Pathology - Research and Practice 215 (2019) 152378

Contents lists available at ScienceDirect

Pathology - Research and Practice

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

Drug repositioning in head and neck squamous cell carcinoma: An integrated pathway analysis based on connectivity map and differential gene expression

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ARTICLE INFO

Keywords:
Head and neck squamous cell carcinoma
Connectivity map
Pathways
Drug repositioning
Candidate drugs
Molecular docking

ABSTRACT

The severe damage to health and social burden caused by head and neck squamous cell carcinoma (HNSCC) generated an urgent need to develop novel anti-cancer therapy. Currently, drug repositioning has risen in responses to the proper time as an efficient approach to invention of new anti-cancer therapies. In the present study, we aimed to screen candidate drugs for HNSCC by integrating HNSCC-related pathways from differentially expressed genes (DEGs) and drug-affected pathways from connectivity map (CMAP). We also endeavored to unveil the molecular mechanism of HNSCC through creating drug-target network and protein-to-protein (PPI) network of component DEGs in key overlapping pathways. As a result, a total of 401 DEGs were obtained from TCGA and GTEx mRNA-seq data. Taking the intersection part of 27 HNSCC-related Kyoto Encyclopedia of Genes and Genomes pathways and 33 drug-affected pathways, we retained 22 candidate drugs corresponding to two key pathways (cell cycle and p53 signaling pathways) of the five overlapping pathways. Two of the hub genes (PCNA and CCND1) identified from the PPI network of component DEGs in cell cycle and p53 signaling pathways were defined as the critical targets of candidate drugs with increased protein expression in HNSCC tissues, which was reported by the human protein atlas (HPA) database and cBioPortal. Finally, we validated via molecular docking analysis that two drugs with unknown effects in HNSCC: MG-262 and bepridil might perturb the development of HNSCC through targeting PCNA. These candidate drugs possessed broad application prospect as medication for HNSCC.

1. Introduction

Head and neck cancer is a highly prevalent tumor in Southeast Asia and Southeast China and ranks first in incidence and mortality rate among malignant head and neck tumors [1,2]. Head and neck squamous cell carcinoma (HNSCC) accounted for approximately 90% of all head and neck cancer cases [3]. Radiotherapy is the primary modality of treatment for HNSCC, which produced better results when combined with chemotherapy [4]. Although extensive efforts have been made to improve the treatment for HNSCC patients, the 5-year survival rate for HNSCC remained under 50%, virtually unchanged in thirty years [5–7]. Therefore, there is an urgent need of developing more effective therapeutic agents for HNSCC.

Considering the tremendous time consumption and high failure rate in developing new drugs [8], drug repositioning has been proposed as an innovative strategy for discovering new drugs. During drug repositioning, new indications of known drugs are identified [9,10]. As a widely used database in drug repositioning, connectivity map (CMAP) contained more than 7000 gene expression profiles of cancer cell lines treated with and without 1309 small bioactive molecules [11–13]. Based on gene expression changes between treatment group and control group, CMAP analyzed the relationship between drug, disease and gene

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https://doi.org/10.1016/j.prp.2019.03.007
Received 31 December 2018; Received in revised form 7 February 2019; Accepted 2 March 2019
0344-0338/

Table 1
Top ten significantly enriched GO terms.

Description	P value	P adjust	Q value	Count
serine-type endopeptidase activity	8.79E-14	8.57E-11	7.37E-11	68
serine hydrolase activity	4.13E-13	2.01E-10	1.73E-10	71
serine-type peptidase activity	7.14E-13	2.32E-10	1.99E-10	70
endopeptidase activity	1.03E-12	2.52E-10	2.16E-10	102
extracellular matrix structural constituent	2.45E-12	4.77E-10	4.10E-10	32
antigen binding	1.23E-10	1.99E-08	1.71E-08	51
actin binding	3.86E-10	5.37E-08	4.62E-08	84
collagen binding	5.37E-10	6.55E-08	5.63E-08	26
growth factor binding	1.79E-09	1.94E-07	1.66E-07	38
glycosaminoglycan binding	1.36E-08	1.33E-06	1.14E-06	49

Note: GO: gene ontology.

expression signatures by adopting rank-based pattern-matching strategy [14,15]. The employment of CMAP has contributed to re-purposing drugs in multiple cancers including ovarian cancer, diffuse large b-cell lymphoma and renal cell carcinoma [16–18]. However, the application of CMAP for drug repositioning in HNSCC has not been reported.

The present study is the first to screen candidate drugs for treating HNSCC by integrating HNSCC-related pathways and drug-affected pathways from CMAP. HNSCC-related pathways corresponded to significant pathways enriched by differentially expressed genes (DEGs) from the cancer genome atlas (TCGA) and genotype-tissue expression (GTEx) mRNA-seq data. Candidate drugs selected from this study may provide insights on alternative therapeutic options for HNSCC patients.

