Functional Module Connectivity Map (FMCM): A Framework for Searching Repurposed Drug Compounds for Systems Treatment of Cancer and an application to Colorectal Adenocarcinoma

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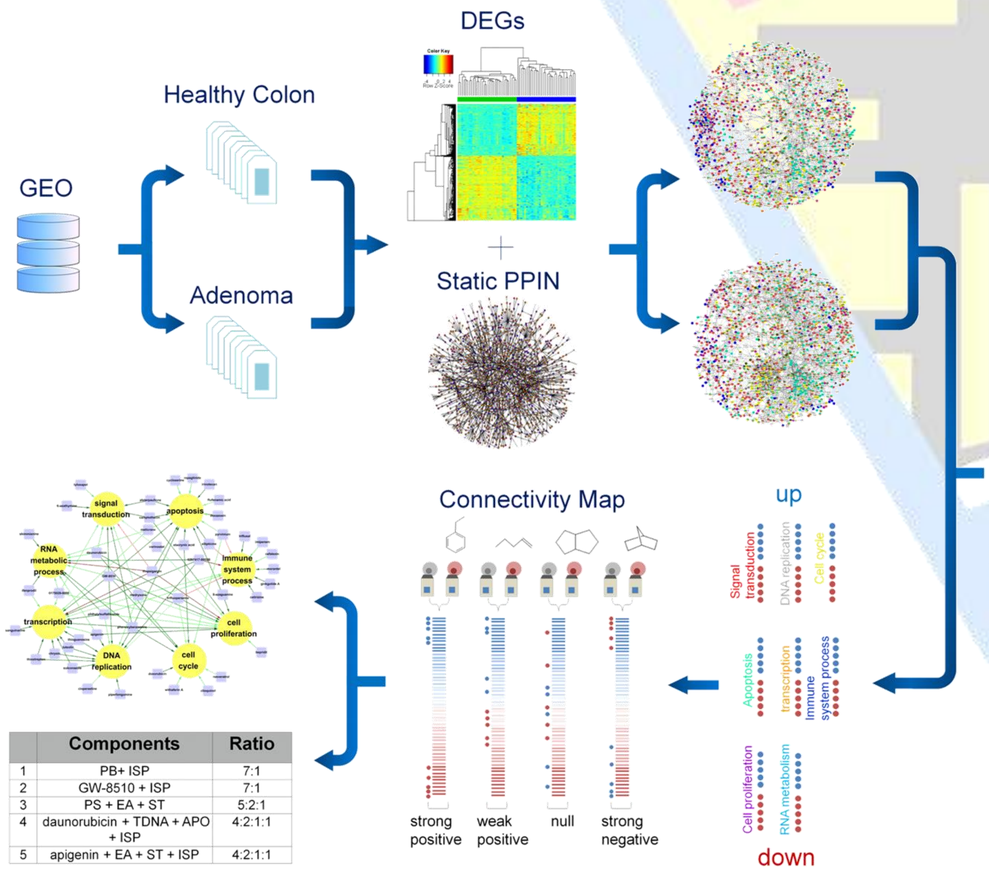
Introduction

Notwithstanding advances made in the treatments in some types of cancers, progress achieved in the last 40 years in reducing the overall cancer mortality rate has been disappointing. At the same time, many previously thought successful drugs have been withdrawn, mostly due to side-effect issues. Cancer is now recognized as is a disease caused by a breakdown of a large part of the biological system in the tumor, not just of the failure of one or two of its biological functions. Here, we devised Functional Module Connectivity Map (FMCM) for the discovery of repurposed drug compounds for systems treatment of complex diseases, and applied it to colorectal adenocarcinoma. FMCM used multiple functional gene modules to query the Connectivity Map (CMap).

Materials & Methods

Samples were two different types of frozen colonic biopsies, from prospectively collected adenomas and from normal mucosa of 32 individuals, which microarray data were downloaded from GEO database (GEO accn. GSE08671). The protein-protein interaction experimental data derived from Human Protein Reference Database (HPRD) [1] and Gene Ontology database [2] were used for network analysis and functional gene sets construction. The Connectivity Map (Cmap) [3], a collection of genome-wide transcriptional expression data of bioactive drugs and small molecules on cultured human cells, was used for drug search. Gene selection by trend-of-progression procedure (ToP [4]) to identify complexly connected and highly expressed hub genes were used.



