Topology-based protein–protein interaction analysis of oral cancer proteins

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Oral cancer is a common type of head and neck cancer that affects majority of the population worldwide. The present study focuses on the network-based protein–protein interaction (PPI) approach for the identification of oral cancer targets and systems biology approach-based analysis. Totally 47 oral cancer gene targets were extracted from the BioXpress database, Oral Cancer Gene Database and HNC database. The related protein networks were explored and visualized using Cytoscape v3.7.2. Topology predictions were performed by Molecular Complex Detection tool and Biological Networks Gene Ontology tool (BiNGO) plug-in from Cytoscape v3.7.2. The comprehensive study using MCODE are three clusters of 15 common oral cancer genes. The predicted proteins were GSK-3β, PKM, Catenin-β1, Tp53, SMAD-3, MYC, LDHA, HIF1-α, PDPK-1, AKT3, PIK3CA, ILK, UBC, E2F1 and SKP. The 15 oral cancer genes with their significant *P*-value < 0.05 are responsible for the development of oral cancer. These 15 proteins obtained from network-based interaction analysis can be a potential solution of anti-cancer drug molecules against multiple targets of oral cancer.

Keywords: Cluster analysis, gene ontology, oral cancer, protein–protein networks, topology analysis.

CANCER is currently one of the most common diseases affecting the human population worldwide. Oral squamous cell carcinoma (OSCC) is among the most prevalent head and neck region cancers primarily affecting the squamous cells of the oral cavity. Cigarette smoking, betel-nut chewing, quid and khat (*Catha edulis*) chewing, and alcohol intake are the common risk factors for OSCC[1]. According to a recent World Health Organization (WHO) survey, the number of head and neck squamous cell carcinoma (HNSCC) cases is increasing and will reach 535,000 by 2040 (ref. 2).

Various advanced biomedical techniques such as surgery, chemotherapy and radiation therapy are now used to treat cancer, but they are unable to detect oral cancer in its early stages. Despite major advances in cancer care and management, OSCC-related mortality remains unchanged. Oral cancer is typically diagnosed in its later stages and the rate of recurrence is often high; so early detection and diagnostic techniques for disease control and prevention may play a critical role. In the last decade several studies have been performed to understand the molecular basis of invasive OSCC. However, production and metastasis remain unclear, which are crucial for understanding the mechanism of its activation[3]. A study performed in 2019 revealed the relationship between chronic periodontitis, *Porphyromonas gingivalis* and cancer with the help of protein–protein interaction (PPIs) network analysis[4]. It identified *IL6*, *STAT1*, *LYN*, *BDNF*, *C3*, *CD274*, *PDCD1LG2* and *CXCL10* as potential candidate genes that might facilitate the prevention and treatment of OSCC[4]. Another study on the oral cancer cell signalling pathway showed that the TP53 protein is responsible for the signalling mechanism involved in the progression and development of oral cancer cells[5]. Various studies have also been performed to identify of potential genes of oral cavity cancer using network analysis-based approaches[6–8].

In recent years, identifying the genes and their functions associated with this complex disorder has become a critical feature. Experimental approaches, e.g. genetic linkage association studies[9], expression profiling[10] and genome-wide association studies[11] have been found to be successful in identifying high relative risk genes for diseases like cancer[12], asthma[13], diabetes[14], etc. For early detection and diagnosis of oral cancer, systems biology-based approaches for PPI analysis are important. While individual proteins are not involved in disease progression, recent studies have shown that several proteins are collectively involved in cancer. The mechanism of oral cancer can be easily understood by cell signaling-based information derived from interaction networks. The PPI network involves disease-related protein analysis in a systematic interaction pattern study[15].

Diseases are oftenly caused by mutations that disrupt the binding interface or induce biochemically abnormal allosteric changes in proteins. Protein interaction networks can therefore identify the molecular mechanisms causing the disease, which in turn can help in prevention, diagnosis and therapy[16].

The present study considers PPIs using a topology-based method, but it does not reveal whether the proteins interact or not, and, if so, what are their interaction affinities. It also does not define PPI sites and cannot predict the end products of PPI reactions.

The main objective of this study was to identify the potential oral cancer proteins which are involved in the progression

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of the disease. Previous studies also predicted several oral cancer targeted genes by combining top-ranked genes with related gene ontology (GO) terms[3]. A recent study identified the significant role of TP53 protein in oral cancer network with other 29 neighbours, but the contribution of the neighbouring protein in the backbone was not included[5]. Most of the published studies consider one or two database systems for the analysis of oral cancer proteins using PPI network, which restricts the number of proteins for network development and affects the final results. Though there are several studies on oral cancer potential gene identification using PPI network, only a few studies showed statistical significant analysis. Here, we have chosen three-different oral cancer-specific database systems: BioXpress database, Oral Cancer Gene Database (OrCGDB) and HNC database (HNCDB) for the PPI network-based study to identify potential oral cancer proteins. We have selected the BioXpress database oral cancer data with a significance level of 0.05 of upregulated genes, which has increased the reliability of the results. Statistical analysis helped identify 15 important oral cancer proteins that can be used not only as disease markers at the early stages, but also as targeted proteins for drug design.

