

A functional network model of the metastasis suppressor RKIP and its regulators in breast cancer cells

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Outline

Introduction

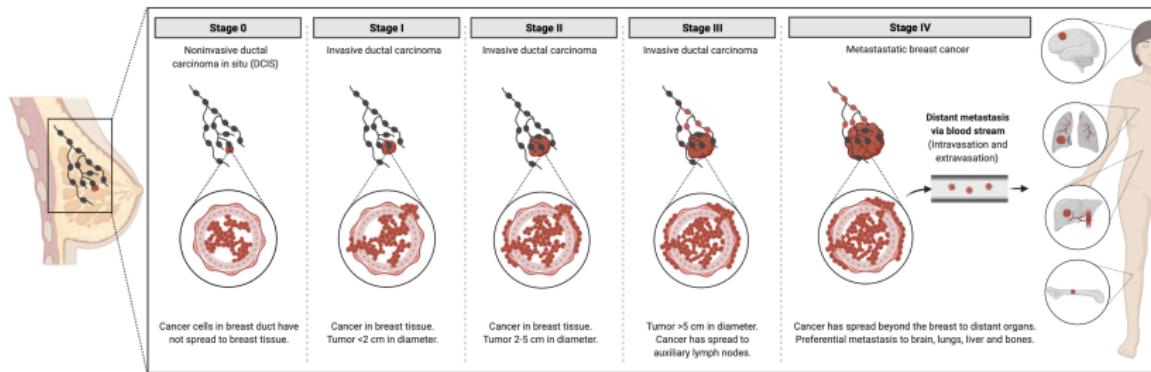
Materials and Methods

Results

Summary

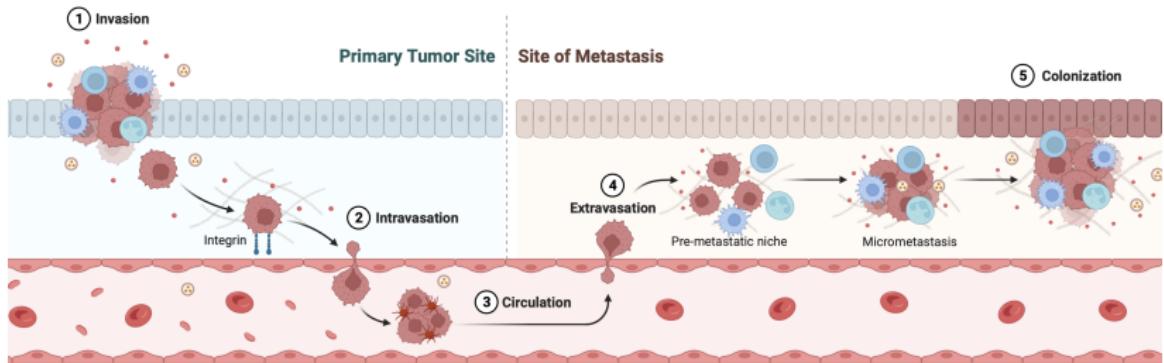
Introduction

STAGES OF BREAST CANCER

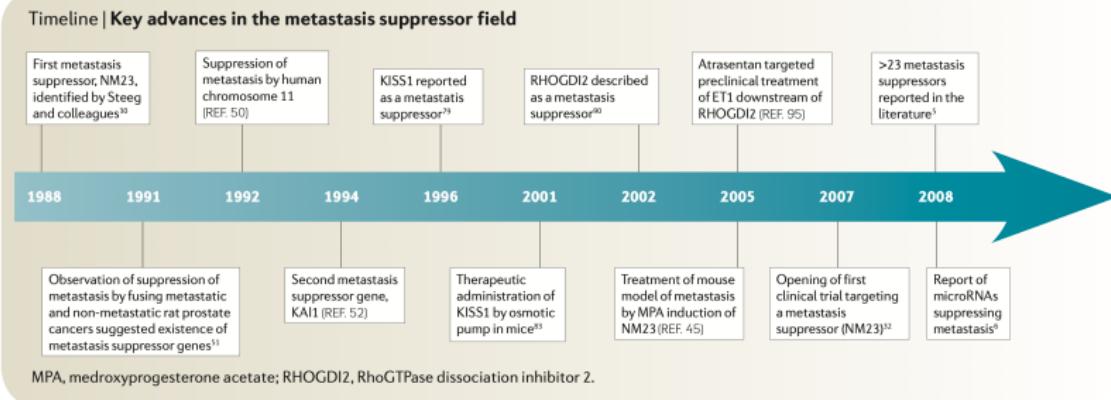


Introduction

Overview of Metastatic Cascade

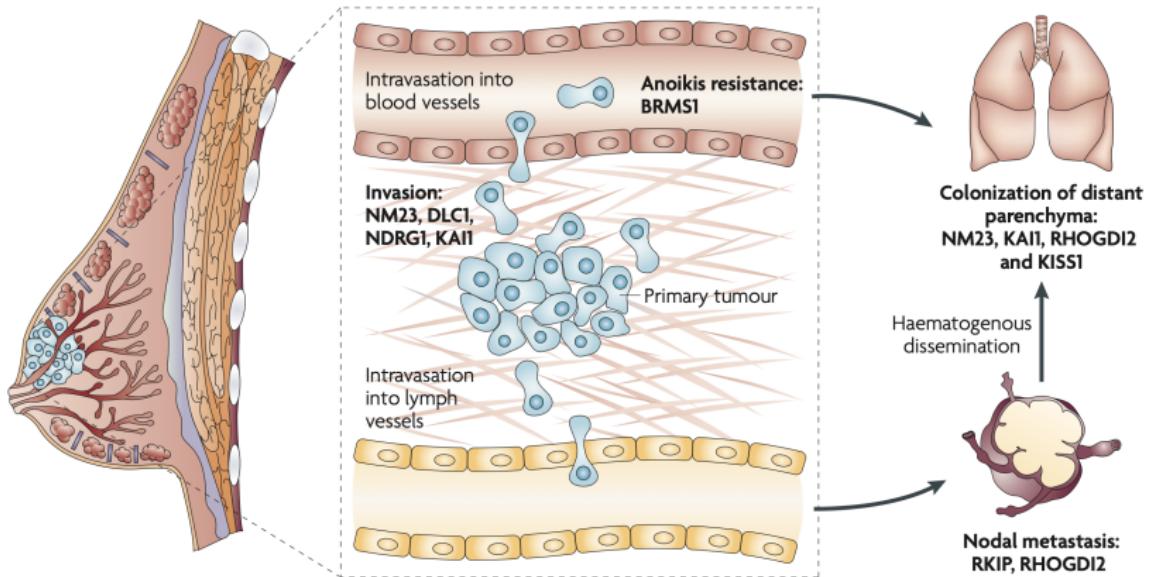


Introduction



[Smith and Theodorescu, 2009]

Introduction



Metastasis suppressor genes and steps in the metastatic cascade in human cancer [Smith and Theodorescu, 2009]

Introduction

Symbol	Alias(es)	Function(s)	Potential targeting strategy
BMP4	BMP2B	Soluble cytokine	Direct therapeutic administration of suppressor protein*
BRMS1	None	Chromatin and transcriptional regulation; regulation of gap junctions	None published at present
CTGF	CCN2, IGFBP8	Soluble cytokine	None published at present
DLC1	ARHGAP7	Regulation of RhoGTPase signalling	Re-induction of endogenous gene through HDAC inhibition ⁶⁹
KAI1	CD82, kangai 1	Inhibition of EGFR signaling; induction of senescence through interaction with DARC	Therapeutic re-induction of endogenous gene by plant extracts ⁶⁰ ; viral ⁶² and non-viral ⁶¹ gene therapy
KISS1	KiSS-1, metastin	Soluble ligand for G-protein-coupled receptor	Direct therapeutic administration of suppressor protein ⁸³ ; possibly small molecule mimetics ⁸⁴
