Article

Meta-analysis of cancer transcriptomes: A new approach to uncover molecular pathological events in different cancer tissues

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Abstract

To explore secrets of metastatic cancers, individual expression of true sets of respective genes must spread across the tissue. In this study, meta-analysis for transcriptional profiles of oncogenes was carried out to hunt critical genes or networks helping in metastasizing cancers. For this, transcriptomic analysis of different cancerous tissues causing leukemia, lung, liver, spleen, colorectal, colon, breast, bladder, and kidney cancers was performed by extracting microarray expression data from online resource; Gene Expression Omnibus. A newly developed bioinformatics technique; Dynamic Impact Approach (DIA) was applied for enrichment analysis of transcriptional profiles using Database for Annotation Visualization and Integrated Discovery (DAVID). Furthermore, oPOSSUM (v. 2.0) and Cytoscape (v. 2.8.2) were used for in-depth analysis of transcription factors and regulatory gene networks respectively. DAVID analysis uncovered the most significantly enriched pathways in molecular functions that were 'Ubiquitin thiolesterase activity' up regulated in blood, breast, bladder, colorectal, lung, spleen, prostrate cancer. 'Transforming growth factor beta receptor activity' was inhibited in all cancers except leukemia, colon and liver cancer. oPOSSUM further revealed highly over-represented Transcription Factors (TFs); Broad-complex_3, Broad-complex_4, and Foxd3 except for leukemia and bladder cancer. From these findings, it is possible to target genes and networks, play a crucial role in the development of cancer. In the future, these transcription factors can serve as potential candidates for the therapeutic drug targets which can impede the deadly spread.

Keywords metastatic cancer; transcriptional analysis; oPOSSUM; transcription factor; DAVID.

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1 Introduction

Cancer is not a single disease, it is a phrase used to identify immense number of similar diseases resulting from the interplay of gene(s) and environmental factors (Emilsson et al., 2008). Although, cancer can affect

every organ or tissue in the human body, the basic pathology remains the same. As abnormal proliferation and failure to cell death in cancer occurs due to the accumulation of mutations in oncogenes or tumor suppressor genes.

The great majority of cancer deaths occur due to the ability of the cancer to metastasize to other organs. Over the last century, biological research has generated a wealth of knowledge about the cellular structural and functional attributes of cancer that lead to initiation and metastasis. Cancerous cells acquire metastatic abilities due to alterations that surpass their physiological barriers as they separate from their original developmental fate and environment (Nguyen and Massague, 2007). Thus, tumor cells proliferate and penetrate into new tissues, which eventually result in organ dysfunction and death. Understanding the metastatic process is far more challenging than early phases of cancer due to its complex cell-to-cell interaction and microenvironment that promote the process (Bardelli et al., 2003). Application of bioinformatics can help to uncover the molecular processes involved in metastatic cancer in a more systems oriented fashion.

Gene expression profiling and other high throughput technologies have helped in understanding and performing systematic analyses of many complex diseases (Schadt et al., 2005). Microarrays put forth a platform to identify biomarkers along with mechanisms of toxicity and pathogenicity (Waring et al., 2002) and disease subtypes (Mootha et al., 2003; Schadt et al., 2003; van 't Veer et al., 2002). Bioinformatics tools further allow to re-construct gene networks by the integration of genetic data with gene expression to decipher the cellular dynamics during tumorigenesis (Zhu et al., 2004).

Rhodes et al. (2004) have attempted to recognize cancer type-independent gene expression signatures and their corresponding transcriptional factors by comparative meta-profiling of microarrays of a wide range of cancers. They have characterized universally regulated transcriptional profiles in both well-differentiated and undifferentiated cancers. In another study, Wang et al. (2009) have identified gene-to-gene co-expression networks in liver among multiple species by performing semi-parametric meta-analysis strategy to study relationships among common human diseases. Using meta-profiling, researchers have revealed that down regulated genes in Duchenne Muscular Dystrophy (DMD) are involved in a single shared pathway that is responsible for creating disruption in activity of muscle related transcription factors (TF), which contribute to the severity of DMD and these TF can be potential candidates for drug targets (Kotelnikovaet al., 2012).

In this study, we have identified tissue specific and conserved molecular pathways in different metastatic cancerous tissues e.g., lung, prostate, colorectal, kidney, breast, spleen, liver, colon, bone and bladder based on freely available gene expression data of these cancers from the GEO data base using the newly-developed ‘Dynamic Impact Approach (DIA) (Bionaz et al., 2012). Furthermore, for in-depth analysis of the identified molecular mechanisms, their association with differentially expressed TFs was evaluated as a means to potentially uncover potential drug targets (Blancafortet al., 2004; Dunker andUversky, 2010; Smith andBirrer, 1996). We speculate that simultaneous analysis of pathological events of cancers in different tissues can reveal conserved networks across different tissues that can highlight the main biological processes involved in pathogenesis and metastatic abilities of cancer.

2 Methods

Gene Expression Omnibus database (GEO) at National Centre for Biotechnology Information (NCBI) stores high throughput functional genomics datasets (Edgar and Barrett, 2006) and was created by adopting both microarray and sequence technologies. For this study, the data for different cancers were extracted from GEO on the basis of population, gender, age, pathological grade and primary site (Online material, file S1). All selected microarray experiments were performed on human genome U133 plus 2.0. The list of differentially

expressed genes (DEGs) with the cut off P-value was <0.001 and FDR 0.05 was used for all the down stream analysis. All the supplementary information is available at http://www.ncbi.nih.gov/geo of colon (GSM38055), lung(GSM38058), spleen (GSM38056), liver (GSM46848), breast (GSM38109), papillary renal cell carcinoma (GSM46847) and prostate cancer. The clinical parameters of the samples are reported in Table S1, file S1 (Supplementary material). The data for bladder leukemia and bladder cancer was extracted from Savli et al. (2012) and Zhu et al. (2011).

The scheme of the study is presented in Fig. 1. The data analysis was divided into three parts, which include the following: (1) Enrichment Analysis by DIA (2) Transcription Factor Analysis performed by using the web server oPOSSUM (v.2.0) and (3) Gene Regulatory Networks generated by Cytoscape (v.2.8.2). Enrichment analysis was performed by using the DIA with the help of a freely available webserver, DAVID (v.6.7) (The Database for Annotation, Visualization and Integrated Discovery (DAVID version 6.7; http:// david.abcc.ncifcrf.gov/) to identify affected pathways, biological processes, and molecular functions within the DEGs. The detailed method for the analysis has been published previously (Bionaz et al., 2012). Briefly, the entire microarray data sets with associated statistical P-values were imported into DAVID. The 'Impact' refers to the absolute perturbation in a biological process (i.e., overall dynamics within a term/pathway). It gives the magnitude of change that occur (due to treatment) within a pathway/function in either direction weighted by the percent DEGs that hit the term/pathway. The 'Flux' (activation or inhibition) refers to the overall direction in which a term/pathway is impacted after treatment (Bionaz et al., 2012). The length of the bars depicts the degree of the impact, and the intensity of the color (e.g., from dark green denoting highly down regulated to dark red denoting highly upregulated) was used to highlight the degree of activation or inhibition of pathways and terms. As DIA is not a statistical approach; thus, it is not possible to apply cut-offs in order to determine if terms have a biological significance. Furthermore, the results (i.e., the impact) can be considered an indication of the effect of changes in the transcriptome on the biological terms. In this sense, the impact between two terms can be compared (i.e., one term being more impacted than another) in a relative fashion. Because the impact is an 'absolute' value, the same term also can be compared between two different experiments. As an example, biological terms that have an impact over 100 were considered as substantially affected by the treatment (i.e., a pathway with an impact of 100 needs to have on average 25% of protein-genes being significantly affected with a minimum of 2-fold change and 10^{-4} P-value) (Bionaz et al., 2012).

