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Shared genetic and epigenetic mechanisms between chronic periodontitis and oral squamous cell carcinoma

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ABSTRACT

Objectives: To analyze bioinformatic datasets for detecting genetic and epigenetic mechanisms shared by chronic periodontitis (CP) and oral squamous cell carcinoma (OSCC).

Materials and methods: Datasets from GEO and TCGA databases reporting mRNAs, miRNAs or methylation expression in human CP and OSCC tissues were analyzed. Differential expression, functional enrichment and protein-protein interaction (PPI) network analyses were performed. Differentially expressed miRNAs (DEmiRNAs) and genes (DEG) in CP and OSCC were determined. DEmiRNA-target and DEmiRNA-DEG networks were constructed. Directly and indirectly interacting cross-talk genes were screened, and their prediction accuracy and association with OSCC prognosis was determined.

Results: 3 DE-miRNAs (miR-375, miR-3609 and miR-3652) expressed in both CP and OSCC critically regulated most DEGs. Among 12 directly interacting cross-talk genes, NCAPH was significantly related with the prognosis of OSCC. NR2F2 had highest differential expression in CP and OSCC. Among 4 cross-talk genes (FN1, MPPED1, NDEL1, and NR2F2) differentially expressed in CP, 3 (FN1, MPPED1, NDEL1) were also expressed in OSCC. Among 12 indirectly interacting cross-talk genes differentially expressed in OSCC, 3 genes (CDCA8, HIST1H3J, and RAD51) were significantly related to its prognosis. Significant pathways involved in CP and OSCC included: chemokine receptors, class I PI3K signaling events, epithelial-to-mesenchymal transition and signaling events by VEGFR1 and VEGFR2, EGF receptor (ErbB1).

Conclusion: Bioinformatic analysis of available datasets implicated 1 directly interacting cross-talk gene (NCAPH), 4 indirectly interacting cross-talk genes (NCAPH, NR2F2, FN1, and MPPED1) and 3 DE-miRNAs (hsa-miR-375, miR-3609 and miR-3652) as shared genetic and epigenetic expression patterns between CP and OSCC.

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Introduction

Chronic infection and persistent inflammation are associated with an increased risk of cancer [1]. 15–20% of cancer is reported to originate from chronic inflammation [1]. For oral cancer, chronic periodontitis (CP) has been documented as a significant and independent risk factor [2]. Oral squamous cell carcinoma (OSCC) is the most common type of oral malignancy, accounting for 80–90% of oral malignancies [3]. Systematic reviews [4,5] have shown a significant association between CP and OSCC. Shared risk factors for CP and OSCC include smoking, socioeconomic status, diabetes, age, gender, and ethnicity, as well as genetic and epigenetic alterations [6].

Altered expression of DNA methylation, messenger RNAs (mRNAs), microRNAs (miRNAs), and signaling pathways have been reported to play critical roles in the onset and progression of both CP and OSCC. Genetic and epigenetic modifications may be caused by carcinogenic metabolites produced by various periodontopathogenic bacteria harbored in periodontal pockets, and signaling pathways such as ERK1/2-Ets1, p38/HSP27, PAR2/NF-KB and β-catenin are suggested to link CP with OSCC [2]. A significant overlap of altered DNA methylation patterns between CP and OSCC has been reported [7]. The expression and functions of various mRNAs, miRNAs, and signaling pathways in both oral inflammation and tumorigenesis have been documented. Aberrant expression of cytokine mRNAs [8] and chemokine mRNAs [9] caused by chronic inflammation can potentially alter tissue microenvironment and thus promote tumorigenesis. MicroRNAs might be used as diagnostic biomarkers and are emerging therapeutic targets in both CP and oral cancer [10,11].

Despite the genetic and epigenetic similarities reported between CP and OSCC, knowledge in this domain is still very limited. Bioinformatic analysis of available datasets from human tissue from CP [12] and OSCC [13] offers the possibility of uncovering putatively shared genetic and epigenetic expression patterns. Recently, a paper [7] only focusing on DNA methylation used bioinformatics techniques to explore the molecular mechanisms linking chronic periodontal inflammation and oral tumor predisposition. This manuscript concluded that a significant overlap exists between the altered DNA methylation patterns in CP and OSCC. However, to the authors' knowledge, there is no study exploring the overlapped genetic and epigenetic mechanisms of these two diseases, from a comprehensive perspective including different levels, e.g., genes, miRNAs, DNA methylated regions, and signaling pathways. Such findings in this study can be used to predict the risk of suffering OSCC in chronic periodontitis patients, and also form a basis for primary research towards development of diagnostic and therapeutic targets. Therefore, the current study, aimed to analyze publicly available bioinformatic datasets and capture the genetic, epigenetic expression patterns and signaling pathways shared between CP and oral squamous cell carcinoma.