MKK4	MAP2K4	Signal transduction	Antibody-mediated activation pathway upstream of MKK4 (REF. 122)
NDRG1	CAP43, DRG1, RTP	Unknown	Induced by iron chelators ¹²³ , p53 (REF. 124) and PTEN expression ¹²⁵
NM23	NME1, NM23-H1	Histidine kinase activity to KSR1, decreasing Ras signalling; regulation of downstream gene expression	Re-induction of endogenous gene ^{42,47,48} ; viral gene therapy ⁴⁹ ; inhibition of downstream genes ⁴⁰
RHOGDI2	ARHGDIIB, LyGDI, GDID4	Regulation of Rho family member activation; regulation of downstream gene expression	Inhibition of downstream genes ⁹⁵
RKIP	PEBP1	Binds to and inhibits Raf kinase activity and downstream signalling	Epigenetic re-induction of endogenous gene ⁶⁷

Metastasis suppressor genes: functions and reported targeting strategies [Smith and Theodorescu, 2009]

Materials and Methods

- Protein-protein interactions, literature search and biological expression language (BEL)
- Knockdown of metastasis suppressors and transcription factors
- Pharmacological perturbations of breast cancer cell lines
- Network Perturbation Amplitude (NPA)
- Measures of consistency
- Software environment and reproducibility

Metastasis suppressor genes (MSG) [Marino et al., 2014]

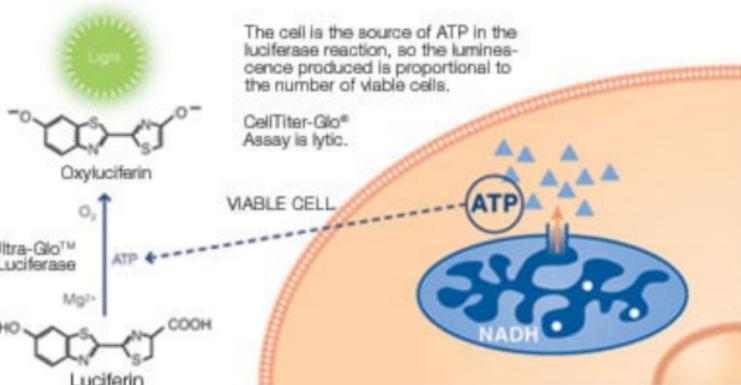
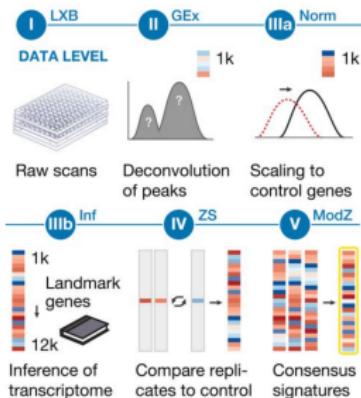
- **Cell-cell adhesion:** CD44, CD82, CDH11, CDH2, CDH1 & GSN
- **Scaffolding:** AKAP12
- **MAPK:** MAP2K6, MAP2K4, MAP2K7 & MAPK14
- **Transcription:** NME1 & BRMS1
- **GTP-binding:** ARGHDIB & DRG1
- **Other:** RRM1 & PEBP1

