A genome-wide association scan of biological processes involved in oral lichen planus and oral squamous cell carcinoma

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Abstract

Background: In this study, the molecular mechanisms underlying malignant transformation from oral lichen planus (OLP) to oral squamous cell carcinoma (OSCC) were examined.

Methods: High-throughput sequencing of long noncoding RNAs (lncRNAs) and mRNAs of normal subjects and patients with OLP and OSCC was conducted. RNA-seq reads were mapped, lncRNA and mRNA transcripts were assembled, and expression levels were estimated. The targets of lncRNAs were predicted. Finally, Gene Ontology (GO) and pathway enrichment analyses of differentially expressed genes (DEGs) and lncRNA targets were performed.

Results: High-quality sequence data were generated and the mapping ratios for OSCC, normal, and OLP samples were high. In total, 820, 656, and 582 DEGs were obtained from OPL vs. normal, OSCC vs. normal, and OSCC vs. OPL, respectively. A total of 1721 known lncRNAs and 133 predicted lncRNAs and targets were obtained. Keratinization was significantly enriched by OSCC-related DEGs, but not OPL-related DEGs. The pathway of olfactory transduction was enriched by OPL- and OSCC-related DEGs. Defense response to virus and viral carcinogenesis were enriched by DEGs and lncRNA targets in all comparisons. GO term related to the metabolic process was enriched by lncRNA targets in the OPL vs normal comparison, and antigen processing and presentation via MHC class I was significantly enriched by lncRNA targets in the other 2 comparisons.

Conclusion: Keratinization and MHC class I antigen processing and presentation were activated during the malignant transformation from OLP to OSCC. Additionally, the olfactory transduction pathway may be important for OSCC.

Abbreviations: ALG3 = ALG3, alpha-1,3- mannosyltransferase, ANAPC5 = anaphase promoting complex subunit 5, CASP14 = caspase 14, CPC = coding potential calculator, DEGs = differentially expressed genes, EIF4G1 = eukaryotic translation initiation factor 4 Gamma 1, GO = gene ontology, KEGG = kyoto encyclopedia of genes and genomes, lncRNAs = long noncoding RNAs, MYH9 = myosin heavy chain 9, OLP = oral lichen planus, OSCC = oral squamous cell carcinoma, scaRNA = small Cajal body-specific RNAs, snoRNA = small nucleolar RNAs, SYNE2 = Spectrin Repeat Containing Nuclear Envelope Protein 2.

Keywords: differentially expressed genes, lncRNAs, oral lichen planus, oral squamous cell carcinoma

1. Introduction

Oral squamous cell carcinoma (OSCC), which accounts for >95% of all head and neck cancer cases,[1] is one of the most common cancers in the world.[2] Human papilloma virus infection is becoming a leading risk factor for oral cavity and oropharyngeal cancers.[3] Oral lichen planus (OLP) is one of the most common chronic oral mucosal diseases and is considered a precancerous lesion of OSCC.[4] The mechanisms underlying malignant transformation from OLP to OSCC have been investigated by extensively researchers.[5–7]

A reduced apoptotic rate and increased proliferative activity of epithelial cells and inflammatory cells in OLP are thought to contribute to malignant transformation.[8] The total antioxidant capacity/malondialdehyde ratio, which is used as an index of oxidative stress status, is significantly lower in patients with OSCC than in patients with OLP or normal subjects.[9] Melatonin has oncostatic effects in cancers, including OSCC, potentially because of its antioxidant properties.[10] Oral microbial colonization densities in both lesions and healthy sites of patients with OSCC are higher than those of patients with OLD and healthy individuals; moreover, the level of carcinogenic acetaldehyde produced by the oral microbiome is increased in patients with OSCC.[11] In addition, chronic inflammatory and immune activation results in malignancy in OLP via multiple processes, including the PI3k/Akt/mTOR pathway, which is

activated in chronic oral disorders and potentially correlated with carcinogenic potential.[12,13] However, the molecular mechanisms of malignant transformation are unclear, and an efficient biomarker to predict malignant outcomes has not been identified.

