Chapter 7

Prediction of key regulators in ovarian cancer network with their roles

7.1 Introduction

Ovarian cancer (OC) is an assorted cancer that begins in an ovary. Most of these tumors are low malignant potential (or non-cancerous Malignant) ovarian tumors can metastasize (spread) to other parts of the body and can be fatal. In 2016, it was reported 22,280 will receive a new diagnosis of ovarian cancer and 14,240 women will die from ovarian cancer [331,332]. As the eighth-most common cause of death, OC is considered as the ‘silent killer’ due to lack of symptoms in its initial stages [331,332]. In the precedent few decades, genetically studies have retrieved some genetic alterations that are also crucial in the pathogenesis of ovarian cancer. The swift growth of next-generation sequencing technologies recently has allowed the possibility for identifying many somatic alterations (genetic) in OC. These somatic alterations can be assessed as the passengers, which on the other hand pose challenge in classifying any cancer [333]. Identification of molecular drivers
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associated with a specific cancer type/sub-type is crucial and at the same time important for understanding its heterogeneity to seek treatment. In recent studies, network based calculation have been implicated on multiple data sources to identify driver genes, including copy number variation, microRNA expression [334].

Epithelial ovarian cancers (EOC) remained the most lethal cancer among developed nations. From its various sub-types mEOC (mucinous epithelial ovarian cancers) represent approx 3% of EOC. It can be divided into the more common, type II (aggressive) and type I (slow-growing) cancers [335]. Low-grade i.e. type I is present in young women and have a high prevalence of KRAS and BRAF mutations, but low in relation to TP53 mutations (that characterize to type II). These prevalent mutations qualify as a favourable prognostic factor for type I EOC. While at the same time identifying MAPK mutations is useful in guiding clinical treatment [336]. The most of the new cases present with advanced stage of disease have initial treatment which consists of a cyto-reductive surgery and chemotherapy [337]. The most of the patients develop advanced EOC are the ones having pre clinical diminution after primary therapy. Though, long term cure resides in the patients exposed for multiple chemotherapeutic agents [338]. Thus the identification of malignant ascites is the most common consequence of EOC. It causes significant symptoms and impact on the patient’s life, more than ever in cases where women have regular ovarian cancer [339].

The current paradigm for studying OC revolves around the identification of critical regulators in the Transcriptional factor (TNF) networks present in OC cells. As these TNFs might be important therapeutic targets [340]. To understand the mechanism and predict the complex interaction within the complex biological network and how various fundamental functions are exhibited by the organization of the components between them. The large scale data from the omics have interestingly been used to map genes with specific diseases [341]. Net-
Network theory is been proposed for being an important approach to understanding topological properties and the dynamics of complex systems, to correlate to their functional modules [148]. Most of the existing networks fall into one of them, namely, scale-free, small world, random and hierarchical network [146]. Amongst them, hierarchical type of network is of special interest to biologists as it includes the appearance of modules and sparsely distributed hubs regulate the network [146]. The appearance of modules in this network type is of picky interest because they may correspond to independent functional components in the network obeying their own laws [146]. Therefore, we focus our study of ovarian cancer network constructed from experimentally identified ovarian cancer genes and their interactions to explore possible regulatory genes. We also aim at to understand its topological properties from which we try to predict important key regulators among which some are of fundamental importance, their activities and regulating mechanism.

7.2 Processing of data and network construction

The detailed workflow of the ovarian cancer network and analysis is given in Figure 7.1 and detail techniques are described below.

7.2.1 Acquisition of ovarian cancer data

We have integrated six highly cited resources for cancer in order to obtain list of ovarian cancer genes. The different resources focus on different aspects of cancer biology; 1. COSMIC database: it stores mutated genes information causing cancer, 2. Ovarian kaleidoscope database: It provides information of human genes related to their genetic and functional information, 3. Gene cards database: It provides information regarding the complex biological function, regulation of genes
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expressed in the ovary and their mutation and expression pattern. 4. Dragon database of ovarian cancer: It stores functional information on genes in ovarian cancer. 5. Curated ovarian database: it contains manually curated data for gene expression from meta-analysis of patients suffering from ovarian cancer. 6. OCGene database: contains the list of experimentally verified ovarian cancer-related genes. From the above assimilated repositories we have got 2000 genes. The protocol we followed in this process is a simple workflow stared with the mining of the list of genes (associated with ovarian cancer) from all of the six defined databases. These lists were subjected to CGI-Perl codes (developed locally) for the removal of duplication of both in terms of redundancy of names and aliases used for gene names. The method of removal involves pattern matching and searching globally in Gene card (http://www.genecards.com) database. This method

