Functional genomics reveal that the serine synthesis pathway is essential in breast cancer

Results

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http://www.amsbio.com/expression-ready-lentiviral-particles.aspx

Overview

Background

- Serine biosynthetic pathway
- *In vivo* functional negative selection shRNA screen

  -- *in vivo* orthotopic model

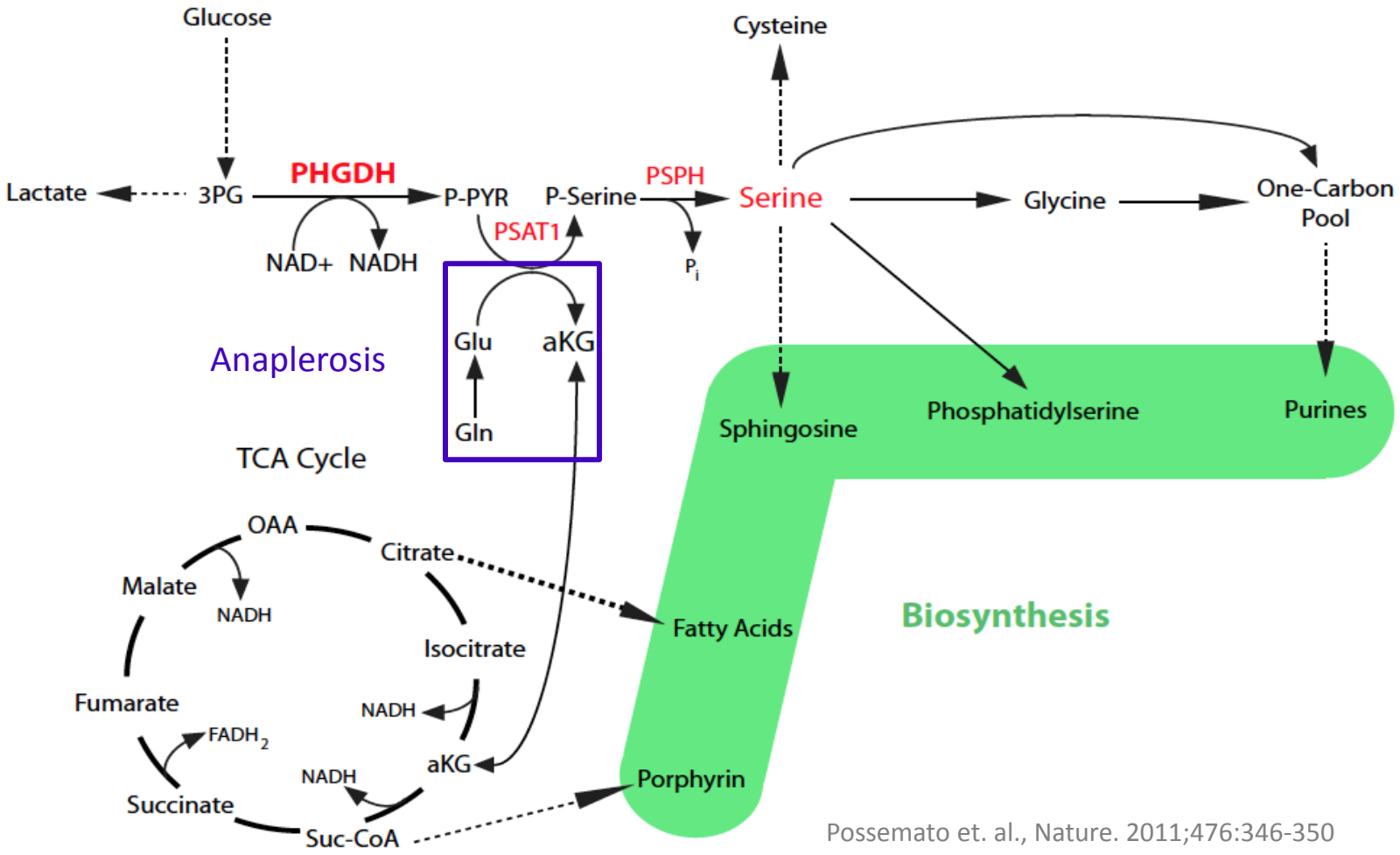
  -- negative selection

Results

- shRNA screen design & identification of essential metabolic genes in tumorigenesis-phosphoglycerate dehydrogenase (PHGDH)
- Correlation of high PHGDH expression with aggressive breast cancer
- Metabolic consequences of increased PHGDH expression level in cancer

Serine biosynthetic pathway
phosphoglycerate dehydrogenase (PHGDH)

Anaplerotic reaction (Anaplerosis): form intermediates of a metabolic pathway



Possemato et. al., Nature. 2011;476:346-350

Choice of mouse model
orthotopic: MCF10DCIS.com inject in mouse mammary fat pad

- Tumor growth rate
- Tumor formation frequency
- Metastases formation

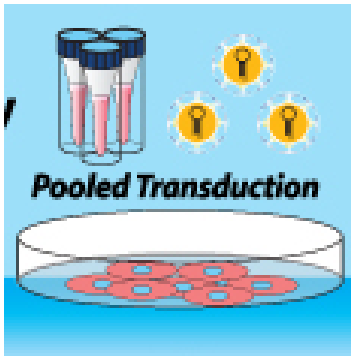
A comparison of xenograft (subcutaneous and orthotopic)

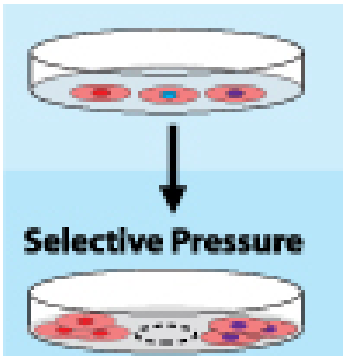
Subcutaneous xenograft	Orthotopic xenograft
Easy to set up	Need surgical expertise
Relatively inexpensive	More expensive
Labor economic	Labor intensive
Time economic	Longer time needed
Easy to monitor tumor burden and progression	Not as easy to monitor tumor burden and progression
Gene expression is not organ specific	Organ-specific gene expression
Least relevant tumor cell and host organ interactions	Relevant tumor cell and host organ interactions
Lack of natural metastasis	Metastasis can be studied

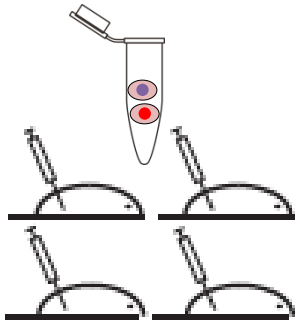
Adapted from: Liu M and Hicklin D. Cancer Drug Discovery and Development. 2011. pg 99-124.

