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RESEARCH ARTICLE

MAPPING OF ANTI-INFLAMMATORY PHYTOCHEMICALS ON CANCER DRUG TARGET NETWORK USING SYSTEMS BIOLOGY APPROACH

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ABSTRACT

In the field of cancer biology, the drug and their targets holds a role of paramount importance. Biological network helps in the evaluation and validation of cancer drugs and their targets. It is usually created to simplify the studies. In the present study we have created a protein-ligand interaction network where ligands and proteins are anti-inflammatory phytochemicals and cancer inducing drug targets respectively. The objective of the study was to identify the highest interacting cancer druggable target protein in the protein-ligand network and an appropriate anti-inflammatory phytochemicals against it. In order to procure the cancer inducing drug targets, we found anti-inflammatory drugs using literature survey. In total, 49 anti-inflammatory drugs were identified as the most critical. Drugbank was used to obtain the targets of all the anti inflammatory drugs collected. 35 protein targets were identified. Cancer inducing property of these targets was evaluated using Human Cancer Protein Interaction Network (HCPIN) database. 16 proteins were found to be cancer inducing proteins. We obtained structures of 11 proteins from Protein Data Bank (PDB) in the form that can be easily docked using ligands in Quantum3.3.0. These 11 proteins served as cancer inducing target proteins for our study. The anti-inflammatory phytochemicals were collected from a wide range of publishers and databases. The survey resulted in 157 anti-inflammatory phytochemicals. All these phytochemicals were subjected to multi receptor docking using Quantum3.3.0 docking software where cancer inducing drug targets served as receptors. The most suitable drug-like compounds were mapped along with the drug targets using VisANT. The protein found to take part in most inter-network interactions was Beta- Catennin 1. The phytochemicals that had the best interactions with Beta- Catennnin 1 were Chicoric acid and Digoxin. Still further investigation with respect to pharmacological and phytochemical profile of these plant derived compounds needs to be carried out to concrete evidence of their drug like behavior against Beta- Catennnin 1 induced cancer.

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INTRODUCTION

Analysis of Drug-Target interaction network is a crucial step in drug development procedure. It is both time consuming and costly to determine compound-protein interactions or potential drug-target interactions by wet lab experiments alone. Conversely, In silico research in medicine is thought to have the potential to speed the rate of discovery while reducing the need for expensive lab work and clinical trials (Sharangdhar *et al.,* 2009; Adam Smith, 2002; Dahiya *et al.,* 2007). Computational methods, today, pave the way for effective prediction of protein ligand interaction and its role in cancer development or cure. In this study, using the network analysis, we analyzed systematic relationship between ligand-protein interaction and protein-protein interaction that can aid in effective cancer cure (Biplab Bhattacharjee *et al.,* 2010).

Cancer results from a multistage carcinogenesis process that involves 3 distinguishable but closely connected stages: initiation (normal cell → transformed or initiated cell), promotion (initiated cell → preneoplastic cell), and progression (preneoplastic cell → neoplastic cell) (Rajesh *et al.,* 2006). Carcinogenesis is a process of outgrowth of clonal population of cells from tissues (Seth Rakoff-Nahoum, 2006). This process start's from sites of infection, chronic irritation and inflammation (Werb, 2003). Over the past few years, inflammation's role in tumor progression has been keenly studied (Ting-Ting Tan and Lisa Coussens, 2007). In fact, Inflammation has a role to play in all 3 stages of cancer (Rajesh *et al.,* 2006). Substantial evidence for the role of inflammation in cancer can be understood by Wnt5a induced endothelial inflammation via beta-catenin-independent signaling (Kim *et al.,* 2010). Beta-catenin is a protein associated with the cytoplasmic region of E-cadherin (Morin, 1999). This complex is often termed as E-cadherin-catenin adhesion complex. The disturbance in this protein-protein interaction is one of the main events in the early and late steps of cancer development (Wijnhoven, 2000). Beta-catenin has been shown to perform two apparently unrelated functions: it has a crucial role in cell-cell adhesion in addition to a signaling role as a component of the Wnt/wg pathway. Wnt/wg signaling results in beta-catenin accumulation and transcriptional activation of specific target genes during development. It is now apparent that deregulation of beta-catenin signaling is an important event in the genesis of a number of malignancies, such as colon cancer, melanoma, hepatocellular carcinoma, ovarian cancer, endometrial cancer, medulloblastoma pilomatricomas and prostate cancer (Morin, 1999). Chemoprevention is the use of a chemical substance of either natural or synthetic origin to prevent, hamper, arrest, or reverse a disease (Rajesh, 2006). A new horizon in chemoprevention research is the recent discovery of molecular links between inflammation and cancer. A wide variety of chemopreventive and chemoprotective agents can alter or correct undesired cellular functions caused by abnormal proinflammatory signal transmission. The modulation of cellular signaling by anti-inflammatory phytochemicals provides a rational and pragmatic strategy for molecular target–based chemoprevention (Young-Joon Surh *et al.,* 2005).