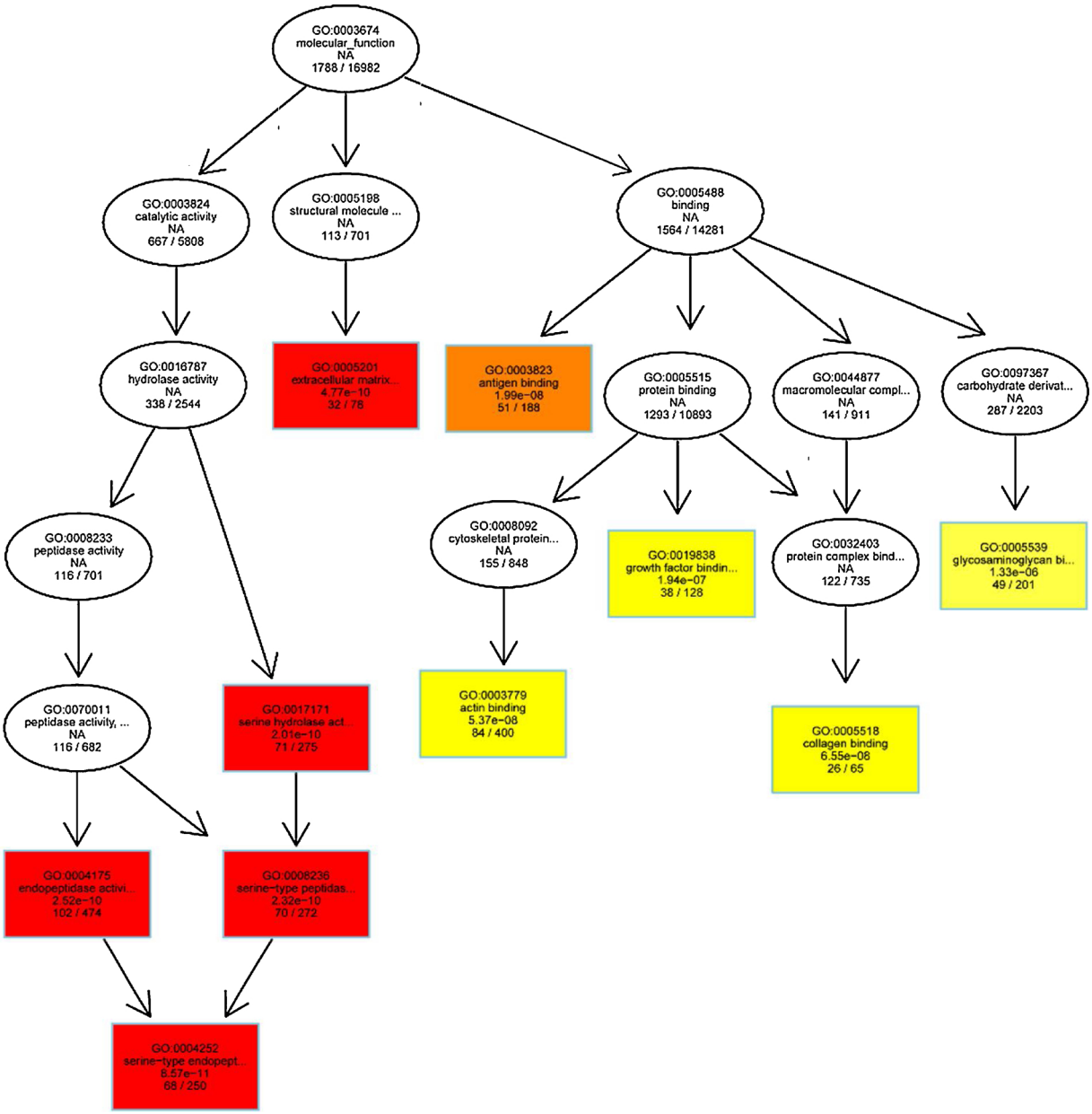

Fig. 1. Dendogram for gene ontology (GO) enrichment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
The dendogram consisting of rectangles and arrows depicted the cascade relationships between the top ten GO terms. Significance of gene ontology GO terms increased with the color of squares changing form yellow to red.

Table 2
Significant KEGG pathways enriched by DEGs.

ID	Description	Count	P value
hsa04512	ECM-receptor interaction	34	1.98E-12
hsa04510	Focal adhesion	55	9.66E-11
hsa04110	Cell cycle	39	9.70E-10
hsa05165	Human papillomavirus infection	72	2.97E-09
hsa04974	Protein digestion and absorption	31	4.51E-09
hsa03030	DNA replication	17	7.06E-08
hsa05323	Rheumatoid arthritis	28	2.96E-07
hsa05222	Small cell lung cancer	28	6.31E-07
hsa05146	Amoebiasis	28	1.29E-06
hsa00532	Glycosaminoglycan biosynthesis - chondroitin sulfate / dermatan sulfate	10	2.02E-05
hsa04933	AGE-RAGE signaling pathway in diabetic complications	26	2.46E-05
hsa05166	HTLV-I infection	50	5.33E-05
hsa05205	Proteoglycans in cancer	41	1.01E-04
hsa04914	Progesterone-mediated oocyte maturation	24	1.95E-04
hsa04115	p53 signaling pathway	18	3.86E-04
hsa04670	Leukocyte transendothelial migration	25	5.63E-04
hsa04380	Osteoclast differentiation	27	8.78E-04
hsa04151	PI3K-Akt signaling pathway	59	1.01E-03
hsa04060	Cytokine-cytokine receptor interaction	47	1.45E-03
hsa05418	Fluid shear stress and atherosclerosis	28	1.50E-03
hsa04218	Cellular senescence	31	1.67E-03
hsa05162	Measles	27	1.80E-03
hsa05414	Dilated cardiomyopathy (DCM)	20	2.06E-03
hsa04610	Complement and coagulation cascades	18	2.52E-03
hsa04657	IL-17 signaling pathway	20	3.10E-03
hsa04978	Mineral absorption	13	3.48E-03
hsa05410	Hypertrophic cardiomyopathy (HCM)	18	4.45E-03

Note: KEGG: Kyoto Encyclopedia of Genes and Genomes; DEG: differentially expressed genes.

2. Materials and methods

2.1. Screening of DEGs from TCGA and GTEx mRNA-seq data

As large consortium projects that produce a wealth of RNA sequencing data for more than 9000 cancer samples and over 8000 normal samples, TCGA and GTEx were highly appraised for facilitating data mining of cancer genomics [19]. In this study, Gene Expression Profiling Interactive Analysis (GEPIA), an online tool that delivered expression analysis functions based on re-calculated TCGA and GTEx mRNA-seq data of HNSCC samples and normal samples from UCSC Xena was utilized for screening out DEGs between HNSCC and normal samples [19–21]. Analysis of DEGs was carried out through one-way ANOVA analysis. The threshold of DEGs were $|\text{Log2 (fold change)}| > 2$ and $q < 0.01$.

2.2. Functional annotation for DEGs

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for DEGs were performed in R clusterprofile package v.3.4.2. $P < 0.05$ was set as the threshold for significant GO and KEGG terms.

2.3. Drug-related pathways and candidate drugs for HNSCC

Wang JY et al. have identified 104 drug-affected subpathways for each small molecule in CMAP using SubpathwayMiner [22]. In this study, we took the intersection of entire pathways corresponding to 104 drug-affected subpathways provided by Wang JY et al., and significant KEGG pathways for DEGs. Small molecules that belonged to the overlapping parts of entire drug-affected pathways and significant KEGG pathways for DEGs were considered as potential drugs for HNSCC. If more than 10 overlapping genes were commonly shared by two pathways, the two pathways were selected as key pathways. We hypothesized that small molecules that influenced the two key overlapping pathways might play crucial roles in interfering the progression of HNSCC. To investigate the interaction between small molecules and component genes of key overlapping pathways, drug-target networks comprised of small molecules and component genes were constructed for each key overlapping pathway through Cytoskape v.3.5.0. Small molecules that corresponded to the key overlapping pathways with false discovery rates of less than (FDR) 0.01 were singled out as candidate drugs for HNSCC.

2.4. Protein-to-protein (PPI) network for key pathways and protein expressions of hub genes

PPI networks of each key pathway were also created by Cytoskape v.3.5.0 with medium confidence values of 0.9. In PPI network, nodes and links between them represented the component genes and the relationship between these genes. Nodes with highest connectivity degrees were defined as hub genes. Additionally, we imported the hub genes into human protein atlas (HPA) to evaluate the immunohistochemical staining of these genes between HNSCC tissues and normal nasopharynx or oral mucosa tissues. HPA database is an important resource for studying human proteom through combining antibody-based approach and transcriptomics data [23]. Immunohistochemistry or immunofluorescence images generated from HPA databases underwent rigorous assessment and were compared with available experimental data of gene or protein characterization [23]. Expression profiles of these genes in HNSCC tissues based on z scores from cBioPortal were also downloaded for analyzing the protein expression levels of these genes in HNSCC tissues.

2.5. Verification of the targeting regulatory relationship between candidate drugs and hub genes through molecule docking

To validate the targeting regulatory relationships between candidate drugs and hub genes, we conducted molecular docking via the Surflex-Dock program in Sybyl X-2.0 (Tripos Inc.). The structural formulas of candidate compounds and proteins of hub genes in key overlapping pathways were downloaded from protein data bank (PDB). Docking scores were generated automatically following the input of structural formulas of proteins, which was the basis for assessing the binding affinity between candidate drugs and proteins of hub genes. A docking score of more than 6 indicated strong binding affinity between proteins.

