Identification of potential therapeutic target genes and mechanisms in head and neck squamous cell carcinoma by bioinformatics analysis

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Received February 26, 2015; Accepted March 8, 2016

DOI: 10.3892/ol.2016.4358

Abstract. The present study aimed to identify the potential target genes and underlying molecular mechanisms involved in head and neck squamous cell carcinoma (HNSCC) by bioinformatics analysis. Microarray data of a Gene Expression Omnibus series GSE6631 was downloaded from the Gene Expression Omnibus database, which was generated from paired samples of HNSCC and normal tissue from 22 patients, and was used to identify differentially expressed genes (DEGs). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed to investigate the functions of the identified DEGs. Furthermore, the protein-protein interaction (PPI) network of these DEGs was constructed using Cytoscape software. Between HNSCC and normal samples there was a difference in 419 DEGs, including 196 upregulated and 223 downregulated genes. The upregulated DEGs were mainly enriched in GO terms of cell adhesion, extracellular matrix (ECM) organization and collagen metabolic process, while the downregulated DEGs were mainly associated with epidermis development and epidermal cell differentiation. The DEGs were enriched in pathways such as ECM-receptor interaction, focal adhesion and drug metabolism. Fibronectin 1 (FN1), epidermal growth factor receptor (EGFR), collagen type I alpha 1 (COL1A1) and matrix metallopeptidase-9 (MMP-9) were hub nodes in the PPI network. These results suggested that cell adhesion and drug metabolism may be associated with HNSCC development, and genes such as FN1, EGFR, COL4A1 and MMP-9 may be potential therapeutic target genes in HNSCC.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer in the world (1). It is an epithelial cancer arising in the upper aerodigestive tract, including the pharynx, larynx and oral cavity (2). Furthermore, the head and neck region contains several distinct structures, such as the lips, nasopharynx, oropharynx and hypopharynx, which result in the large heterogeneity of HNSCC (2,3). In total, >600,000 novel cases of HNSCC are diagnosed annually (1). Currently, chemotheraphy or radiotherapy with locoregional treatment is used for HNSCC patients (4,5). However, the survival rate of this disease is only 40-50% within 5 years after diagnosis and treatment (6).

Numerous studies have explored the pathological mechanism underlying the development of HNSCC (7,8). Several genes have been identified to participate in the progression of HNSCC. For example, Zhang et al (9) reported that fos-related activator-1 could be used as a potential therapeutic target gene in oral squamous cell carcinoma, while transgelin 2 has an oncogenic function (10). For example, Pedrero et al (10) reported that dysregulation of the nuclease VIII-like 1 gene has a critical role in the progression and development of HNSCC (8). Certain signaling pathways have also been demonstrated to be important in HNSCC. For example, Pedrero et al (10) reported that dysregulation of the phosphatidylinositol-4,5-bisphosphate 3-kinase/AKT/phosphatase and tensin homolog signaling pathway may contribute to early HNSCC tumorigenesis. In addition, cyclooxygenase-2 (COX-2) signaling pathway is closely associated with tumor angiogenesis in HNSCC, and COX-2 overexpression predicts a shorter survival in patients with head and neck cancer (11). The coactivation of the mitogen-activated protein kinase and IκB kinase signaling pathways may suppress the mechanism of signal transduction by regulating the secretion of interleukin-8 and vascular endothelial growth factor in human HNSCC (12). Although various factors have been identified to contribute to HNSCC, the pathogenic mechanisms of HNSCC remain to be clearly demonstrated in order to identify potential target genes for the treatment of HNSCC.

In the present study, the differentially expressed genes (DEGs) between HNSCC and normal samples were analyzed.
to gain a better insight of HNSCC. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of DEGs were performed, and the protein-protein interaction (PPI) network of these DEGs was constructed. The purpose of the present study was to explore the underlying mechanisms of HNSCC and to identify novel potential target genes for HNSCC therapy.

Materials and methods

Affymetrix microarray data. Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) is a database repository of high throughput gene expression data, which segregates data into three principle components: Platform (GPL), series (GSE) and sample (GSM). The array data of GSE6631, based on the GPL8300 Affymetrix Human Genome U95 Version 2 Array platform (Affymetrix, Inc., Santa Clara, CA, USA) was downloaded from the GEO database, which was deposited by Kuriakose et al (13). The dataset was generated from paired (from the same patient) samples of tumor and normal tissues from 22 patients with histologically confirmed HNSCC by Kuriakose et al (13).

Data preprocessing and DEGs analysis. The original probe-level data in CEL files (raw probe level data) were converted into gene expression values. Data were normalized using the Bioconductor R package affy version 1.32.0 (Affymetrix, Inc., Santa Clara, CA, USA) (14). Nonspecific probes were filtered. If multiple probes corresponded to the same gene, the average expression value was calculated to represent the expression levels of that gene. The samr package (version 2.0; cran.r-project.org/web/packages/samr/index.html) in R (www.r-project.org) (15) was applied to identify DEGs between HNSCC and normal samples. $\Delta=1.3$ and fold-change $>1.5$ were used as the cutoff criteria, based on the experience of the present authors.

