Identification of Potential Genes both Differentially Expressed and Regulated by Differential Methylation Regions in Oral Squamous Cell Carcinoma

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Research Article

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Abstract

Background: The aberrant methylation included hypermethylation and hypomethylation plays significant role in the progression of many kind of cancers but poorly investigated in oral squamous cell carcinoma (OSCC).

Methods: GSE123781 and GSE87053 were used to identify the differential methylation regions (DMRs) by R software. the targeted genes regulated by DMRs were predicted by wANNOVAR. The biological process, cellular component, molecular function and cell pathways of common targeted genes between GSE123781 and GSE87053 were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analysis. The hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways were identified by protein to protein interaction network (PPI). Finally, the expression level of hub genes and correspondence relationship with head and neck squamous cell carcinoma (HNSCC) patient survival were confirmed by The Cancer Genome Atlas (TCGA) dataset.

Results: There are 372 common targeted genes regulated by DMRs between GSE123781 and GSE87053 and RUNX family transcript factor 1 (RUNX1), phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma (PIK3CG), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3CD), laminin subunit beta 1 (LAMB1), integrin subunit alpha M (ITGAM), interleukin 2 receptor subunit alpha (IL2RA), interleukin 10 (IL10), FYN proto-oncogene, Src family tyrosine kinase (FYN), cholinergic receptor muscarinic 1 (CHRM1) and C-C motif chemokine receptor 1 (CCR1) are hub genes in common targeted genes. Furthermore, RUNX1, PIK3R1, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 differentially expressed in HNSCC tissues and the overexpressed genes PIK3CD, LAMB1 and IL10 could lead to the decrease of HNSCC patient survival.

Conclusions: PIK3CD, LAMB1 and IL10 both differentially expressed and regulated by DMRs are quite associated with the progression of OSCC and the survival of HNSCC patients, which has much potential to be new biomarkers in targeted therapy of OSCC.

Background

Oral squamous cell carcinoma is the most common oral cancer which accounted for 95% of head and neck squamous cell carcinoma [1]. The surgery combined with adjunctive therapy was regarded as the main treatment method of OSCC patients [2]. Although the advancement in diagnosis and therapy, the survival rate of OSCC patients has not increased obviously resulting in the low five-year survival and high recurrence [3, 4]. In order to improve the outcome of OSCC patients, it is quite necessary to study the biomarkers associated with the progression of cancer.

DNA methylation, as one kind of epigenetic mechanism, has significant effect on the interaction between genes and phenotype in many kinds of cancers [5–7]. The DNA methylation occurs in the cytosine-

phosphate-guanine (CpG) islands of human genome and aberrant DNA methylation results in the occurrence of cancer [8]. Aberrant DNA hypermethylation may lead to the decrease of genes expression while aberrant DNA hypomethylation results in the increase of genes expression [9, 10]. The hypermethylation of CpG islands lead to the down-regulation of BARX homeobox 2 (BARX2) associated with the proliferation and invasion of gastric cancer cells [11]. DNA methylation could also silence of miR-486-5p, which lead to the promotion of proliferation and migration of colorectal cancer (CRC) by activation of PLAGL2/IGF2/β-catenin signal pathways [12]. Therefore, DNA methylation could influence the progression of many kinds of cancer by regulating some targeted genes or targeted miRNA. However, whether the DMRs among OSCC tissues, oral lichen planus (OLP) tissues and normal tissues could regulate the progress of OSCC by targeting differentially expressed genes has been poorly investigated.

In this study, the methylation data of GSE123781 and GSE87053 were used to analysis the DMRs and corresponding targeted genes. the biological process, cell component, molecular function and cell pathway of common targeted genes between GSE123781 and GSE87053 were analyzed by GO and KEGG enrichment analysis. The hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways were identified by PPI network. Finally, the expression level of hub genes and correspondence relationship with HNSCC patient survival were confirmed by TCGA dataset. All of them can provide new targeted genes both differentially expressed and regulated by aberrant methylation regions, which has much potential to be new biomarkers in targeted therapy of OSCC.

Methods

Datasets.

The methylation expression profiles of GSE123781 and GSE87053 were obtained from The Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo). The microarray data of GSE123781 submited by Ammerpohl O and Gassling V was based on GPL13534 Illumina HumanMethylation450 BeadChip (HumanMethylation450_15017482), which included 15 OSCC, 8 lichen planus and 18 control samples. The microarray data of GSE87053 submitted by Chatterjee R was based on GPL13534 Illumina HumanMethylation450 BeadChip (HumanMethylation450_15017482), which included 11 OSCC tissues and 10 normal tissues. The gene expression profiles and clinical sample information of HNSCC were downloaded from TCGA, which included 501 HNSCC tissues and 43 normal tissues.

