Int J Clin Exp Med 2016;9(4):7471-7480
www.ijcem.com /ISSN:1940-5901/IJCEM0019390

Original Article

Analysis of the gene expression profile for Oral tongue squamous cell carcinoma

Yuanyuan Li[1,2*], Zhiguo Chai[3*], Lucas Wan[2], Yin Ding[1]

[1]Department of Orthodontics, School of Stomatology, Fourth Military Medical University, Xi'an 710032, PR China; [2]Department of General Dentistry, Guangzhou Military Logistics Department Outpatient Department, Guangzhou, 510080, PR China; [3]Department of Prosthodontics, School of Stomatology, Fourth Military Medical University, Xi'an 710032, PR China. [*]Equal contributors.

Received November 8, 2015; Accepted February 10, 2016; Epub April 15, 2016; Published April 30, 2016

Abstract: Oral tongue squamous cell carcinoma (OTSCC) is the most common cancer diagnosed in the oral cavity and represents one of the main causes of cancer-related death worldwide. The aim of this study was to explore novel biomarkers with diagnostic and therapeutic potentials in OTSCC. The micro-array data GSE9844 were downloaded from Gene Expression Omnibus (GEO), including 26 OTSCC samples and 12 control samples. The differentially expressed genes (DEGs) between OTSCC and normal samples were screened. We performed GO enrichment and KEGG pathway analysis on the DEGs. Then the top 20 DEGs were subjected to do principal component analysis. Finally a PPI (protein-protein interaction) network of top 20 DEGs was constructed by String software. A total of 634 DEGs were identified, including 289 up-regulated and 345 down-regulated ones. The top 20 DEGs could well distinguish OTSCC samples from normal samples in principal component analysis. DEGs were mainly associated with immunoreaction analyzed through gene expression profile. The pivotal genes including collagen proteins (such as COL4A1, COL4A2, COL4A6, COL5A1, COL5A2 and COL11A1), interleukin (like IL8) and metallopeptidase (such as MMP1 and MMP9) in three KEGG pathways were also significantly over-expressed in OTSCC. In the PPI network of the top 20 DEGs, interactions between only 6 proteins were found. Hereinto, furthermore interleukin (IL) and metallopeptidase played important roles. The important genes like IL-8 and MMP9 obtained in this study may help identifying new biomarkers with diagnostic and therapeutic potentials in OTSCC. However, additional studies were still needed.

Keywords: OTSCC, differentially expressed genes, functional annotation, biomarker

Introduction

Oral cancer, ranking the sixth prevalent cancer, is known as a severe increasing health and social problem around the world [1]. While oral tongue squamous cell carcinoma (OTSCC) is the most common type diagnosed in the oral cavity, which accounts for nearly 30% of the oral cancer [2]. The conventional treatments for OTSCC include surgery, radiotherapy and chemotherapy, however their effects on patients with OTSCC is unsatisfactory. Although great advances have been made in OTSCC therapy in the past few years, the survival of OTSCC patients has not been improved much with 5-year survival rate less than 40-50% [3, 4]. It is necessary to explore new and available treatments for OTSCC, and look for new sensitive and specific biomarkers with diagnostic or therapeutic significance to improve the survival rate and quality of life.

Nowadays, high-throughput genomic, transcript-tomic and proteomic studies are important methods to appreciate a large number of novel molecular markers with prognostic potentials [5, 6]. For example, gene expression profiling is a valuable tool to identify differentially expressed genes (DEGs) in human cancers so as to find potential critical genes or transcription factors that play important roles in the regulation of cancer development and progression [7]. Furthermore, numerous previous studies have identified some genes which may be used as diagnostic markers or therapy targets for OTSCC. Such as CXCL8, a neutrophil chemotactic factor coming from CXC-chemokines, is reported being able to stimulate growth of squamous cell carcinoma cells. Moreover, over-expression of CXCL8 was found in head and neck cancer cells [8]. In addition, as CXCL8 could contribute to the proliferation and motility of head and neck cancer cells, it may be consid-