For the analysis of sequences and key regulatory networks that regulate transcriptional responses under specific environment, oPOSSUM version 2.0 Human Site Analysis (link: http://www.cisreg.ca/cgi-bin/oPOSSUM/opossum/) (Huang et al., 2006) and Cytoscape (Lopes et al., 2010) were used. oPOSSUM has been reported to identify the TFs that can mediate changes in gene expression through the detection of over-represented TFBSs in a set of differentially expressed genes in comparison with the pre-compiled background gene sets. The identification of over-represented transcription factor binding sites (TFBS) from sets of co-expressed genes provides insights into the mechanisms of regulation for diverse biological contexts. oPOSSUM calculates two statistical measures for binding site over-representation, one at the gene level (Fisher exact test) and the other based on the ratio of TFBSs to nucleotides (Z-score). oPOSSUM accepts input in the form of gene identifiers e.g. Entrez ID. Against the background set, oPOSSUM compares number of hits for each selected TFBS in the target gene set. The database applies two statistical significance measures, which are Z-score and Fisher-score. Target gene hits and their respective TFs are retrieved through oPOSSUM. Cytoscape version 2.8.2 was used to create networks of the over expressed TF and their target gene list for the network analysis.

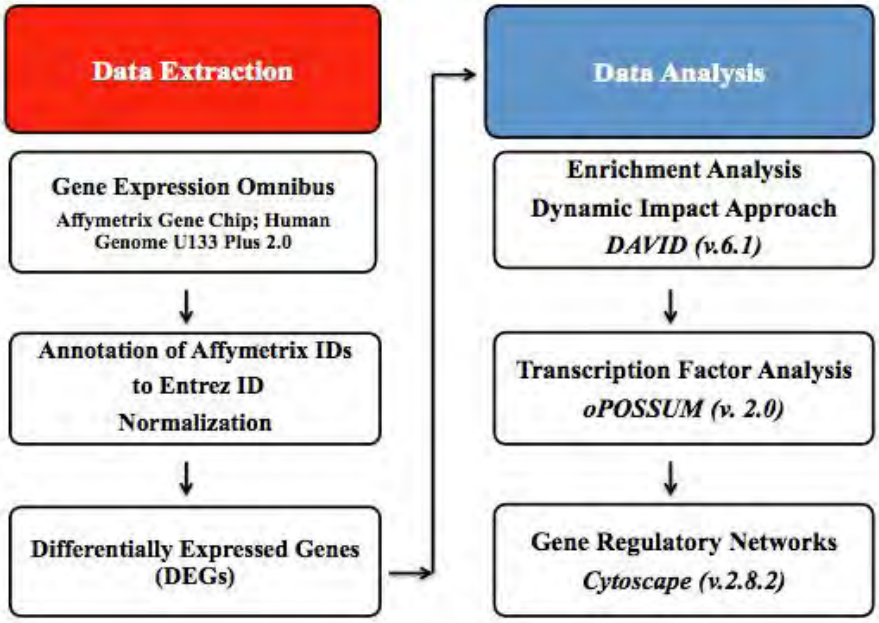

Fig. 1 Scheme of the study.

3 Results

Meta-analysis of transcriptional profiles of different cancers was performed to examine the expression of genes in response to cancer. A total of differential upregulated and downregulated genes with the cutoff as defined above fromselected microarray experiments are presented in Table 1. The aggregated number of DEGs identified in blood, lung, prostate, colorectal, papillary renal, breast, spleen, liver, colon and bladder cancers was160, 1015, 314, 610, 1823, 307, 309, 278, 220, 2018, respectively. For computational analysis with the DIA approach, we used all the 28 categories within DAVID but only Molecular Function (GOTERM_ MF_FAT) are reported here (Online material, file S2). Details of these categories were already reported elsewhere (Bionaz et al., 2012).

Table 1 Number of differentially expressed genes in different cancer tissues with cut off*p*-value<0.001 and FDR 0.05.

DEGs	Lung	Prostate	Colorectal	Renal	Breast	Spleen	Liver	Colon	Leukemia	Bladder
Up	200	200	312	201	201	201	201	201	71	1026
Down	815	114	298	1622	106	108	77	19	89	992
Total	1015	314	610	1823	307	309	278	220	160	2018

With the cut off value stated above, our analysis clearly showed that cancer in all the different tissues sharesseveral molecular events. The most enriched molecular functionsin all the selected cancer profiles are reported in Fig. 2. A global pattern of transcriptional responses in all the cancers, as determined from analysis of cancers in different tissues is shown in file S1 in the form of heat map. The top-most GO terms uncovered by DIAwere ‘Extracellular matrix structural constituent’, ‘Lipase inhibitor activity’, ‘Collagen binding’, ‘Integrin binding’, Metalloendopeptidase inhibitor activity’, ‘Metalloenzyme regulator, ‘Metalloenzyme

inhibitor''Fibroblast growth factor 2 binding', 'Fibrinogen binding', 'Oxidoreductase activity, NAD or NADP', 'Alcohol dehydrogenase activity, zinc dependent' and 'Fibronectin binding' (Fig. 2).

