Genomic analyses identify signaling pathways and biological processes in Human papillomavirus (HPV) infected oral cancer patients

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Research Article

Keywords:

Posted Date: January 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1293225/v1>

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Abstract

Human papillomavirus (HPV) is a critical cause of the head and neck squamous cell carcinoma. However, the molecular mechanism and biological function of HPV in oral cancer remains unknown. In this study, our aim is to identify the significant molecules and pathways by analyzing the RNA-seq data. The GSE184616 was produced by the Illumina NovaSeq 6000 (Homo sapiens). The KEGG and GO analyses showed PI3K–Akt signaling pathway and Extracellular matrix are the main processes during oral cancer with HPV infection. Moreover, we identified the top ten key molecules including FN1, ACTB, COL3A1, COL5A1, COL4A1, COL4A2, COL5A2, MMP9, COL6A2, BGN. Therefore, our study may provide new insights into the treatment of oral cancer.

Introduction

Head and neck squamous cell cancers initiated from the epithelium are the most common malignancies in the head and neck¹. The rich prevalence of HNSCC is due to the consumption of specific cancer-inducing products such as tobacco, alcohol, and areca nut products, and the rising rates of HPV infection have recently resulted in the high prevalence of HNSCC in the US². Men have higher risks than women in the development of oral cancer³. The survival rates for oral cancer have improved, for example, the 5-year survival rate has increased to 66% in the period 2002-2006 by analyzing across all age groups⁴.

Most oral cancers are linked to the infection with oncogenic strains of human papillomavirus (HPV) including HPV-16 and HPV-18⁵. HPV-positive oral cancer shows a more favorable prognosis than HPV-negative oral cancer, and recent studies show that lower-dose chemoradiation is effective in HPV-positive diseases⁶. Oral cancer is characterized by genetic instability. The transition from dysplasia to carcinoma is related to the loss of 3p21, 17p13, 11q13, 13q21, and 14q32 in the site of TP53². However, whether the progression of oral cancer is closely dependent on the sequence alteration or accumulation remains unresolved.

In this study, we analyzed the effects of HPV infection on oral cancers by using the RNA-seq data. We identified a variety of DEGs and significant biological processes. We also performed the gene enrichment and the protein-protein interaction (PPI) network to obtain the interacting molecules. The biological genes and pathways in our study will improve the treatment of oral cancer.

Methods

Data resources

Gene dataset GSE184616 was obtained from the GEO database. The data was produced by the Illumina NovaSeq 6000 (Homo sapiens) (School of Mathematics and Statistics, University of Sydney, City Road, Camperdown, NSW, Australia). The analyzed dataset includes 5 of controls (HPV negative patients) and 5 of HPV positive patients.

Data acquisition and processing

The data were organized and conducted by R package as previously described⁷⁻⁹. We used a classical t-test to identify DEGs with $P < 0.01$ and fold change ≥ 1.5 as being statistically significant.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO)

KEGG and GO analyses were conducted by the R package (ClusterProfiler) and Reactome. $P < 0.05$ was considered statistically significant.

Protein-protein interaction (PPI) networks

The Molecular Complex Detection (MCODE) of Cytoscape was used to create the PPI networks. The significant modules were produced from constructed PPI networks. The pathway analyses were performed by using Reactome (<https://reactome.org/>), and $P < 0.05$ was considered significant.

Results

Identification of DEGs in oral cancer between HPV-negative and HPV-positive patients

To determine the effects of HPV on oral cancer, we analyzed the RNA-seq data from the HPV-negative and -positive oral squamous cell carcinoma Patients. A total of 575 genes were identified with the threshold of $P < 0.01$. The top up- and down-regulated genes were indicated by the heatmap and volcano plot (Figure 1). The top ten DEGs were listed in Table 1.

Enrichment analysis of DEGs between HPV-negative and HPV-positive patients

To further understand the potential biological processes, we performed the KEGG and GO analyses (Figure 2). The significant KEGG items includes "PI3K-Akt signaling pathway", "Human papillomavirus infection", "Focal adhesion", "ECM-receptor interaction", "Small cell lung cancer", "Amoebiasis", and "Arrhythmogenic right ventricular cardiomyopathy". We identified the BP of GO including "Extracellular matrix organization", "Extracellular structure organization", "External encapsulating structure organization", "Connective tissue development", "Collagen metabolic process", "Collagen fibril organization", "Endodermal cell differentiation", "Endoderm formation", "Basement membrane organization", and "Protein hydroxylation". We identified the CC of GO including "Collagen-containing extracellular matrix", "Endoplasmic reticulum lumen", "Focal adhesion", "cell-substrate junction", "basement membrane", "collagen trimer", "lysosomal lumen", "complex of collagen trimers", "microfibril", and "fibrillar collagen trimer". We then identified the MF of GO including "extracellular matrix structural constituent", "growth factor binding", "collagen binding", "metalloendopeptidase activity", "integrin binding", "iron ion binding", "extracellular matrix structural constituent conferring tensile strength", "extracellular matrix binding", "L-ascorbic acid binding", and "platelet-derived growth factor binding".

PPI network analysis

To determine the potential relationship among the DEGs, we constructed the PPI network by using 509 nodes and 1417 edges. We set the combined score > 0.2 as a cutoff by using the Cytoscape software. Table 2 showed the top ten genes with the highest scores. The top two significant modules were indicated in Figure 3. We further analyzed the PPI and DEGs with Reactome map (Figure 4) and identified the top ten functional processes including "Extracellular matrix organization", "Collagen formation", "Assembly of collagen fibrils and other multimeric structures", "Collagen biosynthesis and modifying enzymes", "ECM proteoglycans", "Non-integrin membrane-ECM interactions", "Post-translational protein phosphorylation", "Degradation of the extracellular matrix", "Elastic fibre formation", and "Laminin interactions" (Supplemental Table S1).

