ICSB2014
The Fourth International Workshop on Cancer Systems Biology
June 20-21, Jilin University, Changchun, China

Two types of cancer stem cells – epithelial-mesenchymal transition and proliferation – and their response to repurposed drugs

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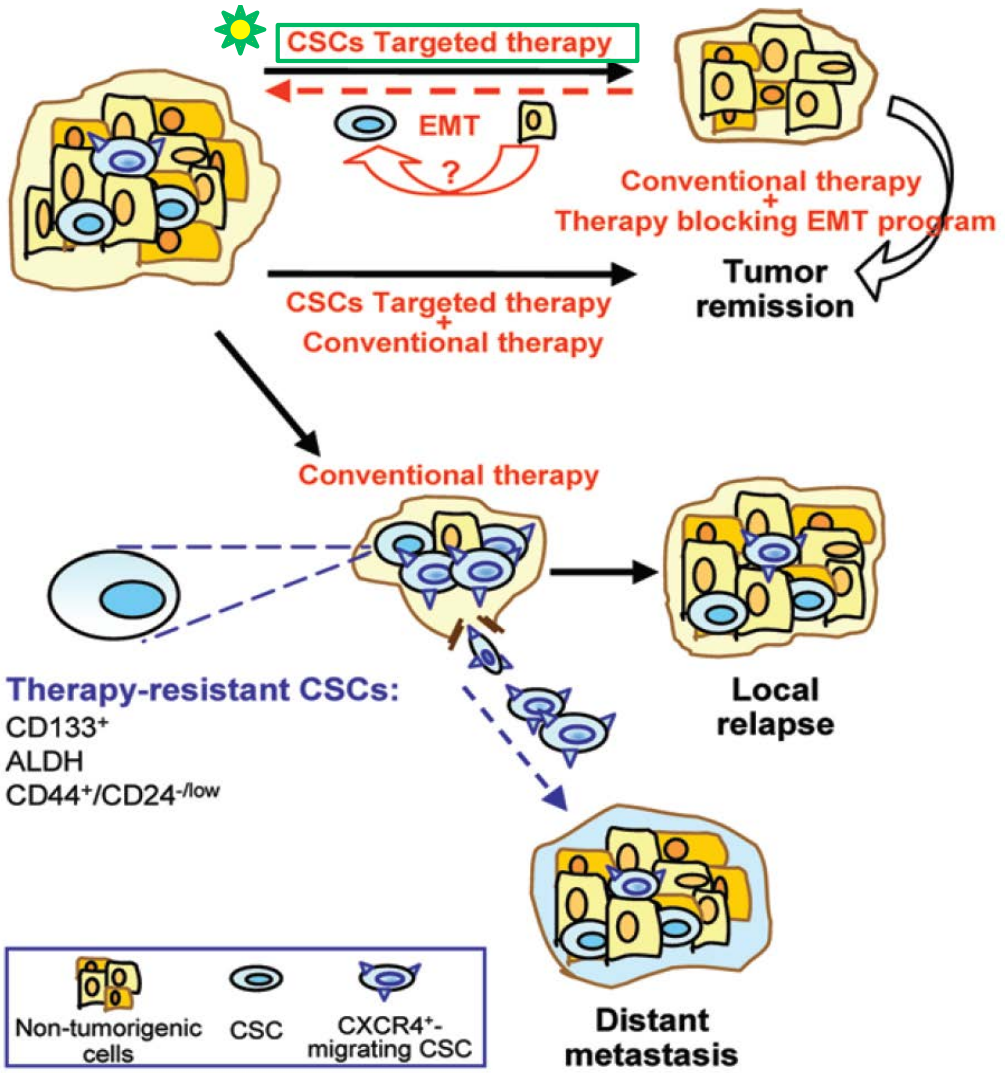
Introduction 1/2

- Mortality from cancers has been steadily declining over the past decade, primarily due to earlier detection, adjuvant therapies and the advent of targeted therapies.

- Disappointing results of standard treatments for preventing cancer relapses, include chemotherapy and radiotherapy, have recently been attributed to the stem cell-like properties of cancer cells.



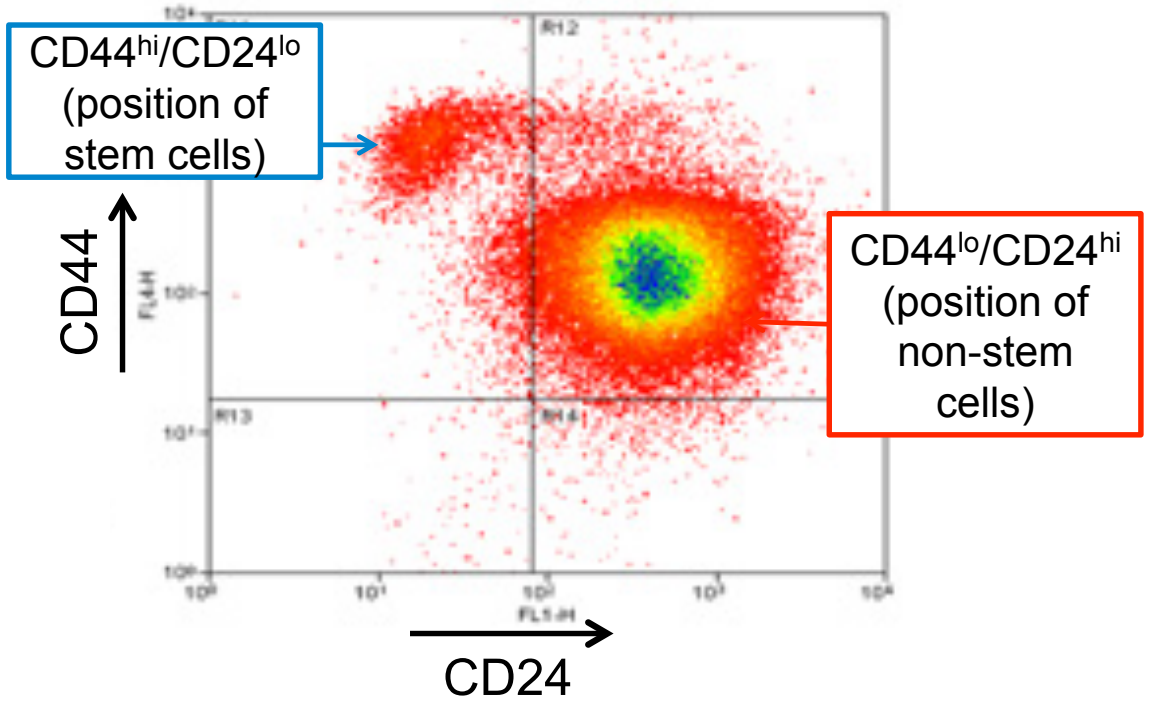
Giulia, et al., Cell Cycle 2010

Characteristics of Cancer Stem Cells (CSCs)