Figure 7.1: Schematic diagram of the workflow of the methods implemented in the study of ovarian cancer network.
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provided the information of unique 660 genes in csv format with their synonymic names. Now, this list of genes is further put into the manual curation followed by the agilent literature search, a plugin of Cytoscape to get the relevant literary background on each gene. Finally, from the whole process, we could able to arrive at the list of 600 genes out of 2000 unique genes. In order to construct primary network of expressed proteins we mapped these genes to UniProt (January, 2016) and got UniProt-ID, names and other functional information associated with them (600 genes).

7.2.2 Construction of Protein-Protein Interaction network

We followed the simple concept of one gene, one protein to build the primary PPI (Protein-Protein Interaction) network of ovarian cancer. The network was constructed using String database and verified and uploaded the file in Cytoscape for further literature verification. We used UniProt, OCgene database verification; this integrative and analytical effort done provided an efficient way to curate and verify experimentally, the PPIs. This gave us a swift exploration of interaction networks (i.e. Graph denoted by G) as it includes certain parameters that weight the reliability of given interaction (i.e. edge denoted by E), and also qualifying the function of any given protein with their interacting partners (i.e. node denoted by V). Here, we cross-checked this with five well studied candidate gene Tp53, BRCA1/2, EGFR, AKT where InAct database when used gave thousands of interaction for them. By combining all the information finally we got a network of 4818 nodes having 16320 connections between them.

7.2.3 Characterization of topological properties of networks

The structural properties of complex networks can be characterized by the behaviours of the topological parameters for detail see chapter 2 section 2.4.1.
7.2.4 Tracking of genes and Knock out experiment

To access the regulation of network we first tried to find out the most influential nodes within the network. This tracing of genes (up to motif level) was done purely on the appearance of the respective genes in various sub modules obtained from the clustering. Then these genes were used to get the picture of change in the organization of the network in their absence. We successively removed all these key genes from the constructed complete network, and calculated the topological properties of the modified network to characterize regulating capabilities of these genes by measuring the degree of structural change due to their absence. We further repeated the knock out experiment at different level of network organization to understand the role of these genes in the network. Every time we calculate the topological properties using Network analyzer, a plug-in in Cytoscape version 3.3.2, while for eigen value calculation we used CytoNCA another plug-in in Cytoscape for topological properties calculation. The result from this plug-in was also helpful in cross checking Network Analyzer.

7.2.5 Community detection/finding: Leading eigenvector method

To perceive and describe the modular nature and their properties in the hierarchical network is important in defining the predicting about the activities of network at various levels of hierarchy and also access the organizing principle of the network in the study. There are many methods to detect communities, of them leading eigen vector method (LEV) gave the promising reliability (in our case) as it calculates the eigenvalue for each link, giving importance to links not nodes. Therefore, with this believe we used LEV detection method in ‘R’ (programming language) from package ‘igraph’. We used this technique to detect modules from complete network, sub-modules from modules at each level of organization, and
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so on until we get only motifs (i.e. 3 nodes and 3 edges). In the whole process, we stuck to the criterion of identifying any sub-module as a community by the presence of at least one motif (defined by $G(3, 3)$).

### 7.2.6 Estimation of network compactness: LCP-DP approach

The LCP-decomposition-plot (LCP-DP) provides one way of portrayal of various topological properties of a network in 2D the space of common neighbors (CN) index of interacting nodes and local community links (LCL) of each pair of interacting nodes in the network. It comprises of information of number, size, and firmness of modules in a network, which can be used as an indicator of self-organization in the network [342]. Mathematically, the CN index between two nodes $x$ and $y$ can be obtained from the measure of overlapping between their sets of first-node-neighbors $S(x)$ and $S(y)$ given by, $CN = S(x) \cap S(y)$. The interaction of the two nodes could possibly take place if there is a significant amount of overlapping between the sets $S(x)$ and $S(y)$ (large value of CN). The increase in CN could be an indication of an increase in compactness in the network, which could provide faster information processing in the network. Further, the LCL between the two nodes $x$ and $y$, whose upper bound is defined by, $\max(LCL) = \frac{1}{2}CN(CN - 1)$, is the number of internal links in the local-community (LC). The two nodes are likely to be linked together if CN of these two nodes are members of LC [342]. LCP-DP generally found to have a linear dependence between CN and $\sqrt{LCL}$.

