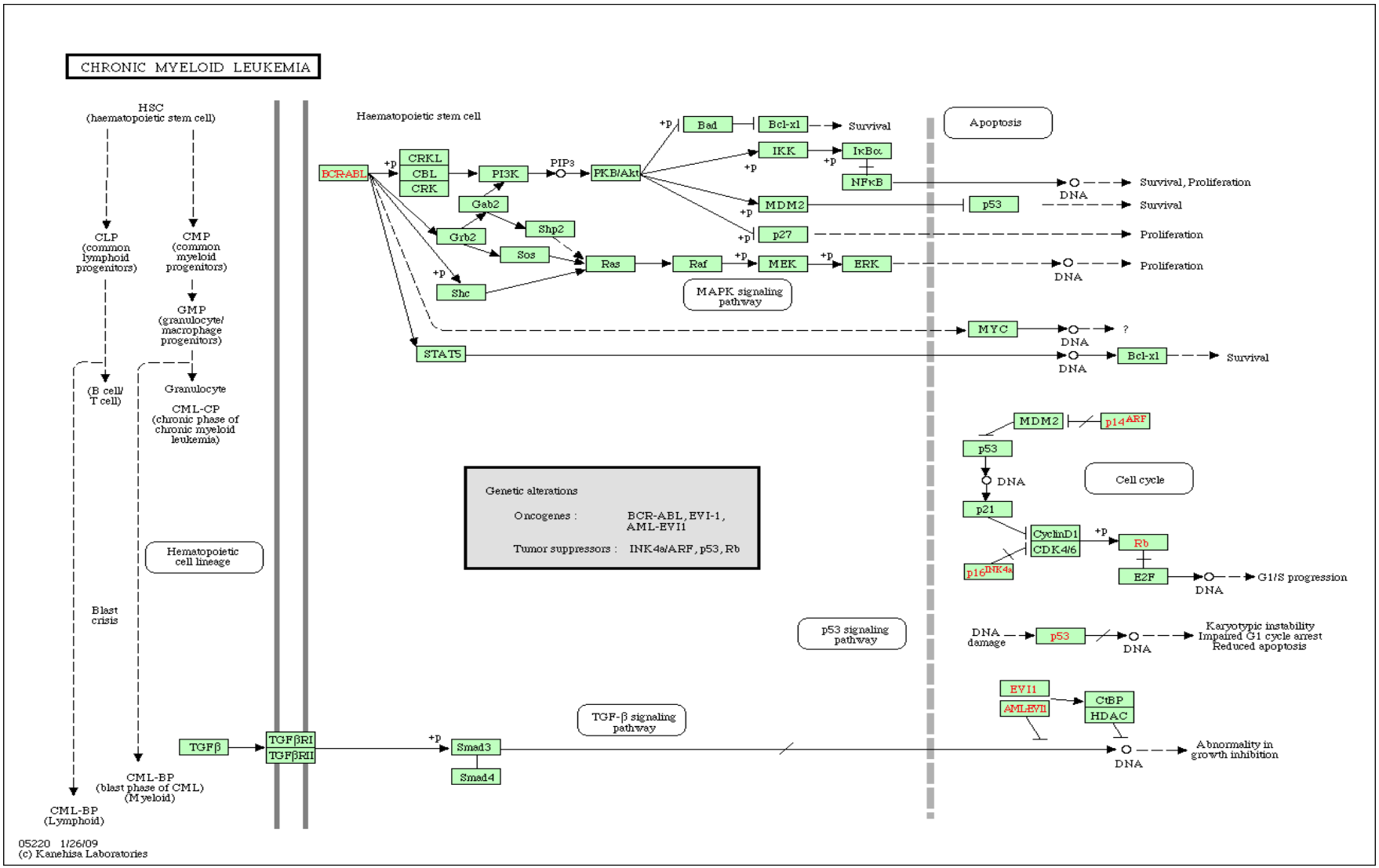
Basal cell carcinoma



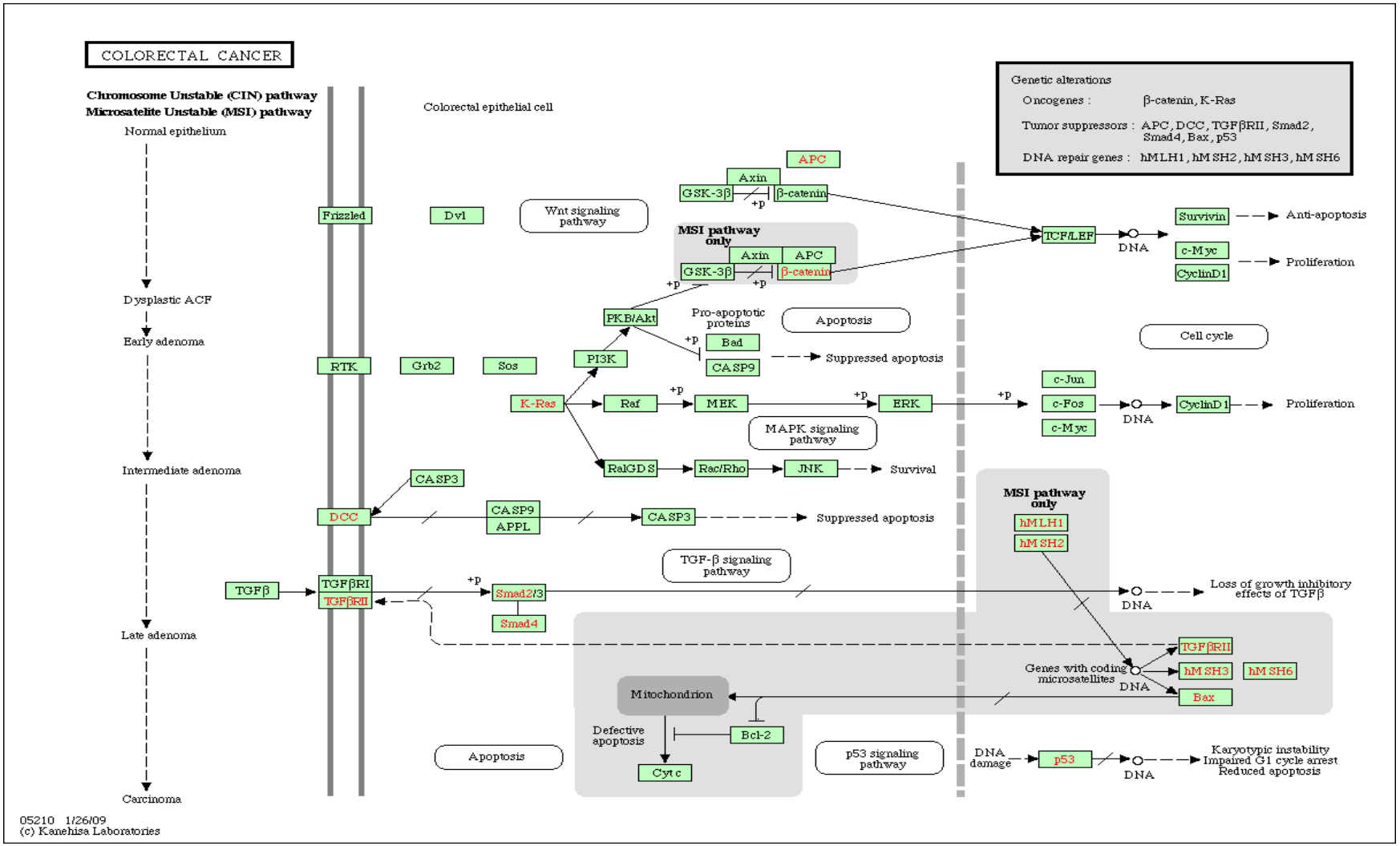
Bladder cancer



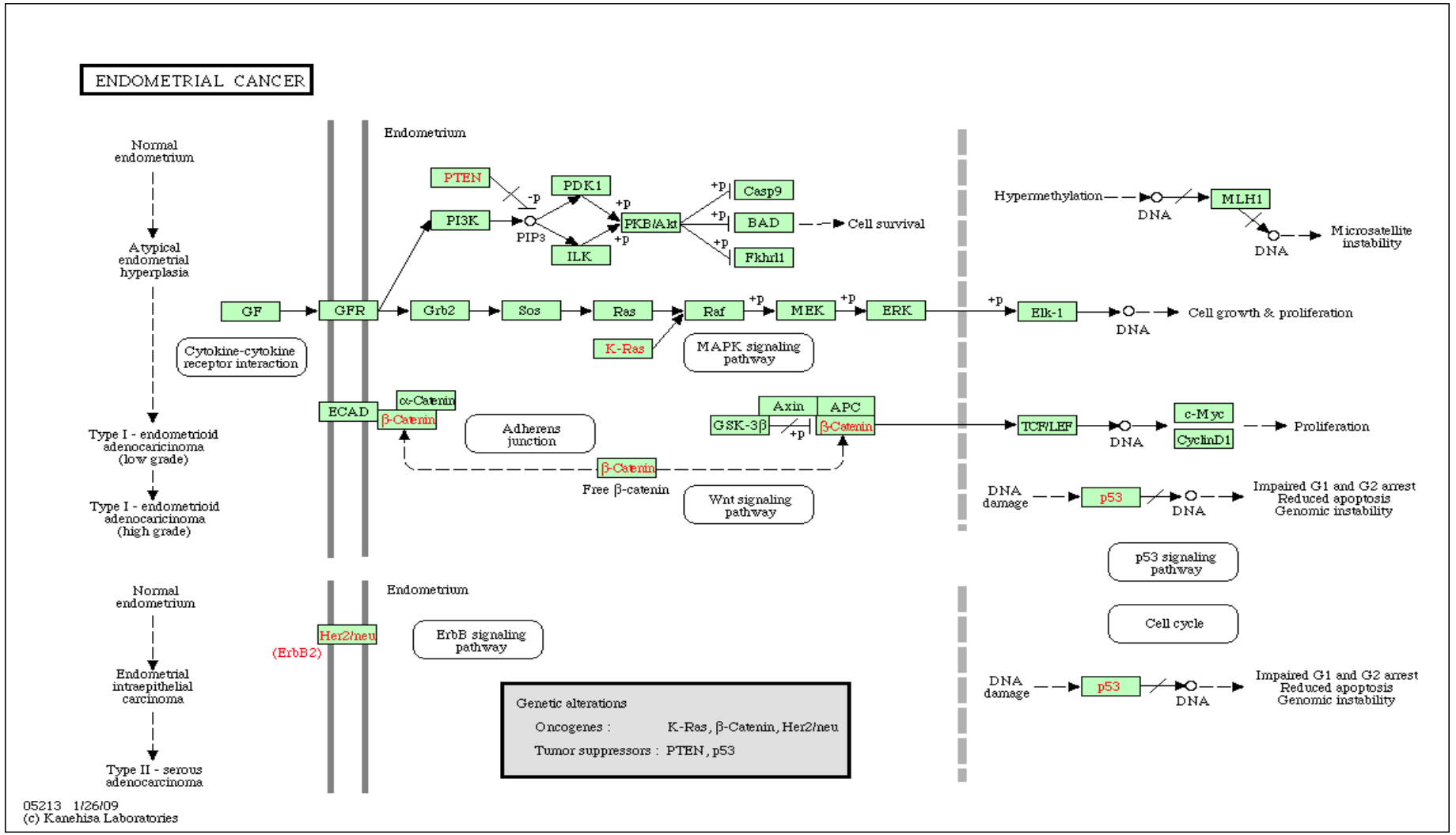
Chronic myeloid leukemia



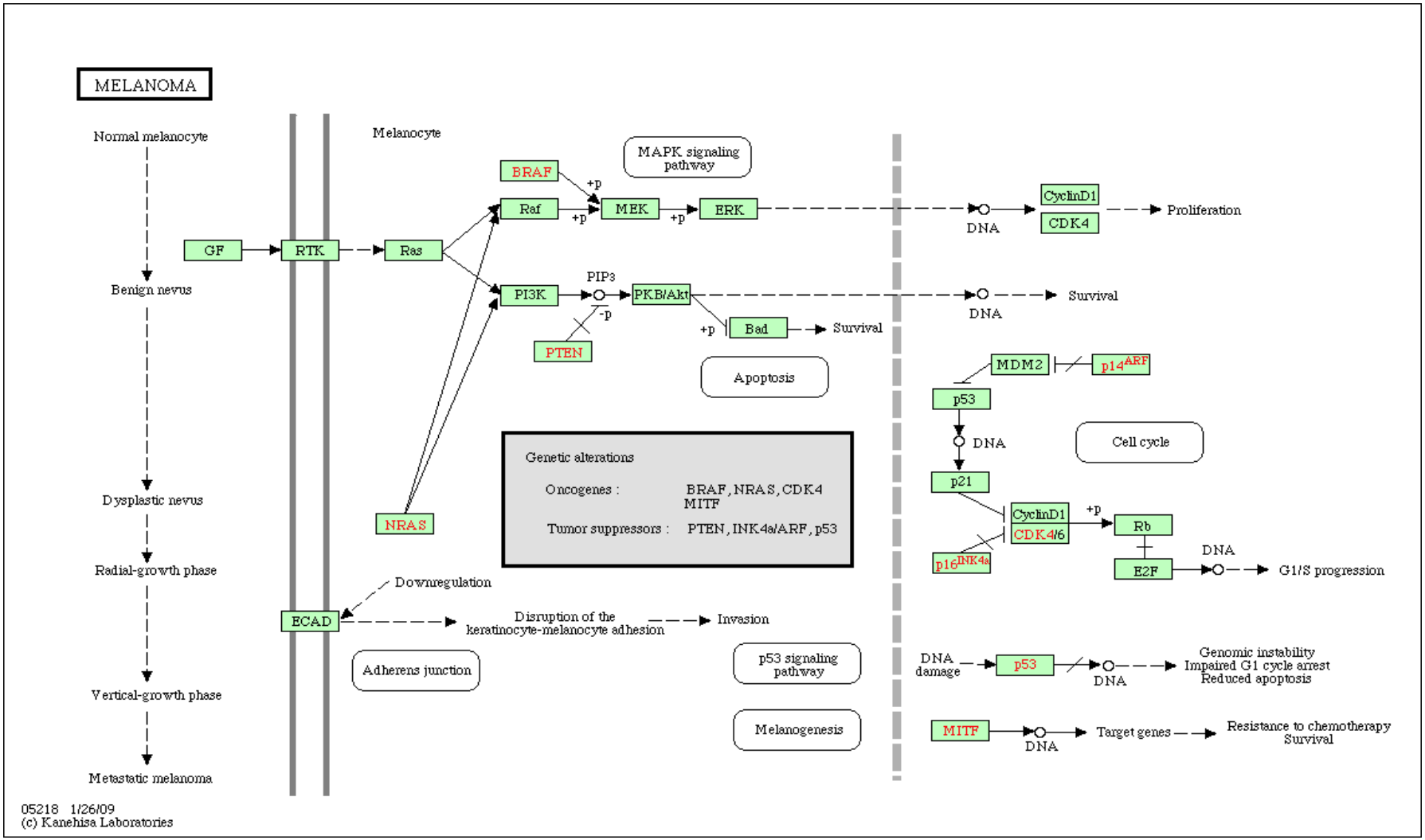
Colorectal cancer



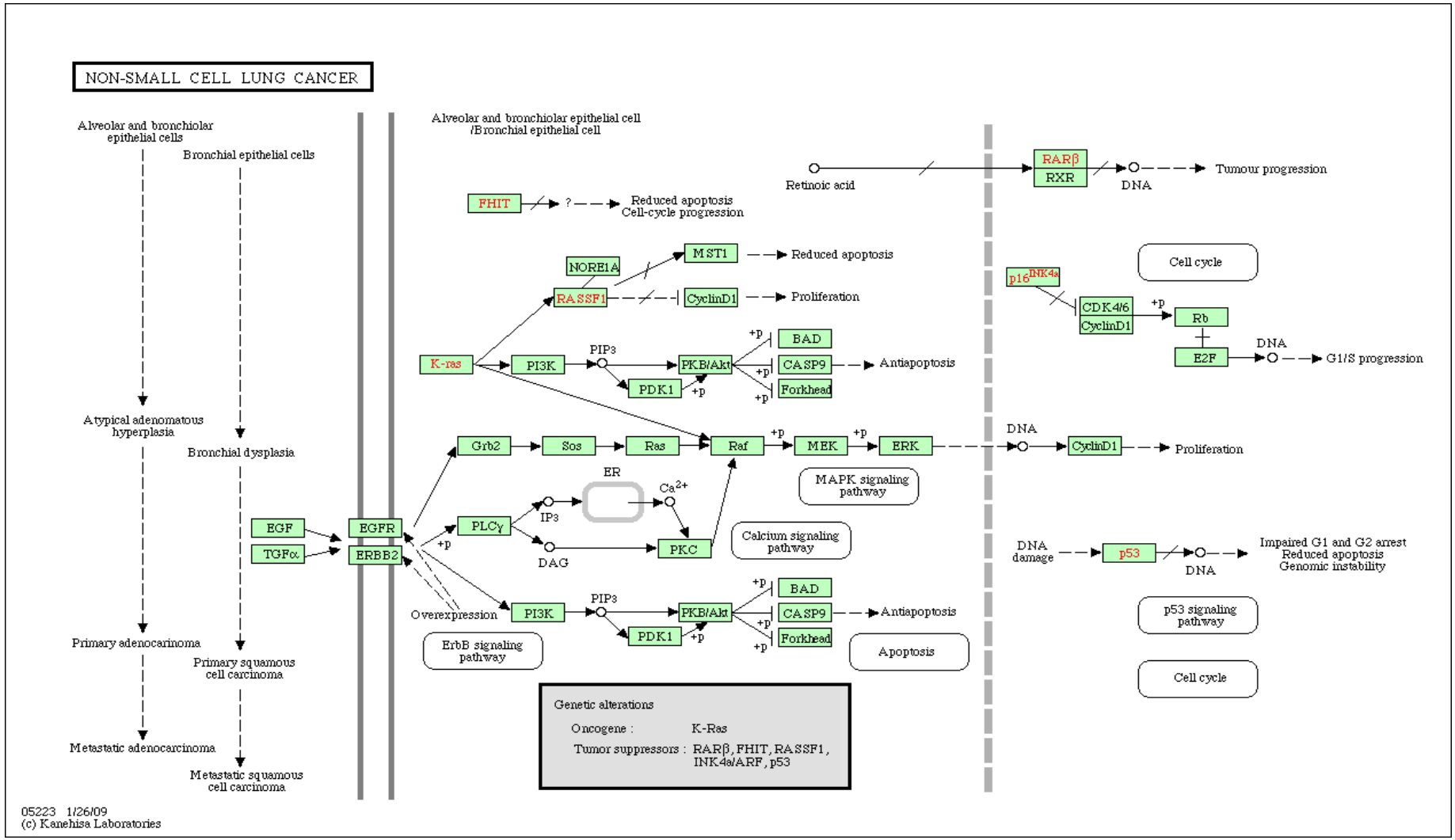
Endometrial cancer



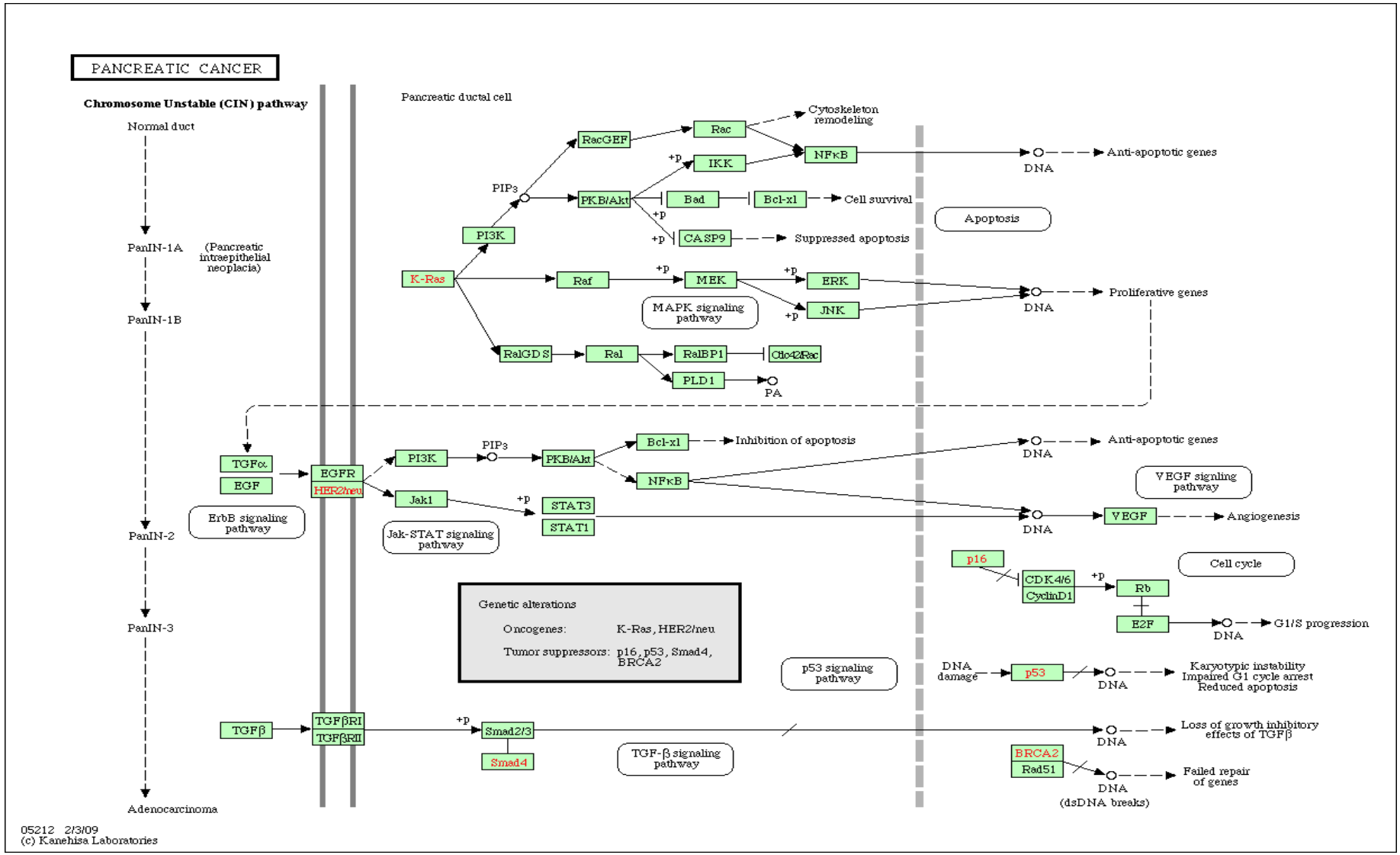
melanoma


MELANOMA
Normal melanocyte
Benign nevus
Dysplastic nevus
Radial-growth phase
Vertical-growth phase
Metastatic melanoma
Melanocyte
GF
RTK
Ras
BRAF
Raf
MEK
ERK
MAPK signaling pathway
+p
PIP3
PI3K
PKB/Akt
PTEN
-p
Bad
Survival
Apoptosis
NRAS
DNA
CyclinD1
CDK4
Proliferation
MDM2
p14ARF
p53
Cell cycle
p21
CyclinD1
CDK4/6
Rb
E2F
G1/S progression
p16INK4a
Genetic alterations
Oncogenes : BRAF, NRAS, CDK4 MITF
Tumor suppressors : PTEN, INK4a/ARF, p53
Downregulation
ECAD
Disruption of the keratinocyte-melanocyte adhesion
Invasion
Adherens junction
p53 signaling pathway
Melanogenesis
DNA damage
Genomic instability
Impaired G1 cycle arrest
Reduced apoptosis
MITF
Target genes
Resistance to chemotherapy
Survival
05218 1/26/09
(c) Kanehisa Laboratories