Genome-wide analyses of gene expression patterns are becoming essential for identifying and analyzing genes involved in diseases. However, few studies have used this method to explore the expression differences between OSCC and OLP. To further investigate the association between the 2 diseases, in this study, we assessed genome-wide transcript profiles. The differential expression patterns of protein-coding genes and long noncoding RNAs (lncRNAs), which are drivers of suppressive and oncogenic functions in cancers, were analyzed. The results of this study further characterize the cellular processes and molecular mechanisms involved in the progression of OLP and OSCC.

2. Methods

2.1. Samples, sequencing, and quality control

High-throughput sequencing of lncRNAs and mRNAs was performed for total RNA obtained from 1 normal oral mucosa, 1 OLP, and 1 human papillomavirus-related OSCC tissue sample by Genenergy Bio-Technology Corporation (Shanghai, China; http://www.genenergy.cn/). Briefly, total RNA was extracted, and paired-end sequencing of 101-bp reads was performed using the Illumina HiSeq 2500 platform. Low-quality reads were removed using Trim Galore (version 0.3.5) and read quality was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

2.2. Data mapping

Clean RNA-seq reads were mapped using TopHat (version 2.0.8, http://tophat.cbcb.umd.edu/) against the human genome (*Homo sapiens* GRCh37), which was downloaded from ftp://ftp.ensembl.org/pub/release-74/fasta/homo_sapiens/dna/. All annotated coding sequences (transcripts) were obtained using Homo_sapiens.GRCh37.74.gtf in the Ensembl database (http://www.ensembl.org/).

2.3. Transcript assembly and prediction

The Cufflinks (http://cufflinks.cbcb.umd.edu/) assembler is generally used to assemble aligned RNA-seq reads to obtain a parsimonious set of transcripts, estimate abundances, and test for differential expression. In this study, this software was used to merge transcripts and estimate the expression level of each transcript.

LncRNA assembly was performed using the Ensembl, Gencode,[14] NCBI RefGene,[15] UCSC lncRNA,[16] LNCipedia,[17] and Noncode databases.[18] LncRNA transcripts satisfying the following criteria were screened: longer than 200 bp, without a Pfam protein domain, a coding potential calculator (CPC) score of <0, and a CPAT probability of ≤.364.

The normalized mRNA abundances were quantified as fragments per kilobase of exon per million reads mapped (FPKM) values using Cuffdiff,[19] and the FPKM values of the filtered transcripts were log-transformed. Transcripts with $P \leq .05$ and fold change (FC) ≥ 2 were considered as differentially expressed genes (DEGs).

2.4. Functional enrichment analysis of DEGs

The Gene Ontology (GO) (http://www.geneontology.org) project provides high-quality electronic and manual annotations for genes, gene products, and sequences.[20] The Kyoto Encyclopedia of Genes and Genomes (KEGG) provides a global map of biological systems in both normal and perturbed states.[21] In this study, hypergeometric enrichment tests were performed to calculate *P*-values for each GO and KEGG pathway class enriched by DEGs.

2.5. Classification of lncRNAs

According to positions of protein-coding transcripts, lncRNAs were divided into 7 categories, that is, sense lncRNA, antisense lncRNA, intronic lncRNA, bidirectional lncRNA, intergenic lncRNA (described by Li et al [22]), enhancer lncRNA (localized close to a coding gene), and sRNA host lncRNA that exhibited overlap with any transcript of an miRNA precursor, small nucleolar RNAs (snoRNA), and small Cajal body-specific RNAs (scaRNA). The sRNA genome was downloaded from http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/wgRna.txt.gz. The genome-wide maps of enhancer elements were obtained from http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/vistaEnhancers.txt.gz.

2.6. Prediction of lncRNA targets

Protein-coding genes close to sense lncRNAs, antisense lncRNAs, intronic lncRNAs, bidirectional lncRNAs, and enhancer lncRNAs were considered candidate cis-targets (near the site of lncRNA production).