The LCP correlation (LCP-corr) is the Pearson correlation co-efficient of CN and LCL defined by $LCP-corr = \frac{\text{cov}(CN, LCL)}{\sigma_{CN}\sigma_{LCL}}$ with $CN > 1$, where $\text{cov}(CN, LCL)$ is the covariance between CN and LCL, $\sigma_{CN}$ and $\sigma_{LCL}$ are standard deviations of CN and LCL, respectively.
Table 7.1: List of experimentally verified genes [343] involved in ovarian cancer which we used for our cancer network construction. The colored (red) genes are FKR s gene which is very important in ovarian cancer regulation.

7.2.7 Distribution of energy in the network: Hamiltonian energy calculation

The energy used in the organization of a network at a certain level/state can be measured by using Hamiltonian energy (HE) of the network at that level/state within the formalism of Constant Potts Model [344, 345]. HE provides energy distribution not only at the global level of a network, but also at modular level, which is in the self-organization of the system. HE of a network or module or sub-module can be calculated by,

$$H^{[c]} = -\sum_{c} \left[ e_{c} - \gamma n_{c}^{2} \right]$$  \hspace{1cm} (7.1)

where $e_{c}$ and $n_{c}$ are the number of edges and nodes in a community ’$c$’ and $\gamma$ is the resolution parameter acting as an edge density threshold. Generally, we have $\gamma \leq \frac{1}{(n_{c})^{2}}$. 

1. ATM  11. AURKA  21. MAPK1  31. RB1  41. CD44  51. HIF1A  61. BRCA2
2. MSH3  12. AKT1  22. ESR1  32. MDM4  42. HRAS  52. CDK4  62. FASLG
3. MSH6  13. AXIN2  23. ERBB3  33. BRAD1  43. DNMT1  53. PARK2  63. EGF
4. MYC  14. NF1  24. ABCB1  34. GSTP1  44. BRIC5  54. MKI67  64. CDKN2A
5. CDH1  15. ERCC1  25. KRAS  35. CDK2  45. CDKN1A  55. SOD2  65. PPARG
6. ATR  16. MAP2K4  26. AR  36. ERBB2  46. BCL2  56. TERT  66. PRKCI
7. MLH1  17. FN1  27. EPCAM  37. NCOA3  47. MCAM  57. INSR  67. CCNE1
8. NBN  18. FANCDD2  28. BRAC1  38. APC  48. ERCC3  58. IGF1R  68. TNF
9. STAT3  19. MVP  29. SMAD4  39. ITGB3  49. CCND1  59. CDKN1B  69. CTSD
10. TP53  20. MAP3K1  30. PIK3CA  40. RAD51  50. E2F2  60. BCL2L1  70. MMP2
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7.3 Results and discussion

7.3.1 Ovarian cancer network follows hierarchical scale free features

The ovarian cancer network, which is the proposed complex regulatory network to be studied, is constructed from the experimentally verified seventy genes (see section 7.2 and Table 7.1). The topological properties of this network, namely, probability of degree distribution \( P(k) \), clustering co-efficient \( C(k) \) and neighborhood connectivity \( C_N(k) \) obey power law behaviours as a function of degree \( k \) (Figure 7.4 first row against Level 0). The power law fits on the data sets of the topological variables of the network are done and verified following a standard statistical fitting procedure proposed by Clauset et al. [169], where, all statistical p-values for all data sets, calculated against 2500 random samplings, are found to be larger than a critical value 0.1, and goodness of fits is found to be less than and equal to 0.33 (Figure 7.4 first row blue fitting line). The values of the exponents are obtained from the power law fittings. The results for the complete network are summarized as follows,

\[
\Gamma(k) = \begin{bmatrix}
\Gamma_1 \\
\Gamma_2 \\
\Gamma_3 \\
\end{bmatrix} = \begin{bmatrix}
P \\
C \\
C_N \\
\end{bmatrix} = \begin{bmatrix}
k^{-\gamma} \\
k^{-\alpha} \\
k^{-\beta} \\
\end{bmatrix} = \begin{bmatrix}
\gamma \\
\alpha \\
\beta \\
\end{bmatrix} \to \begin{bmatrix}
2.16 \\
0.9 \\
0.67 \\
\end{bmatrix} \\
\]