1. *self-renewal*
2. *tumor initiation*
3. *therapy-resistant*
4. *invasive*
5. *metastatic*

CSCs can be identified by FACS

Fluorescence-activated cell sorting (FACS)
fractionation of human mammary epithelial cells



Sendurai A, et al., Cell, 2008.

Data source

- We collected available gene expression CSCs data sets of multiple cancer types from the Gene Expression Omnibus (GEO) database and used a variety of qualitatively different methods to cluster the data sets to establish functional characteristics of cancer specific CSCs.

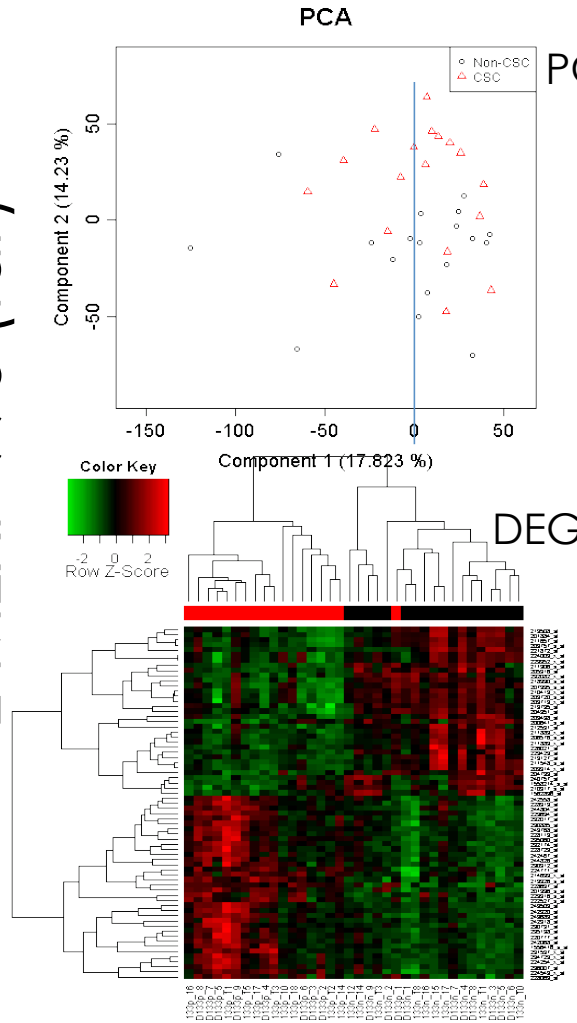
- Data sets were sift by Principle Component Analysis (PCA). 14 CSCs and 4 control data sets were used for the study.

CSC and control datasets were quality assessed

	name	contrast* (no. of chips)	publication	PCA score
CSCs (14)	Breast_CD44_GSE15192	CD44+/CD24- (4) vs CD44-/CD24+ (4)	Bhat-Nakshatri, et al. BMC Cancer 2010	1
	Breast_CD44_GSE36643	CD44+/CD24- (3) vs CD44-/CD24+ (3)	Battula, et al. J Clin Invest 2012	1
	Breast_CD44_GSE7513	CD44+/CD24- (14) vs non-CD44+/CD24- (15)	Chad, et al. PNAS 2009	0.93
	Breast_GD2_GSE36643	GD2+ (3) vs GD2- (3)	Battula, et al. J Clin Invest 2012	1
	Breast_MS_GSE7515	MS (15) vs non-MS (11)	Chad, et al. PNAS 2009	1
	Colon_CD133_GSE24747	CD133+ (3) vs CD133- (3)	---	1
	Glioma_CD133_GSE24716	CD133+ (4) vs CD133- (4)	Shats, et al. Cancer Res 2011	1
	Glioma_CD133_GSE37120	CD133+ (6) vs CD133- (6)	---	0.75
	Glioma_MS_GSE23806	MS (17) vs non-MS (12)	Günther, et al. Oncogene 2008	1
	Glioma-diff_CD133_GSE37120	CD133+ (6) vs CD133- (6)	---	0.83
	Lung_CD133_GSE35603	CD133+ (3) vs CD133- (3)	Yu, et al. Sci Rep 2012	1
	Lung_Chemo_GSE21656	cisplatin-resistant (3) vs cisplatin-senstive (3)	Sun, et al. Int J Radiat Oncol Biol Phys 2012	1
	Ovarian_MS_GSE28799	MS (3) vs non-MS (3)	Wang, et al. Mol Cell Biochem 2012	1
	Prostate_MS_GSE19713	MS (6) vs non-MS (6)	Maria, et al. BMC Genomics 2010	0.92
Control (4)	colon_adenoma_GSE08671	ade (32) vs nor (32)	Sabates-Bellver, et al. Mol Cancer Res 2007	1
	hES_GSE27362	hES (3) vs fbs (3)	Stelzer, et al. Nat Struct Mol Biol 2011	1
	iPS_GSE27362	iPS (8) vs fbs (3)	Stelzer, et al. Nat Struct Mol Biol 2011	1
	TGF-b-lung_EMT_GSE17708	EMT (3) vs non-EMT (3)	Sartor, et al. Bioinformatics 2010	1