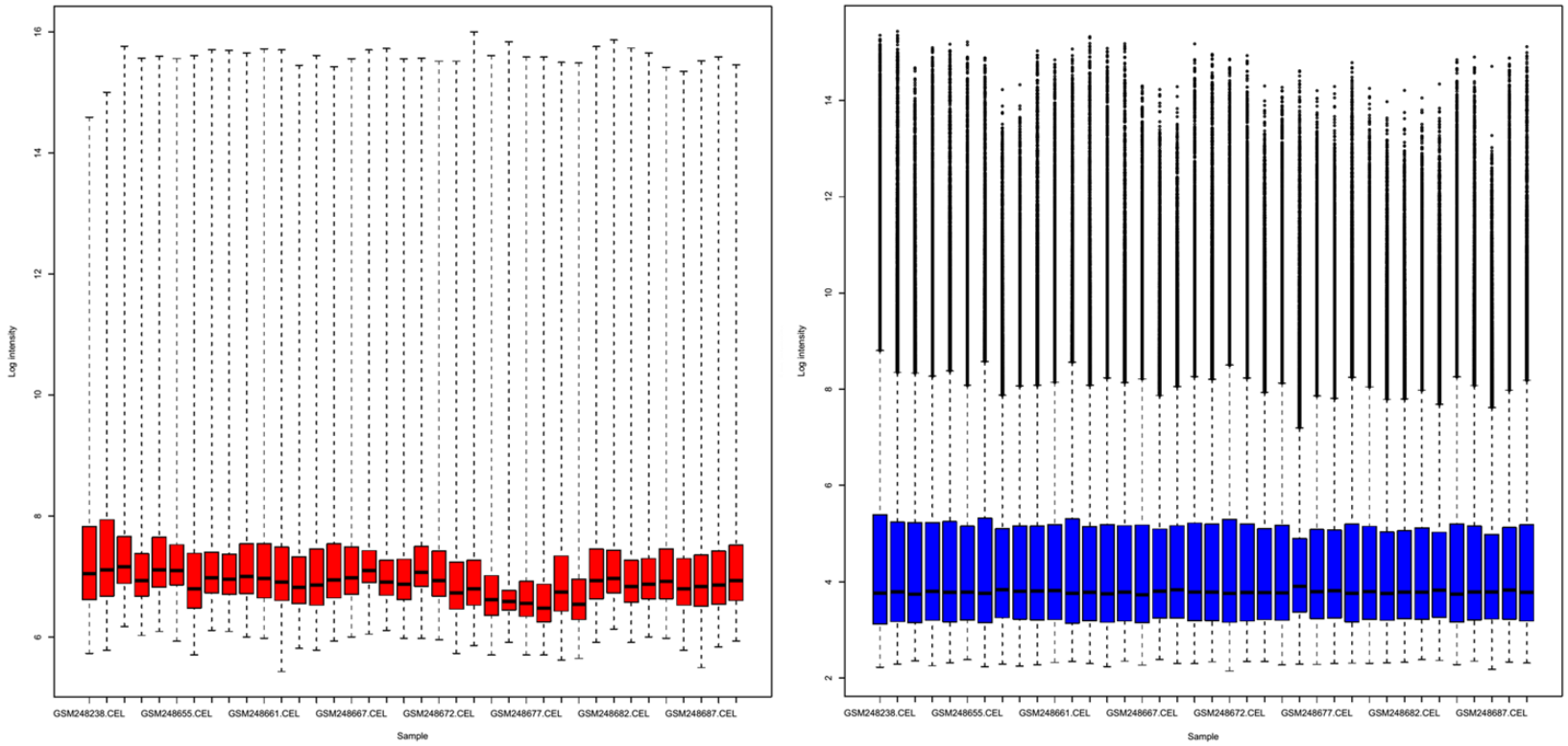

Figure 1. Cartridge of expression values data before and after standardization. The horizontal axis represented sample name, while the vertical axis represented expression value. The black lines in the boxes represented the medians of each set of data. The left and right figures were the cartridges for expression values data before and after standardization respectively.

ered as a potential target for anti-tumor therapeutic strategies [9]. On the other hand, the expression of IL-13 receptor (IL-13R) has been reported to be significantly higher in some human head and neck cancers than in normal samples, and nearly 33% of head and neck squamous cell carcinoma (HNSCC) show high-level expression of IL-13R [10]. A recombinant immunotoxin IL-13-PE that targeted IL-13R has been designed and its effect in HNSCC therapy has also been tested [11]. This trial indicated that molecular targeted therapy might be a good idea for OTSCC as well. Although several genes or proteins have been described to be over-expressed in OTSCC and may be associated with poor diagnosis till now, our understanding of some markers used to guide treatment remains fragmentary.

Therefore, in this study, we screened DEGs between OTSCC and normal samples, and performed functional enrichment analysis on these DEGs. Then, the top 20 DEGs were subjected to do principal component analysis and a protein network for the top 20 DEGs was constructed. We hoped to explore some bio-markers with diagnostic and therapeutic potentials in OTSCC and provide references for novel targeted therapy of OTSCC.

Materials and methods

Data resource and pretreatment

The microarray data GSE9844 (PMID18254958) [12] was downloaded from Gene Expression Omnibus (GEO) database of NCBI (National Center of Biotechnology Information). A total of 38 samples including 26 oral tongue squamous cell carcinoma (OTSCC) samples and 12 control samples were examined in this study. The test platform was GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array). GSE9844 data was preprocessed as follows: firstly, the original data in CEL format was transformed into probe value matrix by ReadAffy [13] of Affy package in R software, and the probe expression values were normalized using RMA function; then R/Bioconductor notes package and Affymetrix Human U133 Plus 2.0 Array were applied to convert the probe numbers into gene names; the probes without gene annotation or having more than one gene annotation were filtered out, the average value of multiple probes corresponding to the same gene was calculated as a unique value of the gene.

Screening of DEGs and hierarchical cluster analysis

The significantly DEGs between OTSCC and control samples were calculated using SAM method in the samr package of R software [14] on the basis of data preprocessing. Gene expression change more than 1.5 times and q value less than 0.1 were the screening thresholds. In order to ensure the screened DEGs could better characterize the OTSCC and control samples, the samples were grouped and hierarchy clustering analysis of the gene expression values were conducted, moreover a clustering dendrogram was drew. At last, the sample grouping of the original data was validated and some samples with unreasonable clustering were filtered out.