Material and methods

Data retrieval, network generation and analysis

BioXpress database[17], OrCGDB[18] and HNCDB[19] help retrieve information on oral cancers and their subtypes. The first step in the analysis was to collect data on genes linked to head and neck cancer from the BioXpress database. It included mRNA data for genes associated with head and neck cancer. The significance level of the data was 0.05, and 2323 upregulated genes were shown to substantially express head and neck cancer data from 41 patient sample sets that were obtained and processed. The second set of information came from the OrCGDB (ACTREC Mumbai). This dataset contains data for 374 oral cancer genes. The third dataset obtain from HNCDB contains 1368 oral cancer genes. The total of 47 oral cancer genes were found to be common in these three databases linked to oral cancer (Figure 1). The Network Analyst tool was used to analyse the 47 common oral cancer genes[20]. It created a sub-network with 790 nodes and 1010 edges using the String v11 software[21]. The networks were analysed using the Cytoscape v3.7.2 tool, which can be used to combine a bimolecular interaction network with high-throughput expression results[22].

Clustering and GO analysis

MCODE is used to find topographically linked regions in a network. The PPI networks with a score greater than 2.0 and at least two nodes were selected as significant prediction according to MCODE. Seed nodes were evaluated as a complex with the highest weighted vertex value in the second stage of the MCODE plug-in analysis (forward and outward)[23]. The GO predicts the functions of each cluster that is generated by the MCODE plug-in. On the basis of the GO hierarchy, the BiNGO plug-in map predicts the leading functional characters of an oral cancer gene dataset in Cytoscape v3.7.2 graph format[24].

Results

Network analysis

After a comprehensive analysis results were obtained for the PPI networks of BioXpress database, OrCGDB and HNCDB. The PPI network had 1010 nodes and 790 edges. In the predicted network, nodes represent proteins and edges denote the physical and functional interactions between proteins. Figure 2 and Table 1 show the oral cancer PPI networks and their properties.

The number of connections associated with a node determines the degree of that node in the network. Hub proteins are nodes with a higher degree that are important for the network structure as well as functionally important nodes. The network analyser operates according to the power law of node degree distribution and betweenness. The network centrality distribution of oral cancer proteins represents proteins with high centrality value. These highly connected nodes and edges are significantly shows that these oral cancer genes are highly involved in the functional and biological process of oral cancer disease generation. Figures 3 and 4 show the degree centrality distribution curve and betweenness centrality distribution curve of the PPI network for oral cancer respectively.

A network with a power law degree distribution is referred to as a scale-free network. These PPI networks have GSK-3β, PKM, Catenin-β1, Tp53, SMAD-3 proteins, MYC, LDHA, HIF1-α, PDPK-1, AKT3, PIK3CA, ILK, UBC, E2F1 and SKP2.

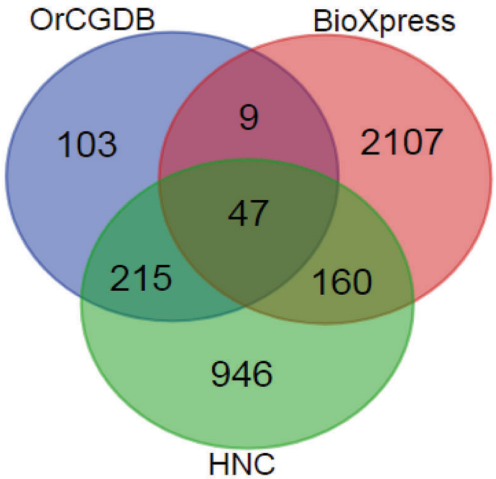


Figure 1. Venn-diagram of oral cancer genes.

Table 1. Properties of oral cancer protein–protein interaction (PPI) networks

Clustering coefficient	0.061	Number of edges	1010
Connected components	1	Number of nodes	790
Network diameter	10	Network density	0.003
Network radius	5	Network heterogeneity	3.384
Network centralization	0.167	Shortest paths	623,310 (100%)
Average number of neighbours	2.557	Characteristic path length	4.084

