Supplementary File: Knowledge-Guided Fuzzy Logic Network Modeling to Detect Alterations in Cancer Signaling Pathways

Hui Liu[1,2], Shital Kumar Mishra[1], Fan Zhang[1], Shuigeng Zhou[3], and Jie Zheng[1,4⋆]

[1] School of Computer Engineering, Nanyang Technological University, Singapore 639798, Singapore
[2] Lab of Information Management, Changzhou University, Jiangsu, 213164 China.
[3] Shanghai Key Lab of Intelligent Information Processing, School of Computer Science, Fudan University, Shanghai 200433, China.
[4] Genome Institute of Singapore (GIS), Biopolis, Singapore 138672, Singapore.

1 Methods

1.1 Fuzzy logic network modeling

We introduced a variable c for each incoming edge to a protein to encode the logical relationship. Intuitively, equal values encode AND and different values encode OR logical operations. For instance, protein j has two input signals from protein i and protein k. If c_{ij} is equal to c_{kj}, AND operation is executed on the input signals $f^{(m)}_{i\to j}$ and $f^{(m)}_{k\to j}$ under mth experimental condition; otherwise OR operation is executed. Note that f is the signal value calculated by using the fuzzy membership function Equation (4). Table S1 shows some examples for the logical relationships and corresponding operations by the fuzzy logic model, in which the signs $\vee$ and $\wedge$ denote OR and AND operations, respectively.

Table S1: Illustrative examples of logical relationships and operations

c values	Logic relationship	Operation
(1, 1)	$f^{(m)}_{1\to j} \wedge f^{(m)}_{2\to j}$	$\min(f^{(m)}_{1\to j}, f^{(m)}_{2\to j})$
(1, 2)	$f^{(m)}_{1\to j} \vee f^{(m)}_{2\to j}$	$\max(f^{(m)}_{1\to j}, f^{(m)}_{2\to j})$
(1, 2, 3)	$f^{(m)}_{1\to j} \vee f^{(m)}_{2\to j} \vee f^{(m)}_{3\to j}$	$\max(f^{(m)}_{1\to j}, f^{(m)}_{2\to j}, f^{(m)}_{3\to j})$
(1, 2, 1)	$(f^{(m)}_{1\to j} \wedge f^{(m)}_{3\to j}) \vee f^{(m)}_{2\to j}$	$\max(\min(f^{(m)}_{1\to j}, f^{(m)}_{3\to j}), f^{(m)}_{2\to j})$
(1, 2, 3, 4)	$f^{(m)}_{1\to j} \vee (f^{(m)}_{2\to j} \vee f^{(m)}_{3\to j} \vee f^{(m)}_{4\to j}$	$\max(f^{(m)}_{1\to j}, f^{(m)}_{2\to j}, f^{(m)}_{3\to j}, f^{(m)}_{4\to j})$
(1, 2, 2, 3)	$f^{(m)}_{1\to j} \vee (f^{(m)}_{2\to j} \wedge f^{(m)}_{3\to j}) \vee f^{(m)}_{4\to j}$	$\max(f^{(m)}_{1\to j}, \min(f^{(m)}_{2\to j}, f^{(m)}_{3\to j}) f^{(m)}_{4\to j})$
(1, 1, 3, 1)	$(f^{(m)}_{1\to j} \wedge (f^{(m)}_{2\to j} \wedge f^{(m)}_{4\to j}) \vee f^{(m)}_{3\to j}$	$\max(\min(f^{(m)}_{1\to j}, f^{(m)}_{2\to j}, f^{(m)}_{4\to j}), f^{(m)}_{3\to j})$

1.2 Constrained integer nonlinear programming

Since the structure space of the signaling network is exponential to the number of participating proteins, exhaustive search for an optimal structure is computationally inhibitive for even moderate-size networks. Heuristic searching methods, such as genetic algorithm and scatter search, can be applicable. We here reformulate the optimization problem described above into an integer nonlinear programming (INLP) and subsequently adopt existing standard software packages to solve it.

Denote by I, C and O the index sets of species corresponding to perturbed Inhibitors, Cytokines and Observed species, respectively. $M{=}P - (I \cup C \cup O)$ is the set of other signaling molecules mediated in the pathway network of interest. Some reasonable assumptions should be made to convert the Boolean model to the INLP formulism. We assume a signaling reaction takes place if and only if all reactants are present. If a reaction takes place, all products are generated [1]. Besides, we have prior knowledge of activation and inhibition effect for some signaling reactions, and expect that the prior information can be encoded into constraints. Considering that the structure space is still large and time-consuming even if the maximum number of input nodes is restricted, we retrieved the protein-protein interactions

⋆ The correspondence author: ZhengJie@ntu.edu.sg

among the p species from STRING databases and added them as constraints to restrict the structure space. Denote by $\tilde{t}_{ij}$ the prior regulation type between species i and j, the binary parameter PPI_{ij} as the indicator of the interaction between species i and j, the INLP problem can be described as :

$$\min \quad \sum_{k=1}^{n}\sum_{i\in O}(x_i^{(m)}-y_i^{(m)})^2+\gamma\sum_{i=1}^{p}\sum_{j=1}^{p}(\lambda-w_{ij})b_{ij}$$

$$s.t.$$

$$t_{ij}\le b_{ij}, \qquad i,j=1,...,p \tag{1}$$

$$t_{ij}\ge b_{ij}\tilde{t}_{ij}, \qquad i,j=1,...,p \tag{2}$$

$$t_{ij}\ge 1-b_{ij}\lceil w_{ij}\rceil+\tilde{t}_{ij}, \qquad i,j=1,...,p \tag{3}$$

$$y_i^{(m)}=x_i^{(m)}, \qquad i\in C, m=1,...,M \tag{4}$$

$$y_i^{(m)}\le 1-x_i^{(m)}, \qquad i\in I, m=1,...,M \tag{5}$$

$$b_{ij}\le \max(k_{ij},PPI_{ij}), \qquad i,j=1,...,p \tag{6}$$

$$b_{ii}=0, \qquad i=1,...,p \tag{7}$$

$$c_{ij}-\sum_{k=1}^{p}b(i,k)\le 0 \qquad i,j=1,...,p \tag{8}$$

$$\sum_{j=1}^{p}c_{ij}-\frac{1}{2}(\sum_{k=1}^{p}b_{i,k})*(\sum_{k=1}^{p}b_{i,k}+1)\le 0 \qquad i,j=1,...,p \tag{9}$$