Functional enrichment analysis of the DEGs

The gene expression profiles of the OTSCC and control samples were re-obtained after hierarchical cluster analysis and sample filtering. Limma package [15] was utilized to screen the filtered DEGs between OTSCC samples and control samples with adj. *P*. Val < 0.05 and |log2 (FC)| > 1. In addition, the online analytical tool DAVID (the Database for Annotation, Visualization and Integrated Discovery) [16] was applied to perform GO and KEGG pathway enrichment analysis of the up-regulated and down-regulated genes, respectively. By this way, the significant biological processes and signal pathways related to OTSCC were identified in GO terms and KEGG pathways ($P < 1^{e-3}$).

Principal component analysis of the top 20 DEGs

Principal component analysis is a mathematical algorithm [17], which can reduce data dimension and keep majority of the variable PCA at the same time. The top 20 most significant DEGs were selected to perform principal component analysis so as to distinguish the OTSCC samples and control samples.

Construction of PPI network of the top 20 DEGs

In order to further study the interaction relationships between the top 20 DEGs in OTSCC

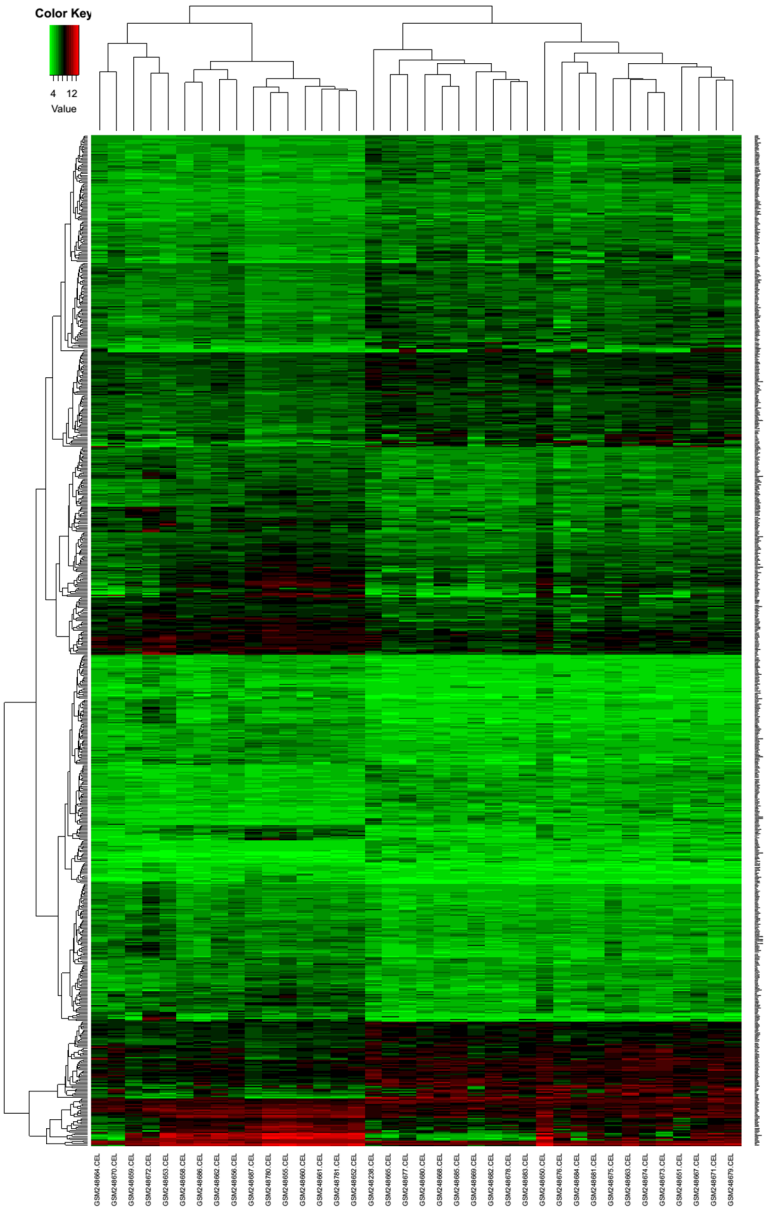

Figure 2. The hierarchical clustering map for DEGs in OTSCC. The horizontal axis below showed sample name, while the horizontal axis above showed sample clustering, the left vertical axis represented gene clustering. The differential genes between OTSCC and normal tissues were mainly clustered into two clusters.

tissues, these genes were directly mapped into the protein interaction database STRING [18] to obtain protein interaction relations. Then, the reliability score of protein interaction relations more than 0.4 was regarded as an index to screen the reliable protein interaction relationship. Finally, a protein interaction network for the top 20 DEGs in OTSCC tissues was constructed.