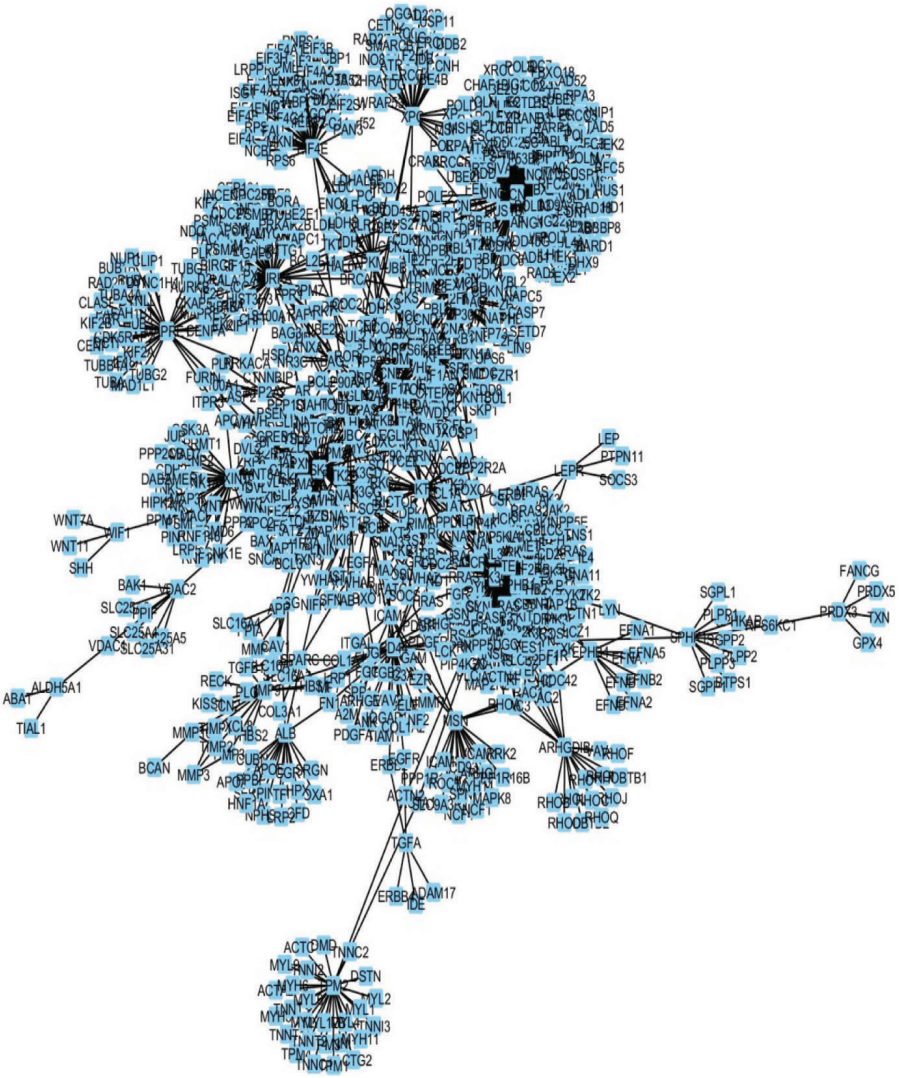


Figure 2. Protein–protein interaction (PPI) network of oral cancer genes obtained from the oral cancer datasets.

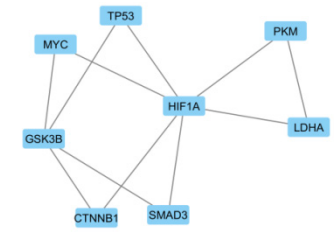
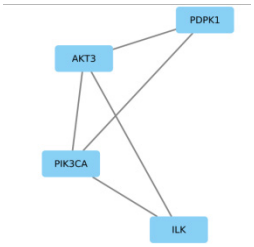
Clustering of the network

The PPIs data of oral cancer gene clusters were analysed using MCODE plug-in. These cluster based approaches have the all nodes in whole weights by local neighbourhood density and determine the highly connected regions in the cluster. These regions show those genes which mainly affect the molecular and functional mechanisms of the disease. Table 2 describes the MCODE plug-in that shows all three sub-networks.

GO analysis

Figures 5–7 show the functional distribution of biological process (BP), molecular function (MF) and cellular component (CC) of oral cancer gene clusters respectively. The binomial test was applied for the construction and analysis of GO of given protein set. Majority of the hub proteins are involved in protein-binding activity, catalytic activity and transcription regulation process. In the gene-enrichment analysis, yellow nodes depict the GO categories that are

Table 2. The three significant clusters of oral cancer PPI network and their properties

	Nodes	8
	Edges	11
	Node proteins	GSK3B, PKM, LDHA, HIF1A, MYC, CTNNB1, TP53, SMAD3
	Seed node	GSK3B
	Seed proteins	GSK3B
	Seed MCODE score	1.33
	Nodes	4
	Edges	5
	Node proteins	PDPK1, AKT3, PIK3CA, ILK
	Seed node	PDKP1
	Seed proteins	PDPK1
	Seed MCODE score	1.79
	Nodes	3
	Edges	3
	Node proteins	UBC, E2F1, SKP2
	Seed node	UBC
	Seed proteins	UBC
	Seed MCODE score	0.48

