Informatics in Medicine Unlocked 9 (2017) 173–180

Contents lists available at ScienceDirect

Informatics in Medicine Unlocked

journal homepage: www.elsevier.com/locate/imu

Hub nodes in the network of human Mitogen-Activated Protein Kinase (MAPK) pathways: Characteristics and potential as drug targets

V.K. MD Aksam [a], V.M. Chandrasekaran [a], Sundaramurthy Pandurangan [b,*]

[a] School of Advanced Sciences, VIT University, Vellore, India
[b] Point Cross Life Sciences, Inc., Bangalore, India

ARTICLE INFO

Keywords:
Cancer drug targets identification
Network of MAPK pathways
Side effects
Essential proteins
Graph theory

ABSTRACT

Proteins involved in the cross-talk between ERK1/2, ERK5, JNK, and P38 signalling pathways integrate the network of Mitogen-Activated Protein Kinase (MAPK) pathways. Graph theory-based approach is used to construct the network of MAPK pathways, and to observe the network organisational principles. Connectivity pattern reveals rich-club among the hubs, enabling structural ordering. A positive correlation between the degree of the nodes and percentage of essential protein showed hubs are central to the network architecture and function. Furthermore, attributes like connectivity, inter/intra-pathway class, position in the pathway, protein type and subcellular localization of the essential and non-essential proteins are characterizing complex functional roles. Shared properties of 34 cancerous essential proteins lack to be drug targets. We identified the seven nodes overlapping properties of the hub, essential and causing side effects on targeting them. We exploit the strategy of cancerous, non-hub and non-essential proteins as potential drug targets and identified 4EBP1, BAD, CHOP10, GADD45, HSP27, MKP1, RNPK, MLTKa/b, cPLA2, eEF2K and elF4E. We have illustrated the implication of targeting hub nodes and proposed network-based drug targets which would cause less side effect.

1. Introduction

Network-based approaches are gaining prominence to mine drug targets in the current scenario [1–4]. Targeting a key protein in the disease pathway can impair the entire signalling network and may have unanticipated consequences. The outcome may be due to the central role and immediate neighbour's acting as controller proteins [5–7]. Most of the targeted proteins cause side effects leading to chronic diseases such as cardiovascular, neuro and psychiatry, respiratory, diabetes, etc. [8,9] To avoid targeting essential and side effect causing nodes, we have developed the network-based drug target identification approach in the network of MAPK pathways.

MAPK pathways play a vital role in the development and diseases like cancer [10,11]. ERK1/2, ERK5, JNK and P38 pathways are not isolated, and cross-talk between the pathways was integrated to form the network with 83 nodes and 183 interactions [12] (Fig. S1) Targeting the prime proteins in the MAPK pathways to treat cancer will cause chronic side effects [13–15]. A network of MAPK pathways is treated as a subnetwork of the human PPI network. With the perspective of drug target identification, we are hypothesized to study the topological and functional properties of the pathways. Previous works on the protein protein interaction (PPI) network illustrates the relationships between degree and side effects by Hase et al. [16], hub and essentiality by Jeong et al. [17] and among degree, essential and side effects by Wang et al. [18].

Hubs, the high degree nodes are the topological organizer of the network as they have more interacting partners. Targeting hub nodes would lead to the unexpected side effects due to the joint dysfunction of the interconnected functional nodes [1] (Table S1). The prevalence of more hubs in the network keeps unbiased and error-free interactions. Moreover, their target removal would lead to the distraction in the network [19]. Decision on this edge parameter defining the hubs have been made in different ways in a different context and is not yet universally confirmed [19].

In the literature, following two criteria exist to define hubs.

1. Network topology based: Cut-off or relative cut-off degree of 5 [20,21], 8 [22], 20 [23], etc., and greater than of those (or) the top 20% [23], 50% [24], 95% [24], etc., of the nodes were defined as hubs.
2. Functional annotation based: Functional annotations based classification of proteins in several species are utilised to define hubs, even

* Corresponding author.
E-mail addresses: mdaksam.vk@vit.ac.in (V.K. MD Aksam), vmcsn@vit.ac.in (V.M. Chandrasekaran), sundaramurthy@pointcross.com (S. Pandurangan).

http://dx.doi.org/10.1016/j.imu.2017.08.006
Received 6 July 2017; Received in revised form 30 August 2017; Accepted 30 August 2017
Available online 3 September 2017

without knowing the corresponding PPI. Machine learning methods employed as hub classifier can predict highly connected nodes [25].

In the present work, our objective is to find relationship between hubs, essential and side effects caused nodes in the network of MAPK pathways and to identify drug target, which may cause fewer side effects.