Potential trans-target genes of lncRNAs were screened using RNAplex (http://www.bioinf.uni-leipzig.de/Software/RNAplex/) to identify possible hybridization sites for short, highly stable RNA–RNA interactions.[23,24]

2.7. Functional enrichment analysis of lncRNA-target genes

As described above, GO and KEGG pathway enrichment analyses of lncRNA-target genes were performed by hypergeometric enrichment tests.

3. Results

3.1. Quality control and location of sequencing data

The raw and preprocessed sequences were shown in Table 1. Reads aligned to the human reference genome GRCh37 were shown in Table 2. The mapping ratios for OSCC, normal, and OLP reads were 72.30%, 65.30%, and 81.90%, respectively.

In total, 26,679 transcripts of 551,753 known genes were obtained from 3 different samples. There were 24,958 protein-coding genes, 1721 known lncRNAs, and 133 predicted lncRNAs.

3.2. Differential expression of genes

As shown in Fig. 1A, the normalized mRNA abundances in different groups were quantified as FPKM values and these values were log-transformed. A total of 820 DEGs (547 upregulated and 273 downregulated), 656 DEGs (454 upregulated and 212 downregulated), and 582 DEGs (233 upregulated and 349 downregulated) were obtained from OPL versus normal, OSCC versus normal, and OSCC versus OPL, respectively. The top upregulated DEG for OPL versus normal was Anaphase Promoting Complex Subunit 5 (*ANAPC5*) ($\log_2$FC = 893.598) and the top downregulated DEG was Spectrin Repeat Containing Nuclear Envelope Protein 2 (*SYNE2*) ($\log_2$FC = −1026.15). The top upregulated DEG for OSCC versus normal was ALG3,

Table 1

Summary of the quality control procedure for raw sequencing data for each group.

Sample	Raw reads	Raw bases	Trim reads	Trim bases	Average length	Trim reads %	Trim bases %
Normal	100217370	10121954370	81959072	7494718324	91.44	0.818	0.740
OLP	87356096	8822965696	77746400	7331570314	94.30	0.890	0.831
OSCC	79835924	8063428324	66754732	6087961474	91.20	0.836	0.755

OLP = oral lichen planus, OSCC = oral squamous cell carcinoma.

Table 2

Sequencing reads aligned to the human reference genome GRCh37.

Sample	Total reads	Total mapped	Mapped ratio (%)	Multiply mapped	Uniquely mapped	Reads properly paired
Normal	81959072	53529855	65.30%	3492342	50037513	42128686
OLP	77746400	63674055	81.90%	2979371	60694684	52761594
OSCC	66754732	48292145	72.30%	3068746	45223399	38605610

OLP = oral lichen planus, OSCC = oral squamous cell carcinoma.

Alpha-1,3- Mannosyltransferase (*ALG3*) ($\log_2 FC = 1020.7$) and the top downregulated DEG was Caspase 14 (*CASP14*) ($\log_2 FC = -631.516$). The top upregulated DEG for OSCC versus OPL was Eukaryotic Translation Initiation Factor 4 Gamma 1 (*EIF4G1*) ($\log_2 FC = 579.426$) and the top down-regulated DEG was Myosin Heavy Chain 9 (*MYH9*) ($\log_2 FC = -719.821$). Previous studies have examined whether the differential expression profile could be used as a phenotypic discriminator by principal component analyses [25,26]; thus, we also performed a principal component analysis and observed good separation of different samples (Fig. 1B).

3.3. Functional enrichment analysis for DEGs

The top 5 enriched GO (biological process) and pathway terms for the DEGs obtained in each comparison were shown in Table 3. The GO (biological process) term "detection of chemical stimulus involved in sensory perception of smell" was significantly enriched by the DEGs in the OPL versus normal comparison, and both the OSCC versus normal and OSCC versus OPL comparison were enriched in keratinization. The olfactory transduction was significant enriched by both DEGs of OPL versus normal and OSCC versus normal.