Differential methylation regions.

The data of GSE123781 and GSE87053 was normalized and qualitied control to analyze DMRs by R software. The genes regulated by DMRs were predicted by wANNOVAR software. And the common targeted genes regulated by DMRs among OSCC tissues, OLP tissues and normal tissues were identified by Venny 2.1.0.(https://bioinfogp.cnb.csic.es/tools/venny/index.html).

GO enrichment analysis and KEGG enrichment analysis.

The biological process, cell component, molecular function and cell pathways of common targeted genes regulated by DMRs were analyzed by GO enrichment analysis and KEGG enrichment analysis through DAVID. And the results were presented by bubble charts which were made by R software. $P < 0.05$ was considered as cutoff value. The common targeted genes both associated with cancer biological process and cancer cell pathways were identified by Venny 2.1.0. (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

PPI network analysis.

The hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways were identified by PPI network through STRING (http://string-db.org/) and cytoscape3.7.2. The co-expression score ≥ 0.5 was considered as significant. The present times of genes ≥ 5 was considered as hub genes.

Verification the expression level of hub genes and correspondence relationship with HNSCC patient survival by TCGA dataset.

The expression level of hub genes in TCGA dataset were presented by Violin pictures, which were made by Graphpad prism 8.0.1. The relationship between the expression level of hub genes and the survival of HNSCC patients was presented by Kaplan–Meier (KM) survival curves, which were made by SangerBox.

Statistical analysis.

The statistical analysis was conducted by GraphPad Prism 8.0.1 software (San Diego, CA, USA). All data are shown as mean ± s.d. (s.d., standard deviation). The comparison of data between two groups were analyzed by the t-test. Values of at least $P < 0.05$ were considered statistically significant.

Results

There are 372 common targeted genes regulated by DMRs between GSE123781 and GSE87053.

The data of GSE123781 about the methylation sites among OSCC tissues, OLP tissues and normal tissues were normalized by R software (Fig. 1A, B). The data of GSE87053 about the methylation sites between OSCC tissues and normal tissues were normalized by R software (Fig. 1C). The DMRs in GSE123781 and GSE87053 were analyzed by R software and the genes regulated by DMRs in

GSE123781 and GSE87053 were predicted by wANNOVAR. Finally, the common targeted genes regulated by DMRs between GSE123781 and GSE87053 were analyzed by Venn picture, which indicated that there are 372 common targeted genes regulated by DMRs between GSE123781 and GSE87053 (Fig. 1D).

The cancer associated biological process, cellular component, molecular function and cell pathways of common targeted genes regulated by DMRs between GSE123781 and GSE87053 were analyzed by Go enrichment analysis and KEGG enrichment analysis.

The biological process, cell component and molecular function of common targeted genes were identified by GO enrichment analysis (Fig. 2A, B, D), which demonstrated that the common targeted genes mainly enriched in cancer associated biological process such as signal transduction, positive regulation of GTPase activity and cell adhesion. In addition, the cell pathways of common targeted genes were analyzed by KEGG enrichment analysis, which indicated that the common targeted genes mainly enriched in cancer associated cell pathways such as pathways in cancer, PI3K-AKT signal pathway and focal adhesion (Fig. 2C).

RUNX1, PIK3R1, PIK3CG, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 are hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways.

The common targeted genes both associated with cancer biological process and cancer cell pathways were identified by Venn, which indicated that there are 51 common targeted genes both associated with cancer biological process and cancer cell pathways (Fig. 3A). Furthermore, the hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways were analyzed by PPI (Fig. 3B), which demonstrated that RUNX1, PIK3R1, PIK3CG, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 are hub genes. All of these data suggested that RUNX1, PIK3R1, PIK3CG, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 are hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways.

RUNX1, PIK3R1, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 differentially expressed in HNSCC tissues.

The expression level of hub genes was confirmed by TCGA datasets, which suggested that RUNX1, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN and CCR1 up-regulated in HNSCC tissues (Fig. 4A, D, E, F, G, H, I and K). PIK3R1 and CHRM1 down-regulated in HNSCC tissues (Fig. 4B and J). the expression level of PIKCG has no difference between HNSCC and normal tissues (Fig. 4C). All of these data suggested that RUNX1, PIK3R1, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 differentially expressed in HNSCC tissues.

The differentially expressed genes PIK3CD, LAMB1 and IL10 are associated with HNSCC patient survival.