High-quality chips yielded more DEGs

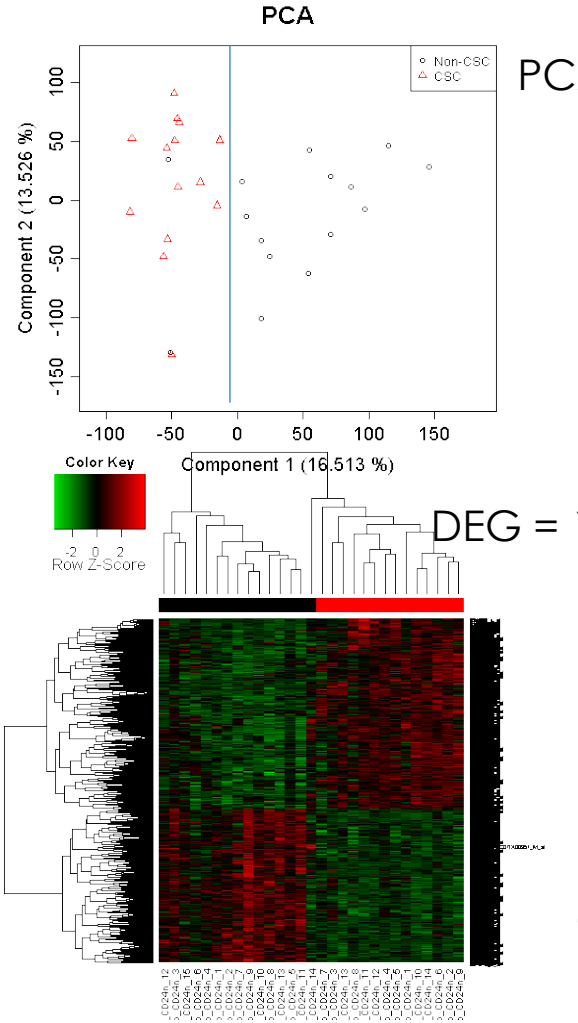
E-MEXP-993 (fail)



PCA score = 0

DEG= 69

GSE7513 (success)



PCA Score = 0.93

DEG = 1549

DEG: Differentially expressed genes

Few DEGs overlapped on CSC datasets

DEG overlaps (%)

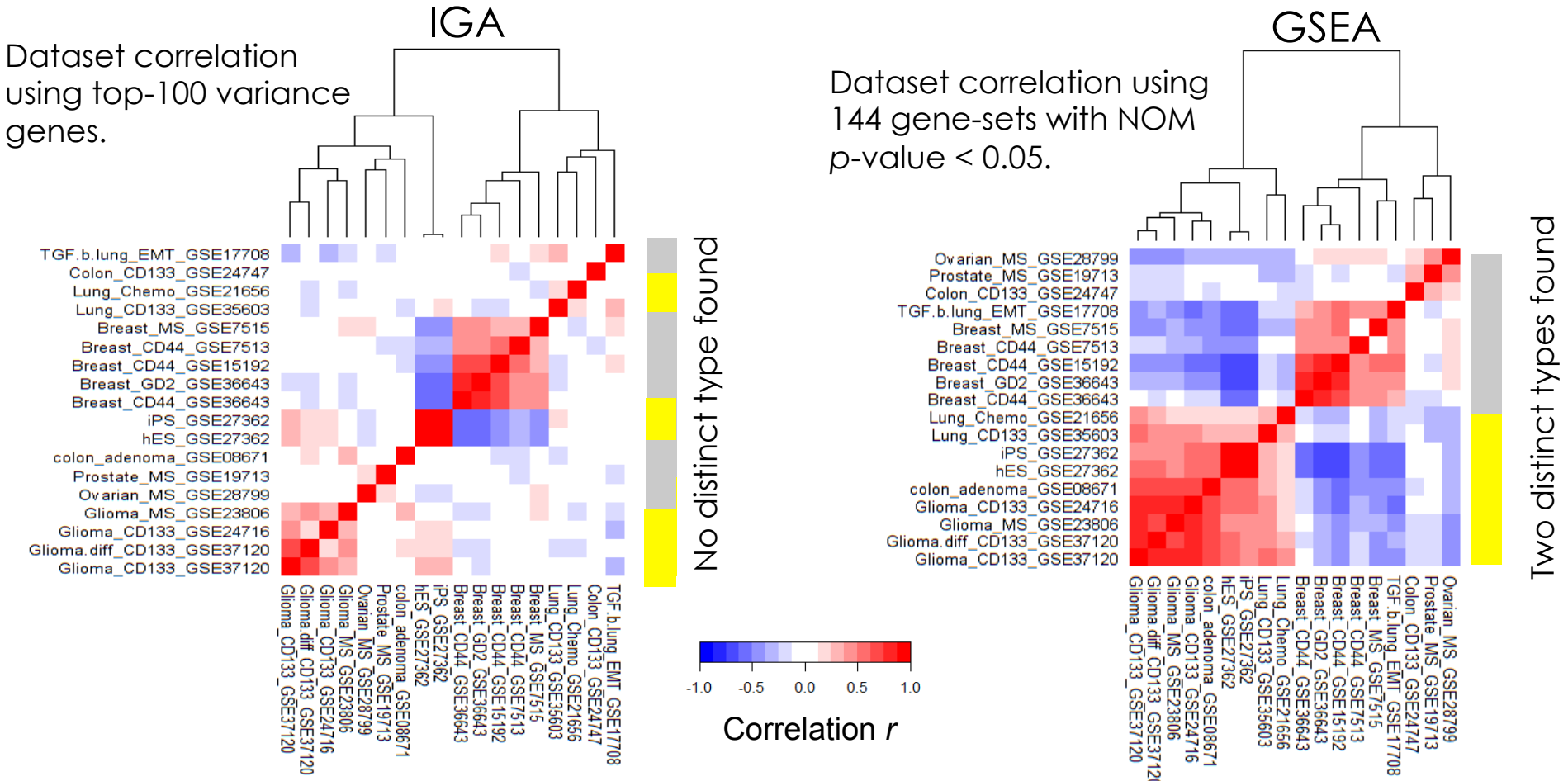
	Breast_CD44_GSE15192 (2343)	Breast_CD44_GSE36643 (910)	Breast_CD44_GSE7513 (1031)	Breast_GD2_GSE36643 (373)	Breast_MS_GSE7515 (2507)	Colon_CD133_GSE24747 (223)	Glioma_CD133_GSE24716 (489)	Glioma_CD133_GSE37120 (1186)	Glioma_MS_GSE23806 (1685)	Glioma-diff_CD133_GSE37120 (1099)	Lung_CD133_GSE35603 (4307)	Lung_Chemo_GSE21656 (161)	Ovarian_MS_GSE28799 (1582)	Prostate_MS_GSE19713 (561)	colon_adenoma_GSE08671 (1507)	hES_GSE27362 (3702)	iPS_GSE27362 (3704)	TGF-b-lung_EMT_GSE17708 (1643)
Breast_CD44_GSE15192 (2343)	100	60	24	65	20	11	8	10	11	9	11	1	17	12	9	0	0	22
Breast_CD44_GSE36643 (910)	23	100	13	70	11	7	5	5	4	4	5	1	9	4	4	0	0	9
Breast_CD44_GSE7513 (1031)	11	15	100	19	7	8	6	4	3	4	6	1	6	8	2	0	0	8
Breast_GD2_GSE36643 (373)	10	29	7	100	4	4	2	2	2	2	2	1	3	2	1	0	0	3
Breast_MS_GSE7515 (2507)	21	31	17	26	100	13	9	8	18	6	8	1	15	17	13	0	0	20
Colon_CD133_GSE24747 (223)	1	2	2	2	1	100	3	2	1	2	1	1	2	3	2	0	0	2
Glioma_CD133_GSE24716 (489)	2	2	3	3	2	7	100	16	7	14	4	1	4	6	7	0	0	2
Glioma_CD133_GSE37120 (1186)	5	7	5	8	4	13	39	100	17	46	7	1	7	9	13	0	0	5
Glioma_MS_GSE23806 (1685)	8	8	5	8	12	11	24	25	100	28	7	1	8	10	20	0	0	8
Glioma-diff_CD133_GSE37120 (1099)	4	4	4	5	3	8	30	42	18	100	6	1	6	7	11	0	0	5
Lung_CD133_GSE35603 (4307)	19	24	24	26	14	22	35	27	19	24	100	1	21	23	17	0	0	26
Lung_Chemo_GSE21656 (161)	0	0	0	1	0	1	0	0	0	0	0	100	0	0	0	1	1	0
Ovarian_MS_GSE28799 (1582)	11	15	9	14	9	12	11	9	7	8	8	1	100	17	8	0	0	8
Prostate_MS_GSE19713 (561)	3	3	4	3	4	7	7	4	3	3	3	1	6	100	4	0	0	2
colon_adenoma_GSE08671 (1507)	6	6	4	5	8	13	21	17	18	16	6	1	8	10	100	0	0	8
hES_GSE27362 (3702)	0	0	0	1	0	1	0	0	0	0	0	23	0	0	0	100	81	0
iPS_GSE27362 (3704)	0	0	0	1	0	1	0	0	0	0	0	29	0	0	0	81	100	0
TGF-b-lung_EMT_GSE17708 (1643)	16	16	12	12	13	12	7	6	8	8	10	1	8	7	9	0	0	100