Transcription factors targeting metastasis suppressor genes in MCF7 [Feng et al., 2019]

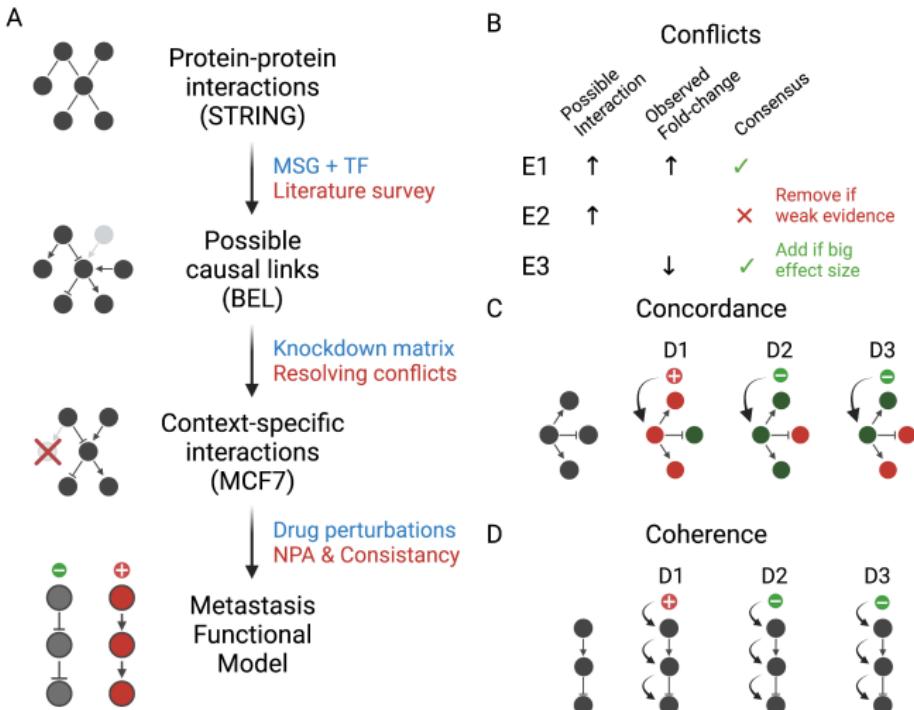
TF	Name	Dataset ID	Ref.
ESR1	Estrogen receptor 1	GSE10061	[Yau and Benz, 2008]
FOS	Fos Proto-Oncogene AP-1 Transcription Factor Subunit	GSE36586	[Dahlman-Wright et al., 2012]
FOXM1	Forkhead Box M1	GSE55204	[Bergamaschi et al., 2014]
GATA3	GATA Binding Protein 3	GSE39623	[Theodorou et al., 2013]
HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha	GSE3188	[Elvidge et al., 2006]
NR5A2	Nuclear Receptor Subfamily 5 Group A Member 2	GSE47803	[Lai et al., 2013]
POLR3A	RNA Polymerase III Subunit A	GSE42239	[Lee et al., 2015]
RARA	Retinoic Acid Receptor Alpha	GSE26298	[Salazar et al., 2011]
SPDEF	SAM Pointed Domain Containing ETS Transcription Factor	GSE40985	[Buchwalter et al., 2013]
TFAP2C	Transcription Factor AP-2 Gamma	GSE26740	[Tan et al., 2011]
YBX1	Y-Box Binding Protein 1	GSE28433	[Lasham et al., 2012]
ZFX	Zinc Finger Protein X-Linked	ENCSR005AHI	[Dunham et al., 2012]

Drug perturbations in MCF7 cell line

[Koleti et al., 2018]



Building a network of the metastasis suppressors and their regulators



Identifying protein-protein interaction (PPI) based on STRING database

The screenshot shows the STRING database homepage. At the top, there is a dark header bar with the text "Version: 11.5" on the left and "LOGIN | REGISTER | SURVEY" on the right. Below the header is a navigation bar with the STRING logo on the left and links for "Search", "Download", "Help", and "My Data" on the right. The main content area has a blue background with a faint image of a DNA double helix. In the center, the text "Welcome to STRING" is displayed above two sub-sections: "Protein-Protein Interaction Networks" and "Functional Enrichment Analysis". Below these sections, there are three large numerical values: "ORGANISMS 14094", "PROTEINS 67.6 mio", and "INTERACTIONS >20 bln". At the bottom center is a large, rounded rectangular button with the word "SEARCH" in capital letters.

