Original Article

Central Nodes in Protein Interaction Networks Drive Critical Functions in Transforming Growth Factor Beta-1 Stimulated Kidney Cells

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Abstract

Objective: Despite the huge efforts, chronic kidney disease (CKD) remains as an unsolved problem in medicine. Many studies have shown a central role for transforming growth factor beta-1 (TGFβ-1) and its downstream signaling cascades in the pathogenesis of CKD. In this study, we have reanalyzed a microarray dataset to recognize critical signaling pathways controlled by TGFβ-1.

Materials and Methods: This study is a bioinformatics reanalysis for a microarray data. The GSE23338 dataset was downloaded from the gene expression omnibus (GEO) database which assesses the mRNA expression profile of TGFβ-1 treated human kidney cells after 24 and 48 hours incubation. The protein interaction networks for differentially expressed (DE) genes in both time points were constructed and enriched. In addition, by network topology analysis, genes with high centrality were identified and then pathway enrichment analysis was performed with either the total network genes or with the central nodes.

Results: We found 110 and 170 genes differentially expressed in the time points 24 and 48 hours, respectively. As the genes in each time point had few interactions, the networks were enriched by adding previously known genes interacting with the differentially expressed ones. In terms of degree, betweenness, and closeness centrality parameters 62 and 60 nodes were considered to be central in the enriched networks of 24 hours and 48 hours treatment, respectively. Pathway enrichment analysis with the central nodes was more informative than those with all network nodes or even initial DE genes, revealing key signaling pathways.

Conclusion: We introduced a method for the analysis of microarray data that integrates the expression pattern of genes with their topological properties in protein interaction networks. This holistic novel approach allows extracting knowledge from raw bulk *omics* data.

Keywords: Chronic Kidney Disease, Microarray Analysis, Protein Interaction Maps, Systems Biology, Transforming Growth Factor Beta-1

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Introduction

Chronic kidney disease (CKD) is a public health problem and a leading cause of death. Despite using current therapies to slow progression of CKD, respective patients are still reaching the end stage renal disease (ESRD) at alarming proportions (1). The histological feature of this debilitating disorder is excessive deposition of extra-cellular matrix (ECM) defined as renal fibrosis. Recent studies declared that transforming growth factor beta-1 (TGFβ-1) is the major driver of fibrosis in kidney, stimulating a variety of signaling pathways related to deposition of ECM components (2). In spite of enormous researches on the role of TGFβ-1 and

downstream elements in the progression of CKD (3, 4), few studies have employed holistic and computational methods for investigation of kidney disorders. Among these studies, there is an elegant report presented by Jin et al. (5) who employed gene regulatory network concepts to analyze high-throughput gene expression data. They could predict and experimentally validate HIPK2 as a potential drug target in HIV-associated nephropathy.

In this study, we propose a holistic approach to investigate the molecular interactions and signaling pathways in response to TGFβ-1 stimulation in human kidney cells. A microarray dataset has been generated by Walsh et al. (6) that examines the expression profile of human tubular epithelial cells before and after treatment with TGFβ-1 for 24 and 48 hours. However, they only focused on the few top differentially expressed (DE) genes including *GREM1, JAG1* and *HES1*. They identified Notch signaling as a critical pathway in diabetic nephropathy. In the current study, we introduced a new method for the analysis of the same microarray dataset that integrated the expression pattern of genes with their topological location in the gene interaction network. Using this strategy, we could infer more informative signaling pathways related to TGFβ-1 stimulation. This approach could also be employed for other large data to improve our understanding of biological processes by extracting remarkable concepts from bulk *omics* data.

Materials and Methods

Microarray data

This study is a bioinformatics analysis of GSE23338 dataset, originally generated by Walsh et al. (6). mRNA expression profile was downloaded from the Gene Expression Omnibus (GEO) database (7). In this microarray experiment, transcriptional response of human proximal tubule epithelial cells (HK-2) to TGFβ-1 stimulation after 24 and 48 hours was assessed. Using GEO2R tool of GEO, the TGFβ-1 treated cells (24 or 48 hours) were compared to untreated HK-2 cells. Benjamini-Hochberg false discovery rate method was applied for P value adjustment. Genes with adjusted $P \leq 0.05$ were considered as differentially expressed.

Protein-protein interaction network

Using CluePedia plugin (8) of the Cytoscape software version 3.1.0 (9), a protein-protein interaction (PPI) network was constructed for the DE genes in time point of 24 hours or 48 hours. Topology of networks was analyzed by the NetworkAnalyzer tool of Cytoscape software.

Pathway enrichment analysis

Pathway enrichment analysis for DE genes was carried out using ClueGO plugin (10) of Cytoscape. In this analysis, KEGG and Reactome databases were chosen for retrieving data and network specificity was adjusted to medium. Bonferroni step down was applied for P value adjustment and pathways with adjusted $P \leq 0.05$ were chosen.

Results

In this study, we reanalyzed the GSE23338 microarray dataset assessing mRNA expression profile of HK-2 cells after 24 and 48 hours of treatment with TGFβ-1. Analysis by GEO2R revealed that 110 genes after 24 hours and 170 genes after 48 hours were differentially expressed with adjusted $P \leq 0.05$ (Table 1). To investigate the interaction between variably expressed genes, a network was constructed for each time point. Although different kind of interactions (activation, post-translational modification, expression and binding) were allowed to be shown, unexpectedly, few interactions were appeared in both networks (Fig.1A, B). To infer pathways related to the DE genes and understand the down-stream processes controlled by TGFβ-1, pathway enrichment analysis was performed, showing only 12 pathways for 24 hours (Fig.1C) and 10 pathways for 48 hours treatments (Fig.1D), with few connections between the signaling pathways.

The scarcity of interactions in PPI and pathway networks was not unexpected, as they were derived from mRNA microarray data which can only detect genes with altered mRNA level, thus regulated genes at other levels were

missed. Hence, to predict other role players, we enriched both PPI networks by adding one interacting node for each gene. This resulted in expansion from 110 to 199 nodes for 24 hours (Fig.2A) and from 170 to 301 nodes for 48 hours treatment (Fig.2B). PPI networks were reconstructed with the same parameters applied initially. To determine the most central genes in these enriched networks, their topology was assessed by graph theory measures such as degree, betweenness centrality, and closeness centrality. In each network, the genes were sorted based on each of these features. Then, the top 20% genes in 24 hours treatment and 15% genes with higher rank in 48 hours were chosen. Because of overlapping nodes between the above three centrality parameters, a total of 62 genes in time point of 24 hours (Table 2) and 60 genes in time point of 48 hours (Table 3) were finally selected. Again, pathway enrichment analysis was performed with either the central genes or the total genes in these two enriched networks. The central genes in time points 24 and 48 hours networks were related to 29 and 49 pathways, respectively (Fig.3). These pathways were strongly related to CKD and formed a deeply connected network in both time points. Interestingly, pathway enrichment analysis with the total enriched networks genes, only determined 16 and 18 pathways for time points of 24 and 48 hours, respectively. These pathways were less inter-connected compared to those derived from the central genes (Fig.4).