2. Results

2.1. Structure of network of MAPK pathways

Identification of the topological properties of network structures gives the class of nodes to influence as target. A network of MAPK pathway revealed to be scale-free, and identify 25 hub nodes (Fig. S1). Further analysis has shown that most of the hub nodes are directly connected to the hub nodes forming rich-club (Fig. 1). Hub nodes are tightly connected with one another to influence integrated function for the whole system like yeast PPI network [26]. In PPI network, hub proteins are having lower connectivity among themselves than non-hub nodes [27]. More recently statistical and topological properties of PPI network on the human and budding yeast revealed a middle degree nodes are tightly connected forming the backbone to network phenomenon known as ‘‘stratus’’. However, in the same network, high degree nodes are linked to low degree nodes forming ‘‘altocumulus’’ structure. Thus, the promiscuous activity of hubs makes them play many functional activities, and network attain robust against failures (Highly Optimised Tolerance (HOT) network) [16]. But in the network of MAPK pathways, hubs form a core backbone in an organised manner to integrate the whole network topology. Targeting the hubs may dismantle the entire cellular signalling network, and may disturb hub-hub interactions (Table S2). Thus, the promiscuous activity of hubs makes them play many functional activities, and their knock-out results in dysfunctional activities leading to the unexpected side-effects or harmful effects to the cell [16].

2.2. Hubs contribution to the cancer mechanism

Malignant signalling network is attained due to the mutational effects of corresponding disease genes. Mutated cancerous genes perturb different signalling pattern in the network. Proteins having higher interactions (hubs) found as enriched with cancerous mutation and possibly contributes to the multifactorial mechanisms of cancer development. Proteins documented as cancerous mutated in any of the four databases F-Census [29], Bushman Lab [30], NCG 4 [31] and COSMIC [32], are considered as cancerous nodes due to their pathways deregulatory role, which could lead to cancer progression.

There are several observations about the class of cancerous nodes in the PPI network:

1. The degree of cancerous proteins is observed to be higher than non-cancerous proteins [24].
2. Hub proteins and its first neighbours play a role in cancer [33].
3. Cancerous hub proteins prefer to interact with other hub proteins rather than interacting with non-hubs proteins [34].

We checked the similar observations in the network of MAPK pathways:

1. Cancerous proteins found abundantly in both hubs and non-hubs (Table S3). Out of 69 cancerous nodes - 20 are hubs and 49 are non-hubs. Out of 14 non-cancerous nodes - 5 are hubs and 9 are non-hubs.
2. The first neighbour of 25 hubs nodes is 71 nodes (containing both hub and non-hub nodes) (Fig. 1). Out of 25 hubs nodes, 20 are cancerous, and out of 71 nodes, 59 are cancerous.
3. Cancer hubs prefer to interact with other hubs in our network (Table S4). (further analysis carried out in section 2.6)

