Identification of Differential Methylation Regions and Correspondence Targeted Genes in Oral Squamous Cell Carcinoma

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Research article

Keywords: OSCC, DNA Methylation, Differentially expressed genes, Bioinformatics, Progression

Posted Date: May 27th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-27701/v1>

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Abstract

Background

The differential methylation included hypermethylation and hypomethylation plays significant role in the progression of many kind of cancers but study little in oral squamous cell carcinoma (OSCC).

Methods

GSE123781 and GSE87053 was used to analysis the differential methylation regions (DMRs) and predict the target genes in OSCC by R software and wANNOVAR respectively. the biological process 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 (hub genes 1) in common targeted genes associated with cancer biological process was identified by protein to protein interaction network (PPI). In addition, GSE74530 and GSE30784 was used to identify common differentially expressed genes (DEGs) in OSCC by R software. The biological process and cell pathways of common DEGs was analyzed by GO and KEGG enrichment analysis. The hub genes (hub genes 2) in common DEGs associated with cancer biological process was identified by PPI network. The significant hub genes between hub genes 1 and hub genes 2 were identified by Venn picture. Finally, the expression level of significant 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 2146 common targeted genes regulated by DMRs between GSE123781 and GSE87053 and 278 hub genes in common targeted genes associated with cancer biological process. In addition, there are 895 common DEGs between GSE74530 and GSE30784 and 144 hub genes in common DEGs associated with cancer biological process. There are 9 significant hub genes between hub genes 1 and hub genes 2. Finally, these 9 significant hub genes differentially expressed in HNSCC tissues except CCR7 and quite associated with the survival of HNSCC patients.

Conclusions

CCR7, ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7, LGALS3 and CDKN3 both are DEGs and regulated by DMRs in OSCC, which are quite associated with the progression of OSCC and the survival of HNSCC patients. All of these genes have much potential to be new biomarkers in targeted therapy of OSCC.

Backgrounds

Oral squamous cell carcinoma is 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, which results 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, have a significant effect on the interaction between genes and phenotype in many kinds of cancers [5–7]. The DNA methylation occurs in the 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 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 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 differential methylation regions between OSCC tissues and normal tissues could regulate the progress of OSCC by targeting genes has not been investigated.

In this study, the methylation data of GSE123781 and GSE87053 was used to analysis the DMRs and predict the target genes. the biological process 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 associated with cancer biological process was identified by constructing PPI network. In addition, the genes expression data of GSE74530 and GSE30784 was used to identify common DEGs between OSCC tissues and normal tissues. The biological process and cell pathways of common DEGs was analyzed by GO and KEGG enrichment analysis. The hub genes in common DEGs associated with cancer biological process was identified by PPI network. The significant hub genes between hub genes in common targeted genes associated with cancer biological process and hub genes in common DEGs associated with cancer biological process were identified by Venn picture. Finally, the expression level of significant hub genes and correspondence relationship with HNSCC patient survival were confirmed by TCGA dataset. All of them can provide new aberrant DNA methylation regions to regulate the progress of OSCC by targeting genes, which has much potential to be new biomarkers in targeted therapy of OSCC.

Methods

Datasets.

The methylation expression profiles and gene expression profiles of GSE123781, GSE87053, GSE74530 and GSE30784 were obtained from The Gene Expression Omnibus (GEO,

<http://www.ncbi.nlm.nih.gov/geo>). The microarray data of GSE123781 submitted 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 of GSE74530 submitted by Oghumu S, Knobloch T and Weghorst C was based on GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array, which included 6 OSCC tissues and 6 normal tissues. The gene expression profiles of GSE30784 was based on GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array and submitted by Chen C et al, which included 167 OSCC tissues, 17 dysplasia tissues and 45 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 and differential expression analysis.