(7.2)

These topological properties of the ovarian cancer network are very close to ideal hierarchical properties of the network whose values of the exponents are, \( \gamma \approx 2.26 \) (mean-field theoretical value) [146], \( \alpha = 1 \) [155,156] and \( \beta = 0.5 \) [124]. This topological function \( \Gamma_i; \ i = 1,2,3 \) satisfy the Mandelbrot’s classical definition of fractal [327], which is defined by the following self-affine process of any scale factor \( \lambda \),
\[ \frac{\Gamma_i(\lambda k)}{\Gamma_i(k)} = \lambda^p; \quad D = \begin{bmatrix} D_1 \\ D_2 \\ D_3 \end{bmatrix} = \begin{bmatrix} -\gamma \\ -\alpha \\ -\beta \end{bmatrix} \]

where, \( D_i \) corresponds to fractal dimension of \( i^{th} \) topological parameter.

Hence the ovarian cancer network follows the hierarchical scale free or fractal features. The negative values in fractal dimension indicate the enrich randomness in the network organization with sample variability \[327\]. Since the \( C_N(k) \) has a negative power in \( k \) i.e. \( \beta = 0.67 \), the ovarian cancer network exhibits disassortivity nature which means that the rich-club formation of a large number of the leading hubs in the network is unlikely \[124\].

The centrality measurement, namely, betweenness \( C_B \), closeness \( C_C \), and eigen-value centrality \( C_E \), characterize the importance of the hubs, their regulating mechanisms (see chapter 2 section 2.4), and obey the following power law behaviours (Figure 7.4 first row),

\[ \Lambda(k) = \begin{bmatrix} \Lambda_1 \\ \Lambda_2 \\ \Lambda_3 \end{bmatrix} = \begin{bmatrix} C_B \\ C_C \\ C_E \end{bmatrix} = \begin{bmatrix} k^\epsilon \\ k^\eta \\ k^\delta \end{bmatrix} ; \quad \begin{bmatrix} \epsilon \\ \eta \\ \delta \end{bmatrix} \rightarrow \begin{bmatrix} 1.15 \\ 0.083 \\ 1.11 \end{bmatrix} \]

The power law natures of the three centrality measurements are again verified and confirmed using the Clauset \textit{et al.} procedure of statistical power law fitting, where, p-values are found to be more than 0.1 and goodness of fit also larger than 3.5. Since only few numbers of higher degree nodes have large centrality values (for all three centrality measurements), the number of most influencing hubs, which can regulate the ovarian network, is few. Hence, moderately low degree nodes (genes/proteins) dominate the network, and therefore, the regulation, functioning and organization of the network are done mostly by these low degree
genes/proteins. However, the sparsely distributed leading few hubs might take important roles in regulating as well as maintaining the network stability. Further, the power law behaviour of centrality measurements given by equation (7.4) follows the following self-affine process for any scale factor $c$,

$$
Q_N = \frac{1}{N^{1-c}}
$$

Figure 7.2: Identification of fundamental key regulators of ovarian cancer network. (A) Organization of the modules/sub-modules of the network. (B) Plots of $Q_N$ and LCP-corr as a function of network level. (C) Characterization of seventy leading hubs of the network by degree ($k$) distribution and identification of fundamental key regulators. Color codes are popularities of the leading hubs.
7.3.2 Identification of fundamental key regulators and properties

Since ovarian cancer network follows hierarchical scale free nature, the emergence of modules in the network is significant and therefore both these modules and sparsely distributed few leading hubs regulate and organize the network. The modular structure and their arrangement at various levels of organization are done following Newmann and Girvan’s standard community finding algorithm (see section 7.2) \[346\]. Using this algorithm, the ovarian cancer network is found to be hierarchically organized through five different levels of organization (Figure 7.2 A). The corresponding modularity \(Q_N\) and local community paradigm, LCP-correlation per node as a function of levels of organization are found to be decreased as one goes from top to down organization (Figure 7.2 B).

