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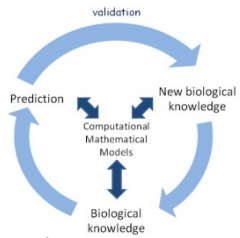


Center for Cancer Systems Biology

Breast Cancer Statistics

In the next 12 months, the American Cancer Society estimates:

>192,000 newly diagnosed cases of breast cancer

>40,000 women will die of breast cancer

One breast cancer death every 13 minutes (on average) in the USA

Jemal, et al., *CA Cancer J Clin* 59:225-249, 2009

Antiestrogens (TAM) and Clinical Outcomes

- ~70% of newly diagnosed cases are ERa positive (ER+) and may benefit from TAM

Age (Menopausal Status)	Risk Reduction[1]
Recurrence: <50 years (ER+)	45 ± 8%
Recurrence: 60-69 years (ER+)	54 ± 5%
Recurrence (ER-)	6 ± 11% (not significant)
Death: any cause <50 years (ER+)	33 ± 6%
Death: any cause 60-69 years (ER+)	32 ± 10%
Death: any cause (ER-)	-3 ± 11% (not significant)

- Reduction in risk is seen irrespective of menopausal status/age
- In postmenopausal patients, this benefit is comparable to that seen for cytotoxic chemotherapy

[1]Proportional reduction in the 10-year risk of recurrences from the Early Breast Cancer Trialists Group meta analyses

Intratumoral	17β-Estradiol
12 studies (all women) n=592	1.28 nM
Postmenopausal (n=34)	1.40 nM
Intratumoral	**Tamoxifen**
Estimated RBA Adjusted	320 nM [drug + metabolites]

Clarke, *et al., Pharmacol Rev* 53: 25-71, 2001

Endocrine Resistant Phenotypes

Two primary estrogen receptor phenotypes

- ERa positive (ER+)
  - about 50% of ER+ tumors are *de novo* resistant (25% if ER+/PgR+)
  - most acquired antiestrogen resistant tumors are ER+
- ERa negative (ER-)
  - almost all ER- tumors are *de novo* resistant
  - some acquired resistant tumors become ER-

Several pharmacological phenotypes

- Pharmacologic phenotypes
  - TAM stimulated (<20% of cases)
  - estrogen inhibited (~3% of cases)
  - antiestrogen unresponsive (>75% of cases)

Clarke *et al., Oncogene* 22: 7316-7339, 2003

Hypotheses

To understand why some ER+ breast cancers are (or become) resistant to endocrine therapies, we invoke an integrated, multimodal, *gene network* hypothesis

- network comprises multiple interacting signaling modules
- exhibits both redundancy and degeneracy

In the face of the stresses induced by endocrine therapies, the modules of interest are those that regulate the cell's choice

- to live or die
- if to live, whether or not to proliferate (cell cycling)
- if to die, how to die (apoptosis, autophagy, senescence, necrosis)

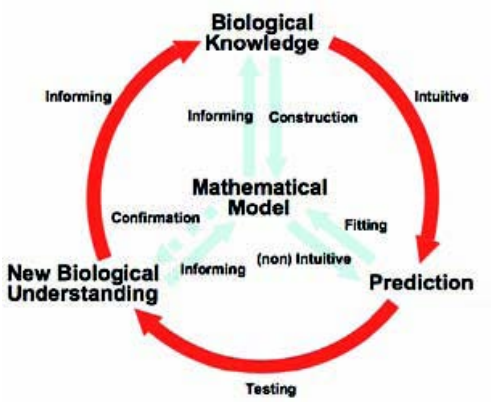
Measuring the expression and regulation of key components of this network, and using these data to construct predictive network models, will improve our ability to predict responsiveness in individual patients and identify new targets for therapeutic intervention

A systems biology approach is required to integrate knowledge from cancer biology with computational and mathematical modeling

Systems Biology in Breast Cancer Research

*Study of an organism viewed as an integrated and interacting network of genes, proteins, and biochemical reactions that give rise to life…**

- Systems biology goals
  - interactions among the components of a biological system
  - how these interactions control system function and behavior
  - integrate and analyze complex data from multiple sources using interdisciplinary tools
  - build *in silico* models of system (network) function