Version: 11.5

LOGIN | REGISTER | SURVEY

STRING

Welcome to STRING

Protein-Protein Interaction Networks

Functional Enrichment Analysis

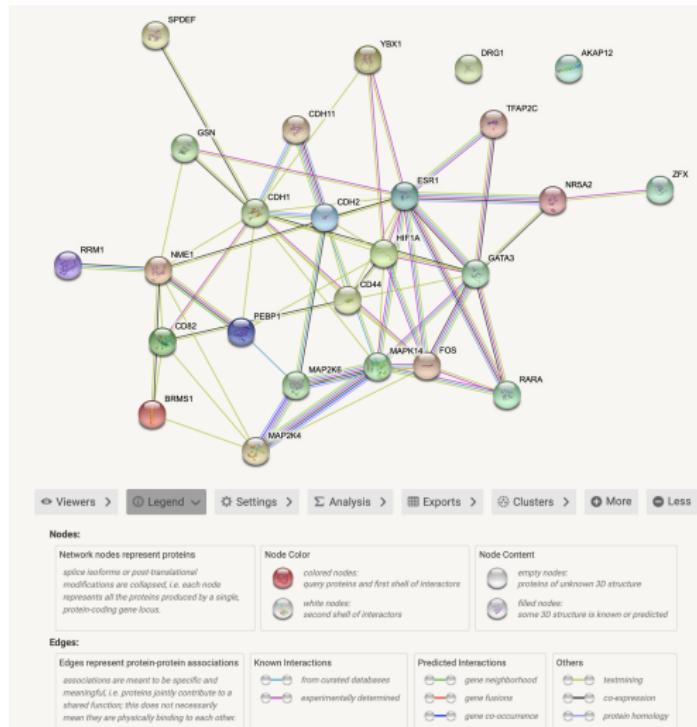
ORGANISMS 14094

PROTEINS 67.6 mio

INTERACTIONS >20 bln

SEARCH

Identifying protein-protein interaction (PPI) based on STRING database



Metastasis suppressor proteins and their regulators on STRINGDB

Identifying protein-protein interaction (PPI) based on STRING database

The screenshot shows a network graph of protein interactions. Nodes represent proteins: PEBP1 (filled purple), NME1 (filled orange), GATA3 (filled green), and others (empty grey). Edges represent interactions, colored by source: blue (curated databases), red (experimentally determined), green (textmining), black (co-expression), and blue (protein homology).

Interaction

PEBP1 [ENSP00000261313]

Phosphatidylethanolamine-binding protein 1; Binds ATP, opioids and phosphatidylethanolamine. Has lower affinity for phosphatidylserine and phosphatidylcholine. Serine protease inhibitor which inhibits thrombin, neutropeptidase and chymotrypsin but not trypsin, tissue type plasminogen activator and elastase (By similarity). Inhibits the kinase activity of RAF1 by inhibiting its activation and by dissociating the RAF1/MEK complex and acting as a competitive inhibitor of MEK phosphorylation

NME1 [ENSP00000337060]

Nucleoside diphosphate kinase A; Major role in the synthesis of nucleoside triphosphates other than ATP. The ATP gamma phosphate is transferred to the NDP beta phosphate via a ping-pong mechanism, using a phosphorylated active-site intermediate. Possesses nucleoside-diphosphate kinase, serine/threonine-specific protein kinase, geranyl and farnesyl pyrophosphate kinase, histidine protein kinase and 3'-5' exonuclease activities. Involved in cell proliferation, differentiation and development, signal transduction, G protein-coupled receptor endocytosis, and gene expression. Required for [...]

Evidence suggesting a functional link:

Neighborhood in the Genome: None, but homologous genes are neighbors in other genomes (score 0.043). [show](#)

Gene Fusions: none / insignificant

Cooccurrence Across Genomes: none / insignificant

Co-Expression: yes (score 0.075). In addition, putative homologs are coexpressed in other organisms (score 0.086). [show](#)

Experimental/Biochemical Data: none, but putative homologs were found interacting in other organisms (score 0.612). [show](#)

Association in Curated Databases: none / insignificant

Co-Mentioned in Pubmed Abstracts: yes (score 0.325). In addition, putative homologs are mentioned together in other organisms (score 0.203). [show](#)

Combined Score: 0.791

Edges:

Edges represent protein-protein associations
associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding to each other.

Known Interactions

from curated databases (blue line)
experimentally determined (red line)

Predicted Interactions

gene neighborhood (green line)
gene fusions (black line)
gene co-occurrence (blue line)

Others

textmining (green line)
co-expression (black line)
protein homology (blue line)

Nodes > Clusters > More Less

Node Content

- empty nodes: proteins of unknown 3D structure
- filled nodes: some 3D structure is known or predicted

Identifying protein-protein interaction (PPI) based on STRING database

TEXTMINING

Relevant publications mentioning your query species (Homo sapiens):

PMID: 21384649: Proteomics data of ovine mastitis associated with *Mannheimia haemolytica*.
Katarekar AI, Toogarje GT, Anagapoutous A, Balins C, Barbagosti MS, Vassilios MG, Spanos SA, Mavrogianni VS, Filimakis GC
Data Brief. 25:104399. 2019.



Abstract:

Proteomic data have been obtained from experimental mastitis in ewes after intramammary challenge with *Mannheimia haemolytica*. Animals were sampled before and sequentially after challenge. Blood plasma and milk whey samples were produced and were subjected to proteomic evaluation by means of two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry. Full protein maps and differential proteomics in sequential samples from blood plasma and milk whey of experimental ewes were presented. Post-challenge, 33 and 89 proteins were identified with differential abundance in blood plasma and milk whey, respectively. Also, 74 proteins were identified with differential abundance between the inoculated and contralateral glands. The data provide further insight in the pathophysiology of mastitis in sheep and indicate potential biomarkers for the disease. The data are further discussed in the research article "Differential quantitative proteomics study of experimental *Mannheimia haemolytica* mastitis in sheep" [1]. Excerpts from full text:

... 10, LACB: Beta-lactoglobulin-1/B, LALBA: Alpha-lactalbumin, MMP1: Intestinal collagenase, NDKA (): Nucleoside diphosphate kinase A (), NDKB: Nucleoside diphosphate kinase B, PDAT: Protein disulfide-isomerase, PDIA3: Protein disulfide-isomerase A3, PEBP1 (): Phosphatidylethanolamine-binding protein 1 (), PERL: Lactoperoxidase, PGAM1...