Depending on the degree of the nodes in the ovarian cancer network, the first seventy leading hubs are identified (Figure 7.2 C). The reasonable question is whether these hubs are actual target genes which regulate the network at a fundamental level. Hence, we propose to define fundamental key regulators (FKRs) as the genes/proteins which are deeply rooted from top to bottom of the network and vice versa, and serve as the backbone of the network organization (Figure 7.2 D).
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C). These FKR s may or may not necessarily be large leading hubs in the network, but randomly change their popularities at various levels of organization. Since the ovarian cancer network qualifies hierarchical characteristics, the removal of the leading hubs will not cause the network breakdown. However, the removal of FKR s from the network may cause maximum local and global perturbations in the network, specially at a deeper level of organization. Then the perturbations will propagate through various levels of organization’s bottom to top or top to

Figure 7.3: Tracing of fundamental key regulators of the network through different levels of the network.
bottom causing topological change in the network. Hence, we propose that these

Figure 7.4: Topological properties of the ovarian cancer network. (A) The behaviours of degree distributions \( P(k) \), clustering co-efficient \( C(k) \), neighborhood connectivity \( C_N(k) \), betweenness \( C_B(k) \), closeness \( C_C(k) \) and eigenvector \( C_E(k) \) measurements as a function of degree \( k \) for original and five FKR knock-out network at different levels of organization. (B) The changes in the exponents of the six topological parameters due to FKR knock-out experiment. (C) Energy distribution in the network quantified by Hamiltonian calculation as a function of network levels. (D) Changes in the network modules/sub-modules due to five FKR knock-out experiment. The dotted modules/sub-modules are the break-down modules/sub-modules.
FKRs could be key target genes of the ovarian cancer.

Following the definition of FKR, we could able to identify five FKRs, namely, AKT1, KRAS, EPCAM, CD44 and MCAM (Figure 7.2 C,7.3,7.5), which are key regulators/organizers of the ovarian cancer network. Surprisingly, the first eleven leading hubs are not found to be FKRs since they fail to reach till the deepest level of organization. Out of these five FKRs few are at low profile/popularity (CD44 and MCAM), but could able to regulate till the bottom level of organization. Further, these FKRs start separating from each other after level three; KRAS and EPCAM go together, AKT1 moves alone and CD44 and MCAM go together till motif (triangular type) level (Figure 7.3,7.5). These FKRs act as signal propagators from top to bottom and vice versa to maintain network stability and inherent properties.

To understand regulating capability of each of the five FKRs, we define a probability $P_y(x^{[s]})$ of a FKR $y$ to have a number of links/edges $x^{[s]}$ at level $s$ out of the total number of links/edges $E^{[s]}$ of the network/module/sub-module in which that FKR is accommodated, which is given by,

$$P_y(x^{[s]}) = \frac{x^{[s]}}{E^{[s]}} \quad \forall E^{[s]} \neq 0 \quad (7.6)$$

The calculated $P_y$s of all the five FKRs show increase in $P_y$ as one goes top to bottom direction (as $s$ increases), and found that $P_y \rightarrow 1$ as $s \rightarrow 5$ (Figure 7.5 lowest panels). This reveals that the regulating capability of each FKR becomes more prominent at deeper levels of organization. Further, the inherent regulating capability of each FKR $P_y^{[I]}$ can be approximately measured by calculating average over $P_y$ as in the following,

$$P_y^{[I]} = \frac{1}{M + 1} \sum_{s=0}^{M=5} P_y(x^{[s]}) \quad (7.7)$$
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The calculated values of $P_y^{[I]}$ shows that $P_{AKT1}^{[I]} > P_{K_RAS}^{[I]} > P_{EPCAM}^{[I]} > P_{CD44}^{[I]} > P_{MCAM}^{[I]}$. The inherent regulating capability of AKT1 is highest, and that of MCAM is lowest.