Results

Data source and preprocessing

A total of 16580 gene expression values from 26 oral cancer samples and 12 control samples were obtained after pretreatment of raw data from GSE9844. The median values of all the gene expression profiles were located in a straight line after normalization, indicating the standardization level was satisfactory (**Figure 1**).

Hierarchical cluster analysis

A total of 724 DEGs between OTSCC and control samples were screened using samr package in R software, including 341 up-regulated and 383 down-regulated ones. In hierarchical clustering analysis of the differential genes, four oral cancer samples numbered GSM248662 GSM248664, GSM248670 and GSM248686 were clustered in control sample group (**Figure 2**). The hierarchical clustering for these 4 samples was unreasonable. Overall, all samples were mainly divided into two clusters including oral cancer samples and control samples in hierarchical clustering analysis.

Screening of DEGs and enrichment analysis

In hierarchical clustering analysis of the differential genes, there were errors in the grouping for four oral cancer samples (GSM248662 GSM248664, GSM248670 and GSM248686). In order to reduce the following interference caused by the system errors of sample grouping, the gene expression values of the 4 samples were filtered out. Then limma package was re-used to screen the DEGs between 22 oral

Table 1. Function enrichment of the up-regulated genes

Category	Term	Count	*P*-Value	Fold Enrichment	FDR
GO_BP	ectoderm development	16	1.4E-06	4.7	2.3E-03
GO_BP	epidermis development	15	2.8E-06	4.8	4.8E-03
GO_BP	collagen metabolic process	7	5.9E-06	14.8	1.0E-02
GO_BP	response to wounding	25	1.0E-05	2.8	1.8E-02
GO_BP	multicellular organismal macromolecule metabolic process	7	1.1E-05	13.3	1.9E-02
GO_BP	multicellular organismal metabolic process	7	3.2E-05	11.2	5.5E-02
GO_BP	immune response	28	3.9E-05	2.4	6.7E-02
GO_BP	regulation of cell proliferation	30	5.9E-05	2.3	1.0E-01
GO_BP	cell cycle phase	20	7.5E-05	2.9	1.3E-01
GO_BP	response to extracellular stimulus	14	9.2E-05	3.8	1.6E-01
GO_BP	cell cycle	29	1.1E-04	2.2	1.9E-01
GO_BP	mitotic cell cycle	18	1.8E-04	2.9	3.1E-01
GO_BP	collagen catabolic process	5	3.1E-04	14.8	5.2E-01
GO_BP	cell adhesion	26	3.2E-04	2.2	5.4E-01
GO_BP	biological adhesion	26	3.3E-04	2.2	5.5E-01
GO_BP	negative regulation of cell proliferation	17	4.2E-04	2.8	7.0E-01
GO_BP	cell proliferation	19	4.3E-04	2.6	7.3E-01
GO_BP	M phase	16	4.7E-04	2.9	7.9E-01
GO_BP	response to nutrient levels	12	5.1E-04	3.6	8.7E-01
GO_BP	defense response	23	7.2E-04	2.2	1.2E+00
GO_BP	multicellular organismal catabolic process	5	8.8E-04	11.4	1.5E+00
KEGG	ECM-receptor interaction	12	1.2E-06	6.7	1.3E-03
KEGG	Pathways in cancer	22	3.7E-06	3.1	4.1E-03
KEGG[a]	Small cell lung cancer	9	3.6E-04	5	4.0E-01

Table 2. Function enrichment of the down-regulated genes

Category	Term	Count	*P*-Value	Fold Enrichment	FDR
GO_BP	Muscle system process	20	9.6E-11	6.8	1.6E-07
GO_BP	Muscle contraction	19	1.6E-10	7.1	2.7E-07
GO_BP	Striated muscle contraction	11	4.9E-09	13.7	8.2E-06
GO_BP	Fatty acid metabolic process	16	1.9E-06	4.6	3.2E-03
GO_BP	Response to wounding	25	1.7E-05	2.7	2.9E-02
GO_BP	Actin filament-based process	16	2.1E-05	3.8	3.5E-02
GO_BP	Inflammatory response	18	5.4E-05	3.2	9.1E-02
GO_BP	Muscle organ development	14	8.1E-05	3.8	1.4E-01
GO_BP	Oxidation reduction	26	1.3E-04	2.3	2.1E-01
GO_BP	Epithelial cell differentiation	11	1.4E-04	4.6	2.3E-01
GO_BP	Muscle cell differentiation	10	2.6E-04	4.7	4.4E-01
GO_BP	Growth	12	3.5E-04	3.8	6.0E-01
GO_BP	Myofibril assembly	5	4.2E-04	13.6	7.1E-01
GO_BP	Cardiac muscle tissue development	7	4.5E-04	7	7.5E-01
GO_BP	Leukotriene metabolic process	5	5.1E-04	13	8.5E-01
GO_BP	Actin cytoskeleton organization	13	5.9E-04	3.3	1.0E+00
GO_BP	Cellular alkene metabolic process	5	6.1E-04	12.5	1.0E+00
GO_BP	Striated muscle cell differentiation	8	8.4E-04	5.2	1.4E+00
KEGG[a]	Drug metabolism	8	2.2E-04	6.3	2.5E-01

Note: red marks indicated the enriched pathways.