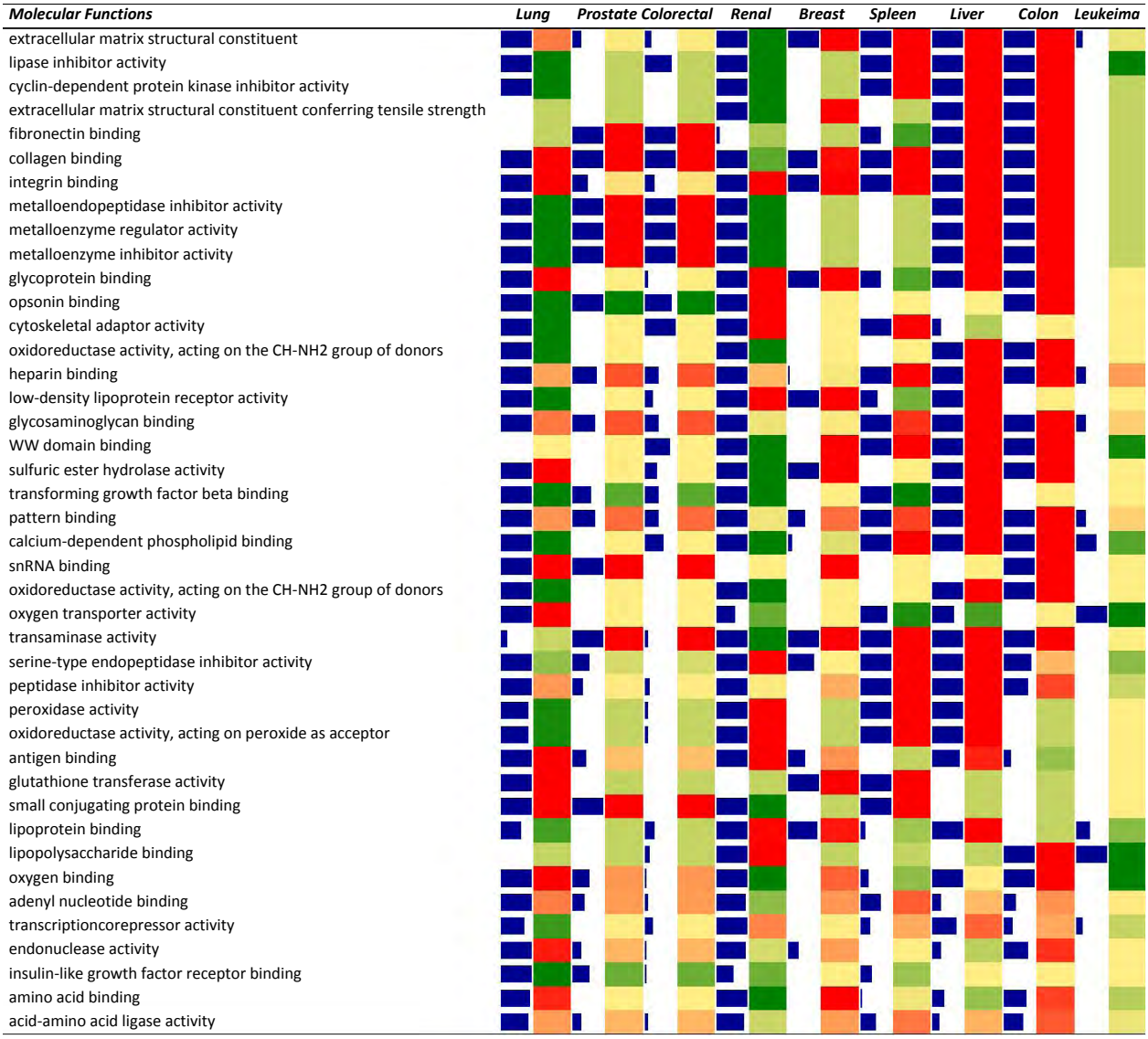

Fig. 2 The most impacted gene ontology terms (GO)as depicted by Dynamic Impact Approach (DIA) in different cancer tissues.On the extreme left are reported the most impacted biological terms. On the right of the column with the biological term is the column of the each tissue typeconsidered in this study. The column presents two sub-columns. In the left sub-column it is reported the horizontal blue bar that denotes the overall impact of the differentially expressed genes on the biological term. Larger the horizontal bar larger the impact. In the right sub-column it is reported a colored square that denotes the direction of the impact (green=inhibited; red=activated). Darker the color larger the activation (if red) or inhibition (if green) of the biological term.

The most impacted GO term identified in all cancers was 'Extracellular matrix structural constituent'. 'Extracellular matrix structural constituent' is strongly induced in lung, breast spleen liver and colon cancer while inhibited in others. 'Collagen binding' that is involved in induction of cellular proliferation in many cancers is namely up regulatedin lung, prostate, colorectal, breast, spleen, liver, and colon cancer. 'Integrin binding' was induced in lung, renal, breast, spleen, liver, and colon cancer. 'Metalloendopeptidase inhibitor activity', metalloenzyme regulator, 'Metalloenzyme inhibitor' and 'Insulin-like growth factor binding' was

inhibited in most of the cancers including lung, prostate, colorectal, renal, spleen, liver, and colon cancer. The pathways of 'Metalloendopeptidase inhibitor activity', metalloenzyme regulator, 'metalloenzyme inhibitor'were induced in prostate, colorectal, liver, and colon cancer, however, they were inhibited in lung, renal, leukemia, breast, and spleen cancer. The GO term 'Transaminase activity'was found induced in six cancer namely prostate, colorectal, spleen, liver and colon cancer. Moreover, 'Transforming growth factor 2 beta receptor activity (TGFB2)' was inhibited in five cancers namely lung, prostate, colorectal, renal, and spleen cancer (Fig. 2).

Fig.3 represents the up regulated molecular functions, which were shared among cancer at different sites. 'Ubiquitin thiolesterase activity' was observed in seven different cancers namely leukemia, breast,bladder, colorectal, lung, prostate, and spleen cancer. 'Phosphoserine phosphatase' was identified in only three cancers; leukemia, colon, and liver cancer. Fig. 4 depicts the down-regulated molecular functions among all cancers. 'Transforming growth factor beta receptor activity'was the most frequently appearing down regulated molecular function, observed in breast, bladder, colorectal, lung, kidney, prostate, and spleen cancer.

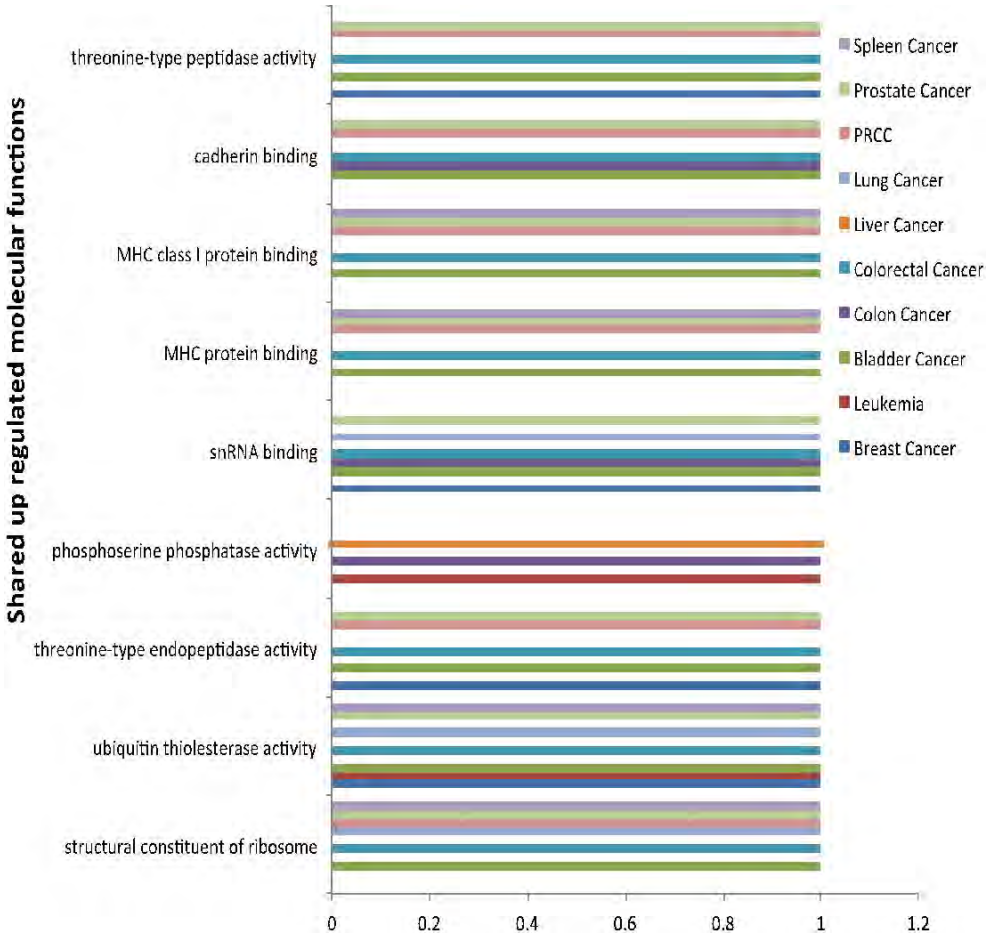

Fig. 3 Graph represents the molecular functions, which were up regulated in different cancers. On y-axis, biological terms aredisplayed and x-axis represents the expression of molecular function in various cancers distinguished by different colors. Legends displayed on the right side of the graph represent the list of cancers. 'Ubiquitin thiolesterase activity' is up regulated in seven different cancers and on the other hand 'phosphoserine phosphatase activity' is up regulated in only threedifferent cancers.