The relationship between the expression level of hub genes and HNSCC patient survival were analyzed by Kaplan–Meier (KM) survival curves, which demonstrated that the overexpressed genes PIK3CD, LAMB1 and IL10 were negatively associated with HNSCC patient survival (Fig. 5D, E and H). In addition, there were no relationship between the expression level of RUNX1, PIK3R1, PIK3CG, ITGAM, IL2RA, FYN, CHRM1 and CCR1 and HNSCC patient survival (Fig. 5A, B, C, F, G, I, J, K). All of these data indicated that the hub genes PIK3CD, LAMB1 and IL10 both differentially expressed in HNSCC tissues and regulated by DMRs among OSCC tissues, OLP tissues and normal tissues are quite associated with HNSCC patient survival.

Discussion

There are 372 common targeted genes regulated by DMRs between GSE123781 and GSE87053 and RUNX1, PIK3R1, PIK3CG, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 are hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways. Furthermore, RUNX1, PIK3R1, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 differentially expressed in HNSCC tissues and the overexpressed genes PIK3CD, LAMB1 and IL10 could lead to the decrease of HNSCC patient survival.

DNA methylation occurs in the CpG enriched regions clustered into CpG islands [13]. DNA methylation includes DNA hypermethylation and DNA hypomethylation. DNA hypermethylation could suppress the gene expression while DNA hypomethylation could promote gene expression but little study in cancer progression [9, 10, 14]. DNA hypermethylation could regulate the progress of cancer by suppressing gene expression. Recent study indicated that DNA hypermethylation undergoes further progression after colorectal cancer cell migrating into liver due to the fact that there is no change of DNA hypermethylation between primary colorectal cancer and colorectal cancer liver metastasis [15]. In addition, the hypermethylation of LIM homeobox 6 (LHX6) could promote the proliferation of human pancreatic cancer [16]. The CpG island hypermethylation could inhibit the expression level of BARX2 resulting in the inhibition of proliferation and invasion of gastric cancer cells [11]. And the promoter hypermethylation of

chondrolectin (CHODL) could promote carcinogenesis and indicate the poor survival of early-stage colorectal cancer [17]. DNA hypomethylation could also regulate the progress of cancer by promoting gene expression. Recent study indicated that the satellite DNA methylation play a significant role in the carcinogenesis of ovarian cancer by targeting cadherin-13 (CDH13) and RNA, ribosomal 45S cluster 1 (RNR1) [18]. The hypomethylation of lncRNA AFAP1-AS1 could promote the invasion and migration of cervical cancer [19]. The promoter hypomethylation results in the up-regulation of MicroRNA-10b-3p, which could promote the progress of esophageal squamous cell carcinoma by targeting forkhead box O3 (FOXO3) [20]. Therefore, the aberrant methylation included hypermethylation and hypomethylation has significant effect on the progression of many kind of cancers. In this study, the methylation data profiles of GSE123781 and GSE87053 were downloaded to identify the different methylation sites among OSCC tissues, OLP tissues and normal tissues. the different methylation sites were changed into DMRs by cluster analysis. The common targeted genes regulated by DMRs in GSE123781 and GSE87053 were predicted by wANNOVAR software. These common targeted genes are mainly enriched in cancer associated biological process such as signal transduction, positive regulation of GTPase activity and cell adhesion and cancer cell pathways such as pathways in cancer, PI3K-AKT signal pathway and focal adhesion. RUNX1, PIK3R1, PIK3CG, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 are hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways. Finally, RUNX1, PIK3R1, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 differentially expressed in HNSCC tissues and the overexpressed genes PIK3CD, LAMB1 and IL10 could decrease HNSCC patient survival.

The class Ⅰ PI3Ks includes four catalytic subunits: PIK3CA, PIK3CB, PIK3CG and PIK3CD. PIK3CD, also known as PI3Kδ and p110δ, plays a significant role in the progression of cancer [21]. PIK3CD could induce the CRC growth and invasion by AKT/GSK-3β/β-catenin signal pathways [22]. microRNAs could also regulate the progression of many kinds of cancers by targeting PIK3CD. Recent study indicated that microRNA-30b could suppress the progression of CRC by targeting PIK3CD [23]. And microRNA663 could inhibit the progression of human Glioblastoma by targeting PIK3CD [24]. In addition, PIK3CD is also a potential gene regulated by differentially methylation sites in HNSCC [25]. In this study, we found that the PIK3CD not only regulated by the DMRs in OSCC but also differentially expressed in HNSCC. Furthermore, the overexpressed PIK3CD leads to decrease of the HNSCC patient survival. Therefore, PIK3CD both regulated by DMRs in OSCC and differentially expressed in HNSCC is a potential gene associated with the progression of OSCC. Laminins constitutes the ECM to affect the biological process of many kind of cancers. LAMB1, as the β-subunit of Laminins, also constitutes the ECM and promote the invasion and metastasis of cancer [26]. Recent study indicated that LAMB1 is quite associated with sentinel lymph node metastasis of primary cutaneous melanoma [27]. microRNA-124-5p could inhibit the growth of high-grade gliomas by regulating posttranscription of LAMB1 [28]. However, the role and function of LAMB1 in OSCC has poorly investigated. In this study, we found that the LAMB1 not only regulated by the DMRs in OSCC but also differentially expressed in HNSCC. Furthermore, the overexpressed LAMB1 leads to decrease of the HNSCC patient survival. Therefore, LAMB1 both regulated by DMRs in OSCC and differentially expressed in HNSCC is a potential gene associated with the progression of OSCC. IL-10 is a