Functional negative selection of shRNA screen *in vivo*

- Negative selection: identify shRNAs depleted in tumorigenesis, shRNA bar-code screening



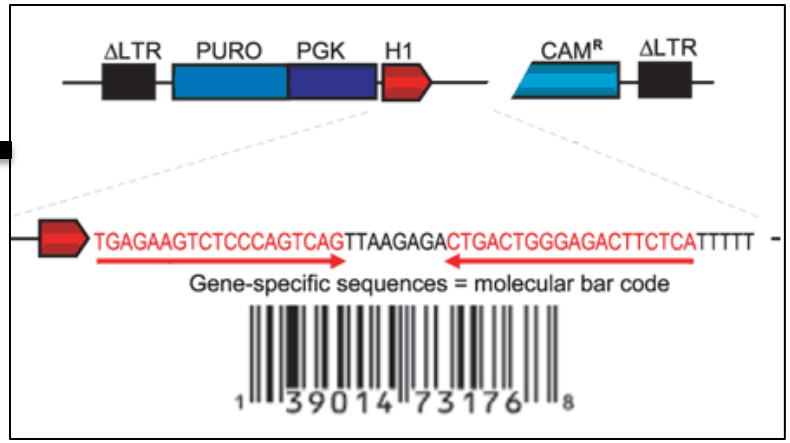




28 days

Orthotopic tumors

- Extract genomic DNA
- PCR amplify shRNA



Deep-sequencing shRNA

Hit identification

Adapted from:
Possemato et. al., Nature. 2011;476:346-350
René Bernards et al., Nature method. 2006; 3:701-706
https://www.openbiosystems.com/RNAi/shRNAmirLibraries/DecodeRNAiviralscreening/

Comprehensive screening of pooled shRNA expression libraries

Considerations:

-- Thoroughly screen pooled shRNA expression libraries
-- Minimize false negatives
-- Obtain reproducible data

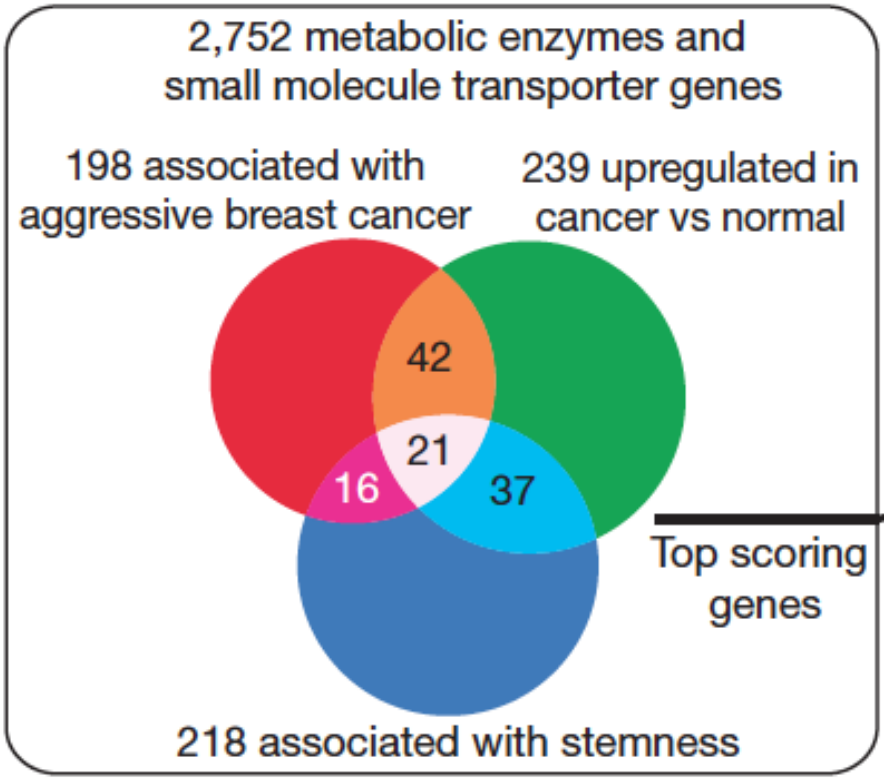
problem	solution
Effectiveness of KD of target gene	~5 distinct shRNA/ gene
Statistical quantification of changes	~500-1000 cells/ distinct shRNA
Avoidance of non- specific effect	Multiplicity of infection (M. O. I.)= 0.3-0.5

Ex: Screen for 100 genes

- Need 100 genes x ~5 shRNA/gene=~500 shRNAs
- Need 500 shRNAs x ~1000 cells/ shRNA =~$5x10^5$ cells
- Need ~3 x ~$5x10^5$ cells = ~$1.5x10^6$ cells