Table 3. The top 20 DEGs between OTSCC and control tissues

Gene	logFC	t	adj.P.Val	Gene description
MMP1	7.307887	15.9779	2.95E-13	matrix metallopeptidase 1 (interstitial collagenase)
PRR9	3.944511	5.04224	0.000625811	proline rich 9
KRT17	3.893319	9.580644	5.14E-08	keratin 17
MMP3	3.748095	10.11051	1.44E-08	matrix metallopeptidase 3 (stromelysin 1, progelatinase)
S100A7A	3.644265	5.805865	0.000131497	S100 calcium binding protein A7A
CXCL1	3.64044	7.610828	3.20E-06	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
IL8	3.523241	9.361302	7.66E-08	interleukin 8
IL24	3.321078	7.23816	6.70E-06	interleukin 24
NELL2	3.275322	6.272496	4.99E-05	NEL-like 2 (chicken)
IFI6	3.271333	5.907391	0.000107211	interferon, alpha-inducible protein 6
ABCA8	-3.55762	-11.0302	3.23E-09	ATP-binding cassette, sub-family A (ABC1), member 8
FAM3B	-3.65228	-4.84821	0.000928679	family with sequence similarity 3, member B
CRISP3	-3.66789	-4.38462	0.002340741	cysteine-rich secretory protein 3
CLCA4	-3.83502	-5.7866	0.000137343	chloride channel accessory 4
MYH2	-4.4079	-4.15011	0.003868734	myosin, heavy chain 2, skeletal muscle, adult
KRT4	-4.41399	-6.78452	1.65E-05	Keratin 4
KRT13	-4.6749	-4.69728	0.001244965	keratin 13
CRNN	-5.03701	-4.78604	0.001055319	cornulin
MAL	-5.37701	-5.91454	0.000107211	mal, T-cell differentiation protein
TMPRSS11B	-5.90986	-7.82821	1.98E-06	transmembrane protease, serine 11B

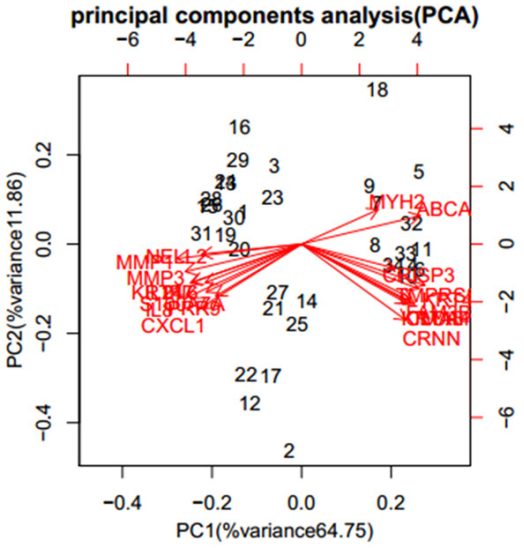

Figure 3. Principal component analysis for the top 20 DEGs. Horizontal axis showed the score of the first principal component, ordinate axis showed the score of the second principal component. The number on the left and right sides represented OTSCC (a total of 22) and control samples (a total of 12) respectively, the red line indicated the effects of 20 variables on OTSCC and control samples.

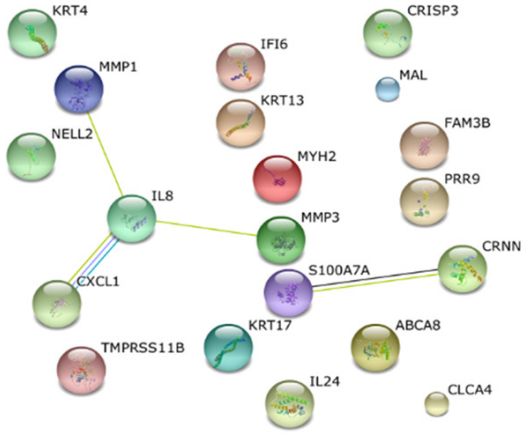

Figure 4. PPI network of the top 20 DEGs. There were six protein existed interaction relationships, lines represented protein interaction pairs corresponding to genes.

cancer samples and 12 control samples with screening threshold adj. *P*.V al < 0.05 and |log2 (FC)| > 1. Finally a total of 634 DEGs including 289 up-regulated and 345 down-regulated ones were obtained. Furthermore, through enrichment analysis by DAVID online tools, we found the up-regulated gene were significantly enriched in 21 biological processes including epidermal cell growth and trauma, immune response, ECM - receptor interaction and two cancer-related metabolic pathways (**Table 1**); while the down-regulated genes were significantly enriched in 18 biological processes which were mainly associated with muscle development and drug metabolism (**Table 2**).