3.4. Classification and annotation of lncRNAs and lncRNA-target networks

Figure 2 displayed the percentage of lncRNAs in each of 7 categories; intergenic lncRNAs were most common at 76.2% (697), followed by sense lncRNAs at 16.5% (151).

There were 326 cis-targets (1535 edges) and 212 trans-targets (394 edges) in the network of OPL versus normal, 182 cis-targets (813 edges) and 331 trans-targets (693 edges) in the network of
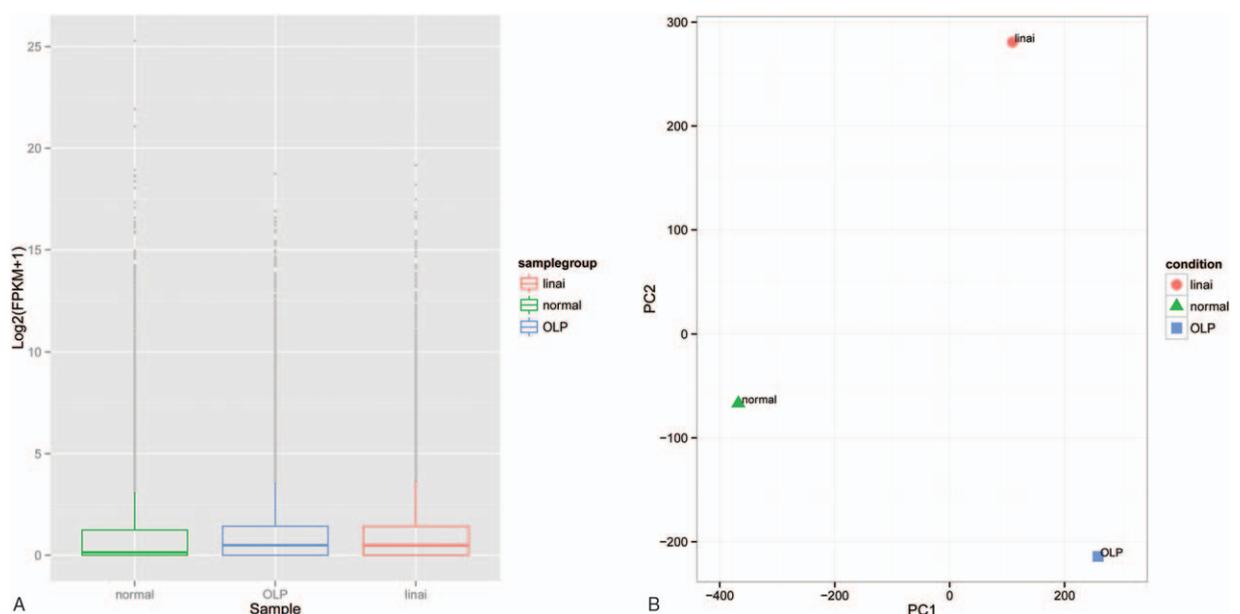

Figure 1. Log-transformed FPKM values (A) and results of a principal component analysis (B) for different samples.

Table 3

Gene ontology (GO) biological process (BP) terms and pathways enriched by differentially expressed genes (DEGs) in 3 comparisons.

Category	GO_ID	GO_term	Count	P
OPL vs normal				
Biological_process	GO:0050911	Detection of chemical stimulus involved in sensory perception of smell	40	0.000147496
Biological_process	GO:0007608	Sensory perception of smell	40	2.13E−07
Biological_process	GO:0009593	Detection of chemical stimulus	42	2.26E−07
Biological_process	GO:0050907	Detection of chemical stimulus involved in sensory perception	40	2.40E−07
Biological_process	GO:0050906	Detection of stimulus involved in sensory perception	41	6.66E−07
KEGG	hsa04740	Olfactory transduction	39	9.76E−05
OSCC vs normal				
Biological_process	GO:0031424	Keratinization	12	2.18E−05
Biological_process	GO:0030216	Keratinocyte differentiation	15	1.12E−03
Biological_process	GO:0051607	Defense response to virus	20	2.04E−03
Biological_process	GO:0045069	Regulation of viral genome replication	10	2.04E−03
Biological_process	GO:0019079	Viral genome replication	11	2.26E−03
KEGG	hsa04740	Olfactory transduction	393	0.000419911
KEGG	hsa03018	RNA degradation	70	0.049751529
OSCC vs OPL				
Biological_process	GO:0031424	Keratinization	20	1.64E−16
Biological_process	GO:0030216	Keratinocyte differentiation	25	7.91E−14
Biological_process	GO:0008544	Epidermis development	38	1.62E−13
Biological_process	GO:0009913	Epidermal cell differentiation	25	2.82E−12
Biological_process	GO:0030855	Epithelial cell differentiation	38	3.10E−12