MATERIALS AND METHODS

A. Data mining, Target and Lead Identification

In silico approach towards finding out the lead compounds began with the literature survey. The cancer active anti-inflammatory phytochemicals were annotated from a wide range of publishers and databases like Wiley, Medline, Pubchem, Ingenta Connect, Chemfinder, etc. The next step was to find the cancer inducing targets (Biplab Bhattacharjee *et al.,* 2010). To achieve this purpose, an attempt was made to find all the available anti-inflammatory drugs through the data mining process. Drug Bank was used to find the targets for each of the anti-inflammatory drugs found. By this approach we gathered targets for all the anti-inflammatory drugs found. Each target was checked for its cancer inducing property using Human Cancer Protein Interaction Network (HCPIN) (Huang *et al.,* 2008). Only those targets involved in cancer pathways were considered for docking studies. Each anti-inflammatory phytochemical was docked with all targets involved in cancer pathways.

B. Docking studies

The anti-inflammatory phytochemicals found were then subjected to energy minimization using MarvinSketch. Energy Minimization is an essential step in computational approach towards drug discovery. This will lower the overall energy of the target protein molecules. Quantum 3.3.0 was used as a docking tool to carry out the docking operations. Energy value (g-bind) and RMS value for each ligand docked with every protein molecule were noted down.

Table 1. List of the proteins taken as targets for this study

S. No	Protein Name	PDB ID	Swissprot ID
1.	72 kDa type IV collagenase	1CK7	P08253
2.	Arachidonate 5-lipoxygenase	2ABV	P09917
3.	Catenin beta-1	1G3J	P35222
4.	Dihydrofolate reductase	1BOZ	P00374
5.	Glucocorticoid receptor	1M2Z	P04150
6.	Glycogen synthase kinase-3 beta	1GNG	P49841
7.	Inositol monophosphatase(1)	1AWB	P29218
8.	Interlukin-8	1ICW	P10145
9.	Pro-epidermal growth factor	1IVO	P01133
10.	Prostaglandin G/H synthase 2	1V0X	P35354
11.	Prothrombin	1A2C	P00734

B. Network Analysis

Networks are a useful computational tool for representing many types of biological data, such as bimolecular interactions, cellular pathways and functional modules. Here the Protein-Ligand bimolecular interaction network was drawn and analyzed using VisANT. It is a web-based software framework for visualizing and analyzing many types of networks of biological interactions and associations. The most suitable drug-like compounds were mapped along with the drug target using VisANT.