Pathway enrichment analysis with the central genes predicted Notch, TNF, P53, Activin and TGFβ signaling as well as platelet-related pathways, affected after TGFβ-1 treatment in both 24 and 48 hours. However, Hippo, PDGF and FGFR signaling pathways were enriched only in the second time point.

Table 1: Differentially expressed genes in time 24 hours and 48 hours with adjusted P≤0.05. The genes are sorted by log2 of fold change (LogFC)

Time 24			Time 48		
Genes	**adj.P.Val**	**logFC**	**Genes**	**adj.P.Val**	**logFC**
GDF15	0.012817	-4.03492	*GDF15*	0.004294	-3.77276
CRYM	0.046546	-3.35307	*CRYM*	0.020195	-3.74094
SCNN1A	0.012817	-3.19552	*CD9*	0.000557	-3.3273
CD9	0.003455	-2.96886	*SCNN1A*	0.006484	-2.86473
RBM47	0.012817	-2.96538	*RBM47*	0.010066	-2.73215
MAL	0.012817	-2.6579	*MAL*	0.007941	-2.72193
HLF	0.033274	-2.44538	*AREG*	0.014332	-2.71598
DEPTOR	0.011983	-2.38064	*HLF*	0.021497	-2.52256
IMPA2	0.002857	-2.22728	*PLA1A*	0.007423	-2.46499
RTEL1	0.003588	-2.11992	*PDZK1IP1*	0.026161	-2.45799
MEGF9	0.03429	-2.04315	*DUSP5*	0.005251	-2.37922
GSE1	0.011894	-2.04015	*ACSL1*	0.003583	-2.36964
ELOVL6	0.004534	-2.02884	*DEPTOR*	0.014818	-2.23285

Table 1: Continued

Time 24			Time 48		
Genes	**adj.P.Val**	**logFC**	**Genes**	**adj.P.Val**	**logFC**
BIRC3	0.012817	-1.98537	*DEFB1*	0.001178	-2.1258
SLC17A3	0.006063	-1.96502	*IMPA2*	0.001964	-2.11942
SULT1C2	0.045879	-1.93073	*HLA-DMB*	0.036113	-2.11004
DUSP6	0.018789	-1.93001	*FXYD2*	0.002471	-2.09686
CEBPD	0.015951	-1.89181	*RTEL1*	0.003148	-1.99502
DEFB1	0.003455	-1.87388	*CLDN1*	0.002102	-1.9428
ACSL1	0.003455	-1.84878	*BIRC3*	0.008587	-1.93307
PLA1A	0.030906	-1.79724	*SULT1C2*	0.028474	-1.89456
DUSP5	0.011894	-1.78577	*FAS*	0.040775	-1.84699
CA12	0.011983	-1.70822	*CEBPD*	0.014469	-1.81201
CLDN1	0.006732	-1.69617	*SLC17A3*	0.010066	-1.78837
PDZK1IP1	0.031449	-1.66723	*LY6E*	0.003332	-1.70064
ADAMTS3	0.009793	-1.64873	*SERPINA1*	0.021497	-1.68148
CDKN2AIP	0.047829	-1.62696	*SLCO4A1*	0.03808	-1.67053
GULP1	0.049153	-1.55674	*SOD2*	0.003686	-1.65771
ACVR1B	0.019538	-1.47953	*TSPAN1*	0.011747	-1.65484
ID2	0.018571	-1.45204	*PLIN2*	0.026161	-1.62099
EPAS1	0.049153	-1.42294	*MEGF9*	0.024224	-1.61932
SOD2	0.016073	-1.41158	*RAB20*	0.026161	-1.59433
ANXA4	0.047613	-1.37096	*CLU*	0.002471	-1.54936
RAB20	0.015265	-1.34593	*SLC4A4*	0.03487	-1.50061

Table 1: Continued

Time 24			Time 48		
Genes	**adj.P.Val**	**logFC**	**Genes**	**adj.P.Val**	**logFC**
MMD	0.030004	-1.33753	*GULP1*	0.047026	-1.46306
CLU	0.01997	-1.32415	*EPAS1*	0.038561	-1.42677
BDNF	0.018571	-1.26903	*ACVR1B*	0.013621	-1.3911
EPCAM	0.015265	-1.26628	*GPRC5C*	0.026161	-1.34555
NR2F2	0.044918	-1.26334	*GSE1*	0.041643	-1.32532
TMEM159	0.047829	-1.25784	*LRRC61*	0.020785	-1.32277
FAS	0.019538	-1.23999	*ANXA4*	0.038789	-1.31199
LY6E	0.014942	-1.20673	*CDKN2AIP*	0.03949	-1.30584
LRRC61	0.033972	-1.17462	*MMD*	0.021485	-1.29784
PPP2R5A	0.023781	-1.16917	*PPP2R5A*	0.019989	-1.25554
SERPINA1	0.039821	-1.09323	*NR2F2*	0.012081	-1.22902
IL24	0.011983	-1.09102	*GLRX*	0.035692	-1.22902
HGD	0.019538	-1.08015	*SERPINA6*	0.00653	-1.22661
ELF3	0.026977	-1.07437	*EMP1*	0.030041	-1.22491
GCH1	0.032261	-1.0672	*MAPKAPK3*	0.037211	-1.20559
ALDH5A1	0.030004	-1.05748	*IFI30*	0.039032	-1.1775
FXYD2	0.020961	-1.02587	*EPCAM*	0.014332	-1.17347
TRIM38	0.043165	-0.92721	*SYS1-DBNDD2*	0.039499	-1.16256
NHLRC2	0.018571	-0.92091	*ADAMTS3*	0.014586	-1.12871
TBL1X	0.040887	-0.88595	*SHMT1*	0.036579	-1.12397
LAD1	0.04193	-0.87726	*GGT2*	0.007492	-1.10696
GLRX	0.035216	-0.87251	*LAD1*	0.014332	-1.09515
TPM1	0.030916	0.782848	*FOSL1*	0.023626	-1.08872
AMIGO2	0.032261	0.803279	*ELF3*	0.022045	-1.078
MISP	0.030916	0.808838	*ID2*	0.03219	-1.07757
ACLY	0.030778	0.809032	*SMAD3*	0.042933	-1.05481
FN1	0.03429	0.860918	*IL24*	0.030041	-1.03178
LYPD1	0.046955	0.922314	*SH2B2*	0.020195	-1.00971
RALA	0.030004	0.95394	*DUSP6*	0.038561	-0.98235
EFNB2	0.030004	0.9589	*ITPR3*	0.021485	-0.9804
SMURF2	0.044772	1.000129	*PDLIM1*	0.044481	-0.96321
TFPI2	0.019538	1.042945	*ALDH5A1*	0.019989	-0.95377
MARCH3	0.026013	1.048251	*FAM3C*	0.039499	-0.93464
NREP	0.031449	1.121914	*REPIN1*	0.038561	-0.9095