Count = number of genes enriched in each term, KEGG = Kyoto Encyclopedia of Genes and Genomes. OLP = oral lichen planus, OSCC = oral squamous cell carcinoma.

OSCC versus normal, and 100 cis-targets (445 edges) and 135 trans-targets (263 edges) in the network of OSCC versus OPL.

The hub lncRNAs in the 3 networks were NONHSAG054274 (degree = 138), ENST00000439233 (degree = 121), and NONHSAG04920 (degree = 98). The networks of OPL versus normal, OSCC versus normal, and OSCC versus OPL are shown in Supplementary Figures 1-3, http://links.lww.com/MD/B728.

3.5. Functional terms of lncRNA targets

The enrichment results for lncRNA targets were shown in Table 4. The lncRNA targets of OPL versus normal were enriched in GO terms of metabolic process and epidermis development, and pathways of virus infection. Targets of OSCC versus normal

Figure 2. Percentage of lncRNAs in 7 categories. Each color represents a distinct lncRNA category.

were enriched in the GO terms of antigen processing and presentation of exogenous peptide antigen, etc., and pathways of virus infection and viral carcinogenesis. Targets of OSCC versus OPL were enriched in the GO terms of hair and molting cycle and pathways of virus infection and antigen processing.

4. Discussion

OLP, a chronic autoimmune disease, has malignant potential, generally developing into OSCC.[27] The focus of this study was to further explore the complex mechanism underlying malignant transformation from OLP to OSCC. Accordingly, high-quality sequence data were generated and DEGs and lncRNAs that differentiate sample types were obtained. Finally, genes and lncRNAs in comparisons among normal, OLP, and OSCC samples were enriched in multiple functional terms.

OSCC-related DEGs, but not OPL-related DEGs, were significantly enriched in keratinization. In 1998, Schultz et al[28] indicated that keratin is abnormally distributed in poorly differentiated SCC and keratin pearl formation is only observed in well-differentiated oral/oropharyngeal SCC. Orthokeratinization-related factors could be involved in the mechanism of OSCC, and the keratin gene expression was changed in poorly differentiated SCC.[29] Our findings demonstrated again that keratinization could be very important during the transformation from OLP to OSCC. The sensory perception of smell is associated with the occurrence of OLP. Additionally, the pathway of olfactory transduction was enriched by DEGs for both OPL versus normal and OSCC versus normal. Previous studies have suggested that the olfactory transduction pathway is associated with a high risk of pancreatic cancer.[30] Sanz et al[31] found that olfactory receptor stimulation could enhance metastasis emergence and spread.

Furthermore, similar results were obtained in a functional enrichment analysis of lncRNA targets. Defense response to virus and viral carcinogenesis are very important in the development of
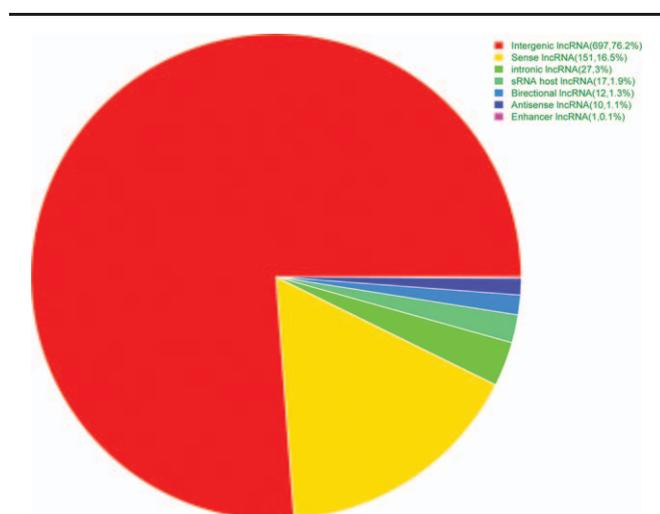
Table 4