### 7.3.3 Local perturbations driven by key regulators

The knock-out experiment of five FKRs from the ovarian cancer network could able to highlight the local perturbations driven by these key regulators, and their effect on global network properties. The removal of these FKRs from the complete network does cause significant changes in the topological properties of the network (Figure 7.4 A first row), where, $\gamma$ and $\alpha$ change significantly in complete network level (Figure 7.4 B), whereas $\beta$ change slightly. Similarly, the changes in the exponents of centrality measurements ($\epsilon$, $\eta$ and $\delta$) also show significant (Figure 7.4 B). Since, all the five FKRs are present in a single module/sub-module upto third level of organization, we only consider that module/sub-module for the five FKRs knock-out experiments (Figure 7.4 second, third and fourth rows). It is evident from the changes in the exponents of topological parameters (Figure 7.4 B) that as one goes to deeper level i.e. top to down direction the network perturbation increases. After the third level, the removal of these FKRs almost breakdown the sub-modules present in the remaining deeper levels (Figure 7.4 D). This indicates that local perturbation caused by five FKRs together is maximum at deeper levels, and propagates the perturbation through other levels from bottom to top.

We then calculate Hamiltonian of the respective complete network and modules /sub-modules in the five FKRs knockout experiments (Figure 7.4 C) in order to understand change in energy distributions in the respective network and module/sub-modules at various levels of organization (see section 7.2). If $\Delta H_s = H_s^{[O]} - H_s^{[R]}$ is the change in Hamiltonian functions due to removal of five FKRs at level $s$, where, $H_s^{[O]}$ and $H_s^{[R]}$ are the Hamiltonian functions for origin-
nal and removed networks respectively, and corresponding modules/sub-modules, then we obtain,

\[
\Delta H_s > 0, \forall s; \begin{cases} 
\frac{\Delta H_s}{H_s} \rightarrow 0 & (\text{for } s \leq 3) \\
\frac{\Delta H_s}{H_s} \rightarrow 1 & (\text{for } s > 3)
\end{cases}
\] (7.8)

Figure 7.5: Network/modules/sub-modules at different network levels which accommodate leading hubs and fundamental key regulators. The probability distributions of the FKRs as a function of level.
where, $H_s = H_s^{[O]}$. This indicates that the removal of FKRs cause enormous lost of wiring/rewiring energy which is propagated throughout the levels of network organization.

### 7.3.4 Network compactness preserves self-organization in ovarian cancer

The compactness of the network/modules/sub-modules with size are calculated using LCP-DP algorithm which is expressed $\sqrt{\text{LCL}}$ (local community links) as a function of CN (common neighborhood) (see section 7.2) and found that the number of strongly connected networks/modules/sub-modules ($LCP - corr \geq 0.8$) are larger than the number of loosely connected network/modules/sub-modules ($LCP - corr < 0.8$) at upper level of organization ($s = 1$) (Figure 7.6). The size of the modules at $s = 1$ ranges from 10 to 180 nodes. However, as one moves from top to bottom ($s > 1$), the number of strongly connected modules becomes decrease as compared to loosely connected modules/sub-modules. Since the ovarian cancer network, is tightly bound at the upper level and complete network, the network itself is organized to maintain its own properties against any external and internal perturbations (both local and global) in the network.

Now the analysis of LCP-DP of the network/modules/sub-modules shows that, except one particular module and its corresponding sub-modules at various levels, all the other modules/sub-modules becomes loosely packed with decrease in size as one moves from top to bottom levels. The particular module/sub-modules, whose size and compactness do not change much till third level (LCP-corr ranges from 0.936-0.994, and size 175-180), is the module/sub-module in which all the five FKRs are accommodated. This means that the module/sub-module is tightly regulated by these five FKRs along with their connecting nodes in them (Figure 7.6 second panel in each row). However, the removing of these five FKRs do not
cause network breakdown (Figure 7.6). Hence, this module/sub-module still try to preserve its own properties against any local and global perturbations.

7.3.5 Centrality-lethality is rule out in cancer network

The breast cancer network obeys close to ideal hierarchical network and hence the emergent modules/sub-modules are compact at upper levels. The removing of FKRIs do not cause the network breakdown. Even though one module in which the five FKRIs are accommodated and its corresponding few sub-modules breakdown after the third level, other modules/sub-modules remain stable to preserve the network properties. Hence, ovarian cancer network rule out centrality-lethality rule.