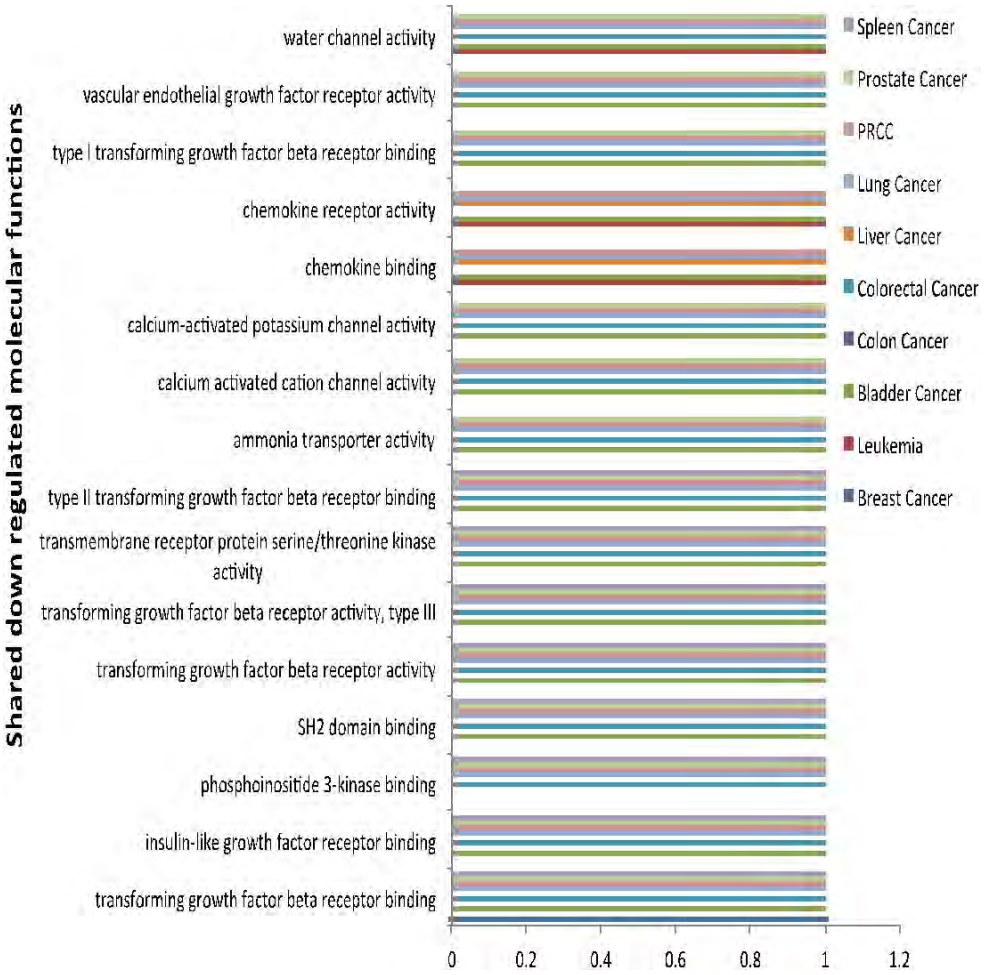

Fig. 4 Graph represents the molecular functions, which were down regulated in different cancers. On y-axis, biological terms aredisplayed and x-axis represents the expression of molecular function in various cancers distinguished by different colors. Legends displayed on the right side of the graph represent the list of cancers. Down regulation of 'Transforming growth factor beta receptor activity' is frequently appearing molecular function observed among seven different cancers.

The prediction of over represented TF binding sites (TFBS) and transcription factors (TF) in different metastatic cancers was accomplished by using oPOSSUM. A complete list of TFBS for all the cancers is provided in file S2. Table 2 enlists the top ranked TFBS in all the cancer with maximum Z scores. Table 3 gives an overview of all TFBS that were shared (appeared in more than one cancer) and/or appeared uniquely (that were tissue specific and found only in one cancer) in all cancers.

We analyzed the list of DEGs for all cancerous data sets with oPOSSUM and found that the binding sites of Broad complex-3 and Broad complex-4, SRY were indeed the most significantly over-represented (Table 2 and 3). Broad complex-3 and Broad complex-4, SRY sites were identified in most of the target gene in all the cancer. Broad complex 3 and Broad complex 4 were present in all the cancers except, leukemia and bladder cancer. Hb, MYB.ph3, NKx 2-5 and Sox 2 were shared among seven cancers while Dof2 and Prrx2 were shared among five cancers. PBF was found unique for colorectal cancer, FOX 11 for renal cancer, and NF-kappaB for leukemia. Agamous, AGL3, Hand1-Tcfe2a, SP1 and TBP were listed only in bladder cancer (Table 3).

Network analysis was done by using Cytoscape (v2.8.2). Genes were mapped against their respective TF,

represented by two different colors i.e. yellow color for TFsand pink color for genes expressed in different cancers. In network analysis, 100 Gene regulatory networks between TF and genes were generated. We have displayed the network of leukemia, which shows an apparently different result from other cancers, and a network of SRY (spleen cancer). It is a common TF observed among the majority of cancers (Fig. 5-6).

Table 2 Statistically over-represented TF binding sites in gene expression data sets of different cancers.

TF	TF Class	Z-score	Fisher Score	TF	TF Class	Z-score	Fisher Score
A: Lung Cancer				**F: Spleen Cancer**			
Broad-complex_3	ZN-FINGER, C2H2	51.8	4.66E-17	Broad-complex_3	ZN-FINGER, C2H2	36.59	1.1E-10
Nkx2-5	HOMEO	49.56	5.14E-08	SRY	HMG	33.25	0.0000003
SRY	HMG	49.42	2.71E-09	Sox5	HMG	32.93	1.55E-08
Broad-complex_4	ZN-FINGER, C2H2	46.51	2.55E-17	Broad-complex_4 _	ZN-FINGER, C2H2	32.58	1.65E-11
B: Prostate Cancer				**G: Liver Cancer**			
Broad-complex_3	ZN-FINGER, C2H2	41.67	1.47E-11	Broad-complex_3	ZN-FINGER, C2H2	36.98	6.87E-14
Nkx2-5	HOMEO	37.63	0.000011	Sox5	HMG	33.48	1.56E-09
SRY	HMG	37.51	0.000004	SRY	HMG	32.77	1.52E-08
Broad-complex_4	ZN-FINGER C2H2	37.28	3.88E-11	Broad-complex_4	ZN-FINGER C2H2	32.29	3.63E-13
C: Colorectal Cancer				**H: Colon Cancer**			
Broad-complex_3	ZN-FINGER, C2H2	12.98	0.01	Broad-complex_3	ZN-FINGER, C2H2	44.69	2.15E-14
SRF	MADS	11.84	0.0118	Broad-complex_4	ZN-FINGER C2H2	40.12	6.27E-15
NR3C1	NUCLEAR RECEPTOR	9.665	0.000568	SRY	HMG	39.56	2.11E-08
MYB.ph3	TRP-CLUS-TER	9.284	0.00396	Nkx2-5	HOMEO	39.18	0.000001
D: PPRC				**I: Leukemia**			
SRY	HMG	28.69	0.00000185	RREB1	ZN-FINGER	12.43	0.0118
Broad-complex_3	ZN-FINGER C2H2	26.43	2.38E-09	ELF5	ETS	9.968	0.00459
Nkx2-5	HOMEO	25.06	0.0000118	RELA	REL	8.161	0.00008
Broad-complex_4	ZN-FINGER C2H2	24.04	8.23E-09	REL	REL	8.064	0.00931
E: Breast Cancer				**J: Bladder Cancer**			
Broad-complex_3	ZN-FINGER, C2H2	32.5	1.35E-09	SRF	MADS	22.4	3.56E-10
Sox5	HMG	28.06	0.0000011	FOXD1	FORKHEAD	18.69	3.37E-08
Broad-complex_4	ZN-FINGER C2H2	26.54	2.41E-09	TBP	TATA-box	17.83	0.000013
SRY	HMG	25.66	0.0000136	SRY	HMG	16.32	0.0000356