Gene Set Enrichment Analysis (GSEA)

- We used an alternative approach called as Gene Set Enrichment Analysis (GSEA), proposed by Subramanian in 2005, to seek general trends for CSCs.

- Basic idea
  - Use gene-sets, instead of using genes, as units for analysis
  - Analysis quantified by KS-test based enrichment score (ES)
  - Use ES to hierarchically cluster samples

Content overlap based on gene-set analysis (GSEA), but not individual gene analysis (IGA), showed two distinct CSC groups



Content overlap by two other gene-set based approaches, PAGE & GAGE, showed same two distinct groups

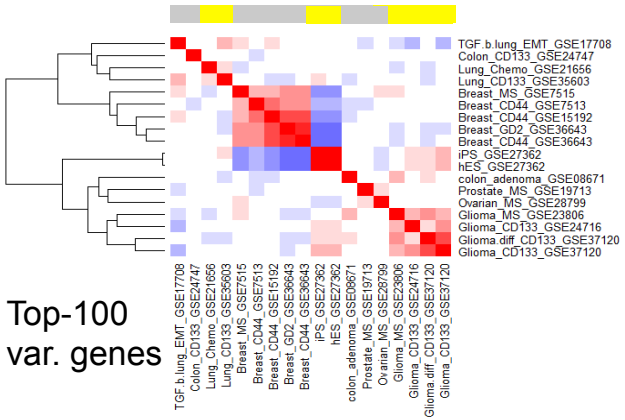
IGA: Individual gene analysis

GSEA: Gene-set enrichment analysis

PAGE: Parametric analysis of gene set enrichment

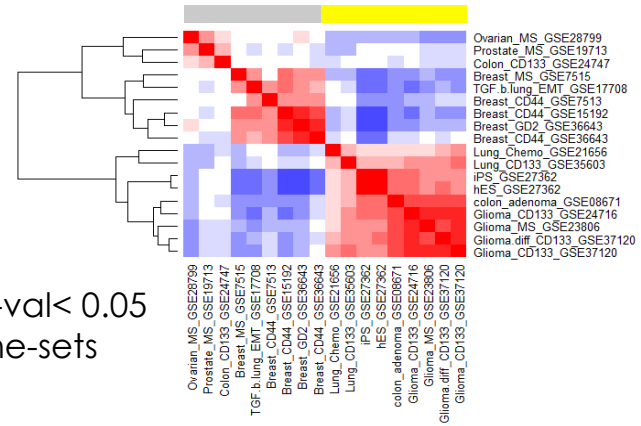
GAGE: Generally applicable gene-set enrichment