	Components	Ratio
1	PB+ ISP	7:1
2	GW-8510 + ISP	7:1
3	PS + EA + ST	5:2:1
4	daunorubicin + TDNA + APO + ISP	4:2:1:1
5	apigenin + EA + ST + ISP	4:2:1:1

Figure 1. Flowchart of methodology

Results

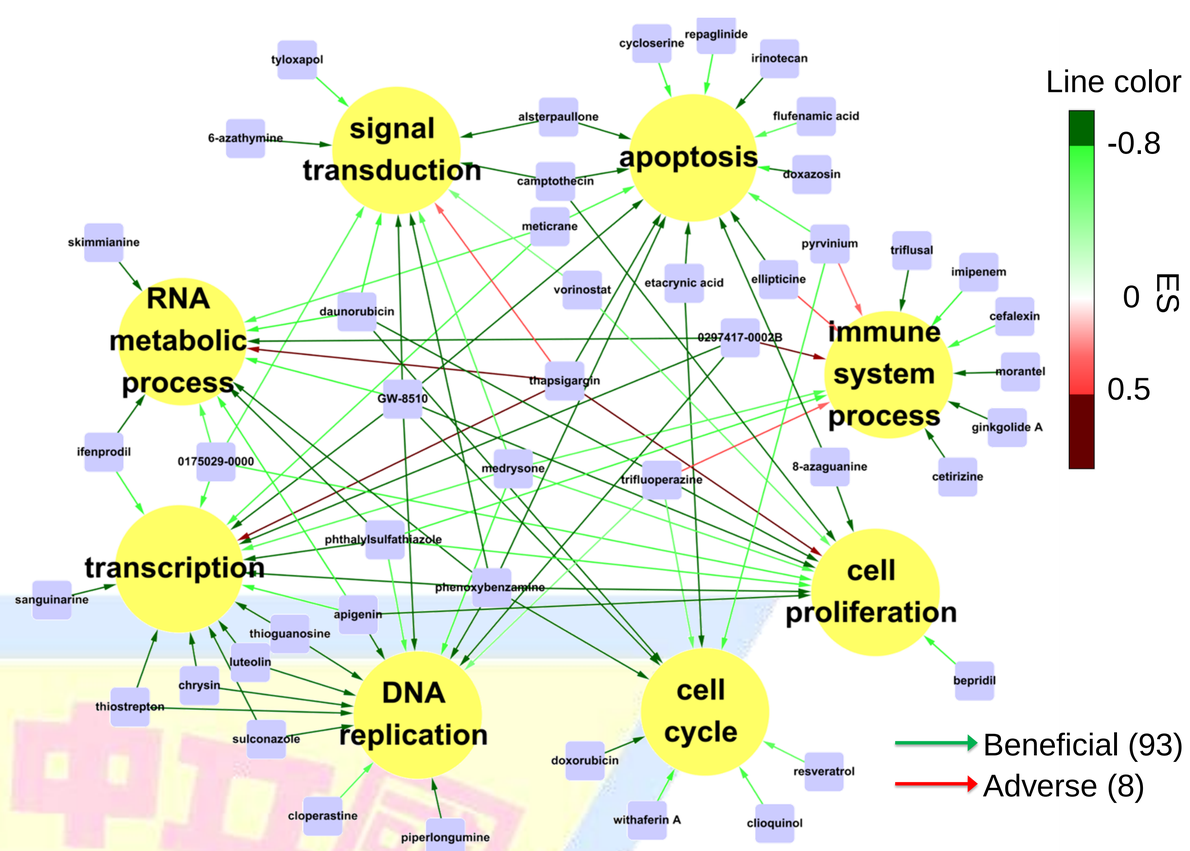


Figure 2. Drug-functional association network. Beneficial links have p-value < 0.001 (by randomization) and enrichment score < -0.8; adverse links have ES > 0.5.

