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Article

Explore novel biomarkers and associated key pathways to perform as potential prognostic biomarkers and therapeutic targets in Oral Cancer

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Abstract: Background: Oral cancer (OC) is serious health concerning issue that has a high fatality rate. The oral cavity has seven kinds of OC, including the lip, tongue, and floor of the mouth, as well as the buccal, hard palate, alveolar, retromolar trigone, and soft palate. The goal of this study is to look into new biomarkers and important pathways that might be used as diagnostic biomarkers and therapeutic candidates in OC.

Methods: Publicly available repository the Gene Expression Omnibus (GEO) was responsible to collect OC-related datasets. GSE74530, GSE23558, and GSE3524 microarray datasets were collected to apply analysis. Minimum cut-off criteria of $|\log$ fold-change (FC)$| > 1$ and adjusted $p < 0.05$ were applied to figure out the up-regulated and down-regulated differential expression genes (DEGs) from the three datasets. After that only common DEGs in all three datasets were collected to apply further analysis. Gene ontology (GO) and Pathway analysis were implemented to explore functional behaviors of DEGs. Then protein-protein interaction (PPI) networks were built to identify the most performed genes, clustering algorithm was also implemented to identify complex parts of PPI. TF-miRNA networks were also constructed to study deeply about OC-associated DEGs. Finally, top gene performers from PPI networks were used to apply drug signature analysis.

Results: After applying filtration and cut-off criteria 2508, 3377, and 670 DEGs were found for GSE74530, GSE23558, and GSE3524 respectively, and 166 common DEGs were found in every dataset. The GO annotation remarks that most of the DEGs were associated with the terms of type I interferon signaling pathway. The pathways of KEGG reported that the common DEGs are related

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with the Cell cycle and Influenza A. The PPI network holds 88 nodes and 492 edges and CDC6 had the highest number of connections. 4 clusters were identified from the PPI. Drug signatures doxorubicin and resveratrol showed high significance according to the hub genes. We anticipate that our bioinformatics research will aid in the definition of the pathophysiology and the development of new therapies for OC.

Keywords: Biomarkers; Drug Signature Identification; Key pathways; Oral Cancer; Oral Squamous Cell Carcinoma.

1. Introduction

OC, often known as mouth cancer, is the world's sixth most prevalent kind of cancer. OC refers to a tumor that develops in a part of the mouth, it may be found on the surface of the tongue, the inside of the cheeks, the roof of the mouth (palate), the lips, or gums. Among all the types of OC, oral squamous cell carcinoma (OSCC) is the most usual type (more than 90 percent of OC are OSCC) and lethal also [1]. OC develops from the oral mucosa and was responsible for over 350,000 new diagnoses and over 175,000 documented deaths globally in 2018 [2]. The classification of oral cavity cancer is based on seven subtypes in the oral cavity (lip, tongue, the floor of the mouth, buccal, hard palate, alveolar, retromolar trigone, and soft palate) [3]. OC is more common due to ethnic variations and sociocultural risk factors such as chewing tobacco leaves, alcohol intake, reverse smoking, HPV infection, and lower eating of fruits and vegetables [4-7]. The location of involvement, the timing of diagnosis, and the stage of the tumor all influence the prognosis and therapy [8]. In patients with head and neck cancer, the stage at the time of diagnosis is the most important predictor of survival [9]. A high proportion of oral malignancies are detected late, as is to be expected. Delayed diagnosis raises costs, increases invasiveness, complicates therapy and reduces survival and quality of life [10, 11]. The signs and symptoms of oral cancer vary depending on where the tumor is located, but they usually appear as thin, uneven white spots in the mouth [12]. It's also possible for them to be a combination of red and white spots. A persistent rough area with ulceration and a raised border that is mildly unpleasant is the traditional warning sign. The ulcer is more usually crusting and dry on the lip, and it is more commonly a mass in the throat. A white patch, loose teeth, bleeding gums, chronic earache, numbness in the lip and chin, and swelling are all possible symptoms [13]. OC claimed the lives of 135,000 people in 2013, up from 84,000 in 1990. In 2018, it struck roughly 355,000 individuals all over the world for the first time, resulting in 177,000 fatalities, including 246,000 men and 108,000 women [14]. People from low- and middle-income nations are more likely to get oral cancer [15]. As of 2015, the overall 5-year survival rate for oral cancer in the United States was 65%. This ranges from 84 percent when it is confined to 66 percent when it has progressed to lymph nodes in the neck and 39 percent when it has gone to distant areas of the body. The location of the illness in the mouth also influences survival chances. In 2017 Nasibeh Khayer, Mona Zamanian-Azodi et al. published a research article where the author reveals the protein interaction network, hub genes including TP53, AKT1, EGFR, MYC, JUN, CDH1, CCND1, and CTNNB1, and clustering algorithm analysis for Oral squamous cell cancer associated esophageal adenocarcinoma [16]. Another research article, Fengxue Meng, Qingxuan Wang, et al. published in 2019 where they proposed a network-based study about Oral Cancer in Response to Chronic Infection with Porphyromonas gingivalis. In this study, the authors analyzed protein interaction networks, hub genes, ontology analysis, etc. The author has been extracted STAT1, CXCL10, MX1, IFIT1, GBP1, IL6, OAS1, MAPK12, LYN, and IFI35 genes as hub genes [17]. A research study has been published in a reputed journal in 2020 on the Fusobacterium nucleatum for revealing genes associated with oral cancer. This study has worked on different analyses such as PPI network construction and analysis, hub genes identification, functional analysis, etc. In this study, the author proposed 10 hub genes including JAK2, FYN, RAF1, FOS, CREB, ATM, NCOA3, VEGFA, CREM, and ATF3 [18].

In this study, a comprehensive network-based approach has been established to reveal the gene expression pattern influence of OC and how these are could affect to promote other disorders. In the beginning, we peruse various gene expression patterns of three datasets and after filtration of minimum criteria, all common genes have been taken for further analysis. The protein interaction network is the major outcome of this study based on which hub genes, GO terms, disease pathways,

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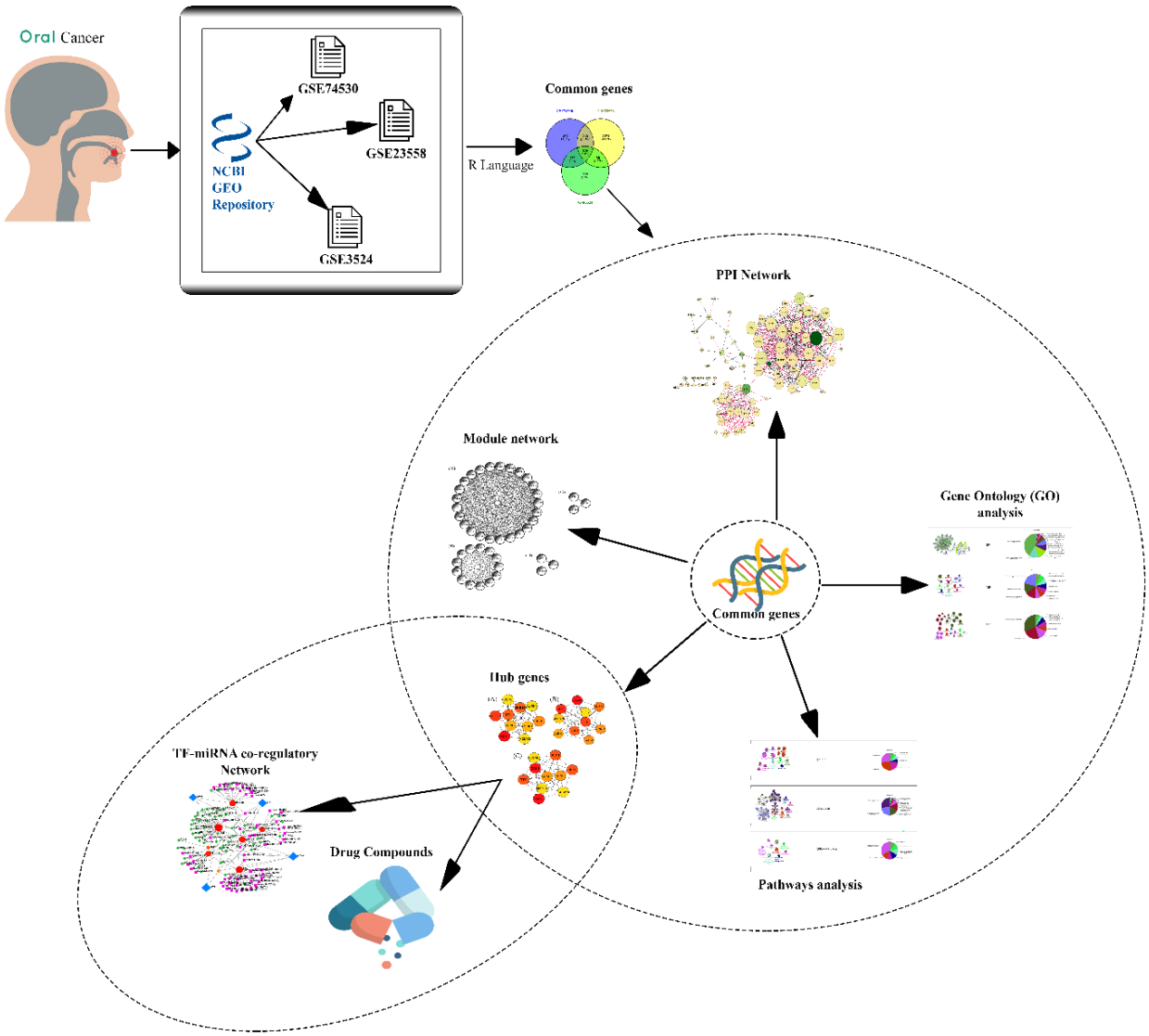
and cluster analysis have been employed. All the footprints of the study are demonstrated in **Figure 1.**