IGA



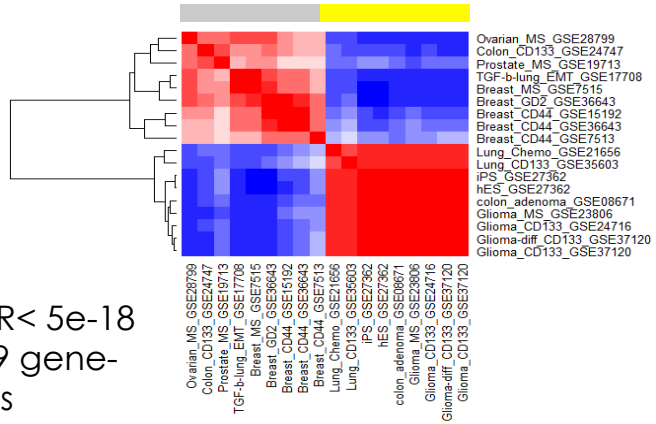
Top-100 var. genes

GSEA

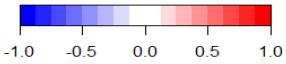


NOM p-val< 0.05
144 gene-sets

PAGE

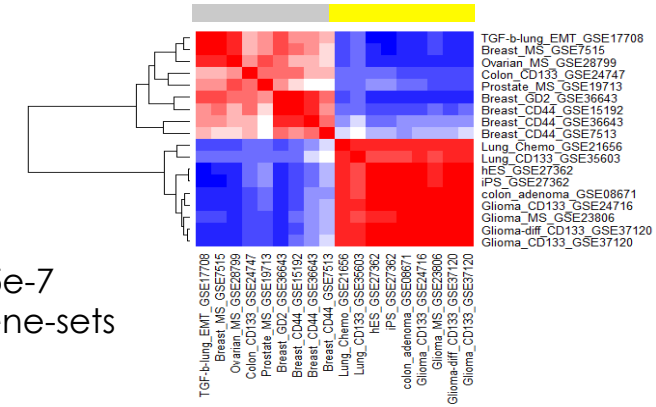


FDR< 5e-18
209 gene-sets



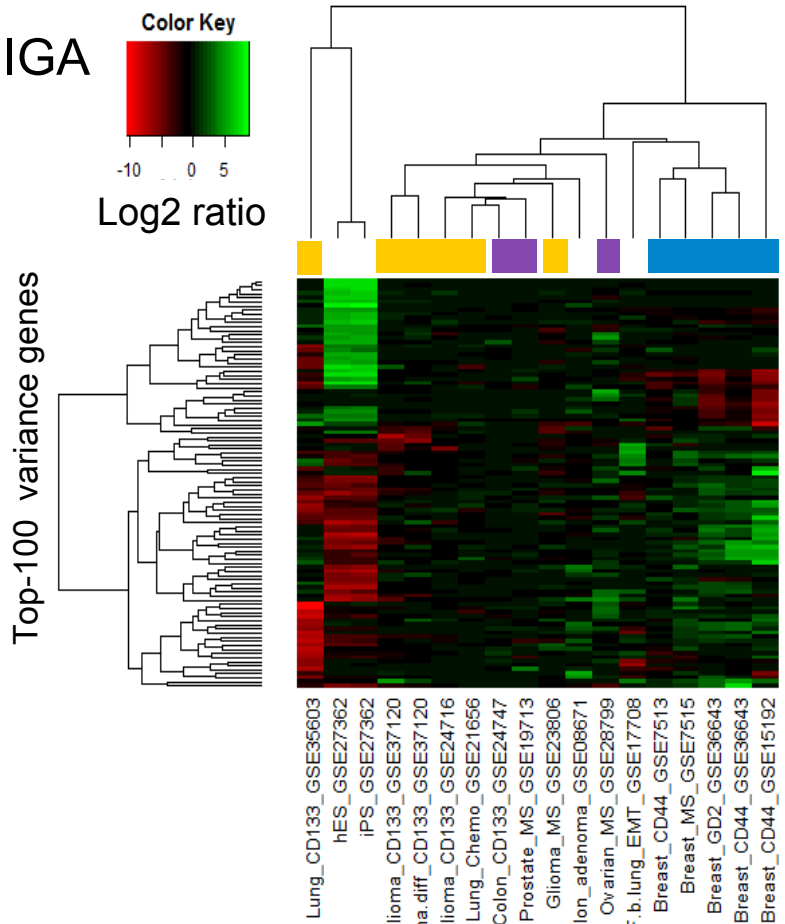
Correlation *r*

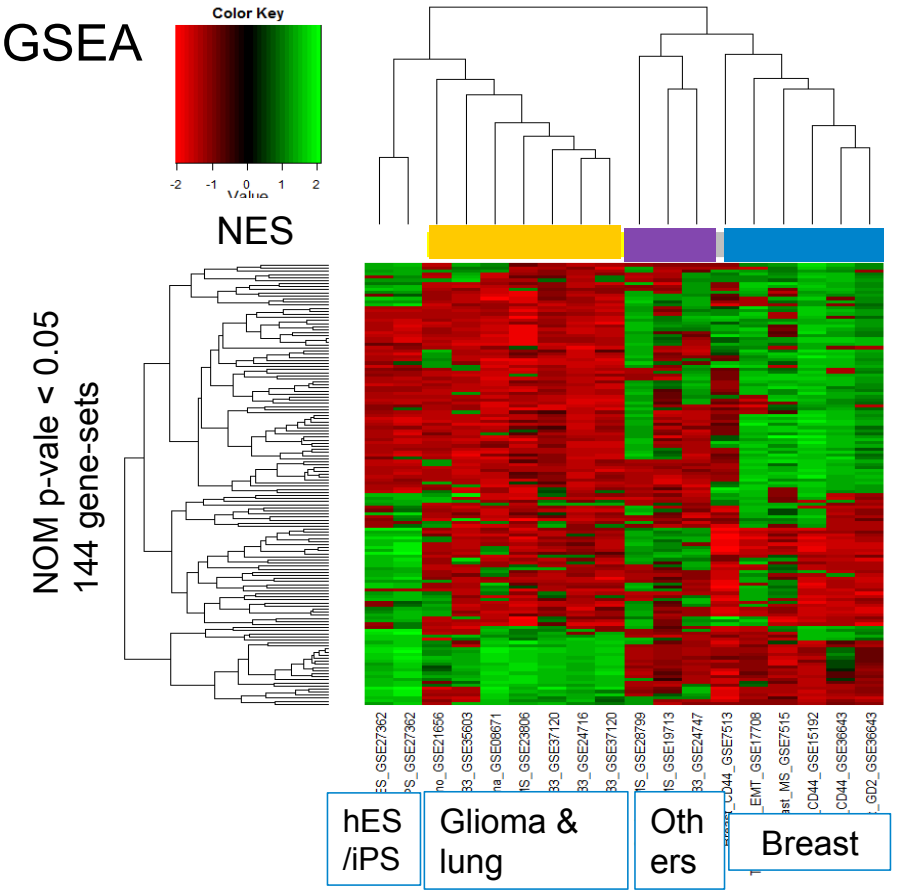
GAGE



FDR< 5e-7
196 gene-sets

Two-way clustering using gene-sets (but not DEGs) classifies the two types into three CSC tissue-specific subtypes