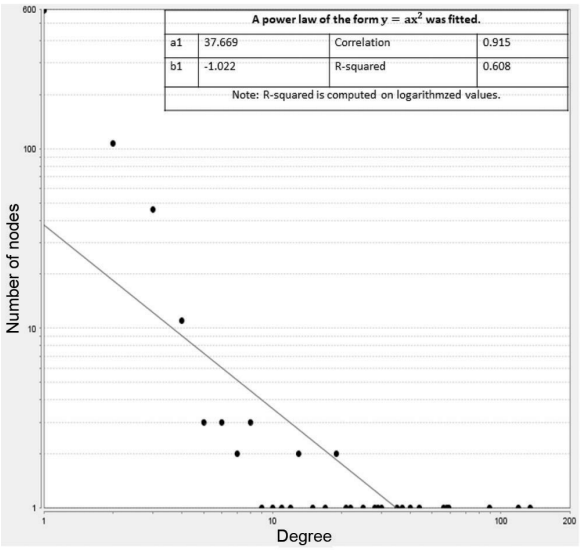


Figure 3. Degree centrality distribution curve of oral cancer PPI network.

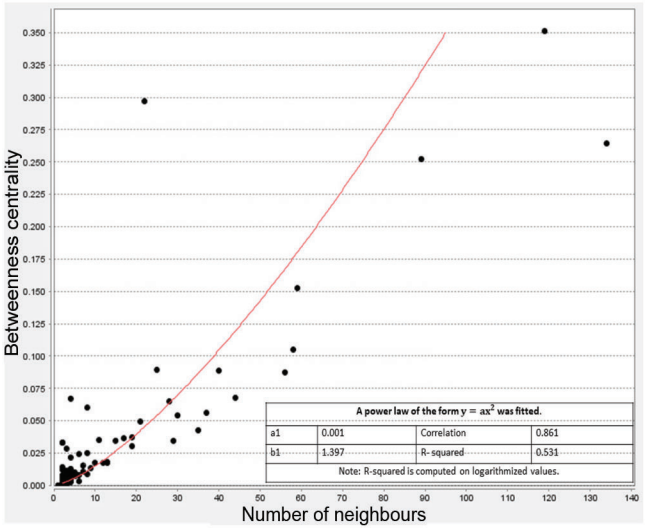


Figure 4. Betweenness centrality distribution curve of oral cancer PPI network.

overexpressed at significance level. It also shows that more significant the P-value, the gene node colours are increasingly more orange which indicates potential functional hub proteins.

Biological processes: GO study describes the main BPs involved in the oral cancer signalling pathway. These biological process analysis explain the role of particular genes highly involved in oral cacner related to biological activity. Table 3 shows the oral cancer genes involved in the BPs.

Molecular functions: These MF based analysis reveal the involvement of particular genes in molecular activity of oral cancer. The obtained oral cancer genes mainly participate in binding and catalytic activity of proteins which are essential in the oral cancer cell signalling process. Table 4 lists the cancer genes involved in oral cancer signalling pathways, as well as their MFs and how they are involved in cancer cell metastasis and progression.

Cellular component analysis: The CC analysis describes parts of the cell or its extracellular environment that plays

