

Kinless hubs are potential target genes in prostate cancer network

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ABSTRACT

Complex disease networks can be studied successfully using network theoretical approach which helps in finding key disease genes and associated disease modules. We studied prostate cancer (PCa) protein-protein interaction (PPI) network constructed from patients' gene expression datasets and found that the network exhibits *hierarchical scale free topology* which lacks *centrality lethality rule*. Knockout experiments of the sets of leading *hubs* from the network leads to transition from *hierarchical* (HN) to *scale free* (SF) topology affecting network integration and organization. This transition, HN → SF, due to removal of significant number of the highest degree hubs, leads to relatively decrease in information processing efficiency, cost effectiveness of signal propagation, compactness, clustering of nodes and energy distributions. A systematic transition from a disassortative PCa PPI network to assortative networks after the removal of top 50 hubs then again reverting to disassortativity nature on further removal of the hubs was also observed indicating the dominance of the largest hubs in PCa network intergration. Further, functional classification of the *hubs* done by using *within module degrees* and *participation coefficients* for PCa network, and leading hubs knockout experiments indicated that *kinless hubs* serve as the basis of establishing links among constituting modules and heterogeneous nodes to maintain network stabilization. We, then, checked the essentiality of the hubs in the knockout experiment by performing *Fisher's exact test* on the hubs, and showed that removal of kinless hubs corresponded to maximum lethality in the network. However, excess removal of these *hubs* essentially may cause network breakdown.

1. Introduction

In complex diseases, finding the key disease genes is the most important task in understanding the regulatory mechanisms of the disease, potential drug targets and disease biomarkers. In recent years, network medicine-based studies on biological complex networks have become one of the most emerging areas of disease research understanding the disease modules and identification of disease biomarkers and drug targets [1]. In complex networks, high degree nodes, *hubs*, play the most significant roles in maintaining their structures, regulating functional dynamics and stabilizing the networks [2,3]. Many of such real-world networks exhibit scale free topology, where *hubs* play central role, and disturbing these *hubs* compromise the stability and signal propagation in the network following the *centrality lethality rule*, which could lead to the collapse of the network [3–5]. Further,

complementation of such networks with *assortativity* network topology associated with *rich club* formation among the high degree *hubs* make them more vulnerable to such targeted disruptions [4,6–11]. A recent study on breast cancer protein-protein interaction (PPI) complex network suggest its conformation to *hierarchical scale free* topology which raises the question on these networks not following the *centrality lethality rule* where the network stability and dynamics are disturbed but not totally compromised when the *hubs* are targeted [12–14]. This could be due to the hierarchical arrangement of modules/sub-modules at different topological levels in the complex networks and other biological networks where they are associated with specific functions [2,3,15,16]. *Hubs* are therefore distributed among these modules maintaining specific functions and help in establishing links among them to coordinate functionalities in the network system [17]. In addition, most *hubs* in PPI networks of eukaryotic organisms are essential

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genes which are necessary for the proliferation of the cells and development and knockout of such genes becomes lethal to the organism [5,18]. Thus, functional classification of the *hubs* and investigating their activities at the modular levels are critical, specially in understanding the roles of the different *hubs* in the analysis of complex biological networks.

Prostate cancer (PCa) is the second most common cancer after lung cancer and fifth leading killer among men worldwide [19], aggravated with the emergence of highly metastatic castration resistant metastatic prostate cancer. This is either due to mutations and other alterations in *Androgen receptor (AR)*, or signalling pathways leading to its elevated expression [20] with a recent study showing *AR gene* knockout reducing cell invasion and migration in PCa cells [21]. In this study, we used the network theorem to understand the structural topology of the PPI network of PCa associated genes, including AR, particularly the role of the high degree nodes, *hubs*, in regulating the complex disease network. The PCa PPI network was shown to exhibit hierarchical scale free topology with modular organization. Knockout analysis of PCa PPI network was performed to reveal the indispensability of the *hubs* and network stability. Owing to an inevitable relevance of *hubs* in hierarchical PCa PPI network, this study classifies *hubs* on the basis of their *participation coefficients* (P_i) and *within module degree* (*z score*) (Z_i). The *hubs* knockout study showed that the *kinless modular hubs* were the most important among the *hubs* maintaining the stability of PCa system. Subsequently, the study reported a maximum enrichment of essential genes among top 100 hubs in PCa.

2. Materials and methods

2.1. Flowchart of the method

We summarized the methods and algorithms we implemented in this work by the flowchart given in the Fig. 1.

2.2. Construction of PPI network and knockout of nodes

Protein-protein interaction network of 2,960 nodes and 20,372 edges was extracted from *GeneMANIA* interactome of 3,871 Prostate cancer associated genes which are overexpressed according to *BioXpressv3.0* ($FC > 1$ and adjusted $p - value < 0.05$) (<https://hive.biochemistry.gwu.edu/bioxpress>) and mutated in PCa patients according to *COSMIC* (<https://cancer.sanger.ac.uk/cosmic>) [22–24]. This

represents the PCa PPI primary network representing a graph $G(N, M)$, $N = \{n_i\}; i = 1, 2, \dots, N$, the set of nodes and M the set of edges with $M = \{m_{ij}\}; i, j = 1, 2, 3, \dots, N$.

Knockout of the nodes was carried by removing the nodes and their respective edges with first randomly generated list of genes from the PCa PPI network and subsequent deletion of top 10,30,50,70,100,200,300,400,500,600,700,800,900 and 1000 highest degree nodes from the network. Sub-networks formed after removing of the nodes and their respective edges were further analyzed for their topological properties.

2.3. Topological analyses of the networks

Calculation of degree of the nodes (k), centralities (*eigenvector centrality* C_E , *betweenness centrality* C_B , *closeness centrality* C_C , *subgraph centrality* C_S), *node degree distribution* ($P(k)$), *clustering coefficients* ($C(k)$) and *node neighborhood connectivity* ($C_N(k)$) for the primary network and knockout experiment on the networks/modules were done using *Network analyzer* and *CytoNCA* in *Cytoscape3.6.0* [25–27]. *Modularity* (Q) was calculated using *Igraph* modularity function based on the *Louvain community detection algorithm* [28]. *Rich club coefficients* (ϕ), *participation coefficients* (P_i) and *within-module degree* or *z score* (Z_i) were calculated using *Igraph* package “*brainGraph*” (<https://github.com/cwatsone/brainGraph>) in R. *Local community paradigm correlation (LCP – corr)* was calculated using *Matlab* pakage for monopartite graphs [29].

Degree (k): It is the total number of connections a node has in a network given by,

$$k_i = \sum_{ij}^N A_{ij} \quad (1)$$

where, k_i is the degree of i^{th} node and A_{ij} denotes the adjacency matrix elements of the graph in graph $G = (N, M)$, where N denotes the set of nodes and M set of edges.

Probability of degree distribution, $P(k)$: It is the probability of distribution of degrees in the whole network and defines the characteristic topology of a network whether a *scale free*, *hierarchical*, *small world* or *random network* according to its distribution against degree in the network [2–4]. It is calculated as,