7.3.6 AKT1 plays central role in regulating ovarian cancer network

AKT1, which is a modulator of apoptotic signal and important therapeutic target gene in ovarian cancer [347], is found to be tightly bound with other important leading ovarian cancer regulator genes with large extension of network/modular sizes 400 to 100 depending on the network level of organization indicated by LCP-DP calculations (see section 7.2, Figure 7.6). In these calculations, the network module/sub-module in which AKT1 is accommodated are considered, where LCP-correlations of these networks/modules/sub-modules are found to be in the range [0.986-0.994] revealing strong compactness of these networks/module/sub-module at various levels of organization. Further, AKT1 is found to act as a main key regulator which allows to cross-talk among other remaining FKRIs (CD44, MCAM, KRAS and EPCAM) and Tp53 (Figure 7.6 C). Since the clustering coefficient of all five FKRIs in the extracting network of these five FKRIs are one (Figure 7.6 C), the identified five FKRIs are again found to be interacting strongly
which is the signature of rich-club formation in the network [348]. However, if we consider the whole network, we could not able to capture the signature of this rich-club formation of these FKRs as evident from network connectivity property

Figure 7.6: Properties of AKT1. (A) The tracing of AKT1 in network/modules/sub-modules at various network levels. (B) The variation of $LCL$ as a function of $CN$ for different levels. (C) Organization of five FKRs with Tp53. (D) Directional tracing of AKT1 at various network levels. Rich-club parameter as a function of $k$. $P_H$ and $P_{LCP}$ as a function of level.
dependence on negative exponent $C_N(k) \sim k^{-\beta}$ [124, 125] (Figure 7.4 A), and negative exponent dependence on rich-club parameter $R$ on degree $k$, $R \sim k^{-\theta}$ [348]. Since the rich-club data ($R$ versus $k$) for all networks, module and sub-modules scale the same scaling functional dependence, $R \sim k^{-\theta}$, the network organization exhibits absence of central controlling mechanism by AKT1 and its rich-club with FKR. Hence, even though AKT1 is significantly important FKR in ovarian cancer network, it never tries to dominate the network organization at various levels of organization.

Now, to understand relative energy AKT1 can have at various levels of network organization can be obtained as follows. Consider, $H_s$ is the Hamiltonian function at any level of network organization $s$, where, $s = 0, 1, ..., 5$ (network corresponding to $s = 0$ is the complete network). If $m_s$ is the number of modules/sub-modules at level $s$, then Hamiltonian function per module/sub-module at level $s$ is given by, $H_s = -\frac{1}{m_s} \sum_{j=1}^{m_s} \sum_{c_j} \left( e_{c_j} - \gamma n_{c_j}^2 \right)$, where, $n_{c_j}$ is the size of the $j^{th}$ module/sub-module at level $s$. Then the Hamiltonian function of AKT1 at the module it belongs to can be obtained as, $H^{[AKT1]} = - \left( e^{[AKT1]} - \gamma n_s^{[AKT1]} \right)$, where, $e^{AKT1}$ and $n_s^{[AKT1]}$ are the number of edges AKT1 has and the size of the module/sub-module where AKT1 belongs to respectively. Now the relative energy AKT1 can have at any level $s$ can be obtained by,

$$U_{AKT1}(s) = \frac{H_{AKT1}}{H_s} \sim e^{-\phi s}; \forall s \in I$$

(7.9)

where, $\phi$ is a constant. This relative energy of AKT1, $U_{AKT1}$ represents the energy associated with AKT1 constrained by the level of organization which could be related to the activities of AKT1 at various levels $s$. In an ovarian cancer network, the activity of AKT1 decreases as one goes down from top to bottom of the network (Figure 7.6 F) indicating its important regulating activity at complete network level than at a basic level.
Further, we calculate the relative compactness of the module/sub-module which accommodates ATK1 at various levels by using,

\[ W_{LCP} = \frac{L_{AKT1}}{\sum_{j=1}^{m_s} L_j} \cdot L \rightarrow LCP - corr \forall s \in I \]  

(7.10)

where, the sum is over non-zero LCP-correlations of the modules/sub-modules at each level \( s \). The calculated values of \( W_{LCP} \) (7.6 F) show that the relative compactness increases as one goes down from top to bottom level indicating the strong interaction of nodes at a lower level of organization.