Systems Biology Research Cycle
Endocrinologist 94: 13, 2010

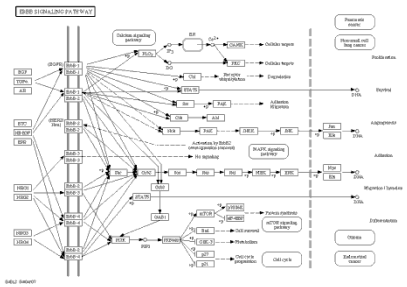
Biological cycle

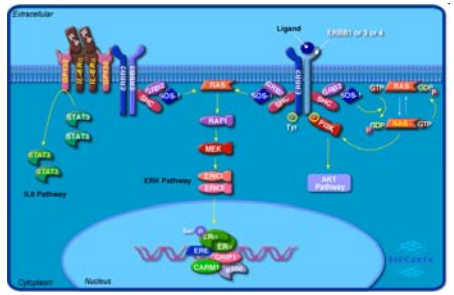
Integration with modeling

*Institute for Systems Biology

Signaling Networks

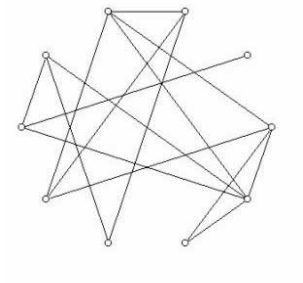
not KEGG



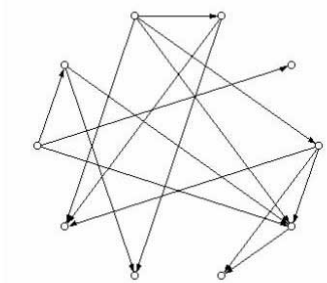


not BIOCARTA

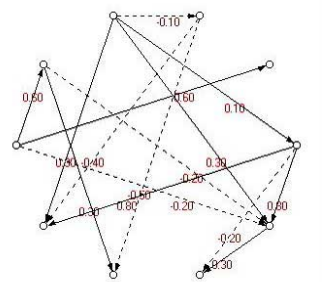
- Network is a graph with nodes/vertices and edges
- Nodes can represent different properties
  - gene, protein, transcription factor (TF), TF target
- Edges that connect nodes have directionality and weight
  - distance, strength of interaction, frequency of use



Nodes and Edges



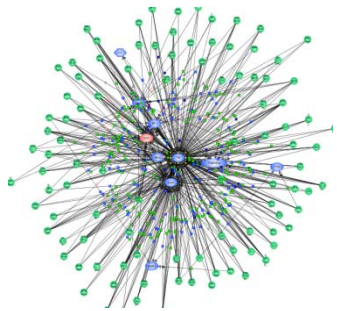
Nodes and Directed Edges



Nodes and Directed Edges with Weights

Network Modeling: Wicked but Tractable Problem

- Module(s) of interest is a subnet within the entire human interactome
- Search space is immense (very high dimensionality)
  - about 30,000 genes in the human genome
  - perhaps 650,000 protein interactions alone
  - many latent variables (sparce data)
- We don't know all of the genes/proteins involved
  - their properties/functions/connectivity
  - topology of the subnetwork module(s) is unknown
  - effect of cellular context on connectivity and function
- Network will be high dimensional (even with few nodes)
  - curse of dimensionality
  - confound of multimodality
- Large networks have unique properties
  - scale free
  - small world
  - critical threshold

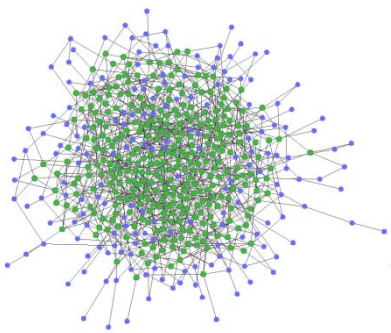


All connections from only 11 seeds

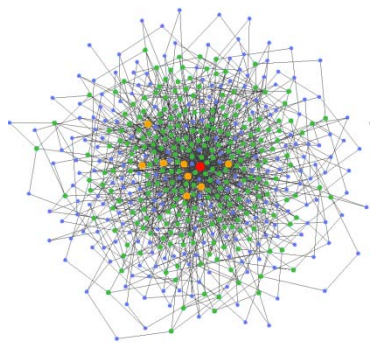
Clarke *et al., Nature Rev Cancer* 8: 37-49, 2008
Stumpf *et al., PNAS* 2008

Scale Free Networks and Connectivity

- Scale-free networks (thought to include most biological systems)
  - connectivity (probability that a node interacts with *k* other nodes) follows a power-law distribution $P(k)=k^{-\gamma}$
  - most nodes are connected to a small proportion of other nodes
  - small proportion of nodes are highly connected (hubs)
  - *modular* with a hierarchical structure linking modules
- Individual nodes are very stable to disruption
  - stay interconnected even with high nodal failure rates (error-tolerant)
- Vulnerable to attacks of the hubs
  - may be good candidate biomarkers of network integrity and for drug discovery
  - failure to target multiple hubs will lead to poor responses and/or short response duration with (often rapid) onset of drug resistance



Random



Scale-free

Addressing Network Modeling Challenges

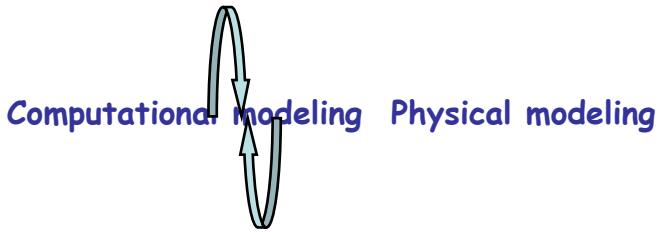
- Top Down (ease curse of dimensionality)
  - reduce dimensionality
  - identify knowledge enriched gene pool subsets/modules
  - allow more than one module to represent a function (degeneracy)

- Bottom Up (learn local node-edge-hub topology)
  - start with small number of select nodes (often from top down approach)
  - allow genes to be in more than one module (redundancy and multimodality)

- Model Properties
  - robust, reproducible, valid across closely related conditions
  - propose testable hypotheses for validation in wet lab studies
  - incorporate knowledge from multiple sources

Approaches to Network Modeling

- For endocrine resistance, our ultimate goal is to model how ER regulates molecular signaling and cellular functions to affect the responsiveness of breast cancer cells to these therapies



- We apply both computational and mathematical modeling tools
  - computational models can find local topologies or modules within high dimensional data using multiple different methods (top down)
  - mathematical models can represent local topologies or modules by a series of differential equations, stochastic reaction networks, *etc.* (bottom up)
  - data from patient specimens, chemically-induced rodent models, xenografts, and breast cancer cell lines