Table 4. The key genes in KEGG pathway

ENTREZ GENE ID	Gene Title	Gene Symbol
1026	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	*CDKN1A*
1163	CDC28 protein kinase regulatory subunit 1B	*CKS1B*
1282	Collagen, type IV, alpha 1	*COL4A1*
1284	Collagen, type IV, alpha 2	*COL4A2*
1288	Collagen, type IV, alpha 6	*COL4A6*
1289	Collagen, type V, alpha 1	*COL5A1*
1290	Collagen, type V, alpha 2	*COL5A2*
1301	Collagen, type XI, alpha 1	*COL11A1*
284217	Laminin, alpha 1	*LAMA1*
3091	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	*HIF1A*
3371	Tenascin C	*TNC*
3576	Interleukin 8	*IL8*
3655	Integrin, alpha 6	*ITGA6*
3914	Laminin, beta 3	*LAMB3*
3918	Laminin, gamma 2	*LAMC2*
4312	Matrix metallopeptidase 1 (interstitial collagenase)	*MMP1*
4318	Matrix metallopeptidase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)	*MMP9*
637	BH3 interacting domain death agonist	*BID*
650	Bone morphogenetic protein 2	*BMP2*
6513	Solute carrier family 2 (facilitated glucose transporter), member 1	*SLC2A1*
6696	Secreted phosphoprotein 1	*SPP1*
6722	Serum response factor (c-fos serum response element-binding transcription factor)	*SRF*
7039	Transforming growth factor, alpha	*TGFA*
8323	Frizzled homolog 6 (Drosophila)	*FZD6*
8722	Cathepsin F	*CTSF*
8900	Cyclin A1	*CCNA1*
898	Cyclin E1	*CCNE1*

Genetic variations of principal component analysis

In principal component analysis of the top 20 most significantly DEGs (**Table 3**), these DEGs could distinguish the OTSCC and contrast samples directly (**Figure 3**). The first principal component explained 64.75% of the variance of 20 variables, while the second principal component explained 11.86% of the variance, and the cumulative variance interpretation degree was 76.61%. The variable directing of another 20 DEGs showed significant impacts on OTSCC tissues. In addition, there were 10 and 10 genes markedly over-expressed and down-expressed in OTSCC tissues, respectively.

PPI network for the top 20 DEGs

In the PPI network of top 20 significantly DEGs, only 6 proteins of the top 20 genes presented interactions (**Figure 4**). Among which, interleukin and metallopeptidase displayed important interactions, speculating that these two proteins had important functions during the pathogenesis of oral cancer. Moreover, a total of 27 cancer-related genes including COL4A1, COL4A2, COL4A6, IL8, MMP1 and so on enriched in 3 KEGG pathways were further extracted, and collagen, interleukin and metal peptide enzyme genes were found to be the pivotal genes based on gene function annotations (**Table 4**).

Discussion

OTSCC with 5-year survival rate less than 40% was a major death cause of cancer around the world. Despite that great advances have been made in the past few years, the early diagnosis and treatment methods still remained poor [19]. Exploring novel biomarkers may contribute to the diagnosis and therapy of OTSCC. In

our study, we screened a total of 634 DEGs in OTSCC, including 289 up-regulated and 345 down-regulated ones. Through gene expression profile analysis, we found that the over-expressed genes were mainly associated with immunoreaction, and that the pivotal genes including COL4A1, COL4A2, COL4A6, IL8, MMP1 and so on in three KEGG pathways were also significantly over-expressed in OTSCC. Collagen type IV (COL4), one of the main components in basement membrane [20], had been identified playing important roles in the regulation of cellular adhesion, gene expression, and differentiation and so on [21]. And COL4 was found to be key protein in the progress of morphogenesis [22]. Furthermore, some specialized forms of morphogenesis such as angiogenesis attracted more and more attention for the importance of blood vessel formation in tumor development. In this study, COL4A1 and COL4A2 were identified up-regulated, indicating that there might be similar roles of these genes in pathogenesis of OTSCC. In addition, recent studies have suggested that proteolytic enzymes like MMP family (MMP1 and MMP9) also played a significant role in angiogenesis [23]. These findings may help us define new biomarkers used for OTSCC treatment.

In order to further study these DEGs, we mainly analyzed the top 20 DEGs that could distinguish OTSCC samples from normal samples, and constructed a protein network. From which, we found the interactions only existed among 6 proteins, furthermore interleukin (IL) and metallopeptidase protein (MMP) played important roles in this network.