kind of immune regulatory cytokines having anti-inflammatory and immunosuppression function. High expression level of IL10 could predict the poor prognosis of OSCC patients [29]. In addition, IL-10 plays a significant role in the process that chronic inflammation promoting the progress of OSCC [30]. CAF in OSCC tumor microenvironment could secret IL-10 to induce the formation of M2 macrophage, which could contribute to an immunosuppressive microenvironment [31]. In this study, we found that the IL-10 not only regulated by the DMRs in OSCC but also differentially expressed in HNSCC. Furthermore, the overexpressed IL10 leads to decrease of the HNSCC patient survival. Therefore, IL10 both regulated by DMRs in OSCC and differentially expressed in HNSCC is a potential gene associated with the progression of OSCC.

Conclusion

In conclusion, PIK3CD, LAMB1 and IL10 both associated with cancer biological process and cancer cell pathways are regulated by DMRs in OSCC and overexpressed in HNSCC. The overexpressed PIK3CD, LAMB1 and IL10 could lead to the decrease of HNSCC patient survival. Therefore, PIK3CD, LAMB1 and IL10 have much potential to be new target in targeted therapy of OSCC.

Abbreviations

OSCC: oral squamous cell carcinoma; DMRs: differential methylation regions; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes pathway; PPI: protein to protein interaction network; HNSCC: head and neck squamous cell carcinoma; TCGA: The Cancer Genome Atlas; RUNX1: RUNX family transcript factor 1; PIK3R1: phosphoinositide-3-kinase regulatory subunit 1; PIK3CG: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma; PIK3CD: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta; LAMB1: laminin subunit beta 1; ITGAM: integrin subunit alpha M; IL2RA: interleukin 2 receptor subunit alpha; IL10: interleukin 10; FYN: FYN proto-oncogene, Src family tyrosine kinase; CHRM1: cholinergic receptor muscarinic 1.

Declarations

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Author contributions

YN designed this experiment and conducted data analysis; CS wrote this article; QS revised this article.

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Availability of data and material

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interests.