$$b_{i,j},t_{i,j},\tilde{t}_{i,j}\in\{0,1\} \qquad i,j=1,...,p \tag{10}$$

$$w_{i,j}\in(0,1) \qquad i,j=1,...,p \tag{11}$$

$$c_{ij}\in N \qquad i,j=1,...,p \tag{12}$$

The constraints (1) is added because the regulation type is unnecessarily considered if there is no interaction between node i and node j. The constraints (2) and (3) enforce the regulation type to be consistent with the prior knowledge when both b_{ij} and w_{ij} are non-zero. The constraints (4) and (5) ensure that the predicted values are equal to the measured values if the nodes are manipulated under kth experiment. The constraint (6) is added to reduce the structure space by introducing the PPI networks. The constraint (7) is applied because we do not consider the self-feedback reactions. The constraints (8)- (9) is to reduce the number of logic combinations encoded by c_{ij}. Finally, we add the constraints (10)-(12) to restrict the value regions of these variables, i.e., $b_{i,j}$, $t_{i,j}$ and $\tilde{t}_{i,j}$ are binary variables, $w_{i,j}$ is the confidence score falls between 0 and 1, and c_{ij} must be an integer.

2 Analysis on synthetic data

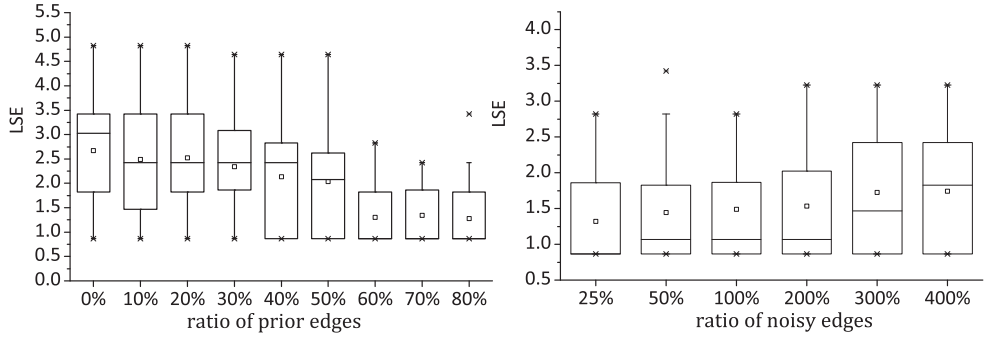

Fig. S1: Boxplot of the LSE under different ratios of prior edges, and full prior edges plus different ratios of noisy edges.

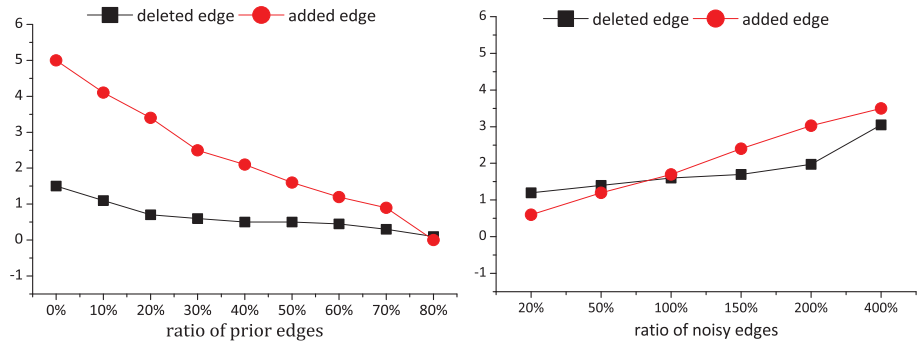

Fig. S2: Curves of the added and deleted edges of the learned networks and true networks under different ratios of prior edges, and 100% prior edges plus different ratios of noisy edges.

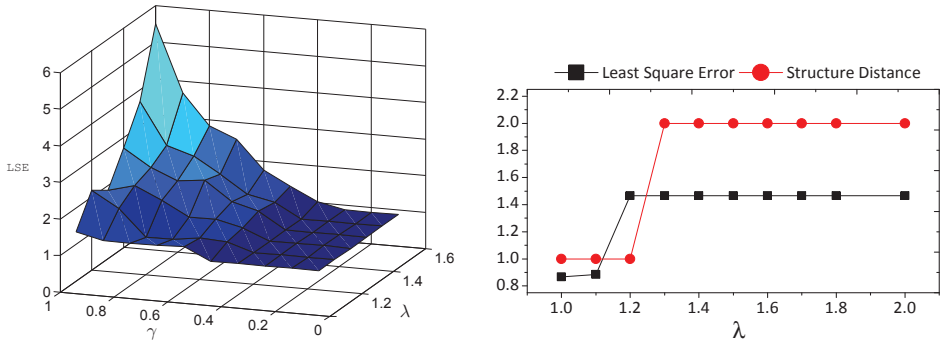

Fig. S3: 3D plot of LSE with respect to λ and γ, and the curves of the best LSE and SD values with respect to λ, given the network with 50% prior edges and 0% noise.

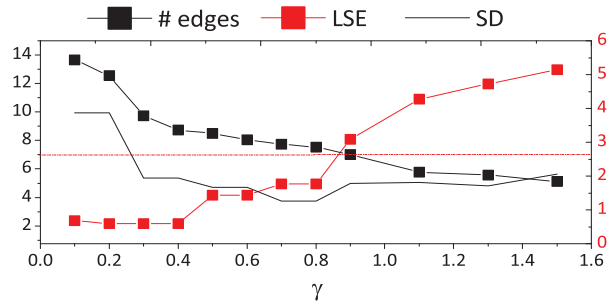

Fig. S4: The curves of least square error (LSE), structure distance (SD) between true and learned network with respect to parameter γ, given 50% prior edges.