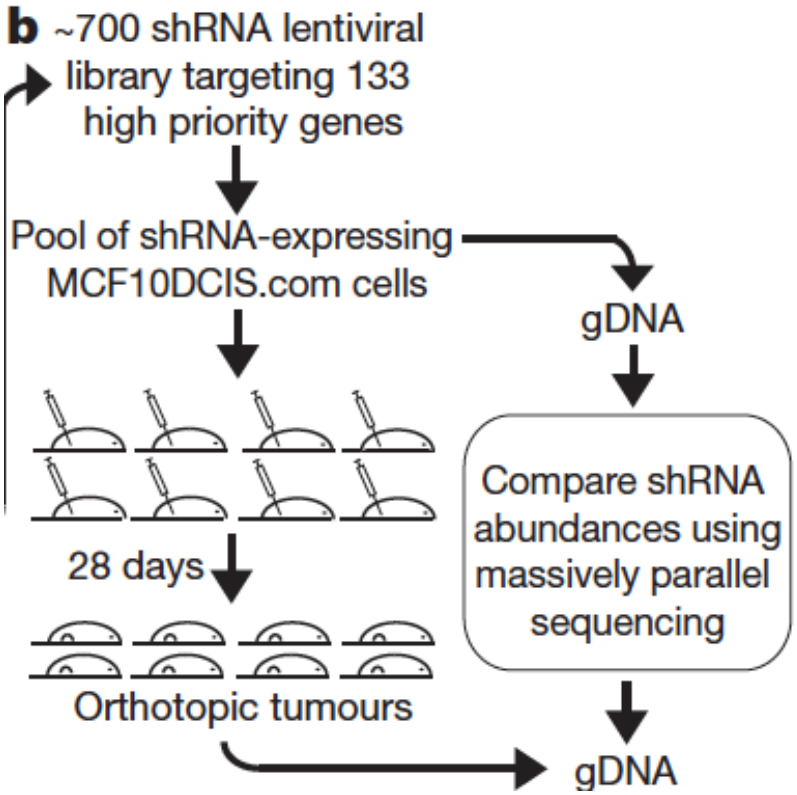
Strategy of *in vivo* pooled shRNA screening

--Determine cancer-relevant subset of metabolic genes for screening



- Cross-reference maps of metabolic pathways with the KEGG database
- Analyze gene expression studies in Oncomine database
  - tumor vs. normal
  - aggressive breast cancer
  - differentiated vs. stem cells
- 133 (43 transporters+ 86 metabolic enzymes) high priority genes scored in 2 categories and the top of each category

In vivo functional pooled shRNA screening



- 2 libraries of shRNA-expressing lentiviruses (Median 5 shRNAs/gene)
  - 47 transporter genes: 235 shRNAs
  - 86 metabolic enzyme genes: 516 shRNAs
- Infect 1.5×10^6 human MCF10DCIS.com cells/library
- Inject shRNA library infected MCF10DCIS cells into mouse mammary fat pads at 2 sites/animal(~500-1000 cells/shRNA; total 10^5-10^6 cells/site)
- Screen for depleted shRNAs (negative selection) during breast tumor formation in mice

PHGDH is identified as essential for tumorigenesis by *in vivo* RNAi screening

16 Hit genes: 75% of shRNA targeting these genes scoring

Enzymes

- *CTPS*
- *GAPDH*
- *GLS2*
- *GMPS*
- *NUDT5*
- *PHGDH*
- *PLA2G7*
- *PYCR1*
- *SEPHS1*
- *SOD2*
- *TALDO1*
- *TPI1*

n=12 tumors

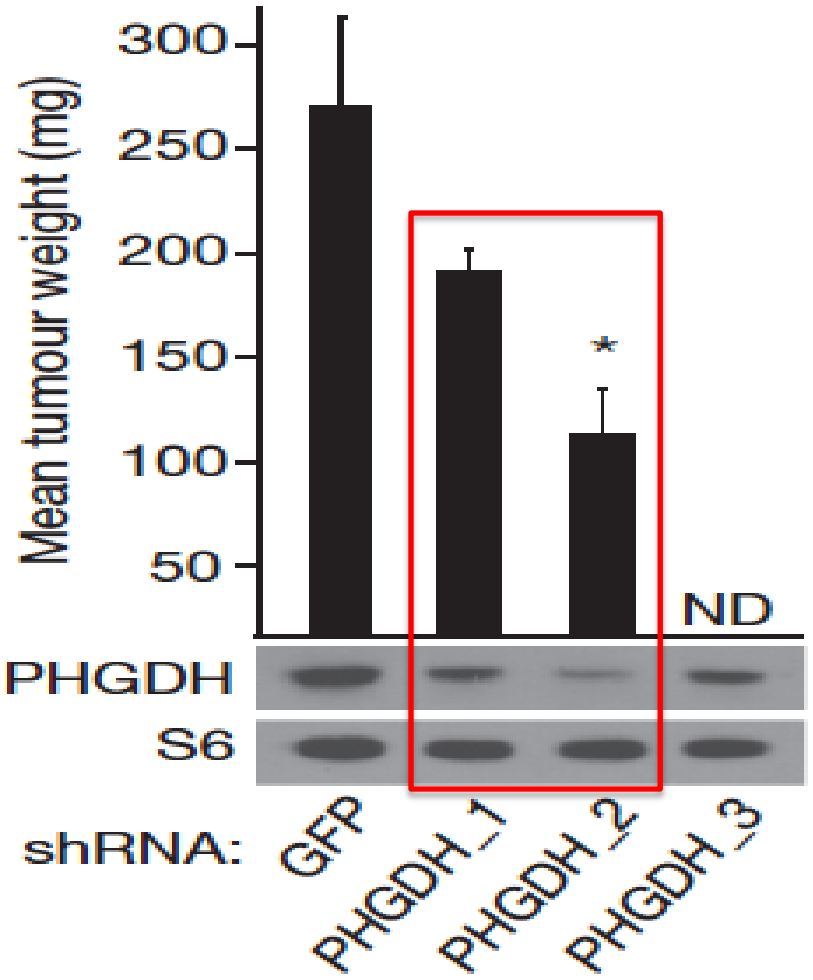
Transporters

- *SLC15A1*
- *SLC16A3*
- *TTYH3*
- *VDAC1*

n=5 tumors

Scoring cutoff:

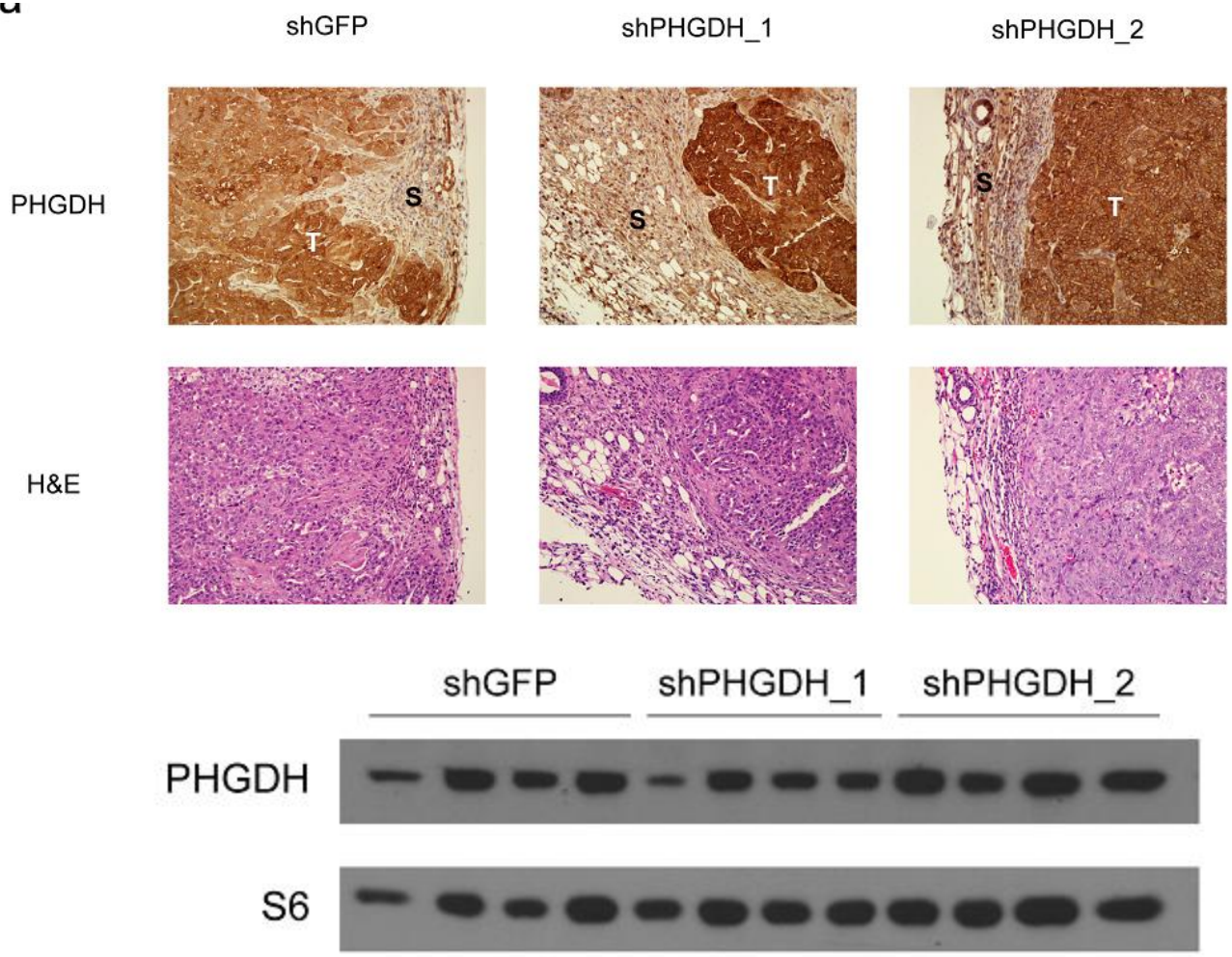
- P value< 0.05
- Log_2 fold change < -1



n=4 tumors for S.E.M error bar

**P* value< 0.05. ND, not done

Tumorigenesis select for lost of shRNA-mediated PHGDH suppression



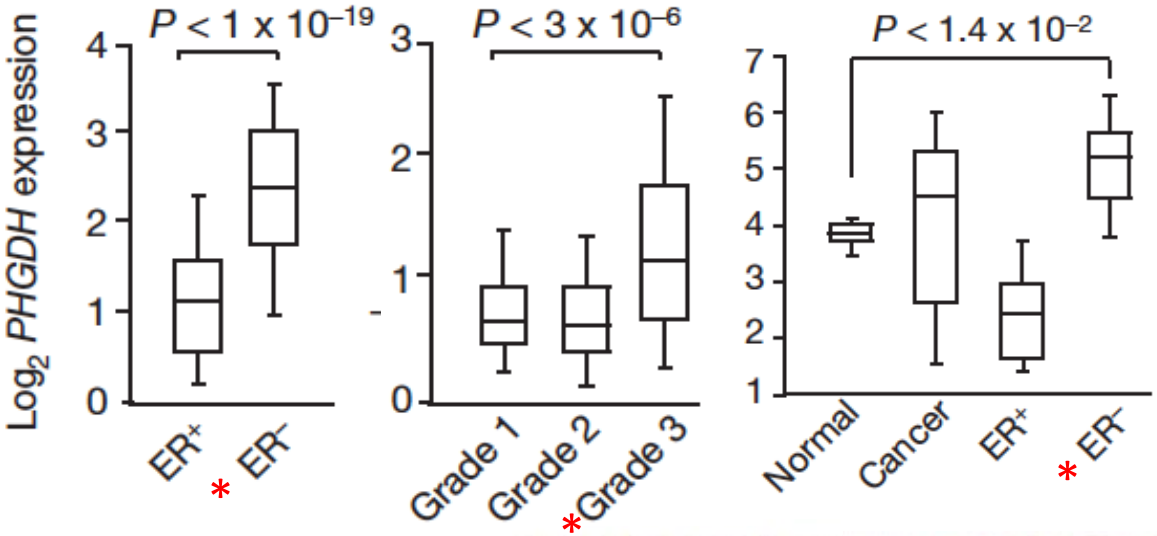
Immunohistochemical staining against PHGDH in tumor