2.3. Hub-centric organisation of the pathways

Proteins are connecting two or more signalling pathways among

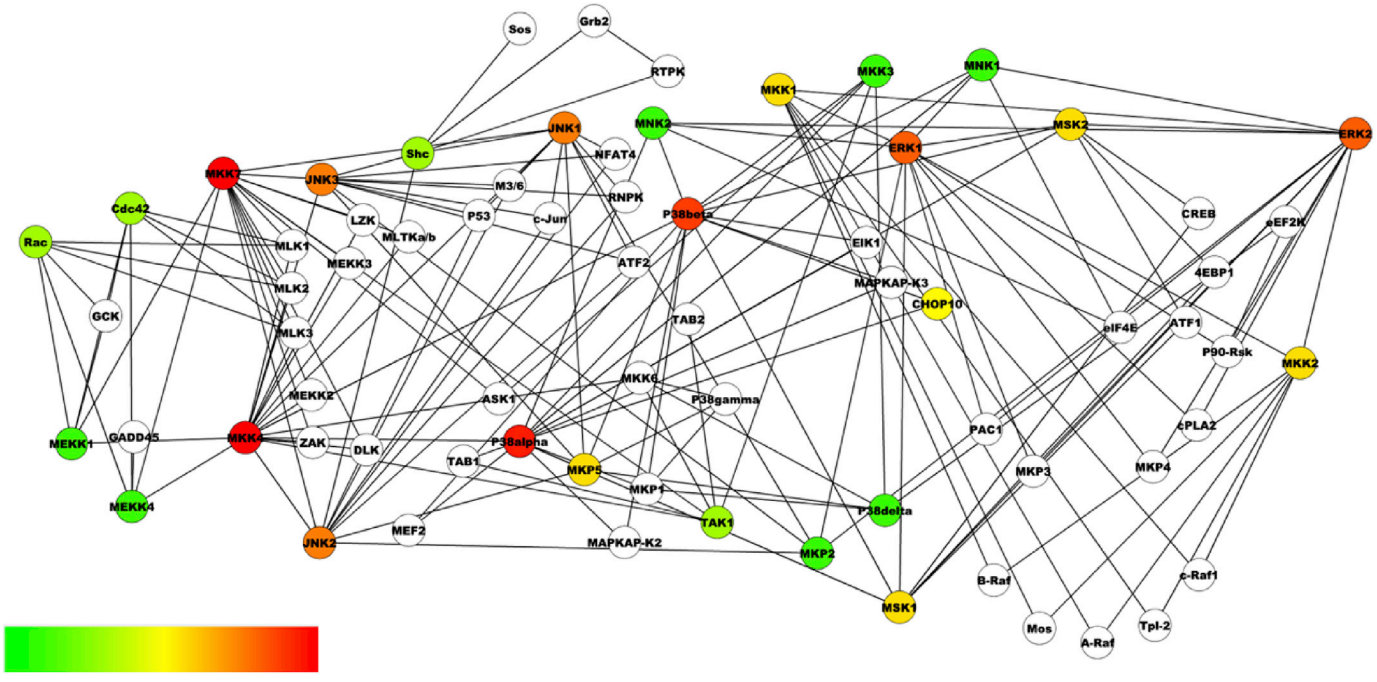


Fig. 1. Hubs with their first neighbours extracted as subnetwork, and hubs are coloured green to red (based on high to the low degree). We observed hub nodes directly connected to the other hub nodes organising rich-club [28]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ERK1/2, ERK5, JNK, and P38 pathways considered as inter-pathway nodes, and intra-pathway nodes confined to a pathway. Inter-pathway hub nodes are the link that organises more than one pathway in the network, these phenomenon known as "hub-centric organisation of a pathway" [35]. Out of 25 hub nodes, 15 are inter-pathway nodes. Among the 58 non-hub nodes, only 18 are inter-pathway nodes (Fig. S3). Thus 60% of the inter-pathway hub nodes contributes to the network formation by cross-activating signalling process in more than one pathway. Due to this, with fewer stimuli, more pathways can be activated to attain diverse functions [36]. Furthermore, the correlation between molecular signalling network complexity and cancer types survivability also states complex role of this juncture well [37]. The highest value of the metric on the degree and information flow (betweenness) cause lower probability of 5-year survival. This work carried out in 14 different cancer sites (Acute myeloid leukaemia, Basal cell carcinoma, Bladder cancer, Chronic myeloid leukaemia, Colorectal cancer, Endometrial cancer, Glioma, Melanoma, Non-small-cell lung cancer, Pancreatic cancer, Prostate cancer, Renal cell carcinoma, Small cell lung cancer, Thyroid cancer).

2.4. Protein types classification in hubs

As per the information is given in Science STKE database hub nodes are classified as 14 protein types [38]. They are (1) kinase, (2) Transcription factor, (3) Adaptor protein, (4) Phosphatases, (5) Receptor, (6) GTP-binding protein, (7) DNA/RNA binding, (8) Receptor-associated Factor, (9) Nucleotide Exchange Factor, (10) Translation inhibitor, (11) Lipase, (12) Chaperone heat/shock protein, (13) Translation initiation factor and (14) DNA Repair.

In our network, kinases are abundant (20/25) among the hub nodes. However, kinases found as smaller degree nodes in the PPI network [39]. 4 out of 14 protein types found as hub nodes, and the non-hub nodes are present in all the 14 protein types. Showing a vital roles played by the kinase hub in the signalling network formation. Kinase, Phosphatase, adaptor protein and Gtp-binding protein are the hubs forming protein types observed as the key players in signal transduction mechanism (Fig. S4).

2.5. Subcellular localization of the hubs

A significant contribution of hub proteins in the network along with the subcellular localization explains signal transduction's dynamic nature. Thus, the multi-localized proteins get activated in various compartments leading to the activation of the other signalling proteins in specific localization. Translocation-associated proteins are found to be a useful drug target [40,41]. Multiple translocations associated proteins flourished in MAPK pathways (in all the four ERK1/2, ERK5, JNK and p38 pathways) from plasma membrane translocation, cytosolic translocation, and nuclear translocation [27,40,42]. Science STKE database [38] is utilised to extract eight subcellular localizations in the network. They are (1) cytosol, (2) nucleus, (3) plasma membrane, (4) cytosolic translocation, (5) nuclear translocation, (6) plasma membrane translocation, (7) mitochondrion, (8) endoplasmic reticulum (Fig. S5).