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Figures

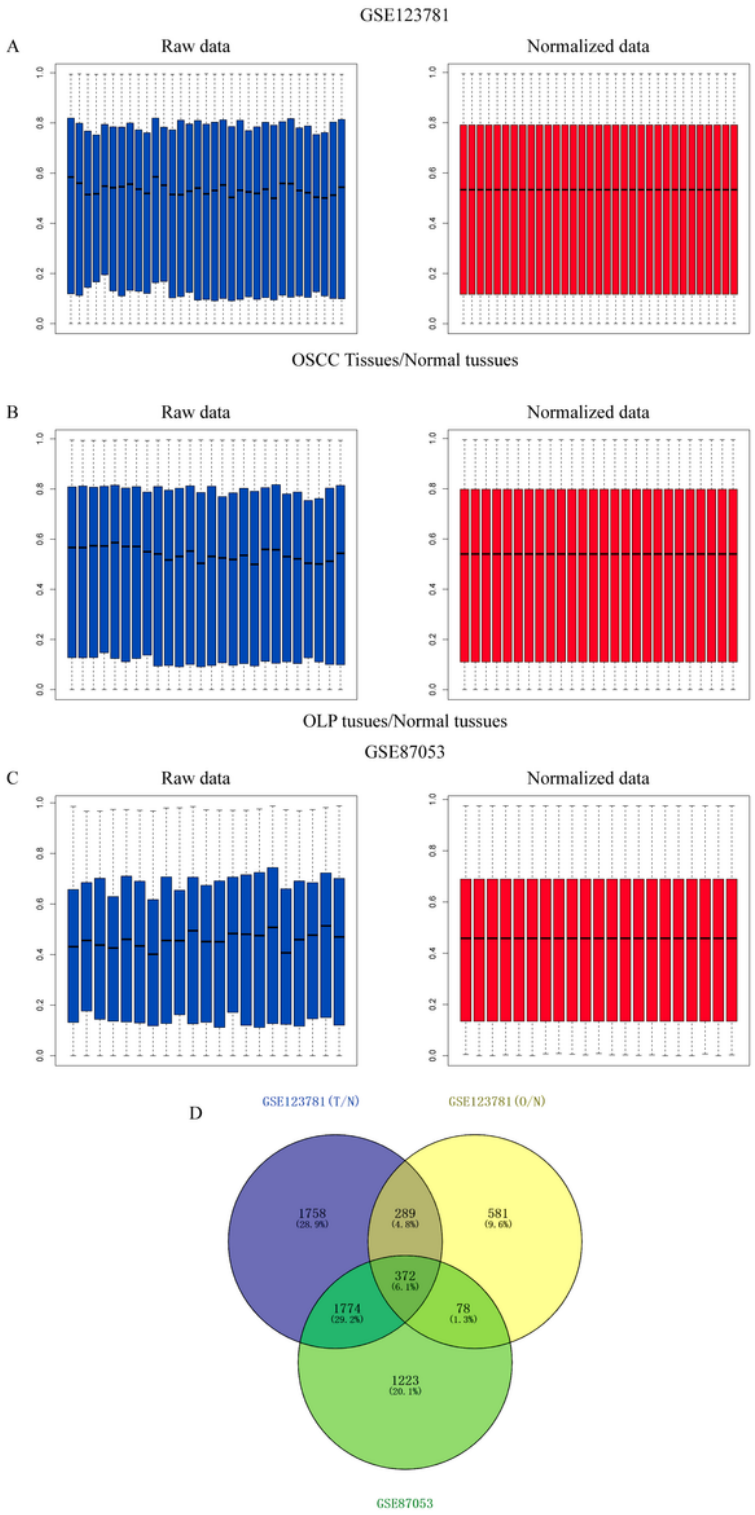

Figure 1

Identification of common targeted genes regulated by differentially methylation regions among OSCC tissues, OLP tissues and normal tissues. (A, B) The data of GSE123781 about the methylation among OSCC tissues, OLP tissues and normal tissues was normalized by R software. (C) The data of GSE87053 about the methylation between OSCC tissues and normal tissues was normalized by R software. (D) The

common targeted genes regulated by the DMRs between GSE123781 and GSE87053 were analyzed by Venn.