- Loss of PHGDH suppression in tumors derived from MCF10DCIS.com cells

Immunoblot for PHGDH and S6 expression from tumor

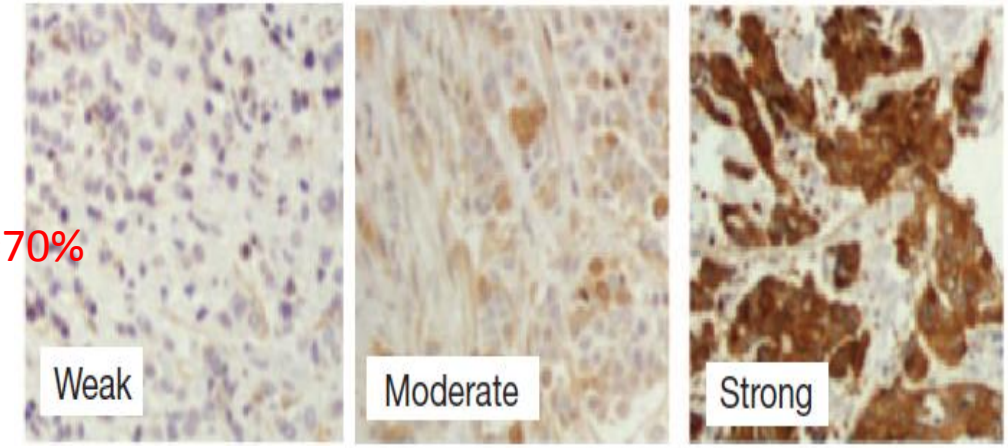
High PHGDH expression associated with aggressive breast cancer

~68% and ~70% of ER$^-$ breast tumors have elevation of PHGDH at the mRNA and protein level, respectively



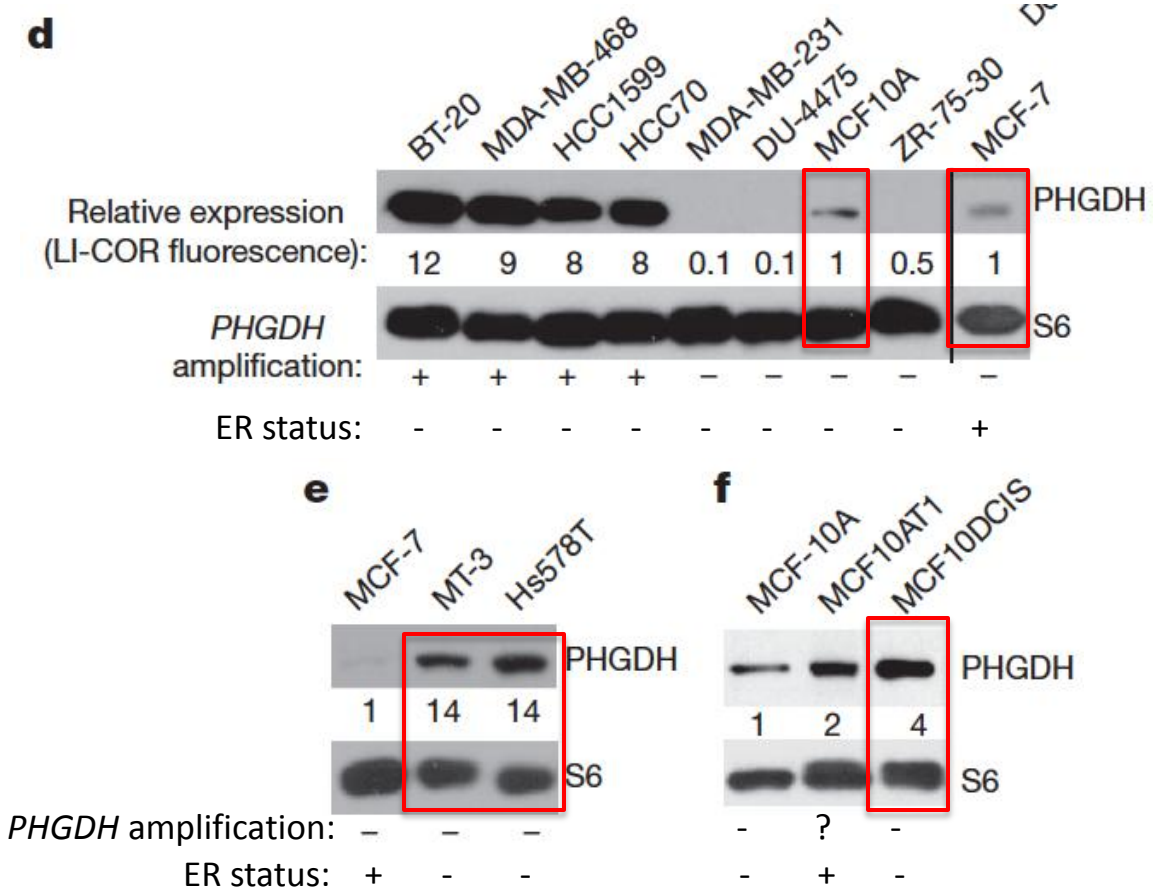
	Staining intensity		
	Weak	Moderate	Strong
ER$^-$, Her2$^-$	0	4	11
ER$^-$, Her2$^+$	2	3	12
ER$^+$, Her2$^-$	13	4	4
ER$^+$, Her2$^+$	12	11	6

* } 23/32=70%



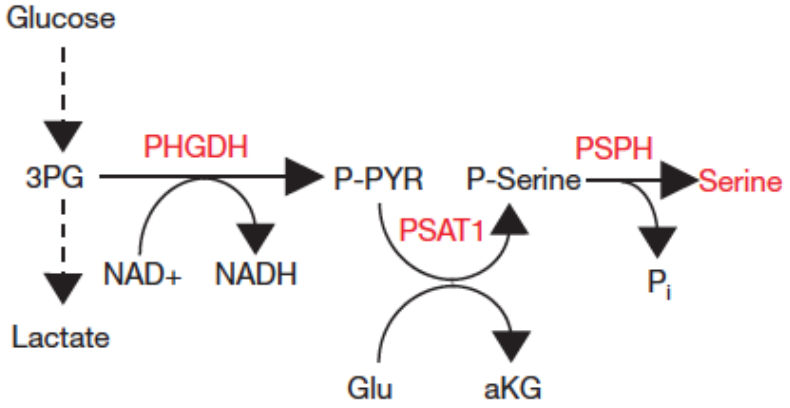
Immunohistochemical staining against PHGDH in tumor