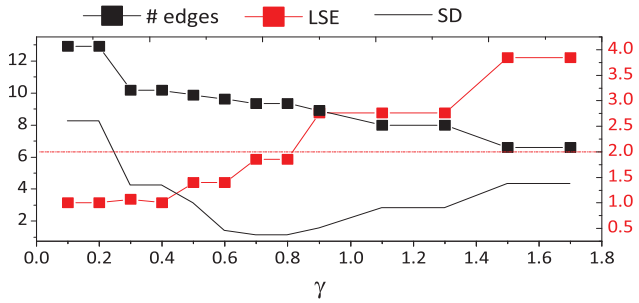

Fig. S5: Impact of parameter γ on the performance in the case of full prior edges. It is found that γ falling into the range [0.5, 0.8] acquires better balance between LSE and model complexity than other values. In terms of structure distance, γ should be set to a value in [0.6, 0.8].

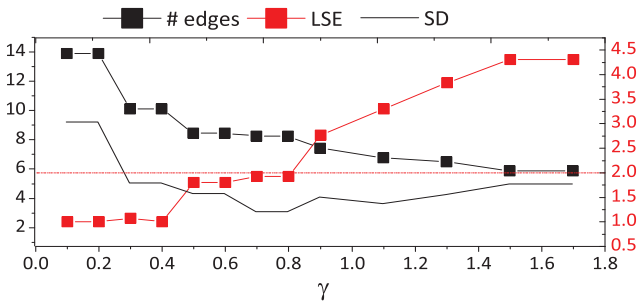

Fig. S6: Impact of parameter γ on the performance in the case of full prior edges and 50% noisy edges. It is found that γ falling into the range [0.5, 0.8] acquires better balance between LSE and model complexity than other values. In terms of structure distance, γ should be set to a value in [0.7, 0.8].

3 Performance evaluation on DREAM 4 challenge data

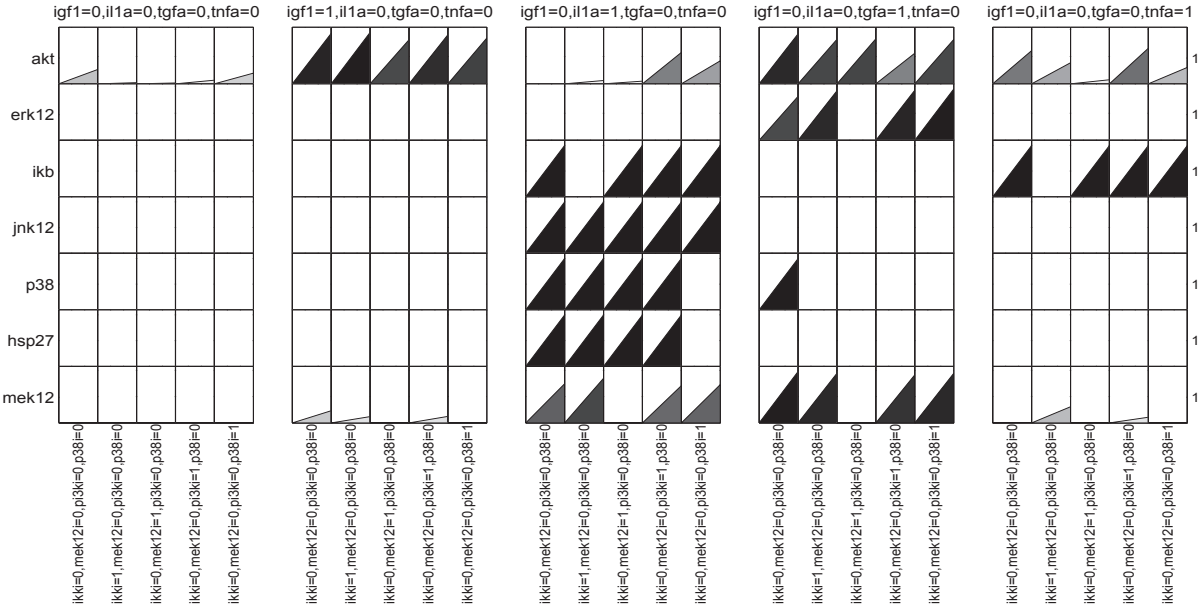

Fig. S7: Plot of normalized phosphoproteomic data of DREMA 4 Challenge for signaling pathway inference. The plot is produced by DataRail developed by Terfve [2].

Table S2: Confidence score of each edge included in the prior network.

prior edge	**confidence score**
tnfa→map3k7	0.99201
tnfa→map3k1	0.36161
tnfa→mkk4	0.26919
tnfa→jnk12	0.23833
tnfa→pi3k	0.66933
il1a→map3k7	0.82746
il1a→map3k1	0.99700
igf1→pi3k	0.14271
igf1→ras	0.56534
tgfa→ras	0.95014
tgfa→pi3k	0.97502
map3k7→p38	0.91839
map3k7→mkk4	0.96900
map3k7→ikk	0.97300
map3k7→hsp27	0.20498
map3k1→ikk	0.98500
map3k1→mkk4	0.99900
map3k1→jnk12	0.97614
ras→pi3k	0.22900
ras→mek12	0.99700
ras→map3k1	0.10000
pi3k→map3k1	0.98801
pi3k→akt	0.99900
pi3k→mek12	0.94656
akt→ikk	0.82717
akt→mek12	0.99201
mek12→erk12	0.99900
erk12→hsp27	0.71459
mkk4→jnk12	0.99900
mkk4→p38	0.18200
ikk→ikb	0.99900
p38→hsp27	0.97417

Table S3: Statistical significance of each edge learned by our method.

learned edge	z**-score**	p**-value**
igf1 → akt	2.8625	0.002102
pi3k-akt	4.01	3.00E-05
pi3k-erk12	3.8235	6.60E-05
p38 → hsp27	3.7689	8.20E-05
ikk → ikb	3.1586	0.000793
map3k1 → ikk	2.8982	0.001877
mkk4 → jnk12	2.8859	0.001951
il1a → map3k1	2.7898	0.002637
tnfa → map3k1	2.792	0.002619
il1a → map3k7	5.1764	0.00001
il1a → mek12	3.3773	0.000366
ras → mek12	2.9765	0.001458
map3k7 → mkk4	4.1638	1.60E-05
map3k7 → p38	3.8736	5.40E-05
ras → pi3k	2.3227	0.010098
tgfa → ras	3.1998	0.000688