The data of GSE123781 and GSE87053 was normalized and qualified control to analyze DMRs by R software. The genes regulated by DMRs were predicted by WANNVAR software. And the common genes regulated by DMRs between OSCC tissues and normal tissues was identified by Venn picture. The data of GSE74530 and GSE30784 was normalized to identify the DEGs by R software. The results of DEGs were presented by volcano pictures which were made by SangerBox. The common DEGs between GSE74530 and GSE30784 was presented by Venn picture, which was made by Venny 2.1.0. (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>). $P < 0.05$ and $|\text{LogFC}| > 1$ was defined as cutoff value to identify differential methylation sites and DEGs.

GO enrichment analysis and KEGG enrichment analysis.

The biological process and cell pathways of common genes regulated by DMRs and common DEGs between OSCC tissues and normal tissues were analyzed by GO enrichment analysis and KEGG enrichment analysis through Cytoscape 3.7.2. and the results were presented by bubble charts which were made by R software. $P < 0.05$ was considered as cutoff value.

PPI network analysis.

The hub genes in common genes both associated with cancer biological process and regulated by DMRs (hub genes 1) and the hub genes in common DEGs associated with biological process (hub genes 2) were identified by constructing PPI network through STRING (<http://string-db.org/>). The co-expression score > 0.5 was considered as significant. The present times of genes > 10 was considered as hub genes. The

significant hub genes between hub genes 1 and hub genes 2 was identified by Venny 2.1.0. (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>).

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

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

Identification of common target genes regulated by DMRs between OSCC tissues and normal tissues.

The data of GSE123781 about the methylation sites between OSCC tissues and normal tissues was normalized (Fig. 1A) and qualified control (Fig. 1B) by R software respectively. The data of GSE87053 about the methylation sites between OSCC tissues and normal tissues was normalized (Fig. 1C) and qualified control (Fig. 1D) by R software respectively. The DMRs in GSE123781 and GSE87053 was analyzed by R software and the genes regulated by DMRs in GSE123781 and GSE87053 were predicted by WANNVAR (Additional file 1). And the common genes regulated by DMRs between GSE123781 and GSE87053 was analyzed by Venn picture (Fig. 1E).

The biological process and cell pathways of common target genes regulated by DMRs in GSE123781 and GSE87053 were analyzed by GO enrichment analysis and KEGG enrichment analysis respectively.

The biological process of common genes regulated by DMRs in GSE123781 and GSE87053 was analyzed by GO enrichment analysis, which indicated that most of genes are enriched in the cancer

associated biological process (Fig. 2A). In addition, the cell pathway of common genes regulated by DMRs in GSE123781 and GSE87053 was analyzed by KEGG enrichment analysis, which demonstrated that the most of genes are enriched in cAMP signal pathway, neuroactive ligand-receptor interaction and cholinergic synapse (Fig. 2B).

The hub genes (hub genes 1) in the common genes both associated with cancer biological process and regulated by DMRs in GSE123781 and GSE87053 was identified by PPI network.

The common target genes mainly enriched in cancer associated biological process and cell pathways such as cell-substrate adhesion, cell migration, cell motility and cAMP signal pathway (Fig. 3A, B). Therefore, the common genes both associated with cancer biological process and regulated by DMRs in GSE123781 and GSE87053 were selected to construct PPI network (Fig. 3C), which suggested there are 278 hub genes in the common genes both associated with cancer biological process and regulated by DMRs in GSE123781 and GSE87053 (Additional file 2).

Identification of common DEGs between OSCC tissues and normal tissues through GSE74530 and GSE30784 datasets.

The data of GSE74530 was normalized to identify the DEGs by R software (Fig. 4A), which indicated that there are 1680 DEGs between OSCC tissues and normal tissues (Fig. 4C). The data of GSE30784 was normalized to identify the DEGs by R software (Fig. 4B), which indicated that there are 1850 DEGs between OSCC tissues and normal tissues (Fig. 4D). Finally, the common DEGs in GSE74530 and GSE30784 were identified by Venny 2.1.0, which indicated that there are 895 common DEGs between GSE74530 and GSE30784 (Fig. 4E, Additional file 3).