Table 1: Continued

Time 24			Time 48		
Genes	**adj.P.Val**	**logFC**	**Genes**	**adj.P.Val**	**logFC**
LTBP2	0.015265	1.133197	*GGT1*	0.036579	-0.893
PLEK2	0.025143	1.137329	*ANXA1*	0.03141	-0.8635
RFTN1	0.014768	1.141252	*UXS1*	0.037211	-0.78881
PRPS1	0.021243	1.212761	*HGD*	0.039499	-0.77866
ADA	0.012817	1.214286	*TBL1X*	0.028029	-0.76181
TNS1	0.027064	1.276677	*MGLL*	0.039499	-0.75719
COL1A1	0.044918	1.349036	*GNPDA1*	0.028029	-0.75096
LAMC2	0.015265	1.448205	*PAX8*	0.031546	-0.73263
CREB3L1	0.004425	1.453935	*TRIM38*	0.026161	-0.69388
TSPAN13	0.030916	1.468138	*PROSC*	0.047627	-0.68991
F3	0.049854	1.537792	*TPM1*	0.045542	0.614444
AKAP12	0.030004	1.541307	*ARL4C*	0.038561	0.67729
HES1	0.015265	1.549119	*IFNGR2*	0.045542	0.695846
SGK1	0.006063	1.584326	*RFTN1*	0.037889	0.727815
PAX6	0.014768	1.602106	*ACLY*	0.021485	0.74412
GREM1	0.004818	1.607941	*EFNB2*	0.026486	0.789092
PTHLH	0.018571	1.651867	*CLTCL1*	0.043748	0.805174
SLN	0.030916	1.66995	*SMURF2*	0.019989	0.813175
ADAM19	0.046955	1.673182	*FAM208B*	0.038561	0.815648
TUFT1	0.01997	1.708363	*TPM4*	0.036579	0.816674
PPP1R13L	0.044622	1.715701	*PLEK2*	0.040607	0.838742
VEGFC	0.006732	1.731189	*FHOD3*	0.043748	0.840283
GPR56	0.005222	1.757315	*CADM1*	0.014818	0.842324
LRP4	0.006732	1.839036	*DLC1*	0.035692	0.861077
SIK1	0.028431	1.847404	*ELK3*	0.037211	0.866603
C1orf106	0.014768	1.852771	*AMIGO2*	0.013633	0.891177
KCNK3	0.019891	1.928548	*PGRMC2*	0.038561	0.892116
WNT5B	0.015265	1.950651	*RAB32*	0.039499	0.911187
SNAI2	0.021356	1.996987	*UAP1*	0.02966	0.914231
GALNT10	0.022735	2.016561	*SKIL*	0.037889	0.927445
GADD45B	0.005222	2.081882	*MAGED2*	0.047466	0.933606
FSTL3	0.006871	2.18737	*DYRK2*	0.045542	0.941228
WNT5A	0.015265	2.199978	*PALLD*	0.039499	0.960395
SCG5	0.006063	2.421762	*MKL1*	0.012081	0.986708

Table 1: Continued

Time 24			Time 48		
Genes	**adj.P.Val**	**logFC**	**Genes**	**adj.P.Val**	**logFC**
TGFBI	0.010068	2.585222	*MARCH3*	0.039989	1.008954
TP53I3	0.018571	2.591672	*LTBP2*	0.007423	1.013795
IL11	0.006063	2.680544	*GABARAPL1*	0.026161	1.018263
PMEPA1	0.002821	2.69133	*TFPI2*	0.045542	1.023787
TAGLN	0.015265	2.807473	*NOV*	0.03219	1.037359
SLCO2A1	0.002821	2.969782	*NUAK1*	0.010962	1.041704
INHBA	0.006732	3.742935	*SLC22A4*	0.021701	1.057375
JAG1	0.012993	4.819474	*PDLIM7*	0.036579	1.075928
			SEMA3C	0.040214	1.084533
			PRPS1	0.018933	1.090259
			COL4A1	0.014469	1.103866
			NREP	0.013884	1.110733
			LYPD1	0.028474	1.112816
			TCF4	0.044016	1.140686
			GADD45B	0.047627	1.201497
			INPP4B	0.003583	1.212552
			SGK1	0.010594	1.225169
			IL15	0.036579	1.22672
			MAP3K4	0.028944	1.263727
			TUFT1	0.037211	1.284833
			SPARC	0.019989	1.288601
			COL7A1	0.00653	1.297757
			ADAM12	0.008731	1.356895
			CREB3L1	0.003148	1.386727
			PTHLH	0.013101	1.415775
			ADAM19	0.026161	1.427201
			IGF1R	0.047026	1.47119
			ARHGEF40	0.01087	1.471459
			WNT5B	0.037889	1.474394
			C1orf106	0.018696	1.482021
			FSTL3	0.010621	1.530293
			LRP4	0.019989	1.533742
			NEDD9	0.040607	1.541275
			HES1	0.019989	1.573046

Table 1: Continued

Time 24			Time 48		
Genes	**adj.P.Val**	**logFC**	**Genes**	**adj.P.Val**	**logFC**
			SPOCK1	0.014586	1.577949
			TSPAN13	0.014818	1.599124
			SPHK1	0.024113	1.599544
			THBS1	0.047872	1.633499
			BCAT1	0.003332	1.666823
			AKAP12	0.010066	1.677861
			SLN	0.015745	1.68979
			DSP	0.048348	1.726407
			FN1	0.004908	1.832317
			SCG5	0.000815	1.864837
			GPR56	0.010962	1.900793
			GALNT10	0.028612	1.917703
			PAX6	0.005251	1.918114
			GREM1	0.001644	1.934697
			SIK1	0.012081	1.972459
			TP53I3	0.035787	1.979721
			VEGFC	0.010621	1.991006
			EFEMP1	0.007516	2.118009
			SLC26A2	0.026357	2.161277
			FBN1	0.019989	2.339046
			WNT5A	0.001354	2.385963
			MMP13	0.024732	2.392553
			TAGLN	0.010066	2.43914
			SNAI2	0.002471	2.45019
			PMEPA1	0.000815	2.475717
			TNS1	0.012081	2.514322
			TGFBI	0.002471	2.622322
			IL11	0.001964	2.698275
			SLCO2A1	0.001145	2.774921
			SLC7A11	0.00226	3.076526
			MMP1	0.021973	3.220728
			SERPINE1	0.000815	3.452134
			INHBA	0.000815	3.757617
			JAG1	0.007516	4.928316