$$P(k) = \frac{n_k}{n} \quad (2)$$

where, n_k is the total number of nodes with degree k and n , total nodes

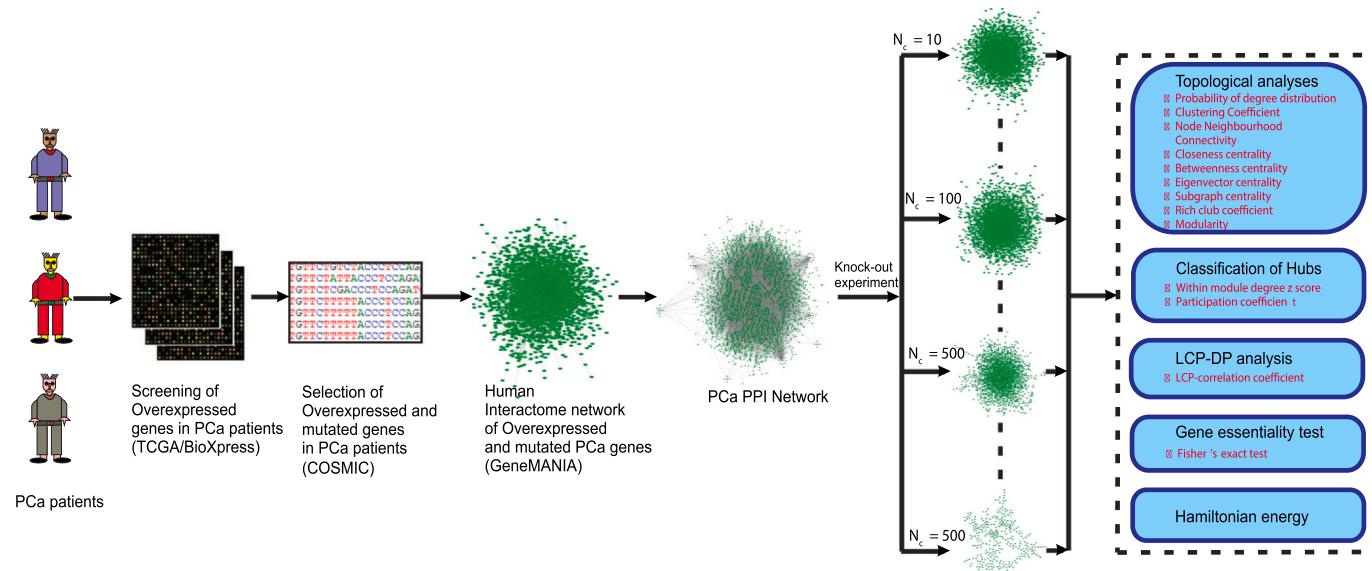


Fig. 1. Flowchart of the methods implemented to study PCa PPI network.

in the network.

Local Clustering coefficient $C_i(k)$: The strength of internal connectivity of a node with its nearest neighbours in a network is measured through its ability to form triangular cliques with its nearest neighbours against the total number of triangular cliques in the network [2–4]. This gives higher values for highly connected among the nodes and is calculated as,

$$C_i(k) = \frac{2m_i}{k_i(k_i - 1)} \quad (3)$$

where, k_i is the degree of i^{th} node and m_i is the total number of edges among its nearest-neighbours.

Average Clustering coefficient $C(k)$: It measures the strength of internal connectivity of the nodes in a network with their nearest neighbours which gives higher values for highly clustering compact network with dense connectivity among the nodes and is calculated as the average of the local clustering coefficients of total number of nodes n in the network [2–4].

$$C(k) = \frac{1}{n} \sum_{i=1}^n C_i(k) \quad (4)$$

Neighborhood connectivity $C_N(k)$: It is the average connectivity of the nearest neighbours of a node in a network which defines the *assortativity* or *disassortativity* mixing in networks [6,7,30] and is calculated as,

$$C_N(k) = \sum_q qP(q|k) \quad (5)$$

where, $P(q|k)$ is conditional probability of the links of a node with k connections to another node having q connections.

Rich club coefficient $\phi(k)$: It is a measure of formation of well connected links between rich high degree nodes among themselves than the lower degree nodes which is in correlation to *assortative* mixing of the network [6,7,16]. For a node with degree k its *rich-club coefficient* is given by

$$\phi(k) = \frac{2m_{>k}}{n_{>k}(n_{>k} - 1)} \quad (6)$$

where, $m_{>k}$ is the number of edges between the nodes of degree $\geq k$, and $n_{>k}$ is the number of nodes with degree $\geq k$.

$$\phi_{norm}(k) = \frac{\phi(k)}{\phi_{rand}(k)} \quad (7)$$

where, $\phi_{rand}(k)$ is the *rich-club coefficient* of *random networks* with similar size and degree sequence, $\phi(k)$ is the *rich club coefficient* with degree k and $\phi_{norm}(k)$ the *normalized rich-club coefficient*.

Closeness centrality C_C : It is the normalized shortest path distance between two nodes in a network and it gives the closeness of a node to other nodes in the network [31–33]. It is calculated as

$$C_C(k) = \frac{n}{\sum_j d_{ij}} \quad (8)$$

where, n is the total number of nodes in the network and d_{ij} is the sum of geodesic path lengths between nodes i and j .

Betweenness centrality C_B : It measures the central controlling ability of node in the network with nodes with higher betweenness centrality have higher control on the network making it more central in the network [32–34]. It is given by

$$C_B(v) = \sum_{i,j:i \neq j \neq k} \frac{d_{ij}(v)}{d_{ij}} \quad (9)$$

where, $C_B(v)$ is the betweenness centrality of node v and $d_{ij}(v)$ denotes the number of geodesic paths from node i to node j passing through node v . Then, **Normalized Betweenness Centrality** C_B is given by,

$$C_B(v) = \frac{1}{M} C_b(v) \quad (10)$$

where, M denotes the number of node pairs, excluding v .

Eigenvector centrality C_E : It measures a node's influence on the network where nodes with higher connectivity to more influential nodes will have higher *eigenvector centrality* and bigger control on signal propagation in the network than others [32,33,35]. It is given by,

$$C_E(i) = \frac{1}{\lambda} \sum_{j=nn(i)} v_j \quad (11)$$

where, $nn(i)$ denotes the nearest neighbours of node i in the network with eigenvalue λ and eigenvector v_i of eigen-value equation, $Av_i = \lambda v_i$ where, A is the network adjacency matrix.

Subgraph centrality C_S : It measures a node's participation in number of subgraphs in the network ultimately more influential nodes will have higher *subgraph centrality* with bigger participation [36]. It is calculated as

$$C_S(i) = \sum_{j=1}^N v_j(i)^2 e^{\lambda_j} \quad (12)$$

where λ_j is the j^{th} eigenvalue and $v_j(i)$, the i^{th} element of the associated eigenvector.

2.4. Parameters to measure compactness of a network

The following parameters along with *average clustering coefficient* $C(k)$ can be used to measure the strength of clustering and compactness of a network.

Modularity Q : It measures a networks capability to divided into *modules/communities* and higher *modularity* networks have nodes with more intra-modular connections forming sparse and lose connections between the modules/communities [15]. *Modularity* is calculated as

$$Q = \frac{1}{2m} \sum_{ij} \left[A_{ij} - \frac{k_i k_j}{2m} \right] \delta(c_i c_j) \quad (13)$$

where, m is the number of edges, A_{ij} adjacency matrix, k_i is the degree of node i , k_j the degree of node j , c_i and c_j are the components of nodes i and j , and δ is 1 if i and j belong to same group and 0 otherwise.

Local-community paradigm correlation coefficient (*LCP – corr*): The assessment of the effect of link prediction and its associated local community paradigm on network topology can be done through *LCP-decomposition-plot* (*LCP – DP*). This is calculated through calculating *Pearson correlation coefficient* between number of *common neighbours* (*CNs*) and to the respective number of *Local community links* (*LCLs*) which are the number of links between the common-first-neighbours between the interacting nodes and plotted on a bidirectional graph [29]. Networks with *LCP-DP correlation coefficient* > 0.80 are highly compact and dynamic with strong self-organization. It is calculated as

$$LCP - corr = \frac{con(CN, LCP)}{\sigma_{CN} * \sigma_{LCL}}, CN > 1 \quad (14)$$

where, $con(CN, LCP)$ is the covariance of the *CN* and *LCP*, and σ_{CN} and σ_{LCL} are the standard deviations of *CN* and *LCP*.

Hamiltonian Energy: *Hamiltonian* (H) characterises the partitioning of a complex network into smaller subgraphs depending on the size and density of the network with the resolution parameter γ [37,38]. Higher density of network has more influence on the resolution parameter resulting into better partition of the network and distinguishable subgraphs and it is given by

$$H = -[m - \gamma n] \quad (15)$$

where, m is the total number of edges in the community/module, n the total nodes and γ the resolution parameter.

2.5. Methods to classify hubs

The following parameters can be used to classify types of *hubs* in a network.