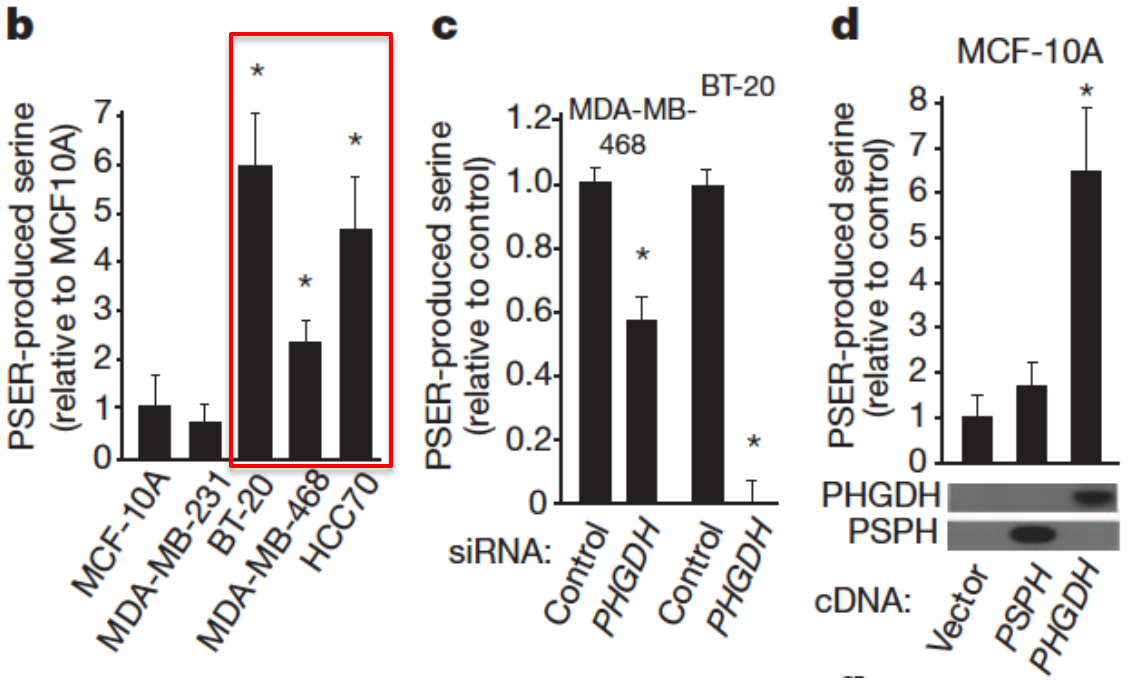
Elevated PHGDH protein expression in some ER$^-$ breast cancer cell lines



- PHGDH protein level increase by 8-12X in *PHGDH* amplified ER$^-$ breast cancer cell lines
- PHGDH protein level increase in *PHGDH* non-amplified, ER$^-$ cell lines
- PHGDH protein expression up-regulated in MCF10DCIS by 4x

PHGDH controls serine biosynthetic pathway in cancer cell lines



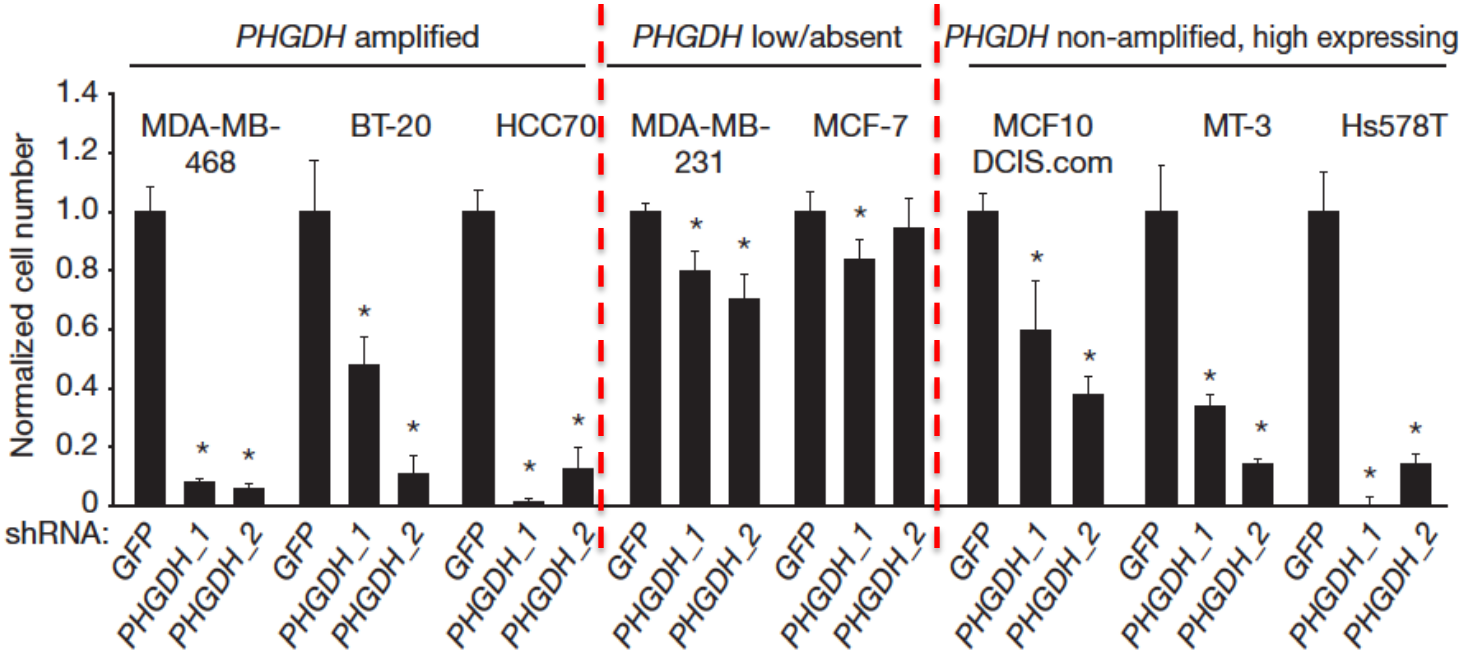


- PHGDH is required for the increased serine pathway flux
- Overexpression of PHGDH is sufficient to drive serine pathway flux