Out of 25 hub nodes, 17(68%) nodes found in the cytosol. In the mammalian cells, most of the proteins are destined to the cytosol to carry out various functions [43]. The other eight hub nodes distributed among plasma membrane, plasma membrane translocation, nucleus, nuclear translocation and endoplasmic reticulum as 1, 2, 2, 2, 1 respectively. The nuclear translocation hub nodes MKP5 and P38 alpha with 7 and 14° respectively. MKP5 translocate and dephosphorylate critical kinase cascade nodes such as JNK1, JNK2, JNK3, p38gamma, p38delta, p38beta, p38alpha maintaining signal transduction in JNK and P38 pathways individually. Hub nodes in the plasma membrane translocation, SHC and TAK1 are having 6° each, found to interact with other nodes by performing various cellular functions. Targeting them may impair the functional activity of the cell. Furthermore, aberrant activation of the hub nodes found in cancer and strategies previously employed as therapeutic restoration like arresting nodes in localization [40,41] can be a better approach. We aim to identify the drug targets, which are free from essential functions and would cause fewer side effects.

2.6. Cancerous hubs conjoin

To know the cancerous hubs interactions in our network, we considered degree, the interaction between cancerous and non-cancerous hubs, and interaction among cancerous hubs (Table S3). We observed as cancerous hubs preferred to interact with the other cancerous hubs. Central hit strategy of targeting hubs to kill the malignant cell has found to be a successful strategy [1,44–48]. To implement revert strategy from pathological to a healthy condition; hubs may not be a potential target. Furthermore, targeting cancerous hub nodes may lead to the lethality or side-effects due to its central position in the network.

2.7. Targeted hubs cause side effects

Previously known targets for various diseases and their corresponding side effects can be found in Dr PRODIS database [49]. We considered cardiovascular, diabetes, neuro & psychiatry and chronic respiratory diseases only as side effects. From the same database, we have collected the number of side effects caused due to targeting the hub and non-hub nodes (Fig. 2). Among the targeted 25 hubs, 19 were causing one (or) more side effects, and the other 6 were side effect free. While among the targeted non-hub nodes, 26 nodes were causing one or more side effects, and 32 nodes were side effects free. Targeting the hub nodes may not be the preferable drug target strategy.

2.8. Connectivity, inter/intra-pathway class, position in the pathway, protein type and subcellular localization of the essential and non-essential proteins are characterized to elucidate complex functional roles

Essential proteins are fundamental to the survival of the organism, and they should remain untouched in the drug target identification process [50]. Database of essential genes (DEG 10) [51] is used to check the nodes are essential or non-essential. Further, we explore percentage of 1. Hub and non-hub nodes, 2. Inter and intra-pathway nodes, 3. Upstream, MAPK cascade and downstream nodes, 4. Protein type and 5. Subcellular localization of the essential and non-essential proteins (Fig. 3). The inner donut ring showcases essential, and the outer ring showcases non-essential nodes. We found 11 hubs and 30 non-hubs as essential, 14 hubs and 28 non-hubs as non-essential. There is no significant difference between hubs and non-hubs among essential and non-essential proteins.

Also, we found 15 inter and 26 intra-pathway nodes as essential, 18 inter-pathway and 24 intra-pathway nodes as non-essential. Thus, there is no significant quantitative difference between intra and inter-pathway nodes presence in essential and non-essential class.

Nodes position in the pathway characterized into three categories: 1. Upstream pathway proteins 2. MAPK cascade proteins and 3. Downstream pathway proteins. We observed essential proteins accumulate in upstream 21(51%) nodes, directed by activation of receptors or first neighbours of the receptors. On the other hand, non-essential proteins are mostly present as downstream 17(41%) nodes, which are activated by cascade nodes and activate transcription factors. However, in the middle cascade level, there is no significant difference observed between essential and non-essential nodes.

Out of 14 proteins types in the network, only 8 types are essential. Nodes in the protein type kinases (20), transcription factors (7), adaptor proteins (5), Gtp-binding proteins (4) and phosphatase (3) are more of essential than non-essential. Receptor, receptor-associated factor and nucleotide exchange factor are having 1 node each in essential category. In the non-essential category, 11 different protein types observed. Highest among non-essential nodes are the kinases with 26(61%).

Influence of proteins localization of essential and non-essential

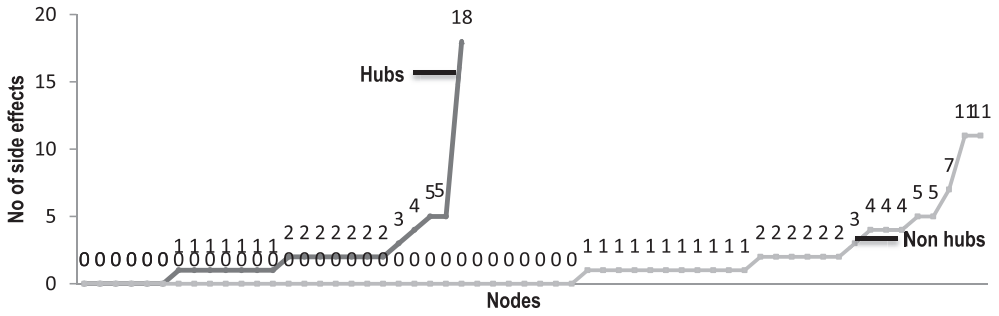


Fig. 2. Hub and non-hub nodes scattered in increasing order versus number of side effects.