Zhang *et al., PLoS ONE,* 5 (4): e10268, 2010
Chen *et al., Bioinformatics,* 26: 1426-1422, 2010
Yu *et al., J Mach Learn Res,* 11;2141-2167, 2010
Wang *et al., BMC Bioinformatics,* 11:162, 2010
Clarke *et al., Nature Rev Cancer* 8: 37-49, 2008
Wang *et al., Bioinformatics,* 23: 2024-2027, 2007

Zhang *et al., Bioinformatics* 25: 526-532, 2009
Chen *et al., Int J Data Mining Bioinformatics,* 3: 365-381, 2009
Zhang *et al., BMC Genomics,* 10:S15, 2009
Zhu *et al., BMC Bioinformatics,* 9: 383, 2008
Wang *et al., BMC Bioinformatics,* 9: S21, 2008
Xuan *et al., EURASIP J Bioinformat System Biol,* 2007

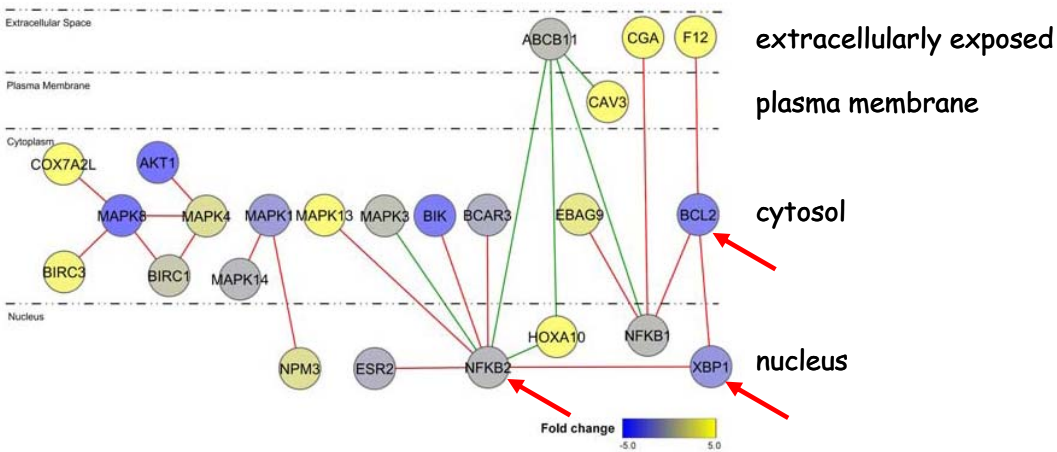
Two Studies of Cell Fate Signaling

Computational Modeling of Hormone Resistance

- Time course data in ER+ breast cancer cell lines treated with 17β-estradiol (E2) and/or Fulvestrant (Lin et al, 2004)
  - hypothesis: that time dependent changes in gene expression identify new topological features of ER-driven signaling
  - Affymetrix platform
- Sensitive (LCC1) vs. resistant (LCC9) human breast cancer variants
  - hypothesis: that differences in gene expression patterns will identify new topological features of hormone resistance signaling
  - Affymetrix, 2D-gel, and SAGE data

Differential Dependency Network Analysis

- Represent the local structures of a network by a set of local conditional probability distributions – decompose the entire expression profile into a series of local networks (nodes and their parents)
  - local dependency is learned
  - local conditional probabilities are estimated from linear regression model
  - allow more than one conditional probability distribution per node
  - Lasso technique is used to limit overfitting
- Identify motifs and "hot spots" within motifs
  - T47D cells ± E2; ±Fulvestrant (data from Lin *et al., Genome Biol* vol 5, 2004)
  - key nodes identified include XBP1, NFκB, BCL2



Wang *et al., Bioinformatics*, 2009

Two Studies of Cell Fate Signaling (#2)

Computational Modeling of Hormone Resistance

- Time course data in breast cancer cell lines treated with 17β-estradiol (E2) and/or Fulvestrant (Lin et al, 2004)
  - hypothesis: that time dependent changes in gene expression identify new topological features of ER-driven signaling
  - Affymetrix platform
- Sensitive (LCC1) vs. resistant (LCC9) human breast cancer variants
  - hypothesis: that differences in gene expression patterns will identify new topological features of hormone resistance signaling
  - Affymetrix, 2D-gel, and SAGE data

Genes Associated with Endocrine Resistance

LCC1 vs. LCC9: Genes selected from our SAGE, gene microarray, and 2D-gel data sets

Gene Name	Gene Symbol[1]	Difference	p-value
Genes Up-regulated in LCC9 vs. LCC1			
Cathepsin D	CTSD	5-fold	<0.001
X-box Binding Protein-1	XBP1	4-fold	<0.001
Heat Shock Protein 27	HSBP1	2-fold	0.001
Nucleophopsmin (numatrin)	NPM1	2-fold	0.01
Vitamin B12 Binding Protein	TCN1	2-fold	0.002
NFκB (p65)	RELA	2-fold	<0.05
Genes Down-regulated in LCC9 vs. LCC1			
Death Associated Protein 6	DAXX	6-fold	0.049
Early Growth Response-1	EGR1	3-fold	<0.05
Interferon Regulatory Factor-1	IRF1	2-fold	<0.05
Tumor Necrosis Factor-α	TNF	2-fold	<0.05
TNF-Receptor 1	TNFRSF1A	2-fold	<0.05