The biological process and cell pathways of common DEGs were identified by GO enrichment analysis and KEGG enrichment analysis.

The biological process of common DEGs was analyzed by GO enrichment analysis, which demonstrated that most of common DEGs are enriched in cancer associated biological process (Fig. 5A). furthermore, the cell pathway of common DEGs was analyzed by KEGG enrichment analysis, which suggested that most of common DEGs are enriched in cancer associated cell pathways such as NF-kappa B signal pathway, IL-17 signal pathway, TNF signal pathway, Focal adhesion and small cell lung cancer (Fig. 5B).

The significant hub genes between hub genes 1 and hub genes 2 was identified by Venn picture.

The common DEGs mainly enriched in cancer biological process such as cell adhesion, cell migration, cell death and cell apoptosis and cancer cell pathways such as TNF signal pathway, small cell lung cancer and IL-17 signal pathway (Fig. 6A, B). Therefore, the common DEGs associated with cancer biological process were selected to construct the PPI network to identify the hub genes (Fig. 6C), which suggest that there are 144 hub genes (hub genes 2) in the common DEGs associated with cancer biological process (Additional file 2). The significant hub genes between hub genes 1 and hub genes 2 was analyzed by Venn picture (Fig. 6D), which indicated there are 9 significant hub genes between hub genes 1 and hub genes 2 (Additional file 2).

The expression level of significant hub genes was confirmed by TCGA dataset.

The expression level of these 9 significant hub genes was confirmed by TCGA dataset, which indicated that ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7 and CDKN3 up-regulated in HNSCC tissues (Fig. 7B, C, D, E, F, G and I) while LGALS3 down-regulated in HNSCC tissues (Fig. 7H). The expression level of CCR7 has no different between HNSCC tissues and normal tissues (Fig. 7A).

The relationship between expression level of significant hub genes and HNSCC patient survival was confirmed by TCGA dataset.

The mRNA expression level of these 9 significant hub genes and correspondence clinical information of samples were downloaded from TCGA dataset. the relationship between the expression level of these 9 significant hub genes and HNSCC patient survival was analyzed by SangerBox, which results suggested that the up-regulated ETS1, RUNX3, CCR1, LAMB1, IRF7 and CDKN3 have negative effect on HNSCC patient survival (Fig. 8B, C, D, F, G and I), while up-regulated CCR7 and C3AR1 have positive effect on HNSCC patient survival (Fig. 8A, E). Finally, the down-regulated LGALS3 has positive effect on HNSCC patient survival (Fig. 8H).

Discussion

There are 2146 common targeted genes regulated by DMRs between GSE123781 and GSE87053 and 278 hub genes in common targeted genes associated with cancer biological process. In addition, there are 895 common DEGs between GSE74530 and GSE30784 and 144 hub genes in common DEGs associated with cancer biological process. There are 9 significant hub genes between hub genes in common targeted

genes associated with cancer biological process and hub genes in common DEGs associated with cancer biological process. Finally, these 9 significant hub genes differentially expressed in HNSCC tissues except CCR7 and quite associated with the survival of HNSCC patients.