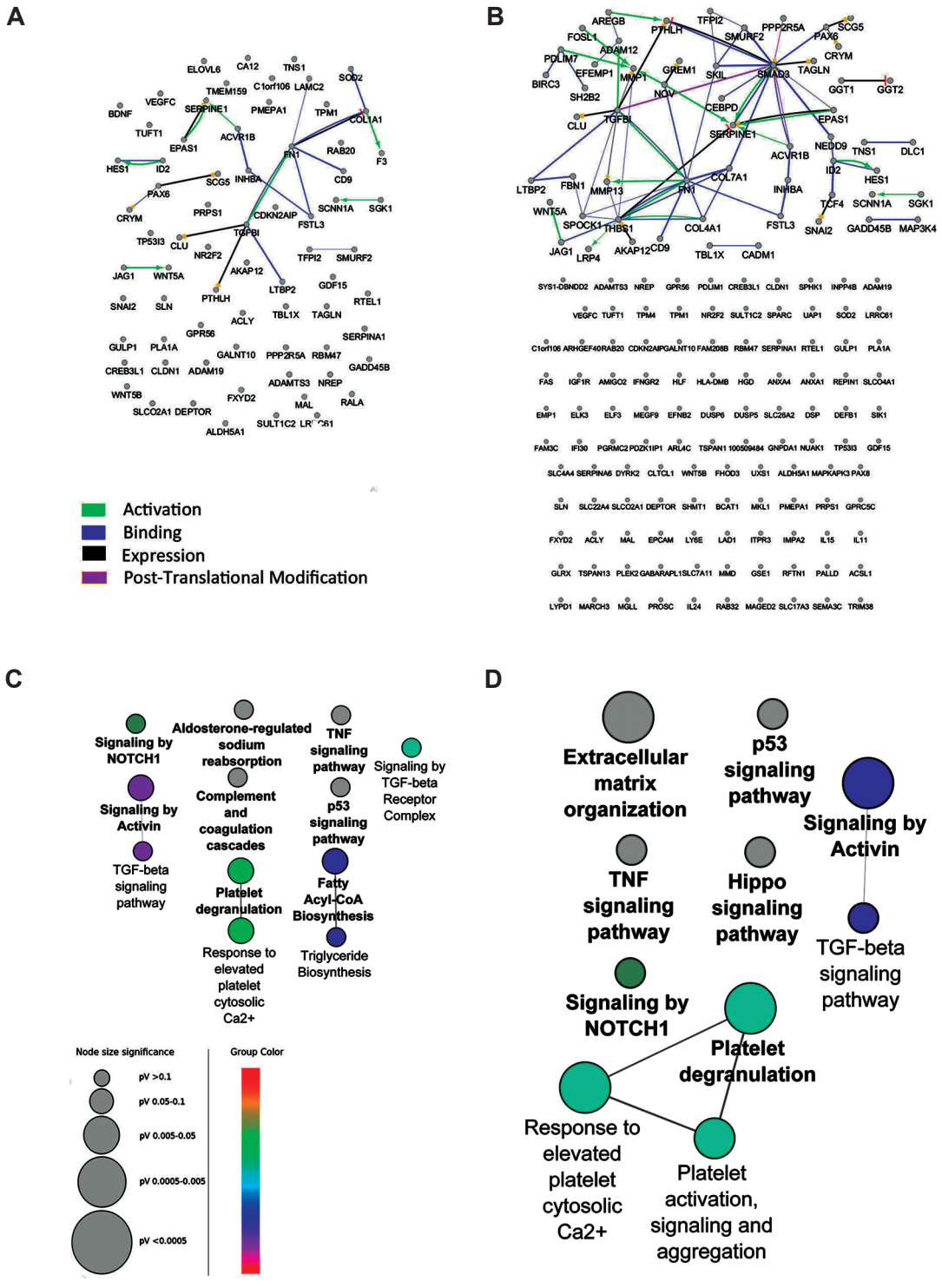


Fig.1: Interaction networks of the DE genes in the microarray dataset were poor and few signaling pathways were enriched. The expression profiles of human kidney cells treated with TGFβ-1 for 24 or 48 hours were compared to untreated cells. The interaction networks of the differentially expressed genes in the time points of **A.** 24 hours and **B.** 48 hours have few edges. In addition, pathway enrichment analysis of these genes in **C.** 24 hours and **D.** 48 hours could not detect key signaling pathways. Pathways with adjusted P≤0.05 are shown. Color represents the gene ontology (GO) term level.
TGFβ-1; Transforming growth factor Beta-1 and DE; Differentially expressed.