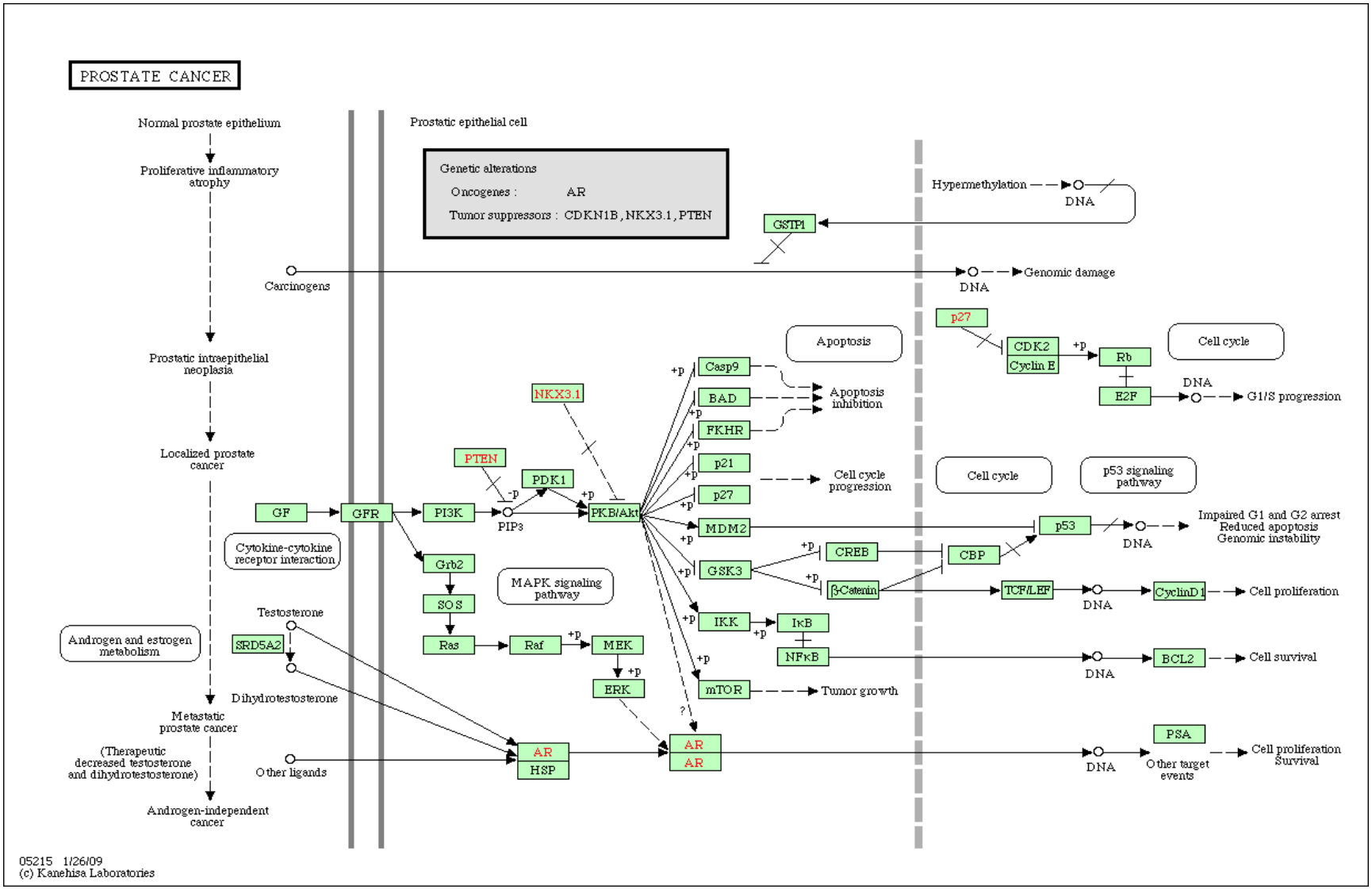
Non-small cell lung cancer



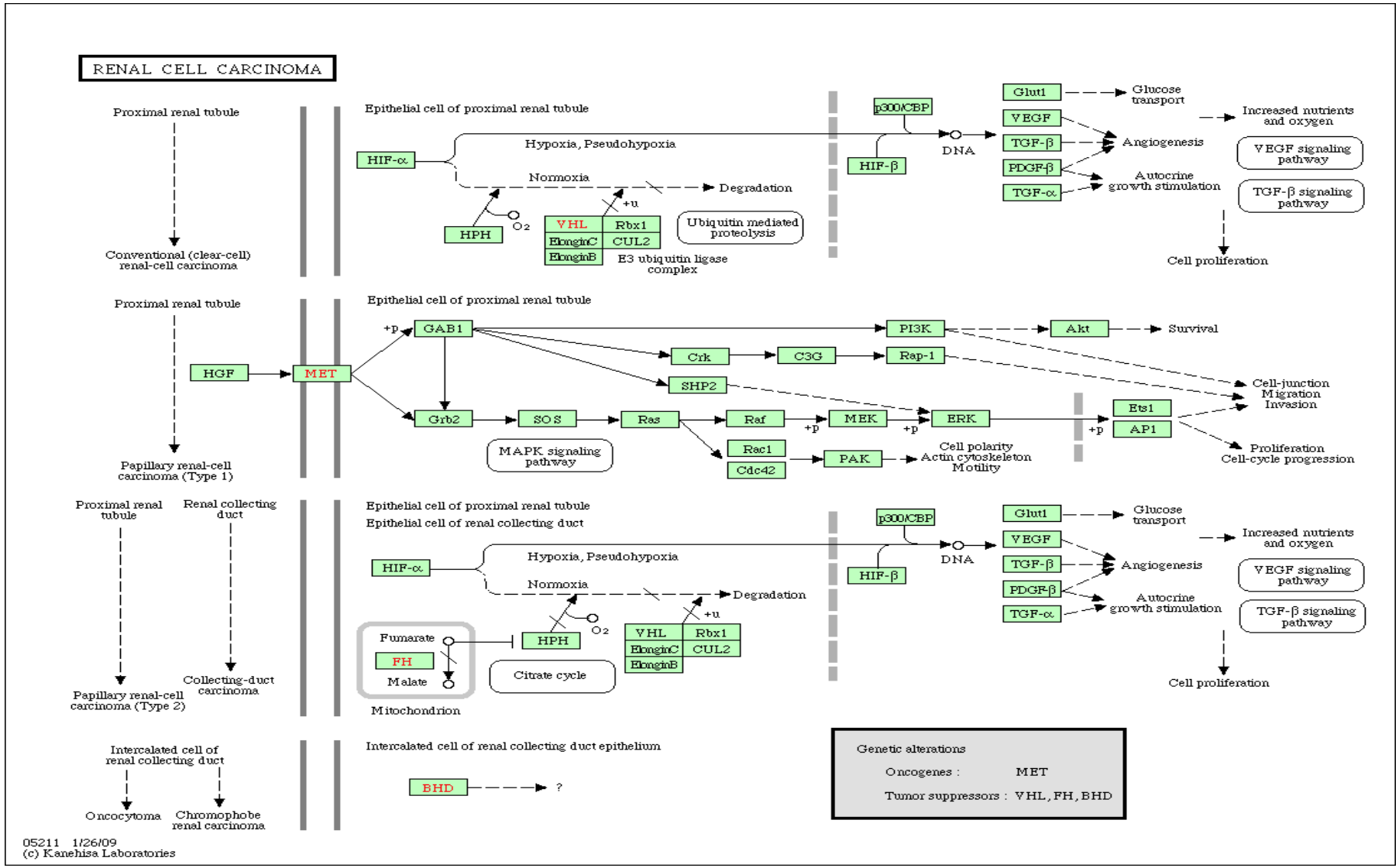
Pancreatic cancer



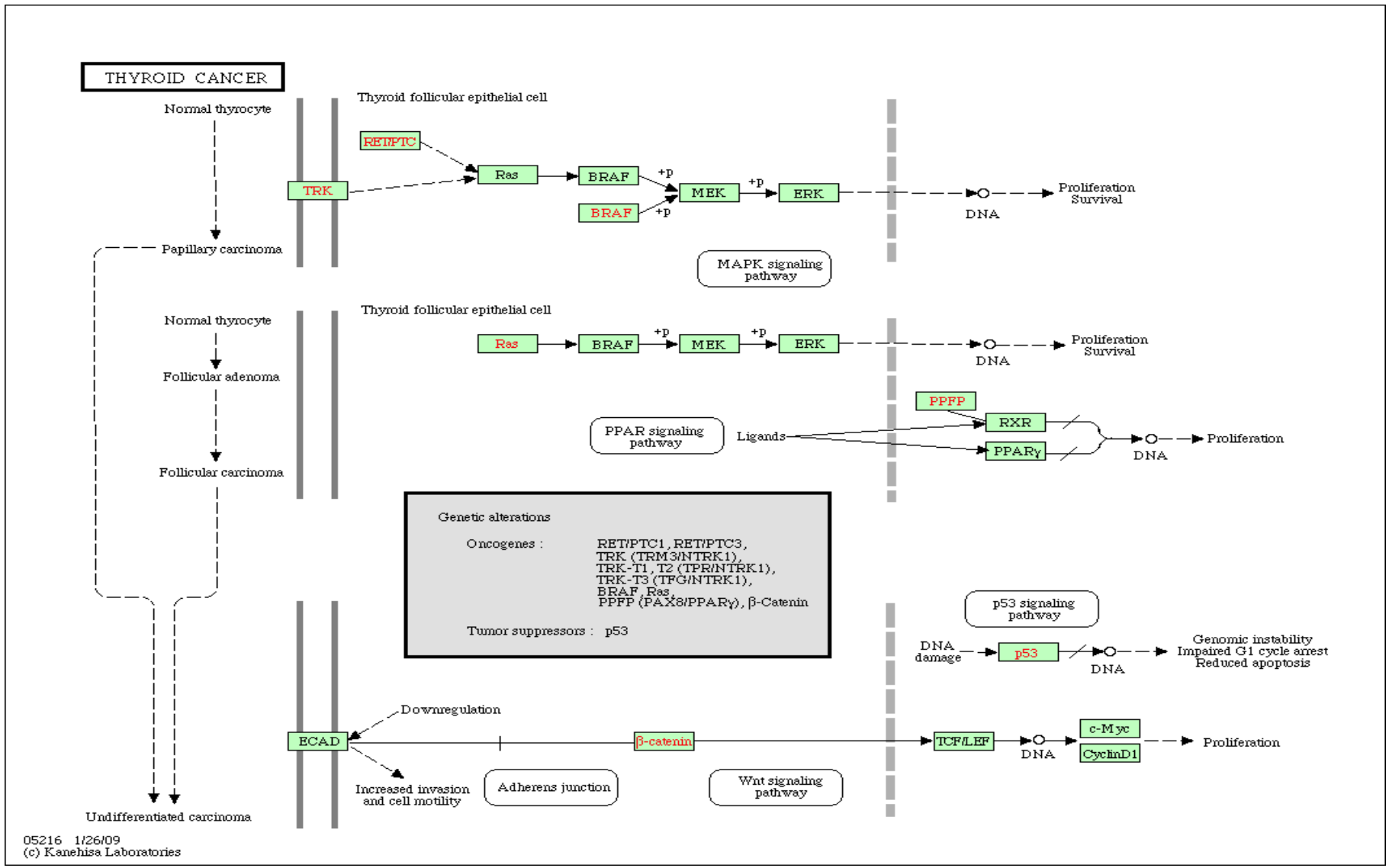
Prostate cancer



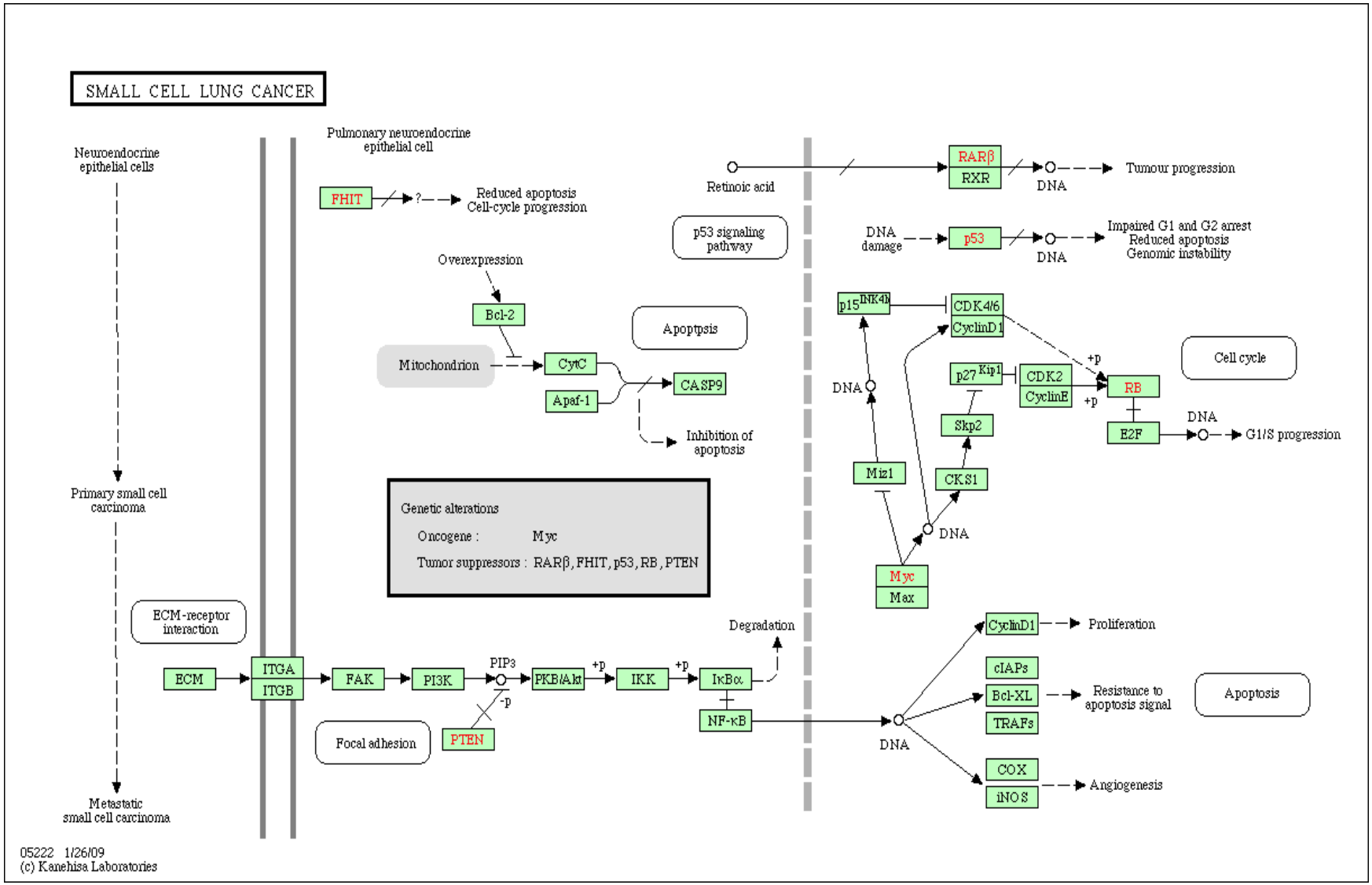
Renal cell carcinoma



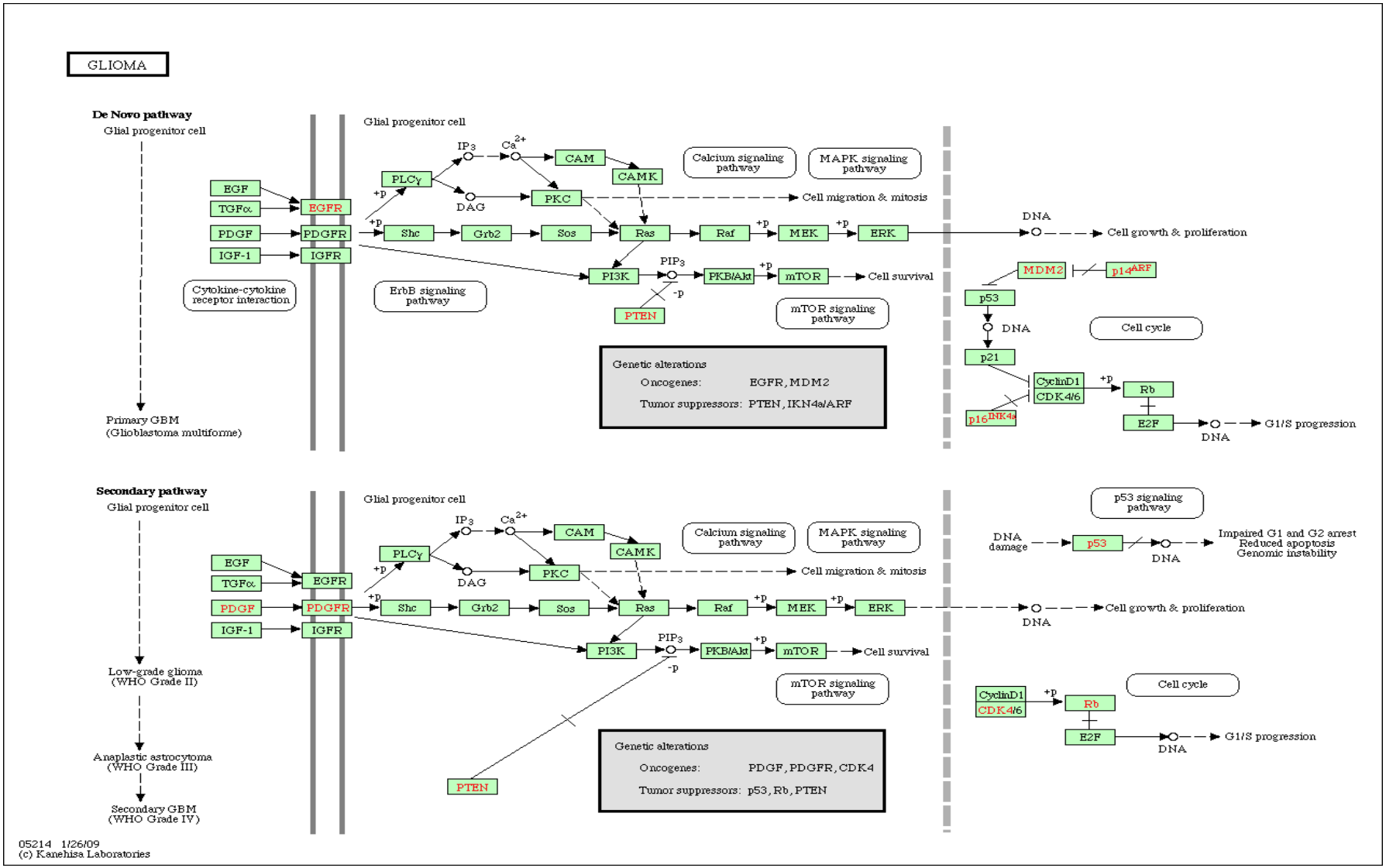
Thyroid cancer



Small cell lung cancer



glioma


GLIOMA
De Novo pathway
Glial progenitor cell
Primary GBM
(Glioblastoma multiforme)
Glial progenitor cell
EGF
TGFα
PDGF
IGF-1
EGFR
PDGFR
IGFR
PLCγ
IP3
Ca2+
CAM
CAMK
PKC
DAG
Shc
Grb2
Sos
Ras
Raf
MEK
ERK
PI3K
PIP3
PKB/Akt
mTOR
PTEN
Calcium signaling pathway
MAPK signaling pathway
Cell migration & mitosis
Cell growth & proliferation
Cell survival
Cytokine-cytokine receptor interaction
ErbB signaling pathway
mTOR signaling pathway
DNA
MDM2
p14ARF
p53
p21
Cell cycle
CyclinD1
CDK4/6
p16INK4a
Rb
E2F
G1/S progression
Genetic alterations
Oncogenes: EGFR, MDM2
Tumor suppressors: PTEN, IKN4a/ARF
Secondary pathway
Glial progenitor cell
Low-grade glioma
(WHO Grade II)
Anaplastic astrocytoma
(WHO Grade III)
Secondary GBM
(WHO Grade IV)
p53 signaling pathway
DNA damage
Impaired G1 and G2 arrest
Reduced apoptosis
Genomic instability
Genetic alterations
Oncogenes: PDGF, PDGFR, CDK4
Tumor suppressors: p53, Rb, PTEN
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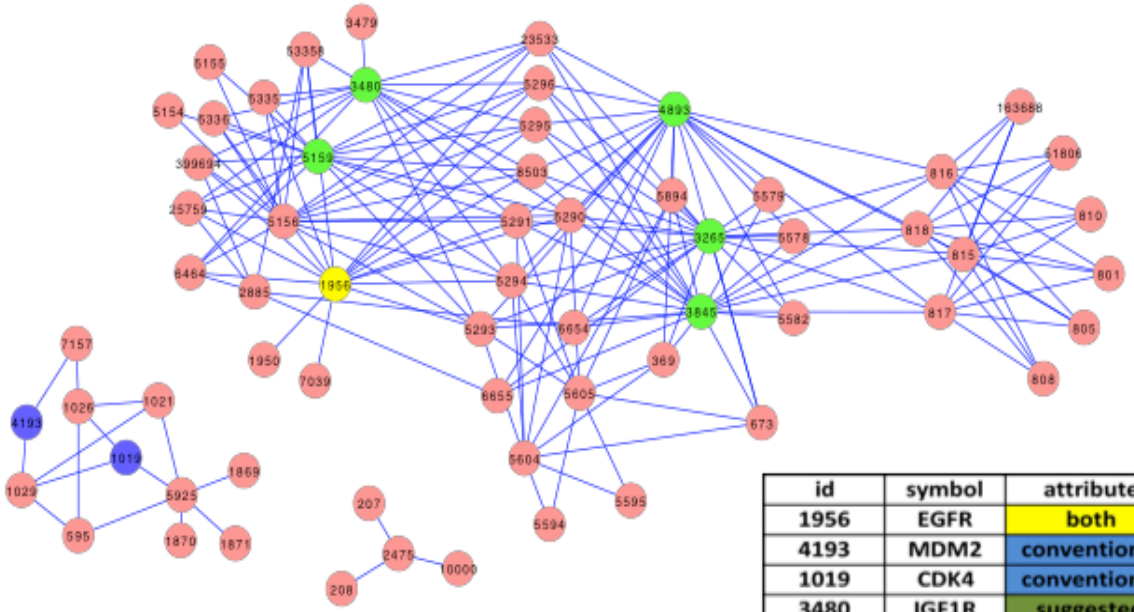
Glioma Carcinoma Protein-Protein Interaction Network



id	symbol	attribute
1956	EGFR	both
4193	MDM2	conventional
1019	CDK4	conventional
3480	IGF1R	suggested
3265	HRAS	suggested
4893	NRAS	suggested
3845	KRAS	suggested
5159	PDGFRB	suggested

Calculating Degree Entropy

The second network topology metric we explored, for which we did find correlation with 5-year survival probability, was network entropy, specifically degree-entropy, which is simply stated as:

$$H = -\sum_{k=1}^{N-1} p(k) \log p(k) \qquad (2)$$

where *N* is the total number of nodes in the network and *p(k)* is the degree (number of incident lines) of node k (Wang, et al. 2006). In words, the degree-entropy provides a measure of the network's heterogeneity and complexity.