Figure 1. Image workflow of this study methodologies. A transcriptomic comparative analysis has been performed between the datasets of OC. Three datasets have been collected for OC and these datasets were filtered to normalize datasets and identify shared DEGs. Using shared genes has been performed for further transcriptomic analysis.



2. Work Strategies

2.1. Dataset consideration and DEGs identification

GEO [19] (https://www.ncbi.nlm.nih.gov/geo/) database has been used to extract the microarray datasets. GEO is a publicly accessible gene expression collection with over 94 000 datasets and over 2 million samples [20], which was founded by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine [21-23]. Three datasets including accession numbers GSE74530, GSE23558, and GSE3524 were extracted for the OC, all the datasets belong to microarray. GSE74530 dataset stand on a single platform GPL570 [HGU133_Plus_2] Affymetrix Human Genome U133 plus 2.0 Array for Homo sapiens. This dataset conducts 6 normal tissue and 6 tumor tissue. GSE23558 dataset also depends on the GPL6480 platform, Agilent-014850 Whole Human Genome Microarray 4x44K G4112F which conducts 27 tumor tissue and 5 normal tissue. GSE3524 dataset conduct 16 tumor tissue and 4 normal tissue which also stands on a single platform GPL96 [HG-U133A] Affymetrix Human Genome U133A Array. Three datasets have been filtered with the

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minimum criteria adjusted p-value < 0.05 and logFc ± 1 and also made a comparison method to identify the common genes between the three datasets using online Venny toll (http://bioinformatics.psb.ugent.be/webtools/Venn/) [24].

2.2. GO and pathways enrichment analysis

The biological activities of the common DEGs were evaluated by the functional analysis. To complete the functional analysis the GO and pathways analysis have been performed through clueGO. ClueGO is a Cytoscape App that pulls typical functional biological information from long lists of genes or proteins. The functional enrichment study is based on the most recent publicly accessible data from several annotations and ontology resources, which ClueGO can automatically retrieve. To make the analysis easier, predefined options for term selection are supplied. The results are shown as networks in which GO terms and pathways are classified according to their biological function [25]. GO terms are divided into three stages, biological process (BP), Molecular function (MF), Cellular component (CC). Three databases KEGG, Wikipathways, and Reactome have been used to extract the pathways-related data.

2.3. PPI network construction and cluster algorithm implementation

The PPI network is the picturesque representation of the protein-protein interaction by edges which helps to identify the critical genes and potential biomarkers [26]. The protein network was constructed based on the physical connections between the proteins for datasets of OC from the STRING database [27] through the NetworkAnalyst free web tool [28]. To develop the PPI network the minimum cutoff confidence score of 0.70 was used. Cytoscape is a free and open-source network visualization, data integration, and analysis software tool. Its research and implementation have mostly focused on the modeling demands of systems biology, but it has also been used in other fields [29]. The MCODE plugin [30] tool has been used to identify the complex network area of the PPI network where the basic parameter was degree cutoff =2, k-core = 2, maximum depth = 100, and node score cutoff = 0.2.

2.4. Hub genes identification and analysis

Hub genes are a highly connected protein in the PPI network that can play a significant role to identify the potential therapeutic biomarker [31]. The three most popular and effective algorithms including Degree [32], Maximal Clique Centrality (MCC) [33], Maximum Neighborhood Component (MNC) [34] have been used to identify the significant genes from the PPI network by the cytoHubba plugin tool of Cytoscape.

2.5. Computational drug signature identification and analysis

Therapeutic targets for chosen hub DEGs were identified using the Drug Signatures Database (DSigDB) [35]. The DSigDB is a new gene set repository for gene set enrichment analysis, which connects medicines and chemicals to their target genes (GSEA). DSigDB presently includes 22527 gene sets with 17389 distinct compounds covering 19531 genes in each. The DSigDB database allows users to search for, browse through, and download medications, chemicals, and gene sets. For drug repurposing and translational research, DSigDB gene sets may be used in GSEA software to correlate gene expression to drugs/compounds [35]. P-value 0.01 and an overlap gene count of >= 9 were used as cut-off criteria for finding pharmaceutical targets.

2.6. TF-miRNA co-regulatory network and analysis

The TF-miRNA co-regulatory network has been extracted from the RegNetwork repository (http://www.regnetworkweb.org/) that may play a significant role to identify novel information about the OC. MicroRNA (miRNA) is a form of non-coding RNA molecule that regulates gene expression after it has been transcribed. MiRNAs have a crucial role in tumor growth, differentiation, and apoptosis, according to recent research [36, 37]. The targets of miRNAs have been linked to tumor development in oral cancer in a number of studies [38]. Some miRNAs are antiapoptotic, whereas others are apoptotic promoters. Transcription factors (TFs) are frequent regulators of genes.

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They are primarily responsible for transcriptional regulation. Cancer subtypes may be identified using data from networks of miRNAs, TFs, and mRNAs. This information sheds light on the processes that control each cancer subtype.

3. Result

3.1. 166 common genes were found

Three datasets have been extracted using the GEO repository from the NCBI open-source database. Initially, GSE74530, GSE23558, and GSE3524 datasets showed 22187, 19563, and 9617 DEGs respectively. Afterward, filtration with minimum criteria 2508, 3377, and 670 DEGs were found for GSE74530, GSE23558, and GSE3524 respectively. A comparative analysis has been used to identify the common DEGs, a total of 166 common DEGs was found [Figure 2].