Numerous previous studies had indicated the roles of IL in OTSCC. For example, IL-6 had been suggested as a valuable biomarker in the diagnosis of OTSCC. IL-6, a multi-functional cytokine, which played key roles in the growth factors and differentiation for several cells including neuronal cells, endothelial cells and so on [24]. Moreover, IL-6 was also an important factor in predicting growth and cell differentiation of tumor cells. It was reported to be increased in OTSCC, renal cell carcinoma and lymphoma [25, 26]. Lotfi t al. thought that the salivary level of IL-6 could be used to predict the progression of OTSCC [27, 28]. The expression of IL-8 has also been identified elevated in the serum of patients with OTSCC [28, 29]. The protein level of IL-8 secreted by OTSCC tumor cell cultures was found significantly increased. So, the potential of IL-8 as a biomarker in early detection of OTSCC was supported [30]. Metallopeptidase was another important protein found in this study, which was a family of protease with metal iron, the most important was matrix metalloproteinases (MMPs). MMPs consisted of collagenases, stromelysins, gelatinases, membrane-type MMPs, matrilysins, and other MMPs [31]. MMPs could regulate cell growth and survival, thus was associated with cancer development and tumor invasion. Certain MMPs, such as MMP-8, also showed a protective role in tumor progressions [32]. While MMP-2 and MMP-9 cleaving type IV collagen and gelatin, were linked to angiogenesis [33]. It was reported in head and neck cancer the production of MMPs was accelerated and its activation was also enhanced, in addition, the high expression of MMPs had been indicated to predict tumor metastasisand invasion [34]. Up-regulation of several MMPs including MMP-1, MMP-2, MMP-3 and MMP-14 was linked to poor tumor differentiation and shorter life expectancy [35]. MMP-2, MMP-9 and MMP-13 were found to be over-expressed in head and neck squamous cell carcinoma (HNSCC) [36], and the increased expression of MMPs was demonstrated to play a crucial role in HNSCC invasion and metastasis [37, 38]. Many other previous researches had also confirmed the roles of MMPs in tumor development especially in human head and neck cancers. Kurahara et al. [39] identified the expression of MMP-2 and MMP-9 was associated with nodal status in OTSCC. Similarly, Hong et al. [40] found that MMP-9 expression was significantly correlated with OTSCC metastasis, and Katayama et al. [41] discovered that MMP-9 had predictive potential for tumor metastases in patients with OTSCC. These findings all together indicated that IL or MMPs may be considered as potential targets in the early diagnosis and treatment of OTSCC.

To conclude, the DEGs between OTSCC samples and normal samples were analyzed based on micro-array expression data in this study. Several critical proteins such as COL4A1, COL4A1, IL-8, MMP1, MMP9 and so on that may play important roles in the development of OTSCC were identified. The major proteins (such as IL and MMPs) screened in our study were consistent with previous studies, which may help us better understand the pathogenesis of OTSCC and contribute to explore novel

biomarkers for early diagnosis and treatment. However, additional studies were still needed to clarify the detail roles and action mechanisms of these biomarkers in OTSCC.

Disclosure of conflict of interest

None.

Address correspondence to: Yin Ding, Department of Orthodontics, School of Stomatology, Fourth Military Medical University, Changle Xi Road, No. 145, Xi'an 710032, Shaanxi, PR China. E-mail: dingyin195807@126.com

References

[1] Rodriguez T, Altieri A, Chatenoud L, Gallus S, Bosetti C, Negri E, Franceschi S, Levi F, Talamini R and La Vecchia C. Risk factors for oral and pharyngeal cancer in young adults. Oral Oncol 2004; 40: 207-213.

[2] Regezi JA, Sciubba JJ and Jordan RC. Oral pathology: clinical pathologic correlations. Elsevier Health Sciences 2012.

[3] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008; 58: 71-96.

[4] Cognetti DM, Weber RS and Lai SY. Head and neck cancer. Cancer 2008; 113: 1911-1932.

[5] Ralhan R. Diagnostic potential of genomic and proteomic signatures in oral cancer. International Journal of Human Genetics 2007; 7: 57.

[6] Choi P and Chen C. Genetic expression profiles and biologic pathway alterations in head and neck squamous cell carcinoma. Cancer 2005; 104: 1113-1128.

[7] van den Boom J, Wolter M, Kuick R, Misek DE, Youkilis AS, Wechsler DS, Sommer C, Reifenberger G and Hanash SM. Characterization of gene expression profiles associated with glioma progression using oligonucleotide-based microarray analysis and real-time reverse transcription-polymerase chain reaction. Am J Pathol 2003; 163: 1033-1043.

[8] Miyazaki H, Patel V, Wang H, Ensley JF, Gutkind JS and Yeudall WA. Growth factor-sensitive molecular targets identified in primary and metastatic head and neck squamous cell carcinoma using microarray analysis. Oral Oncol 2006; 42: 240-256.

[9] Miyazaki H, Patel V, Wang H, Edmunds RK, Gutkind JS and Yeudall WA. Down-regulation of CXCL5 inhibits squamous carcinogenesis. Cancer Res 2006; 66: 4279-4284.

[10] Kawakami M, Kawakami K, Kasperbauer JL, Hinkley LL, Tsukuda M, Strome SE and Puri RK. Interleukin-13 receptor alpha2 chain in human head and neck cancer serves as a unique diagnostic marker. Clin Cancer Res 2003; 9: 6381-6388.

[11] Joshi BH and Puri RK. Optimization of expression and purification of two biologically active chimeric fusion proteins that consist of human interleukin-13 and Pseudomonas exotoxin in Escherichia coli. Protein Expr Purif 2005; 39: 189-198.

[12] Ye H, Yu T, Temam S, Ziober BL, Wang J, Schwartz JL, Mao L, Wong DT and Zhou X. Transcriptomic dissection of tongue squamous cell carcinoma. BMC Genomics 2008; 9: 69.

[13] Gautier L, Cope L, Bolstad BM and Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.

[14] Tusher VG, Tibshirani R and Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A 2001; 98: 5116-5121.