3. Results

3.1. DEGs from TCGA and GTEx data

A total of 401 DEGs comprised of 201 up-regulated genes and 200 down-regulated genes were reserved from 519 HNSCC samples and 44 normal samples (Online Resource 1).

3.2. Functional annotation for DEGs

According to the results of GO enrichment analysis, a total of 90 GO terms were significantly enriched by DEGs ($P < 0.05$). The top ten significantly enriched GO terms were listed in Table 1, which revealed that the 401 DEGs in HNSCC were mainly clustered in molecular functions such as serine-type endopeptidase activity, serine hydrolase activity, serine-type peptidase activity and endopeptidase activity (Fig. 1). With respect to KEGG analysis, DEGs in HNSCC were significantly assembled in 27 pathways, with ECM-receptor interaction, focal adhesion and cell cycle ranking as the top three KEGG pathways (Table 2) (Fig. 2A).

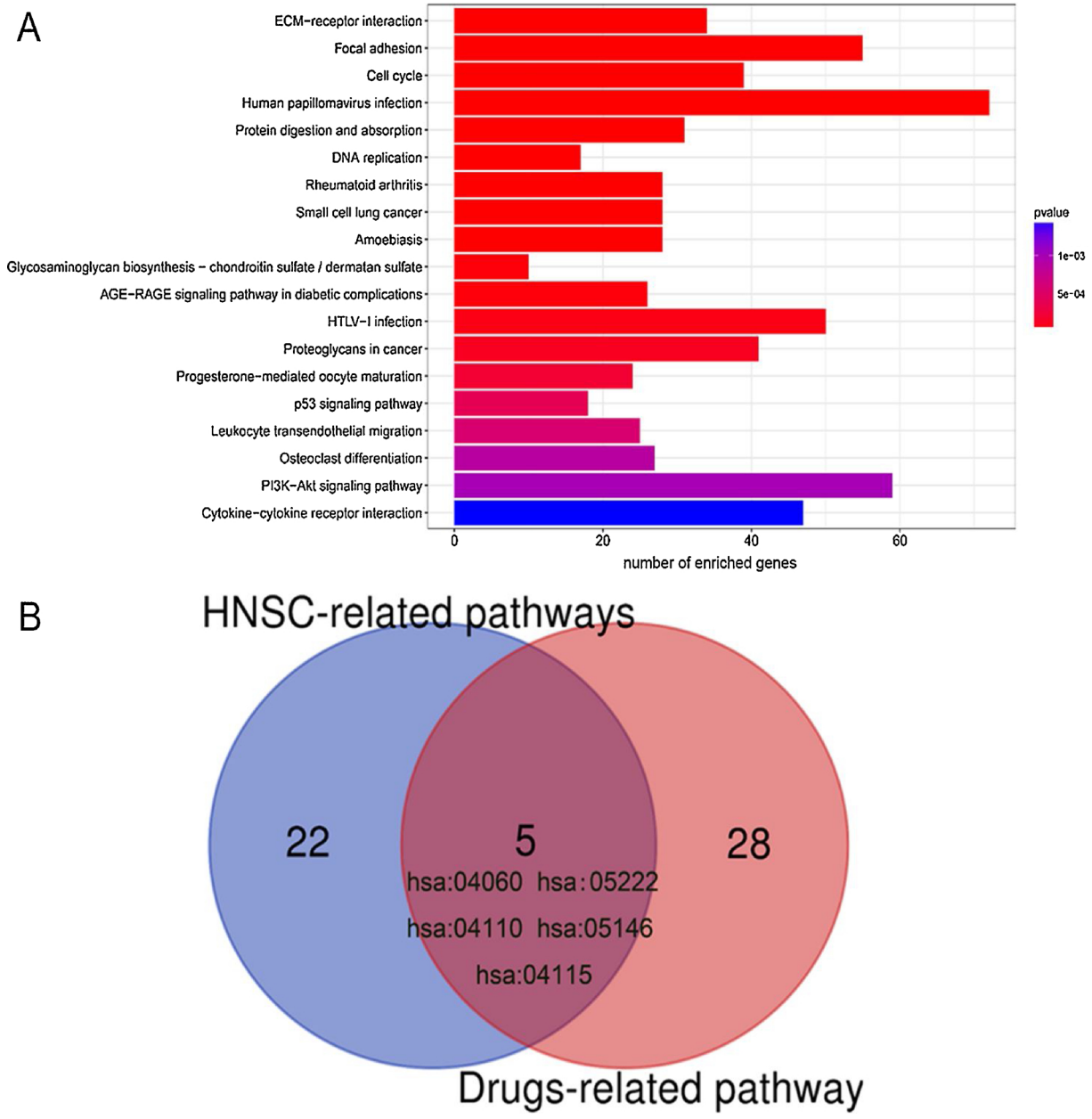

Fig. 2. Bar diagram and venn diagram for Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
A. All the 27 KEGG pathways were arranged in the bar diagram according to the level of significance, with the X-axis indicating the number of component genes for each pathway and the Y-axis the pathway name. The significance of P values was represented by the color of the bars. B. The intersection results of head and neck squamous cell carcinoma-related pathways (blue circle) and drug-affected pathways (orange circle) were the overlapping parts of two circles.

Table 3
Five overlapping pathways.

ID	Description	Count	P value	Q value
hsa04110	Cell cycle	39	9.700000E-10	8.410000E-08
hsa05222	Small cell lung cancer	28	6.310000E-07	2.050000E-05
hsa05146	Amoebiasis	28	1.290000E-06	3.720000E-05
hsa04115	p53 signaling pathway	18	3.855060E-04	6.682110E-03
hsa04060	Cytokine-cytokine receptor interaction	47	1.452119E-03	1.951328E-02

3.3. Drug-related pathways and candidate drugs for HNSCC

In the study of Wang JY et al., the 104 drug-affected subpathways for each small molecule in CMAP belonged to 33 entire pathways. A Venn diagram in Fig. 2B exhibited the overlapping parts of the 33 entire pathways for compounds from CMAP and the 27 HNSCC-related KEGG pathways. The detailed information of the five overlapping pathways including cytokine-cytokine receptor interaction, small cell lung cancer, cell cycle, amoebiasis and p53 signaling pathway were summarized in Table 3. Specifically, cell cycle and p53 signaling pathway were selected as the key overlapping pathways because more than ten overlapping genes were affiliated to both of them. Based on the drug-related pathway analysis in previous study, a total of 20 and 43 small molecules were associated with cell cycle and p53 signaling pathway, respectively. Two drug-target networks in Fig. 3 illustrated the interactions between these drugs and the component genes of the two key overlapping pathways. Ultimately, 22 drugs that corresponded to the two key overlapping pathways with FDR < 0.01 were identified as