Within module degree or z score, (Z_i): Exploring the role of nodes at the level of modules in a complex network can be done through their functional classification in the modules. *Within module z score* (Z_i) of a node is derived from its modular degree and accordingly the nodes are classified as *modular hubs* (with $Z_i \geq 2.5$) which have higher connections and having more influence in their modules than other *non-hub modular* nodes ($Z_i < 2.5$) [17]. It is calculated as

$$Z_i = \frac{k_i - \bar{k}_{s_i}}{\sigma_{k_{s_i}}} \quad (16)$$

Where k_i is the degree of node i in its module s_i and \bar{k}_{s_i} represents the average of degree k of all nodes in the module s_i , and $\sigma_{k_{s_i}}$ denotes the standard deviation of degree k in s_i .

Participation co-efficient (P_i): Further local and global functional roles of the modular nodes are defined through their participation in establishing links between the nodes within their own modules or outside their modules. Higher $P_i > 0.70$ of a node is associated with its more participation in linking different modules.

$$P_i = 1 - \sum_{s=1}^{N_M} \left(\frac{k_{is}}{k_i} \right)^2 \quad (17)$$

Where, k_{is} is the degree of node i to nodes in s module, k_i is the total degree of node i and N_M is the total number of modules in the network.

P_i along with Z_i classify the *modular hubs* as *modular kinless hubs* (with $Z_i \geq 2.5$ & $P_i > 0.70$) which establish connections between nodes among all other modules, *modular connector hubs* (with $Z_i \geq 2.5$ & $0.30 < P_i \leq 0.75$) which has maximum links with nodes outside own modules and *modular provincial hubs* (with $Z_i \geq 2.5$ & $P_i \leq 0.30$) [17].

2.6. Gene essentiality analysis

A total of 7,168 essential genes in humans had been identified experimentally by OGEE v2 (<http://ogee.medgenius.info>) out of a total of 21,556 genes [39]. These genes are essential for the survival, development and proliferation of cells which were confirmed in 7 different cancer cell lines [39]. Taking the sets of top 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 highest degree genes from the primary PPI PCa network, the percentage of these essential genes in every set were calculated and their enrichment analysis against the whole human dataset of 21,556 genes were done using two tailed Fisher's exact test [40]. The odds ratio along with the 95 % CI values and their p value were calculated for every genes set of highest degree genes.

3. Results

Topological properties of PCa PPI network, $P(k)$, $C(k)$ & $C_N(k)$ (Equations (2), (3), (4) in Methods) followed power law behaviors, $P(k) \sim k^{-\gamma}$, $C(k) \sim k^{-\alpha}$ & $C_N(k) \sim k^{-\beta}$, with $\gamma \sim 1.92$, $\alpha \sim 0.30$ and $\beta \sim 0.006$ (Eq. (18), Fig. 2). Since this power law behavior is the signature of fractal or scale freeness in the network, and this network is organized into modules and sub-modules at different levels of organization, thus PCa, PPI network follows hierarchical scale free network topology [2,3,41].

3.1. Transition of network topologies driven by hubs

Hubs, highest degree nodes, which have key responsibilities in signal propagation and maintaining network stability, might have various roles in organization/re-organization of the network system when some of them are removed from the network. This could reflect

the possible importance of the *hubs* in network organization, such as, in the optimization of signal processing, change in the network properties, variation in system level organization etc. Hence, to study significant obligation of the *hubs* in the network, we carried out knockout experiment of the first leading *hubs* ($10 < N_C < 1000$ as given in Methods) from the primary PCa PPI network and analyzed the topological properties of the resulting networks (Fig. 2). From the analysis, we found that the networks and sub-networks followed *hierarchical-scale free* topologies, where, $P(k)$, $C(k)$ and $C_N(k)$ still exhibiting power law nature against degree k with negative exponents (Eq. (18)). The slopes of the fitted power law curves on the resulting data of $P(k)$ varied and increased systematically as the number of knockout *hubs* was increased ($10 < N_C < 700$ as in Fig. 2 first upper panels). Since the value of γ for *hierarchical network* is $\gamma \sim 1 + \frac{\ln(4)}{\ln(3)} \sim 2.26$ [3] and for *scale free* network $2 < \gamma < 3.4$ [4], we could identify three different distinct regimes, where, the network properties were significantly different. First, the *hierarchical regime*, where, the value of γ was approximately in between 1.9 and 2.3, $1.9 < \gamma < 2.3$, with corresponding number of knockout *hubs* were in between 0 and 200, $0 < N_C < 200$. In this regime, emergence of *modules* in the network dominates the emergence of *hubs* in it [2] and the system level organization of *modules* and *sub-modules* at various levels of organization are much more prominent in the network. Here, the network lacks central control mechanism, and hence, *centrality-lethality rule* [5] is violated and the network is generally self-organized. Second regime, which can be termed as *strong scale free regime*, was with $2.3 < \gamma < 4$ which corresponded to the removal of *hubs* $200 < N_C < 500$ from the network. In this regime, the function of *hubs* could be much more prominent/dominant than the role of *modules* [3]. In this regime, removing of *hubs* could be more lethal to the network leading to significant breakdown of network organization. Third, *network breakdown regime*, where, the value of γ was very large, $\gamma > 4$ with removal of *hubs* $N_C > 500$, leading to random breakdown of the network.

$$\begin{bmatrix} P(k) \\ C(k) \\ C_N(k) \end{bmatrix} \sim \begin{bmatrix} k^{-\gamma} \\ k^{-\alpha} \\ k^{-\beta} \end{bmatrix}; \begin{bmatrix} \gamma \\ \alpha \\ \beta \end{bmatrix} \rightarrow \begin{bmatrix} 1.92 - 4.73 \\ (-0.52) - 0.86 \\ (-0.04) - 0.29 \end{bmatrix} \quad (18)$$

The behavior of the *clustering coefficients* at the three regimes are mentioned in the following. In the *hierarchical regime*, the range of α was $0.10 < \alpha < 0.86$, and in the *strong scale free regime* α decreases as N_C increases with $0.86 > \alpha > 0.5$, whereas, in the *network breakdown regime*, the values of α becomes random and sometimes negative showing an almost *random sparse network topology* (Fig. 2 middle row panels). On the other hand, the results of neighborhood connectivity $C_N(k)$, which obeyed power law behavior, showed two distinct behaviors and their transition driven by *hubs' activities* (Fig. 2 lowest row panels). First, observation of *assortativity* in the network topology when the knockout of *hubs* range is $0 < N_C < 100$ ($k \geq 85$), where, the values of exponent β are in the range $-0.04 > \beta > 0.1$. In this case, significant role of *hubs* showed up in the network making densely connection among them, the phenomenon which is termed as *rich-club formation*, where, apart from modular organization, few *hubs* start working together probably to control the network [16], to provide functional diversity in the network [42] and to minimize energy cost in the network organization [43]. Hence, removing one or few such *hubs* becomes lethal/toxic to the network [18]. In such situation, the network carries the characteristics of *scale free network* with central or negative *rich-club topology* [42]. Further calculation of *rich club coefficients* (ϕ) and *normalized rich club coefficients* (ϕ_{Norm}) of these knockout networks using eqs. (6) and (7) in Methods showed the evidence of *rich club* formation in the higher nodes in these networks corresponded to intermediate degree nodes ($19 \leq k \leq 107$) in PCa network (Fig. 3). Second, observation of *disassortativity* in the network topology when the knockout hubs range was in between $100 < N_C < 1000$, where, the range of β was in between $0.01 < \beta < 0.29$ [30]. In this case, the *scale*