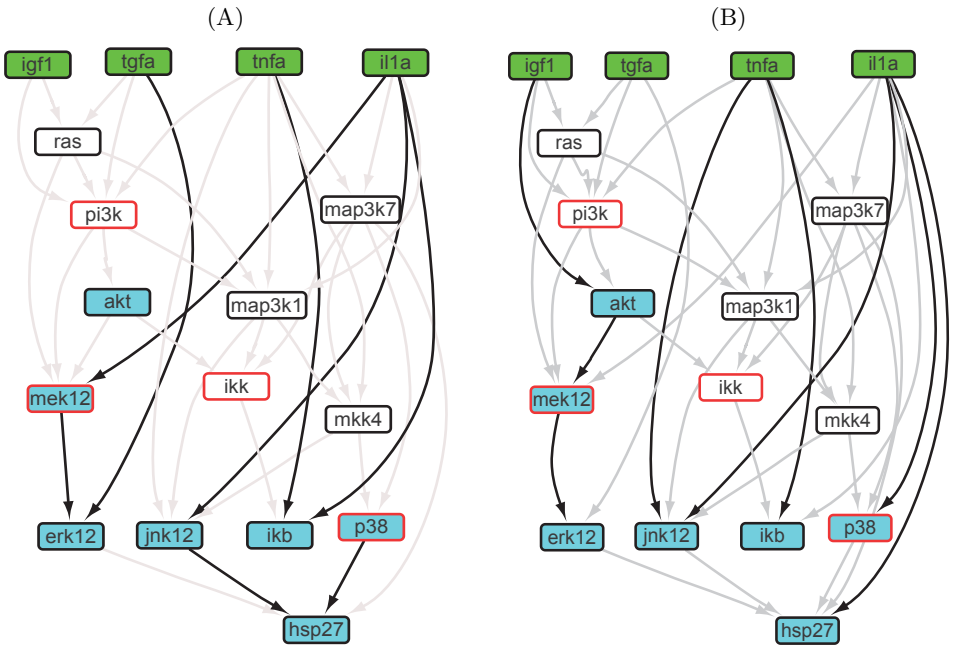

Fig. S8: DREAM 4 challenge signaling pathway learned by the data-driven methods. (A) Signaling pathway learned by the classical Bayesian network algorithm MMHC. (B) Signaling pathway inferred by Markov blanket algorithm HITON_PC. The significance threshold for independence tests of both methods is set to 0.01. Note that the direction of the edges are oriented according to the prior knowledge.

4 Apoptotic signaling pathway induced by sequential drug treatments

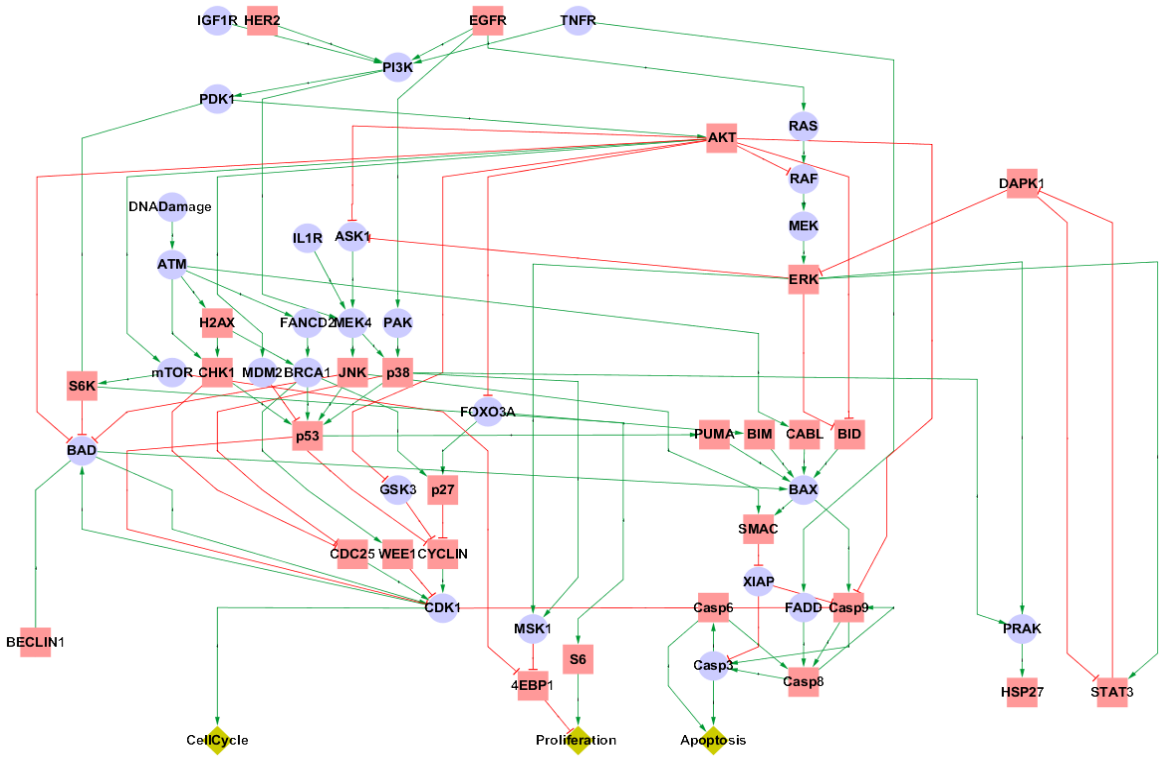

Fig. S9: Primary signaling pathway involving in apoptosis and DNA damage signaling pathway, which was proposed in our previous study [3].