Calculating Betweenness-Centrality

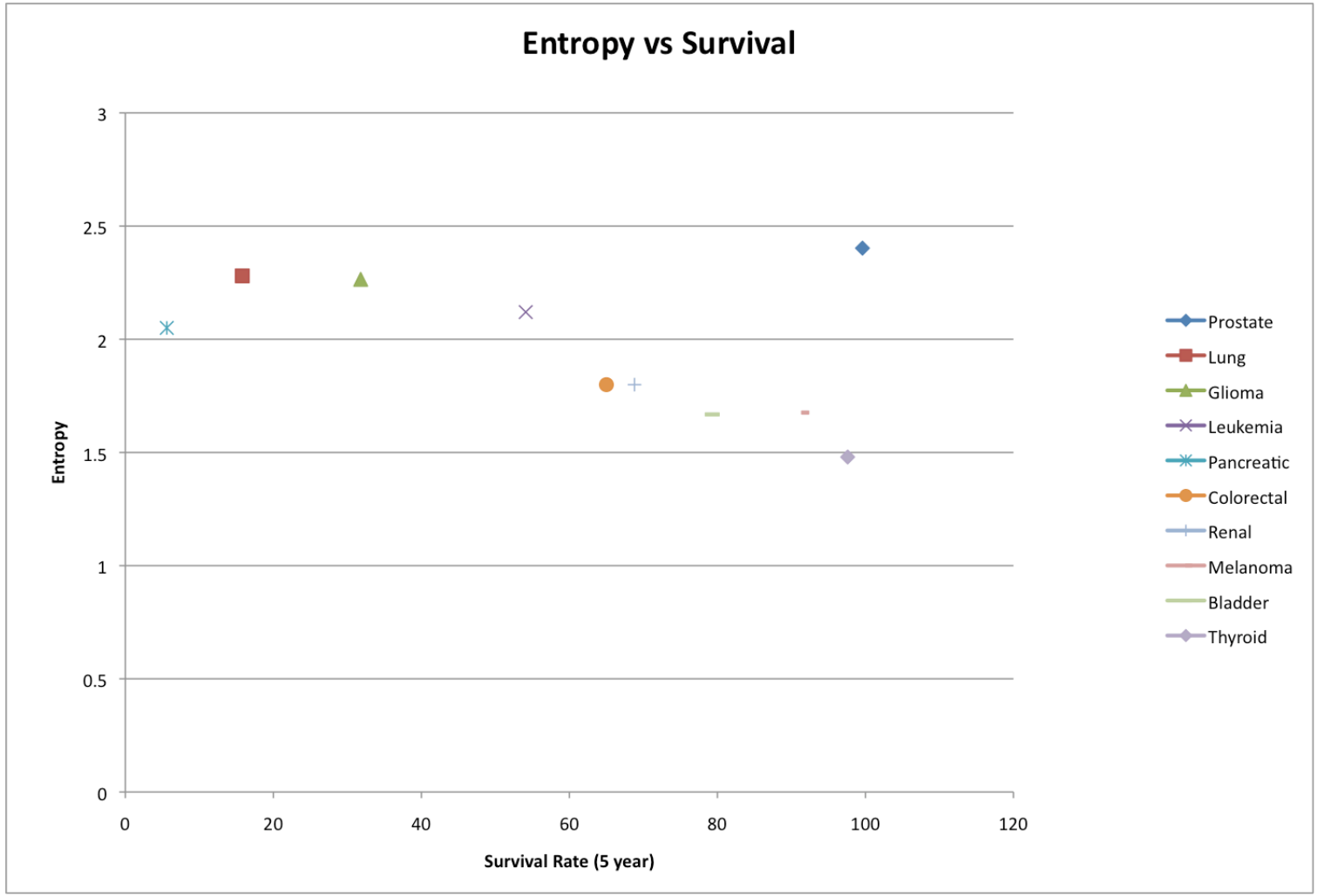
Betweenness centrality, or just betweenness, is a network topological metric and a measure of the centrality of a node, v_i. Specifically, it is the sum of the fractions of shortest paths that pass through v_i. The relation is given by

$$c_B(v) = \sum_{s \neq v \neq t} \frac{\sigma_{st}(v)}{\sigma_{st}} \qquad (1)$$

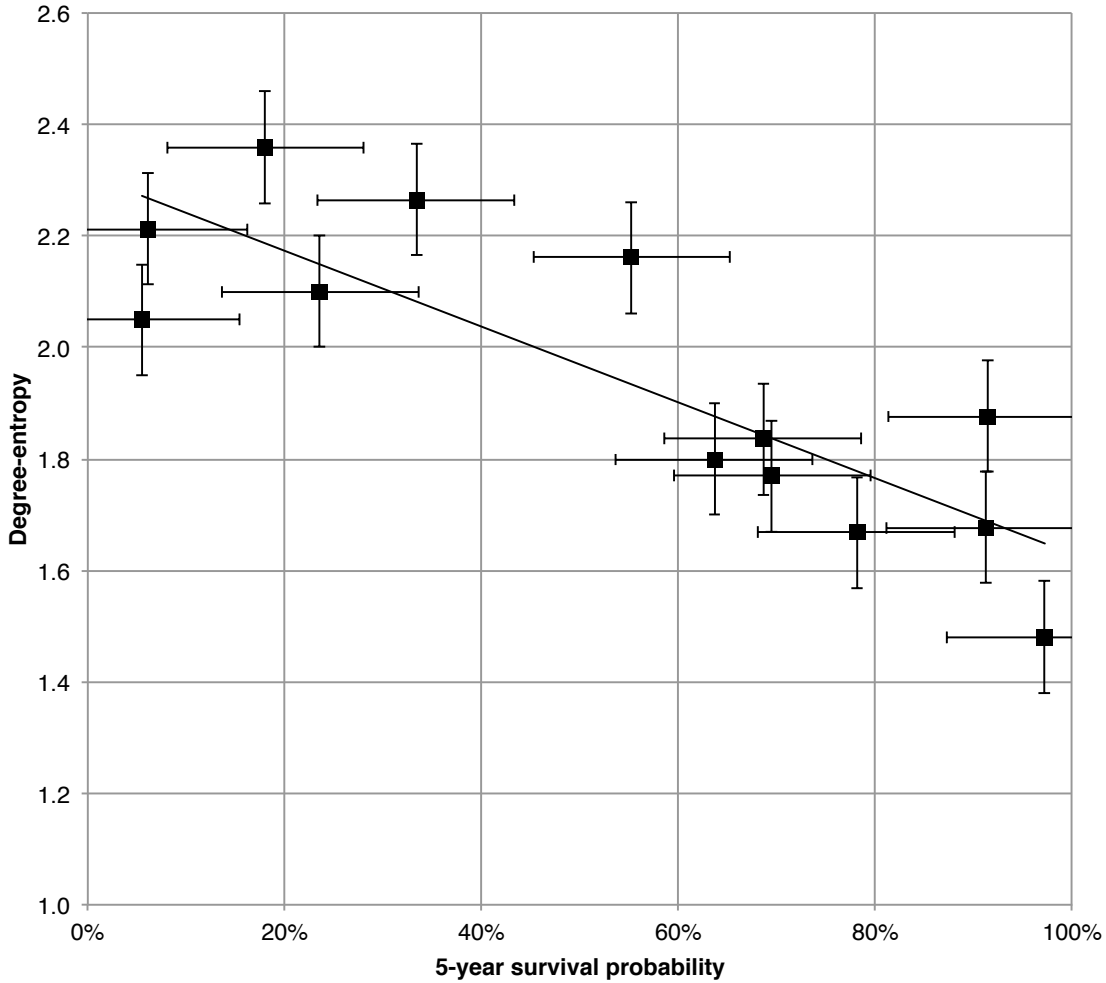
where σ_{st} is the number of shortest paths between two nodes (s,t) and $\sigma_{st}(v)$ is the number of those paths passing through v_i (Newman, 2010). In other words, betweenness centrality is a measure of the extent that a node lays on the paths between other nodes. This is important because it may indicate the influence within the network that this node plays in controlling information transfer between other nodes.

cancer	SEER	H	nodes	edges	degree	B1	B2	B3
AML	23.6	2.0998	60	170	5.533	2322	6688	3728
CML	55.2	2.1607	73	185	5.041	2885	4193	9846
colorectal	63.6	1.7994	62	104	3.3548	3845	5900	1499
glioma	33.4	2.2646	65	189	5.8154	1956	3480	5159
melanoma	91.2	1.6761	71	281	7.4648	4893	5604	5595
NSCL	18	2.3584	54	124	4.6481	3845	11186	595
renal	69.5	1.7691	70	109	3.1143	2549	5981	5594
SCL	6.2	2.212	84	219	5.2262	4792	5747	595
thyroid	97.2	1.4798	29	49	3.379	3265	4893	3845
bladder	78.1	1.668	42	46	2.1905	5605	5604	5595
endometral	68.6	1.8352	52	87	3.2308	2885	105	5170
basal	91.4	1.8768	55	310	11.273	2932	1499	2735
pancreatic	5.5	2.0501	70	137	3.9143	3845	10928	3716
prostate	99.4	2.4025	89	295	6.6292	2885	2932	207

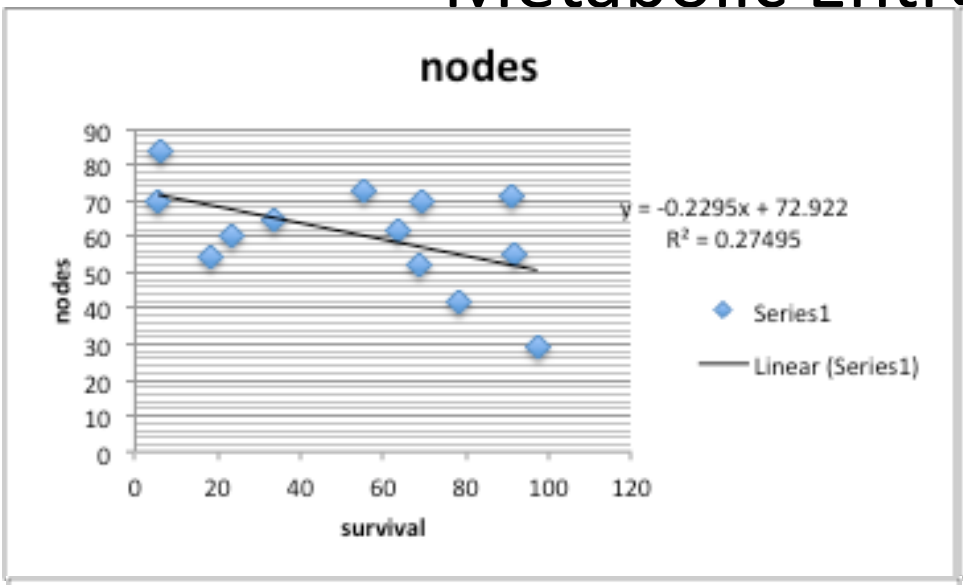
Table 1. Cancer, survival probability, network statistics. Here, H stands for degree-entropy; nodes for the number of nodes; edges for the number of edges; degree for the average degree. The symbols B1, B2, B3 indicate the Entrez ID's for the top three betweenness centrality nodes, respectively.

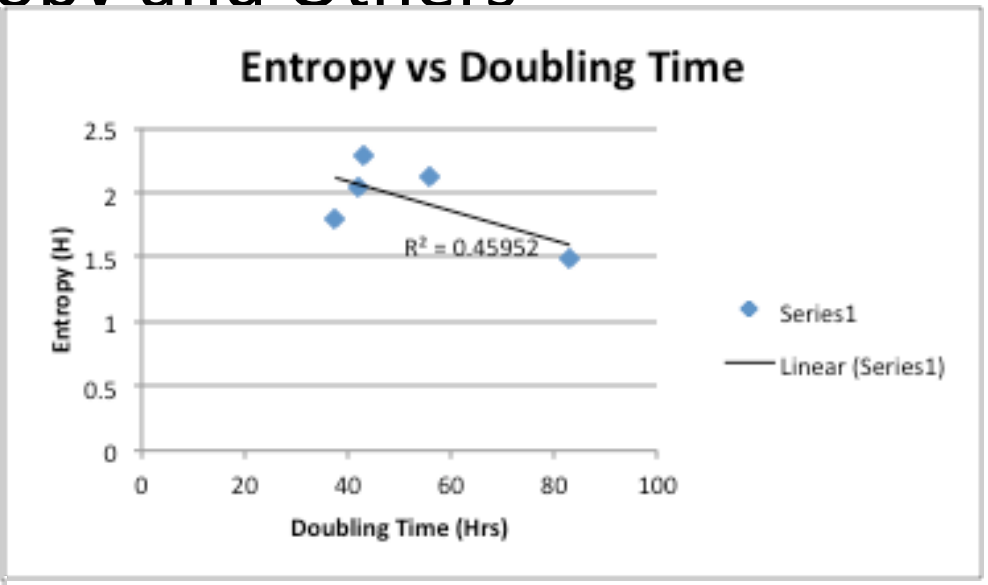

Entropy vs Survival
Entropy
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
Survival Rate (5 year)
Prostate
Lung
Glioma
Leukemia
Pancreatic
Colorectal
Renal
Melanoma
Bladder
Thyroid

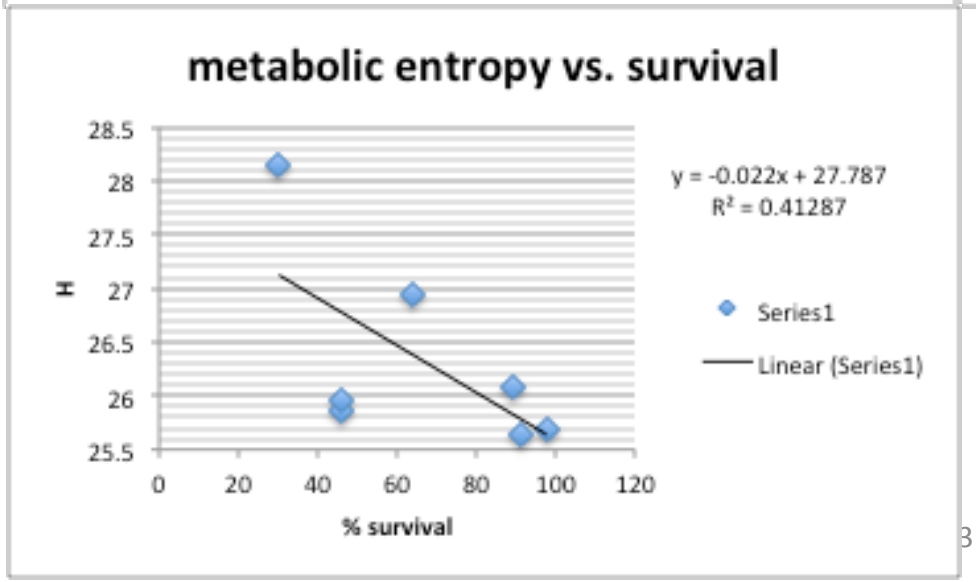
Main Result:
Pathway Network Degree Entropy vs. Survival
(correlation $R^2 = 0.702$)