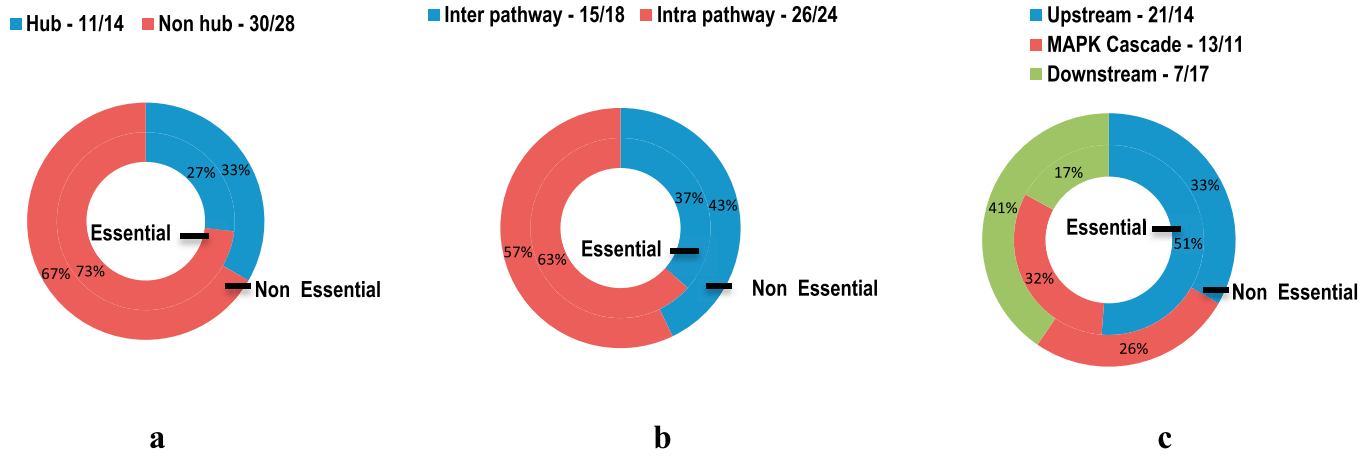


Fig. 3. Percentage of a) Hub and non-hub nodes, b) Inter and intra-pathway nodes, c) Upstream, MAPK cascade and downstream nodes, d) Protein type and e) Subcellular localization in the essential and non-essential proteins. The inner donut ring showcases essential, and the outer ring showcases non-essential nodes.

proteins are studied to understand the substrate activation role confined to cellular components or translocation. Previously translocations of nodes are observed to be playing essential signalling network [42]. We noted that plasma membrane and cytosolic translocation are having 3 and 2 nodes respectively in the essential category, and there are no nodes found in non-essential category. However, nuclear translocation has 1 essential and 3 non-essential nodes. Cytosolic proteins are 22(64%) in essential and 27(54%) in non-essential nodes. There is no significant difference between essential and non-essential nodes in the nucleus, having 8, 9 nodes respectively. Most of the essential proteins clustered around the cytosol.

2.9. Centrality-lethality rule

The network of MAPK pathway are scale free, and random removal of nodes in the network tolerate errors. Jeong et al. [17] in the protein-protein interaction network defined a centrality-lethality rule as "on the average lower degree nodes are to be less essential than higher degree nodes". In our network we examine centrality-lethality rule, we found 41 out of 83 nodes as essential as per DEG 10 [51]. Furthermore, the correlation between degree and percentage of essential proteins showed r = 0.936 - strong positive Karl Pearson's correlation coefficient (Fig. 4). Out of 58(70%) nodes with degree less than five, 29 are essential, and 29 are non-essential, and out of 25 hub nodes, 12 are essential, and 13 are non-essential. Implies that the network follows the centrality-lethality rule with higher degree hub nodes is mostly essential than the non-hub nodes (Table S5).