Data are mean values of the relative level of expression for each gene to the nearest integer; [1]Gene Symbols as approved by HUGO

Gu *et al., Cancer Res* 62: 3428-3437, 2002
Other unpublished data

Genes Regulated by XBP1(s) Overexpression

Symbol	*Gene Name*	*Change*	*p-value*	*# CREs*
APBB2	amyloid beta (A4) precursor protein-binding	-1.3	0.001	1
BCL2	**B-cell CLL/lymphoma-2**	**3.1**	**0.029**	**3**
CRK	v-crk sarcoma virus CT10 oncogene homolog	-2.0	0.003	2
ESR1	**estrogen receptor alpha (ERα)**	**2.8**	**0.040**	**0***
IL24	interleukin 24	-9.7	<0.001	1
MYC	v-myc myelocytomatosis viral oncogene homolog	1.6	0.04	1
PHLDA2	pleckstrin homology-like domain, family A, member 2	-3.3	0.004	2
S100A6	S100 calcium binding protein A6 (calcyclin)	2.3	0.001	1
XRCC6	X-ray repair complementing defective repair 6	1.6	0.016	1

*several ATF6 sites that may be regulated by ATF6:XBP1 heterodimers

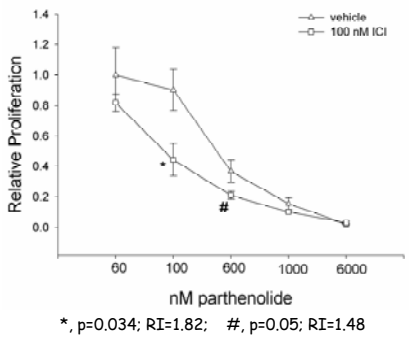
Gomez *et al., FASEB J* 21:4013-27, 2007

Key Nodes are Found by Different Methods

Different methods and different comparisons find some common genes

Gene Symbol	Global LCC1:LCC9	mNCA LCC1:LCC9	DDN T47D	Local MCF7/XBP1	Local MCF7/IRF1	Validated LCC1:LCC9
BCL2			X	X	X	X
BCL2L2					X	
EGR1	X	X				
ERα		X		X		X
IRF1	X					X
NFkB	X		X			X
NPM	X		X			X
MYC		X		X		
XBP1	X		X			X

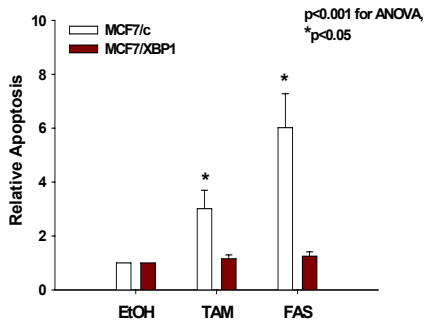
Inhibiting NFκB Restores ICI Sensitivity



*, p=0.034; RI=1.82; #, p=0.05; RI=1.48

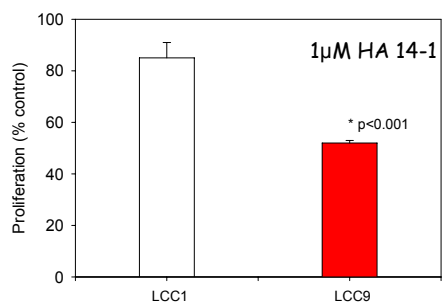
Riggins *et al., Mol Cancer Ther* 4: 323-412, 2005

XBP1 confers antiestrogen resistance



Gomez *et al., FASEB J* 21:4013-27, 2007

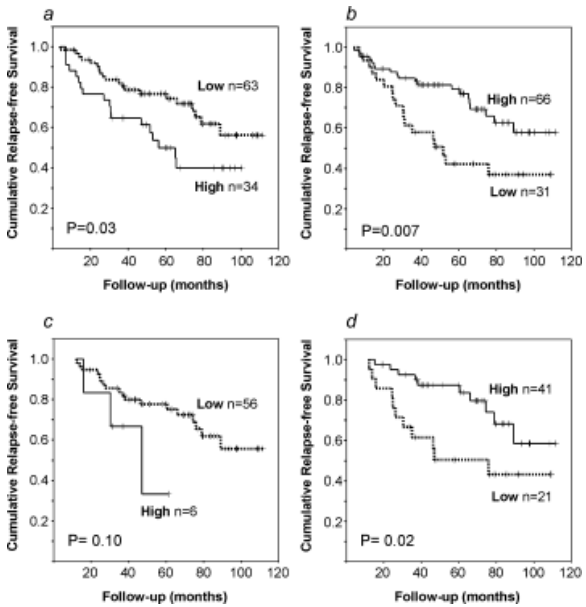
LCC9 cells are sensitive to BCL2 inhibition