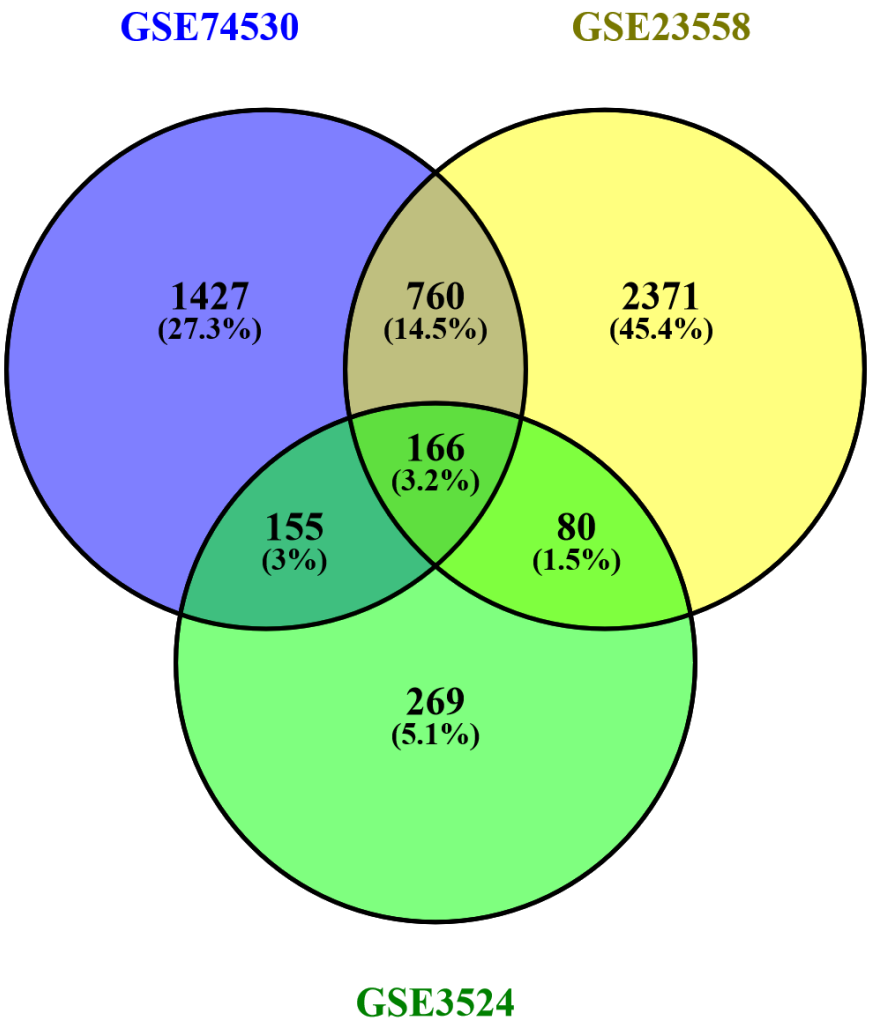

Figure 2. The gene expression datasets of OC were analyzed to identify the common differentially expressed genes (DEGs) between the datasets. A total of 166 genes were regarded as the common DEGs between the datasets of OC.

3.2. The ClueGO analysis for gene ontology and pathways enrichment

The GO annotation remarks that the terms of type I interferon signaling pathway, type I interferon signaling pathway, cellular response to type I interferon, response to type I interferon, mitotic sister

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chromatid segregation, sister chromatid segregation, regulation of mitotic metaphase/anaphase transition, etc. are highly significant in BP. Also, the terms spindle, mitotic spindle, chromosome, centromeric region, kinetochore, chromosomal region, spindle midzone, condensed chromosome, contractile actin filament bundle, stress fiber, condensed chromosome kinetochore, etc. are highly associated with the CC. The MF is also related to the terms that are peptidase activator activity, integrin binding, ATP-dependent microtubule motor activity, peptidase activator activity involved in the apoptotic process, microtubule motor activity, transmembrane receptor protein tyrosine kinase activity, motor activity, DNA replication origin binding, non-membrane spanning protein tyrosine kinase activity, chaperone binding, etc. [Table 1, Figure 3] On the other hand, the pathways of KEGG reported that the common DEGs are connected with the Cell cycle, Influenza A, Progesterone-mediated oocyte maturation, Epstein-Barr virus infection, Oocyte meiosis, Measles, Serotonergic synapse, Hepatitis C, ECM-receptor interaction, Small cell lung cancer, etc. Also, the Reactome pathways showed the common DEGs are mostly connected with Expression of IFN-induced genes, CDK1 phosphorylates CDCA5 (Sororin) at centromeres, Formation of Cyclin B: Cdc2 complexes, CDK1: CCNB phosphorylates, CDK1 phosphorylates, ISGylation of host proteins, etc. The Wikipathways reveals the common DEGs were associated with the Cell Cycle, Overview of nanoparticle effects, Non-genomic actions of 1,25 dihydroxy vitamin D3, DNA Replication, Type II interferon signaling (IFNG), The human immune response to tuberculosis, Type I Interferon Induction and Signaling During SARS-CoV-2 Infection, Host-pathogen interaction of human coronaviruses - Interferon induction, etc [Table 2, Figure 4]

Table 1. Significant gene ontological terms of the common DEGs associated with the common DEGs. The table consists of five attributes including GO ID, GO Terms, Terms P-Value, Group P-Value, and Terms. The measures of Terms P-Value is less than 0.05 have taken to select the important GO terms.

GO ID	GO Terms	Terms P-Value	Group P-Value	Categories
GO:0060337	type I interferon signaling pathway	1.809E-13	4.480E-07	BP
GO:0071357	cellular response to type I interferon	2.095E-13	4.480E-07	BP
GO:0034340	response to type I interferon	4.251E-13	4.480E-07	BP
GO:0000070	mitotic sister chromatid segregation	3.548E-10	2.742E-06	BP
GO:0000819	sister chromatid segregation	3.930E-09	2.742E-06	BP
GO:0030071	regulation of mitotic metaphase/anaphase transition	4.689E-09	1.616E-07	BP
GO:0007091	metaphase/anaphase transition of mitotic cell cycle	6.237E-09	1.616E-07	BP
GO:0140014	mitotic nuclear division	7.428E-09	2.742E-06	BP
GO:1902099	regulation of metaphase/anaphase transition of cell cycle	8.216E-09	1.616E-07	BP
GO:0010965	regulation of mitotic sister chromatid separation	9.397E-09	1.616E-07	BP
GO:0044784	metaphase/anaphase transition of cell cycle	1.072E-08	1.616E-07	BP
GO:0051306	mitotic sister chromatid separation	1.221E-08	1.616E-07	BP
GO:1905818	regulation of chromosome separation	2.556E-08	1.616E-07	BP
GO:0005819	spindle	2.740E-11	4.636E-10	CC
GO:0072686	mitotic spindle	1.981E-07	4.636E-10	CC
GO:0000775	chromosome, centromeric region	2.277E-07	4.133E-08	CC
GO:0000776	kinetochore	4.474E-07	4.133E-08	CC
GO:0098687	chromosomal region	7.160E-07	4.133E-08	CC
GO:0051233	spindle midzone	7.456E-07	4.636E-10	CC

GO:0000779	condensed chromosome, centromeric region	9.731E-07	4.133E-08	CC
GO:0097517	contractile actin filament bundle	2.813E-06	1.143E-05	CC
GO:0001725	stress fiber	2.813E-06	1.143E-05	CC
GO:0000777	condensed chromosome kinetochore	4.721E-06	4.133E-08	CC
GO:0032432	actin filament bundle	5.672E-06	1.143E-05	CC
GO:0042641	actomyosin	6.158E-06	1.143E-05	CC
GO:0000228	nuclear chromosome	1.917E-05	4.133E-08	CC
GO:0016504	peptidase activator activity	3.497E-05	3.497E-05	MF
GO:0005178	integrin binding	6.853E-05	6.853E-05	MF
GO:1990939	ATP-dependent microtubule motor activity	2.390E-04	9.072E-04	MF
GO:0016505	peptidase activator activity involved in apoptotic process	6.662E-04	3.497E-05	MF
GO:0003777	microtubule motor activity	7.133E-04	9.072E-04	MF
GO:0004714	transmembrane receptor protein tyrosine kinase activity	7.391E-04	1.067E-03	MF
GO:0003774	motor activity	9.072E-04	9.072E-04	MF
GO:0003688	DNA replication origin binding	1.635E-03	1.635E-03	MF
GO:0004715	non-membrane spanning protein tyrosine kinase activity	1.979E-03	1.067E-03	MF
GO:0051087	chaperone binding	2.370E-03	2.370E-03	MF
GO:0043394	proteoglycan binding	5.449E-03	5.449E-03	MF
GO:0003725	double-stranded RNA binding	6.270E-03	6.270E-03	MF

Table 2. Significant pathways terms of the common DEGs associated with the common DEGs. The table consists of five attributes including Pathway ID, Pathway Terms, Terms P-Value, Group P-Value, and Database. The measures of Terms P-Value is less than 0.05 have taken to select the important pathways terms.