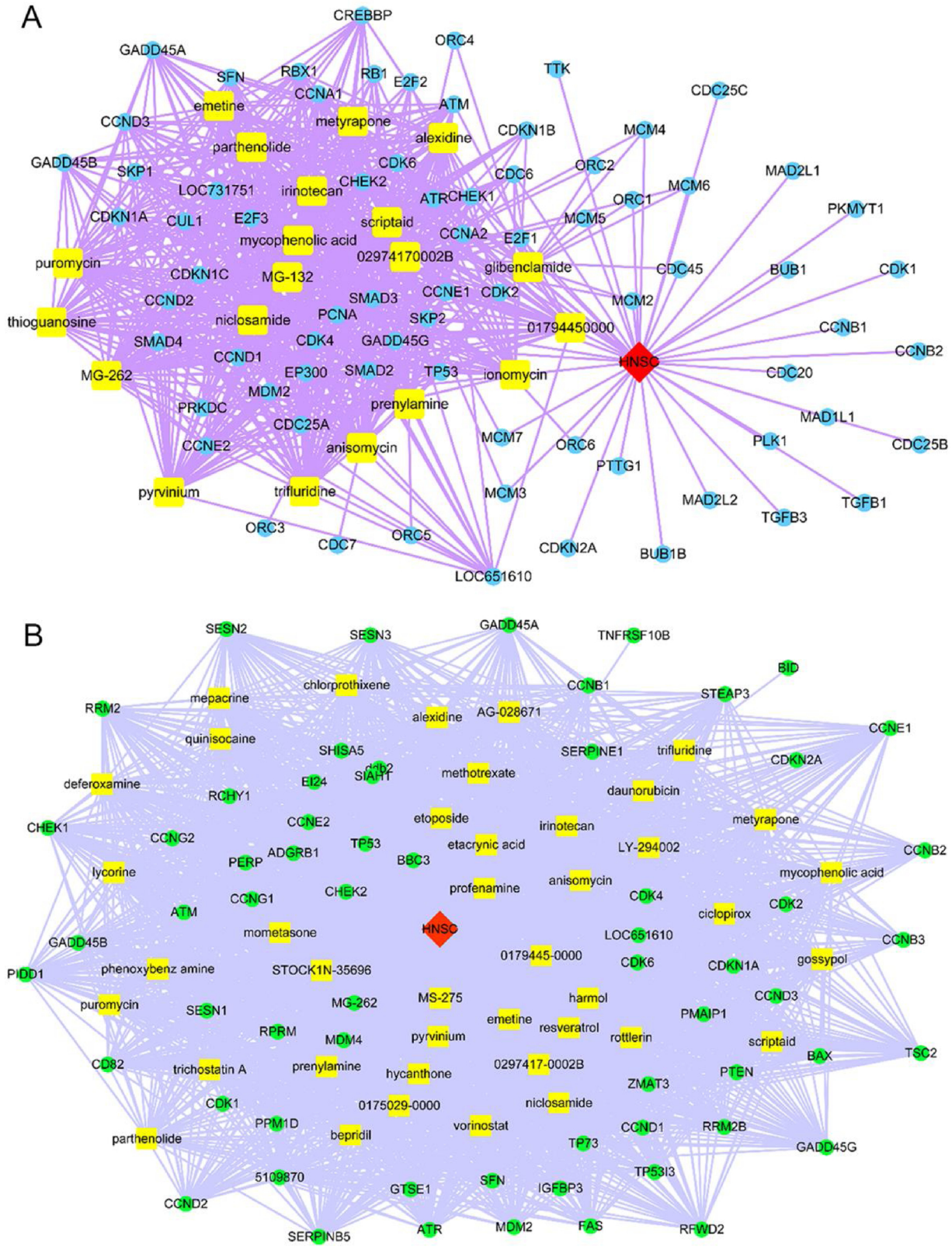

Fig. 3. Drug-target network for cell cycle and p53 signaling pathway. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A. The yellow rectangle nodes and blue circle nodes in the network represented drugs and component genes of cell cycle pathway, respectively. Purple links reflected the relationships between drugs and component genes of cell cycle pathway. B. The yellow rectangle nodes and green circle nodes in the network represented drugs and component genes of p53 signaling pathway, respectively. Blue links reflected the relationships between drugs and component genes of p53 signaling pathway.

Table 4
Candidate drugs for HNSCC corresponding to cell cycle and p53 signaling pathways.

Drug. ATC	Drug name	Pathway name	Pathway ID
NA	0297417-0002E	Cell cycle	path:04110_5,path:04110_2 1
L	alexidine	Cell cycle	path:04110_1 8
NA	anisomycin	Cell cycle	path:04110_1 9,path:04110_1 7
L	irinotecan	Cell cycle	path:04110_7
NA	MG-262	Cell cycle	path :04110_7,path :04110_1 7,path:04110_1 8,path:04110_1 9
P	niclosamide	Cell cycle	path:04110_7,path:04110_1 7, path:04110_1 9
C	prenylamine	Cell cycle	palh:04110_7,path:04110_1 7,path:04110_1 9
NA	pyrvinium	Cell cycle	palh:04110_7,path:04110_1 7,path:04110_1 9
NA	scriptaid	Cell cycle	path:04110_2 6
NA	MG-262	p53signaling pathway	path :04115_1, palh :04115_2
C	bepridil	p53signaling pathway	path:04115_1,palh:04115_2,pa1h:04115_3,path:04 115_4,path:04115_7
L	vorinostat	p53signaling pathway	path :04115_1, pa1h :04115_3
L	irinotecan	p53signaling pathway	path:04115_1,palh:04115_2,palh:04115_3,path:04 115_4,path:04115_7
D	ciclopirox	p53signaling pathway	path :04115_1,path :04115_2,path :04115_3,path:04 115_4,path:04115_7
P	mepacrine	p53signaling pathway	path:04115_1,pathib4115_2,palh-b4115_3,path:04 115_4,path:04115_7
V	resveratrol	p53signaling pathway	path:04115_1,pathib4115_2,palh-b4115_3,path:04 115_4,path:04115_7
A	hycanthone	p53signaling pathway	path:04115_1,palh:04115_2,palh:04115_3,path:04 115_4,path:04115_7
NA	AG-028671	p53signaling pathway	path:04115_1,palh:04115_2,palh:04115_3,path:04 115_4,path:04115_7
NA	emetine	p53signaling pathway	path:04115_1,palh:04115_2,palh:04115_3,path:04 115_4,path:04115_7
V	metyrapone	p53signaling pathway	path:04115_1,palh:04115_2,palh:04115_3,path:04 115_4,path:04115_7
L	alexidine	p53signaling pathway	path:04115_1
L	etoposide	p53signaling pathway	path:04115_1,palh:04115_2,palh:04115_3,path:04 115_4,path:04115_7
NA	5109870	p53signaling pathway	path :04115_1, pafh:04115_2,pofri:04115_3,path:04 115_4,path:04115_7
N	profen amine	p53signaling pathway	path:04115_1,pathib4115_2,palh-b4115_4,path:04 115_7
NA	pyn/inium	p53signaling pathway	path:04115_1,palh:04115_2,palh:04115_3,path:04 115_4,path:04115_7
C	prenylamine	p53signaling pathway	path:04115_1
NA	rottlerin	p53signaling pathway	path:04115_1

Note: ATC: Anatomical Therapeutic Chemical. A: alimentary tract and metabolism; C: cardiovascular system; D: dermatologicals; L: antineoplastic and immunomodulating agents; N: nervous system; P: antiparasitic products, insecticides and repellents; V: various; NA: not available.

candidate drugs for HNSCC (Table 4). The molecular structure diagrams for the 22 drugs downloaded from PubChem Compound of National Center for Biotechnology Information (NCBI) were shown in Fig. 4.