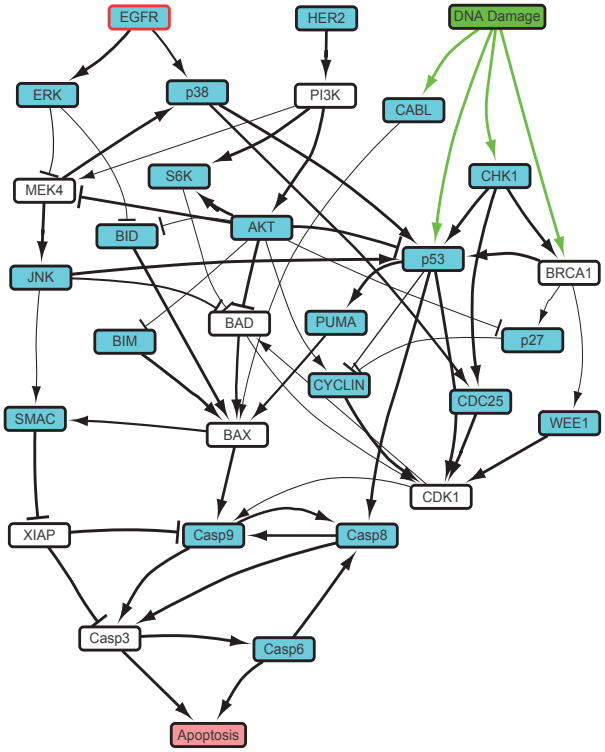

Fig. S10: Prior network compressed from the apoptosis and DNA damage signaling pathway, and the width of each edge is proportional to its prior confidence score that is computed based on the PPI comprehensive scores of STRING database release 9.1.

Table S4: Confidence score of each edge included in the prior network.

source	interaction type	target	confidence score
AKT	-	BAD	0.998
AKT	+	BID	0.26
AKT	+	BIM	0.1
AKT	+	CYCLIN	0.379
AKT	+	MEK4	0.947
AKT	+	p27	0.1
AKT	+	p53	0.947
AKT	+	S6K	0.986
BAD	+	BAX	0.855
BAD	+	CDK1	0.297
BAX	+	Casp9	0.969
BAX	+	SMAC	0.75
BID	+	BAX	0.99
BIM	+	BAX	0.999
BRCA1	+	p27	0.1
BRCA1	+	p53	0.999
BRCA1	+	WEE1	0.274
CABL	+	BAX	0.1
Casp3	+	Apoptosis	0.9
Casp3	+	Casp6	0.984
Casp6	+	Apoptosis	0.9
Casp6	+	Casp8	0.994
Casp8	+	Casp3	0.999
Casp8	+	Casp9	0.875
Casp9	+	Casp8	0.875
Casp9	+	Casp3	0.994
CDC25	+	CDK1	0.999
CDK1	-	Casp9	0.369
CDK1	+	BAD	0.297
CHK1	-	CDC25	0.999
CHK1	+	p53	0.999
CHK1	+	BRCA1	0.966
CYCLIN	+	CDK1	0.999
DNA Damage	+	BRCA1	NA
DNA Damage	+	CABL	NA
DNA Damage	+	CHK1	NA
DNA Damage	+	p53	NA
EGFR	+	ERK	0.973
EGFR	+	p38	0.735
ERK	-	BID	0.1
ERK	+	MEK4	0.419
HER2	+	PI3K	0.946
JNK	-	BAD	0.674
JNK	+	p53	0.999
JNK	+	SMAC	0.38
MEK4	+	JNK	0.999
MEK4	+	p38	0.969
p27	-	CYCLIN	0.1
p38	-	CDC25	0.876
p38	+	p53	0.994
p53	-	CDK1	0.994
p53	-	CYCLIN	0.474
p53	+	PUMA	0.98
PI3K	+	MEK4	0.318
PI3K	+	AKT	0.998
PI3K	+	S6K	0.997
PUMA	+	BAX	0.783
S6K	-	BAD	0.173
SMAC	-	XIAP	0.999
WEE1	-	CDK1	0.977
XIAP	-	Casp3	0.999
XIAP	-	Casp9	0.999

5 Drug induced alteration of EGFR pathway

We also evaluated the proposed method on another real case: the signaling pathway rewiring induced by anticancer drug treatment on transformed hepatocytic cell line HepG2 [1]. The four drugs are the dual EGFR/ErbB-2 inhibitor Lapatinib, two potent EGFR kinase inhibitors Erlotinib and Gefintinib, and the Raf kinase inhibitor Sorafenib. We aim to identify drug effects by detecting drug-induced topological alterations based on the perturbation data provided by [1]. The cell-type specific signaling pathway constructed by [1] was used as the prior network. For computational efficiency, we compressed the signaling pathway by removing unidentifiable nodes applying the rules proposed by [4]. The resulting knowledge model includes 23 reactions among 22 proteins, among which there are six stimuli, four inhibitors and twelve observed proteins, as shown in Fig. S11. For each drug, the experimental dataset includes the measured levels under 11 different perturbation conditions. The data are normalized to the range between 0 and 1 by using the logistic transformation proposed by [4]. Considering that the prior network is refined from a large primary signaling pathway using the cell-type phosphoproteomic data, we assigned the confidence scores of prior edges to 1. The values of parameter λ and γ were empirically set to to 1.2 and 0.3, respectively.

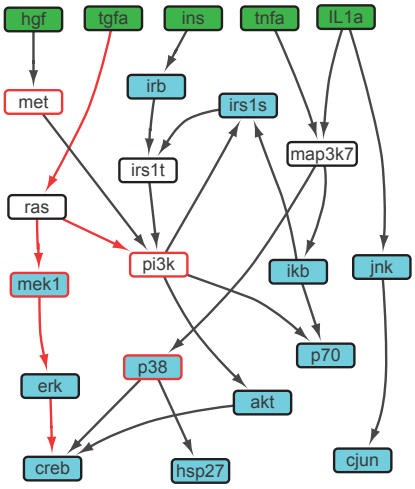

Fig. S11: Signaling pathway alteration induced by EGFR inhibitor Lapatinib. Red edges represent the drug effect that EGFR is inhibited by Lapatinib and downstream proteins are inactivated accordingly.