7.3.7 Ovarian cancer network exhibit active regulating mechanism of FKRs with modules

Since ovarian cancer network follows hierarchical network features, the emerged modules/sub-modules become important regulating units at various levels of the organization along with active participation of FKRs in network phenotypes. The multi-functionality of the network could be the manifestation of the interacting emerged module/sub-modules at each network level to keep the network properties stable. The FKRs could be important workers of integrating the components in each module/sub-module they belong to for efficient functioning, through optimal signal processing among the components organized by these FKRs. The five identified FKRs in fact form rich-club phenomena at each level of organization, however, the impact of this rich-club activity at each level is weak enough such that this perturbation could not able to cause a significant change in the overall network topological properties. Besides the network/modules/sub-modules are mostly tightly bound due to strong interaction among the nodes/genes. Hence, the removing of these important FKRs do not cause network breakdown indicating absence of central control system, which is a signature of self-organization [130].

The network also exhibits topological properties close to the ideal hierarchi-
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The network indicated by equation (7.4) (Figure 7.4) and therefore the regulating mechanism in the network is active (far from equilibrium) in order to maintain network properties. At the same time the topological properties of the network exhibit power law behaviour indicating the network obeys fractal nature. This fractal nature due to self-affine process in the network could be a signature of self-organization in the network [350].

Figure 7.7: LCP correlation as a function of CN for different modules/sub-modules and their distribution.
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The knock out experiment of the five FKRs from the original network indicates that the change in the network properties due to knock out of five FKRs do not cause significant change in the network topology. This indicates that the system do not prefer to change due to perturbation imparted by FKRs knock out from the network. The network then reorganize itself and adapted to the changed topological properties. The ability to adapt to the change for a better network organization without breakdown of the system is another signature of self-organization in the network [349].

7.4 Conclusion

Complex ovarian regulatory network constructed from experimentally verified set of genes show hierarchical features, which allows the genes to organize in a number of different pathways (modules/sub-modules) in a complicated way, to exhibit multi-functionality of the system. Since the network is hierarchical, individual gene activities are not much important, but their co-ordination exhibit various important functional special deeds. At the same time, the leading hubs have significantly important functions, for example, integration of lower degree nodes for organizing regulating activities, means of inter and intra cross-talk among various other essential genes, maintaining network properties and stability, and optimizing the network signal processing. However, out of these leading hubs, few hubs, which we term as fundamental key regulators, take significantly more important roles in keeping network properties in better perspectives (ability to adaptation to fit change). In ovarian cancer network, out of seventy leading hubs in the network, we could able to explore five such FKRs which are AKT1, CD44, MCAM, KRAS and EPCAM. These FKRs are deeply rooted in the network, they serve as the backbone of the network for any network activities and regulations, and could be a possible target genes for this disease control mechanisms. Surprisingly,
the first few largest hubs (eleven hubs) do not fall in FKRs and these FKRs need not necessarily be most popular hubs, but some of them keep a low profile in the network. These FKRs form tightly bound rich-club, but the regulating activity of this rich-club could not able to show up in the network properties because the number of members in the rich-club is negligibly small as compared to the whole network. Further, these five FKRs fall in a single module/sub-module upto fourth level of organization indicating closer working of the FKRs and then start separating afterwards. Some of these identified FKRs have experimentally been shown important backbone genes in ovarian cancer. For example, AKT1 is experimentally found therapeutic target gene [347], CD44 is found to be target gene, which serve as the backbone for paclitaxel prodrugs [351], MCAM is reported to be an important metastasis marker and invasion of ovarian cancer cells [352], KRAS is identified as important genetic marker of ovarian cancer [353]. However, even though EPCAM is involved in ovarian cancer regulation [354], we propose that this EPCAM could be an important gene for possible target gene in ovarian cancer.

Since the network possesses hierarchical properties, removing of these FKRs does not cause network breakdown, rather reorganize the network to another perspective and adapted to it. Since the five FKRs are associated with a single module/sub-module, one can target (possible drug target genes) these FKRs and accommodating module/sub-module in ovarian cancer. But removing of FKRs from the module they belong to cause modular breakdown after a certain level of organization in the network. Hence, one needs to look into this module/sub-module for the critical target of this disease.

The ovarian cancer network is a tightly bound network, and follows certain properties: first, the network rules out centrality-lethality rule (no central control system); second, network topology obeys fractal laws; third, out of FKRs AKT1
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plays central role in regulating ovarian cancer system. However, we need to study large scale analysis of the dynamical network, which involves various biologically well defined modules to understand the time evolution of the ovarian cancer, and for spatio-temporal target genes.