Pathways ID	Pathways Terms	Terms P-Value	Group P-Value	Database
KEGG:04110	Cell cycle	8.389E-07	5.580E-05	KEGG
KEGG:05164	Influenza A	9.510E-05	7.551E-05	KEGG
KEGG:04914	Progesterone-mediated oocyte maturation	1.017E-04	5.580E-05	KEGG
KEGG:05169	Epstein-Barr virus infection	3.345E-04	7.551E-05	KEGG
KEGG:04114	Oocyte meiosis	4.923E-04	5.580E-05	KEGG
KEGG:05162	Measles	7.698E-04	7.551E-05	KEGG
KEGG:04726	Serotonergic synapse	1.544E-03	1.544E-03	KEGG
KEGG:05160	Hepatitis C	1.570E-03	7.551E-05	KEGG
KEGG:04512	ECM-receptor interaction	2.652E-03	3.054E-03	KEGG
KEGG:05222	Small cell lung cancer	3.218E-03	3.054E-03	KEGG
KEGG:05146	Amoebiasis	5.007E-03	3.054E-03	KEGG
KEGG:00350	Tyrosine metabolism	6.907E-03	6.907E-03	KEGG
KEGG:04115	p53 signaling pathway	8.122E-03	5.580E-05	KEGG
R-HSA:1015702	Expression of IFN-induced genes	4.799E-13	4.799E-13	Reactome

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R-HSA:2468287	CDK1 phosphorylates CDCA5 (Sororin) at centromeres	5.845E-07	7.830E-07	Reactome
R-HSA:170057	Formation of Cyclin B:Cdc2 complexes	1.058E-06	8.867E-05	Reactome
R-HSA:170055	Myt-1 mediated phosphorylation of Cyclin B:Cdc2 complexes	4.201E-06	8.867E-05	Reactome
R-HSA:170161	Dephosphorylation of cytoplasmic Cyclin B1/B2:phospho-Cdc2 (Thr 14, Tyr 15) complexes by CDC25B	4.201E-06	8.867E-05	Reactome
R-HSA:2984220	CDK1:CCNB phosphorylates NEK9	4.201E-06	8.867E-05	Reactome
R-HSA:4086410	CDK1 phosphorylates BORA	4.201E-06	8.867E-05	Reactome
R-HSA:9624800	CDK1 phosphorylates LBR	4.201E-06	8.867E-05	Reactome
R-HSA:1678841	Regulation of protein ISGylation by ISG15 deconjugating enzyme USP18	4.921E-06	4.196E-07	Reactome
R-HSA:1169406	ISGylation of host proteins	1.325E-05	4.196E-07	Reactome
R-NUL:2422970	Phosphorylation of Gorasp1, Golga2 and RAB1A by CDK1:CCNB	3.594E-05	8.867E-05	Reactome
R-HSA:179410	Association of Nek2A with MCC:APC/C	5.539E-05	4.052E-07	Reactome
WP:179	Cell Cycle	1.024E-04	1.024E-04	Wikipathways
WP:3287	Overview of nanoparticle effects	1.430E-03	1.430E-03	Wikipathways
WP:4240	Regulation of sister chromatid separation at the metaphase-anaphase transition	3.225E-05	3.225E-05	Wikipathways
WP:4341	Non-genomic actions of 1,25 dihydroxyvitamin D3	1.589E-03	1.589E-03	Wikipathways
WP:466	DNA Replication	1.562E-03	1.562E-03	Wikipathways
WP:619	Type II interferon signaling (IFNG)	7.336E-05	7.336E-05	Wikipathways
WP:4197	The human immune response to tuberculosis	2.525E-03	2.864E-04	Wikipathways
WP:4868	Type I Interferon Induction and Signaling During SARS-CoV-2 Infection	3.733E-04	2.864E-04	Wikipathways
WP:4880	Host-pathogen interaction of human corona viruses - Interferon induction	6.200E-04	2.864E-04	Wikipathways

3.3. PPI network analysis and cluster algorithm implementation

The PPI network is the most significant outcome of this research study. The STRING database has been used to construct the PPI network which is modified by the Cytoscape application. The PPI network stands on a total of 88 nodes and 492 edges where nodes represent the genes and edges represent the connection between the genes [Figure 5]. In addition, the MCODE algorithm has been implemented to identify the complex network area of the PPI network. There are four complex networks (modules) that have been reported by the MCODE plugin algorithm [Figure 6]. The first module is built by 26 nodes and 269 edges where most of the hub genes are interconnected, the second module conducted 13 nodes and 70 edges, the third and fourth modules both stand on the three nodes and three edges.

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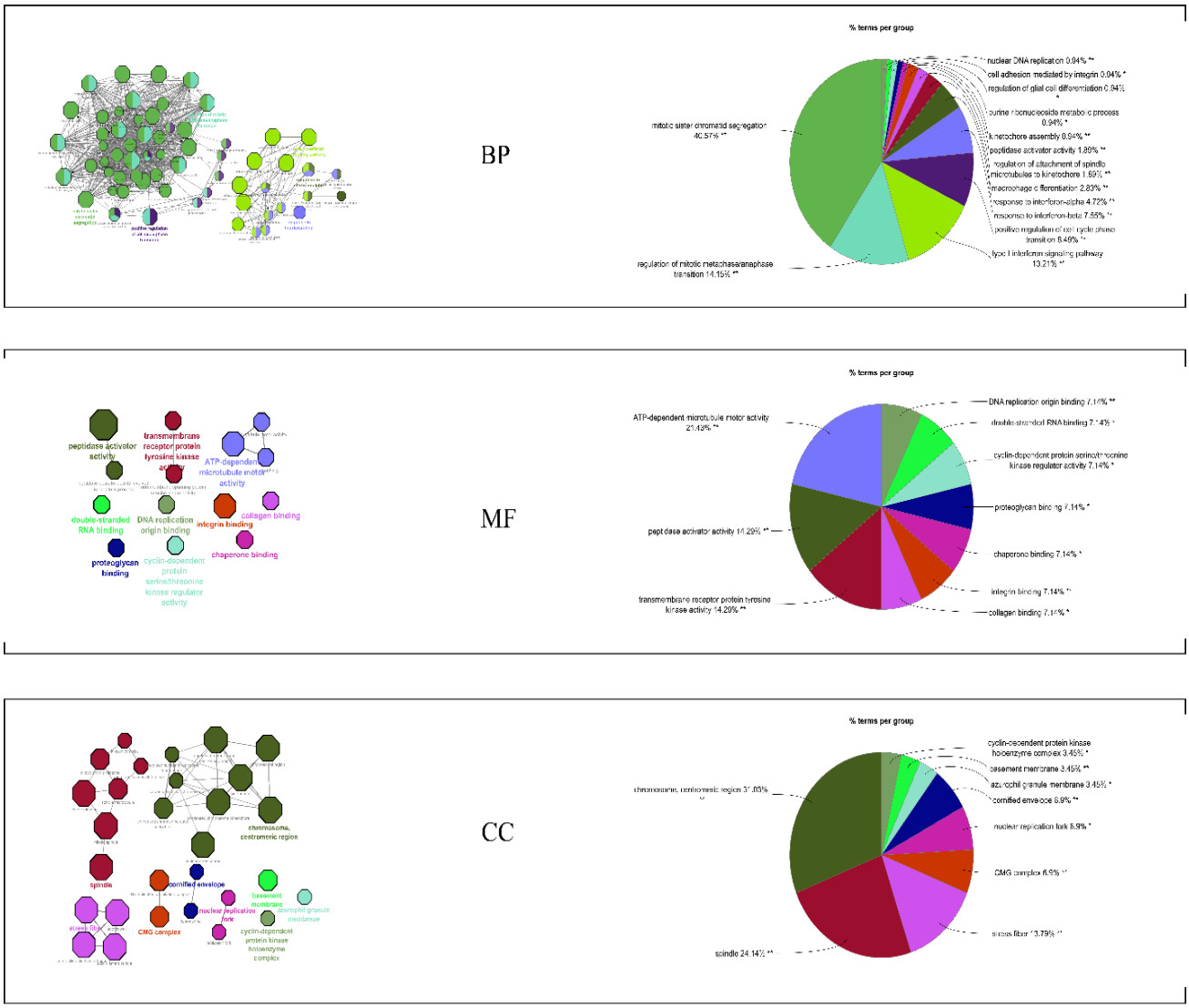

Figure 3. Gene ontological analysis for biological process (BP), cellular component (CC), molecular function (MF). A group-wise terms analysis has been used to observe the GO annotation. On the left side of the figure, different groups of terms consist of different colors. The nodes refer to the terms and edges refer to the connections between the terms of groups. On the right side of the figure, the pie chart indicates the percent-wise evaluation of the terms.