Gene ontology (GO) biological process (BP) terms and pathways enriched by long noncoding RNAs (lncRNAs) in 3 comparisons.

GO_category	GO_ID	GO_term	Count	P
OPL vs normal				
Biological_process	GO:0008152	Metabolic process	328	5.12E−04
Biological_process	GO:0090304	Nucleic acid metabolic process	159	5.41E−04
Biological_process	GO:0008544	Epidermis development	18	5.80E−04
Biological_process	GO:0010467	Gene expression	155	6.27E−04
Biological_process	GO:0007010	Cytoskeleton organization	39	7.33E−04
KEGG	hsa05132	Salmonella infection	9	0.000368
KEGG	hsa05169	Epstein-Barr virus infection	14	0.000707
KEGG	hsa05203	Viral carcinogenesis	13	0.001493
KEGG	hsa05205	Proteoglycans in cancer	14	0.002244
KEGG	hsa05219	Bladder cancer	5	0.003406
OSCC vs normal				
Biological_process	GO:0002480	Antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent	4	5.55E−05
Biological_process	GO:0051220	Cytoplasmic sequestering of protein	5	3.60E−04
Biological_process	GO:0002474	Antigen processing and presentation of peptide antigen via MHC class I	9	1.36E−03
Biological_process	GO:0046602	Regulation of mitotic centrosome separation	2	2.07E−03
Biological_process	GO:0060449	Bud elongation involved in lung branching	2	2.07E−03
KEGG	hsa05203	Viral carcinogenesis	15	5.24E−05
KEGG	hsa05169	Epstein-Barr virus infection	11	8.50E−03
KEGG	hsa05168	Herpes simplex infection	10	1.25E−02
KEGG	hsa00260	Glycine, serine, and threonine metabolism	4	1.30E−02
KEGG	hsa04621	NOD-like receptor signaling pathway	5	1.33E−02
OSCC vs OPL				
Biological_process	GO:0042633	Hair cycle	6	4.06E−04
Biological_process	GO:0042303	Molting cycle	6	4.06E−04
Biological_process	GO:0032507	Maintenance of protein location in cell	6	7.30E−04
Biological_process	GO:0001961	Positive regulation of cytokine-mediated signaling pathway	3	1.17E−03
Biological_process	GO:0051651	Maintenance of location in cell	6	1.29E−03
KEGG	hsa05168	Herpes simplex infection	8	0.000736
KEGG	hsa04612	Antigen processing and presentation	5	0.000797
KEGG	hsa05169	Epstein-Barr virus infection	7	0.00553
KEGG	hsa05416	Viral myocarditis	4	0.006803
KEGG	hsa05330	Allograft rejection	3	0.006866

Count = number of genes enriched in each term, KEGG = Kyoto Encyclopedia of Genes and Genomes, OLP = oral lichen planus, OSCC = oral squamous cell carcinoma.

OLP and OSCC.[32] This is consistent with our results for the human papilloma virus-related OSCC tissue sample. It is interesting to note that the GO term "metabolic process" was enriched by the lncRNA targets in OPL versus normal, whereas antigen processing and presentation via MHC class I was significantly enriched by lncRNA targets in the other 2 comparisons. A metabolomics-based diagnostic approach can be used to distinguish between OSCC and precancerous lesions.[33] Moreover, glucose metabolism disturbance is highly presented in patients with OLP.[34] Previous studies have found that HLA class I antigen processing machinery associated with antigen processing plays an important role in head and neck SCC,[35] and HLA class I molecules are downregulated in OSCC.[36] The overexpression of MHC class I chain-related protein A in OSCC is related to enhanced cytotoxicity to target tumor cells.[37]

However, the study had some limitations. One of it was the small sample size; accordingly, it is necessary to validate the experiments using a larger sample size. Additionally, sequencing was performed on a single microarray platform.