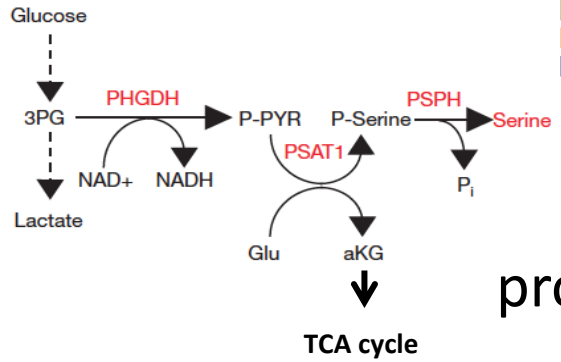
w/ amplification: BT-20, MDA-MB-468, HCC70

w/o amplification: MCF-10A, MDA-MB-231

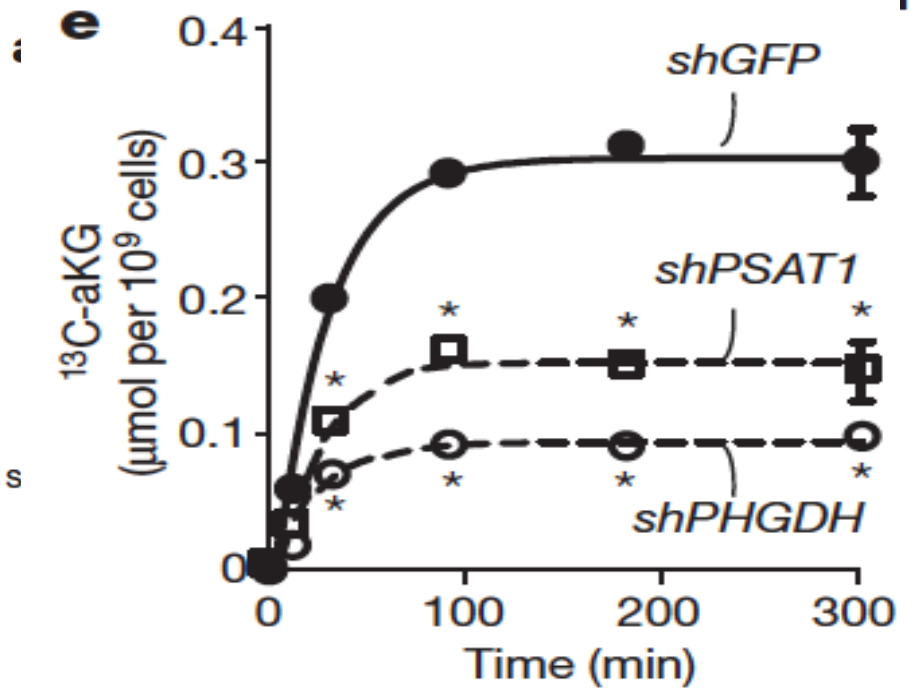
Dependence of elevated PHGDH expression in a subset of ER$^-$ cancer cell lines proliferation

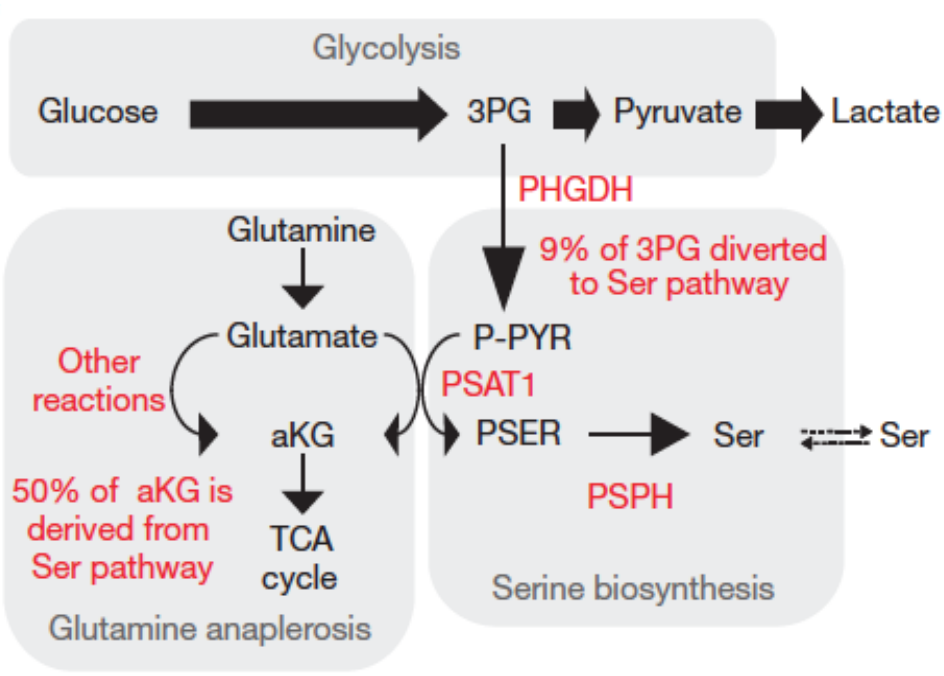


- A subset of ER$^-$ cancer cells addicted to flux through serine biosynthesis pathway for proliferation