Crawford *et al., PLoS ONE, 2010*

Key Nodes are Associated with Clinical Outcome

XBP1 and TAM recurrence
n=100 cases

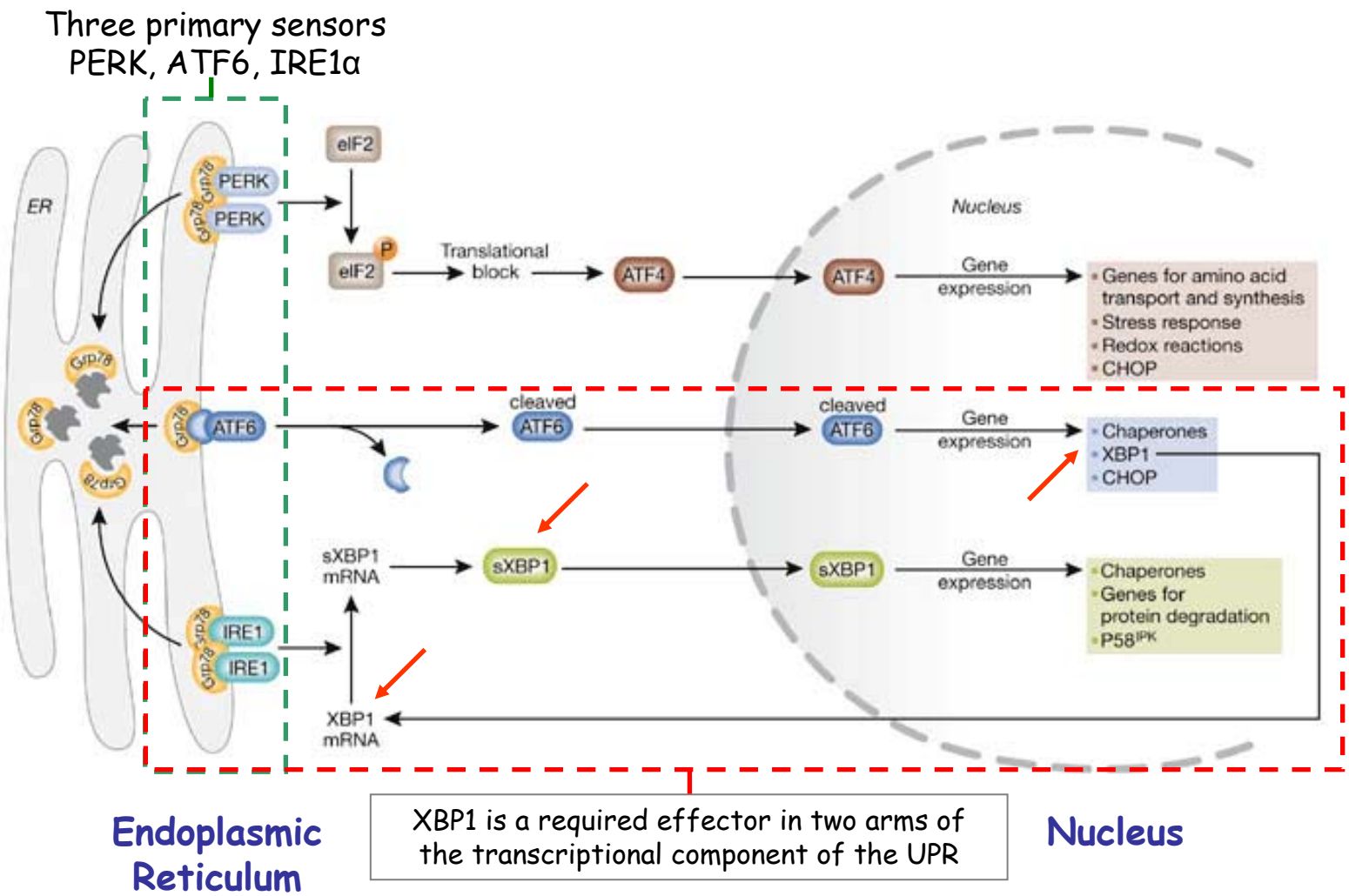


Davies *et al.*, Int J Cancer (2008)

Gene Symbol	Clinical Assoc.
BCL2	√
BCL2L2	ND
EGR1	ND
ERa	√
IRF1	√
NFκB	√
NPM	√
MYC	√
XBP1	√

ND=no data (yet!)

Unfolded Protein Response (UPR)

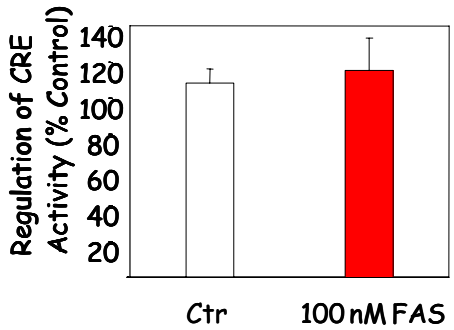


Adapted from Szegezdi *et al.* 2006

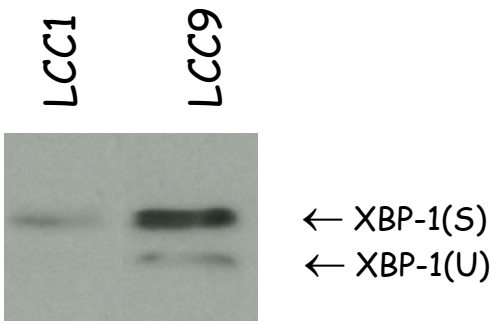
Endogenous XBP1 in Endocrine Responsiveness

- XBP1 estrogenic regulation is lost in LCC9 cells
- Most XBP1 is present as the spliced form XBP1(S)
- XBP1 transcriptional activity is increased 4-fold in LCC9 vs. LCC1 cells p<0.001