DNA methylation occurs in the cytosine-phosphate-guanine (CpG) enriched regions, which 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 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 [17]. And the promoter hypermethylation of CHODL could promote carcinogenesis and indicate the poor survival of early-stage colorectal cancer [18]. 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 CDH13 and RNR1 [19]. The hypomethylation of lncRNA AFAP1-AS1 could promote the invasion and migration of cervical cancer [20]. The promoter hypomethylation results in the up-regulation of MicroRNA-10b-3p, which could promote the progress of esophageal squamous cell carcinoma by targeting FOXO3 [21]. Therefore, the aberrant methylation included hypermethylation and hypomethylation has significant effect in the progression of many kind of cancers. In this study, we downloaded the methylation data profiles of GSE123781 and GSE87053 and analyzed the different methylation sites between OSCC tissues and normal tissues. the different methylation sites were changed into DMRs by cluster analysis. The biological process and cell pathways of targeted genes regulated by DMRs were analyzed by GO enrichment analysis and KEGG enrichment analysis and the hub genes in the targeted genes associated with cancer biological process was analyzed by PPI network. Furthermore, the gene data profiles of GSE74530 and GSE30784 was downloaded to analyze the common DEGs and hub genes in the common DEGs associated with cancer biological process. The genes CCR7, ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7, LGALS3 and CDKN3 both differentially expressed in OSCC and regulated by the DMRs in OSCC. these genes also differentially expressed in HNSCC except CCR7 according to TCGA datasets, which may relevant with the number of patient samples. In addition, the different expression level of CCR7, ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7, LGALS3 and CDKN3 is quite associated with the survival of HNSCC patients. Therefore, the genes CCR7, ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7, LGALS3 and CDKN3 both differentially expressed and regulated by DMRs have much potential to regulate the progress of OSCC and have a significant effect on the survival of OSCC patients.

Recent study suggested that CCR7/CCL21 interaction can promote the lymph node of OSCC directly [22]. And CCR7/CCL21 interaction could also enhance the EMT process and promote stemness of OSCC by activating JAK2/STAT3 signal pathway [23]. Therefore, CCR7 is quite associated with the progression of

OSCC. ETS-1, as a crucial member of transcript factor, could regulate the MMP9 expression, which has a significant effect on the invasion and progression of breast cancer [24]. ETS1 can also inhibit the growth and metastasis of breast cancer [25]. ETS1 also plays a significant role in the progression of gastric cancer [26]. But the role and function of ETS1 in the progression of OSCC was studied little. Recent study indicated that RUNX3 could inhibit invasion, migration and angiogenesis of OSCC [27]. The loss of RUNX3 could inhibit the bone invasion of OSCC [28]. Therefore, the RUNX3 is quite associated with progression of OSCC. The CCR1 has a significant effect on the colorectal cancer liver metastasis [29]. The loss of SMAD4 could promote the accumulation of MDSCs around the colorectal cancer through CCL15-CCR1, which could promote the progression of colorectal cancer [30]. MiR-126-3p could inhibit the growth, invasion and migration of NSCLC by targeting CCR1 [31]. But the role and function of CCR1 has little investigated in OSCC. C3AR1 studied little in OSCC. LAMB1, as extracellular matrix protein, plays a significant role in the progression of cancer. Recent study demonstrated that MiR-124-5p could inhibit the growth of high-grade gliomas through LAMB1 [32]. But the role and function of LAMB1 in the progression of OSCC has not been investigated. Recent study suggested that MicroRNA-762 could promote the proliferation and invasion of breast cancer by targeting IRF7 expression [33]. Circular RNA circ-EGLN3 could promote the proliferation and aggressiveness of renal cell carcinoma via miR-1299-mediated IRF7 activation [34]. And the IRF7 also has a significant effect on the immunological dormancy of breast cancer [35]. But the role and function of IRF7 in the progression of OSCC has not been investigated. LGALS3 could influence the progression of colorectal cancer by Wnt/ β -catenin pathway [36]. LGALS3 also an important prognostic marker in breast cancer [37]. The overexpression of LGALS3 also a predictor of poor prognosis of cervical cancer [38]. Therefore, LGALS3 is an important prognostic marker in many kinds of cancers but little study in OSCC. Recent study demonstrated that YY1 could inhibit the proliferation and migration of pancreatic ductal adenocarcinoma by regulating CDKN3/MdM2/P53/P21 signal pathway [39]. The knockdown of CDKN3 could inhibit the proliferation, invasion and promote the apoptosis of human ovarian cancer [40]. CDKN3 could also promote the progression of esophageal cancer [41]. Therefore, CDKN3 is quite associated with the progression of many kinds of cancer but study little in OSCC.