Receptors	1G3J	1G3J	2ABV	2ABV	1ICW	1ICW	1VOX	1VOX	1CK7	1CK7	1GNG	1GNG	1BOZ	1BOZ	1IVO	1IVO	1M2Z	1M2Z	1A2C	1A2C	1AWB	1AWB
Ligands	G-BIND	RMS val	G.BIND	RMS VAL	G.BIND	RMS V	G.BIND	RMS V	G.BIND	RMS VAL	G.BIND	RMS VAL	G.BIN	RMS V	G.BIND	RMS VA	G.BIN	RMS V	G.BIN	RMS V	G.BIN	RMS VA
1,8-cineole	-14.94	99.97	-14.9	168.04	-11.1	53.6	-14.4	50.14	-20.83	171.58	-9.94	153.22	-17.2	32.39	-13.95	105.7	-20	33.16	-11.9	14.41	-17.7	65.93
5-demethylr	-21.48	102.4	-18.2	170.2	-20.9	36.6	-21.8	57.17	-22.94	208.18	-16.6	145.75	-5.25	30.64	-26.87	103.8	101	34.44	-15.5	18.8	-26.5	57.34
5-MeO-DMT	-13.75	105.8	-22.5	164.09	-11	50.3	-20.5	46.68	-22.59	179.11	-13.2	146.09	-23.9	33.52	-17.82	111.8	-24.8	37.55	-15.1	22.08	-26.9	60.74
aconitine	-20.17	103.52	-18.6	37.74	-21.7	39.8	5.65	43.69	-29.22	188.9	-19.9	139.99	-19.4	26.84	-26.45	98.33	3348	32.2	-15.2	19.97	114	55.53
agunuside	-14.97	88.44	-28.7	171.65	-20.2	31.8	-23.6	44.15	-18.29	161.62	-15.7	143.57	-26.5	30.25	-22.47	106.4	65.5	32.79	-25.6	12.41	-6.68	60.39
allicin	-13.48	110.46	-13.3	169.63	-13.2	36.3	-15	37.82	-11.93	168.94	-11.3	141.59	-13.7	31.45	-14.5	104.9	-17.4	33.48	-12.4	22.24	-16.2	65.39
allyl isithiocy	-12.93	98.64	-13.7	147.44	-11.1	43.1	-12.2	53.73	-15.91	163.75	-7.21	139.09	-13	26.79	-11.27	96.25	-12.2	34.63	-11.9	15.08	-18.7	61.32
alpha linoler	-23.75	111.51	-10.8	166.32	-19	48	-8.92	40.89	-17.27	178.17	-11	140.19	-26.9	31.09	-18.67	99.66	-15.2	34.16	-20	13.38	-17.4	60.75
anabasine	-15.49	93.08	-14.6	163.47	-13.6	32.4	-15.2	45.12	-18.22	200.4	-12.5	145.88	-19.9	30.42	-17.72	101.7	-20.1	31.91	-13.1	16.07	-25.6	60.48
apigenin	-17.23	99.47	-17.6	171	-16.7	57.9	-23.7	47.63	-22.09	186.91	-16.7	142.72	-23.3	35.92	-23.16	105.9	-22.1	35.43	-14.5	19.47	-24.5	60.55
arecoline	-13.57	102.64	-14	176.23	-12.3	60.3	-15.8	45.16	-14.33	198.36	-10	143.98	-14.1	28.48	-11.25	139.1	-18.6	35.04	-15.4	30.24	-17.3	61.05
astaxanthine	-15.87	138.99	-16.5	171.98	-15.7	35.1	-22.2	53.07	-23.2	160.32	-14.3	141.6	-19	28.44	-16.78	144.2	-23.9	35.65	-20.1	41.38	-15.7	73.98
atropine	-16.07	125.53	-17.8	170.48	-14.3	49.2	-20.1	44.54	-19.69	168.4	-15.9	144.37	-17.5	43.19	-15.49	143.4	-20.1	37.03	-20.3	30.53	-24.7	59.98
aucubin	-12.34	126.31	-18.4	158.82	-19	47.4	-22.3	48.03	-19.24	187.83	-14.3	146.72	-19.9	32.93	-16.25	145.9	173	36.53	-17.3	28.76	-17.3	28.76
baicalein	-15.19	118.32	-15.1	156.39	-17.6	29.3	-20.6	44.08	-16	159.16	-14.7	139.42	-16.9	22.96	-13.1	143.3	-9.24	37.77	-15.5	30.15	-23.4	57.67
berberine	-17	123.27	-22.9	175.42	-21.3	38.2	-25	40.58	-23.32	161.66	-13.8	149.76	-16.1	30.91	-19.76	137.2	64.8	36.92	-4.85	40.6	-28.6	63.72
biochanin ac	-16.52	100.72	-19.5	170.38	-16.1	45.6	-23.8	32.33	-17.91	164.43	-13.5	139.5	-17.2	29.86	-16.56	138.1	-26.5	30.49	-16.9	33.39	-28.6	63.72
boswellic aci	-22.71	119.46	-26.1	181.51	-18.8	50.7	-27	47.08	-20.08	177.32	-18.2	151.92	-19.8	29.32	-16.2	145.9	-21	40.43	-22.4	19.34	-16.5	70.1
bromelain	-21.54	122.39	21.36	152.32	-18.6	55.1	-20.7	48.06	-28.75	177.92	-16.4	140.76	-26	31.5	-16.62	149.8	2391	35.45	-23.3	19.55	-11.7	67.21
harmaline11	-16.35	115.65	-21.4	168.14	-19.1	34.8	-23	41.68	-14.69	164.42	-16.3	136.53	-21.8	27.44	-15.77	150.2	-16.6	40.19	-15.3	11.97	-19.8	66.76
harmine8	-15.71	95.36	-21	164.96	-15.3	56	-25.8	40.2	-20.12	186.51	-14.4	139.63	-20.7	32.91	-16.03	160	-23.3	30.98	-13.7	29.42	-21.2	64.79
hesperidine1	-24.21	98.44	-24.1	185.4	-24	46.4	-33.3	45.29	-23.63	175.94	-20.9	142.06	-30	33.12	-21.37	158	670	42.75	-22.3	21.27	71.9	63.97

Fig. 1. Screenshot of the excel sheet showing G-bind and RMS values for the protein-ligand interaction for first few ligands.

molecule making it flexible enough to fit in the active sites of the protein molecule with greater compatibility during the protein-ligand docking (May and Zacharias, 2008). Drugs generally exhibit their action by binding to certain target receptor (Jones *et al.,* 2002)**.** The energy required for the drug-receptor binding directly correlates to the effective binding of the drug to the target receptor. This energy at the expense of which protein-Ligand interaction occurs is denoted as G-bind value (Hyung-June Woo and Benoît Roux, 2005; Abhilash, 2010). The energy minimized compounds were ready to get docked with the