TFBSs detected by oPOSSUM with the top 4 mostly highly ranked Z-scores or with Fisher P-value < 0.01. TFs over-expressed or inhibited in gene expression studies.

Table 3 Summary of over-represented TF binding sites in gene expression data sets of all cancers.

List of TF	Lung	Prostate	Colorectal	Renal	Breast	Spleen	Liver	Colon	Leukemia	Bladder
Agamous	-	-	-	-	-	-	-	-	-	Yes
AGL3	-	-	-	-	-	-	-	-	-	Yes
Broad-Complex_3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	-
Broad Complex _4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	-
Dl_1	-	-	-	-	-	-	-	-	Yes	-
Dl_2	-	-	-	-	-	-	-	-	Yes	-
Dof2	-	Yes	Yes	-	Yes	Yes	Yes	-	-	-
Dof3	-	-	-	-	Yes	Yes	Yes	-	-	-
ELF5	-	-	-	-	-	-	-	-	Yes	-
FOXD1	-	-	-	-	-	-	-	-	Yes	Yes
Foxd3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-	-
FOXF2	-	-	Yes	-	-	-	-	-	-	Yes
FOXI1	-	-	-	Yes	-	-	-	-	-	-
Hand1-Tcfe2a	-	-	-	-	-	-	-	-	-	Yes
Hb	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	-
MYB.ph3	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	-	-
NF-kappaB	-	-	-	-	-	-	-	-	Yes	-
Nkx2-5	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	-
NR3C1	-	-	Yes	-	-	-	-	-	-	-
PBF	-	-	Yes	-	-	-	-	-	-	-
Pdx1	Yes	-	-	Yes	-	-	-	-	-	-
Prrx2	Yes	Yes	Yes	Yes	-	-	-	Yes	-	-
REL	-	-	-	-	-	-	-	-	Yes	-
RELA	-	-	-	-	-	-	-	-	Yes	-
RREB1	-	-	-	-	-	-	-	-	Yes	-
RUNX1	-	-	-	-	-	-	-	-	Yes	-
Sox5	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	-
SP1	-	-	-	-	-	-	-	-	-	Yes
SQUA	-	-	-	-	-	-	-	Yes	-	Yes
SRF	-	-	Yes	-	-	-	-	-	Yes	Yes
SRY	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	-	Yes
TBP	-	-	-	-	-	-	-	-	-	Yes

Set of genes in the included target set from which TFBS was identified is listed under Target gene hits

Z-score is the possibility that the number of TFBS nucleotides predicted in the included target genes is significant as compared to the TFBSnucleotides predicted in the Background set.

Fisher Score is the possibility that the number of hits vs. non-hits in the included target genes could have occurred in random chance based on the number of hits vs. non-hits of the background.

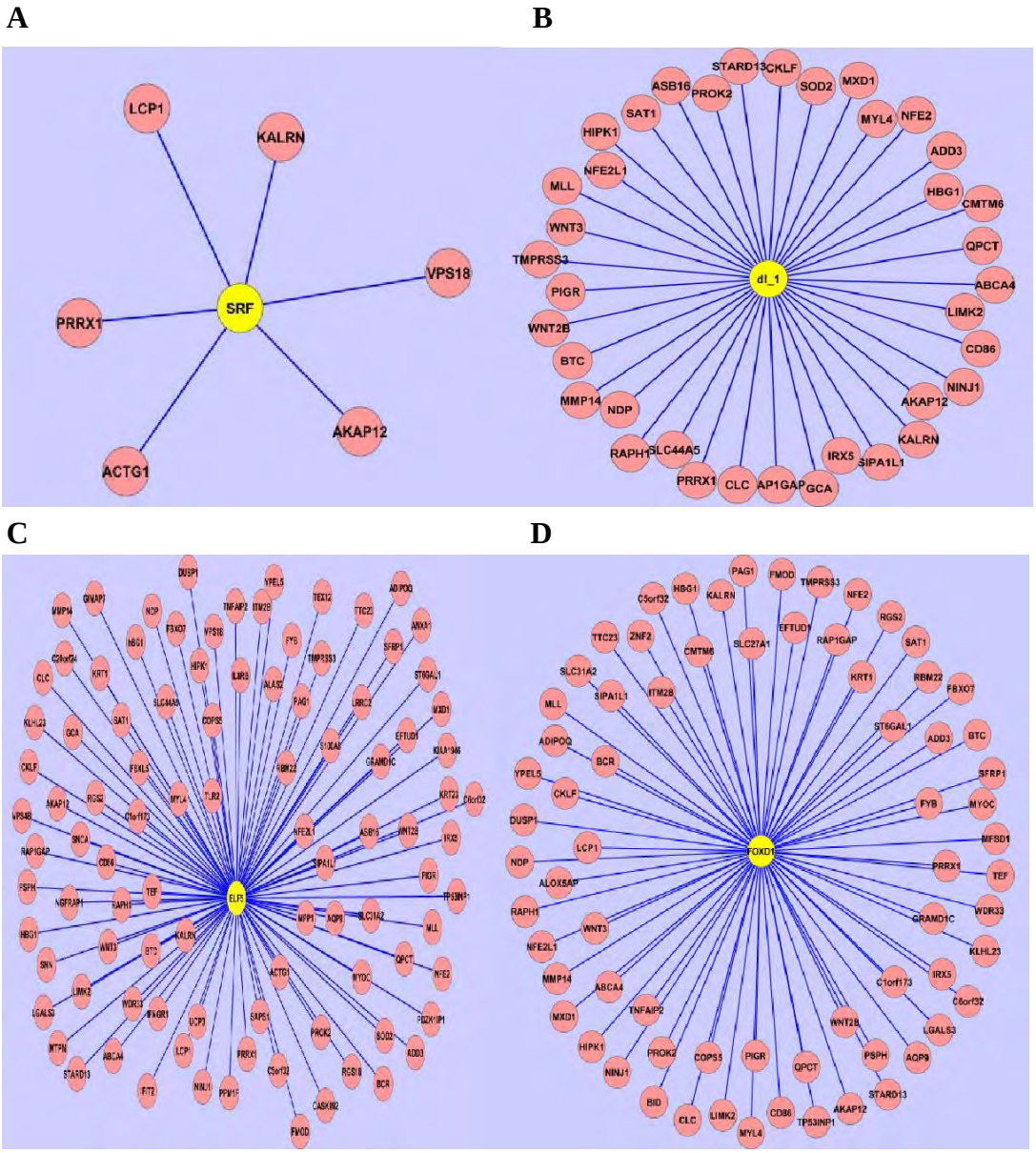

Fig. 5 A, B, C and D networks of leukemia representing association of transcription factors SRF, dl_1, ELF5 and FOXD1(yellow color) with its respective genes (pink color), generated by using Cytoscape (v. 2.0)

4 Discussion

According to the objectives of our study, we primarily analyzed the microarray data sets of metastatic cancers at different sites to visualize the most impacted biological terms. The analysis revealed not only a set of the most impacted biological terms in all selected cancers, but also uncovered a list of TF and their biological networks in the datasets. The data further uncovered the biological terms that were shared by most of the cancers as well as the set of genes that were unique to a particular tissue.