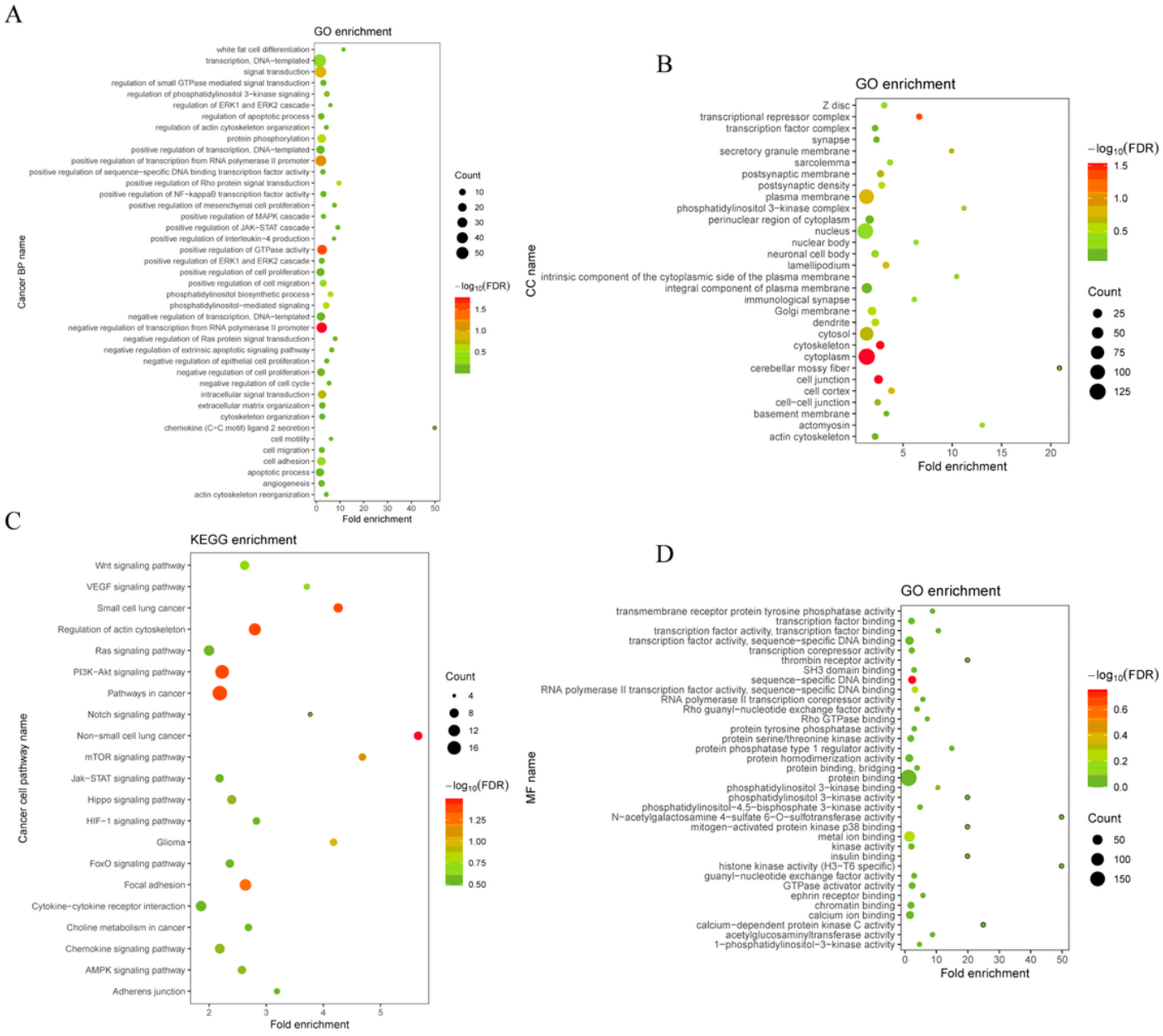

Figure 2

Identification of common targeted genes regulated by differentially methylation regions among OSCC tissues, OLP tissues and normal tissues. (A, B) The data of GSE123781 about the methylation among OSCC tissues, OLP tissues and normal tissues was normalized by R software. (C) The data of GSE87053 about the methylation between OSCC tissues and normal tissues was normalized by R software. (D) The common targeted genes regulated by the DMRs between GSE123781 and GSE87053 were analyzed by Venn.

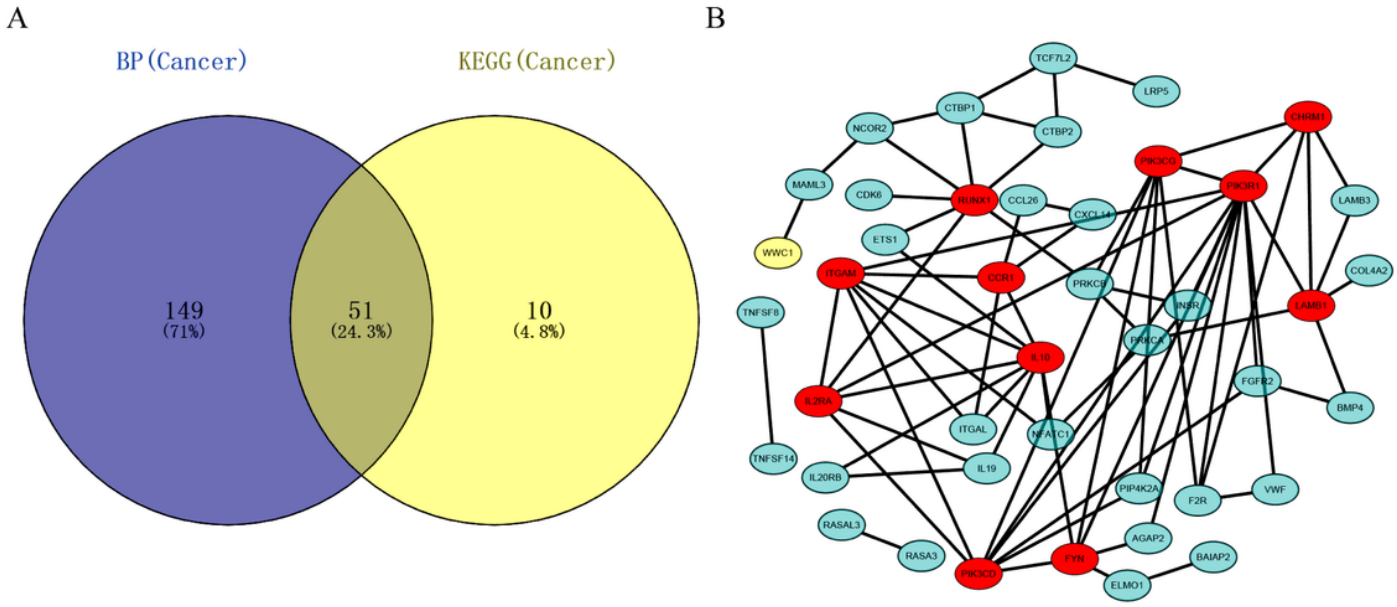

Figure 3

The hub genes in the common targeted genes both associated with cancer biological process and cancer cell pathways were analyzed by PPI network. (A) The common targeted genes both associated with cancer biological process and cancer cell pathways were analyzed by Venn. (B) The hub genes in common targeted genes both associated with cancer biological process and cancer cell pathways were identified by PPI network.