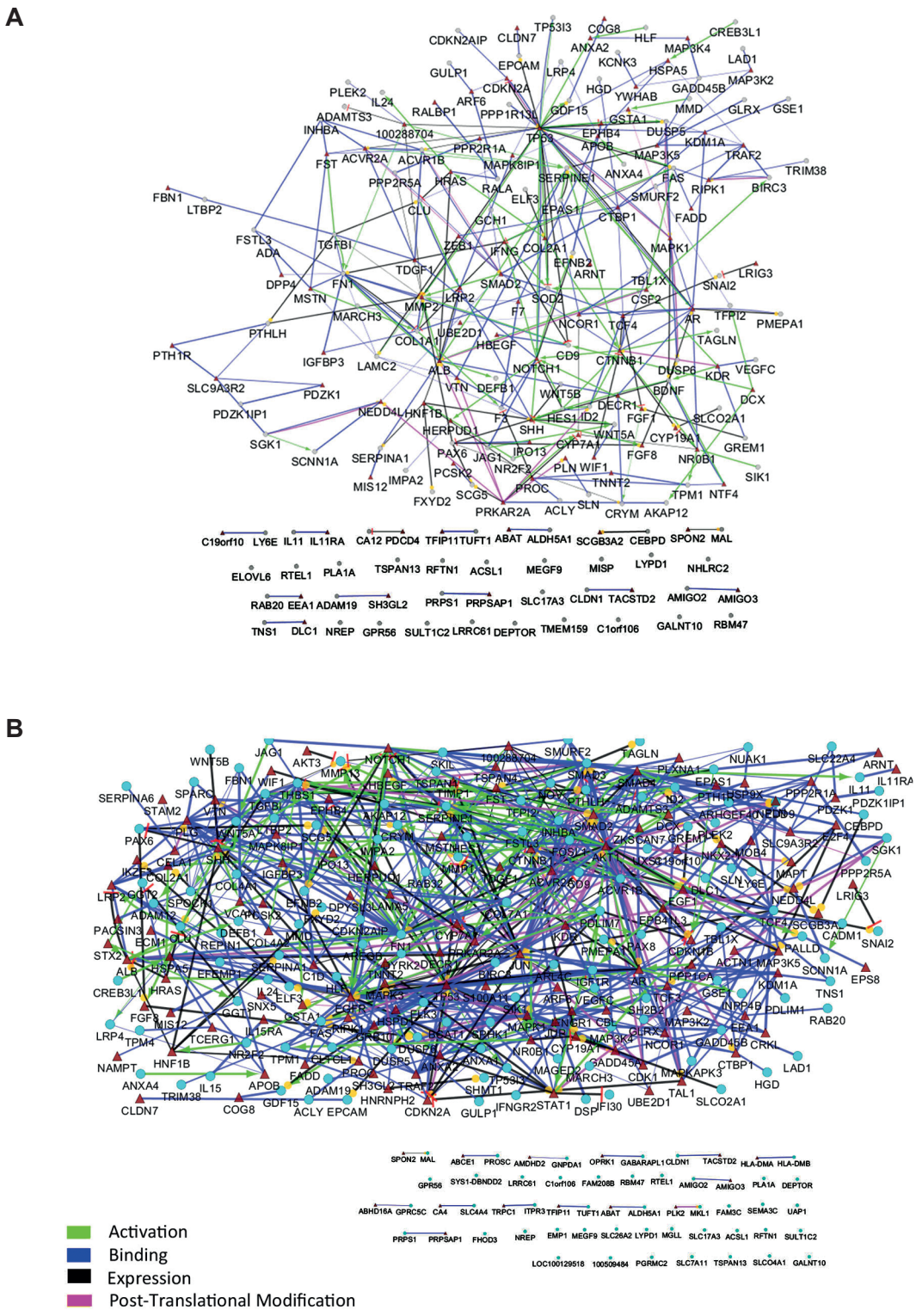


Fig.2: Enrichment of the protein-protein interaction (PPI) network is an efficient method to predict the missed interacting nodes. The networks of **A.** 24 hours and **B.** 48 hours treatment were enriched. The selected nodes from microarray experiment are depicted with ellipse and enriched nodes with triangle.

Table 2: The top 20% genes with the best rank in degree, betweenness centrality, and closeness centrality parameters in the enriched protein-protein interaction (PPI) network of time 24 hours

Genes	Degree	Genes	Betweenness	Genes	Closeness
TP53	35	*TP53*	0.338181	*TP53*	0.397906
FN1	16	*MMP2*	0.171374	*MMP2*	0.38191
CTNNB1	15	*ALB*	0.145517	*NOTCH1*	0.361045
MMP2	15	*CTNNB1*	0.125341	*ALB*	0.356808
ALB	14	*NOTCH1*	0.11946	*AR*	0.35023
AR	14	*SERPINE1*	0.100643	*CTNNB1*	0.347032
NOTCH1	14	*AR*	0.085536	*SERPINE1*	0.344671
SHH	13	*FN1*	0.075905	*SMAD2*	0.334802
SMAD2	11	*SHH*	0.069747	*FN1*	0.333333
SERPINE1	10	*SMAD2*	0.069443	*ACVR1B*	0.326882
COL1A1	9	*PRKAR2A*	0.067309	*ACVR2A*	0.326882
PRKAR2A	9	*HSPA5*	0.067049	*SHH*	0.325482
MAPK1	9	*MAP3K5*	0.049224	*CD9*	0.324094
TGFBI	8	*PTHLH*	0.047677	*MAPK1*	0.319328
ACVR1B	8	*HRAS*	0.045799	*NCOR1*	0.316667
IFNG	8	*TGFBI*	0.044403	*LAMC2*	0.31405
TCF4	8	*HNF1B*	0.040631	*VTN*	0.312115
ACVR2A	8	*CDKN2A*	0.039982	*FAS*	0.310838
FAS	7	*NCOR1*	0.039374	*TCF4*	0.310204
BDNF	7	*PAX6*	0.038418	*SOD2*	0.308943
CD9	6	*CD9*	0.038417	*CTBP1*	0.307692

Table 2: Continued

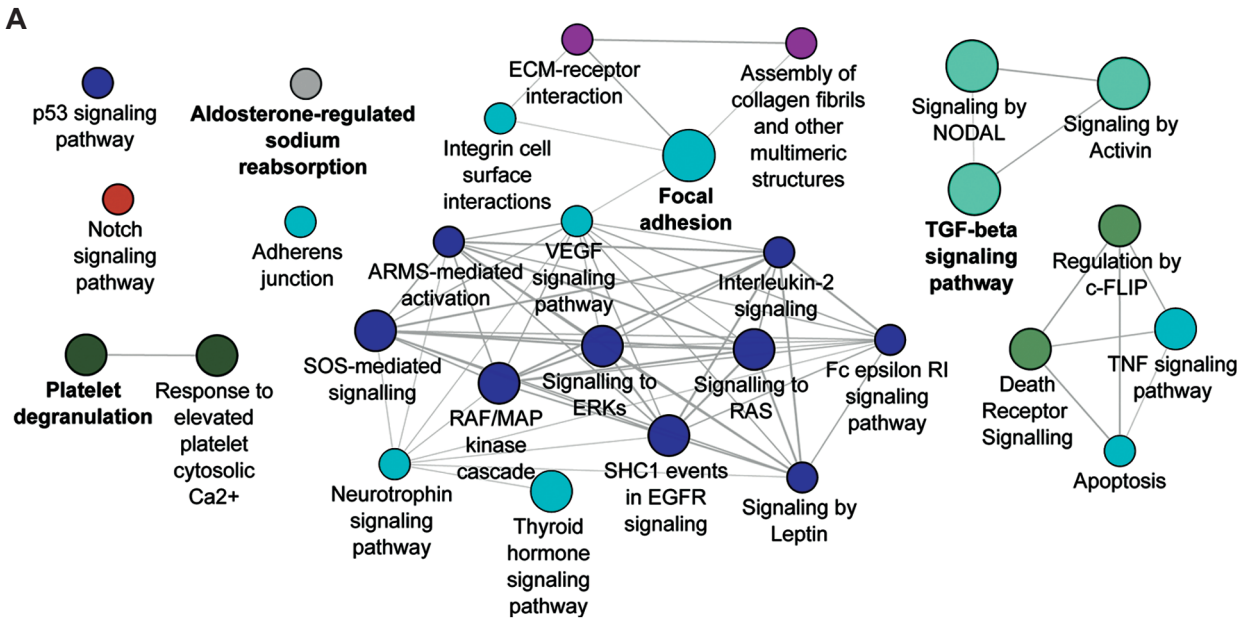
Genes	Degree	Genes	Betweenness	Genes	Closeness
TP53	35	*TP53*	0.338181	*TP53*	0.397906
LAMC2	6	*TCF4*	0.037912	*PAX6*	0.306452
CTBP1	6	*NR0B1*	0.035918	*HES1*	0.306452
PAX6	6	*FAS*	0.035234	*HSPA5*	0.305835
HES1	6	*MAPK1*	0.031996	*IFNG*	0.305221
CSF2	6	*NEDD4L*	0.030261	*KDM1A*	0.305221
NR0B1	6	*SLC9A3R2*	0.028821	*TGFBI*	0.304609
HNF1B	6	*IFNG*	0.028734	*CSF2*	0.304609
LRP2	6	*CSF2*	0.027151	*PRKAR2A*	0.304
TRAF2	6	*ANXA2*	0.026874	*DECR1*	0.303393
RIPK1	6	*PROC*	0.026277	*PPP2R1A*	0.302187
NCOR1	5	*KDR*	0.024832	*DECR1*	0.303393
VTN	5	*CTBP1*	0.024626	*PPP2R1A*	0.302187
SOD2	5	*APOB*	0.024534	*COL1A1*	0.30099
HSPA5	5	*TRAF2*	0.024347	*BDNF*	0.298625
CDKN2A	5	*F3*	0.022625	*TDGF1*	0.295146
HRAS	5	*BDNF*	0.022597	*F7*	0.294574
CYP7A1	5	*LRP2*	0.022317	*NR0B1*	0.293436
KDR	5	*COL2A1*	0.022231	*HNF1B*	0.292308
ID2	5	*GSTA1*	0.021794	*CDKN2A*	0.291747
MAP3K5	5	*VTN*	0.021145	*DUSP5*	0.290631
CLU	5	*ARF6*	0.020175	*LRP2*	0.290076
NEDD4L	5	*YWHAB*	0.01996	*ANXA2*	0.289524
FST	5	*ACVR1B*	0.018667	*F3*	0.288425
MSTN	5	*ACVR2A*	0.018667	*PTHLH*	0.287335
PROC	5	*RALA*	0.018621	*HRAS*	0.286792