Extracellular matrix (ECM) not only contributes to the structural integrity of the tissues but also mediates the signal transduction processes that conduct growth, differentiation and cell migration. Expression and activation of some tumor related genes is known be associated directly with ECM (Adams and Watt, 1993;

Howlett et al., 1994). The exact mechanism of ECM action is still not known, but deregulation of ECM promotes metastasis by destabilizing the cell structural organization and basement membrane that allow the passage of tumor cell. As the result of such degradation, primary tumor proliferates and penetrates into new tissue habitats (Nerenberg et al., 2007). Growth kinetics of the cell highly depends on the type of growth factor (GF) that binds the ECM and its microenvironment (Petersenet al., 1992). Upregulation of ECM constituents in current study might have promoted the specific set of GFs that might have positively influenced the proliferation and cell invasion. Furthermore, collagen is the primary constituent of physiological barriers, and it has assumed to be the primary ground upon which biochemical events of metastasis take place (Nerenberg et al., 2007). Verhoeven et al. (1993) have reported an increased expression of type IV collagen in periductal breast cancer. The induction of collagen binding in most of the cancers might be indicative of the fact that important physiological changes occur in non-basement membrane of ECM.

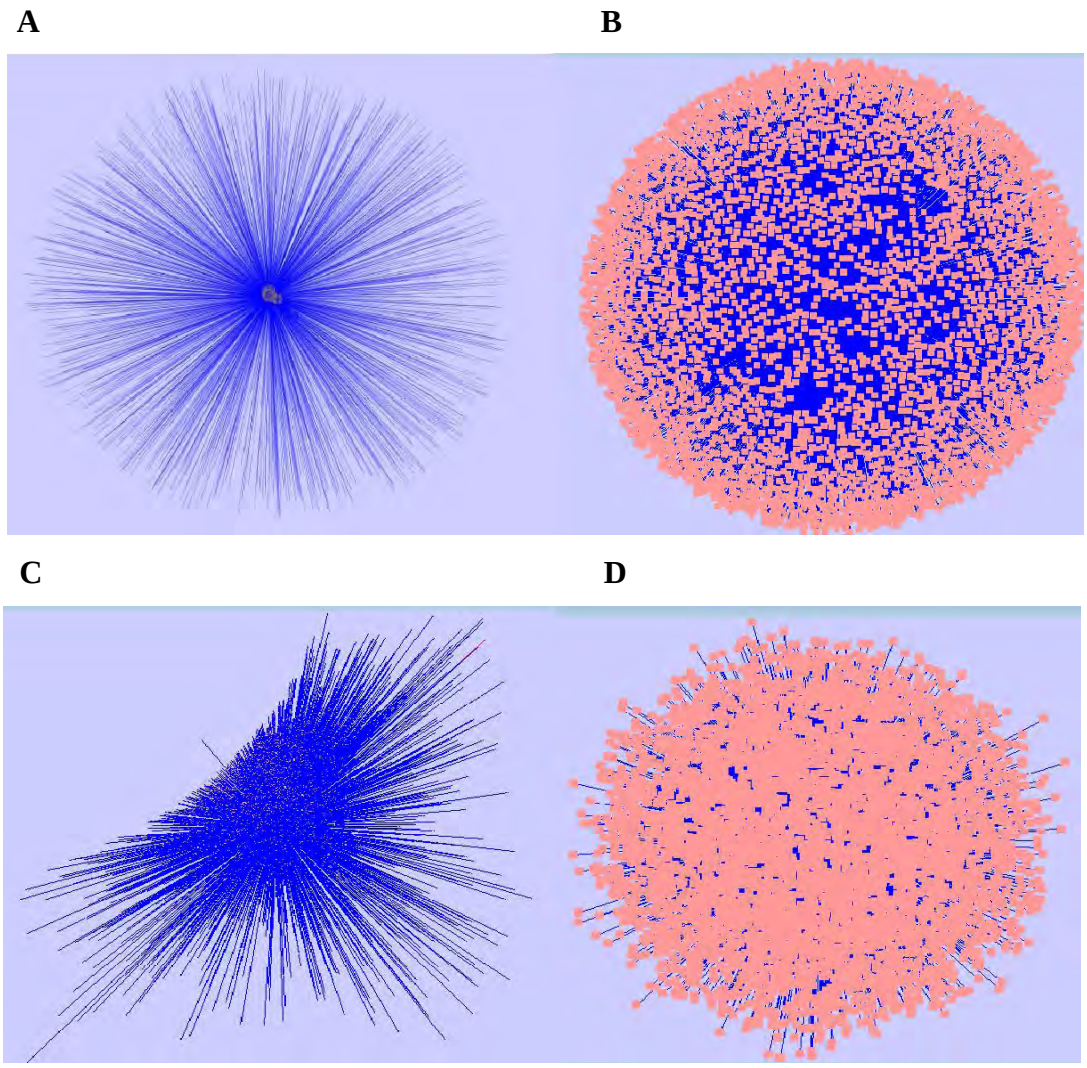

Fig. 6 A, B, C and D networks of spleen cancer generated by using Cytoscape (v. 2.0),represents association of transcriptionfactors SRY, Broad-complex_3, Broad-complex_4 and Foxd3 (yellow color) with its respective genes (pink color).

The function of metalloendopeptidase inhibitor is to inhibit the matrix metalloendopeptidase (MMP) that is associated with the extracellular matrix (ECM) turn over. Metalloenzyme regulator and metalloenzyme inhibitor are the proteins that regulate and inhibit the metal containing enzymes that can modulate many diseases including cancer. These inhibitors play an important role in the homeostasis of ECM, which is a crucial player of tumor invasion and metastasis (Jiang et al., 2002). Deregulation of the metallo-endopeptidase inhibitor activity is reported to be associated with migration of the tumor cell and neovascularization. Prolyloligopeptidase activity is elevated in various cancers and plays an important role in the promotion of angiogenesis (Christiansen et al., 2013). Transminase activity is the catalyst for stimulating the transfer of amino group to acceptor, an aid to detection of liver metastases (Kim et al., 1977).