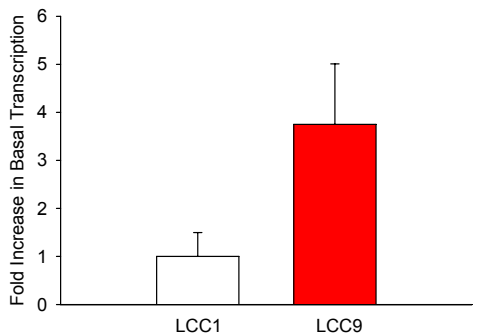
LCC1 cells are TAM and FAS sensitive
LCC9 cells are TAM and FAS crossresistant



XBP1 Western



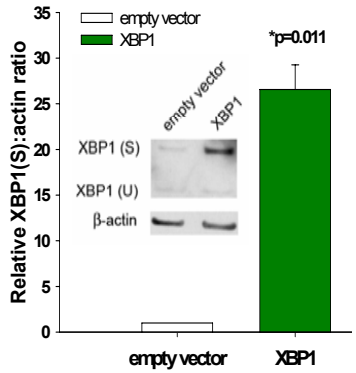
Basal XBP1 (CRE) Activity



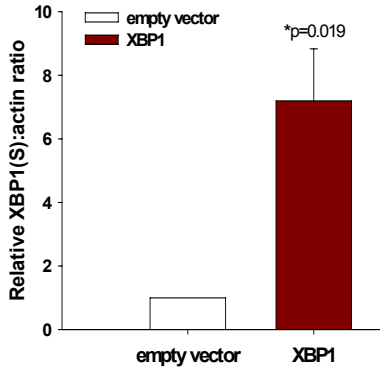
FAS = Faslodex; Fulvestrant; ICI 182,780
TAM = Tamoxifen

XBP1(s) Modulates Endocrine Responsiveness

MCF-7 and T47D cells transfected with the full length (*unspliced*) cDNA primarily generate the spliced variant XBP1(S)

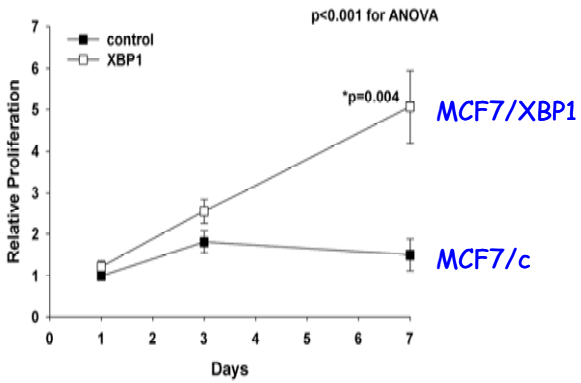


T47D cells



MCF-7 cells

XBP1(s) confers *Estrogen Independence*



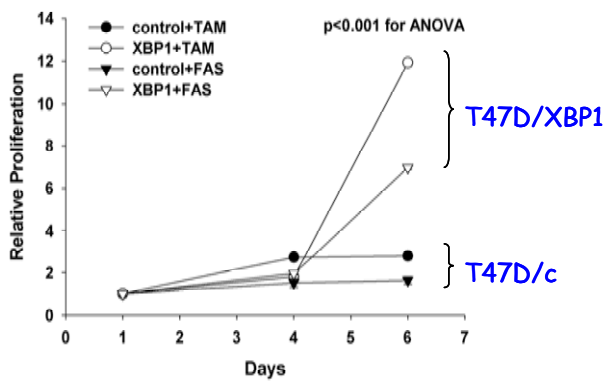
MCF7/XBP1

MCF7/c

Estrogen-independence is phenotypically similar to aromatase inhibitor resistance

FAS = Faslodex; Fulvestrant; ICI 182,780
TAM = Tamoxifen

XBP1(s) confers *Antiestrogen Resistance*



T47D/XBP1

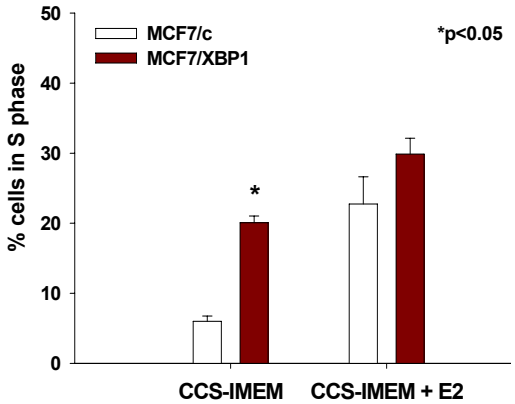
T47D/c

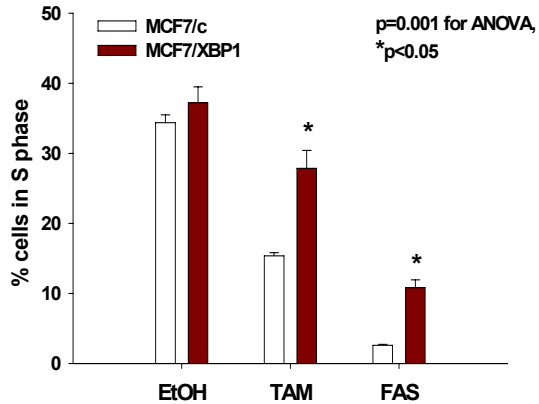
Gomez et al., FASEB J 21:4013-27, 2007

Lombardi
COMPREHENSIVE
CANCER CENTER at GEORGETOWN UNIVERSITY

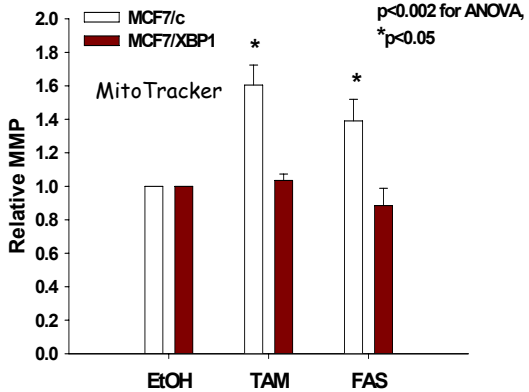
XBP1(s) Modulates Endocrine Responsiveness