2.10. Essential versus cancerous proteins

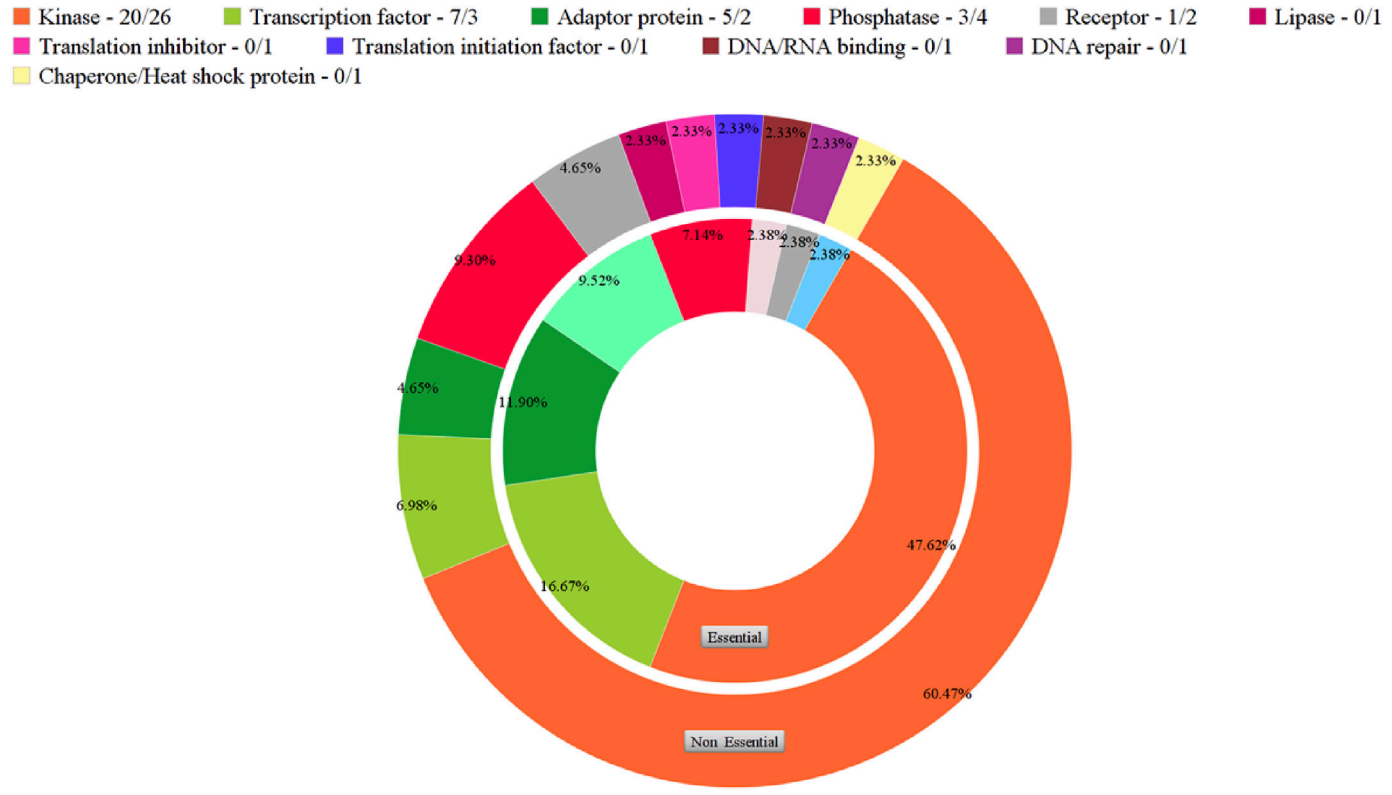
Disease proteins are found to be non-essential and are hubs in human PPI network [52]. Venn diagram is drawn between essential and cancerous nodes to obtain overlapping nodes. 34 cancerous essential proteins cannot be drug targets, due to their essential roles (Fig. 5). We observed half of the cancerous proteins are essential. Out of 35 cancerous non-essential proteins, 26 are non-hubs, and 9 are hubs. Thus, 26 cancerous non-essential non-hub proteins may serve to be a good target for anti-cancer drugs.

2.11. Essential versus side effects

Consequences of considering essential nodes as drug targets may cause side effects due to its functional role. So, we explore essential, and the unexpected side effects caused due to targeted effects. Classified essential and non-essential, with their number of side effects, are scattered in increasing order (Fig. S5). To see the chronic side effects caused by targeted proteins, we took four chronic diseases like cardiovascular, diabetes, neuro & psychiatry and respiratory as documented in DR. PRODIS database [49]. The majority of the targeted essential proteins are having no side effects as shown in Fig S5. 16 (39%) targeted essential and 29(69%) targeted non-essential nodes are causing one (or) more side effects. The Strong relationship between essential versus hubs and the removal of the hub are prone to cause more side effects [17]. In our MAPK pathways network, targeted essential hub nodes produce on an average 2.3 side effects, and 4 non-essential nodes are found to be more (>=7) side effects causing.

2.12. Relationship among hubs, essential proteins and side effects

Hubs contribute the network architecture by connecting to the other hub nodes as rich-club. Further, Inter-relationship among hub, essential and side effects integrate to form complex system to understand (Fig. 6).