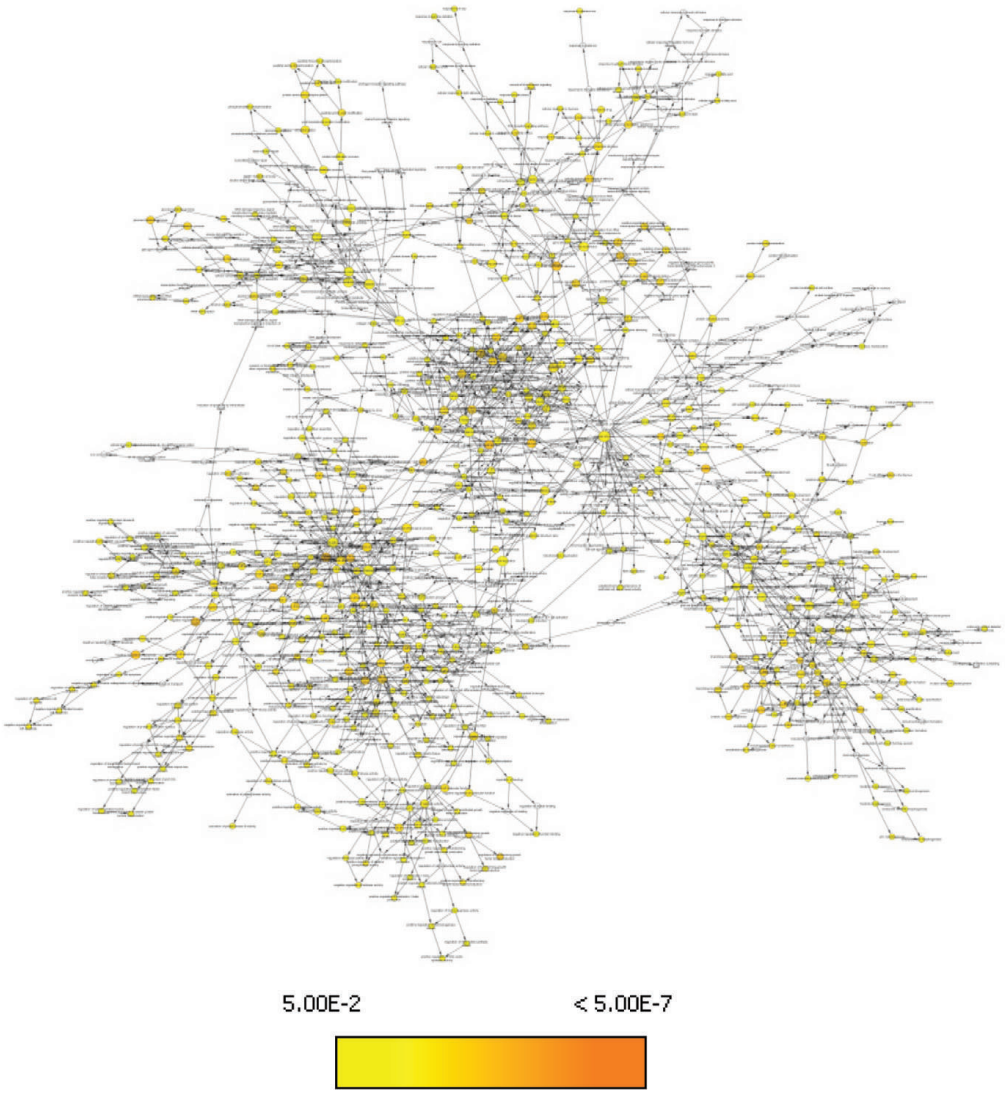


Figure 5. Biological processes of oral cancer PPI network obtained from BiNGO plug-in.

Table 3. Oral cancer genes involved in biological processes

Gene ontology (GO) description	*P*-value	Genes
Cell surface receptor linked signalling pathway	0.030882	*GSK3B*, *SMAD3*, *ILK*, *CTNNB1*
Centrosome organization	0.030864	*CTNNB1*
Regulation of dephosphorylation	0.030864	*SMAD3*
Positive regulation of protein complex assembly	0.030864	*GSK3B*
Regulation of protein kinase B signalling cascade	0.030864	*ILK*
Synaptic vesicle transport	0.030864	*CTNNB1*
Embryonic digit morphogenesis	0.030864	*CTNNB1*
Positive regulation of endothelial cell proliferation	0.030864	*HIF1A*
Positive regulation of signalling process	0.030016	*ILK*, *CTNNB1*
Regulation of catabolic process	0.030016	*SMAD3*, *HIF1A*

a significant role in cancer cell signalling. The GO analysis reveals that particular genes are involved in the particular types of CC. Table 5 shows the CCs are involved in the oral cancer signalling process, as well as the proteins involved in cancer cell signalling mechanism.

Discussion

The three gene clusters that play a crucial role in oral cancer research have been identified using systems biology approach. The first cluster predicts *GSK3B*, *PKM*, *LDHA*, *HIF1A*,