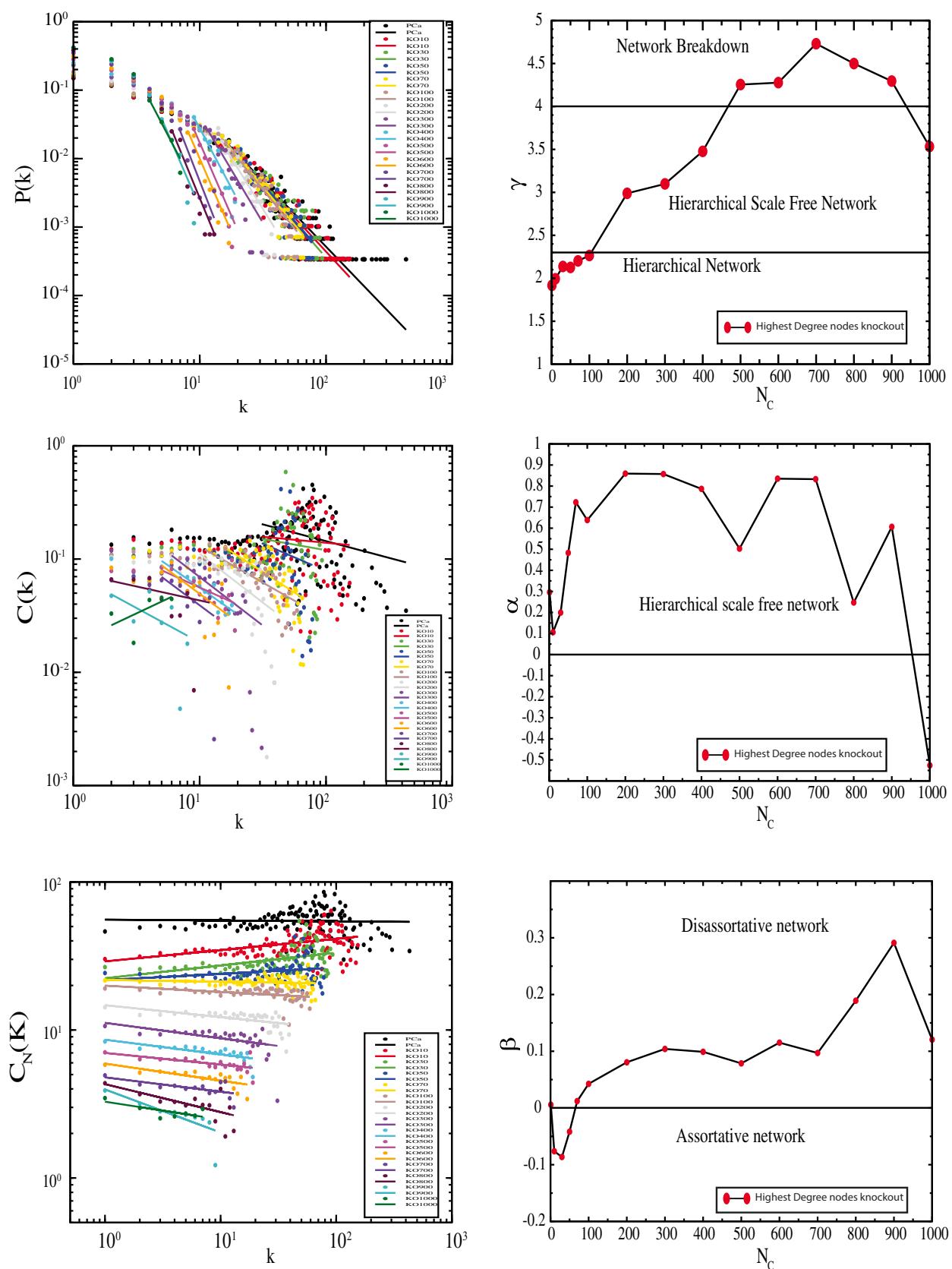


Fig. 2. PCa PPI network and sub-networks followed hierarchical-scale free topologies.

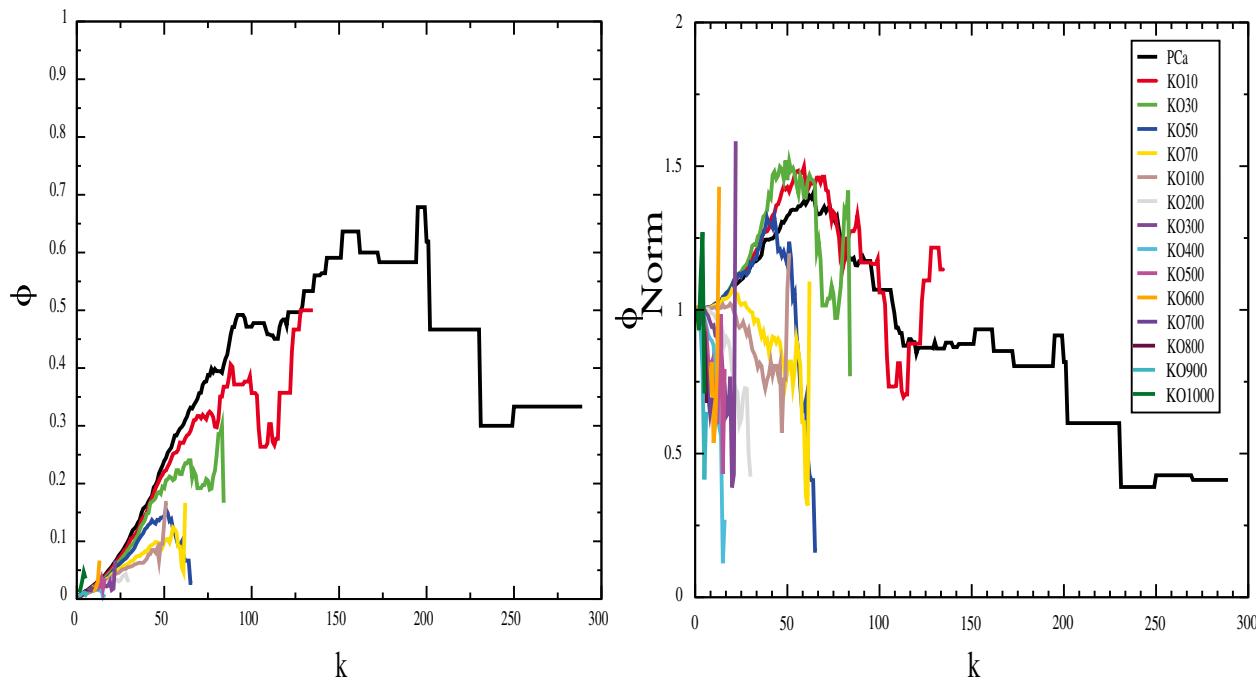


Fig. 3. Rich club analysis of PCa PPI network and sub networks.

free topology lost *rich-club* signature, where, the network loses functional diversity and energy cost in network organization is increased [42,43].

Then, we studied the change in the information processing in the network due to knockout of *hubs* from the PCa network measured by centrality parameters, namely, *closeness* C_C , *betweenness* C_B , *eigenvector* C_E and *subgraph* C_S centralities (see Methods), and found that these centrality parameters obeyed power law, $C_C \sim k^\epsilon$, $C_B \sim k^\eta$, $C_E \sim k^\delta$ and $C_S \sim k^\zeta$ respectively, where, ϵ , η , δ and ζ were the corresponding exponents (Fig. 4). The results of the knockout experiment with $0 < N_C < 1000$ is summarized in Eq. (19). The values of ϵ , η , δ and ζ were calculated by fitting the respective data points with the corresponding power law equations where the goodness of fit ranges from $r^2 = 93\% - 98\%$. Since the values of C_C increases as k increases (Fig. 4 first row left panel), it showed that the larger *hubs* were closer to the other nodes in the network indicating closer cross-talks of the *hubs* with other nodes enhancing fast information propagation among the nodes. Further, we also found that as the number of knockout *hubs* N_C increases, ϵ was increased allowing to increase in C_C values (Fig. 4 first row right panel). Hence, as N_C increases ($0 < N_C < 500$) faster the information propagation in the network. Then for $N_C > 500$ information propagation became slow and decreased drastically ($N_C > 700$) which might indicate breakdown of network organization. Again, C_B with exponent η also increases as k increases indicating *hubs* had the capability of central control ability via fast signal processing (Fig. 4 second upper row left panel). In the knockout experiment, removal of *hubs*, first, leads to the decrease in η as N_C increases ($N_C < 50$), then η increases monotonically with N_C ($50 < N_C < 150$), followed by a steady decrease in η with N_C ($150 < N_C < 500$) (Fig. 4 second upper row right panel). Further increase in N_C ($N_C > 500$), η fluctuates almost random. However, the scenario was quite different in the case of δ with respect to N_C , where, δ first increases as N_C increases ($0 < N_C < 50$), then decreases monotonically with N_C ($50 < N_C < 100$) followed by maintaining constant with N_C ($100 < N_C < 500$), after which δ became nearly random. Similar behavior was also observed for C_S with respect to k as well as ζ with respect to N_C (Fig. 4 lowest row panels). Since the values of these exponents were positive, hence, leading *hubs* (large k nodes)

had large values of respective centrality values indicating *hubs* were key nodes for fast information processing in the network which includes inter-modular, intra-modular, local and global signal propagations.