3.4. Protein-to-protein (PPI) network for key pathways and protein expressions of hub genes

We constructed PPI network for cell cycle and p53 signaling pathways (Fig. 5), which reflected the relationships between component genes of the two key pathways. A total of eight genes including CDK2, CDKN1A, CCNA2, CDKN1B, RB1, PCNA, CCNA1 and CDC6 were defined as the hub genes for cell cycle pathway due to a connectivity degree of more than 30. As for p53 signaling pathway, 11 genes including TP53, CDK2, CDK1, CCNB1, CDK4, CDKN1A, CDK6, MDM2, CCNB2, CCNE1 and CCND1 were singled out as the hub genes with a connectivity degree of more than ten. We retrieved the protein expression data of these hub genes in HPA and cBioPortal. Three of the 17 hub genes including PCNA, TP53 and CCND1 demonstrated both protein upregulation in cBioPortal and strong immunoreactivity in HNSCC tissues from HPA. According to the OncoPrint module of cBioPortal, a total of 357 patients and samples were profiled in Protein expression Z-scores (RPPA). The threshold for Z-score was ± 2. Protein upregulations of PCNA, TP53 and CCND1 were detected in ten, three and five of 357 sequenced cases, respectively (Fig. 6). Apart from this, immunostaining results displayed in Fig. 7 from the HPA database suggested the elevated expression of PCNA (antibody HPA030521), TP53 (antibody CAB002973) and CCND1 (antibody HPA000024) in HNSCC tissues. Compared with the weak or moderate immunostaining intensity of the three proteins in normal nasopharynx and oral mucosa tissues, strong immunostaining intensity was observed in HNSCC tissues.

3.5. Verification of the targeting regulatory relationships between candidate drugs and hub genes through molecule docking

We selected seven small molecules (Anisomycin, MG262, Pyrvinium, Bepridil, Hycanthone, Emetine and Rottlerin) and one of the hub genes: PCNA for molecule docking. The results revealed that Bepridil and MG262 showed strong binding affinity to PCNA with a connection score of more than 6 (7.836 for Bepridil and 9.436 for MG262) (Fig. 8) (Table 5).

4. Discussion

With the advent of databases archiving profiles of transcriptional responses to small molecular treatment, drug repurposing has become a feasible way of discovering anti-cancer therapy for human cancers. In this study, we attempted to select candidate drugs with anti-HNSCC properties through integrating significant KEGG pathways clustered by DEGs of HNSCC and drug-affected pathways from CMAP. Drugs associated with the overlapping pathways were considered to have potential perturbation effect on the initiation and development of HNSCC. The subsequent bioinformatics analyses such as drug-target networks and PPI networks indicated the underlying molecular mechanism of candidate drugs and hub DEGs in the tumorigenesis of HSNCC. Furthermore, binding affinity between one of the hub genes and several candidate drugs were validated by molecular docking.

We firstly calculated DEGs using the mRNA-seq data of HNSCC and normal samples from TCGA and GTEx. To have an overview of the engagement of DEGs in biological events of HNSCC, we annotated the molecular functions of DEGs through GO and KEGG enrichment analysis. Results from GO analysis implied that these DEGs might participate in the occurrence and progression of HNSCC with active roles in molecular functions such as serine-type endopeptidase activity, serine hydrolase activity, serine-type peptidase activity, endopeptidase activity. KEGG pathway analysis revealed three top significant HNSCC-

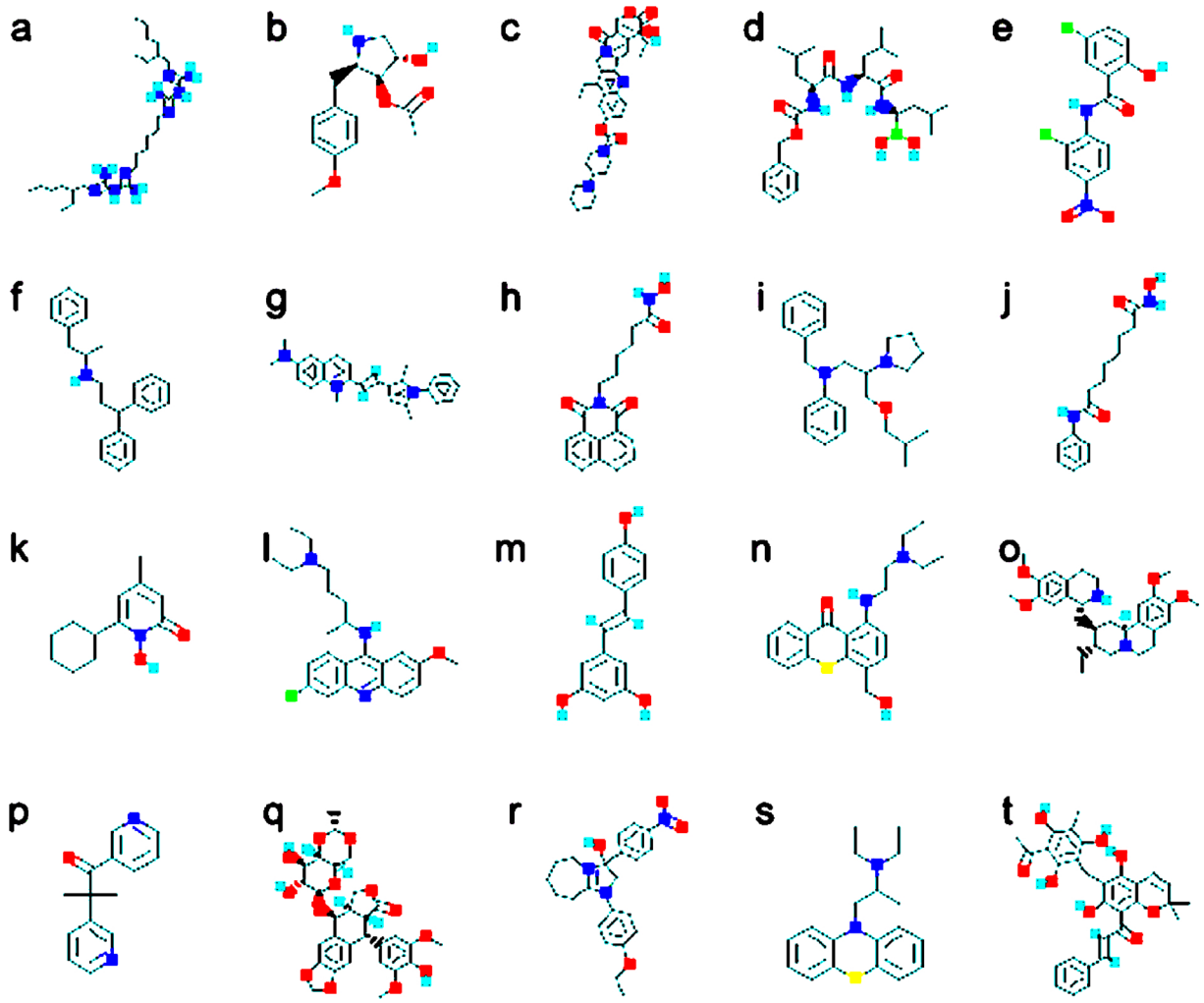

Fig. 4. Molecular structure diagrams for candidate drugs.
a: alexidine; b: anisomycin; c: irinotecan; d: MG-262; e: niclosamide; f: prenylamine; g: pyrvinium; h: scriptaid; i: bepridil; j: vorinostat; k: ciclopirox; l: mepacrine; m: resveratrol; n: hycanthone; o: emetine; p: metyrapone; q: etoposide; r: 5109870; s: profenamine; t: rottlerin. Two candidate drugs: 0297417-0002B and AG-028671 were not in the panel because there were no available molecular structure diagrams for them in National Center for Biotechnology Information PubChem Compound.