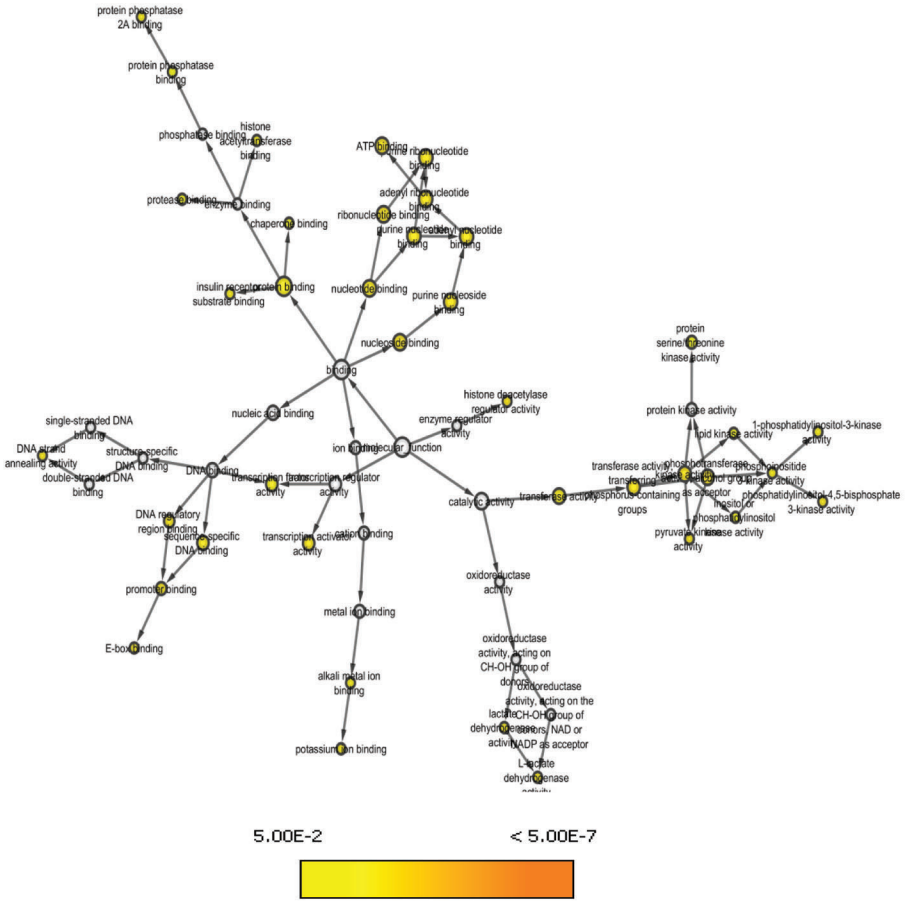


Figure 6. Molecular functions of oral cancer PPI network obtained from BiNGO plug-in.

Table 4. Oral cancer genes involved in molecular functions

GO description	*P*-value	Genes
Kinase binding	7.8284E-07	*GSK3B*, *SMAD3*, *PDPK1*, *CTNNB1*, *TP53*
Promoter binding	2.7055E-06	*SMAD3*, *MYC*, *CTNNB1*, *TP53*
DNA regulatory region binding	3.1029E-06	*SMAD3*, *MYC*, *CTNNB1*, *TP53*
Transcription factor binding	3.5408E-06	*GSK3B*, *SMAD3*, *E2F1*, *CTNNB1*, *HIF1A*, *TP53*
Enzyme binding	0.000010607	*GSK3B*, *SMAD3*, *PDPK1*, *CTNNB1*, *HIF1A*, *TP53*
Sequence-specific DNA binding	0.000010703	*SMAD3*, *MYC*, *E2F1*, *CTNNB1*, *HIF1A*, *TP53*
Protein kinase binding	0.000015068	*GSK3B*, *SMAD3*, *PDPK1*, *TP53*
Phosphotransferase activity, alcohol group as acceptor	0.000016234	*GSK3B*, *PKM*, *PIK3CA*, *PDPK1*, *AKT3*, *ILK*
Kinase activity	0.00002621	*GSK3B*, *PKM*, *PIK3CA*, *PDPK1*, *AKT3*, *ILK*
Transcription activator activity	0.000026833	*SMAD3*, *MYC*, *E2F1*, *CTNNB1*, *TP53*

MYC, *CTNNB1*, *TP53* and *SMAD3* as common connected nodes. In this cluster, the seed protein is GSK-3β. GSK-3β is a serine/threonine kinase involved in several physiological processes and is a highly conserved region that belongs to the CMGC family of protein kinases. GSK-3β belongs to the cyclin D1 family. Cyclin D1 belongs to potent proto oncogenes and has been expressed in various cancer pathways such as OSCC[25]. GSK-3β also shows serine/threonine kinase activity, which is prominently involved in the glycogen metabolic process[26]. TP53 is an important protein for cell apoptosis and oral cancer progression. LDH levels were studied as important biomarkers for cancer. The high levels of LDH involved in oral sub-mucous fibrosis indicate that it is responsible for tissue breakdown. Two types of LDH, viz. LDH-A and LDH-B are involved in metabolic interactions, metabolic fuel exchange and serve as potential targets for anticancer drug development. The downregulated level of HIF is responsible for the transcription of target cancer genes, which play an important role in the regulation of various cancer cell processes such as angiogenesis, cell proliferation, glucose metabolism, pH regulation and migration processes[27]. Increased levels of RAS,