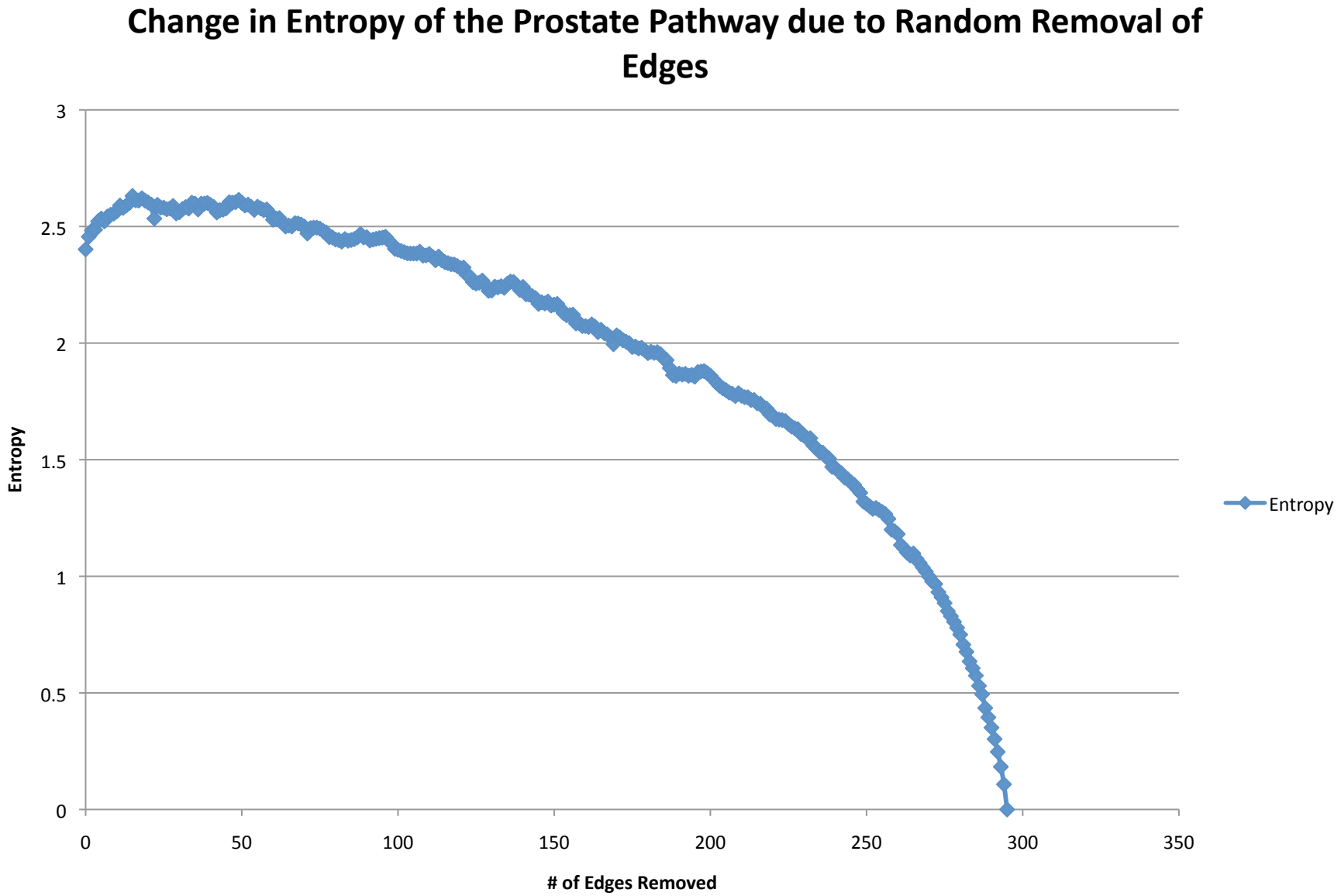


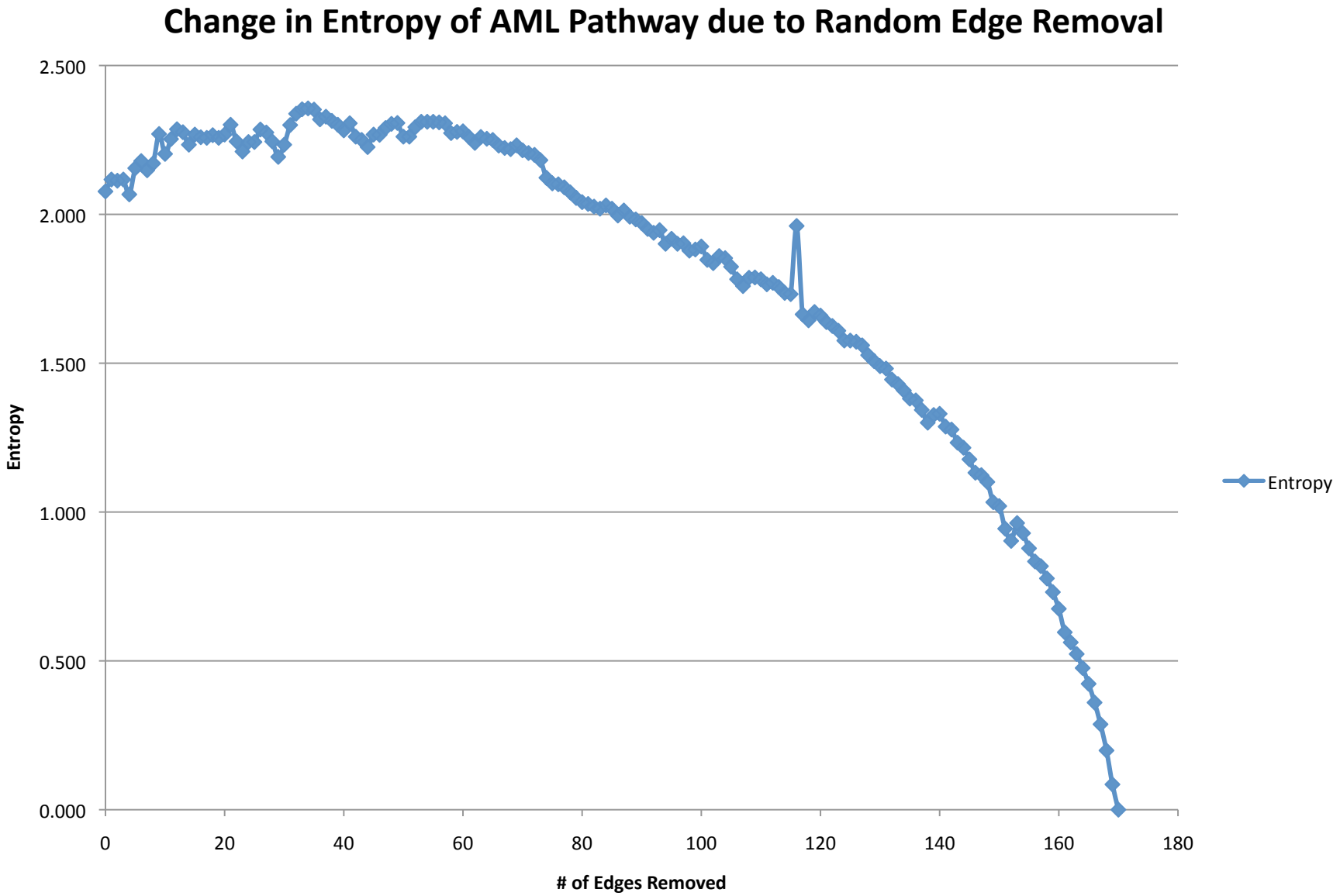
Preliminary Work in Progress: Metabolic Entropy and Others