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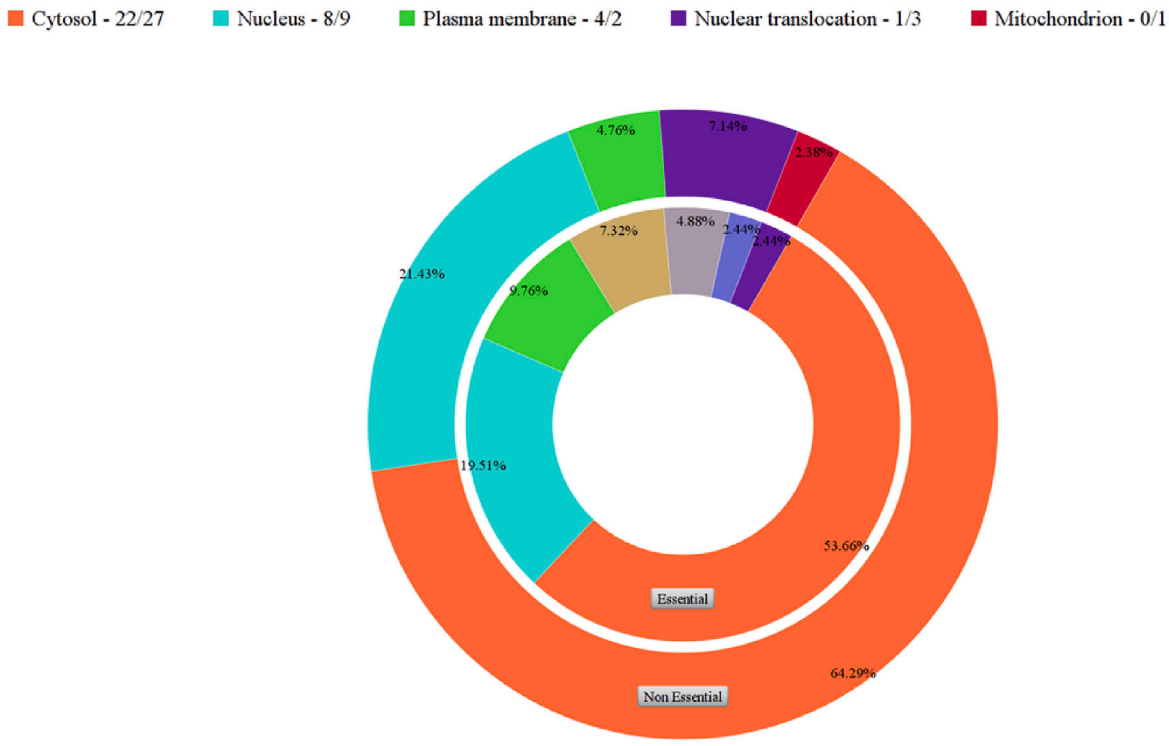

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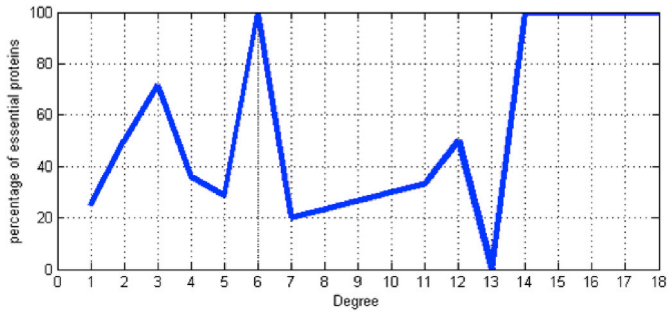

Fig. 4. Strong positive Karl Pearson's correlation coefficient r = 0.936 observed between degree and percentage of essential proteins.

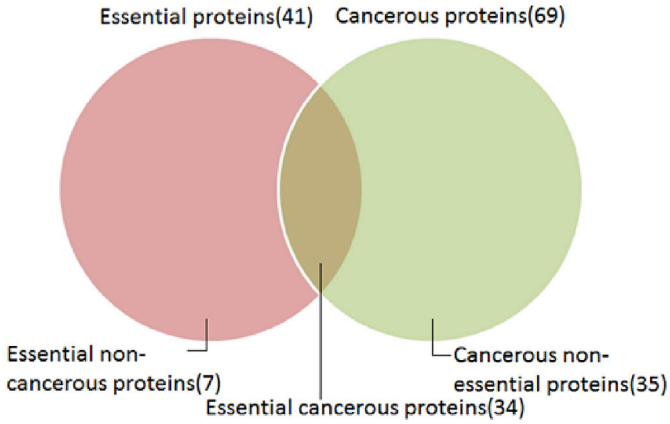

Fig. 5. Relationship between cancerous and essential proteins.

Thus, hub nodes non-overlapping with side effects and essentiality is only 2 nodes, and the other hub nodes intersect with the essentiality and side effects. Out of 25 hub nodes topological organising the network, 11 nodes share essential property, and 19 nodes found causing one or more side effects. Whereas, out of 41 essential proteins, 16 nodes found to cause one or more side effects. The nodes leading to phenotypic consequence due to at least one chronic side effect are found to be 45 nodes. Overall hub, essential and side effects causing nodes cannot be considered as a good target due to each of their topological, functional and phenotypic roles respectively. Targeting some of the cancerous non-hub non-essential nodes might cause fewer side effects, can be searched among the other 11 nodes not falling in any of the above mentioned three node categories. Hubs overlapping with essentiality and side effects are the key factors to be considered while attempting to identify drug targets, which would cause less adverse effects (Fig. 7).