Insulin-like growth factor (IGF) binding promotes cell migration and has been shown to either inhibit or stimulate growth-promoting effects of Insulin-like Growth Factors (IGFs). They alter the interaction of IGFs with their cell surface receptors that triggers the cascade of molecular events, and ultimately lead to malignancy (Sala et al., 2005; Samani et al., 2007). Inhibition of IGF binding in most of the cancers in our study dictates a diverse role of this pathway in controlling the cell migration and abnormal proliferation pathways. Transforming growth factor 2 beta receptor activity (TGFB2) regulates a plethora of physiological and pathological processes such as cell cycle arrest in epithelial and hematopoietic cells, control of mesenchymal cell proliferation and differentiation, wound healing extracellular matrix production immunosuppression and carcinogenesis. TGFB2 plays an important role in tumor invasion and metastasis (Ganapathy et al., 2010). Microtubule plus end binding is known as the most crucial player in many cellular functions, such as cell migration. The regulation of cell migration is more activated in cancer cells when cancer cells become metastatic (Scolz et al., 2012).

Phosphoserine phosphatase activity plays a fundamental role in the regulation of a number of signaling pathways whose deregulation can contribute to cancer (Eichhorn et al., 2009). The most important pathways include Notch, Wnt and Hedgehog. Notch signaling is the key regulator of development and cell fate in various tissues. Notch signaling activates proto-oncogenes; Notch 1 and -3, they play a crucial role in carcinogenesis. Similarly, Wnt signaling produces a proto-oncogene Wnt1 that stimulates oncogenesis. Hedgehog signaling is mostly identified in basal cell carcinomas (Malhotra et al., 2011). Threonine type endopeptidase activity has associated pathological processes such as carcinogenesis. This endopeptidase activity through its upregulation plays a central role in tumor growth and the multistep processes of invasion and metastasis, as it destabilize the cell stability (Gialeli et al., 2011; Yu et al., 2010). These evidence supports the upregulation of phosphoserine phosphatase activity in many cancers in this study. Upregulation of phosphoserine phosphatase activity, threonine type endopeptidase activity in conjunction with down regulation of TGF beta-receptor activity that is a tumor suppressor gene, are combined factors that lead to malignancy.

Ubiquitin thiolesterase activity is involved in the enhancement of proliferation in multiple types of cells. Kabuta et al has probed this activity by using various mutants of ubiquitin thiolesterase. Inhibition of the interaction between ubiquitin thiolesterase activity and cell cycle-associated CDK resulted in the destruction of ubiquitin thiolesterase-induced enhancement of cell proliferation (Kabuta et al., 2013). Ubiquitin thiolesterase activity is observed in the majority of cancers in our study, which emphasizes its important role in boosting cellular proliferation. Besides this, phosphoserine phosphatase activity, although identified as up regulated in few cancers in our study, because it plays a discrete role in signals pathway regulation, also has tumor suppressor characteristic.

Cadherins are calcium-dependent cell adhesion proteins that trigger loss of cell–cell adhesion; cadherin might also deliver signals that actively induce tumor-cell invasion and metastasis (Vleminckx et al., 1991).

Cadherin binding that was observed as an up regulated function in bladder, colon, breast, kidney, prostate cancer, is pivotal in maintaining epithelial tissue integrity and are the major barrier for epithelial cancer metastasis (Imai et al., 2000; Lu, 2010). It has been observed as a key element of tumor progression in many studies, serving as a suppressor of invasion and metastasis (Hajra and Fearon, 2002). Cadherin binding is also the important molecular function observed in many cancers in our study as it actively induces and supports tumor development.

Major histocompatibility complex I (MHC) Class I and MHC protein binding presented the same response in many cancers in our study. These are well known to trigger the immune system in fighting against cancerous cells. MHC Class I protein binding is involved in the presentation of foreign antigens to the immune system (Watkins et al., 1990). MHC Class I A is commonly expressed in carcinoma cell lines. It is phenotypically detected mostly in T cell leukemia cell lines. Proper functioning of MHC I molecules are essential in fighting cancer (Evans et al., 1999) for recognition of cancerous cell in order to generate a proper immune response (Pende et al., 2002). MHC protein binding plays a role in the natural control of cancer cells. Cancer cells contain many mutated proteins that may be presented by MHC to alert the immune system. Tumor cells may also express normal proteins but in rare places or in abnormal amounts, providing a potential signal to mobilize an immune response (Goodsell, 2005).

snRNA-binding is a molecular function that plays different yet an important role. It plays a role in aggravating cancer by overexpression of genes during splicing. snRNA binding is among the increasing list of splicing factors that have been found to be upregulated or downregulated in cancers, as compared with the equivalent normal tissues. Cancer-associated genetic instability is likely to have an important role in this process. Overexpression of splicing factor like SF2/ASF was reported to associate with amplification of the gene encoding these splicing factors, thus, enhancing the genetic instability (Karni et al., 2007), whereas reduced expression of many genes like RBM5 in lung cancer correlates with deletion of its gene locus at chromosomal region 3p21.3 (Oh et al., 2006).

Structural constituent of ribosome exhibits its role in biological processes including cell adhesion, differentiation, migration, signaling, neurite outgrowth and metastasis (Hao et al., 2004). Ribosomal proteins control cell cycle functioning such as checkpoints of cell cycle and cell proliferation. They play a crucial role in translational regulation and control of cellular transformation, tumor growth, aggressiveness and metastasis (Chattopadhyay et al., 2007).

Water channel activity includes specific membrane proteins that play important roles in numerous physiological processes such as adhesion and migration. Studies indicated that abnormal expression or activity of a number of ion channels e.g. voltage-gated K+, Na+, Ca2+ channels, TRP channels, and epithelial Na+/degenerin family of ion channels, are involved in the growth/proliferation, migration or invasion of cancer cells (Li and Xiong, 2011). In our study, water channel activity is found down regulated in many cancers as it enhances abnormal cell growth and expression.

Vascular endothelial growth factor (VEGF) is associated with tumor angiogenesis and poor prognosis in human colorectal cancer. VEGF receptor-1 is a high-affinity receptor for VEGF and is typically considered specific to endothelial cells. VEGFR-1 was expressed in all colorectal cell lines. Both VEGF-A and -B led to significant induction of cell motility and invasiveness of colorectal cells. VEGF family ligands can activate processes involved in tumor progression and metastasis (Fan et al., 2005). VEGF was observed to be upregulated in many cancers, however, unexpected down regulation was observed in, colorectal, kidney, lung and prostate cancer.

Transforming growth factor-β (transforming growth factor-β (TGF-β) has emerged as a family of growth factors involved in essential physiological processes, including embryonic development, differentiation,

tissue repair and cell growth control (Javelaud and Mauviel, 2004). Alterations in the TGF-β signaling pathway, including mutation or deletion of members of the signaling pathway and resistance to TGF-β-mediated inhibition of proliferation are frequently observed in human cancers (Elliott and Blobe, 2005). Down regulation in our study supports these findings. TGF is down regulated in bladder, colorectal, leukemia, prostate and spleen cancer. The role of transforming growth factor beta (TGF-beta) in carcinogenesis is quite complex, with tumor suppressor and pro-oncogenic activities depending on the particular tumor cell and its stage in malignant progression. Smad2/3, important players in TGF signaling, played a prominent role in regulating tumor suppressor effects on well-differentiated breast cancer cell lines resulting in the formation of larger tumors with an increased proliferation and more malignant histologic features (Tian et al., 2004). VGEF, TGF-beta and SH2 binding are the three important molecular functions mentioned above were identified as down regulated in many cancers. This may be due to their almost similar role in inhibiting cancer progression because all three highly promote proto-oncogenic activities that's why they need to be down regulated to maintain the balance in a cell.