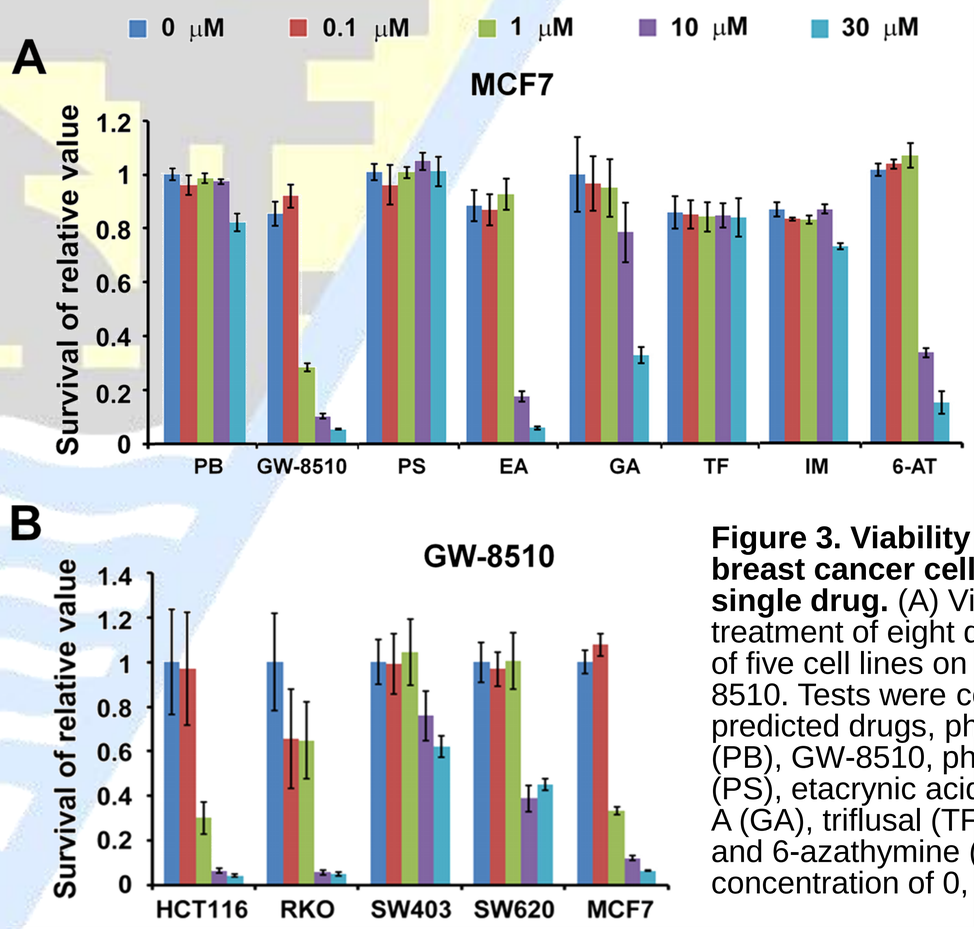


Figure 3. Viability test of colon and breast cancer cells treated with single drug. (A) Viability of MCF7 on treatment of eight drugs. (B) Viability of five cell lines on treatment of GW-8510. Tests were conducted on predicted drugs, phenoxybenzamine (PB), GW-8510, phthalylsulfathiazole (PS), etacrynic acid (EA), ginkgolide A (GA), triflusal (TF), imipenem (IM), and 6-azathymine (6-AT), with concentration of 0, 0.1, 1, 10, 30 μM.

Table 1. Predicted functional specific drugs.