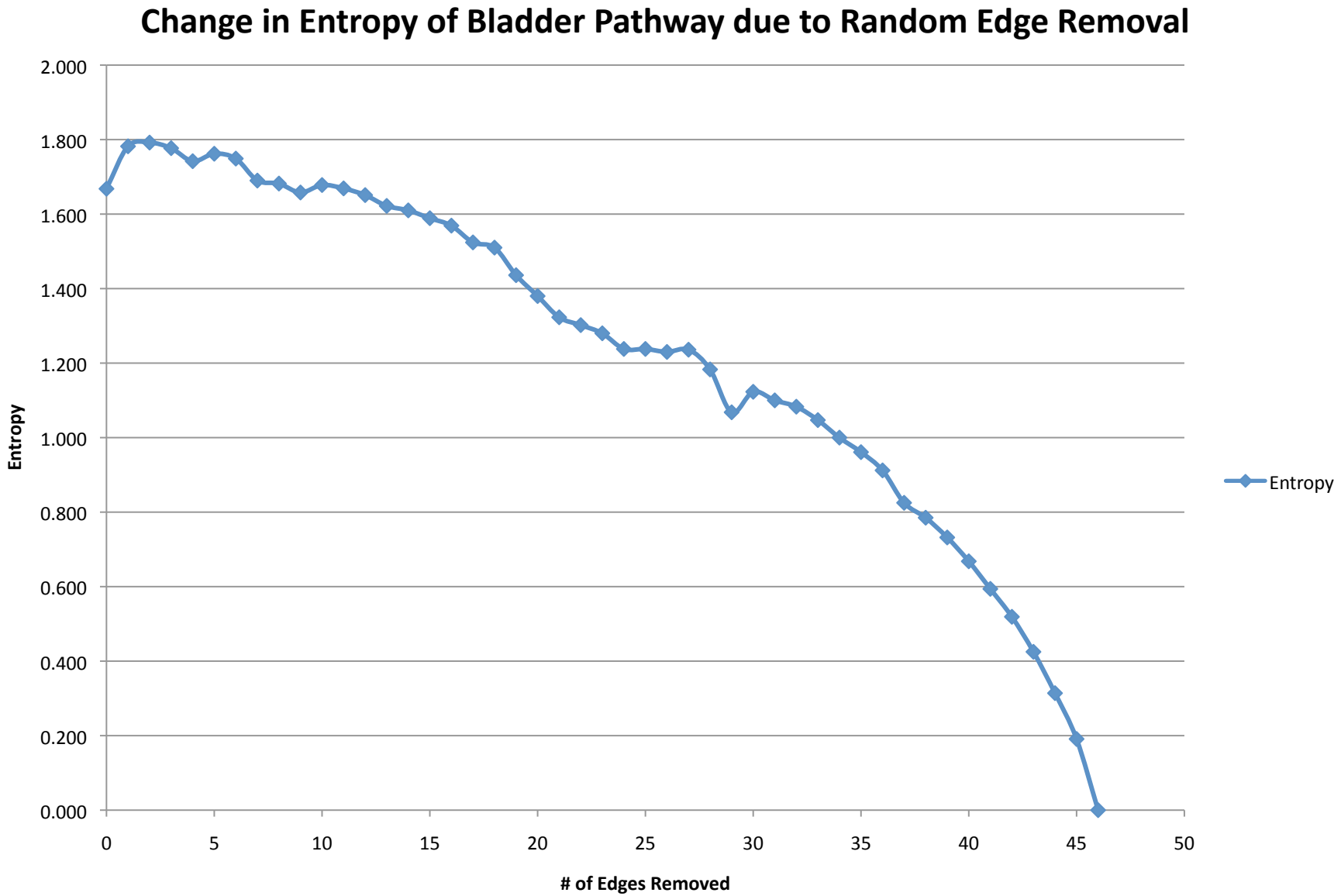


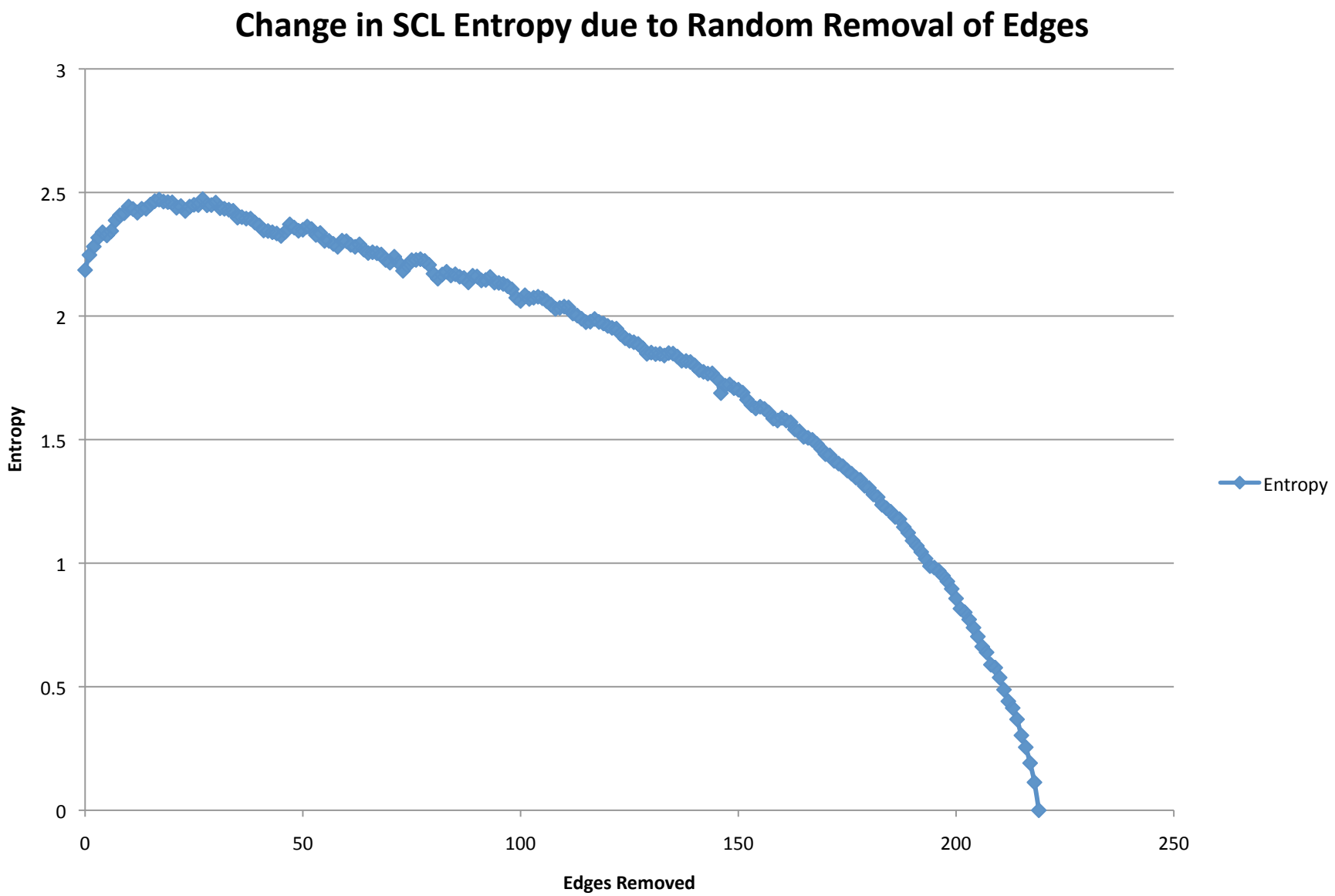


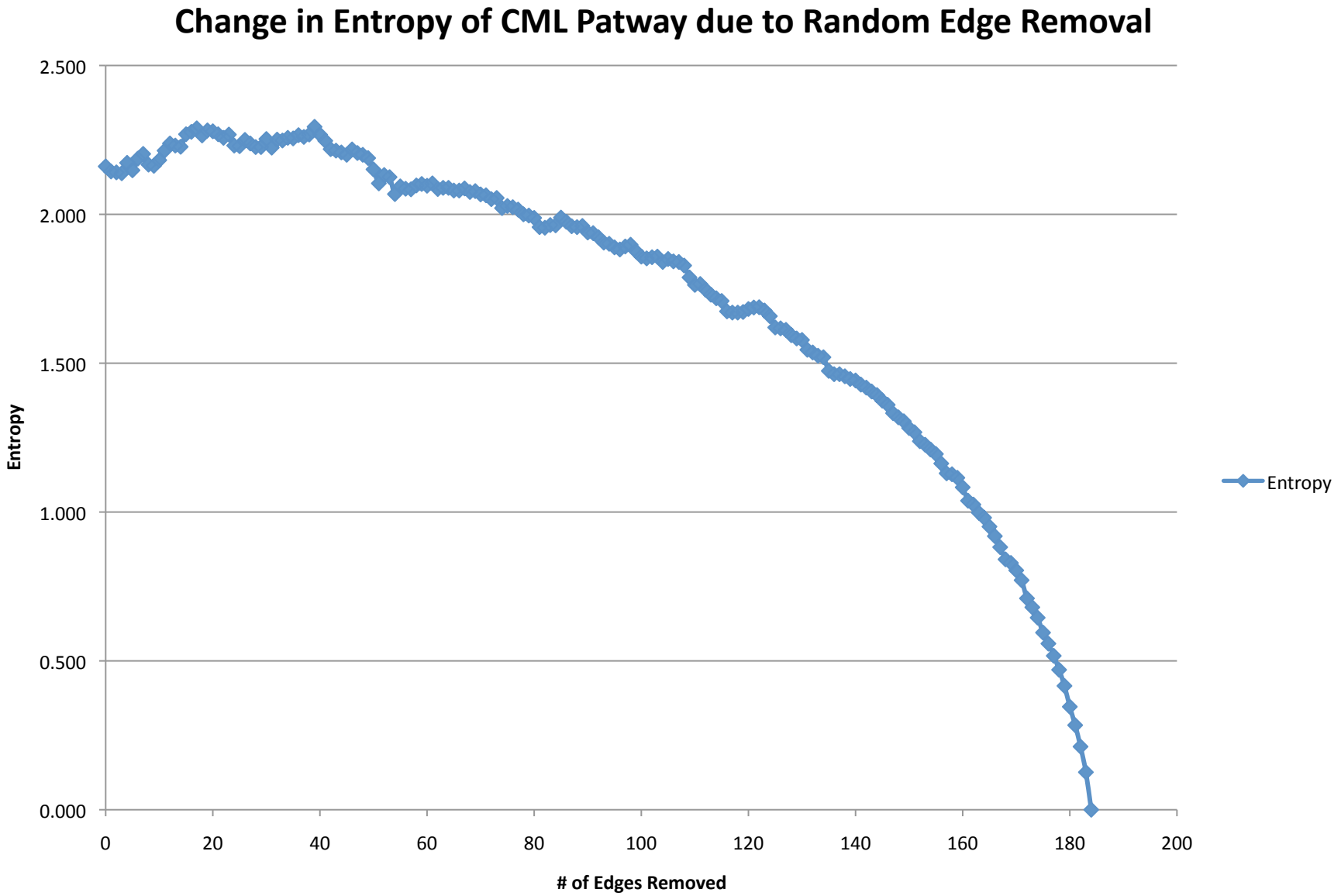



Change in Entropy of the Prostate Pathway due to Random Removal of Edges
Entropy
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
300
350
# of Edges Removed
Entropy


Change in Entropy of AML Pathway due to Random Edge Removal
2.500
2.000
1.500
1.000
0.500
0.000
Entropy
0
20
40
60
80
100
120
140
160
180
# of Edges Removed
Entropy


Change in Entropy of Bladder Pathway due to Random Edge Removal
2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Entropy
0
5
10
15
20
25
30
35
40
45
50
# of Edges Removed
Entropy

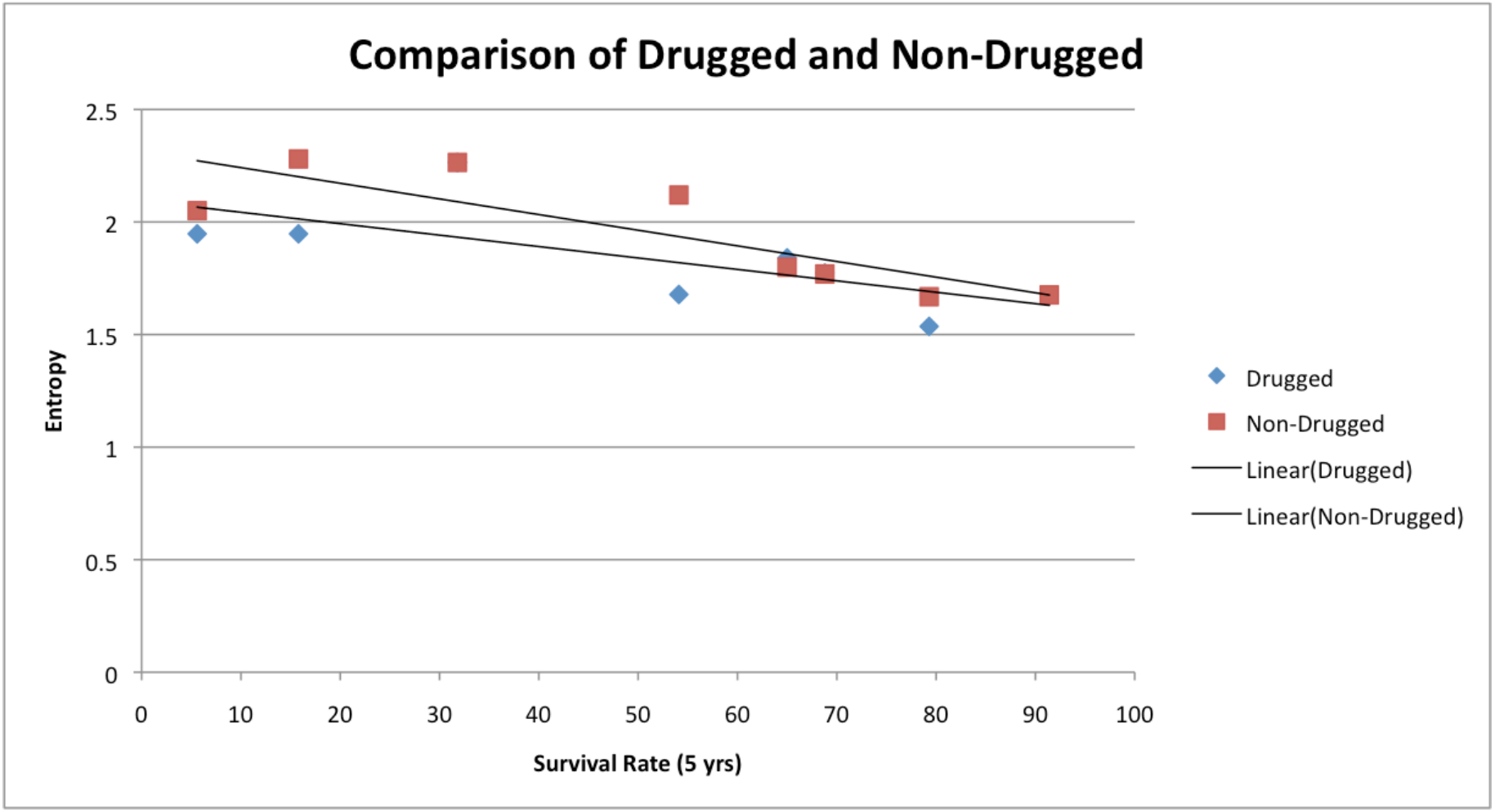

Change in SCL Entropy due to Random Removal of Edges
Entropy
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
Edges Removed
Entropy


Change in Entropy of CML Patway due to Random Edge Removal
2.500
2.000
1.500
1.000
0.500
0.000
Entropy
0
20
40
60
80
100
120
140
160
180
200
# of Edges Removed
Entropy

- A possible correlation between entropy and lethality is seen.
- These survival rates however, take into account all methods of treatment.
- To improve both the reliability and clarity of this correlation a few things are being done:
- Survival statistics of patients who refused treatment and those that only received chemotherapy will be used.
- Check how the random deletion of edges affects the entropy of each pathway.

Drugged Pathways

- Went through each of the pathways and altered them depending on the drugs that inhibited certain interactions.
- Inhibition was represented as the removal of certain parts of the graph that could only be reached by the inhibited interaction.
- The results were less than extraordinary, only a few pathways were altered by more than about $\Delta H=0.4$


Comparison of Drugged and Non-Drugged
Entropy
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
70
80
90
100
Survival Rate (5 yrs)
Drugged
Non-Drugged
Linear(Drugged)
Linear(Non-Drugged)

Examples of combinations

- POMP (prednisone, methotrexate, 6-mercaptopurine, vincristine)
- MOPP (nitrogen mustard, vincristine, procarbazine, prednisone)
- FAC (5-fluorouracil, doxorubicin, cyclophosphade)
- TAC (docetaxel, doxorubicin, cyclophosphamide)
- CMF (cyclophosphamide, methotrexate, fluoruracil)
- AC (doxorubicin, cyclophosphamide)
- FOLFOX (5-fluoruracil, leucovorin, oxaliplatin)

Table 1. Cancer survival probabilities and network statistics for 14 cancer types. The columns B1, B2, and B3 give the HGNC gene symbols[19] for the top three betweenness centrality nodes. Table reproduced from Breitkreutz *et al*[12].

Cancer Type	B1	B2	B3
Acute myeloid leukemia	FLT3	SPI1	JUP
Basal cell carcinoma	GSK3B	CTNNB1	GLI1
Bladder cancer	MAP2K2	MAP2K1	MAPK3
Chronic myeloid leukemia	GRB2	MDM2	GAB2
Colorectal cancer	KRAS	RALGDS	CTNNB1
Endometrial cancer	GRB2	ADARB2	PDPK1
Glioma	EGFR	IGF1R	PDGFRB
Melanoma	NRAS	MAP2K1	MAPK3
Non small-cell lung cancer	KRAS	RASSF1	CCND1
Pancreatic cancer	KRAS	RALBP1	JAK1
Prostate cancer	GRB2	GSK3B	AKT1
Renal cell carcinoma	GAB1	RFC1	MAPK1
Small cell lung cancer	NFKBIA	PTK2	CCND1
Thyroid cancer	HRAS	NRAS	KRAS

EGFR	Cetuximab	Trastuzumab	Lidocaine	Gefitinib
LAMA5	Alteplase	Reteplase	Anistreplase	Tenecteplase
FN1	Alteplase	Reteplase	Anistreplase	Tenecteplase
LAMB1	Alteplase	Reteplase	Anistreplase	Tenecteplase
LAMC1	Alteplase	Reteplase	Anistreplase	Tenecteplase
LAMA1	Alteplase	Reteplase	Anistreplase	Tenecteplase
LAMA3	Alteplase	Reteplase	Anistreplase	Tenecteplase
CSF3R	Pegfilgrastim	Filgrastim		
CSF2RA	Sargramostim			
RB1	Insulin recomb	Insulin Lyspro	Insulin Glargir	Insulin_ porcir
IGF1R	Insulin recomb	Insulin Lyspro	Insulin Glargir	Insulin_ porcir
FGFR2	Palifermin			
FGFR1	Palifermin			
FGFR3	Palifermin			
GRB2	Pegademase bovine			
TGFB1	Hyaluronidase			
ERBB2	Trastuzumab	Letrozole	Lapatinib	
PDGFRB	Becaplermin	Sorafenib	Imatinib	Dasatinib
THBS1	Becaplermin			
PDGFRA	Becaplermin	Imatinib	Sunitinib	
PLD1	Choline			
NOS3	L-Arginine	L-Citrulline	Tetrahydrobiop	Rosuvastatin
EGLN1	Vitamin C			
EGLN3	Vitamin C			
CREB1	Adenosine mo	Naloxone	Bromocriptine	
PIM1	Adenosine monophosphate			
GSTP1	Glutathione	Ethacrynic acid		
PRKCA	Phosphatidylse	Vitamin E		
PTGS2	gamma-Homo	Icosapent	Mesalazine	Acetaminophe
NOS1	L-Citrulline			
PPARG	Icosapent	Rosiglitazone	Atorvastatin	Pioglitazone
PPARD	Icosapent	Treprostinil	Sulindac	
ARAF	Adenosine triphosphate			
AKT1	Adenosine trip	Arsenic trioxide		
APAF1	Adenosine triphosphate			
ACVR1B	Adenosine triphosphate			
ABL1	Adenosine trip	Imatinib	Dasatinib	
RAC1	Pravastatin	Simvastatin		
RARA	Adapalene	Acitretin	Alitretinoin	Tazarotene
RXRB	Adapalene	Bexarotene	Acitretin	Alitretinoin
RARB	Adapalene	Acitretin	Alitretinoin	Tazarotene
RXRG	Adapalene	Acitretin	Alitretinoin	Tretinoin
RXRA	Adapalene	Acitretin	Alitretinoin	
SLC2A1	Etomidate			
BRAF	Sorafenib			
FLT3	Sorafenib	Sunitinib		
RAF1	Sorafenib			
KIT	Sorafenib	Imatinib	Dasatinib	Sunitinib
PDPK1	Celecoxib			
AR	Flutamide	Oxandrolone	Testosterone	Nilutamide
CALM1	Dibucaine	Fluphenazine	Isoflurane	Loperamide
SRD5A2	Azelaic Acid	Dutasteride		
JUN	Vinblastine	Irbesartan	Arsenic trioxide	
HRAS	Sulindac			
EGF	Sulindac			
MMP2	Sulindac	Marimastat		
MAPK3	Sulindac	Simvastatin	Isoproterenol	Arsenic trioxid
HSP90AA1	Rifabutin			
HSP90B1	Rifabutin			
RET	Imatinib			
NTRK1	Imatinib			
CSF1R	Imatinib	Sunitinib		
IL6	Simvastatin	Bicalutamide	Arsenic trioxid	Ginseng
IL8	Simvastatin	Zileuton	Salbutamol	Ketoprofen
BMP2	Simvastatin			
MMP9	Simvastatin	Marimastat	Minocycline	Glucosamine
CASP3	Simvastatin	Minocycline	Melatonin	
RHOA	Simvastatin	Atorvastatin		
FGF1	Pentosan Polysulfate			
FGF4	Pentosan Polysulfate			
FGF2	Pentosan Polysulfate			
ITGA2B	Tirofiban			
IKBKB	Auranofin	Arsenic trioxide		
CYCS	Minocycline	Melatonin		
NFKB1	Thalidomide	Dexamethasor	Pranlukast	
PIK3R1	Isoproterenol			
BCL2	Melatonin	Fludarabine	Paclitaxel	Docetaxel
STAT1	Fludarabine			
SRD5A1	Dutasteride	Finasteride		
CCND1	Arsenic trioxide			
MAPK1	Arsenic trioxide			
STAT5B	Dasatinib			
CTNNB1	Lithium			
GSK3B	Lithium			
MTOR	Everolimus	Temsirolimus		
HDAC1	Vorinostat			
HDAC2	Vorinostat			

Implications for Chemotherapy

- Three main avenues of application:
- The standard chemotherapeutic treatments can be investigated.
- Important nodes of the graphs may be ideal targets for new drugs.
- An accurate model of drug inhibition would allow for the development of new synergistic chemotherapy regimens.