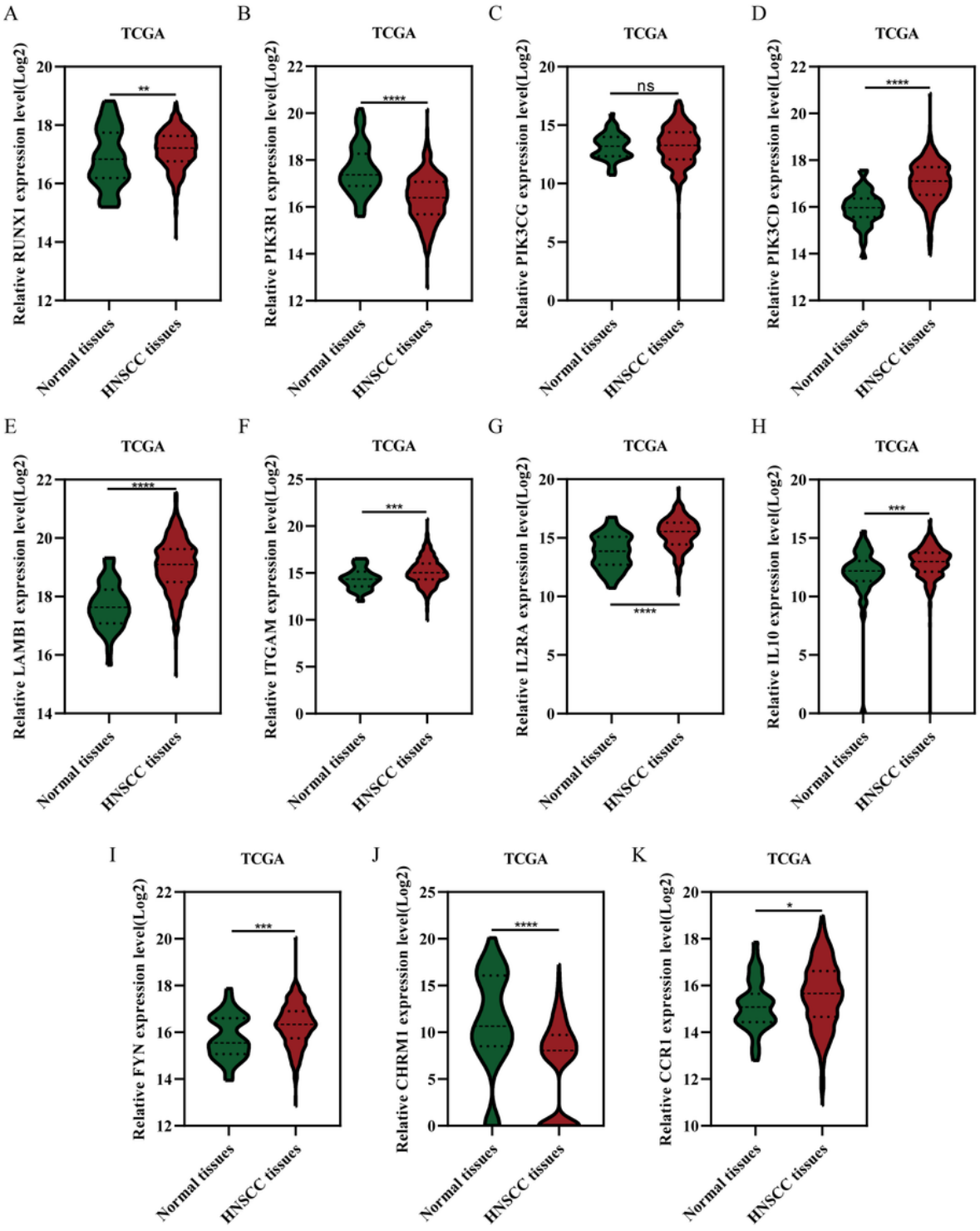

Figure 4

The expression level of hub genes was confirmed by TCGA datasets. (A, B, C, D, E, F, G, H, I, J and K) The expression level of RUNX1, PIK3R1, PIK3CG, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1 and CCR1 in TCGA datasets respectively. Data are shown as mean ± s.d. (s.d., standard deviation). Statistical analysis was performed by Student's t-test. ns means P>0.05, *Indicates P<0.05, **indicates P < 0.01, ***indicates P<0.001, ****indicates P<0.0001.