Fig. S11 shows the resulting network trained on the experimental data of Lapatinib, which confirms that the downstream nodes of TGFa branches are removed: (TGFa→RAS→MEK1→ERK) and (TGFa →RAS→PI3K). This is consistent with the fact that Lapatinib specifically blocks the TGFa-induced phosphorylation of RAS, MEK1 and ERK [5]. The downstream branches of PI3K→AKT and PI3K→P70 are not removed, since HGF and INS would activate PI3K and its downstream proteins, even though TGFa is blocked by Lapatinib. Moreover, the logical gate (MET OR RAS OR IRS-1t) →PI3K is correctly inferred, which is consistent with TGFa, HGF or INS-independent activation of PI3K. Our results are consistent with previous studies [5, 6]. The edge P38→CREB is not removed, this is also consistent with the observation that TGFa and IL1a independently activate CREB via the MAP3K7→P38→CREB pathway, in the case that TGFa→RAS→ ... →CREB pathway is blocked [7, 8]. For the other two EGFR inhibitors, Gefitinib and Erlotinib, we obtained results similar to Lapatinib, as shown in Fig. S11-S12. It can be found that TGFa→RAS→MEK1→ERK and TGFa→RAS→PI3K pathways are definitely removed, and the reaction P38→CREB is reserved. The result of Sorafenib differs largely from that of the other three drugs mentioned above. Three edges P38→CREB, P38→HSP27 and JNK→CJUN are blocked by Sorafenib. It is assumed that Sorafenib blocks MEK/ERK pathway by repressing RAF activity and inhibiting TGFa→RAS [9]. Interestingly, the MEK/ERK pathway was not blocked in the learned network, as shown in Fig. S13. Close inspection showed that HGF activates MEK1 and downstream proteins, whereas TGFa→RAS is blocked. This is consistent with previous studies that Sorafenib does

not inhibit the activation of the MEK/ERK pathway in human tumor cell lines [10]. Furthermore, we have found a list of supporting evidence from the literature for each edge inferred to be blocked by each of the four anticancer drugs (please refer to the Supplementary File for more detail).

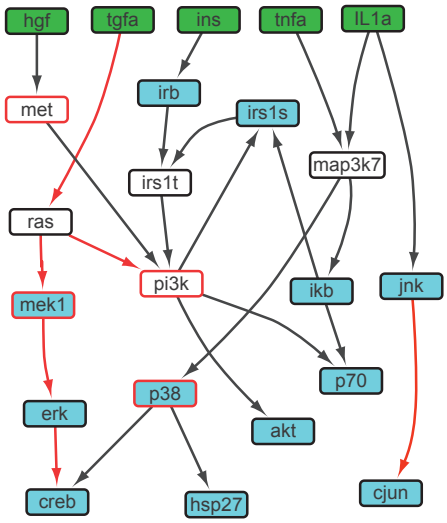

Fig. S12: Signaling pathway alterations induced by Gefitinib. Red color edges represent the drug effect that EGFR is inhibited by Lapatinib and downstream species are also inactivated. Beside, jnk→cjun is also blocked by Gefitinib.

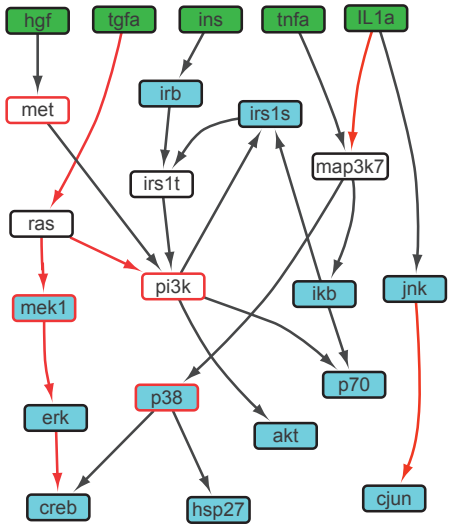

Fig. S13: Signaling pathway alterations induced by Erlotinib. Red color edges represent the drug effect that EGFR is inhibited by Erlotinib and downstream species are also inactivated. Beside, jnk→cjun and il1a→map3k7 are also blocked.

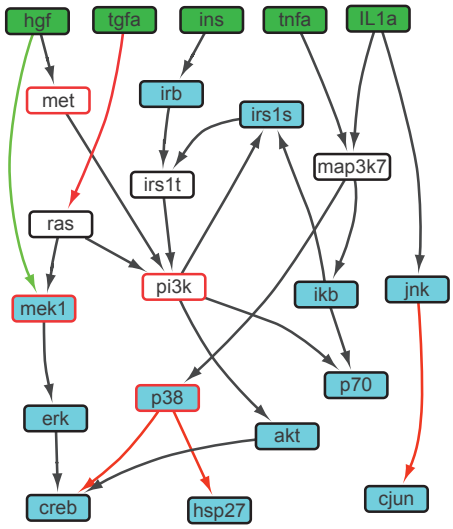

Fig. S14: Signaling pathway alterations induced by Sorafenib. Red color edges represent the drug effect that jnk→cjun, p38→creb and p38→hsp27 are also blocked. While tgfa→ras is blocked, but hgf is inferred to activate mek1 and downstream species.

1) Validation of the Pathway rewiring induced by EGFR inhibitor Lapatinib/Gefitnib/Erlotinib

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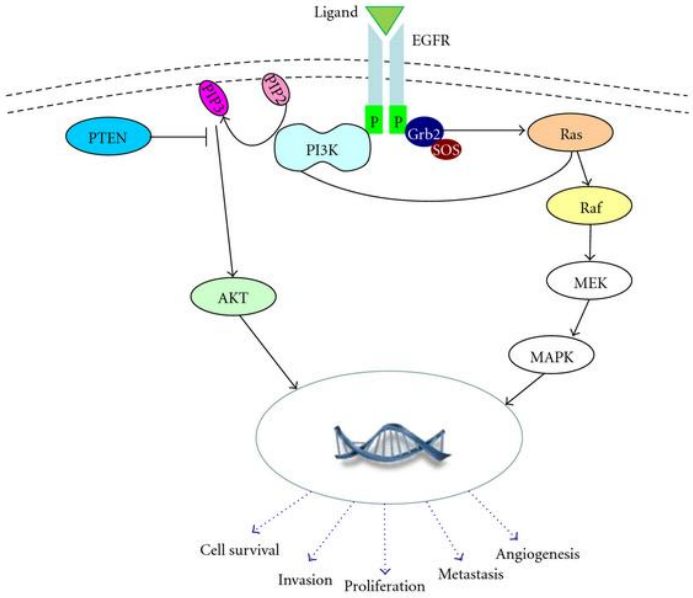

Fig.S12 Generic EGFR signaling pathway [Alyssa M. Krasinskas, PR Int., 2011]

a. Tgfa-ras

EGFR and ligands such as transforming growth factor-α and amphiregulin are over-expressed in a large subset of primary breast carcinomas [1,2]. TGF-α is a member of the epidermal growth factor (EGF) family. TGF-α, basically a ligand for EGFR, activates a signaling pathway for cell proliferation, differentiation and development. Lapatinib is an orally active drug for breast cancer and other solid tumours.[3] It is a dual tyrosine kinase inhibitor which interrupts the HER2/neu and epidermal growth factor receptor (EGFR) pathways[4]. The effect of Lapatinib, the most selective and specific EGFR inhibitor, is the complete removal of the downstream reactions of the TGFα branch: TGFα→GRB2→SOS→RAS→PI3K and RAS→RAF1→MEK1/2→ERK1/2. This resulted from the fact that Lapatinib blocks the TGFα induced MEK1/2, ERK1/2, and AKT phosphosignals [5]. Since Lapatinib inhibits EGFR, the EGFR-Ras-Raf-MEK-ERK signaling network will be suppressed [6].