$$\begin{bmatrix} C_C \\ C_B \\ C_E \\ C_S \end{bmatrix} \sim \begin{bmatrix} k^\epsilon \\ k^\eta \\ k^\delta \\ k^\zeta \end{bmatrix}; \begin{bmatrix} \epsilon \\ \eta \\ \delta \\ \zeta \end{bmatrix} \rightarrow \begin{bmatrix} 0.09 - 0.13 \\ 1.15 - 1.72 \\ 0.99 - 1.73 \\ 0.85 - 2.77 \end{bmatrix} \quad (19)$$

The knockout of *hubs* experiment clearly showed the significant change of network topology driven by *hubs* in the network. The observed clear transition from *hierarchical* to *scale free* properties and vice versa was due to the dominance of activities of *modules* over *hubs* in the network and vice versa. However, both *modules* and *hubs* probably might take up constructive responsibilities to maintain network stability and organization and excess removal of *hubs* from the network or if there is no leader(s) in the system, the network will not function or may break down.

3.2. Hubs perturbation compromise self-organization in PCa network

Hubs perturbation is quite sensitive to the network which could be lethal to the network system, but the magnitude of lethality injected into the system network is dependent on the network topology. For example, *hubs* perturbation in scale free network may leads to network breakdown [5,18]. So, we performed comparative knockout analyses on the PCa network in two ways, first by randomly deleting the nodes from the PCa network, and, second by sequentially removing the leading *hubs* from the network. Then, we analyzed the network properties by calculating *average clustering coefficients* ($C(k)$), *modularity* (Q), *local community paradigm correlation coefficient* ($LCP - Corr$) and *Hamiltonian energy* (H) (H was calculated with $\gamma = 0.5$) of the PCa network and the sub-networks after knockouts of the nodes using the eqs. (4), (13), (14), and (15) respectively. The behavior of *average clustering coefficient* $C(k)$ as a function of N_C indicated that systematic removal of leading *hubs* in between $N_C \rightarrow [0 - 1000]$ showed significant change with respect to *hubs* removal where it monotonically decreases as N_C increases (Fig. 5 upper left panel). However, where N_C nodes are randomly removed from the network did not show significant change in

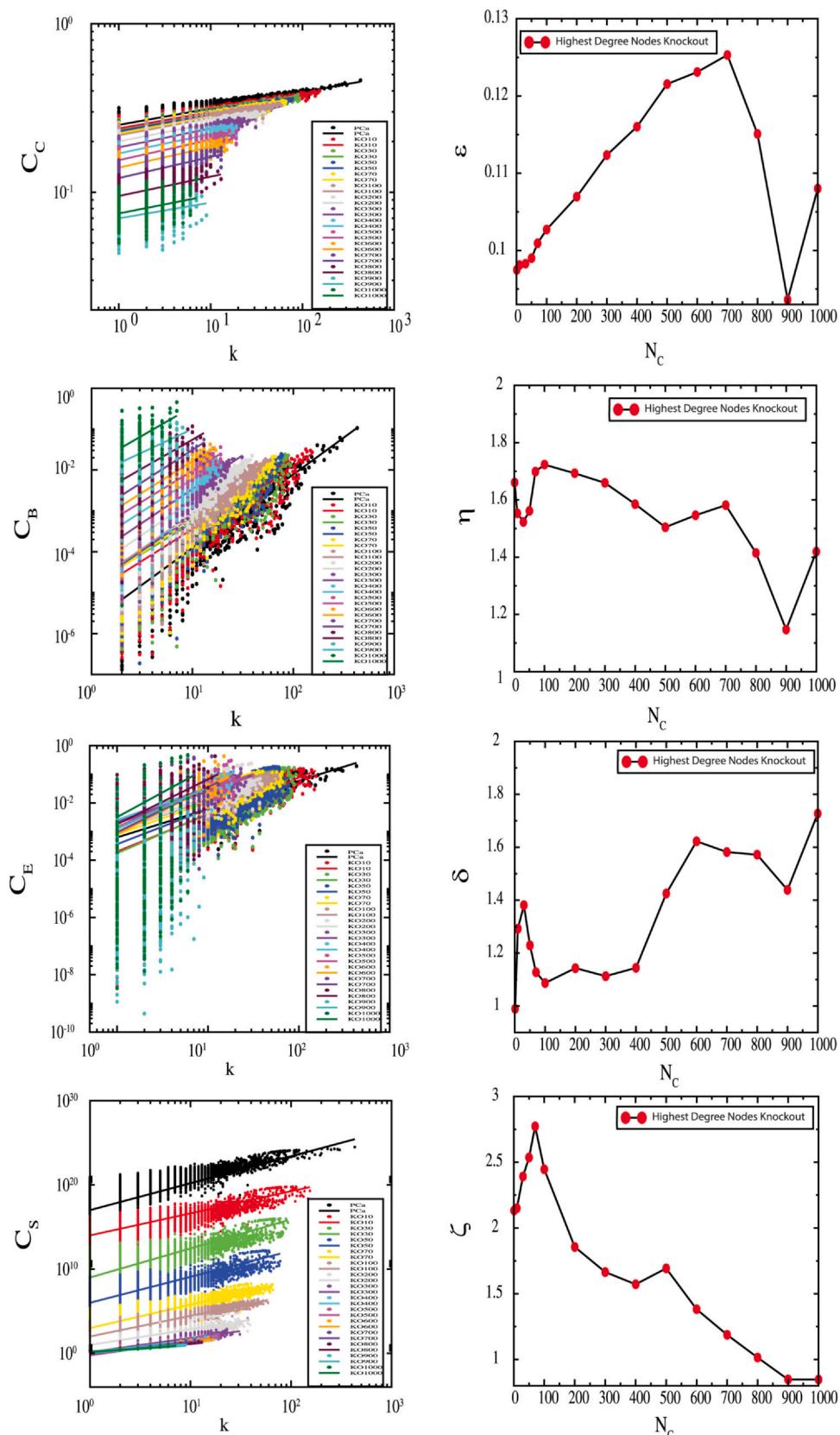


Fig. 4. High degree hubs are important for effective Signal propagation.