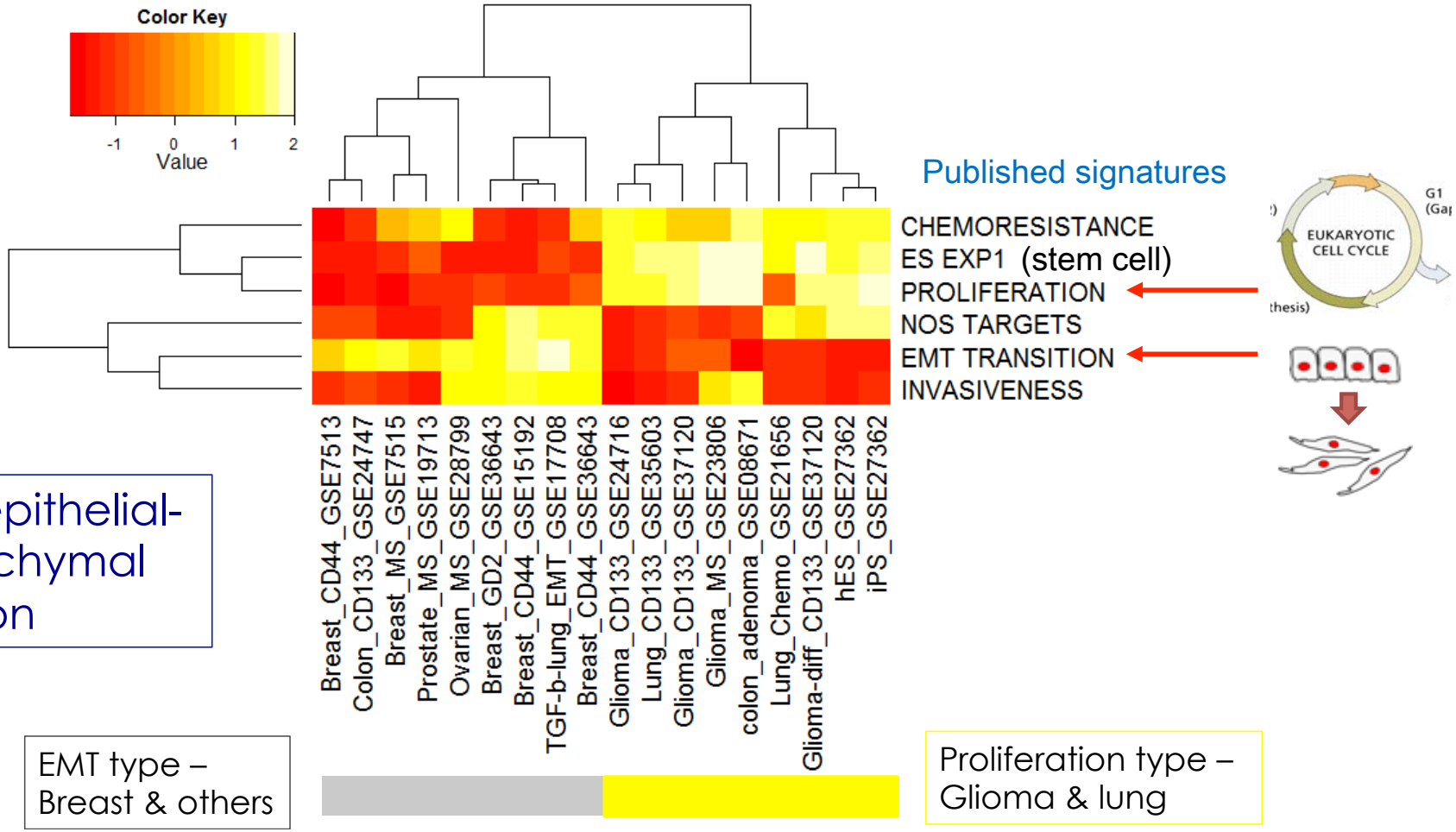


Summary I

- The t-test based IGA was not able to classify the 14 CSCs data-sets into subtypes.

- All three gene-set-based methods tested, GSEA (NOM *p*-value < 0.05), PAGE (FDR< 5e-18), and GAGE (FDR< 5e-7), made the same division of the 14 CSCs data-sets into two types.

Hierarchical clustering with 6 cancer/SC signatures shows two types characterized by two signatures: proliferation & EMT