1. Salomon DS, Brandt R, Ciardiello F, Normanno N: Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995, 19:183-232.

2. Torregrosa D, Bolufer P, Lluch A, Lopez JA, Barragan E, Ruiz A, Guillem V, Munarriz B, Garcia CJ: Prognostic significance of c-erbB-2/neu amplification and epidermal growth factor receptor (EGFR) in primary breast cancer and their relation to estradiol receptor (ER) status. *Clin Chim Acta* 1997, 262:99-119.
3. Burris HA (2004). "Dual kinase inhibition in the treatment of breast cancer: initial experience with the EGFR/ErbB-2 inhibitor lapatinib". Oncologist. 9 Suppl 3: 10–5.

4. Higa GM & Abraham J (September 2007). "Lapatinib in the treatment of breast cancer". Expert Review of Anticancer Therapy (log in required) (Future Drugs) 7 (9): 1183–92.

5. Mitsos A, Melas IN, Siminelakis P, Chairakaki AD, Saez-Rodriguez J, et al. (2009) Identifying Drug Effects via Pathway Alterations using an Integer Linear Programming Optimization Formulation on Phosphoproteomic Data. PLoS Comput Biol 5(12): e1000591.

6. Alyssa M. Krasinskas, "EGFR Signaling in Colorectal Carcinoma," Pathology Research International, vol. 2011, Article ID 932932, 6 pages, 2011.

b. ras- mek1

Ras is a key downstream effector of the epidermal growth factor receptor (EGFR), which is mutationally activated and/or overexpressed in a wide variety of human cancers. Ras activates Raf and Raf activates the MAPK/ERK kinase (MEK)1/2 dual-specificity protein kinases, which then activate ERK1/2. Following the inhibition of EGFR by Lapatinib, Ras gets inhibited. Since ras inhibits Raf, Raf will also get inhibited which in turn inhibits MEK1/2. Since Lapatinib inhibits EGFR, the the EGFR-Ras-Raf-MEK-ERK signaling network will be suppressed.

Reference: P.J. Roberts, C.J. Der, Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer Oncogene, 26 (2007), pp. 3291–3310

c. ras- pi3k

EGFR and HER2 are expressed in circulating tumor cells. The phosphoinositide-3 kinase (PI3K)/Akt pathways operates downstream of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER)2. This pathway is implicated in cell migration and survival. EGFR ligand binding triggers the activation of downstream signaling pathways, such as the phosphoinositide-3 kinase (PI3K)/Akt pathway (among others), which control cell proliferation, survival, and migration [1]. Since EGFR and Ras are inhibited by Lapatinib, the activity of PI3K will be inhibited [2]. The study conducted by Long et al suggest that lapatinib may alter the malignant phenotype of osteosarcoma cells via downregulation of the activity of the HER2-PI3K/AKT-FASN signaling pathway in vitro [3].

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3. Long et al, Lapatinib alters the malignant phenotype of osteosarcoma cells via downregulation of the activity of the HER2-PI3K/AKT-FASN axis in vitro. Oncol Rep. 2014 Jan;31(1):328-34.

d. mek1-erk

Gottfried et al have shown the dose-dependent and time-dependent activity of lapatinib on ERK phosphorylation in SK-BR-3 and BT474 cells. Both cell lines were treated with increasing doses of

lapatinib (0.2-20 µmol/L) for 1 hour (dose-dependent) or for increasing duration (10-60 minutes) with 2 or 0.2 µmol/L lapatinib (time-dependent). The results show that the Exposure of SK-BR-3 and BT474 human breast cancer cells to lapatinib resulted in a dose- and time-dependent reduction of phosphorylation of ERK [1]. in another study Sylvia et al have reported Lapatinib treatment of HER2-overexpressing cells inhibited MEK/ERK signaling, leading to up-regulation of the pro-apoptotic protein Bcl-2-interacting mediator of cell death (BIM) [2].

1. Konecny, G. E. *et al.* Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. *Cancer Res.* **66**, 1630–1639 (2006)

2. Sylvia et al, MEK inhibition increases lapatinib sensitivity via modulation of FOXM1. Curr Med Chem. Jun 1, 2013; 20(19): 2486–2499.

e. erk- creb

The cyclic (c)AMP responsive element binding protein (CREB) plays a key role in many cellular processes, including differentiation, proliferation, and signal transduction. CREB overexpression was found in tumors of distinct origin and evidence suggests an association with tumorigenicity. in HER-2/neu–transformed cells, treatment with Lapatinib leads to CREB downregulation. The downregulation in turn caused morphologic changes, reduced cell proliferation with G_0–G_1 cell-cycle arrest. The study further reports that lapatinib decreased the phosphorylation of CREB.

Reference: Steven et al. **HER-2/neu mediates oncogenic transformation via altered CREB expression and function**. Mol Cancer Res 2013; 11: 1462-1477.

2) Validation of the Pathway rewiring induced by Raf inhibitor Sorafenib.