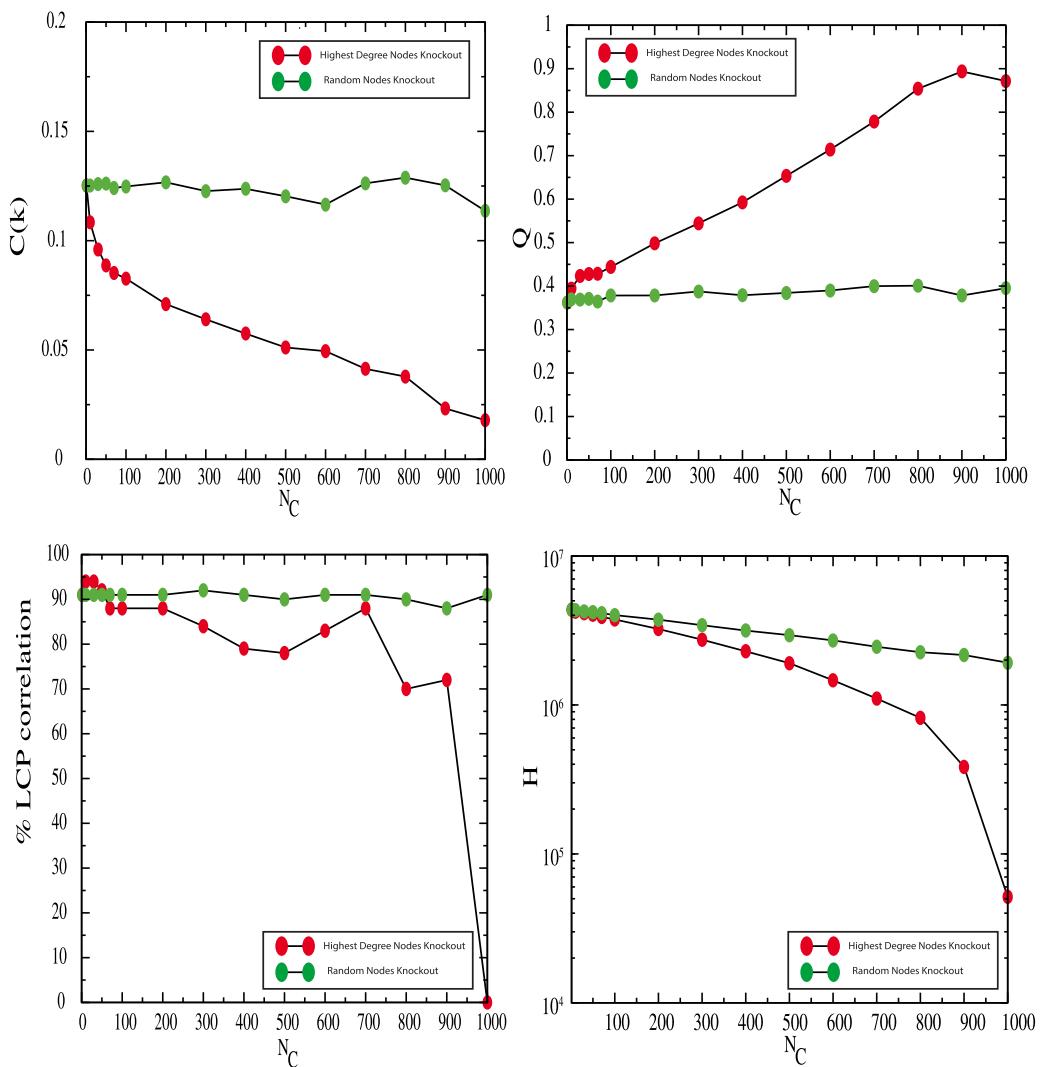


Fig. 5. High degree hubs are important for network stability and integrity.

$C(k)$ with respect to N_C . This indicated that significant role of *hubs* in maintaining network clustering and density. Then, *modularity* Q as a function of N_C showed similar behavior for random nodes removal process from the network, whereas, for systematic *hubs* removal process, Q increases as N_C increases (Fig. 5 upper right panel), showing an increase in intra-modular connections but decrease in inter-modular connections [15]. Hence, *hub* nodes took more responsibility in intra as well as inter modular organization in the PCa network. Next, we also found similar nature of % of *LCP correlation* which did not changed significantly, and fluctuates roughly around 90%, with respect to N_C for random nodes removal experiment (Fig. 5 lowest left panel), whereas, it drastically decreased from 95% to down 0% as N_C was increased in systematic removal of leading *hubs*. These results revealed that *hubs* again play significant role in maintaining network compactness. We, then, analyzed the energy distribution measured by *Hamiltonian energy function* (H) in the networks as a function of N_C (Fig. 5 lowest right panel) for both cases of knockout experiments. It was found that H decreased as a function of N_C much faster in the case of systematic removal of leading *hubs* knockout process than the random removal of nodes case. Thus, *hubs* exhibit a major role in energy distribution in the network for maintaining network organization/reorganization.

From the random and systematic knockout experiments on PCa network we can deduce that it is *hubs* which take significantly major role in maintaining network stability, optimization of signal

propagation in it and network organization/reorganization. Generally in *hierarchical networks*, *hubs* are distributed at the level of the modules and are specially classified as of *kinless*, *connector* and *modular hubs* [17], and are very sensitive to the network perturbation. They serve as the linkers of intra and inter modular nodes, energy distributors in the network and many other roles locally as well as globally. Removal of such *hubs* from the network may be lethal to the network, or may lead to network topological transformation and even cause network breakdown.

3.3. Kinless hubs are key to the PCa network integrity

Functional classification of the nodes in PCa PPI network was performed through the calculation of the *within module degree (z score)*, Z_i , and the corresponding *participation coefficient*, P_b , of the constituting nodes of the networks [17]. The values of Z_i and their corresponding P_i of the constituting nodes of PCa PPI network and each sub-network from leading *hubs* N_C ($0 < N_C < 1000$) systematic knockouts were calculated using eqs. (16) and (17) as described in *Methods*. The analysis of the calculated Z_i and P_i of PCa and resulting sub-networks showed that the *kinless* (R7 in Fig. 6 upper middle panel) and *connector* (R6 in Fig. 6 upper middle panel) *hubs* were maximum in the primary PCa network as well as in the networks after knockout of leading *hubs* $N_C \rightarrow [300 - 400]$ and $N_C \rightarrow [600,700]$ respectively, whereas, the

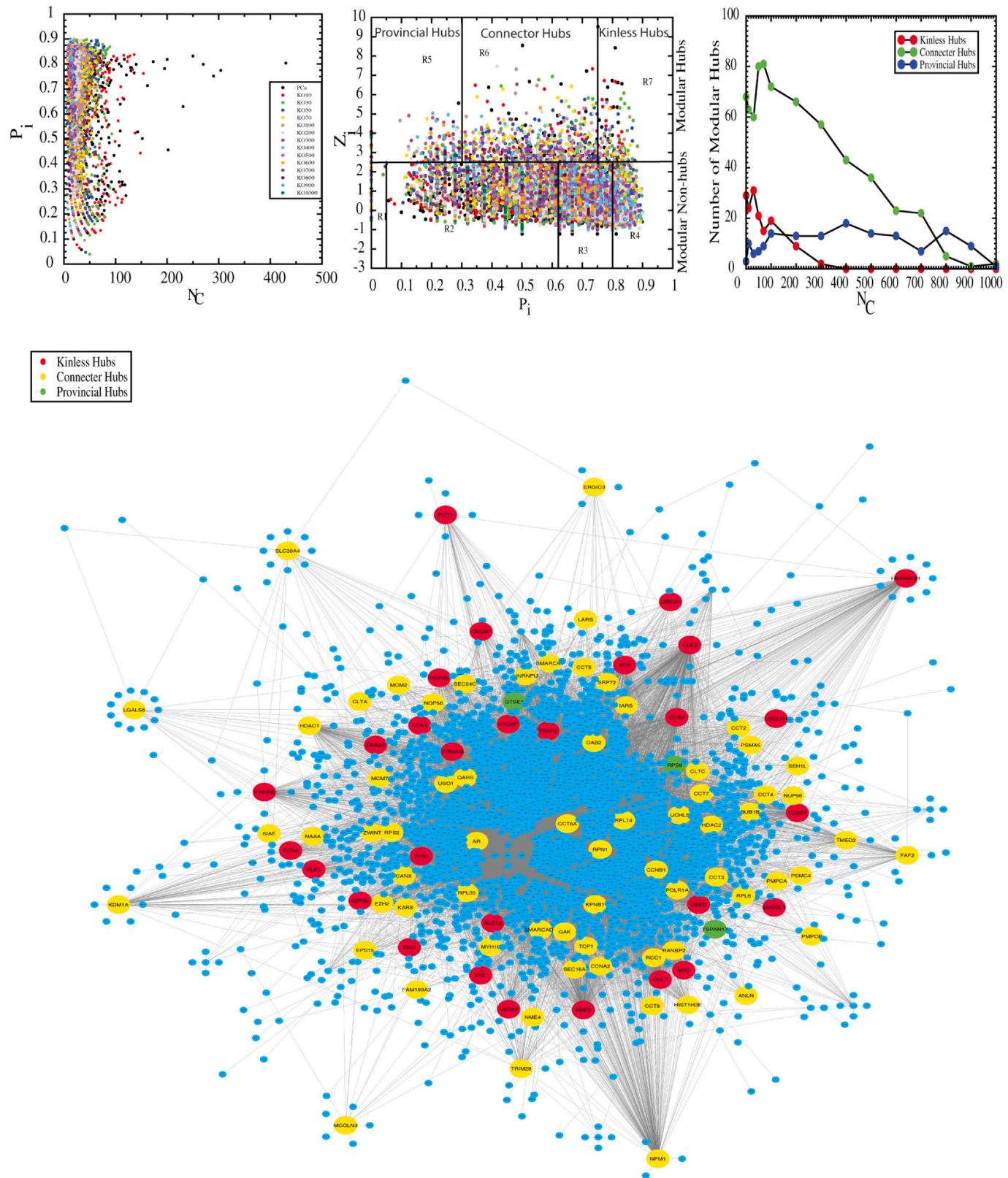


Fig. 6. Classification of modular hubs in PCa PPI network.

provincial (R5 in Fig. 6 upper middle panel) hubs remained almost same throughout all calculations of the networks. These results indicated that *kinless and connector hubs* were key linkers of the intra-nodes in each module as well as inter-nodes among the constituting modules in the PCa network. Further, these *kinless hubs* were sensitive to the change in

the network topology due to removal of leading *hubs* N_C and performed almost similar responsibility in all the networks, primary PCa PPI network as well as resulting networks after knockout of N_C hubs. Hence, *kinless hubs* were accountable for local as well as global network integration to maintain network stabilization, optimal signal processing

Table 1
Modular Hubs of PCa PPI network.