Discussion

Recent publications showed that HPV does not lead to genital and anal cancer but is a direct cause for the progression of oral cancer¹⁰. Interestingly, several studies also found that HPV DNA is present in the OSCC, but their interactive relationships are still unclear¹. Thus, further studies focusing on larger genomic data are needed to decipher risk factors for oral cancers.

The KEGG and GO analyses showed that "PI3K-Akt signaling pathway" and "Extracellular matrix" are the main processes during the oral cancer with HPV infection. Sadhna Aggarwal et al found PI3K inhibitors (PI-828 and PI-103) inhibit the growth and proliferation of oral cancer cells by activating the apoptosis and changing the cell cycles¹¹. An-Jie Jhou et al found chlorpromazine promotes the G2/M phase arrest and apoptosis through mediating the PI3K/ATK pathways in human oral cancer¹². Moreover, they also found chlorpromazine represses tumor growth in zebrafish and mouse oral cancer models¹². Franziska E Marquard et al found 90% of oral cancer showed the activation of PI3K/AKT/mTOR signaling. And the activated PI3K/AKT signaling is associated with radiotherapy and drug resistance¹³. One feature of cancer is the degradation of the extracellular matrix¹⁴. For example, the matrix metalloproteinases in oral cancers break down the extracellular matrix to allow cancer cells to spread. Moreover, the increased expression of MM9 is a marker of poor prognosis¹⁵. Elias Sundquist et al also found that the neoplastic extracellular matrix environment boosts cancer in vitro studies¹⁶.

In addition, we identified the significant interactive molecules by creating the PPI network in oral cancer with HPV infection, which may help to explain the effects of HPV infection. Yoshihiro Morita et al found that fibronectin 1 increases the expression of VEGF-C, lymphangiogenic, and lymph node metastasis in oral cancer¹⁷. Interestingly, the upregulation of fibronectin 1 results in oral cancer by the activation of FAK, which suggests that fibronectin 1-FAK signaling may be a potential target for oral cancer¹⁷. ACTB is closely related to a number of cancers, which is disordered in the liver, melanoma, renal, colorectal, gastric, lung, and breast cancers. The dysregulated ACTB causes the malfunction of the cytoskeleton to further promote the invasiveness and metastasis of cancers¹⁸. Su Young Oh et al found COL3A1 is significantly decreased in the saliva of oral cancer patients, which may be a potential biomarker for the

prediction of oral cancer¹⁹. GPCR and RGS proteins play essential roles in different diseases including heart, brain, lung, bone diseases, and a variety of inflammatory diseases^{20–22} and cancers^{23–31}. Interestingly, GPR56, a GPCR from the family of adhesion GPCRs, can interact with COL3A1 to inhibit neural migration³². Hung-Chih Chen et al found COL5A1 is upregulated in tumor tissues and a high level of COL5A1 is related to the late stage of oral cancer and lymph node metastasis³³. Moreover, they also found that the high expression level of COL5A2 was associated with better disease-free survival in oral cancer patients³³. Xiao-Zhi Lü et al found that COL4A1, COL4A2, and COL5A2 mRNAs in the oral cancer tissues are increased in comparison to the normal samples³⁴. Li-Sung Hsu et al found KLF6 can inhibit oral cancer migration and invasion by decreasing the MMP9 activities³⁵. Stéphane Grauzam found NEDD9 induced MMP9 secretion is invadopodia formation in oral cancer cells³⁶. Circadian clocks and their downstream pathways play key roles in cellular functions (proliferation, differentiation, and secretion) and human diseases (fat, metabolic syndrome, arthritis, and aging)^{37–48}. Jian Wang found MMP9 is a critical downstream of Bmal1, which is able to promote the invasion and metastasis of cancer⁴⁹. Yang Liu et al found the biglycan is increased in endometrial cancer, which is correlated with histopathological grade and poor prognosis of endometrial cancer⁵⁰.

In summary, our study discovered the significant effects of HPV on oral cancers. The “PI3K–Akt signaling pathway” and “Extracellular matrix” signaling pathways are the major affected processes during the development of oral cancer. Our study provides valuable guidance for the treatment of oral cancer.

Declarations

Author Contributions

Fang Wang, Tingxiang Chang, Hongmei Guo: Methodology and Writing. Hanming Gu, James Liu: Conceptualization, Writing- Reviewing and Editing.

Funding

This work was not supported by any funding.

Declarations of interest

There is no conflict of interest to declare.

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Tables 1-2

Tables 1-2 are available in the Supplementary Files section.

Figures

Figure 1

Heatmap and volcano plot were created in oral cancer between HPV-negative and HPV-positive patients

(A) Heatmap of significant DEGs. Significant DEGs ($P < 0.01$) were used to construct the heatmap.

(B) Volcano plot for DEGs in oral cancer between HPV-negative and HPV-positive patients. The most significantly changed genes are highlighted by grey dots.

Figure 2

KEGG and GO analyses of DEGs in oral cancer between HPV-negative and HPV-positive patients

(A) KEGG analysis, (B) Biological processes, (C) Cellular components, (D) Molecular functions.

Figure 3

The PPI network analyses of DEGs in oral cancer between HPV-negative and HPV-positive patients

The cluster (A) and cluster (B) were constructed by MCODE.

Figure 4

Reactome map representation of the significant biological processes in oral cancer between HPV-negative and HPV-positive patients

Supplementary Files

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- [SupplementalTableS1.csv](#)
- [Onlinefloatimage5.png](#)

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