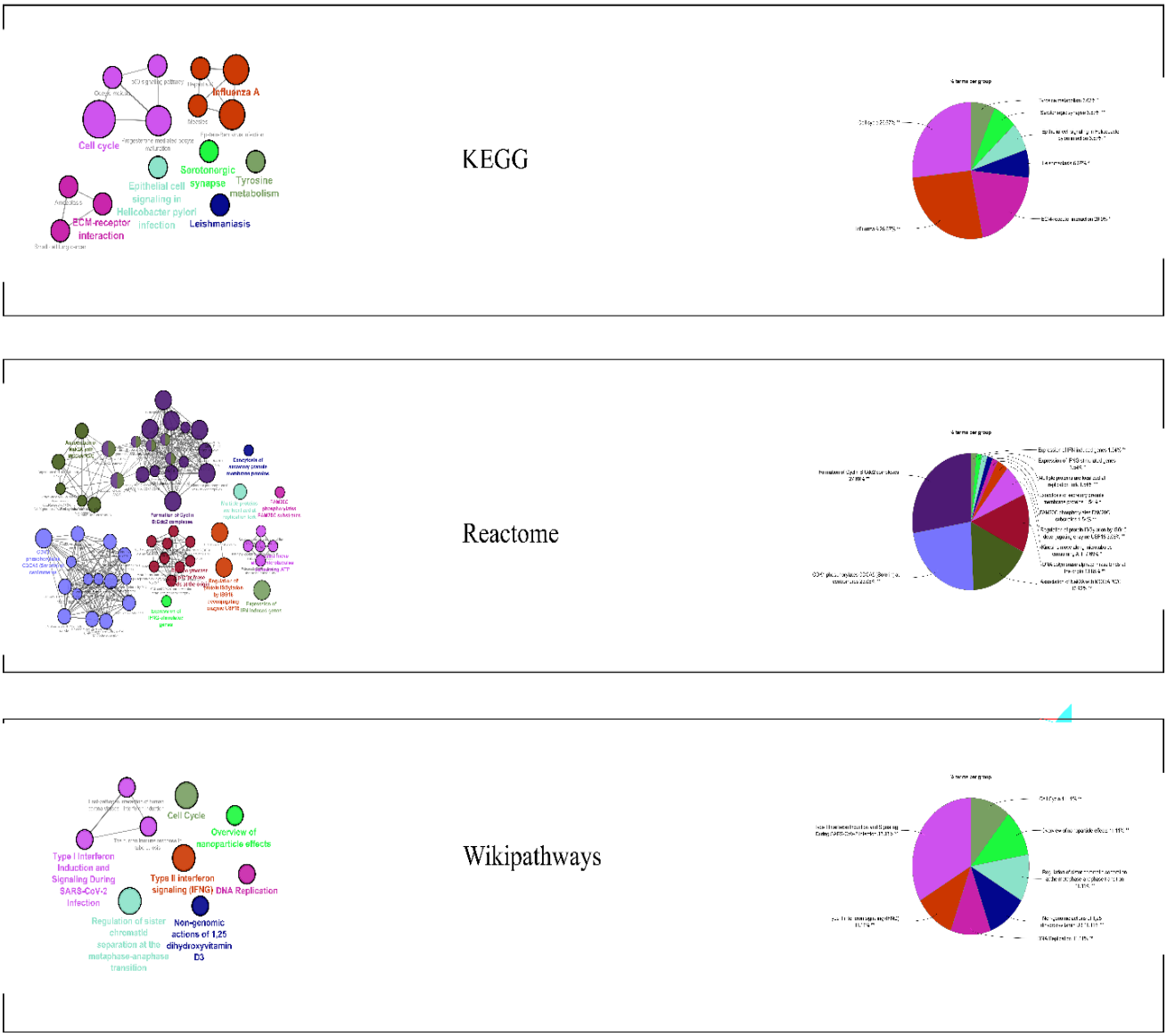

Figure 4. Pathways enrichment analysis observes for KEGG, Reactome, and Wikipathways. A group-based pathways analysis has been used to observe the pathway's connectivity with the genes. On the left side of the figure, different groups of pathways consist of different colors. The nodes refer to the pathways and edges refer to the connections between the groups of pathways. On the right side of the figure, the pie chart indicates the percent-wise evaluation of the pathways terms.

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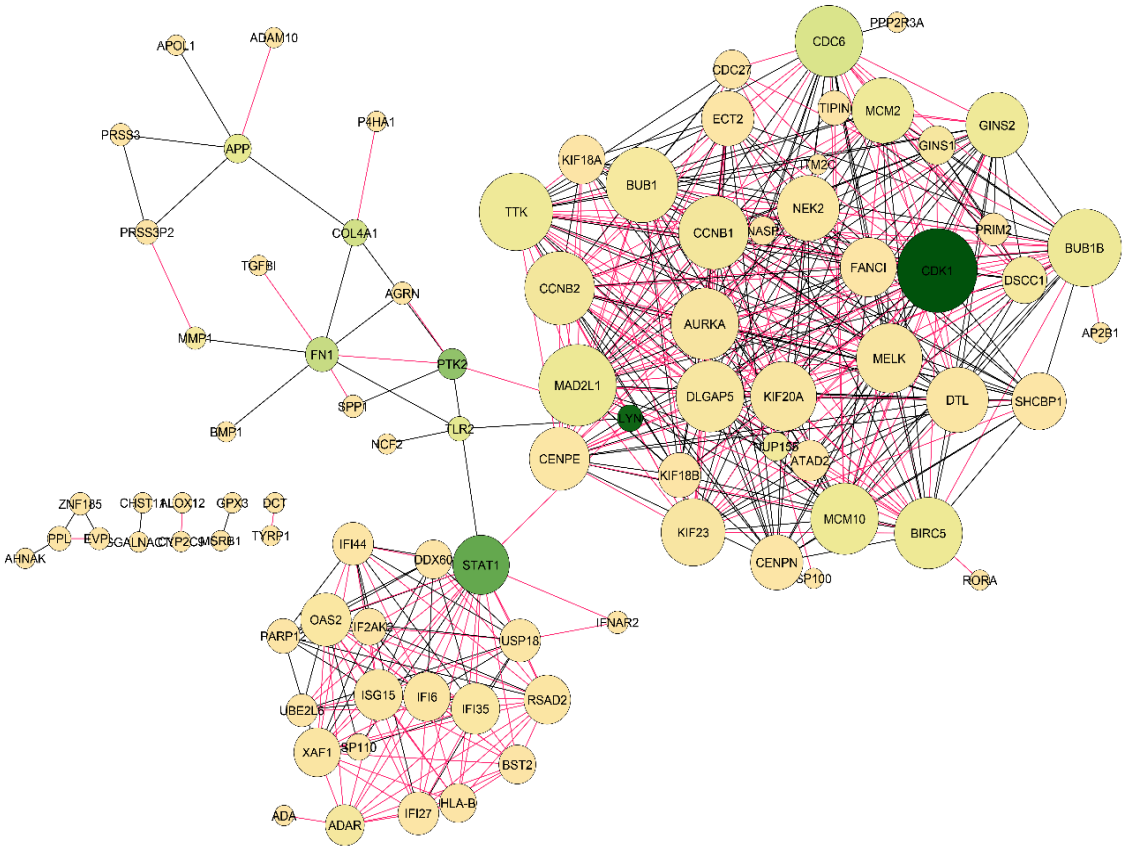

Figure 5. The protein interaction network has been created with nodes and their connections. In the figure, the sizes of the nodes have been stand on their degree (connectivity) value. The network consists a total of 88 nodes and 492 edges. According to the figure CDK1, and MAD2L1 are highly connected proteins of the network.