Drug/molecule	degree	Functional module (ES)	Drug function	TTD	Carcinogen / immune	Anticancer agents
phenoxybenzamine	7	CC (-0.987), DR (-0.983), At (-0.977), CP (-0.962), Ts (-0.905), ST (-0.886), RM (-0.81)	an α-adrenergic-antagonist	DAP000478	[1, 2]	
GW-8510	7	CP (-0.972), ST (-0.936), DR (-0.882), At (-0.867), CC (-0.834), Ts (-0.822), RM (-0.791)	a CDK2 inhibitor that protects hair-loss in chemotheraply	DNC004631		[3]
thapsigargin	5	At (-0.918), ST (0.4), Ts (0.521), CP (0.528), RM (0.887)	a nonselective inhibitor of endoplasmic reticulum Ca^{2+} ATPase	DNC014889	[4-7]	[8-11]
daunorubicin	4	CC (-0.867), CP (-0.844), RM (-0.8), ST (-0.786)	a chemotherapeutic antibiotic	DAP000788		[12-14]
apigenin	4	DR (-0.896), CP (-0.837), Ts (-0.796), RM (-0.784),	a flavone that have the chemopreventive action in vegetables	DNC004714		[15-18]
pyrvinium	3	CC (-0.75), At (-0.694), IS(0.314)	anthelmintic	---		[19, 20]
trifluoperazine	3	CC (-0.604), DR (-0.501), IS(0.415)	a typical antipsychotic of the phenothiazine chemical class.	DAP000034	[21]	[22, 23]
camptothecin	3	At (-0.953), CP (-0.935), ST (-0.878)	a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I	DNC000385		[24-26]
ellipticine	2	At (-0.827), IS(0.422)	an antineoplastic agent which inhibits the DNA enzyme toposiomerase II	DNC000599	[27-29]	[27-29]
8-azaguanine	2	At (-0.87), CP (-0.83)	a purine analog that shows antineoplastic activity	DNC002551		[30, 31]
etacrynic acid	2	At (-0.891), CC (-0.875)	GST Inhibitor-2, diuretics	DAP000748		[32]
alsterpaullone	2	ST (-0.874), At (-0.866)	CDK inhibitor	DNC000188		[33]
vorinostat	2	CP (-0.592), ST (-0.503)	HDAC inhibitor, antineoplastic agent	DAP001082		[34-36]
thioguanosine	2	DR (-0.935), Ts (-0.811)	antineoplastic agent	---		[37, 38]
chrysin	2	Ts (-0.934), DR (-0.913)	a naturally occurring flavone, antineoplastic agent	DNC004715		[39, 40]
thiostrepton	2	DR (-0.837), Ts (-0.816)	a natural cyclic oligopeptide antibiotic	DNC001438		[41-43]
luteolin	2	Ts (-0.856), DR (-0.811)	a flavonoid, antioxidant, anti-inflammatory, and an antineoplastic agent	DNC000896		[44-46]
ifenprodil	2	RM (-0.839), Ts (-0.779)	a selective inhibitor of the NMDA receptor, vasodilator	DNC000779		[47]
doxazosin	1	At (-0.804)	an α1a-selective alpha blocker, treat high blood pressure	DAP000381		[48-50]
flufenamic acid	1	At (-0.665)	a non-steroidal anti-inflammatory drug.	DNC002446		[51-53]
irinotecan	1	At (-0.871)	inhibition of topoisomerase 1, antitumor agent	DAP000647		[54-56]
resveratrol	1	CC (-0.627)	a stilbenoid, anticancer, anti-inflammatory	DNC001205		[57-59]
withaferin A	1	CC (-0.799)	inhibit agiogenesis and tumorigenesis	---		[60-62]
clioquinol	1	CC (-0.719)	an antifungal drug and antiprotozoal drug	DNC011356		[63-65]
doxorubicin	1	CC (-0.874)	anthracycline antibiotic, TOP2 inhibitor, antitumor agent	DAP000192		[66-68]
bepridil	1	CP (-0.791)	a calcium channel blocker once used to treat angina	DAP000525		[69-71]
piperlongumine	1	DR (-0.956)	a natural product which have anti tumor activities	---		[72]
ginkgolide A	1	IS(-0.834)	Anti-platelet-activating factor	DNC007171		[73]
triflusal	1	IS(-0.891)	a platelet aggregation inhibitor	---		[74]
imipenem	1	IS(-0.791)	an intravenous β-lactam antibiotic	DAP000459		[75, 76]
6-azathymine	1	ST (-0.813)	Immunodeficiency disease, antitumor agent	---		[77]
sanguinarine	1	Ts (-0.959)	Anti-bacterial, anti-Trypano-soma and anti-tumor	---		[78-80]

Conclusions

1. The present program of combining functional gene sets determined by two-cohort gene expression data with Cmap allows us to find repurposed drug compounds for treating colorectal cancer with predicted strong beneficial effects on all eight biological functions and no adverse effect on any.
2. In cell viability tests, we identified four repurposing drugs – GW-8510, etacrynic acid, ginkgolide A, and 6-azathymine – as having high inhibitory activities against cancer cells.
3. FMCM is expected to be useful for other systems diseases.

References

[1] Keshava Prasad, T.S., et al., *Human Protein Reference Database--2009 update.* Nucleic Acids Res, 2009. **37**(Database issue): p. D767-72.
[2] Ashburner, M., et al., *Gene ontology: tool for the unification of biology. The Gene Ontology Consortium.* Nature genetics, 2000. **25**(1): p. 25-9.
[3] Lamb, J., et al., *The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease.* Science, 2006. **313**(5795): p. 1929-35.
[4] Chung, F.H., et al., *ToP: A Trend-of-Disease-Progression Procedure Works Well for Identifying Cancer Genes from Multi-State Cohort Gene Expression Data for Human Colorectal Cancer.* PLoS One, 2013. **8**(6): p. e65683.