Functional enrichment analysis of DEGs. The GO database (geneontology.org/page/go-database) (16) is a collection of numerous gene annotation terms. The knowledge contained in the KEGG database (www.genome.jp/kegg/) (17) was applied to identify functional and metabolic pathways. The Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.7 (National Cancer Institute at Frederick, Frederick, MD, USA) (18) was used as a gene functional enrichment analysis tool to understand the biological meaning of the results of bioinformatics analysis. GO and KEGG enrichment analyses for the upregulated and downregulated identified DEGs were performed with DAVID. P<0.05 and false discovery rate <0.01 were selected as the cutoff criteria.

Construction of PPI network and disease enrichment analysis. The Search Tool for the Retrieval of Interacting Genes/Proteins (version 9.05; string-db.org) (19) is an online database that contains comprehensive information of proteins. This online tool was applied to analyze the interactions of protein pairs. PPI network of DEGs was constructed using Cytoscape software (version 3.0.1; Cytoscape Consortium San Diego, CA, USA) (20). The degree of connectivity was analyzed and used to obtain the hub proteins in the PPI network.

Results

Identification of DEGs. As represented in Fig. 1, the raw expression data were preprocessed and normalized. A total of 419 DEGs were identified between HNSCC and normal samples, including 196 upregulated genes and 223 downregulated genes.
The pathways of these upregulated and downregulated genes are indicated in Table II. The upregulated genes were mainly involved in ECM-receptor interaction, focal adhesion and small cell lung cancer. Genes such as fibronectin 1 (FN1), epidermal growth factor receptor (EGFR) and collagen type I alpha 1 (COL1A1) were identified in the focal adhesion pathway. By contrast, the downregulated DEGs were enriched in drug metabolism. Cytochrome P450 3A5 (CYP3A5) was identified in the drug metabolism pathway.

PPI network construction and disease enrichment analysis. The results of the PPI network analysis are represented in
Fig. 2. The upregulated genes FNI, EGFR, COL1A1, matrix metallopeptidase-9 (MMP-9), COL5A2, COL1A2, COL3A1, transforming growth factor, beta-induced and cyclin B1 were selected as hub nodes.

Discussion

In the present study, gene expression profile data were downloaded from the GEO database to identify DEGs in HNSCC using bioinformatics analysis. A total of 419 DEGs between HNSCC and normal samples, including 196 upregulated and 223 downregulated genes, were selected. The results of functional enrichment analysis revealed that the upregulated genes, including FNI, EGFR and COL1A1, were associated with GO term of cell adhesion, while the downregulated DEGs, including CYP3A5, were enriched in drug metabolism pathways. According to the results of the PPI network analysis, FN1, EGFR, COL1A1 and MMP-9 were identified as hub nodes. Therefore, these DEGs and their interacting partners may be involved in HNSCC development.

Cell adhesion is the process of binding of a cell to a surface or substrate, such as the ECM or another cell (21).
the present study, the majority of the upregulated DEGs were enriched in pathways of ECM-receptor interaction and focal adhesion. Previous studies have indicated that ECM-receptor interaction and focal adhesion were associated with cell adhesion (22). Recent evidence suggests that cell adhesion is mediated by several genes, including FN1, EGFR and COL4A1 (23-25). FN1 is an ECM glycoprotein (26) involved in cell adhesion (27), which corresponds to the pathway identified in the present study. It was previously reported that FN1 acts as a tumor suppressor gene, playing a critical role in migration and invasion of laryngeal carcinoma (23), which is the most common type of HNSCC (28). EGFR was also indicated to be associated with HNSCC (29). EGFR is the cell-surface receptor of the EGF family (30). In the present study, EGFR was enriched in GO terms of cell adhesion and pathway of focal adhesion, which was consistent with previous studies that reported that EGFR contributed to transduce extracellular signals to intracellular responses, thus influencing adhesion and proliferation in tumor cells (24,31). Rubin Grandis et al (32) reported that EGFR was overexpressed in HNSCC. High expression levels of EGFR have been associated with reduced survival and increased risk of recurrence in HNSCC (33). COL4A1 is a member of the collagen family, and is also associated with cell adhesion (25). The adhesion of cells to collagen is mediated by fibronectin (25). Tanaka et al (34) indicated that the differential expression of type IV collagen chains was associated with the invasive potential of cell carcinoma. The results of the present study indicated that FN1, EGFR and COL4A1 were upregulated genes in HNSCC and hub nodes in the PPI network, which suggests that FN1, EGFR and COL4A1 may regulate cell adhesion in HNSCC. Thus, cell adhesion may participate in HNSCC through multiple genes, including FN1, EGFR and COL4A1, which may be potential therapeutic target genes in HNSCC.

In conclusion, a total of 419 DEGs were identified between HNSCC and normal samples, and the present study indicates that cell adhesion and drug metabolism may be closely associated with HNSCC development. Genes such as FN1, EGFR, COL4A1 and MMP-9 may be potential therapeutic target genes in HNSCC. However, further studies are required to confirm the present results.

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