Conclusion

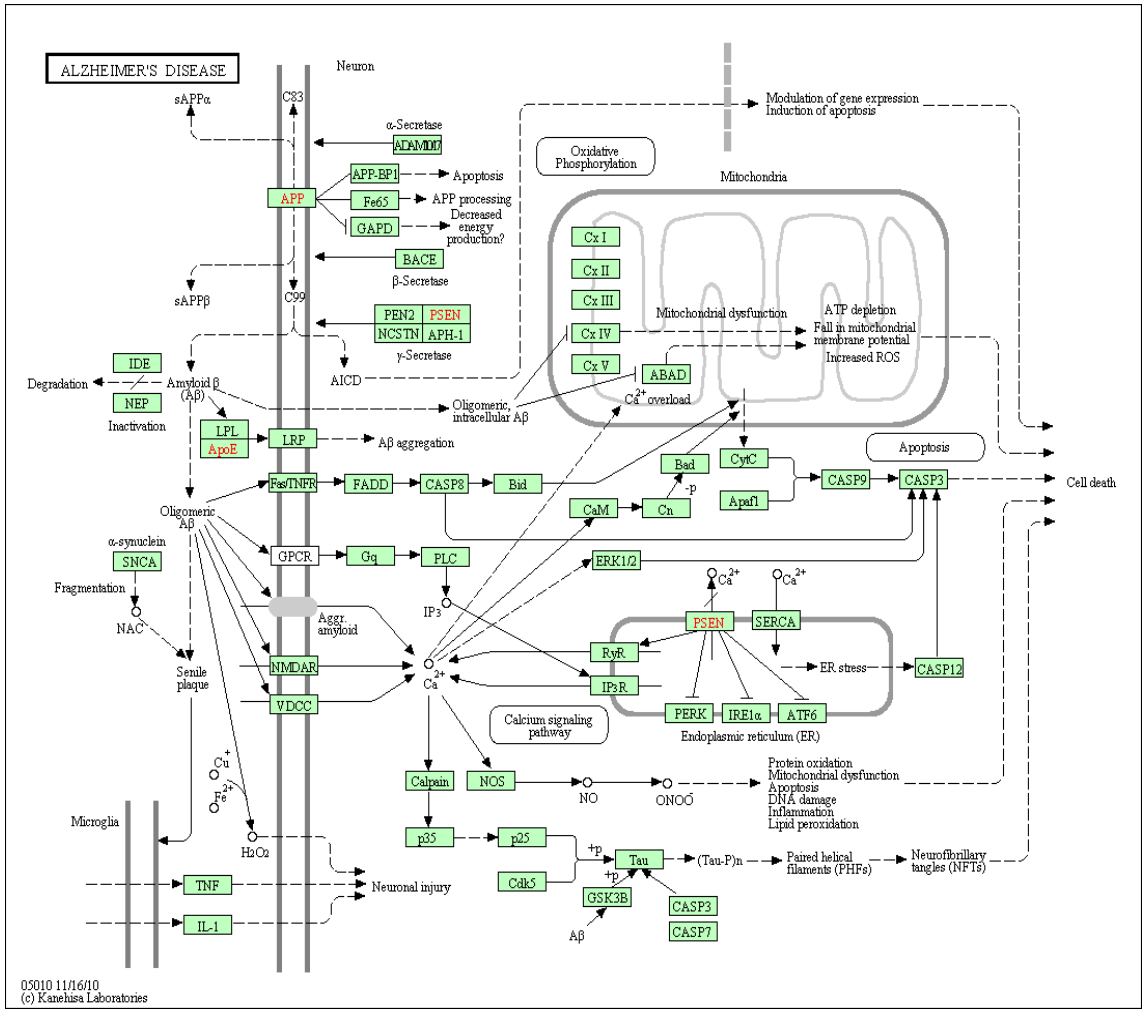
- Entropy looks like a promising quantitative measure of the robustness of biological networks.
- The next step will be to see how our current arsenal of cancer drugs affects these pathways.
- It may eventually be possible to use these pathways to create new synergistic chemotherapy regimens.

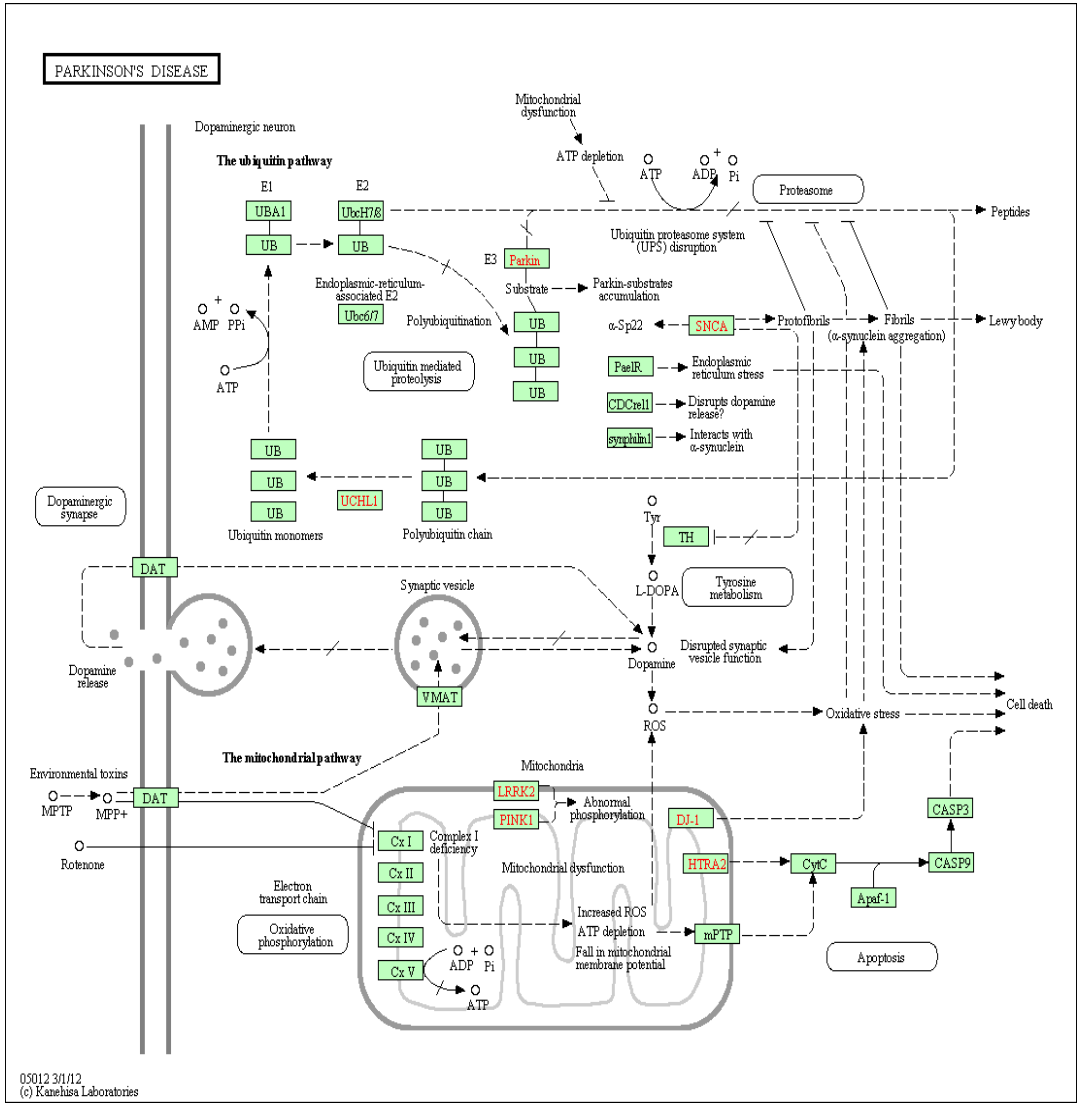
Table 2. Summary of the epidemiological data for the key neurodegenerative diseases.

Disease	Prevalence
Parkinson's Disease	0.3% of people in industrialized nations[21]
Amyotrophic Lateral Sclerosis	Affects 1 (or 2) per 100 thousand people[22]
Huntington's Disease	5–10 cases per 100 thousand persons[23]
Alzheimer's Disease	4.5 million US citizens[24]

Table 4. The top three betweenness centrality values for each NDD, along with the names of the associated protein with that value.

	First (B1)	Second (B2)	Third (B2)
Parkinson's	0.00775 (Cytochrome C)	0.00258 (Caspase 9)	0.00074 (Ubiquitin B)
Huntington's	0.00714 (Huntingtin)	0.00070 (Guanine Nucleotide Binding Protein)	0.00045 (Glutamate Receptor, NMDA-1)
Alzheimer's	0.00051 (Cyclin-dependent Kinase 5, reg. subunit 1 (p35))	0.00051 (Microtubule assoc. protein tau)	0.00043 (Guanine Nucleotide Binding Protein)
ALS	0.34392 (Superoxide dismutase, Cu-Zn Family)	0.23048 (Neural Filament Light Protein)	0.14824 (Apoptosis Regulator BCL-2)


ALZHEIMER'S DISEASE
Neuron
sAPPα
C83
α-Secretase
ADAM10?
APP
APP-BP1
Apoptosis
Fe65
APP processing
GAPD
Decreased energy production?
BACE
β-Secretase
sAPPβ
C99
PEN2
PSEN
NCSTN
APH-1
γ-Secretase
AICD
IDE
Degradation
Amyloid β (Aβ)
NEP
Inactivation
LPL
ApoE
LRP
Aβ aggregation
Oligomeric, intracellular Aβ
Modulation of gene expression
Induction of apoptosis
Oxidative Phosphorylation
Mitochondria
Cx I
Cx II
Cx III
Cx IV
Cx V
Mitochondrial dysfunction
ATP depletion
Fall in mitochondrial membrane potential
Increased ROS
ABAD
Ca2+ overload
Apoptosis
Bad
CytC
-p
Apaf1
CaM
Cn
CASP9
CASP3
Cell death
FasTNFR
FADD
CASP8
Bid
Oligomeric Aβ
α-synuclein
SNCA
Fragmentation
NAC
Senile plaque
GPCR
Gq
PLC
ERK1/2
Aggr. amyloid
IP3
Ca2+
PSEN
SERCA
RyR
IP3R
ER stress
CASP12
NMDAR
VDCC
PERK
IRE1α
ATF6
Endoplasmic reticulum (ER)
Calcium signaling pathway
Cu+
Fe2+
Microglia
H2O2
Calpain
NOS
NO
ONOO
Protein oxidation
Mitochondrial dysfunction
Apoptosis
DNA damage
Inflammation
Lipid peroxidation
p35
p25
Cdk5
+p
Tau
(Tau-P)n
Paired helical filaments (PHFs)
Neurofibrillary tangles (NFTs)
GSK3B
CASP3
CASP7
Aβ
TNF
IL-1
Neuronal injury
05010 11/16/10
(c) Kanehisa Laboratories