Sorafenib is used to treat advanced renal cell carcinoma (RCC; a type of cancer that begins in the kidneys). Sorafenib is also used to treat hepatocellular carcinoma (a type of liver cancer) that cannot be treated with surgery and a certain type of thyroid cancer that has spread to other parts of the body and cannot be treated with radioactive iodine. Sorafenib is in a class of medications called kinase inhibitors. It works by blocking the action of an abnormal protein that signals cancer cells to multiply. This helps stop the spread of cancer cells.

http://www.nlm.nih.gov/medlineplus/druginfo/meds/a607051.html

Addition:

a) Hgf-mek1

The study by Gedaly, R. *et al*. has shown that Sorafenib may activate HGF/HGFR signaling via activation of PI3K. sorafenib stimulated HGF secretion in HCC cells and promoted c-Met, S6K and 4EBP1 phosphorylation[1]. Increased HCC activation and mobility by HGF was reported to be mediated through PI3K signaling. Hepatocyte growth factor leads to activation of MEK and PI3K signal pathways which contributes to expression of Proangiogenic Cytokines Interleukin-8 and VEGFR in several types of carcinoma[2].

1. Gedaly, R. *et al.* PI-103 and sorafenib inhibit hepatocellular carcinoma cell proliferation by blocking Ras/Raf/MAPK and PI3K/AKT/mTOR pathways. *Anticancer Res.* **30**, 4951–4958 (2010)

2. Dong, G., Chen, Z., Li, Z.Y., Yeh, N.T., Bancroft, C.C., Van Waes, C.(2001) Hepatocyte growth factor/scatter factor-induced activation of MEK and PI3K signal pathways contributes to expression of proangiogenic cytokines interleukin-8 and vascular endothelial growth factor in head and neck squamous cell carcinoma. Cancer Res. 61:5911–5918

Deletions:

a) Tgfa-ras

The extracellular ligand (e.g. EGF) binds to the membrane receptor (e.g. EGFR), starting the cascade of signals. In this cascade, Ras activates MAP3K (e.g. Raf), which activates MAP2K, which activates MAPK. MAPK can now activate a transcription factor, such as myc. Basically, activated Ras activates the protein kinase activity of RAF kinase[1]. RAF kinase phosphorylates and activates MEK (MEK1 and MEK2). MEK phosphorylates and activates a mitogen-activated protein kinase (MAPK). Sorafenib is a small molecular inhibitor of Raf kinases (more avidly C-Raf than B-Raf).[2][3] Sorafenib also inhibits some intracellular serine/threonine kinases (e.g. C-Raf, wild-type B-Raf and mutant B-Raf)[4]. Thus, Sorafenib treatment leads to inhibition of the Ras-Raf-MEK-ERK pathway [5].

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3. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M (October 2008). "Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling". Mol. Cancer Ther. 7 (10): 3129–40.
4. Keating GM, Santoro A (2009). "Sorafenib: a review of its use in advanced hepatocellular carcinoma". Drugs 69(2): 223–40.
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I think a better way to show blocking for this rewiring will be to show deletion of an edge containing raf protein. But since, we don't have RAF protein in our diagram; we can link deletion of the Ras protein with Sorafenib.

b) jnk-cjun

RAF-1 is a mitogen-activated protein kinase (MAPK) kinase, which phosphorylates and activates the serine/threonine-specific extracellular signal-regulated protein kinases ERK1 and ERK2 [Avruch et al., 1994]. In the nucleus, phosphorylated ERK activates transcription factors such as ELK-1 and c-JUN, leading to cell proliferation and survival. so if Raf is inhibited, it will also reduce the activity of C-Jun[1]. In another study, Vin et al report that Sorafenib suppresses JNK-dependent apoptosis through inhibition of ZAK. Sorafenib suppresses UV-induced apoptosis specifically by inhibiting c-jun-NH2-kinase (JNK) activation through the off-target inhibition of leucine zipper and sterile alpha motif-containing kinase (ZAK).

1. Yasunobu and Toshifumi ,Sorafenib-Inhibited Signaling: Emerging Evidence of RAF-Independent Pathways as Potential Therapeutic Targets in Hepatocellular Carcinoma. Oncology. Hepatocellular Carcinoma - Future Outlook; licensee InTech. 2013.

2. Vin et al. Sorafenib suppresses JNK-dependent apoptosis through inhibition of ZAK. Mol Cancer Ther. 2014 Jan;13(1):221-9.

c) **p38-hsp27**

Sorafenib (SRF) is a multi-kinase inhibitor that has been shown to have antitumor activity against several types of cancers. There are several literature evidences showing that Sorafenib inhibits p38. For example **(**Edwards and Emens, 2010) have reported that Sorafenib inhibited the activation of the MAP kinase p38 and its downstream target mitogen [1]. It is possible that p38 activity, which is known to be inhibited by sorafenib, may also be required for GRP78 induction [2]. In another study Honma et al. have reported that Sorafenib inhibited both the JNK and p38 pathways in a time- and dose-dependent manner. In addition, sorafenib also inhibited proteasome inhibitor-mediated JNK and p38 activation in both Huh7 and OUMS29 [3].

1. Edwards JP1, Emens LA. The multikinase inhibitor sorafenib reverses the suppression of IL-12 and enhancement of IL-10 by PGE_2 in murine macrophages. Int Immunopharmacol. 2010 Oct;10(10):1220-8.
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3. Honma et al. Sorafenib enhances proteasome inhibitor-induced cell death via inactivation of Akt and stress-activated protein kinases. J Gastroenterol. 2014 Mar;49(3):517-26

d) **map3k7-ikb**

Sorafenib Inhibits the MAPK Pathway in several types of cancer cells. For example, Grazia et al examined the expression of downstream signaling molecules of the Ras/Raf/MAPK pathway. For their studies, the sarcoma cell lines were treated with 100 nmol/L sorafenib for 24 h and examined for inhibition of p-MEK and p-ERK by Western blotting. When compared with no drug controls (−), both p-MEK and p-ERK were inhibited by sorafenib (+) in the sensitive MPNSTs, MPNST and ST8814 cells.

Reference: Ambrosini et al. Sorafenib inhibits growth and mitogen-activated protein kinase signaling in malignant peripheral nerve sheath cells. Mol Cancer Ther. 2008 Apr;7(4):890-6.

inhibition of p-MEK and p-ERK by Western blotting. When compared with no drug controls (−), both p-MEK and p-ERK were inhibited by sorafenib (+) in the sensitive MPNSTs, MPNST and ST8814 cells.

Reference: Ambrosini et al. Sorafenib inhibits growth and mitogen-activated protein kinase signaling in malignant peripheral nerve sheath cells. Mol Cancer Ther. 2008 Apr;7(4):890-6.

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