S.No.	Kinless hub	Degree (k)	S.No.	Connector hubs	Degree (k)	S. No.	Connector hubs	Degree (k)	S. No.	Peripheral hubs	Degree (k)
1.	CUL3	430	1.	NPM1	231	39.	NUP98	44	1.	RPS8	120
2.	RNF2	304	2.	HNRNPU	202	40.	PSMA5	42	2.	GTSE1	37
3.	TP53	290	3.	HDAC1	152	41.	QARS	41	3.	TSPAN17	13
4.	CUL7	270	4.	RPL35	143	42.	SEC24C	38			
5.	CDK2	250	5.	NOP56	118	43.	MYH10	38			
6.	VCP	200	6.	RPL6	113	44.	SLC39A4	37			
7.	MYC	195	7.	SRP72	113	45.	MCM2	37			
8.	HSP90AB	173	8.	RPS2	108	46.	ZWINT	37			
9.	VHL	140	9.	RPL14	107	47.	BUB1B	37			
10.	UBE2I	136	10.	EZH2	91	48.	CCNA2	37			
11.	SUZ12	130	11.	CLTC	87	49.	IARS	35			
12.	YWHAQ	129	12.	HDAC2	87	50.	EPS15	34			
13.	TUBG1	121	13.	AR	86	51.	LGALS8	31			
14.	CDK1	117	14.	CCT3	84	52.	PSMC4	31			
15.	HSPA5	111	15.	TRIM28	79	53.	SEH1L	30			
16.	HSPA8	101	16.	HIST1H3E	74	54.	GAK	29			
17.	YWHAE	96	17.	KPNB1	73	55.	KARS	29			
18.	C1QBP	92	18.	RANBP2	72	56.	ANLN	27			
19.	ADRB2	84	19.	KDM1A	72	57.	CLTA	26			
20.	PLK1	69	20.	FAP2	67	58.	LARS	26			
21.	UBA52	64	21.	RPN1	67	59.	POLR1A	24			
22.	MAD2L1	61	22.	CCT4	66	60.	DAB2	23			
23.	TRAFA2	58	23.	CANX	64	61.	FAM189A2	21			
24.	CD2AP	55	24.	CCT2	63	62.	NME4	21			
25.	POT1	55	25.	SMARCA4	63	63.	ERGIC3	20			
26.	CDK4	51	26.	TMED2	62	64.	PMPCB	20			
27.	BSG	47	27.	RCC1	56	65.	MCOLN3	19			
28.	UBQLN4	40	28.	SMARCAD1	56	66.	PMPCA	19			
29.	UBE2N	37	29.	CCT7	55	67.	NAAA	17			
			30.	CCT6A	54	68.	SLAE	14			
			31.	TCP1	54						
			32.	CCT8	51						
			33.	USO1	50						
			34.	MCM7	49						
			35.	SEC16A	47						
			36.	UCHL5	47						
			37.	CCNB1	46						
			38.	CCT5	46						

and self-organization. This result is in accordance with behavior of transition of the network from *hierarchical* (more compact clustered one) to *scale free topology* (comparatively less compact where role of *hub* (s) is significant), and then to a highly sparse network with decreasing clustering capacity of the nodes and increasing the modularity as in Fig. 5, thereby, loosening the connections among the modules ultimately affecting the overall topology of the network.

We then, estimated the number of these modular *hubs* in leading *hubs* knockout experiment and their role in maintaining network integrity (Fig. 6 upper right panel). The results showed that the fastest decrease of the number of *kinless hubs* (n_{kh}) as N_C increases, where, $n_{kh} \rightarrow 0$ as $N_C \rightarrow 500$ which was the beginning of breakdown of the PCa network. Thus, *kinless hubs* removal caused fast PCa network breakdown. Next more responsible *hubs* were *connector hubs* as evident from the results (Table 1). Hence, *kinless* (mainly) and *connector hubs* are key to PCa network integrity, and attack on them might be lethal to the network. The presentation of these modular *hubs* in the PCa network is shown in Fig. 6 lower panel.

3.4. Fisher's exact test: Top 100 hubs show maximum lethality

We carried out an enrichment analysis of the essential genes among the sets of highest degree nodes in PCa network by applying *Fisher's exact test* [40] to identify the essential *hubs* which may cause lethal to the network upon removal. We did this test to find out the % of essential *hubs* (genes) for every N_C leading *hubs* within $0 \geq N_C \geq 1000$ against the total of 7,168 essential genes reported from a set of human 21,556 genes [39] of the PCa network (Fig. 7). The results showed that in the group of top highest degree 100 nodes 90% are essential genes,

indicating a highest enrichment among other sets of highest degree nodes with lowest p – value and highest *ODDs ratio* value (Fig. 7).

The obtained results show that among the highest degree *hubs*, the first top 100 *hubs* were found to be the most essential genes which were sensitive to the perturbation induced in the network. Interestingly, it was also found that the number of *kinless* and *connector hubs* were maximum within the top 100 *hubs* (Fig. 6 upper row right panel). Hence, the removal of the first top 100 *hubs* will cause maximum lethality in the PCa network which may cause transition of network structure and properties.

4. Discussion

Functional properties of biological molecular systems are generally rendered by interactions between proteins and such organized PPI networks manifest different topologies,[1–3,15]. These PPI networks exhibit either scale free topology where high degree nodes, *hubs* play central role in signal propagation and maintain the stability of the network; or *hierarchical scale free topology* with characteristic system level modular organizations where modules represent different functional/pathway modules, [2,3,15]. The manifestation of the *hierarchical scale free topology* in PCa PPI network indicated that high degree nodes, *hubs* coordinate the specific modular functions/pathways, and establish connections between these modules maintaining the system integrity [16,41]. In such networks, interplay of *hubs* and modules are prominent, and generally these *hubs* are multifunctional. One important finding in this study of PCa PPI network is the observation of the phenomenon of *rich club* formation in the networks formed after removal of the top 50 highest degree hubs ($k \geq 85$), indicated by