related pathways: ECM-receptor interaction, focal adhesion and cell cycle pathway, all of which were closely correlated with the hallmark phenotypes of cancers including cell migration, invasion, proliferation and growth [24–32]. Aberrations of these pathways played essential roles in cancers. Dis-regulations of the three pathways involved by DEGs might also exert important influence on HNSCC.

We conjectured that drugs targeting HNSCC-related pathways might be novel therapeutic agents for HNSCC. Thus we proceeded to select candidate drugs for HNSCC via integrating HNSCC-related KEGG pathways and drug-affected pathways. A total of five pathways came as the intersection results of HNSCC-related KEGG pathways and drug-affected pathways. To further narrow down the scope of candidate anti-HNSCC drugs, we selected key pathways from the five overlapping pathways. It is noticed that more than ten overlapping component genes were commonly shared by cell cycle and p53 signaling pathway, which suggested the remarkable enrichment of the two pathways by DEGs. Previous studies have elaborated on the tumor suppressive function of p53 signaling pathways and the promotive effect of abnormal cell cycle pathway on cancer progression [24,33–38]. Therefore, we focused on drugs corresponding to cell cycle and p53 signaling pathways. According to the criteria of FDR < 0.01, a total of 22 drugs corresponding to the two key pathways were chosen as the candidate drugs for HNSCC. Through literature research of the 22 drugs, nine drugs including alexidine, irinotecan, niclosamide, scriptaid, vorinostat, ciclopirox, mepacrine, resveratrol and etoposide were supported by literature survey to obstruct the malignant progression of HNSCC and four drugs including 0297417-0002B, AG-028671, 5109870 and profenamine were useful for non-cancer diseases [39–48]. We found that the other seven drugs including anisomycin, bepridil, emetine, hycanthone, MG262, pyrvinium and rottlerin demonstrated anti-cancer effects validated by in vivo or in vitro experiments in several human cancers. However, the cancer-combating properties of the seven drugs in HNSCC have not been reported yet. Anisomycin is an antibiotic that delays the synthesis of proteins through inactivating peptidyl transferase in ribosomes [49]. The apoptosis-inducing capacity of anisomycin emerged in various cancers including renal cell carcinoma, leukemia, glioma and melanoma [50–52]. Bepridil, a calcium channel blocker, could potentiate the anti-tumor activity of antiestrogens and cytotoxity of mitoxantrone in brain tumor cells and chronic myeloid leukemia cells [53,54]. Emetine was originally designated as an anti-protozoal drug and an emetic [55]. The prohibitive impact of emetine on migration and invasion of non-small cell lung cancer cells as well as the enhanced tumor necrosis factor-related apoptosis-inducing ligand-induced pancreatic cell apoptosis caused by emetine reflected the possible application of emetine in cancer treatment [56,57]. Hycanthone is a thioxanthenone DNA intercalator originally synthesized for the treatment of human schistosomiasis [58]. Because hycanthone caused side effects such as hepatic necrosis, strong mutagenicity and

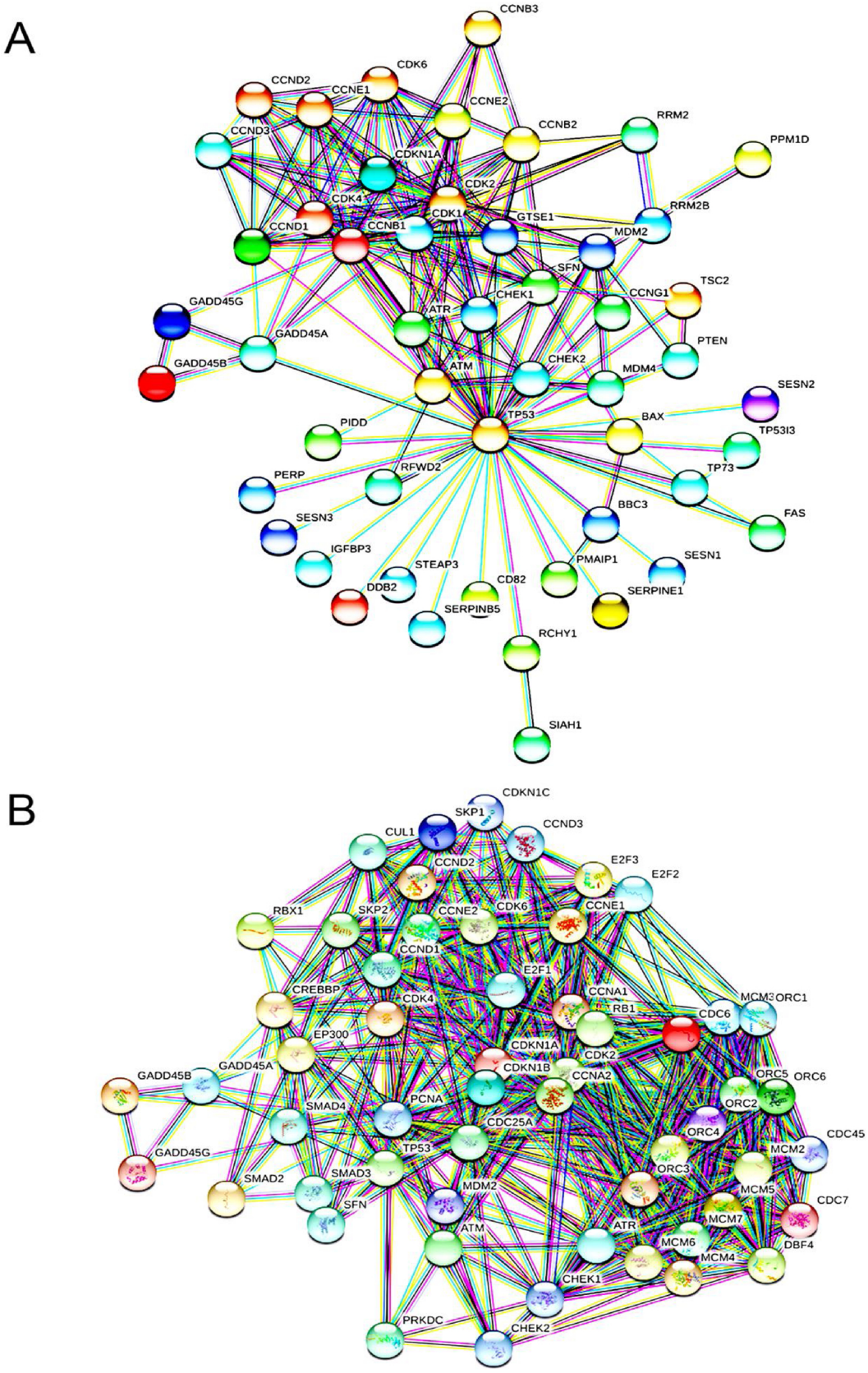

Fig. 5. Protein-to-protein-interaction (PPI) networks for cell cycle and P53 signaling pathway.
A. A total of 55 nodes and 576 edges constituted the PPI network. Nodes with different colors represent different component genes. Edges connecting nodes symbolize the interactions between component genes. B. A total of 57 nodes and 166 edges constituted the PPI network. Nodes with different colors represent different component genes. Edges connecting nodes symbolize the interactions between component genes.