Methods

Data procurement

Datasets from inflamed gingival tissue in human CP were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/gds/). Data pertaining to mRNA and miRNA expression, and DNA methylation were downloaded. Four datasets (GSE54710, GSE23586, GSE59962, and GSE53849) were obtained. The platforms used, experiment types, and numbers of diseased and healthy samples of each database are shown in Table S1. The amount of data analyzed for miRNAs, mRNAs, and methylation expression in CP and healthy control samples are shown in Table S2. RNA-sequencing data, miRNA-sequencing data, methylation data, and clinical meta-data from OSCC samples and healthy adjacent tissue samples were downloaded from the TCGA database (http://cancergenome.nih.gov/).

Differential expression (DE) analysis

mRNA expression profiles from microarray data were analyzed using the limma package (https://bioconductor.org/packages/release/bioc/html/limma.html), and differentially expressed genes (DEGs) were screened. The sequencing data were analyzed using R package (https://www.R-project.org/). The genes with $p < 0.05$ and $|\log FC| > 1.5$ were defined as DEGs.

Protein-protein interaction (PPI) network analysis

Based on HPRD [14] and BioGRID [15] databases, PPI interaction pairs of DEGs expressed in OSCC and CP were obtained to construct a PPI network. This network contained three types of nodes, specifically DEGs expressed in OSCC, DEGs expressed in CP, and DEGs expressed in both OSCC and CP.

miRNAs data analysis

DE-miRNAs expressed in CP and OSCC were analyzed using limma (mentioned above) and edge package (https://bioconductor.org/packages/release/bioc/html/edge.html). For CP, miRNAs with p value < 0.05 were chosen as DE-miRNAs. For OSCC, miRNAs with p value < 0.05 and $|\log FC| > 1$ were chosen as DE-miRNAs. The miRNA-target interaction pairs confirmed by experiments were downloaded from the miRTarBase 6.1 [16] and miRbase 21 [17] databases. The targets of DE-miRNAs were obtained from these miRNA-target interaction pairs.

DNA methylation analysis

Two datasets (GSE59962 and GSE53849) for array-based DNA methylation profiles of CP were obtained and standardized. Methylation sites were standardized using the Illumina Infinium DNA methylation platform, DE-methylation sites were obtained and annotated to corresponding genes. The functions of DE-methylation sites in CP and OSCC were investigated by analyzing the functions of the corresponding genes.

Functional enrichment analysis

Functional analysis was based on five databases (KEGG [18], REACTOME [19], HumanCyc [20], Cell map [21], PID [22]). Statistical enrichment analysis was performed to investigate the functions of DEGs and targets of DE-miRNAs. Here, the p value was determined using hypergeometric testing with the equation:

$$P = 1 - \sum_{t=0}^{x} \frac{\binom{K}{t}\binom{N-K}{M-t}}{\binom{N}{M}}$$

where $\boldsymbol{N}$ represents all genes with biological functions, $\boldsymbol{K}$ represents all genes with specific functions, $\boldsymbol{M}$ represents all DEGs, and $\boldsymbol{\chi}$ represents DEGs expressed in the specific tissue. DEGs within a specific function were considered significantly enriched when a p value of < 0.05 was noted.

Reference database procurement

Genes related to CP and OSCC were downloaded from DisGeNET [23]. MiRNAs related to CP and OSCC were downloaded from the HMDD [24]. MiRNA-target interaction pairs validated by experiments were downloaded from miRTarBase 6.1 [16] and miRbase 21 [17]. Human PPI pairs confirmed by experiments were downloaded from HPRD [14], BIOGRID [15], DIP [25], MINT [26], mentha [27], PINA [28], InnateDB [29], and INstruct [30]. Data for the interaction pairs