[15] Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004; 3: Article3.

[16] Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 2003; 4: P3.

[17] Raychaudhuri S, Stuart JM and Altman RB. Principal components analysis to summarize microarray experiments: application to sporulation time series. Pac Symp Biocomput 2000; 455-466.

[18] Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, Doerks T, Julien P, Roth A, Simonovic M, Bork P and von Mering C. STRING 8--a global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Res 2009; 37: D412-416.

[19] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[20] Mosher DF, Sottile J, Wu C and McDonald JA. Assembly of extracellular matrix. Curr Opin Cell Biol 1992; 4: 810-818.

[21] Goldfinger LE, Stack MS and Jones JC. Processing of laminin-5 and its functional consequences: role of plasmin and tissue-type plasminogen activator. J Cell Biol 1998; 141: 255-265.

[22] Zhang X, Hudson BG and Sarras MP. Hydra cell aggregate development is blocked by selective fragments of fibronectin and type IV collagen. Dev Biol 1994; 164: 10-23.

[23] Xu J, Rodriguez D, Petitclerc E, Kim JJ, Hangai M, Yuen SM, Davis GE and Brooks PC. Proteolytic exposure of a cryptic site within collagen

type IV is required for angiogenesis and tumor growth in vivo. J Cell Biol 2001; 154: 1069-1080.
[24] Forastiere A, Koch W, Trotti A and Sidransky D. Head and neck cancer. N Engl J Med 2001; 345: 1890-1900.
[25] Riedel F, Zaiss I, Herzog D, Gotte K, Naim R and Hormann K. Serum levels of interleukin-6 in patients with primary head and neck squamous cell carcinoma. Anticancer Res 2005; 25: 2761-2765.
[26] St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, Shi W, Qi F, Wu B, Sinha U, Jordan R, Wolinsky L, Park NH, Liu H, Abemayor E and Wong DT. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004; 130: 929-935.
[27] Lotfi A, Shahidi N, Bayazian G, AbdollahiFakhim S, Estakhri R, Esfahani A and Notash R. Serum Level of Interleukin-6 in Patients with Oral Tongue Squamous cell Carcinoma. Iran J Otorhinolaryngol 2015; 27: 207-211.
[28] Korostoff A, Reder L, Masood R and Sinha UK. The role of salivary cytokine biomarkers in tongue cancer invasion and mortality. Oral Oncol 2011; 47: 282-287.
[29] St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, Shi W, Qi F, Wu B, Sinha U, Jordan R, Wolinsky L, Park NH, Liu H, Abemayor E, Wong DT. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004; 130: 929-35.
[30] Chen Z, Malhotra PS, Thomas GR, Ondrey FG, Duffey DC, Smith CW, Enamorado I, Yeh NT, Kroog GS and Rudy S. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. Clin Cancer Res 1999; 5: 1369-1379.
[31] Rosenthal EL and Matrisian LM. Matrix metalloproteases in head and neck cancer. Head Neck 2006; 28: 639-648.
[32] Gutiérrez-Fernández A, Fueyo A, Folgueras AR, Garabaya C, Pennington CJ, Pilgrim S, Edwards DR, Holliday DL, Jones JL and Span PN. Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. Cancer Res 2008; 68: 2755-2763.
[33] Kessenbrock K, Plaks V and Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 2010; 141: 52-67.
[34] Vihinen P and Kähäri VM. Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets. Int J Cancer 2002; 99: 157-166.
[35] Deryugina EI and Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 2006; 25: 9-34.
[36] O-Charoenrat P, Rhys-Evans PH and Eccles SA. Expression of matrix metalloproteinases and their inhibitors correlates with invasion and metastasis in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 2001; 127: 813-820.
[37] Yoshizaki T, Maruyama Y, Sato H and Furukawa M. Expression of tissue inhibitor of matrix metalloproteinase-2 correlates with activation of matrix metalloproteinase-2 and predicts poor prognosis in tongue squamous cell carcinoma. Int J Cancer 2001; 95: 44-50.
[38] Culhaci N, Metin K, Copcu E and Dikicioglu E. Elevated expression of MMP-13 and TIMP-1 in head and neck squamous cell carcinomas may reflect increased tumor invasiveness. BMC Cancer 2004; 4: 42.
[39] Kurahara S, Shinohara M, Ikebe T, Nakamura S, Beppu M, Hiraki A, Takeuchi H and Shirasuna K. Expression of MMPS, MT-MMP, and TIMPs in squamous cell carcinoma of the oral cavity: correlations with tumor invasion and metastasis. Head Neck 1999; 21: 627-638.
[40] Hong SD, Hong SP, Lee JI and Lim CY. Expression of matrix metalloproteinase-2 and -9 in oral squamous cell carcinomas with regard to the metastatic potential. Oral Oncol 2000; 36: 207-213.
[41] Katayama A, Bandoh N, Kishibe K, Takahara M, Ogino T, Nonaka S and Harabuchi Y. Expressions of matrix metalloproteinases in early-stage oral squamous cell carcinoma as predictive indicators for tumor metastases and prognosis. Clin Cancer Res 2004; 10: 634-640.