PARKINSON'S DISEASE
Dopaminergic neuron
Mitochondrial dysfunction
ATP depletion
ATP
ADP
Pi
The ubiquitin pathway
E1
E2
UBA1
UbcH7/8
Proteasome
Peptides
UB
E3
Parkin
Ubiquitin proteasome system (UPS) disruption
Endoplasmic-reticulum-associated E2
Ubc6/7
Substrate
Parkin-substrates accumulation
AMP
PPi
Polyubiquitination
α-Sp22
SNCA
Protofibrils
Fibrils
Lewy body
(α-synuclein aggregation)
Ubiquitin mediated proteolysis
PaeIR
Endoplasmic reticulum stress
CDCrel1
Disrupts dopamine release?
synphilin1
Interacts with α-synuclein
UCHL1
Ubiquitin monomers
Polyubiquitin chain
Dopaminergic synapse
Tyr
TH
DAT
L-DOPA
Tyrosine metabolism
Synaptic vesicle
Dopamine release
Dopamine
Disrupted synaptic vesicle function
VMAT
ROS
Oxidative stress
Cell death
The mitochondrial pathway
Environmental toxins
Mitochondria
MPTP
MPP+
LRRK2
Abnormal phosphorylation
PINK1
DJ-1
CASP3
Cx I
Complex I deficiency
Rotenone
Mitochondrial dysfunction
HTRA2
CytC
CASP9
Cx II
Electron transport chain
Apaf-1
Cx III
Increased ROS
Oxidative phosphorylation
ATP depletion
mPTP
Cx IV
Fall in mitochondrial membrane potential
Apoptosis
Cx V
ADP
Pi
ATP
05012 3/1/12
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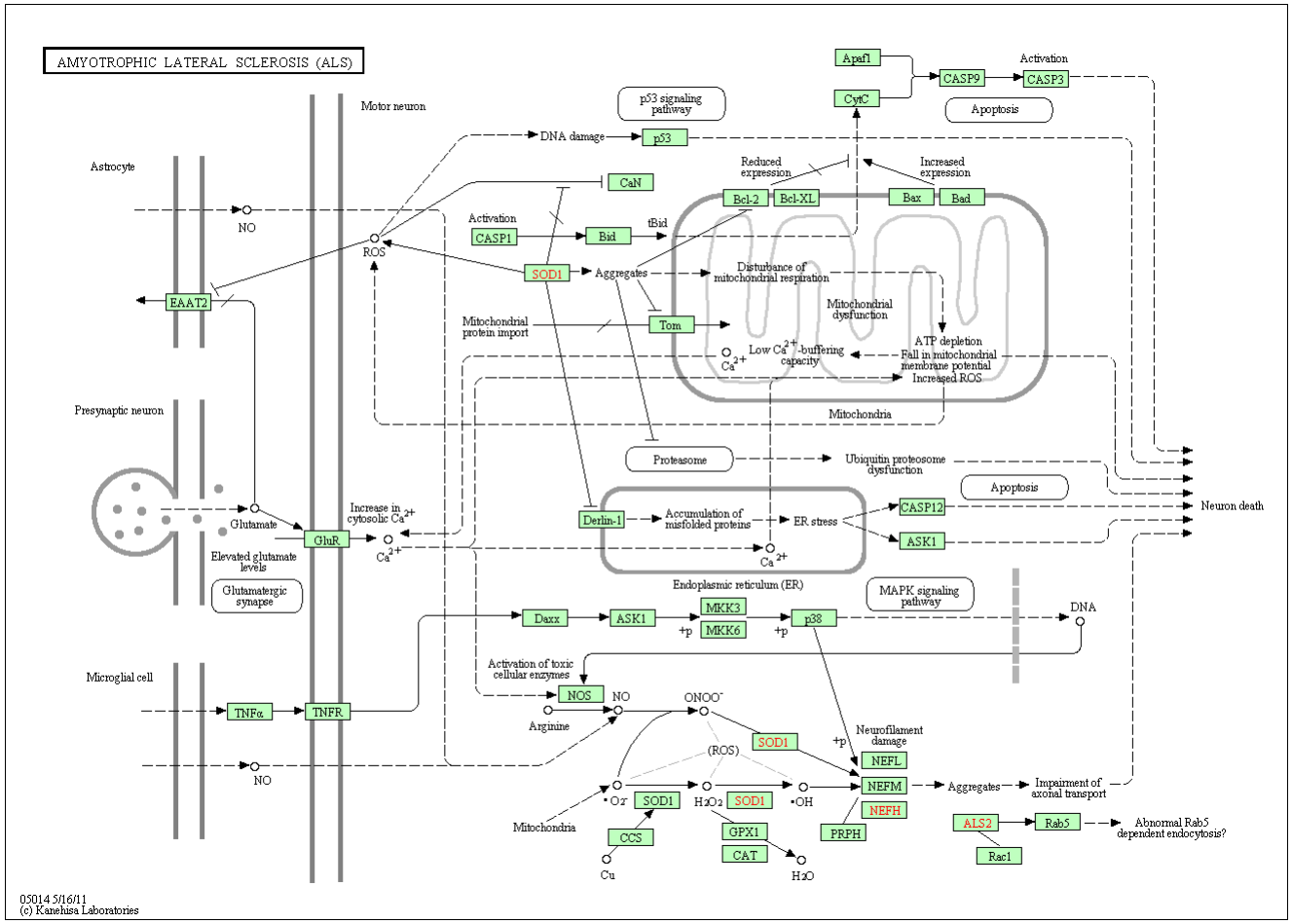
AMYOTROPHIC LATERAL SCLEROSIS (ALS)
Motor neuron
Astrocyte
Presynaptic neuron
Microglial cell
Glutamatergic synapse
Mitochondria
Endoplasmic reticulum (ER)
NO
ROS
EAAT2
Glutamate
Elevated glutamate levels
GluR
Increase in cytosolic Ca2+
Ca2+
TNFα
TNFR
p53 signaling pathway
DNA damage
p53
Apaf1
CytC
CASP9
CASP3
Activation
Apoptosis
CaN
Reduced expression
Increased expression
Bcl-2
Bcl-XL
Bax
Bad
Activation
CASP1
Bid
tBid
SOD1
Aggregates
Disturbance of mitochondrial respiration
Mitochondrial dysfunction
Mitochondrial protein import
Tom
Low Ca2+-buffering capacity
ATP depletion
Fall in mitochondrial membrane potential
Increased ROS
Proteasome
Ubiquitin proteosome dysfunction
Apoptosis
Neuron death
Derlin-1
Accumulation of misfolded proteins
ER stress
CASP12
ASK1
MAPK signaling pathway
DNA
Daxx
ASK1
MKK3
MKK6
+p
p38
Activation of toxic cellular enzymes
NOS
NO
ONOO-
Arginine
(ROS)
SOD1
+p
Neurofilament damage
NEFL
NEFM
NEFH
PRPH
Aggregates
Impairment of axonal transport
ALS2
Rab5
Abnormal Rab5 dependent endocytosis?
Rac1
Mitochondria
•O2-
SOD1
H2O2
SOD1
•OH
GPX1
CAT
H2O
CCS
Cu
05014 5/16/11
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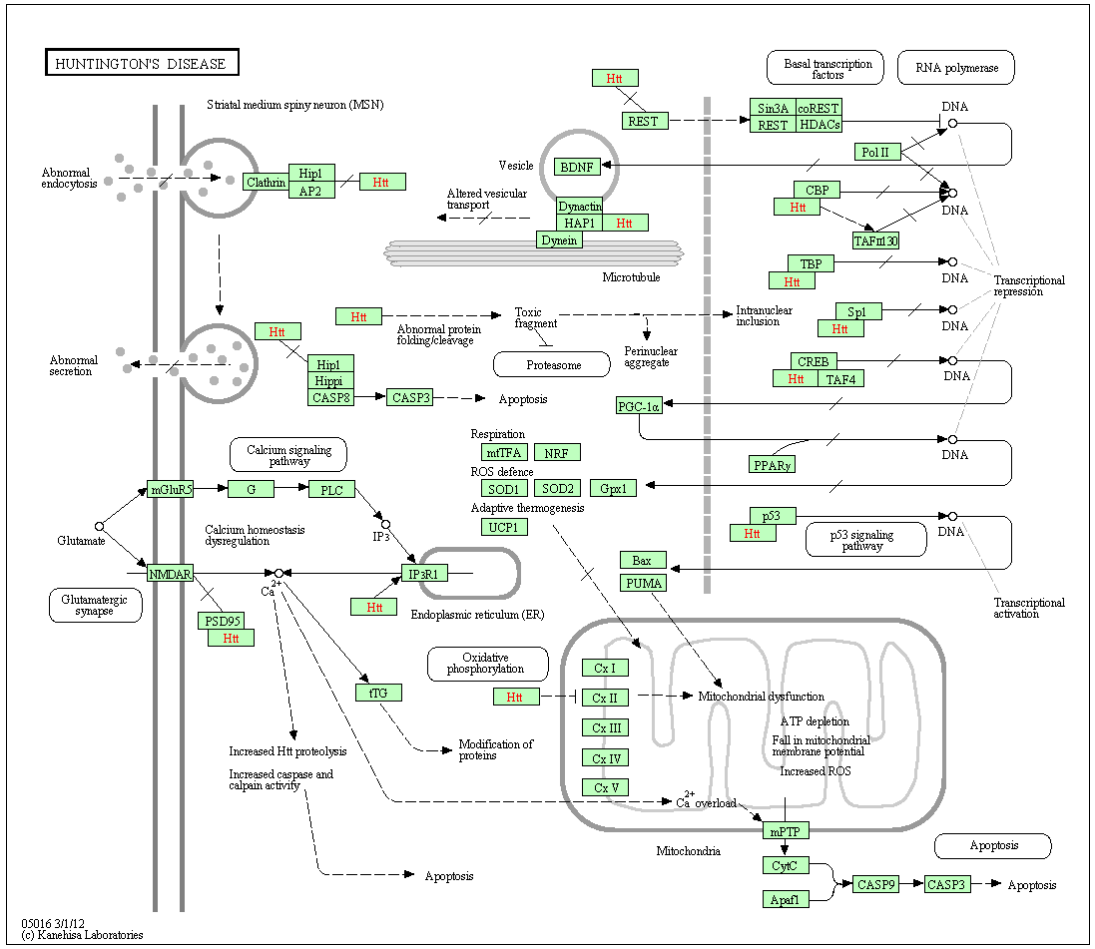
HUNTINGTON'S DISEASE
Striatal medium spiny neuron (MSN)
Abnormal endocytosis
Clathrin
Hip1
AP2
Htt
Vesicle
BDNF
Altered vesicular transport
Dynactin
HAP1
Dynein
Microtubule
Abnormal secretion
Hip1
Huntingtin
CASP8
CASP3
Apoptosis
Abnormal protein folding/cleavage
Toxic fragment
Proteasome
Perinuclear aggregate
Intranuclear inclusion
Basal transcription factors
RNA polymerase
REST
Sin3A
coREST
REST
HDACs
DNA
Pol II
CBP
TAFII 30
TBP
Sp1
CREB
TAF4
PGC-1α
PPARγ
Transcriptional repression
Transcriptional activation
Respiration
mtTFA
NRF
ROS defence
SOD1
SOD2
Gpx1
Adaptive thermogenesis
UCP1
p53
p53 signaling pathway
Bax
PUMA
Calcium signaling pathway
mGluR5
G
PLC
Glutamate
Calcium homeostasis dysregulation
IP3
NMDAR
IP3R1
Ca2+
Glutamatergic synapse
PSD95
Endoplasmic reticulum (ER)
Oxidative phosphorylation
Cx I
Cx II
Cx III
Cx IV
Cx V
Mitochondrial dysfunction
ATP depletion
Fall in mitochondrial membrane potential
Increased ROS
Ca2+ overload
mPTP
Mitochondria
CytC
Apaf1
CASP9
CASP3
Apoptosis
tTG
Modification of proteins
Increased Htt proteolysis
Increased caspase and calpain activity
Apoptosis
05016 3/1/12
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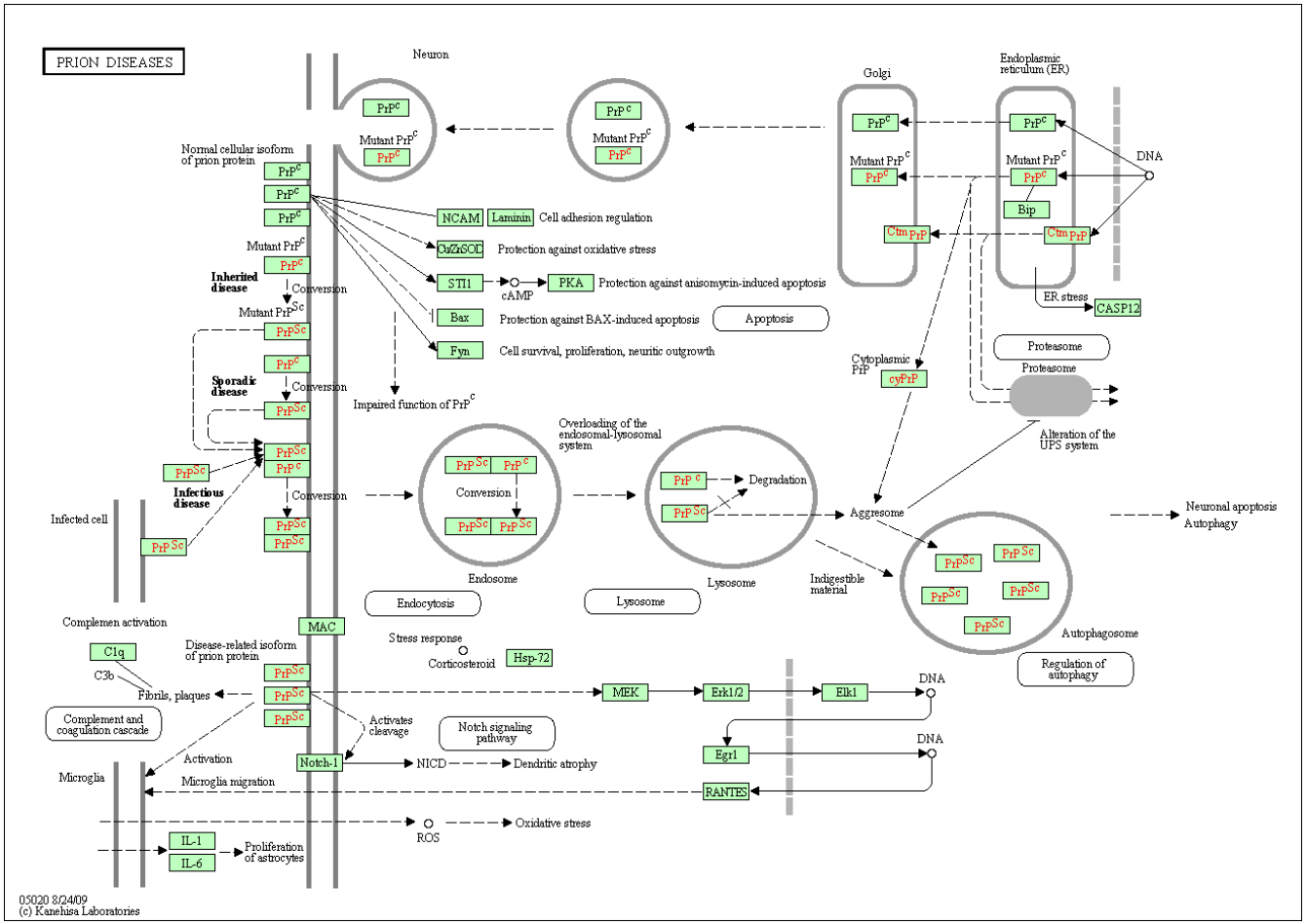
PRION DISEASES
Neuron
Golgi
Endoplasmic reticulum (ER)
Normal cellular isoform of prion protein
Mutant PrP^c
DNA
NCAM
Laminin
Cell adhesion regulation
CuZnSOD
Protection against oxidative stress
STI1
PKA
cAMP
Protection against anisomycin-induced apoptosis
Bax
Protection against BAX-induced apoptosis
Apoptosis
Fyn
Cell survival, proliferation, neuritic outgrowth
Bip
CtmPrP
ER stress
CASP12
Inherited disease
Conversion
Mutant PrP^Sc
Sporadic disease
Impaired function of PrP^c
Cytoplasmic PrP
cyPrP
Proteasome
Alteration of the UPS system
Infectious disease
Infected cell
Overloading of the endosomal-lysosomal system
Endosome
Endocytosis
Degradation
Lysosome
Aggresome
Indigestible material
Autophagosome
Neuronal apoptosis
Autophagy
Regulation of autophagy
Complemen activation
C1q
C3b
MAC
Disease-related isoform of prion protein
Fibrils, plaques
Complement and coagulation cascade
Stress response
Corticosteroid
Hsp-72
MEK
Erk1/2
Elk1
Activates cleavage
Notch signaling pathway
Notch-1
NICD
Dendritic atrophy
Egr1
RANTES
Activation
Microglia
Microglia migration
ROS
Oxidative stress
IL-1
IL-6
Proliferation of astrocytes
05020 8/24/09
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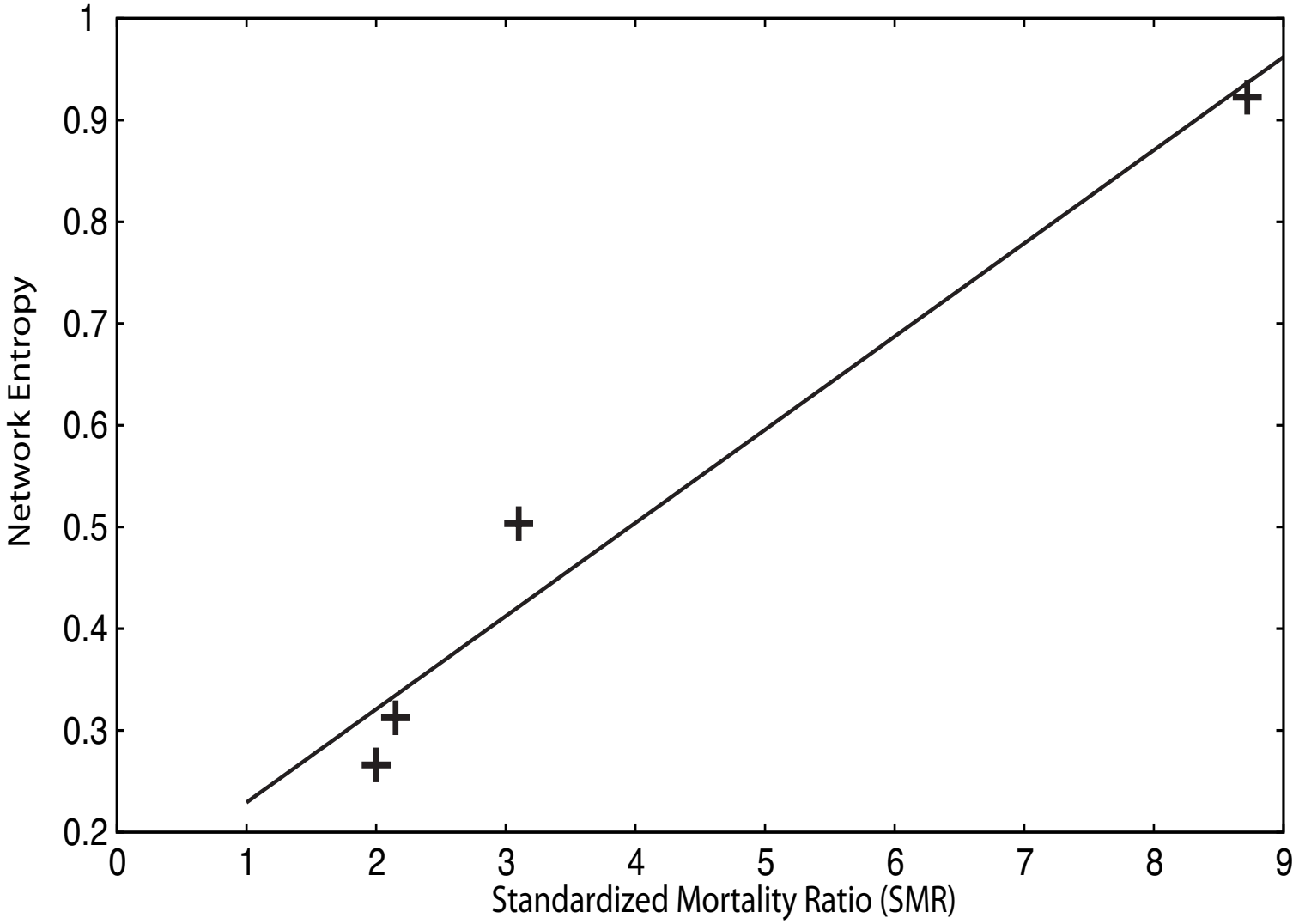
Results of the analysis of SMR as a function of network entropy for directed NDD networks with no nodes removed. The line is a linear fit with $R^2 = 0.96447$



Future Projects:

- build a mathematical model with the presence of both bio-molecules and their inhibitors
- simulate the action of individual drugs as well as their combinations by setting coupled ODE's with respect to time (find parameter values!)
- show why some drug combinations are not effective in stopping cancer due to parallel pathways and redundancies
- predict the optimum efficacy of drug combinations as a function of scheduling and amplitudes