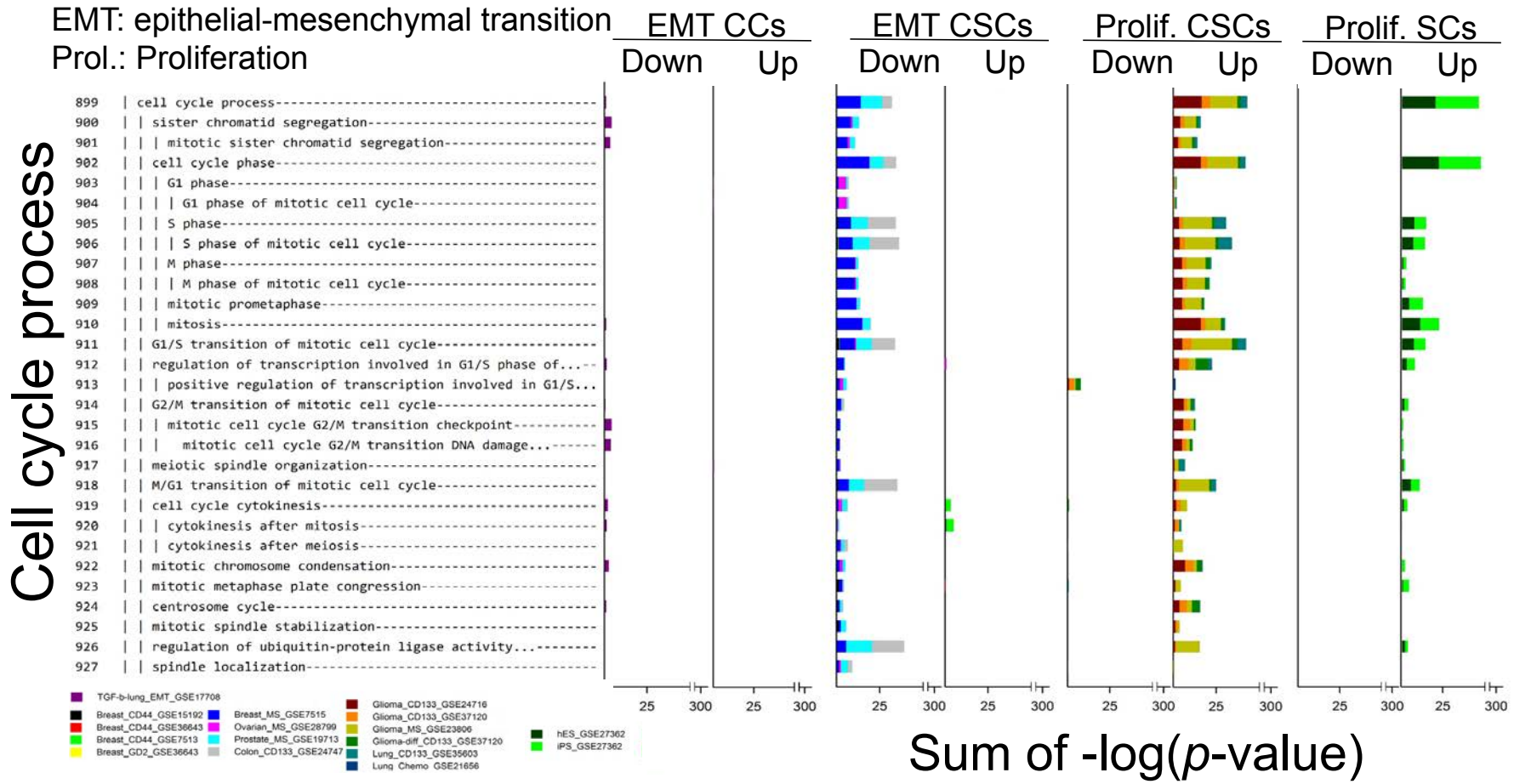
EMT – epithelial-mesenchymal transition

EMT type – Breast & others

Proliferation type – Glioma & lung

Cell cycle process over-enriched in proliferation-type CSCs but depleted in EMT-type CSCs

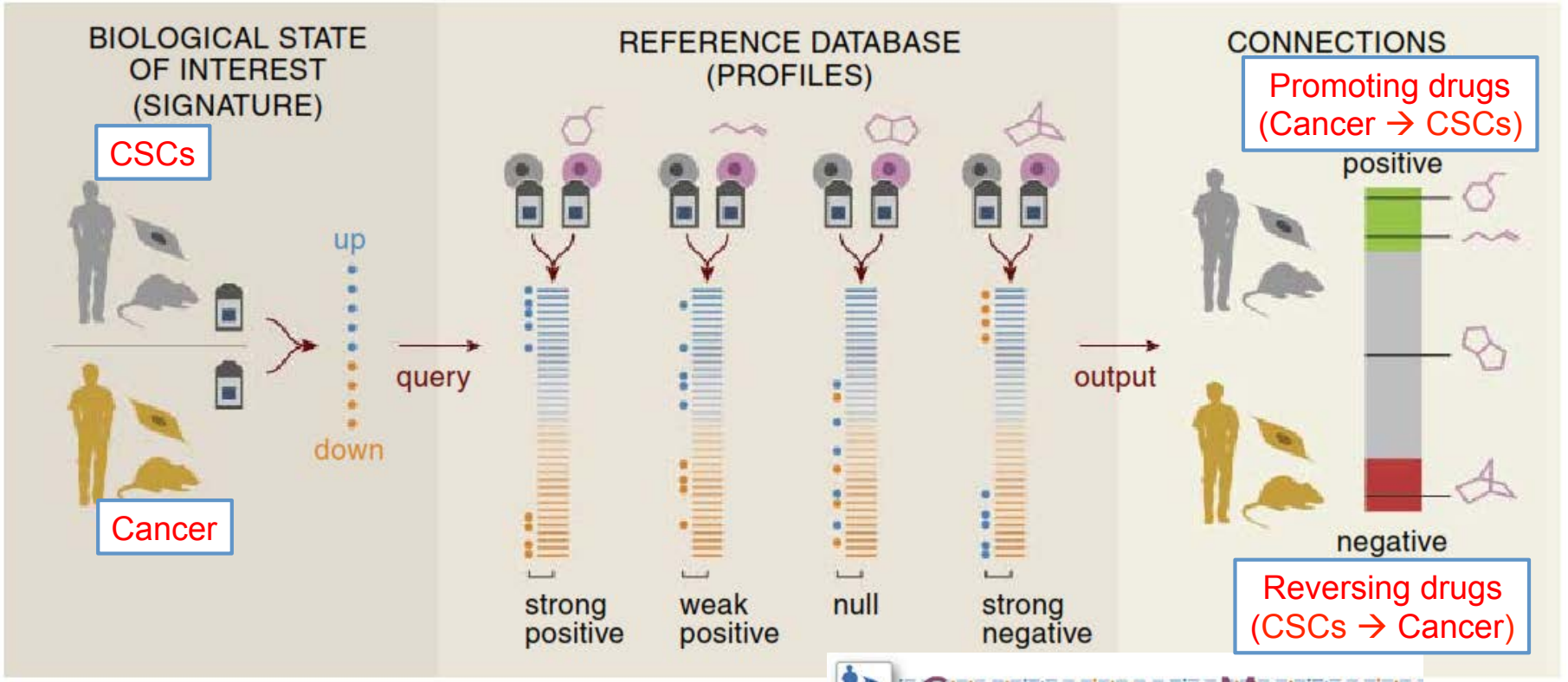
EMT: epithelial-mesenchymal transition
Prol.: Proliferation