PMID: 28967874: Metastasis suppressors: functional pathways.



Khan I, Steer L
Lab Cell. 36(2):158-210. 2018.

© NM23, NM23H1, NMET1 () PEBP1, RKIP; Raf kinase inhibitory protein ...

PMID: 28585830: Multifactorial Regulation of G Protein-Coupled Receptor Endocytosis.



Zhang X, Kim JH, Lee JY
Biosci Ther (Seoul). 23(1):29-43. 2017.

© NM23-H1 () Raf kinase inhibitor protein ...

PMID: 28507050: Tumor Dormancy and Relapse: From a Natural Byproduct of Evolution to a Disease State.



Moroff AH
Cancer Res. 77(10):2564-2569. 2017.

© NM23-H1 () Rkip ...

PMID: 25485823: SNAD2/Stargate gene is silenced in prostate cancer and regulates neuroendocrine differentiation, metastasis-suppressor and pluripotency gene expression.



Espinoza S, Russo MV, Arredondo J, Yupon MG, Sommerville C, Barberito G, Di Mio S, Di Carlo E
Oncotarget. 6(19):17123-17134. 2015.

© NM23-H1 () PBP1, RKIP; Raf kinase inhibitor protein, phosphatidylethanolamine-binding protein 1 ...

[Truncated after 5 items ... view more]

Relevant publications mentioning other organisms:

PMID: 28507050: Tumor Dormancy and Relapse: From a Natural Byproduct of Evolution to a Disease State.
Moroff AH
Cancer Res. 77(10):2564-2569. 2017.



Abstract:

Species evolve by mutations and epigenetic changes acting on individuals in a population, tumors evolve by similar mechanisms at a cellular level in a tissue. This article reviews growing evidence about tumor dormancy and suggests that (i) cellular malignancy is a natural byproduct of evolutionary mechanisms, such as de novo mutations and epigenetic modifications, which is manifested in the form of tumor dormancy in healthy individuals as well as in cancer survivors; (ii) cancer metastasis could be an early dissemination event that could occur during malignant dormancy even before primary cancer is clinically detectable; and (iii) chronic inflammation is a key factor in awakening dormant malignant cells at the primary site, leading to primary cancer development, and at distant sites, leading to secondary stage diseases. On the basis of this evidence, it is conceivable to propose that we are all cancer survivors rather than cancer-free individuals because of harboring dormant malignant cells in our bodies. A better understanding of local and metastatic tumor dormancy could lead to novel cancer therapeutics for the prevention of cancer. (Cancer Res; 77(10): 2564-9. (C2017 AACR))

Excerpts from full text:

... or latency. Each cancer type appears to have distinct metastasis suppressor genes. For instance, NM23 () and BRMS1 are involved in breast cancer; KAI1, MKK4, RKIP (), RHODD2, Drg-1 are involved in prostate cancer, and TXNIP and KISS1 are ...

PMID: 27924059: Plant-based foods containing cell wall polysaccharides rich in specific active monosaccharides protect against myocardial injury in rat myocardial infarction models.



Uhm J, Kim K, Kim JK, Jang JH, Han MJ, Lee J

Sci Rep. 6:38723. 2016.

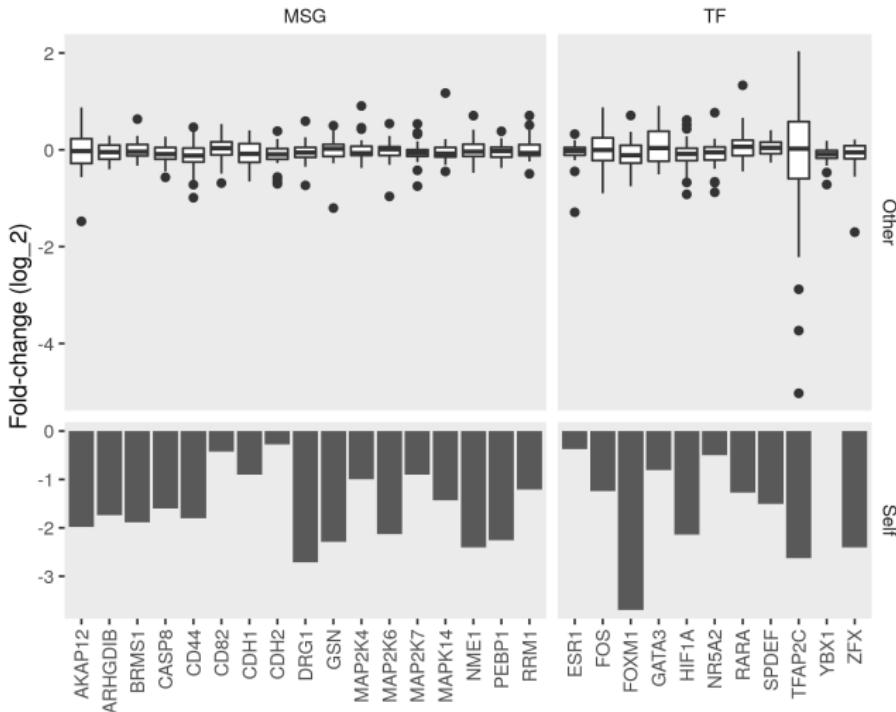
© NM23 () RKIP ...