RESULTS AND DISCUSSION

The literature survey resulted in 157 cancer active anti-inflammatory phytochemicals which includes Curcumin, Epigallocatechin 3-gallate, Quercetin, Indole-3-carbinol, Lactoferrin, Silymarin, Vinblastin, Vincristine, etc. These phytochemicals were taken as targeting agents (ligands). Data mining approach resulted in 49 anti-inflammatory drugs. Targets for these anti-inflammatory drugs were found using Drug Bank which resulted in 35 different targets. Interaction of these target proteins with cancer proteins was predicted using HCPIN. Results from HCPIN show that among 35 target

proteins obtained using Drug Bank only 16 were found to have an interacting protein which confirms their role in cancer pathways. These 16 proteins were considered for further analysis. An attempt was made to retrieve the structures of these 16 proteins using Protein Data Bank (PDB). It was possible to retrieve structures of 11 proteins in the form that can be easily docked using ligands in Quantum3.3.0. These 11 proteins were taken as target proteins for this study. The list of these 11 proteins is given by Table 1. Each of the 157 ligands was docked with all the 11 target proteins in Quantum 3.3.0 and the scores of G-bind and RMS values were noted down as shown in Fig 1. A notepad file consisting of a list of ligands with its interacting protein and RMS value was made. It was then opened in VisANT to obtain the network. Screenshot of that notepad file is given by Fig 2 where as the network obtained is clearly illustrated in Fig 3.

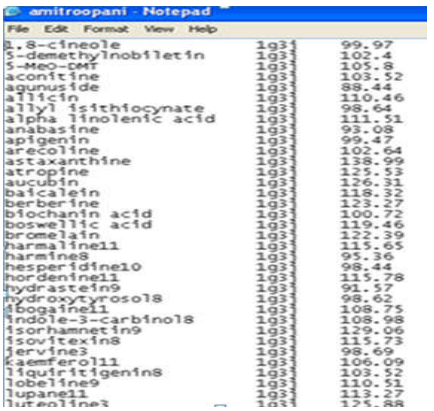

Fig. 2. Screen shot of the notepad file

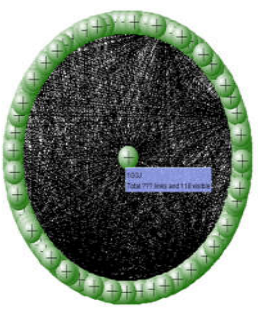

Fig 3. Network obtained after opening the notepad file in VisANT

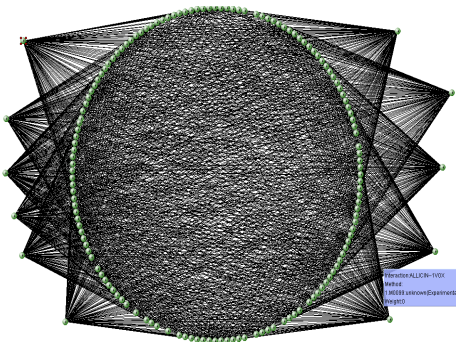

Fig 4. Protein-Ligand interaction network drawn using VisANT

Using VisANT it was found that Catenin beta-1 (1G3J) was the highest interacting protein taking part in most of the interactions with ligands. Based on the network analysis in VisANT, given by Fig 3, Catenin beta-1 (1G3J) was found as the hub of the interaction network. A screening of ligands was done to find phytochemicals that strongly bind to Catenin beta-1 (1G3J). Chicoric acid and digoxin were found to have highest docking scores with Catenin beta-1 (1G3J). A Protein-Ligand interaction network was drawn using a feature of VisANT that enables the user to displace the proteins in the network obtained (Fig 3) to periphery. Interactions of all the 11 proteins present in periphery with the ligands (that make an oval) can be viewed in the protein-ligand interaction network shown in Fig 4.

Conclusion

The anti-inflammatory phytochemicals and cancer inducing proteins were subjected to network interaction, in that the highest interacting protein (hub node) was found to be Catenin beta-1 (1G3J). The vital role played by this protein in various cancers like breast cancer, colon cancer, head and neck cancer, prostate cancer, hepatocellular cancer, etc. has been lucidly studied in the past. Blocking this protein will apparently impede the subsequent reactions in the cancer network, giving promises for cancer prevention. From the docking scores of all the anti-inflammatory phytochemicals docked with this protein it was found that Chicoric acid and Digoxin were the compounds with highest

docking scores. These compounds by inhibiting Catenin beta-1 (1G3J) may stop any kind of Catenin beta-1 (1G3J) induced cancer. We observed an inadequacy of data in the literature that gives a tangible evidence for the specific interaction of these compounds with Catenin beta-1 (1G3J). Further investigation with respect to pharmacological and phytochemical profile of these plant derived compounds needs to be carried out to concrete evidence of their drug like behavior against cancer.

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