Fig. 6. Protein expression profiles of hub genes in head and neck squamous cell carcinoma (HNSCC) from cBioPortal.
a: Protein expression profile of PCNA in HNSCC cases; b: Protein expression profile of TP53 in HNSCC cases; c: Protein expression profile of CCND1 in HNSCC cases. Protein expressions of the three hub genes were profiled in 357 sequenced HNSCC cases based on Z-scores. Genetic alterations were marked with different colors in the second line of grids.

weak carcinogenicity in human [59–61], it was banned from the therapies of schistosomiasis [58]. In the study of Efferth T et al., hycanthone was proved to reverse the multidrug resistance of L1210 leukemia tumor cells in vitro and in vivo [62], which hinted that hycanthone might act as a favorable factor in preventing multidrug resistance in human cancers. MG262 belonged to the family of proteasome inhibitors and was proposed as a novel approach to anti-tumor treatment with its pivotal roles in abrogation of tumor cell growth and promotion of cell apoptosis [63–65]. Pyrvinium, a classic drug of anthelminthic [66], has been recently reported by Esumi H et al. to demonstrate selective toxicity to glucose-starved cancer cells and to suppress tumor development in a hypovascular Panc-1 pancreatic cancer model resistant to hypoglycemia [67]. Rottlerin is a natural inhibitor of PKCδ and showed carcinostatic effects in multiple human cancers including colon cancer, glioma and breast cancer via extensive interactions with molecules in pathways related to cell survival, apoptosis, autophagy, and invasion [68–72]. The seven drugs with unknown pharmacological effects in HNSCC deserve further investigation. For this reason the seven drugs were chosen for molecular docking analysis.

Although the molecular mechanism of HNSCC was characterized by complicated interaction network of numerous genes and proteins, hub genes figure out prominently in the intricate network as leading protagonists in the molecular basis underlying HNSCC. It can be inferred that drugs targeting the hub genes of HNSCC might work against HNSCC effectively. In this study we sought the critical targets of candidate drugs for HNSCC by constructing drug-target networks and PPI networks. The drug-target networks illustrated the interactions between candidate drugs and component DEGs of cell cycle and p53 signaling pathways, which might help elucidate molecular basis underlying the pharmacological influences of candidate drugs on HNSCC. The creation of PPI networks enabled us to identify hub genes with crucial roles in the pathogenesis of HNSCC and we further selected the critical targets of candidate drugs in HNSCC from hub genes in cell cycle and p53 signaling pathways. Since most drugs were designed as the inhibitors of molecules, hub genes with upregulated protein expression in HNSCC were retained after selection. As a result, three hub genes including PCNA, TP53 and CCND1 endured the dual tests from HPA and cBioPortal. Because TP53 was known to influence the formation and development of cancer through mutations rather than upregulation of protein expression [73], we excluded TP53 from the critical targets of candidate drugs in HNSCC. Eventually, PCNA and CCND1 were retained as the critical targets of candidate drugs. PCNA is a protein devoted to DNA replication, chromatin remodeling or DNA repair [74]. Frequently over expressed PCNA in cancer cells were often used as a biomarker for cell proliferation [75]. In a study conducted by Zhang Q and his colleagues, the boosted anti-tumor effects of combined blockade of GRPR and EGFR pathways was accompanied by decrease of PCNA expression [76]. CCND1 serves as a key mediator of the G1 phase of the cell cycle and the overexpression of CCND1 was reported to be positively correlated with chemotherapy resistance and poor survival of HNSCC patients [77]. These findings were suggestive of the participation of PCNA and CCND1 in the carcinogenesis of HNSCC. Then, molecular docking analysis was carried out to further evaluate the targeting regulatory relationships between the seven candidate drugs and PCNA. We failed to assess the targeting regulatory relationship between the seven drugs and CCND1 because there was no available structural formula of CCND1 in PDB. Based on the results of molecular docking, two drugs including bepridil and MG262 showed strong binding affinity with PCNA. We hypothesized that bepridil and MG-262 might perturb the development of HNSCC through targeting PCNA.

Despite the above achievements, in vitro or in vivo experiments were required in future studies to explore the effect of the candidate drugs on the biological processes of HNSCC cancer cells before their safety application in clinical therapy. Moreover, experiments were also