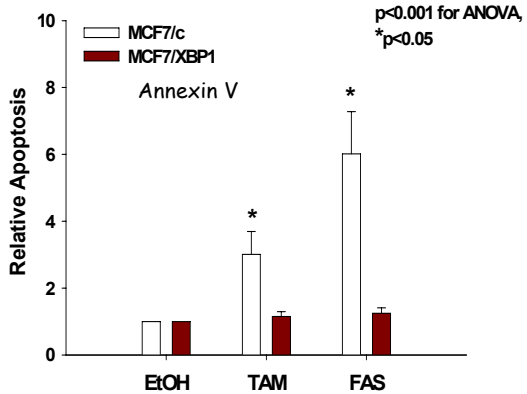
XBP1(s) reduces endocrine-induced cell cycle arrest

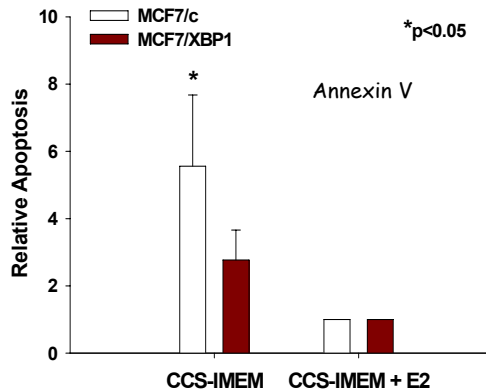




XBP1(s) reduces endocrine-induced apoptosis







Gomez *et al.*, *FASEB J* 21:4013-27, 2007
Shajahan *et al.*, in preparation

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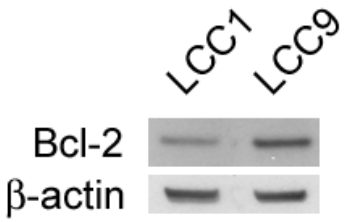
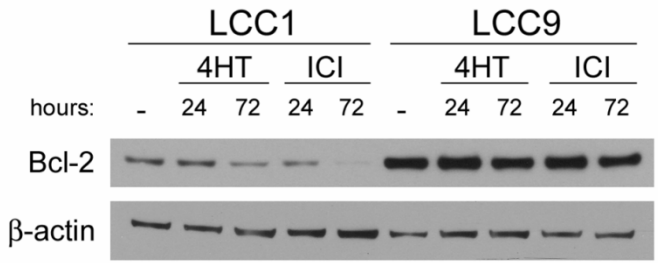
XBP1(s) and BCL2 in Endocrine Resistance

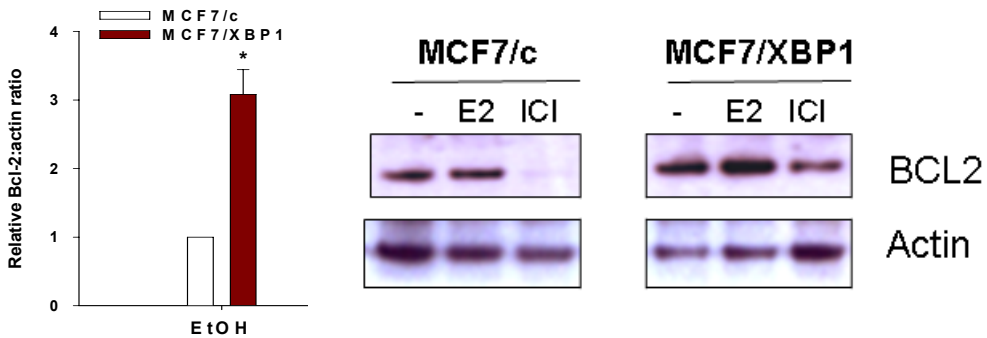
BCL2 is up-regulated in LCC9 cells



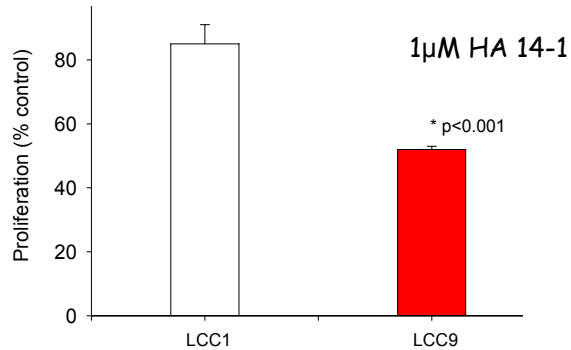
LCC9 cells have lost BCL2 regulation by antiestrogens