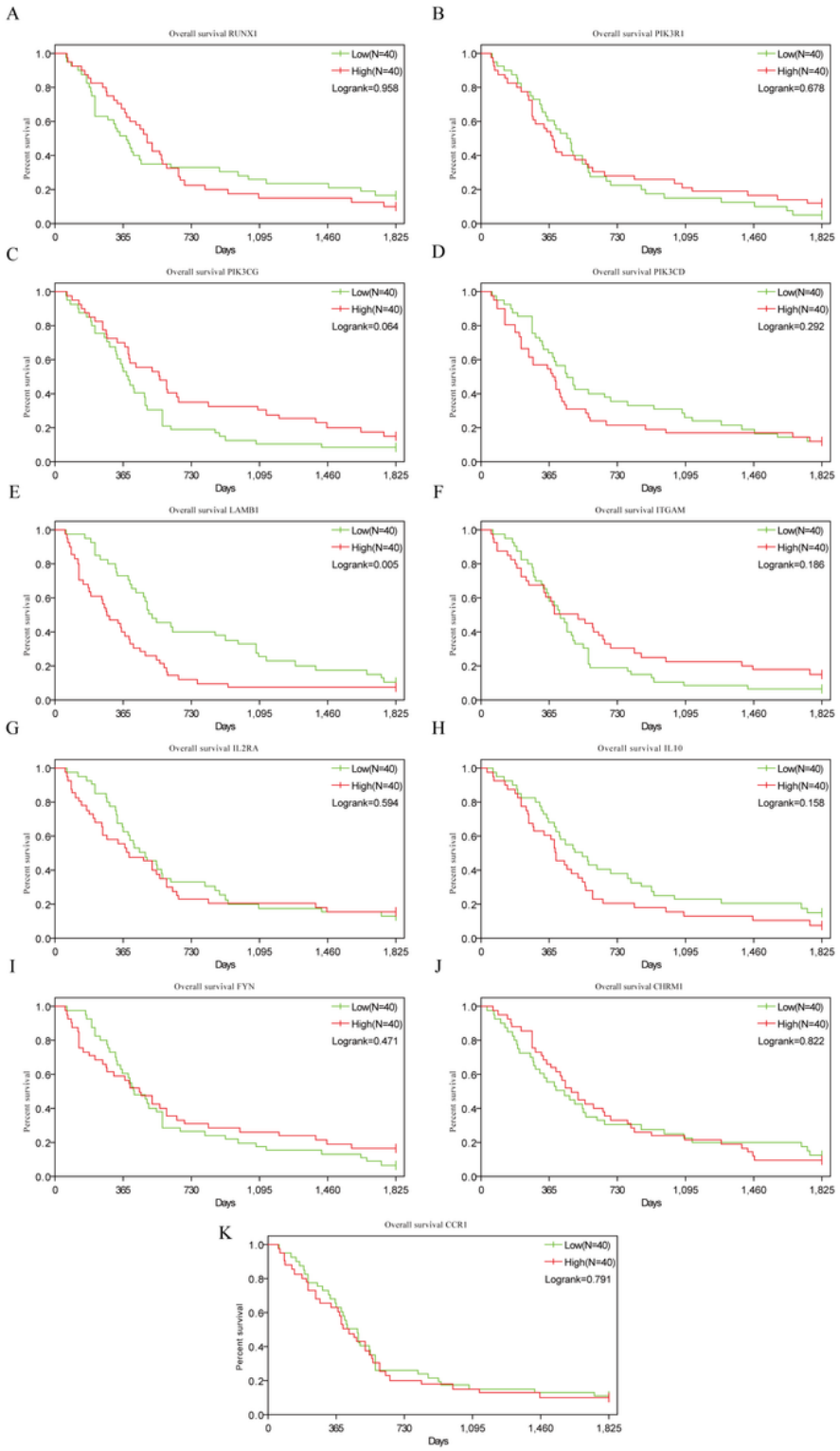

Figure 5

The relationship between expression level of hub genes and patient survival were confirmed by TCGA datasets. (A, B, C, D, E, F, G, H, I, J and K) The relationship between expression level of RUNX1, PIK3R1, PIK3CG, PIK3CD, LAMB1, ITGAM, IL2RA, IL10, FYN, CHRM1, CCR1 and HNSCC patient survival were confirmed by TCGA dataset.