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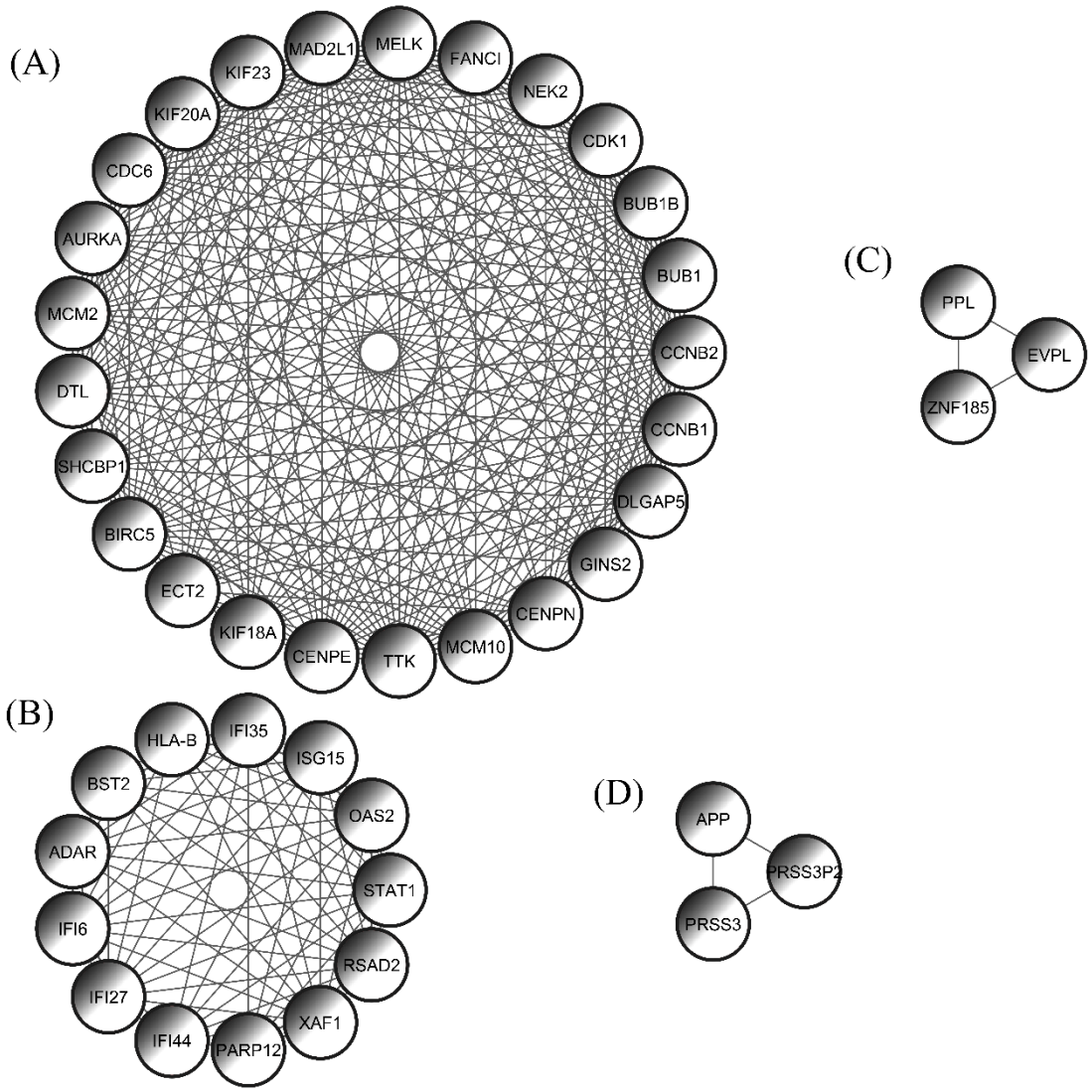

Figure 6. There are 4 protein complex networks (module) that have been observed through the MCODE plugin tool. The first module was built on 25 nodes and 269 edges where most of the hub genes are interconnected. The second module consists of 13 nodes and 70 edges. The third and fourth modules both of consist 3 nodes.

3.4. CDK1, MAD2L1 significant hub genes

To characterize the hub DEGs three algorithms of the cytoHubba app including Degree, MCC, MNC have been implemented, although all algorithms reported almost the same results. The Degree algorithms report CDC6, CCNB1, MCM10, BUB1, CCNB2, TTK, MAD2L1, CDK1, BUB1B, and AURKA as the top ten hub genes. The MCC method showed CCNB1, BUB1, CCNB2, TTK, DLGAP5, MAD2L1, KIF20A, CDK1, BUB1B, and AURKA as the top ten hub genes. On the other hand, the MNC method reveals the top ten hub genes as follows CCNB1, MCM10, BUB1, CCNB2, TTK, DLGAP5, MAD2L1, CDK1, BUB1B, and AURKA [Table 3, Figure 7]. Afterward, a comparison method has been applied and identified the common hub genes as following CCNB1, BUB1, CCNB2, TTK, MAD2L1, CDK1, BUB1B, and AURKA from all the hub genes reported by the Degree, MCC, and MNC method.

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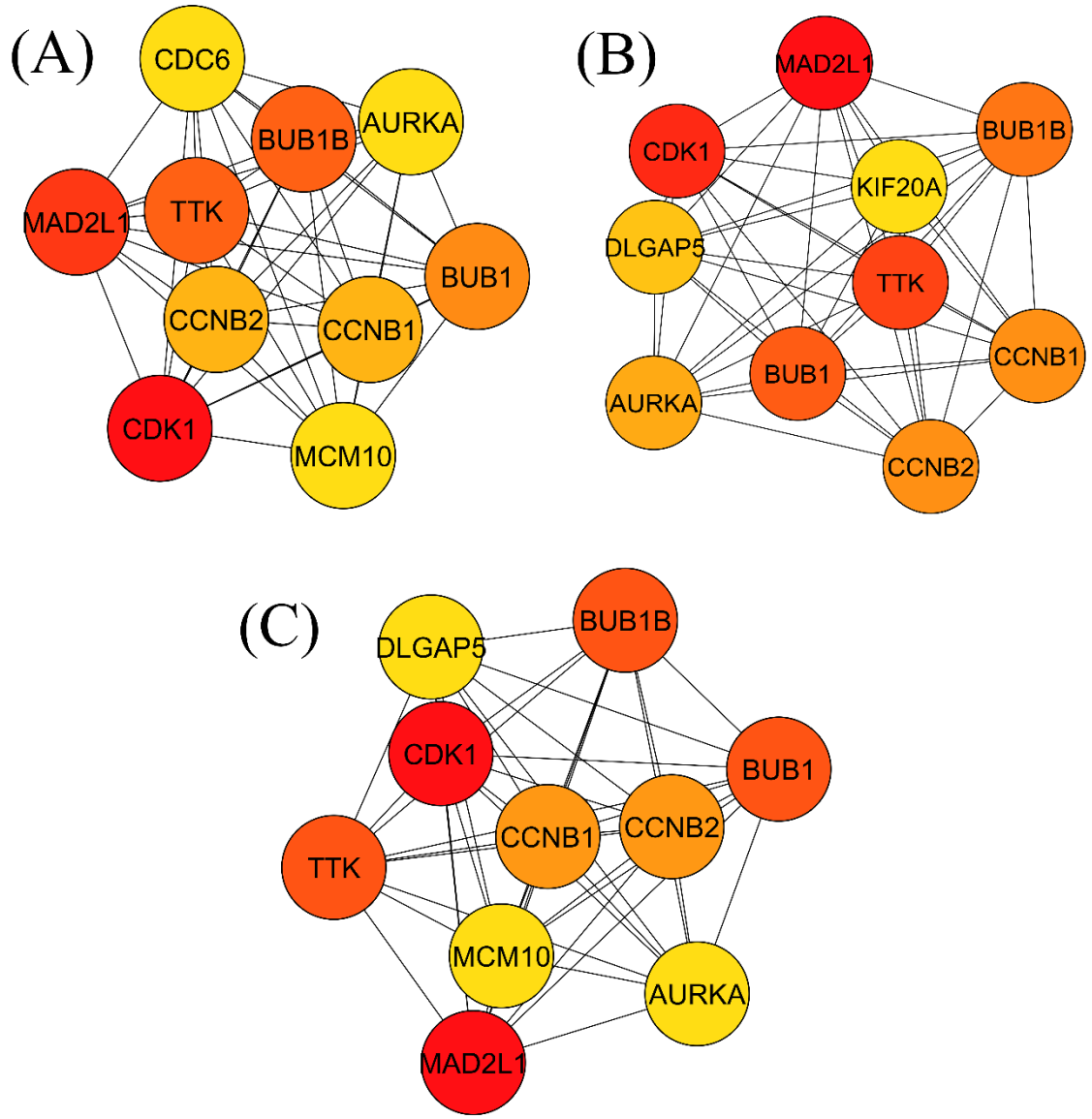

Figure 7. Three methods (Degree, MNC, and MCC) have been used to identify the hub genes. Three methods have shown different hub genes, between them a comparison method has been deployed to identify common genes and the eight common genes selected as hub genes as following CCNB1, BUB1, CCNB2, TTK, MAD2L1, CDK1, BUB1B, and AURKA.