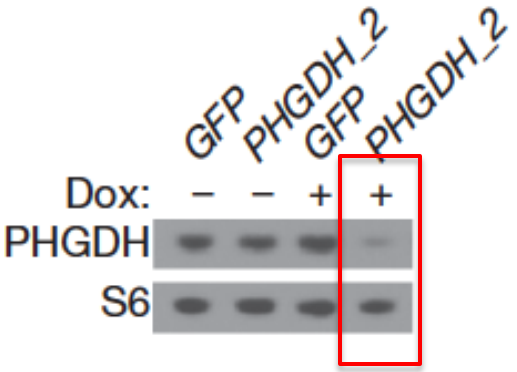
PHGDH pathway can promote cell proliferation by anaplerosis for TCA cycle

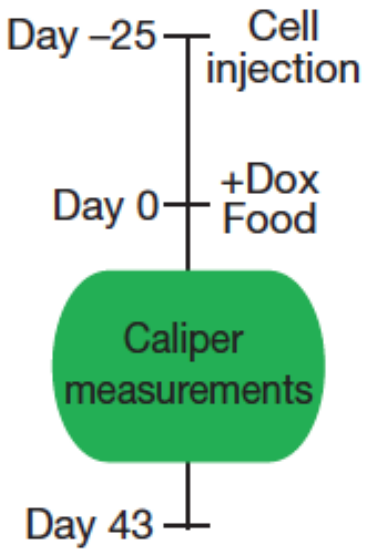


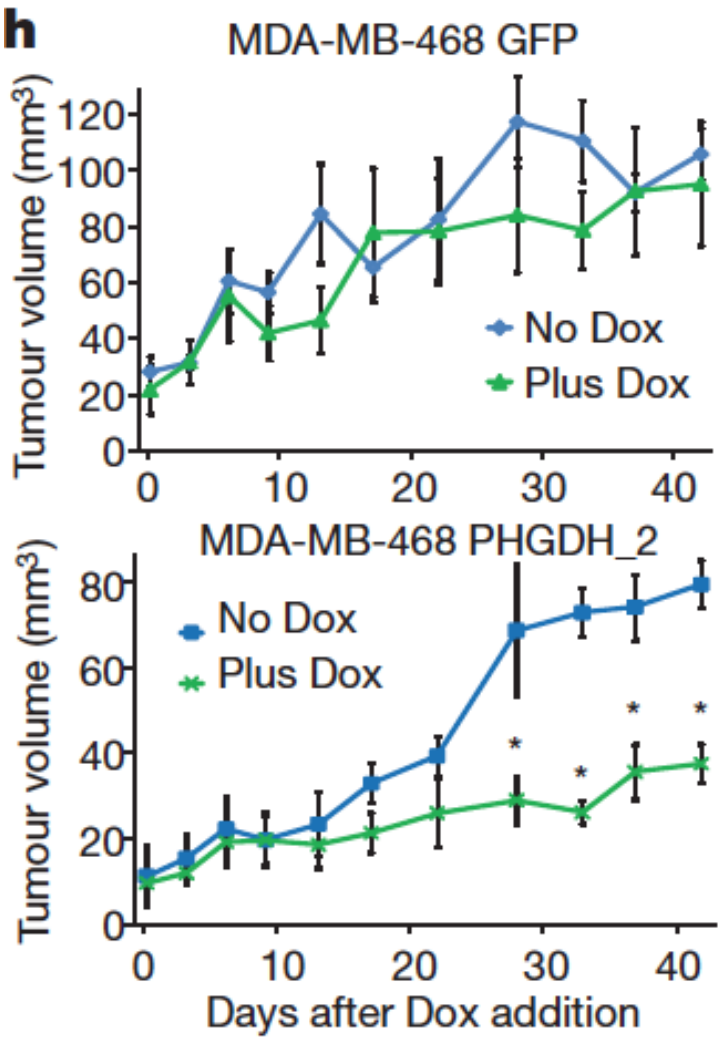


- KD of PHGDH leads to deficiency in anaplerosis of glutamine to αKG

Inducible reduction of PHGDH protein expression suppresses established tumor *growth in vivo*







Conclusion

- Apply *in vivo* negative- selection shRNA screen for finding potential anticancer targets
- Demonstrate the association of elevated PHGDH expression with ER^- breast cancer
- PHGDH is important in increased serine pathway flux
- PHGDH is responsible for a significant portion of anaplerosis of glutamine into TCA cycle in breast cancer cells with high PHGDH expression
- Increased PHGDH expression may contribute to breast cancer
- Demonstrate PHGDH as potential therapeutic target for a subset of ER^- breast cancer patients

Discussion

Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis

Jason W Locasale[1,2], Alexandra R Grassian[3], Tamar Melman[1,2], Costas A Lyssiotis[1,2], Katherine R Mattaini[4], Adam J Bass[5,6], Gregory Heffron[7], Christian M Metallo[8], Taru Muranen[3], Hadar Sharfi[1,2], Atsuo T Sasaki[1,2], Dimitrios Anastasiou[1,2], Edouard Mullarky[1,2], Natalie I Vokes[4], Mika Sasaki[1,2], Rameen Beroukhim[5,6,9], Gregory Stephanopoulos[8], Azra H Ligon[5,10], Matthew Meyerson[5,6,11], Andrea L Richardson[5,10], Lynda Chin[5,12], Gerhard Wagner[7], John M Asara[2], Joan S Brugge[3], Lewis C Cantley[1,2] & Matthew G Vander Heiden[4,5]

Nature Genetics 43, 869–874 (2011)

REVIEW

Succinate dehydrogenase and *fumarate hydratase*: linking mitochondrial dysfunction and cancer

A King, MA Selak and E Gottlieb

Cancer Research UK, The Beatson Institute for Cancer Research, Glasgow, UK

Oncogene (2006) 25, 4675-4682

genetic alterations in metabolic genes selected in tumorigenesis lead to remodeling of cancer metabolism