Summary II

- The two CSC types were respectively characterized by two important cancer/SC signatures, proliferation and EMT
- Through clustering with144 gene-sets (heatmap), the two groups are subdivided into stem cell (control) and three CSC tissue-specific types:
  - Proliferation:
    - Stem cell (control)
    - Type I – glioma and lung
  - EMT:
    - Type II_a (type 2) – breast
    - Type II_b (type 3) – others (colon, prostate, ovary)

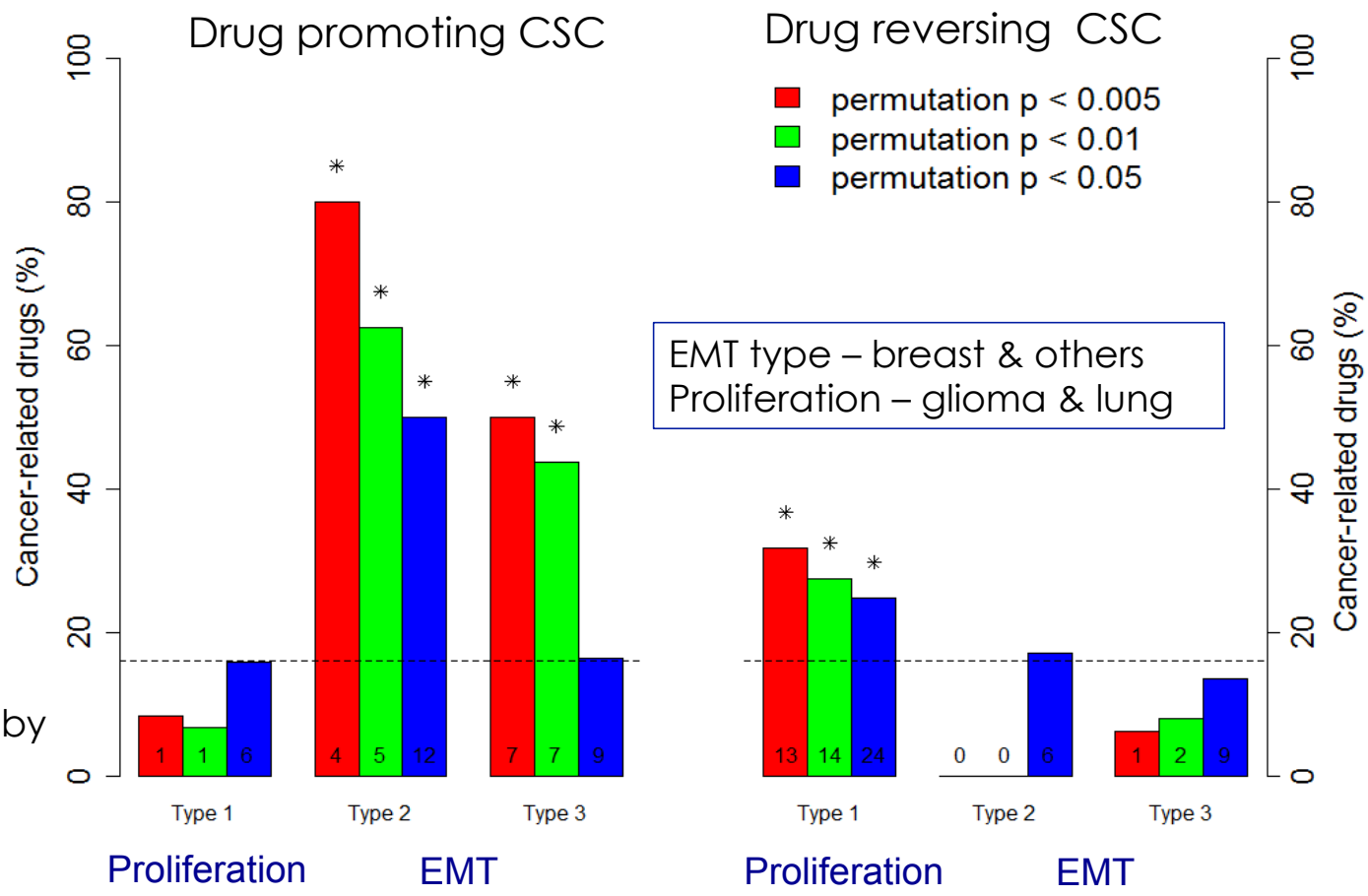
The Connectivity Map (C-Map) was used in GSEA mode for drug discovery



J Lamb et al. Science 2006;313:1929-1935

Drug analysis (CMap) suggests many cancer drugs have tendency to enhance CSC properties in EMT-type CSCs

C-Map queried by type-specific small p-value gene-sets



* represents $p < 0.05$ by Fisher's exact test

Hsu, Chung & Lee (2014)

Summary

- Our analysis suggested CSC has two main types: Type I is proliferative-up and EMT-down, and mostly glioma (plus lung); Type II is the reverse, mostly breast (plus colon, prostate, ovary).
- A preponderant number of drugs selected (through CMap) by EMT-type II CSCs tend to push non-CSC cancer cells to become CSCs.
- Conversely, a preponderant number of drugs selected by proliferation-type I CSCs tend to push CSCs to become non-CSC cancer cells.

Conclusion

- Many known chemo-drugs has the effect of suppressing cell proliferation. These drugs should be beneficial to cancers in Type I CSC (glioma and lung). However, these drugs may have adverse effects for cancers in Type II CSCs (breast, colon, prostate, ovary).

- This suggests a need for novel, type-specific CSCs targeted cancer therapy.

- Work done by:
  - Dr. Feng-Hsiang Chung 鍾豐翔, PDF
  - Jue-Lin Hsu 許爵麟, PhD student
  - Dr. Chih-Hao Chen 陳志浩, PDF

- Supported by
  - Ministry of Education
  - National Research Council/Ministry of Science & Technology
  - National Central University-Cathay General Hospital Joint Research Center
  - Center for Dynamical Biomarkers and Translational Medicine, National Central University

Thank you for your attention