3. Conclusions

Hubs are found to be the topological organizer of the network by having more interacting partners. Further, most of the hub nodes are essential for the survival of the organism. Hub is the organising structural integrity of the network, and the essentiality property made them functional vital. We observed a strong positive correlation between degree and percentage of essential proteins. Rich-club among hub nodes forms the core of the network. Preference of hub target found to best only in rational drug design to kill malignant cells. Cancerous hub nodes should not be the drug targets as most of them are essential. On the other hand, targeting some of the cancerous non-hub non-essential nodes might be the right strategy. Most of the hub nodes are kinases, are located in the cytosol and plays a primary role in cancer mechanism. Essential proteins are shown to be causing fewer side effects when targeted, and since their functional presences are vital for organism's survival, they cannot act as drug targets. Non-essential nodes are causing more side effects than the essential nodes, could be due to their interaction with their adjacent essential nodes. Thus, we can conclude that hub nodes cannot be a good drug target due to its strong relationship between essential and side effects. Cardiovascular, Neuro & psychiatry, respiratory and diabetes chronic diseases were implications of the side effects caused by wrongly targeted nodes. Additionally, we also tried to chalk out attributes like connectivity, inter/intra-pathway class, position in the pathway, protein type and subcellular localization of the essential and non-essential proteins characterizing complex functional roles. Out of 11 identified drug targets, previously known 6 targets (BAD, HSP27, RNPK, MLTKa/b, cPLA2, elF4E) were found to be side effects free. The proposed method identified those previously known targets and adds value to them by tagging them as side effects free. We believe that the remaining five identified drug targets (4EBP1, CHOP10, GADD45, MKP1 and eEF2K) are to be new and side effects free(Table S6). Overall we have found degree centrality measure as hub nodes can be used to avoid side effects by not targeting them. We have illustrated the reasons for the hub nodes not be targeted and showed the ways to minimise the side effects through network-based drug target identification approach.

4. Materials and methods

ERK1/2, ERK5, JNK and P38 Pathways were extracted from Science STKE database [38] and integrated to construct the network of MAPK pathways with 83 nodes and 183 interactions (Fig. S1). We set network topology based arbitrary cutoff for the nodes with degree ≥5 as hubs and less than 5° to define non-hubs [20,21]. Network analysis tool Cytoscape_v3.1.1 [53] is used along with CytoHubba plugin [54] to find the degree of the nodes. We manually checked every hub node to know its interconnectivity in the network of MAPK pathways among cancerous proteins. Cancerous proteins in the network extracted from any of the

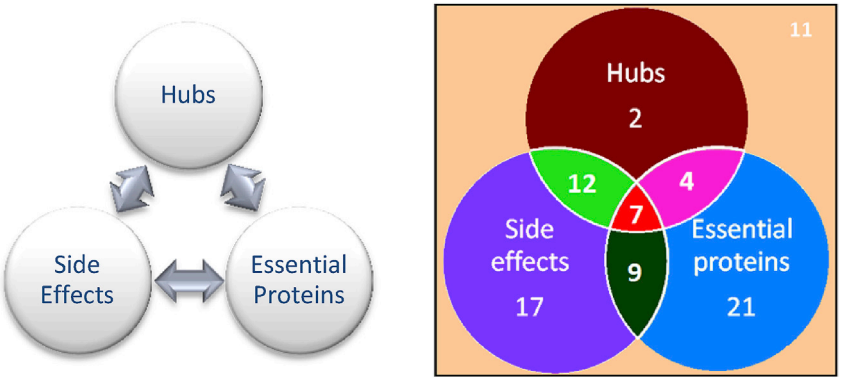

Fig. 6. Inter relationship among hub, essential and side effects.

Fig. 7. Network of MAPK pathways with the hub, essential and side effects causing nodes, and their combinations.

four databases F-Census [29], Bushman Lab [30], NCG 4 [31] and COSMIC [32].

Extraction of functional hub node's protein types, subcellular localizations and their position in inter/intra-pathway is from Science STKE database [38]. Information on essential proteins obtained from Database of Essential Genes (DEG 10) [51]. DR.PRODIS [49] database used to know whether targeted proteins are causing chronic side effects like cardiovascular, neuro and psychiatry, respiratory and diabetes. Correlation between the degree of the nodes and percentage of essential proteins are calculated using SPSS 16.0. Data visualisation performed by using MATLAB R2014a.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.imu.2017.08.006.

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