3.5. Doxorubicin and resveratrol significant drug signature

The DSigDB database has been used to uncover the computational drug signature for the hub genes. There are many signatures that showed significant connectivity with the hub genes [Table 4]. From them, doxorubicin and resveratrol showed high significance. Doxorubicin is a commonly utilized therapy drug for many forms of cancer, according to a large number of research studies [39-42]. This medication binds to DNA and inhibits topoisomerase II activity, preventing the DNA double helix from resealing and stopping replication. Long-term replication halting triggers molecular pathways that lead to cell demise. Long-term replication halts, which trigger molecular pathways that lead to cell death. While it is an effective anticancer drug, its usage has been limited because of the accompanying adverse effects, which include permanent myocardial damage and deadly congestive heart failure [42].

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Table 3. Significant hub genes according to their degree value. CDK1 (cyclin-dependent kinase 1) and MAD2L1 (MAD2 mitotic arrest deficient-like 1 (yeast)) are the most significant genes.

Hub genes	Name	Degree
CDK1	cyclin dependent kinase 1	32
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	31
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	29
TTK	TTK protein kinase	29
BUB1	BUB1 mitotic checkpoint serine/threonine kinase	28
CCNB1	cyclin B1	27
CCNB2	cyclin B2	27
AURKA	aurora kinase A	26

Table 4. Significant therapeutic computational drug target analysis shows the most important drug targets are doxorubicin and resveratrol who are associated with most of the hub genes. According to the table, the doxorubicin is connected with 7 hub genes, and resveratrol is linked with 8 hub genes.

Targets	Overlap	P-value	Adjusted P-value	Genes
doxorubicin CTD 00005874	7	7.85E-10	2.87E-08	CCNB2;CCNB1;CDK1;BUB1B;TTK;MAD2L1;AURKA
resveratrol CTD 00002483	8	1.66E-09	5.00E-08	CCNB2;CCNB1;CDK1;BUB1B;TTK;BUB1;MAD2L1;AURKA
COUMESTROL CTD 00005717	8	4.48E-09	1.09E-07	CCNB2;CCNB1;CDK1;BUB1B;TTK;BUB1;MAD2L1;AURKA
Enterolactone CTD 00001393	7	4.77E-09	1.11E-07	CCNB2;CCNB1;CDK1;BUB1B;TTK;MAD2L1;AURKA
paclitaxel CTD 00007144	6	5.84E-09	1.30E-07	CCNB2;CCNB1;CDK1;BUB1B;MAD2L1;AURKA
5-Fluorouracil CTD 00005987	7	2.11E-08	3.86E-07	CCNB2;CCNB1;CDK1;BUB1B;BUB1;MAD2L1;AURKA
genistein CTD 00007324	7	2.49E-08	4.40E-07	CCNB2;CCNB1;CDK1;BUB1B;TTK;BUB1;AURKA

3.6. TF-miRNA co-regulatory network and analysis

MicroRNAs (miRNAs) and transcription factors (TFs) are essential regulators of gene expression [43]. MiRNAs and TFs may have a dual regulatory role in OC. After aggregating hub genes from the PPI network, we created a full TF-miRNA co-regulatory network by combining anticipated and experimentally proven TF and miRNA targets. The RegNetwork repository was used to create a TF-miRNA co-regulatory network using hub genes. The TF-miRNA co-regulatory network has 131 nodes and 153 edges, including 63 TF candidates, 8 hub nodes, and 60 miRNA candidate nodes [Figure 8]. The hsa-miR-590-3p miRNA is the most significant target that is connected with the 3

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hub genes and a TF gene. In the TF-miRNA co-regulatory network four TF genes including MYB, SP1, NFYA, and MYC traced as highly connected with the hub genes.

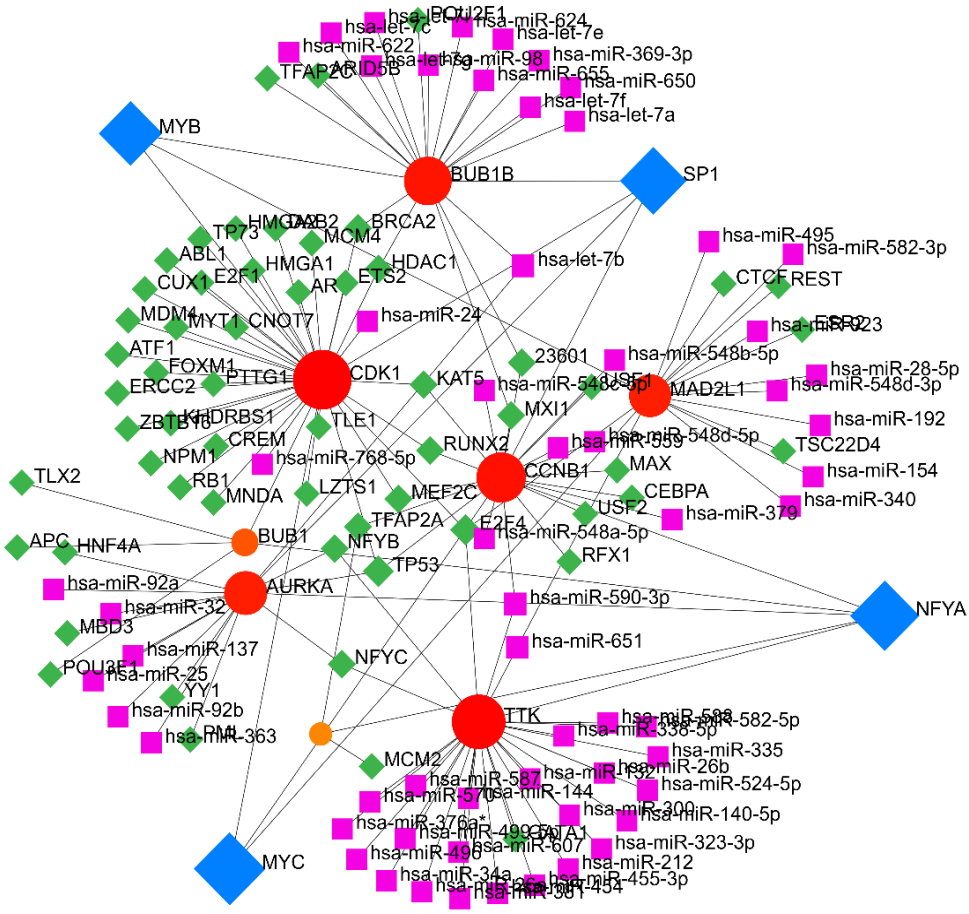

Figure 8. TF-miRNA co-regulatory network has been created using hub genes. In the figure, circular red color nodes refer to hub genes, pink color rectangular nodes indicate miRNA, green and blue colored diamond shape nodes are TF genes. According to the figure MYC, MYB, SP1, and NFYA are significant TF genes on the other hand hsa-mir-590-3p is an important miRNA target.