Converting possible interactions of metastasis suppressors to Biological expression language (BEL)

(Examples)

Subject	Object	Ref.	Interaction
MAPK14	FOS	[Janknecht and Hunter, 1997]	act(p(HGNC:6876!MAPK14), ma(kin)) increases p(HGNC:3796!FOS)
MAP2K4	FOS	[Xue et al., 2018]	p(HGNC:6844!MAP2K4) increases p(HGNC:3796!FOS)
MAPK14	GATA3	[Wan, 2014]	p(HGNC:6876!MAPK14) increases act(p(HGNC:4172!GATA3))
RUNX2	HIF1A	[Lee et al., 2012]	p(HGNC:10472!RUNX2) increases act(p(HGNC:4910!HIF1A))
PEBP1	MAP2K6	[Lai et al., 2017]	p(HGNC:8630!PEBP1) increases act(p(HGNC:6846!MAP2K6))
PEBP1	MAP2K3	[Lai et al., 2017]	p(HGNC:8630!PEBP1) increases act(p(HGNC:6843!MAP2K3))
MAPK14	RUNX2	[Hutchison, 2013]	p(HGNC:6876!MAPK14) increases r(HGNC:10472!RUNX2)
NME1	AKAP12	[McCorkle et al., 2014]	p(HGNC:7849!NME1) increases r(HGNC:370!AKAP12)
NME1	PEBP1	[Berger et al., 2005]	r(HGNC:7849!NME1) increases r(HGNC:8630!PEBP1)
PEBP1	MAPK14	[Lai et al., 2017]	p(HGNC:8630!PEBP1) increases act(p(HGNC:6876!MAPK14))
TNFSF10	CASP8	[Mizamtsidi et al., 2018]	p(HGNC:11925!TNFSF10) increases p(HGNC:1509!CASP8)

Contextualizing metastasis suppressor interactions in Breast cancer cells

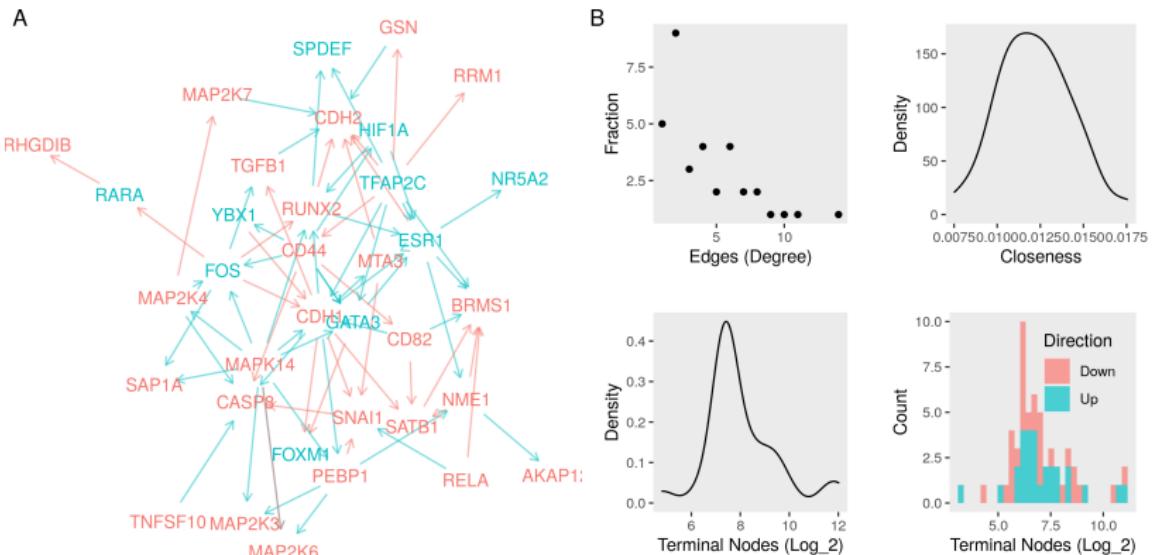


Contextualizing metastasis suppressor interactions in Breast cancer cells

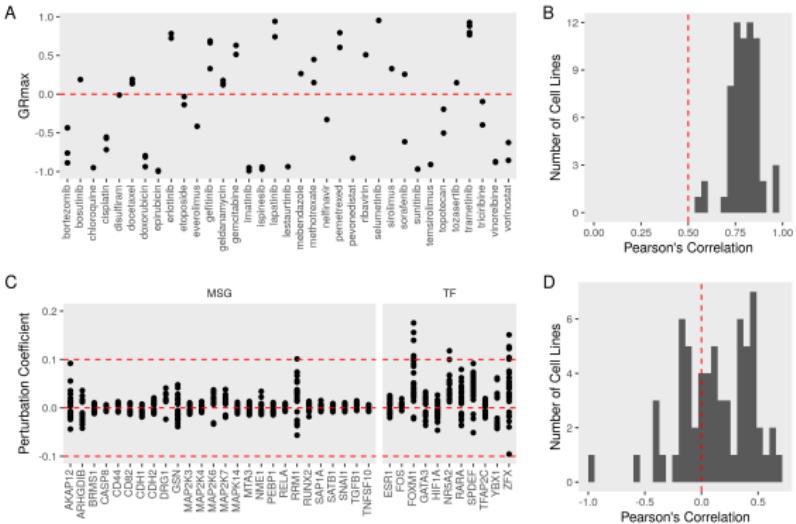
Resolving *conflicts* between possible **interactions** and **fold-changes**:

- No evidence to the contrary → Keep
- Significant change in the opposite direction → Remove
- Positive significant effects of the knockdown of one node on others as an interaction between the mRNA → Add

Contextualizing metastasis suppressor interactions in Breast cancer cells



Evaluating the metastasis model using drug perturbation data



Evaluating the metastasis model using drug perturbation data

The **agreement** between expected and observed direction of change in the subnetworks and paths.

$$\frac{1}{n} \sum_{i=1}^n x_i - x = \begin{cases} 1, & \text{if } u.e = x' \\ 0, & \text{otherwise} \end{cases} \quad u = \begin{cases} 1, & \text{if } u \text{ is activated} \\ -1, & \text{if } u \text{ is repressed} \end{cases}$$

- Where x is the observed and x' is the expected effect on the nodes of the *subnetwork* downstream from u and connected to it by the edges e (**concordance**).
- Where x is the observed and x' is the expected effect on the nodes in a *path* connected by edges e and u is considered moving down the path (**coherence**).

Evaluating the metastasis model using drug perturbation data

TEXTMINING

Relevant publications mentioning your query species (Homo sapiens):

PMID: 9138464; Proteomics data of ovine mastitis associated with *listeria heminola*.
Katsarabasi AL, Toogaripati GT, Anagnostopoulou AK, Balinis C, Barbagosti MS, Vassilou MC, Spanos SA, Mavrogianni VS, Filisakis GC
Data Brief. 25:10439 2019.



Abstract:

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... 10. LACB: Beta-lactoglobulin-1/B, LALBA: Alpha-lactalbumin, MMP1: Intestinal collagenase, NDKA (): Nucleoside diphosphate kinase A (), NDKB: Nucleoside diphosphate kinase B, PDAT: Protein disulfide-isomerase, PDIA3: Protein disulfide-isomerase A3, PEPBP1 (): Phosphatidylethanolamine-binding protein I (), PERL: Lactoperoxidase, PGAM1...

PMID: 28967874; Metastasis suppressors: functional pathways.



Khan I, Steeg PS
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Moroff AH
Cancer Res. 77(10):2564-2569 2017.

© NM23 () Rkip ...

PMID: 25848582; SNAD2/Slug gene is silenced in prostate cancer and regulates neuroendocrine differentiation, metastasis-suppressor and pluripotency gene expression.



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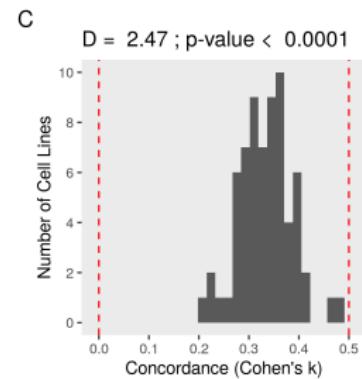
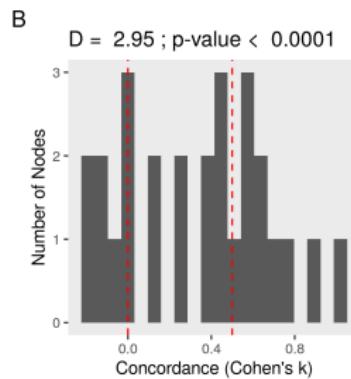
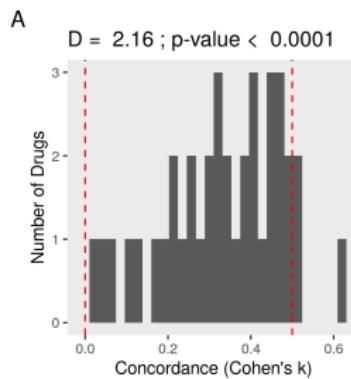


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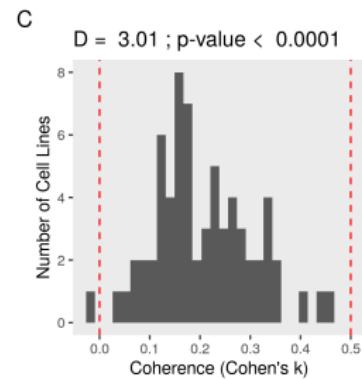
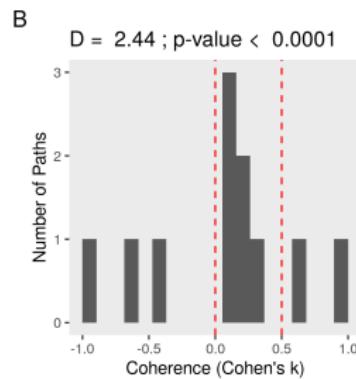
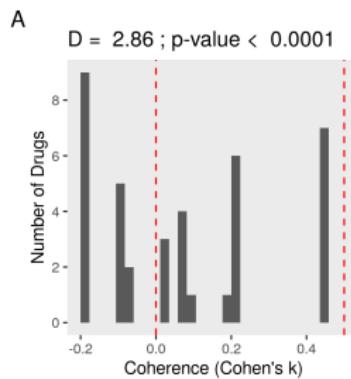
Sci Rep. 6:38723 2016.