Table 3: The top 15% genes with the best rank in degree, betweenness centrality, and closeness centrality parameters in the enriched protein-protein interaction (PPI) network of time 48 hours

Genes	Degree	Genes	Betweenness	Genes	Closeness
TP53	55	*TP53*	0.218425	*JUN*	0.419966
AKT1	49	*AKT1*	0.180618	*TP53*	0.419244
EGFR	33	*EGFR*	0.129406	*AKT1*	0.415673
SMAD3	32	*JUN*	0.121849	*EGFR*	0.403974
JUN	32	*SMAD3*	0.091028	*AR*	0.403306
AR	28	*ALB*	0.08664	*SMAD3*	0.394184
FN1	25	*CTNNB1*	0.077284	*CTNNB1*	0.3904
THBS1	24	*AR*	0.063727	*SMAD4*	0.387917
CTNNB1	23	*SMAD4*	0.059499	*SERPINE1*	0.387917
SMAD2	23	*FN1*	0.056896	*NOTCH1*	0.380655
SERPINE1	20	*THBS1*	0.049411	*THBS1*	0.377709
SMAD4	20	*SHH*	0.0474	*SMAD2*	0.375963
NOTCH1	18	*NOTCH1*	0.044617	*FN1*	0.371951
ALB	16	*SERPINE1*	0.041249	*MMP1*	0.369138
SHH	16	*STAT1*	0.039746	*MAPK1*	0.365269
PLG	15	*HSPA5*	0.037599	*STAT1*	0.364179
MMP1	14	*PLG*	0.035914	*MMP13*	0.359882
TCF4	13	*TRAF2*	0.035805	*ALB*	0.357247
TGFBI	12	*PRKAR2A*	0.03185	*IGF1R*	0.357247
MAPK1	12	*SMAD2*	0.028572	*ACVR1B*	0.350575
ACVR1B	11	*SLC9A3R2*	0.028142	*ACVR2A*	0.350575
CSF2	11	*HSPD1*	0.027604	*CSF2*	0.34907

Table 3: Continued

Genes	Degree	Genes	Betweenness	Genes	Closeness
PRKAR2A	11	*HRAS*	0.025499	*KDR*	0.348074
STAT1	11	*TCF4*	0.024472	*CDK1*	0.348074
TRAF2	11	*PALLD*	0.024455	*CTBP1*	0.347578
IGF1R	10	*TGFBI*	0.024427	*PPP2R1A*	0.346591
CDKN2A	10	*STX2*	0.024388	*SHH*	0.343662
MAP3K5	10	*CDKN2A*	0.022895	*SPOCK1*	0.343662
ACVR2A	10	*CD9*	0.021863	*GRB10*	0.343179
ID2	9	*NCOR1*	0.021568	*NOV*	0.342216
MMP13	9	*MAP3K5*	0.020279	*GSTA1*	0.33936
SKIL	9	*HNF1B*	0.020242	*TCF4*	0.338889
SPOCK1	9	*SPOCK1*	0.018718	*FAS*	0.336088
PDLIM7	9	*CTBP1*	0.018114	*CDKN2A*	0.335626
KDR	9	*TPM1*	0.018056	*NCOR1*	0.335626
LRP2	9	*PTHLH*	0.017657	*TGFBI*	0.335165
TCF3	9	*TBL1X*	0.016927	*VCAN*	0.334705
NOV	8	*CSF2*	0.015788	*HSPA5*	0.334247
PTHLH	8	*GSTA1*	0.015304	*CLTCL1*	0.333333
CDKN1B	8	*KDR*	0.015185	*SKIL*	0.333333
GADD45A	8	*MMP13*	0.014604	*PLG*	0.332879
GRB10	8	*ANXA2*	0.01411	*PTHLH*	0.332879
LAMA5	8	*CLTCL1*	0.013602	*PRKAR2A*	0.332425
VTN	8	*MAPK1*	0.012772	*MAP3K5*	0.330623
CBL	8	*TCF3*	0.012502	*LAMA5*	0.330176