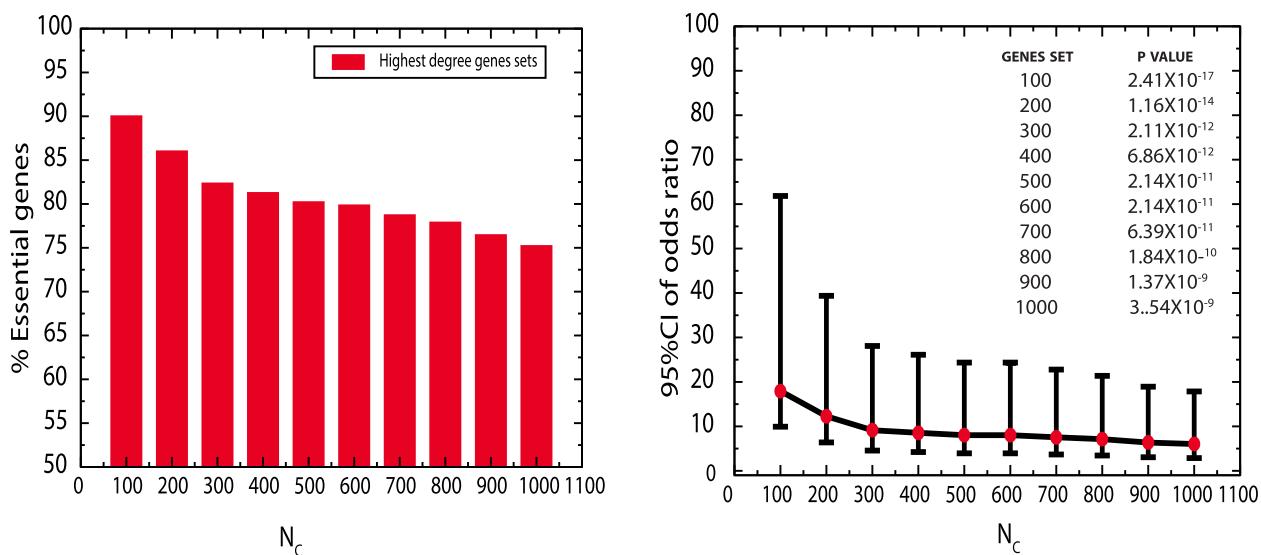


Fig. 7. Essentiality of genes are highest among the top 100 highest degree hubs of PCa PPI network.

signature of network *assortativity* [6,7,30]) and then reverting back to disassortative nature on further removal of the hubs (Fig. 2). This indicates the dominance of the largest hubs in network integrity maintaining the modular organization of the hierarchical PCa PPI network and their deletion induces transformation of the network to a more *assortative* network where the higher degree *hubs* [7, 16] establish strong connections among them making the network more resilient towards random attacks but at the same time it increases their vulnerability to targeted attacks on the *hubs* [3,4,9]. Vulnerability of these hubs and the network integrity were observed with the systematic removal of the high degree nodes from the network disturbing the self-organization of the system. This led to topological transition of a more modular hierarchical network with strong communications between different functions or pathways in the system to, first *scale free topology* then to sparse random and disintegrated system (Fig. 2). During this transition, the information processing efficiency and cost effectiveness of signal propagation in the networks, characterized by centralities (C_B , C_C , C_E and C_S) measurements, were significantly reduced due to removal of the highest degree *hubs* (Fig. 4)[44]. The changes in the centrality measures on deletion of the higher degree nodes in the PCa PPI network indicates the decrease in average path length between any pairs of the nodes in the networks reducing them to *small world* network topology and decreasing participation of the nodes in the network integration encouraging isolation of nodes forming smaller groups [45,46,47,48]. The compactness (measured by LCP-correlation), clustering of the nodes (indicated by network topological parameters) and energy distributions (attributed by Hamiltonian energy, H) in the network are relatively decreased due to knockout of significant number of the highest degree hubs from the original network (Fig. 5). Thus, perturbation of highest degree nodes especially the top 100 hubs transform the PCa network from a compact, highly clusterd, network to more sparse ones with loosely bound nodes with weaker communications between the modules (Fig. 5) [47,48]. Further, the sytemic transition from a *disassortative* to *assortative* nature of the network on removal of the largest hubs, ($k \geq 85$) also indicates the importance of intermediate degree nodes ($19 \leq k \leq 107$) (Fig. 3) [41], specially *modular kinless and connector hubs* (Table 1), in connecting the high degree and lower degree nodes in establishing a strong PCa network integrity and stability. Then, excess removal of high degree hubs led to the network breakdown regime losing network integrity completely. In this case, the network become quite sparse and lost almost all important network topological properties.

The transition from a highly organized structure to sparse random

and disintegrated system was correlated to losing the greatest number of *modular kinless* and *connector hubs* which were responsible for linking different functional modules and pathways (Fig. 6) [17,49,50]. Some *modular kinless hubs* (e.g., c-MYC and p53) and *connector hubs* (Table 1) (e.g., Androgen Receptor, AR) of the PCa PPI network play important roles in prostate tumour development and progression [51]. Gene amplification of the cellular proto-oncogene MYC increases cell proliferation, survival and metastasis specially in androgen-deprived PCa patients [51]. Genome wide association studies also reported several susceptibility loci in *MYCgene* increasing the PCa risk in people with certain mutations in these gene loci [52]. The tumour suppressor gene p53 is the most common gene found to be mutated and lose its tumour suppressor function in various cancers including PCa [51,53]. P53 is involved tumour suppression in cells by inducing cell cycle arrest, DNA repair, apoptosis etc. and the loss of its functionality in cancers is either due to genetic mutation or deletion [53]. Mutated p53 protein is reported in high grade prostate tumours with highly metastatic characteristics and advanced stages [51]. Transition from an androgen-dependent to androgen-independent prostate tumour in prostate tumour tissues is also associated with mutations in this tumour suppressor [51]. The androgen receptor overexpression increasing its transcriptional activity enhances the androgen signalling and dependency in PCa inducing cell growth and proliferation [51–53]. Mutations in AR compelling the receptor to remain stimulated even in lower level of androgen and AR gene amplifications enhancing its expression are associated with resistance to androgen deprivation therapy and found mostly in hormone refractory prostate cancers [51–53].

In addition, among the 19 novel key regulators of PCa PPI network, CUL7, HSPA5 are *modular kinless hubs* and CCT4, RANBP2 and RPL6, *modular connector hubs* [41]]. These proteins are associated to different cancers inducing cell growth and proliferation, inhibiting apoptosis, and have been reported as prognostic markers in several cancers (including PCa) [41]]. Furthermore, among the key regulators in PCa, 17 regulators (ACCT4, EIF3A, HSPA5, NOP2, RANBP2, RPL11, RPL15, RPL19, RPL23A, RPL3, RPL5, RPL6, RPLP0, RPS11, RPS8, RPSA and SNU13) are essential genes responsible for survival in human [,39,41]. Since the highest enrichment of essential genes was observed among the top 100 highest degree nodes, hubs (90%), largest number of kinless hubs (16 out of total 29) in PCa PPI network were also found among these top 100 hubs (Fig. 7). Therefore, the high degree nodes of PCa PPI network corresponded to majority of the *kinless hubs* and essential genes indicating their importance in the sustenance of the system and ultimately survival of the PCa cells [5,18]. Loss of such important *kinless*

and *connector hubs* from the PCa PPI network corresponding to the essential genes may induce a shift in the interactions between the proteins and ultimately disintegration of the functional/pathway modules rendering the PCa cells difficult to survive. Hence, *kinless hubs* are the most important hubs in PCa PPI network which could be potential target candidates for development of drugs against PCa. Mutations in such modular hubs and dysregulation of their expressions could alter the interactions between proteins affecting the multi protein complex formations and signalling pathways disturbing the dynamics of the system leading to tumour development in PCa and other related diseases [54]. Thus, these modular hubs could serve as the driver nodes of the PPI networks and perturbing them could induce changes in the network dynamics altering the phenotypic characteristic of the biological system [55].

5. Conclusion

We constructed PCa PPI network from the gene expression data of PCa patients and studied its topological properties to understand the role/s of the hubs in network regulation and organization. In the study, we found that PCa PPI network follow *hierarchical scale free topology*, with *disassortative* mixing of the nodes indicating that it does not follow *centrality lethality rule*, lacking central control of the hubs in the network organization. On systematic knock out of the highest degree nodes or *hubs* from the network, we observed a systemic transformation from a *hierarchical topology to scale free topology* which later became sparse and random network showing network breakdown on excessive removal of the leading hubs. This network topology transformation was associated with losing self-organization and compactness of the network with decrease in clustering and information processing among the nodes. Further, classification of the hubs into modular *kinless*, *connector* and peripheral hubs and on analysis on them revealed that losing of *kinless hubs*, which are most responsible for interlinking all the modules in the network, corresponded with maximum lethality on the network organization followed by *connector hubs*. Moreover, maximum number of *kinless hubs* was found to be essential genes responsible for survival to keep network stability and properties. Thus, we consider that the *kinless hub* genes can be good potential targets for drug designing and development against PCa.

Declaration of Competing Interest

None declared.

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