© NM23 () RKIP ...

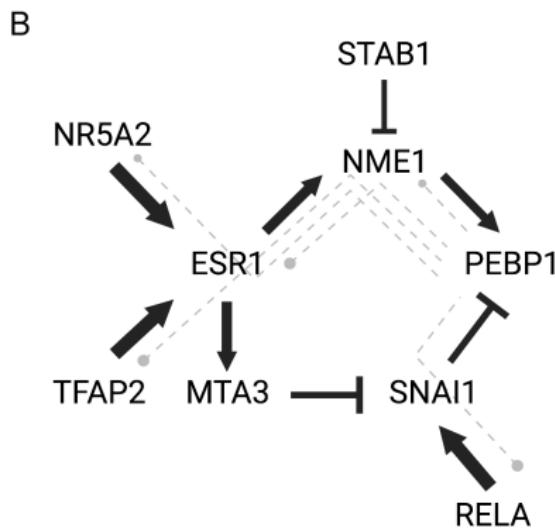
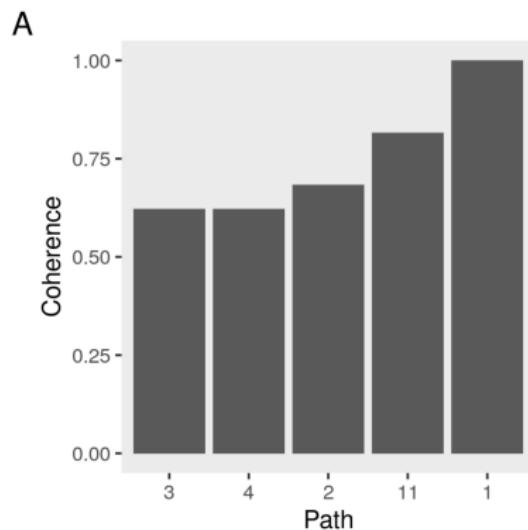
Evaluating the metastasis model using drug perturbation data



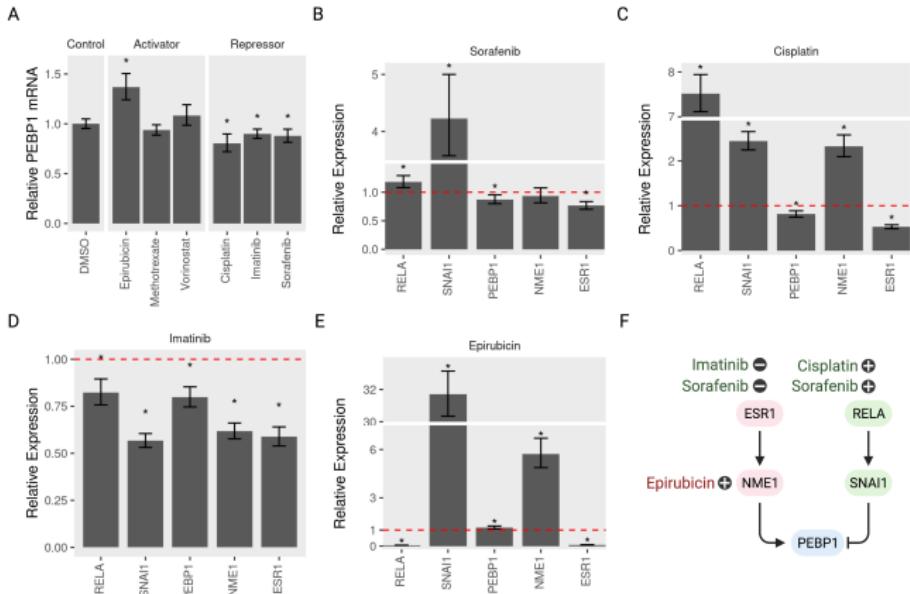
Constructing a model of PEBP1 and its interaction with other metastasis suppressors



Constructing a model of PEBP1 and its interaction with other metastasis suppressors



Results



Summary

- We used text mining datasets and a manual literature search to extract evidence for *possible* interactions between several metastasis suppressors and their regulators.
- We then used the knockdown dataset to filter these interactions in the *context* of the breast cancer cell line MCF7.
- The resulting interactions were coded in the biological expression language (BEL) to build a functional metastasis *model*.
- A reverse causal reasoning approach was used to test and *prioritize* these interactions and extract pathways that are most consistent with drug treatments that inhibit cell growth.
- We suggested that the metastasis suppressor PEBP1 is on the receiving end of two key regulatory *pathways*. One involves P65 (RELA) and SNAI1, which were previously reported to inhibit PEBP1, and the other involves the estrogen receptor (ESR1), which induces PEBP1 through the kinase NME1.

References I

-  Bergamaschi, A., Madak-Erdogan, Z., Kim, Y. J., Choi, Y.-L., Lu, H., and Katzenellenbogen, B. S. (2014). The forkhead transcription factor FOXM1 promotes endocrine resistance and invasiveness in estrogen receptor-positive breast cancer by expansion of stem-like cancer cells. *Breast cancer research : BCR*, 16(5):436.
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