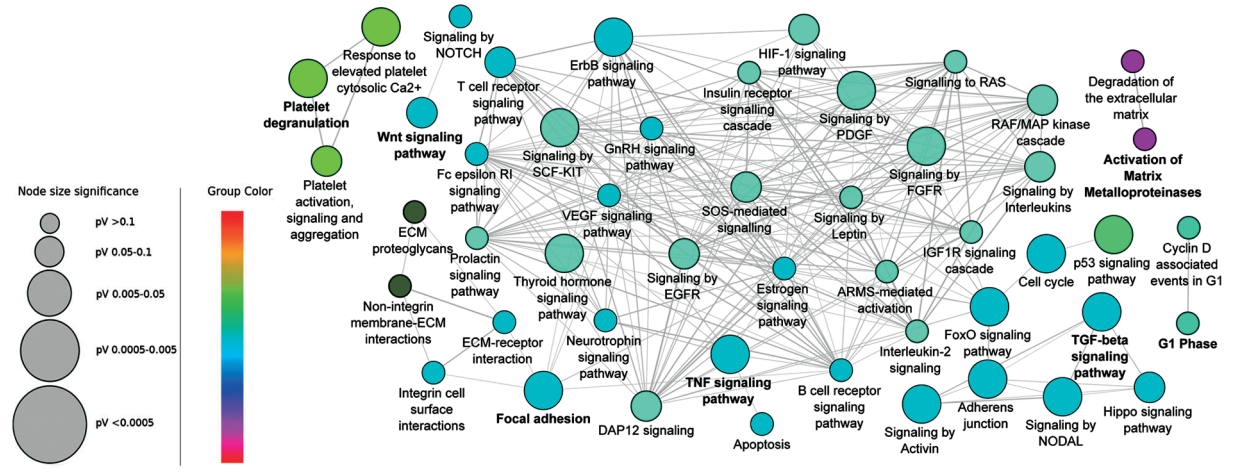


Fig.3: Selection of central nodes for pathway enrichment analysis can detect critical signaling pathways. In the enriched protein-protein interaction (PPI) networks, 62 genes for 24 hours treatment network and 60 genes for 48 hours treatment network were chosen as nodes with high centrality. These central nodes are related to 29 and 49 highly connected pathways in **A.** 24 hours and **B.** 48 hours, respectively. Pathways with adjusted P≤0.05 are shown. Color represents the gene ontology (GO) term level.

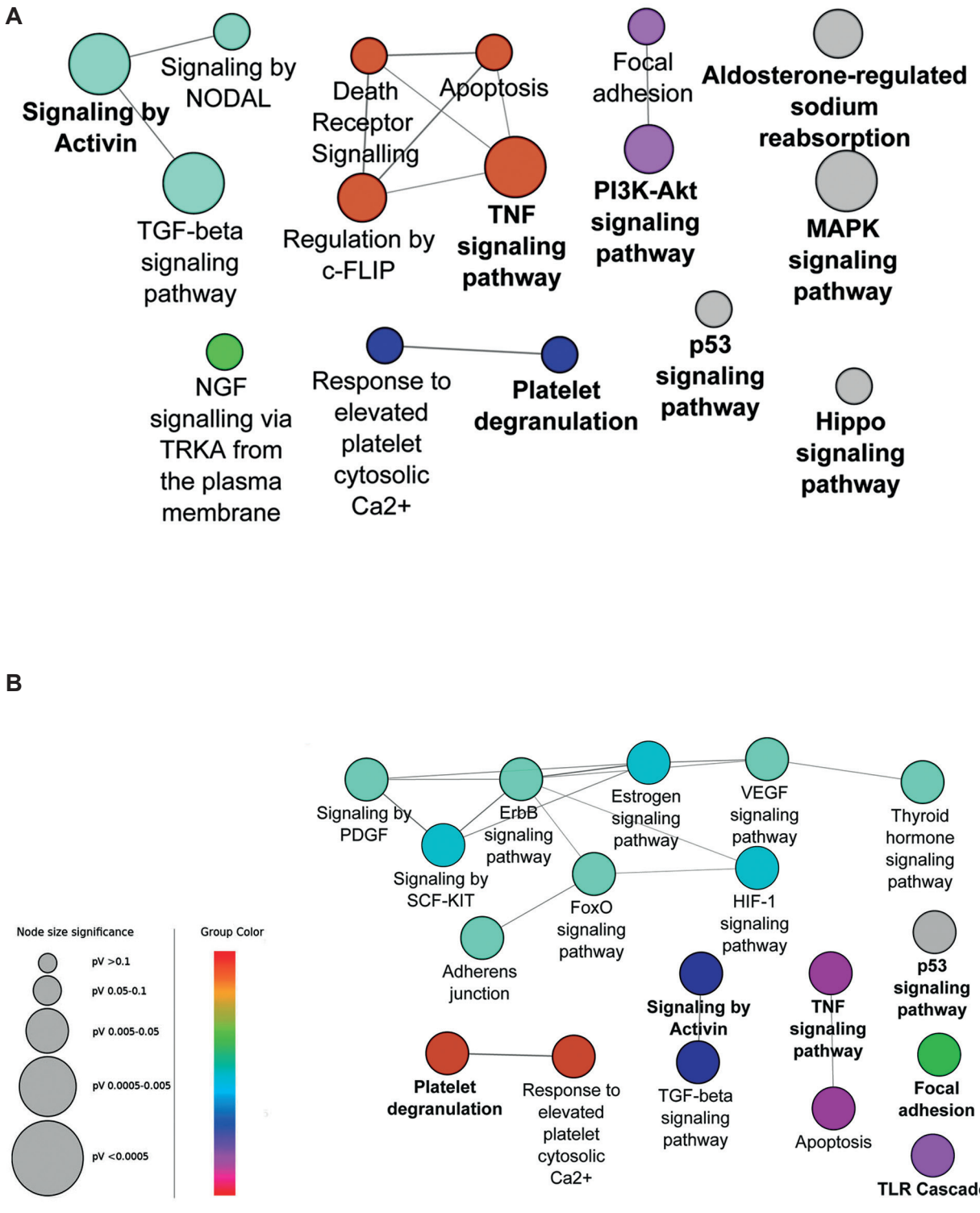


Fig.4: Pathway enrichment analysis with total genes in the enriched network is not informative. Pathway enrichment analysis with all 199 genes in 24 hours, or 301 genes in 48 hours treatment in enriched PPI networks only demonstrated **A.** 16 or **B.** 18 poorly inter-connected pathways, respectively. Pathways with adjusted P≤0.05 are shown. Color represents the gene ontology (GO) term level.

Discussion

In this study, we reanalyzed a microarray dataset to determine gene expression alteration in response to TGFβ-1 in a human kidney cell line. The investigators who originally generated this data emphasized the involvement of Notch signaling pathway based on a few DE genes (6). In contrast, we have constructed PPI networks for DE genes in the time points of 24 and 48 hours treatment. We found that expansion of these networks followed by selection of central nodes for pathway enrichment analysis is an efficient method to recognize key signaling pathways in response to TGFβ-1 stimulation. Our analysis also predicted the potential role of some novel pathways in this *in vitro* model and also pointed out time-dependent activation of particular pathways. Interestingly, the same investigators later repeated the experiment and assessed the mRNA expression profile by RNA-Seq and found that this technique is superior to microarray in identification of the DE genes and altered signaling pathways (11). Noteworthy, the signaling pathways determined by our analysis on the original microarray dataset is similar to the pathways identified with RNA-Seq data.