BCL2 is increased in MCF7/XBP1 cells



LCC9 cells are sensitive to inhibition of BCL2



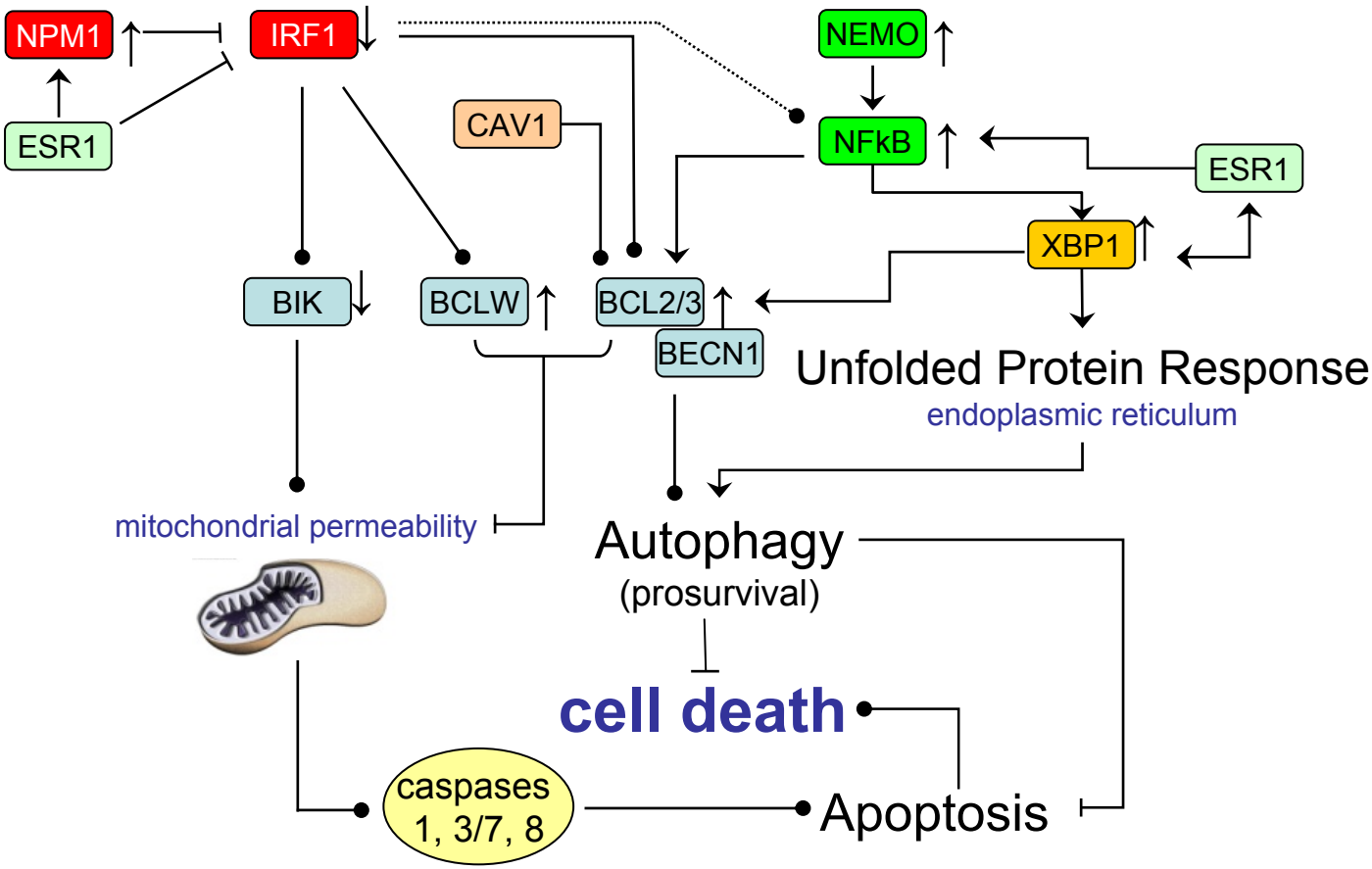
Crawford *et al., PLoS ONE*, 2010
Nehra *et al., FASEB J*, 2010

Mathematical Modeling of Hormone Resistance

- Goals
  - understand development of ER signaling, estrogen independence and antiestrogen resistance
  - generate new hypotheses of signal regulation and flow
  - design informative experiments to test new hypotheses (iterative model building)
- Types of models
  - based on molecular interactions
  - modeled using ordinary differential equations or stochastic reaction networks
- Modular approach
  - build models for individual modules
  - cell cycle, apoptosis, autophagy, unfolded protein response, etc.

Initial "Control Overview" Wiring Diagram

Signaling among subcellular organelles/compartments to guide mathematical modeling



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Conclusions

- Transitional signaling from sensitive to resistant
  - proliferation signaling dominates early responses
  - cell survival signaling dominates stable acquired resistance
  - cells coordinate regulation of metabolic and survival signaling
- Short-term responsiveness (selected nodes - time course study)
  - AKT, BCL2, BIK, BIRCs1/2, MAPKs (n=6), NFkB, NPM3, XBP1
- Long term acquired resistance (selected nodes – LCC1 vs. LCC9 studies)
  - BCL2, BCL2L, BIK, ERa, EGR1, IRF1, MYC, FOXO3a, NFkB, NPM1, XBP1
- Some genes (or closely related gene functions) are common
  - BCL2, NFkB, BIK, NPM, XBP1
- Resistance may not require many new nodes but does change the nature/usage of existing edges among nodes

  (it's mostly the same network of nodes, its just wired differently)
- Consistent with current graph and network theories
  - predict that rewiring for resistance is conferred, at least partly, by the altered regulation of key nodes (*e.g.,* by ER) with only limited linking to new nodes
  - in our resistant models IRF1, NFkB, XBP1, BCL2 lose their endocrine regulation

Acknowledgments

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