Conclusions

In conclusion, the genes CCR7, ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7, LGALS3 and CDKN3 both differentially expressed in OSCC and regulated by DMRs in OSCC, which are quite associated with the progression of OSCC and the survival of OSCC patients. All of these genes have much potential to be new biomarkers in targeted therapy of OSCC.

Abbreviations

CCR7 C-C motif chemokine receptor 7

ETS1 ETS proto-oncogene 1

RUNX3 RUNX family transcription factor 3

CCR1 C-C motif chemokine receptor 1

C3AR1 complement C3a receptor 1

LAMB1 laminin subunit beta 1

IRF7 interferon regulatory factor 7

LGALS3 galectin 3

CDKN3 cyclin dependent kinase inhibitor 3

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

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

Competing interests

The authors declare no conflict of interests.

Funding

Funding information is not applicable.

Author contributions

Yan Niu designed this experiment and conducted data analysis; Qiusheng Shan wrote this article; Yuanling Guo revised this article.

Acknowledgements

Not applicable

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Figures

Figure 1

The biological process and cell pathways of common genes regulated by DMRs in GSE123781 and GSE87053 were analyzed by GO enrichment analysis and KEGG enrichment analysis. A The biological process of common target genes regulated by DMRs in GSE123781 and GSE87053 were analyzed by GO enrichment analysis. B The cell pathway of common target genes regulated by DMRs in GSE123781 and GSE87053 were analyzed by KEGG enrichment analysis

Figure 3

The hub genes (hub genes 1) in the common target genes both associated with cancer biological process and regulated by DMRs in GSE123781 and GSE87053 was analyzed by PPI network. A The cancer biological process of common genes regulated by DMRs in GSE123781 and GSE87053. B The cancer cell pathway of common genes regulated by DMRs in GSE123781 and GSE87053. C PPI network was constructed by the common genes both associated with cancer biological process and regulated by DMRs in GSE123781 and GSE87053

Figure 4

Identification the common DEGs between OSCC and normal tissues. A The data of GSE74530 about the expression of genes in OSCC tissues and normal tissues were normalized by R software. C Analyze the DEGs between OSCC tissues and normal tissues in GSE74530; Green color means down-regulated genes and red color means up-regulated genes. B The data of GSE30784 about the expression of genes in OSCC tissues and normal tissues were normalized by R software. D Analyze the DEGs between OSCC tissues and normal tissues in GSE30784; Green color means down-regulated genes and red color means up-regulated genes. E Identified the common DEGs between GSE74530 and GSE30784

Figure 5

The correspondence biological process and cell pathways of common DEGs were analyzed by GO enrichment analysis and KEGG enrichment analysis respectively. A The biological process of common DEGs were analyzed by GO enrichment analysis. B The cell pathways of common DEGs were analyzed by KEGG enrichment analysis

Figure 6

Identification the significant hub genes between hub genes (hub genes 1) associated with cancer biological process in the common target genes regulated by DMRs in GSE123781 and GSE87053 and hub genes (hub genes 2) associated with cancer biological process in common DEGs between GSE74530 and GSE30784. A The cancer biological process of common DEGs between GSE74530 and GSE30784. B The cancer cell pathway of common DEGs between GSE74530 and GSE30784. C The hub genes (hub genes 2) in common DEGs associated with cancer biological process was identified by PPI network. D The significant hub genes between hub genes1 and hub genes 2 was identified by Venn picture

Figure 7

The expression level of significant hub genes was confirmed by TCGA datasets. A, B, C, D, E, F, G, H and I The expression level of CCR7, ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7, LGALS3 and CDKN3 in TCGA datasets respectively

Figure 8

The relationship between expression level of significant hub genes and patient survival were confirmed by TCGA datasets. A, B, C, D, E, F, G, H and I The relationship between expression level of CCR7, ETS1, RUNX3, CCR1, C3AR1, LAMB1, IRF7, LGASL3 and CDKN3 and HNSCC patients survival were confirmed by TCGA dataset. Data are shown as mean $\pm$ 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 $P < 0.0001$

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