4. Discussion

Oral cancer is a major health issue that has a high morbidity and fatality rate. Early identification and prevention are critical in reducing the global incidence of oral cancer [44]. We looked examined gene expression patterns in three microarray datasets of OC patients using a network-based method and discovered molecular targets that might be exploited as cancer biomarkers. It might also provide critical details regarding their influence on the evolution of diseases or disorders. Expression profiling using high-throughput microarray datasets has shown to be a useful resource for discovering biomarker candidates for a number of disorders in the domains of biomedical and computational biology [45]. The common 166 DEGs had comparable expression across three datasets, according to the OC transcriptomic analysis. The biological significance of 166 common DEGs was investigated using gene ontology and pathway analysis methodologies based on P-values to gain insight into the etiology of OC.

The GO is a gene regulatory framework based on a general conceptual paradigm that makes genes and their interactions easier to grasp. Evolution accumulated biological knowledge about gene

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activities and regulation in a range of ontological areas over time to achieve this [46]. The GO database was used as an annotation source for three different types of GO analyses: BP (molecular activities), CC (gene regulates function), and MF (molecular level activities) [47]. The BP reported that the mitotic sister chromatid segregation, regulation of mitotic metaphase/anaphase transition, type I interferon signaling pathway, positive regulation of cell cycle phase transition, response to interferon-beta, response to interferon-alpha, macrophage differentiation, etc. are significant terms that have been revealed by the group-wise analysis through ClueGO. From these the mitotic sister chromatid segregation is significantly associated with the common DEGs. The groups of terms are chromosome, centromeric region, spindle, stress fiber, CMG complex, nuclear replication fork, cornified envelope, etc. associated with the CC. The MF is related to the groups of terms are ATP-dependent microtubule motor activity, peptidase activator activity, transmembrane receptor protein tyrosine kinase activity, collagen binding, integrin binding, etc.

The most effective approach for reflecting an organism's behavior through internal alterations is pathway analysis. KEGG, Reactome, and WikiPathways were used to compile the pathways of the most common DEGs. The groups of pathways terms are Cell cycle, Influenza A, ECM-receptor interaction, Leishmaniasis, Epithelial cell signaling in Helicobacter pylori infection, etc. are associated with the pathway of KEGG. On the other hand, the pathway group of Reactome reported that the Formation of Cyclin B: Cdc2 complexes, CDK1 phosphorylates CDCA5 (Sororin) at centromeres, Association of Nek2A with MCC: APC/C, DNA polymerase alpha: primase binds at the origin, Kinesins move along microtubules consuming ATP, etc. pathways are connected with the common DEGs. In addition, the Type I interferon induction and signaling during SARS-CoV-2 infection, Type II interferon signaling (IFNG), DNA Replication, Non-genomic actions of 1,25 dihydroxy vitamin D3, etc. are groups of pathways that are related to the Wikipathways.

Using common DEGs a PPI network had been created to understand the biological characteristics in-depth and explore disease biomarkers [48]. Depending on three methods 8 hub DEGs including CCNB1, BUB1, CCNB2, TTK, MAD2L1, CDK1, BUB1B, and AURKA were traced that might be playing a role to identify the therapeutic biomarker [49]. CDK1 is a serine/threonine kinase with a high degree of conservation. With roughly 70 regulatory targets, it plays a critical role in cell cycle control. A variety of target substrates are phosphorylated by CDK1 directly in order to govern cell transcription and advancement in response to various stimuli [50]. CDKs and their modulators have been found to be abnormally active in a variety of cancers. CDK deficiency results in aberrant cell proliferation and genomic instability [51]. All human malignancies are known to be influenced by the D-cyclin-cdk4/6 INK4-Rb pathway [52]. CDK1 has been reported to be overexpressed in a variety of malignancies, including pancreatic adenocarcinoma, hepatoma, colorectal carcinoma, and head and neck cancers [53, 54], indicating that it plays a significant role in cell-cycle regulation and cancer formation.

The hsa-mir-590-3p is an important target in the TF-miRNA co-regulatory network. Previous studies have reported that the hsa-mir-590-3p may play a significant role in pancreatic cancer [54], colorectal cancer [55], prostate cancer [56], and also breast cancer [57] as a therapeutic biomarker. There is no study that has been exposed yet that hsa-mir-590-3p may play any role in OC. The TF genes MYB, SP1, NFYA, and MYC showed significance in the co-regulatory network of TF-miRNA. The use of modern molecular biology and gene modification methods in vitro and in vivo over the last decade has shown the significance of c-MYB in several forms of cancer. Breast [58], ovarian [59], colorectal [60], and colon [61] carcinoma are all examples of cancers where MYB plays a key role in cancer start and maintenance. A high level of MYB expression is thought to be linked to a halt in cellular differentiation as well as continuing proliferation, which leads to oncogenicity [62]. The next experiment revealed that c-Myb may directly decrease miR-1258 production by binding to its promoter. Furthermore, in OSCC tissues, a study discovered a negative correlation between c-Myb and miR-1258 expression. When used together, c-Myb was shown to be responsible for miR-1258 unregulation in OSCC [63]. SP1 appears to have a role in cancer growth, invasion, and metastasis, according to new findings [64]. SP1 accelerated the cell cycle from G1 to S phase, promoting cell proliferation [65]. SP1 seems to enhance cancer progression through altering cell proliferation and invasion, according to these findings. Overexpression of SP1 was found to contribute to OSCC development in prior research, suggesting that targeting SP1 might be a possible therapeutic target in OSCC [66].

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Doxorubicin, often called as Adriamycin, is a *Streptomyces paucities* spp. anthracycline antibiotic. Doxorubicin inhibits topoisomerase II, intercalates DNA, and produces free radicals, resulting in cell death or growth inhibition [67]. Doxorubicin has been widely employed for the treatment of various types of tumors [68, 69] due to its broad-spectrum anti-tumor action and affordable cost. Doxorubicin resistance, on the other hand, is common in advanced cancers with a dismal prognosis [70]. Doxorubicin is a drug that is typically used for breast cancer treatment [71-73], also some studies have been announced that Doxorubicin might play an important in the treatment of colon cancer [74], thyroid cancer [75], as well as oral cancer [76]. A study looked at the effects of resveratrol on numerous targets such as tubulin, protein kinase C alpha (PKC), phosphodiesterase-4D, human oral cancer cell line proteins, DNA sequences containing AATT/TTAA segments, protein kinase C alpha, and lysine-specific demethylase 1 [77]. Resveratrol administration inhibited the rate of cell growth in OSCC cell lines (0–1.5 g/ml) in a concentration- and time-dependent manner [78]. Cell cycle analysis demonstrated that resveratrol administration increased the number of cells in the G2/M phase with a subsequent decrease in the G1 phase in a time-dependent way [79].

5. Conclusion

The discovered biologic areas and regulatory components were quickly addressed in this work, which may hasten clinical activity against OC-related other illnesses. Our study's strength should be considered, as it is the largest transcriptome investigation on OC. This study's transcriptomic analysis yielded a shared ontological entity, pathways, illness connections, and transferrin genes. In OC, the related genes between datasets have been found, resulting in additional molecular results and demonstrating the interaction of DEGs. This research might aid in the development of therapeutic targets and therapies in the future.

Abbreviation

GEO = Gene Expression Omnibus

DEG = Differential Expression Gene

OC = Oral Cancer

GO = Gene Ontology

PPI = protein-protein interaction

TF = Transcription Factor

miRNA = microRNA

KEGG = Kyoto Encyclopedia of Genes and Genomes

OSCC = Oral Squamous Cell Carcinoma

MCC = Maximal Clique Centrality

MNC = Maximum Neighborhood Component

NCBI = National Center for Biotechnology Information

STRING = Search Tool for the Retrieval of Interacting Genes

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, FA, FHA, ZMS, DAA, RAA, TH, MKA, MTH, MRI, KA, RB; methodology, MTH, MRI, KA; software, MTH, MRI, KA; validation, MTH, MRI, KA; formal analysis, MTH, MRI, KA; investigation, MTH, MRI, KA; resources, MTH, MRI, KA; data curation, MTH, MRI, KA; writing—original draft preparation, FA, FHA, ZMS, DAA, RAA, TH, MKA, MTH, MRI, KA, RB; writing—review and editing, FA, FHA, ZMS, DAA, RAA, TH, MKA, MTH, MRI, KA, RB; visualization, X.X.; supervision, MKA, KA; project administration, MKA, KA; funding acquisition, MKA. All authors have read and agreed to the published version of the manuscript."

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