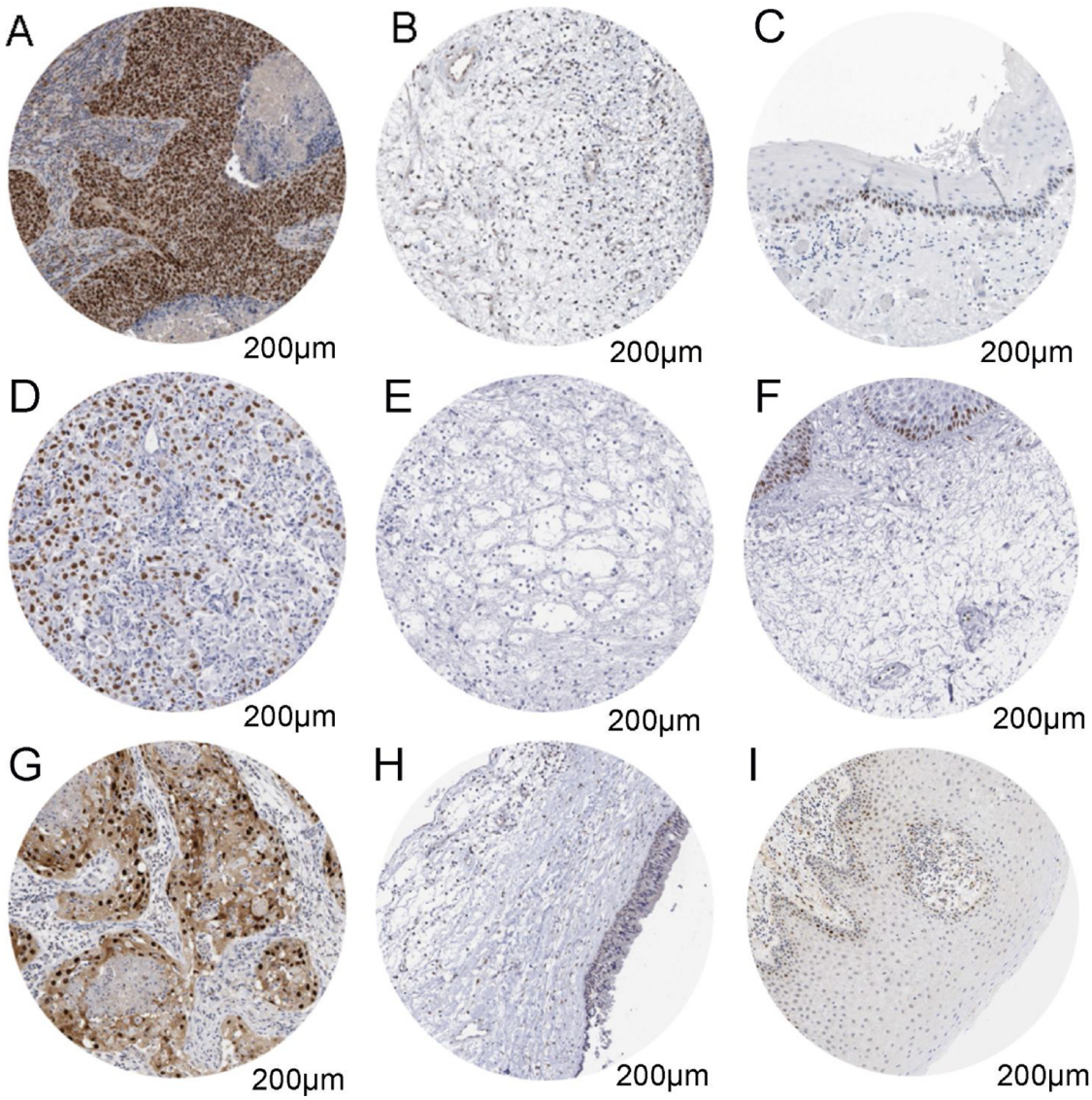

Fig. 7. Immunohistochemistry of the hub genes in head and neck squamous cell carcinoma (HNSCC) tissues and normal tissues from the human protein atlas (HPA) database.
a: PCNA expression in HNSCC tissues (antibody HPA030521). b: PCNA expression in normal nasopharynx tissues (antibody HPA030521). C: PCNA expression in normal oral mucosa tissues (antibody HPA030521). d: TP53 expression in HNSCC tissues (antibody CAB002973). e: TP53 expression in normal nasopharynx tissues (antibody CAB002973). f: TP53 expression in normal oral mucosa tissues (antibody CAB002973). g: CCND1 expression in HNSCC tissues (antibody HPA000024). h: CCND1 expression in normal nasopharynx tissues (antibody CAB000024). i: CCND1 expression in normal oral mucosa tissues (antibody CAB000024). All the three genes presented moderate or strong intensity of immunostaining in HNSCC tissues while weak or medium intensity of immunostaining in normal nasopharynx and oral mucosa tissues.

needed to verify the inhibitory influence of candidate drugs on critical targets in HNSCC cells because molecular docking analysis was virtually a prediction of drug candidate positioning. In this study, we emphasized on seven of the 22 candidate drugs with unknown effect in HNSCC. The other 15 candidate drugs were either cancer-irrelavent compounds or small molecules that have been reported to exhibit anti-tumor activity in HNSCC, they are also worthy of noting in searching efficacious treatment for HNSCC in further studies.

5. Conclusion

In conclusion, we identified 22 candidate drugs for HNSCC via integrating HNSCC-related KEGG pathway and drug-affected pathways. At least two drugs with unknown effects in HNSCC out of the 22 candidate drugs might reduce the susceptibility of head and neck tissues to carcinogenesis through targeting proteins of hub genes in cell cycle and p53 signaling pathways. The bioinformatics analyses in this study were anticipated to shed lights on the molecular mechanism of the tumorigenesis HNSCC and provide new directions of innovative anti-tumor therapy for HNSCC.

Conflict of interest

Gan-guan Wei, Li Gao, Zheng-yi Tang, Peng Lin, Li-bin Liang, Jing-jing Zeng, Gang Chen and Long-cheng Zhang declare that they have no conflict of interest.

Funding

None.

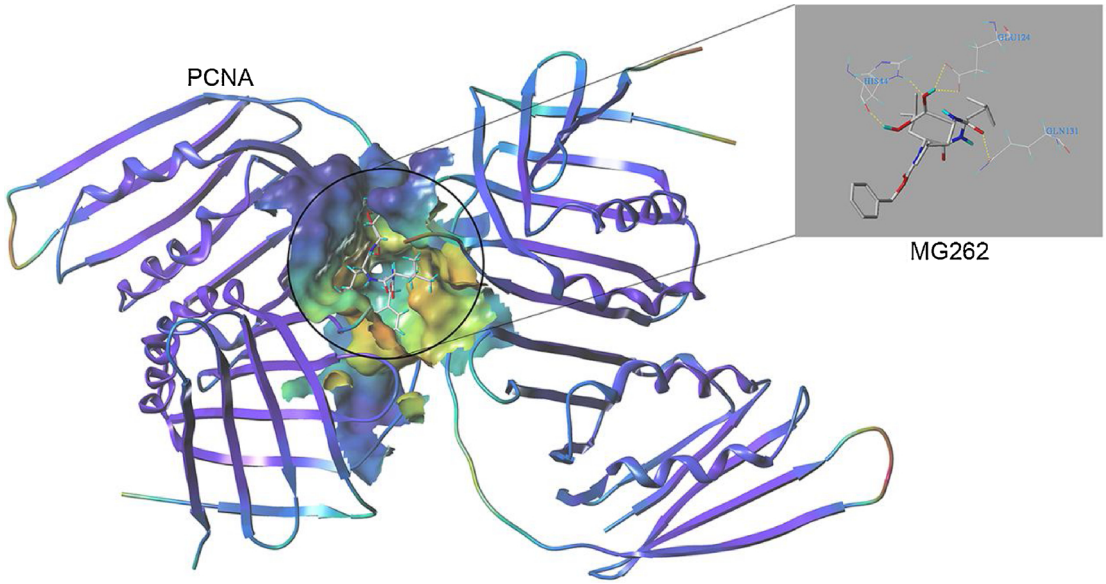

Fig. 8. Molecular docking between PCNA and MG262.
The strong binding affinity between PCNA and MG262 was manifested in the circle. Molecular structure of MG262 was projected in the rectangle at the upper left corner. A macromolecule surrounded by ribbons in the main body of the picture represented PCNA.

Table 5
Molecular docking between candidate drugs and PCNA.

Compounds	Total score	Crash	Polar	Similarity
Anisomycin	4.9061	−2.477	0.0374	0.333
Bepridil	7.8355	−2.673	0.0004	0.428
Emetine	5.4621	−1.6481	0.9517	0.174
Hycanthone	5.6237	−1.3718	1.3568	0.533
MG262	9.4364	−3.4798	3.1274	0.479
Pyrvinium	5.3727	−0.4434	0	0.293
Rottlerin	3.9106	−1.5127	2.9252	0.333

Acknowledgment

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.03.007.

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