Chemokines and their receptors play critical roles in leukocyte trafficking during inflammatory processes. Although the role of chemokine receptors (CKRs) in cancer biology is relatively new, the data suggest that a number of CKRs, including CXCR4, CCR4, CCR7, and CCR10, may play diverse roles in cancer growth, metastasis, and angiogenesis, or the composition of the cancer microenvironment. For example, preclinical models of cancer indicate that cancer antagonists, most notably those for CXCR4, can block cancer growth either directly or by altering the cancer stroma (Wu et al., 2009). Similar to these observations, chemokines activity is identified as down regulated in leukemia, bladder, liver, lung and kidney cancer in our study.

Src homology-2 (SH2) domain containing phosphatases (Shps) are small, highly conserved subfamily of protein-tyrosine phosphatases. It has been identified that mutations in human Shp2 as the cause of the inherited disorder Noonan syndrome. Shp2 mutations might also contribute to the pathogenesis of some leukemia's. These domains are the elements that control the interaction of cytoplasmic signaling proteins (Neel et al., 2003). SH2 binding is down regulated in bladder, breast, lung, kidney, prostate, and spleen cancer. In our study, the SH2 domain binding is observed as down regulated in many cancers because of role in controlling the signaling pathways. But with reference to the study described above, our results for leukemia were found to be different because in our study leukemia is not reported to be down regulated in SH2 binding domain. This may be due to the difference in choosing the cancer type, pathological grade or primary site in our study comparable to their study.

Phosphoinositide kinase 3 binding activity is down regulated in breast, lung, leukemia and spleen cancer. It is crucial for cell invasion and migration, not only for physically tethering cells to the matrix, but also for sending and receiving molecular signals that regulate these processes (Engelman, 2007). It establishes the initiation of an intricate signaling cascade, which eventually results in the mediation of cellular activities such as proliferation, differentiation, chemotaxis, survival, trafficking, and glucose homeostasis (Katso et al., 2001). Broad-complex (BR-C_3 or 4) belongs to class of zinc finger transcription factors, observed in all cancers except leukemia and bladder cancer. It is a primarily ecdysone response gene, which is a key regulator of metamorphosis, which enhances cell growth and differentiation (Karim et al., 1993). On the contrary, FoxD3 was observed in kidney, lung, prostate, colorectal, spleen, breast, liver and colon cancer. FoxD3 belongs to the forkhead family of transcription factors, acts as transcription repressor and activator. Important player required for the maintenance of pluripotent cells in the various embryogenesis stages as it inhibits migration and invasion of cells (Guo et al., 2002).

In conjunction with the other shared transcription factors, SRY is a member of transcription factor class HMG. SRY is a transcriptional regulator that controls a genetic switch in male development. SRY is also

involved in different aspects of gene regulation including activation and repression of tumor. It plays an important role in pre-mRNA splicing. Elevated expression of SRY-related HMG box transcription factor has been reported in various tumors, comprising colorectal cancer, lung cancer, and breast cancer. SRY plays a central role in the development of these malignancies (Vervoort et al., 2013).

ELF5, NF-kappa B, Dl_1, REL, RELA, RREB1 and RUNX1 are predicted only in leukemia. L_1 and dl_1 both belongs to REL family of transcription factors. ELF5 belongs to epithelium specific subclass of the ETS transcription factor family (Zhou et al., 1998). ELF5 regulates the later stages of terminal differentiation of keratinocytes and regulates a number of epithelium-specific genes found in tissue containing glandular epithelium such as salivary glands and prostate (Sharrocks et al., 1997). Likewise, NF-kappa B, REL, RELA all belong to REL family, it is a pleiotropic transcription factor which is present in almost all cell types and is involved in various biological process such as inflammation, immunity, differentiation, cell growth tumorigenesis and apoptosis. NF-kappa B is controlled by various mechanisms of post-translational modification and sub cellular compartmentalization as well as by interactions with other co-factors or co-repressors (Chen et al., 1999). Correspondingly, RREB1 belongs to ZN-FINGER, C2H2 family. RREB1 binds specifically to the RAS-responsive elements (RRE) of gene promoters it may be involved in Ras/Raf-mediated cell differentiation by enhancing calcitonin expression and this further leads to the medullary thyroid carcinoma (MTC) (Carson et al., 1995). RREB1 represses the angiotensinogen gene. It negatively regulates the transcription activity of androgen receptor Stimulates the transcriptional activity of NEUROD1. RUNX1 belongs to RUNT family. It is a heterodimeric transcription factor that binds to the core element of many enhancers and promoters (Zhang et al., 1997). The protein encoded by RUNX1 is involved in the development of normal hematopoiesis. Chromosomal translocations involving this gene were well documented and have been associated with several types of leukemia (Ichikawa et al., 2013).

TBP that was found only in leukemia belongs to TATA-Box family; it is a general transcription factor that functions at the core of the DNA binding multi protein factors TFIIT. Binding of TFIIT to the TATA box is the initial transcriptional step of the peri-initiation complex, playing a major role in the activation of eukaryotic genes transcribed by RNA polymerase II (Peterson et al., 1990). Similarly, SP1 belongs to same family of transcription factor as RREB1 i.e. ZN-FINGER, C2H2 family. SP1 can activate or repress transcription in response to physiological and pathological stimuli. It binds with high affinity to GC-rich motif and regulates the expression of large number of genes involved in a variety of process such as cell growth, apoptosis, differentiation and immune response. SP1 highly regulated by post translational responses. It may have a role in modulating the cellular response to DNA damage. TBP and SP1 both show expression in regulating cellular proliferation and apoptosis (Wimmer et al., 1997).

NR3C1 that appeared only in colorectal cancer belongs to ZN-FINGER and DOF family. NR3C1 gene encodes glucocorticoid response elements in the promoters of glucocorticoid responsive genes to activate their transcription, and as a regulator of other transcription factors. This receptor is typically found in cytoplasm, but upon ligand binding is transported in to the nucleus. It is involved in inflammatory responses, cell proliferation and differentiation in target tissues (Ray et al., 1996).

In papillary renal cell carcinoma, *FOXI1* gene appears unique. FOXI1 belongs to FORKHEAD family of transcription factors, which is characterized by a distinct FORKHEAD domain. FOXI1 plays a major role in the development of cochlea and vestibulum, as well as embryogenesis (Larsson et al., 1995). Deregulation of FOX family transcription factors can alter the cell fate, boost cancer development and progression (Myatt and Lam, 2007). FORKHEAD transcription factors are the crucial player in the regulation of cell cycle arrest, cell death and DNA damage repair (Yang and Hung, 2011).

5 Conclusions

The use of bioinformatics techniques for a holistic understanding of any biological system needs to be addressed with all the biological elements involved in a system like transcriptome, proteome and their regulator networks. The use of system biology to study cancer and its pathological effects needs to go beyond simple functional analysis of transcriptome. Instability and invasiveness of the cancers reported in this study were clearly depicted by deregulation of pathways like extracellular matrix structural constituent, metallo-endopeptidase inhibitor, insulin-like growth factor (IGF), phosphoserine phosphatase activity and cadherins at the transcriptome level. However, to confirm and specify the role of these pathways in the deadly spread of cancer, regulator pattern of these pathways have to be identified at protein level as well to screen candidate for drug targets. This will bring a deeper understanding of complex microenvironment of cancer with the improvement in drug candidates against cancer.

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