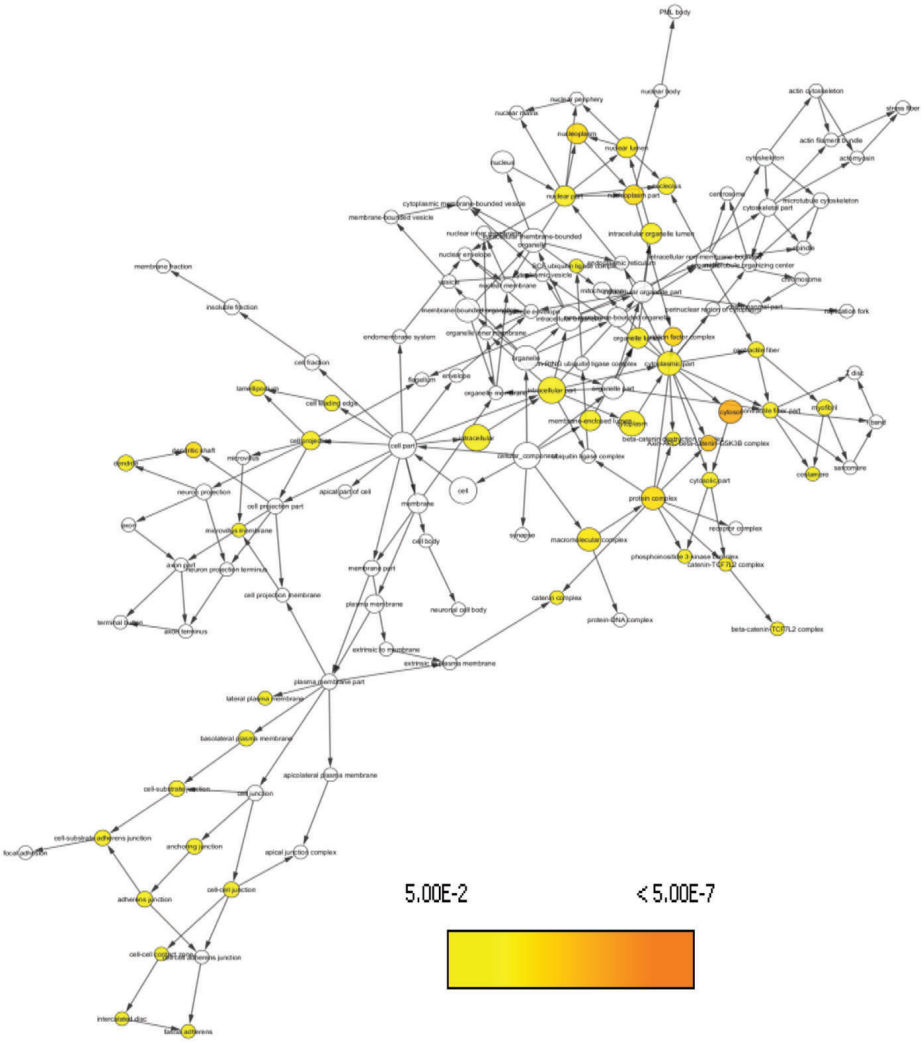


Figure 7. Cellular components of oral cancer PPI network obtained from BiNGO plug-in.

Table 5. Oral cancer genes involved in cellular components

GO description	*P*-value	Genes
Cytosol	1.9217E-07	*GSK3B*, *LDHA*, *SMAD3*, *PKM*, *PIK3CA*, *PDPK1*, *ILK*, *CTNNB1*, *TP53*
Axin–APC–beta-catenin–GSK3B complex	3.0488E-06	*GSK3B*, *CTNNB1*
Transcription factor complex	0.000019405	*SMAD3*, *E2F1*, *CTNNB1*, *HIF1A*
Protein complex	0.000059852	*GSK3B*, *SMAD3*, *PIK3CA*, *E2F1*, *ILK*, *CTNNB1*, *SKP2*, *HIF1A*, *TP53*
Nucleoplasm	0.000067641	*SMAD3*, *MYC*, *E2F1*, *CTNNB1*, *HIF1A*, *TP53*
Nucleoplasm part	0.000088053	*SMAD3*, *E2F1*, *CTNNB1*, *HIF1A*, *TP53*
Dendritic shaft	0.00010895	*ILK*, *CTNNB1*
Macromolecular complex	0.00028788	*GSK3B*, *SMAD3*, *PIK3CA*, *E2F1*, *ILK*, *CTNNB1*, *SKP2*, *HIF1A*, *TP53*
Nuclear lumen	0.00045816	*SMAD3*, *MYC*, *E2F1*, *CTNNB1*, *HIF1A*, *TP53*
Intracellular organelle lumen	0.0015036	*SMAD3*, *MYC*, *E2F1*, *CTNNB1*, *HIF1A*, *TP53*

p53 as well as C-MYC provide information on molecular-level changes in cancer cells. An increased level of c-myc is also a poor prognosis of oral squamous cell cancer. *C-myc* gene amplification has been reported in the head and neck cancers. Overexpressed levels of CDK-4 play an important role, either for gene amplification or in the progression of cancerous tumours through the c-myc regulated pathway. β-Catenin is a multifunctional protein which regulates the cell adhesion process by the e-cadherin mediated pathway. It is a target transcriptional transcription of the Wingless (Wnt) signalling pathway and the *APC* gene product which are involved in the initiation of various

types of cancer. Glucose metabolism mediated by PKM2 fuels overgrowth and proliferation[28]. Other evidences linking PKM2 to tumorigenesis also suggest that PKM2 decreases through cancer cell proliferation, induced cell apoptosis, RNAenference or pharmacological agents. The increased therapeutic sensitivity also decreases the tumorigenicity reported in *in vivo* and *in vitro* studies[29].