An interesting finding in this study was that pathway enrichment analysis with the DE genes in the microarray experiment was not efficient for prediction of key signaling pathways. However, it was expected that all important genes were not regulated at the mRNA level and so they were not detectable by mRNA microarrays. Therefore, to compensate for this limitation, we constructed a PPI network of DE genes and then enriched this network by adding genes that were previously known to be interacting with the initial network nodes. This expanded gene set was more informative for detecting signaling pathways. Indeed, it is perfect to perform multi-level assessments in biological experiments, but for practical reasons it is not commonly feasible. In this case, it is possible to measure changes at one level and then make bioinformatics predictions to fill the gaps at other levels.

Several previous studies have shown that highly connected nodes (hubs) in the networks, determined by degree parameter, are vital for the organism survival (12). Next studies revealed that essential genes in the network can be determined not only by degree but also by other centrality parameters, such as betweenness or closeness centrality (13, 14). Here, we have used a combination of these three network topology parameters to determine the central nodes. Interestingly, pathway enrichment with these central genes was more informative than enrichment with the initial genes or even with the total genes in the expanded PPI networks. This observation is in line with our recent study on diabetic nephropathy showing the central network nodes tend to be present in signaling pathways and cross talks (15).

In pathway enrichment analysis, Hippo, PDGF, and FGFR signaling pathways were detected only in the second time point, 48 hours treatment. Actually, the initial activation of upstream signaling pathways detected in 24 hours treatment may lead to the expression of genes, related to these three pathways after 48 hours. This finding on time-specific expression of genes underscores the importance of time-course designs for gene expression analysis experiments.

Most of the predicted pathways in our analysis such as Notch, TNF, P53, and TGFβ signaling have been previously known to be involved in the pathogenesis of CKD (16-19), whereas, for some others, such as platelet degranulation pathway, there is not currently direct experimental proof for participation in renal fibrosis. However, previous experiments have shown megakaryocytes as mediators of fibrosis in a subset of hematologic malignancies, idiopathic pulmonary fibrosis, as well as bone marrow (20-22). The role of megakaryocytes in kidney fibrosis is an interesting topic for future studies.

Conclusion

We have here employed a holistic approach to assess the consequences of TGFβ-1 stimulation in kidney cells. Although, high-throughput techniques are frequently applied in biological investigations, data interpretation is yet commonly limited to the assessment of most up or down-regulated factors missing the huge effect of interactions for genes with subtle expression change. Systems biology provides novel concepts and methods to infer the underlying mechanisms of biological phenomena from *omics* raw data and hopefully will bring a higher quality of life to those suffering from chronic diseases.

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References

1. Thomas R, Kanso A, Sedor JR. Chronic kidney disease and its complications. Prim Care. 2008; 35(2): 329-344.
2. Lan HY. Diverse roles of TGF-beta/Smads in renal fibrosis and inflammation. Int J Biol Sci. 2011; 7(7): 1056-1067.
3. Bottinger EP. TGF-beta in renal injury and disease. Semin Nephrol. 2007; 27(3): 309-320.
4. Munoz-Felix JM, Gonzalez-Nunez M, Martinez-Salgado C, Lopez-Novoa JM. TGF-beta/BMP proteins as therapeutic targets in renal fibrosis. Where have we arrived after 25 years of trials and tribulations? Pharmacol Ther. 2015; 156: 44-58.
5. Jin Y, Ratnam K, Chuang PY, Fan Y, Zhong Y, Dai Y, et al. A systems approach identifies HIPK2 as a key regulator of kidney fibrosis. Nat Med. 2012; 18(4): 580-588.
6. Walsh DW, Roxburgh SA, McGettigan P, Berthier CC, Higgins DG, Kretzler M, et al. Co-regulation of Gremlin and Notch signalling in diabetic nephropathy. Biochim Biophys Acta. 2008; 1782(1): 10-21.
7. Barrett T, Suzek TO, Troup DB, Wilhite SE, Ngau WC, Ledoux P, et al. NCBI GEO: mining millions of expression profiles--database and tools. Nucleic Acids Res. 2005; 33(Database issue): D562-566.
8. Bindea G, Galon J, Mlecnik B. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. Bioinformatics. 2013; 29(5): 661-663.
9. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11): 2498-2504.
10. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009; 25(8): 1091-1093.
11. Brennan EP, Morine MJ, Walsh DW, Roxburgh SA, Lindenmeyer MT, Brazil DP, et al. Next-generation sequencing identifies TGF-beta1-associated gene expression profiles in renal epithelial cells reiterated in human diabetic nephropathy. Biochim Biophys Acta. 2012; 1822(4): 589-599.
12. He X, Zhang J. Why do hubs tend to be essential in protein networks? PLoS Genet. 2006; 2(6): e88.
13. Raman K, Damaraju N, Joshi GK. The organisational structure of protein networks: revisiting the centrality-lethality hypothesis. Syst Synth Biol. 2014; 8(1): 73-81.
14. Ozgur A, Vu T, Erkan G, Radev DR. Identifying gene-disease associations using centrality on a literature mined gene-interaction network. Bioinformatics. 2008; 24(13): i277-285.
15. Abedi M, Gheisari Y. Nodes with high centrality in protein interaction networks are responsible for driving signaling pathways in diabetic nephropathy. PeerJ. 2015; 3: e1284.
16. Wang W, Huang XR, Li AG, Liu F, Li JH, Truong LD, et al. Signaling mechanism of TGF-beta1 in prevention of renal inflammation: role of Smad7. J Am Soc Nephrol. 2005; 16(5): 1371-1383.
17. Sharma S, Sirin Y, Susztak K. The story of Notch and chronic kidney disease. Curr Opin Nephrol Hypertens. 2011; 20(1): 56-61.
18. Misseri R, Meldrum DR, Dinarello CA, Dagher P, Hile KL, Rink RC, et al. TNF-alpha mediates obstruction-induced renal tubular cell apoptosis and proapoptotic signaling. Am J Physiol Renal Physiol. 2005; 288(2): F406-411.
19. Samarakoon R, Overstreet JM, Higgins SP, Higgins PJ. TGF-beta1 --> SMAD/p53/USF2 --> PAI-1 transcriptional axis in ureteral obstruction-induced renal fibrosis. Cell Tissue Res. 2012; 347(1): 117-128.
20. Papadantonakis N, Matsuura S, Ravid K. Megakaryocyte pathology and bone marrow fibrosis: the lysyl oxidase connection. Blood. 2012; 120(9): 1774-1781.
21. Thachil J. The lung megakaryocytes and pulmonary fibrosis in systemic sclerosis. Med Hypotheses. 2009; 72(3): 291-293.
22. Cripe LD, Hromas R. Malignant disorders of megakaryocytes. Semin Hematol. 1998; 35(3): 200-209.