The second cluster consists of PDPK1, AKT3, PIK3CA and ILK cancer proteins. The seed protein of this cluster is PDPK-1, which is mainly involved in cell proliferation. Akt3 is an important component of the cancer pathway; its increased levels inhibit the tumour development process. It is also the major mediator of oncogenic PI3K signalling[30]. PIK3CA and PI3K contribute to the amplification and enhancement of p110α. Increase PI3K p110α and PIP3 are important for activating the PI3K/AKT signalling pathway. PIP3 is coupled to a C-terminal placestrin homology (PH) domain with phosphoinositide dependent protein kinase-1.

PIK3CA has been reported to participate in tumorigenesis and developmental process of cancer in the form of an oncogene[31]. Previous studies have shown that the PI3K/AKT signalling pathway was often activated by gene mutations to promote tumour growth. PIK3CA regulates PIP3 and is helpful in promoting PIP3 to activate PDK1. AKT activity is regulated by the PIP3 content, allowing its activation by activated membrane PDK1 that binds AKT to the cell membrane. Integrin-linked kinase (ILK) functions to regulate several key biological processes, including cell cycle, growth and angiogenesis; its high expression enhances tumorigenesis. The PDPK1/AKT pathway is helpful in controlling cell proliferation, apoptosis and migration in a variety of cancers[32].

The third cluster obtained in this study consists of UBC, E2F1, SKP2. The seed protein mainly involved in the protein degradation process is ubiquitin-C. The ubiquitin-C mediators are helpful in targeting a variety of oncogenic E3 ligase tumour proteins for degradation. Deregulation of E3 ligase is important in inducing an imbalance between the oncogenic signal and the tumour suppressor pathway. It leads to cellular label changes, tumour progression and metastasis in oral and other types of cancer[33]. E2f1 also plays a key role in the obtained cluster protein oral cancer factors. The transcription factor elicited by E2f1 is dominant in cancer-affected cell-cycle regulation and apoptosis process[34]. Skp2 is a member protein of the F-box family of substrate-recognition subunits of the SCF ubiquitin–protein ligase complex. It plays an important role in ubiquitin-mediated degradation of several regulators of mammalian G1 progression, including the cyclin-dependent kinase inhibitor p27. It is a dose-dependent tumour suppressor protein. Skp-2 and p-27 protein expression provides important information in oral cancer progression, including dysplasia and OSCC[35].

The GO study of 15 oral cancer targets was performed using the BiNGO tool. In the PPI networks, the oral cancer targets demonstrate a major contribution in BPs, CCs and MFs. The BP analysis of oral cancer targets reveals that these proteins play a key role in cell surface receptor linked signalling pathway, centrosome organization and regulation of dephosphorylation, etc. GSK3B, SMAD3, ILK and CTNNB1 are involved in the cell surface receptor linked signalling pathway of oral cancer. MF analysis reveals that the oral cancer target proteins are involved in kinase binding, promoter binding, DNA regulatory region binding, etc. Proteins GSK3B, SMAD3, PDPK1, CTNNB1 and TP53 are involved in the kinase binding of oral cancer BFs. The cytosol, axin-APC-beta–catenin-GSK3B complex and transcription factor complex are involved in the PPI network of oral cancer targets, according with CC analysis. The GSK3B, LDHA, SMAD3, PKM, PIK3CA, PDPK1, ILK, CTNNB1 and TP53 proteins are involved in cytosol CC in oral cancer.

Conclusion

Based on the systems biology approach, target potential cancer proteins that are responsible for the alteration of oral cancer pathways have been predicted. This analysis reveals 47 proteins that are common in head and neck cancer and oral cancer. The PPI-based study predicts that 15 potential proteins are involved in oral cancer PPI network construction. In this analysis, the proteins were predicted on the basis of significance value 0.05 and belonged to three different protein clusters. The predicted proteins mainly affect the whole signalling process of the cancer cells; so by activation or inhibition, it is easy to analyse the whole cancer cell signalling mechanism. The analysis is helpful in the development of new treatment strategies for oral cancer.

Based on network analysis and gene ontology analysis of 15 genes, it can be concluded that these genes play an important role in oral cancer development. The screened cancer targets play an important role in oral cancer biomarker development and computer-aided drug design.

Conflict of interest: The authors declare that they have no conflict of interest.

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