Based on the evidence presented above, we reached the general conclusion that the molecular mechanisms underlying malignant transformation from OLP to OSCC involve multiple biological processes. Keratinization and MHC class I antigen processing and presentation were activated. Additionally, the sensory perception of smell and olfactory transduction may be associated with a high risk of malignant transformation in OLP. Moreover, disturbed metabolic processes may be more frequent in OLP than in OSCC. Future studies will focus on candidate genes and lncRNAs with significant effects on the malignant transformation of OLP.

References

[1] Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol Lett 2014;8:7–11.
[2] Jithesh P, Risk J, Schache A, et al. The epigenetic landscape of oral squamous cell carcinoma. Brit J Cancer 2013;108:370–9.
[3] Tommasino M. The human papillomavirus family and its role in carcinogenesis. Seminars in Cancer Biology 2014;Elsevier,
[4] Payeras MR, Cherubini K, Figueiredo MA, et al. Oral lichen planus: focus on etiopathogenesis. Arch Oral Biol 2013;58:1057–69.
[5] Yang Q, Guo B, Sun H, et al. Identification of the key genes implicated in the transformation of OLP to OSCC using RNA-sequencing. Oncol Rep 2017;37:2355–65.
[6] National Research CouncilGuide for the Care and Use of Laboratory Animals. Vol 103. 2011;National Academies Press, 1072–1073.
[7] Segura S, Rozasmuñoz E, Toll A, et al. Evaluation of MYC status in oral lichen planus in patients with progression to oral squamous cell carcinoma. Brit J Dermatol 2013;169:106–14.
[8] Bascones-Ilundain C, Gonzalez-Moles MA, Esparza-Gómez G, et al. Importance of apoptotic mechanisms in inflammatory infiltrate of oral lichen planus lesions. Anticancer Res 2006;26:357–62.

[9] Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, et al. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. J Oral Pathol Med 2012; 41:736–40.

[10] Cutando A, López-Valverde A, De Vicente J, et al. Action of melatonin on squamous cell carcinoma and other tumors of the oral cavity (Review). Oncol Lett 2014;7:923–6.

[11] Marttila E, Uittamo J, Rusanen P, et al. Acetaldehyde production and microbial colonization in oral squamous cell carcinoma and oral lichenoid disease. Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:61–8.

[12] Mignogna MD, Fedele S, Russo LL, et al. Immune activation and chronic inflammation as the cause of malignancy in oral lichen planus: is there any evidence? Oral Oncol 2004;40:120–30.

[13] Sonis ST, Amaral Mendes R. Could the PI3K canonical pathway be a common link between chronic inflammatory conditions and oral carcinogenesis? J Oral Pathol Med 2016;45:469–74.

[14] Harrow J, Frankish A, Gonzalez JM, et al. GENCODE: the reference human genome annotation for The ENCODE Project. Genome Res 2012;22:1760–74.

[15] Pruitt KD, Tatusova T, Maglott DR. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Aacids Res 2007;35(suppl 1):D61–5.

[16] Schneider KL, Pollard KS, Baertsch R, et al. The UCSC archaeal genome browser. Nucleic Acids Res 2006;34(suppl 1):D407–10.

[17] Volders P-J, Helsens K, Wang X, et al. LNCipedia: a database for annotated human lncRNA transcript sequences and structures. Nucleic Acids Res 2013;41:D246–51.

[18] Bu D, Yu K, Sun S, et al. NONCODE v3. 0: integrative annotation of long noncoding RNAs. Nucleic Acids Res 2011;40:D120–5.

[19] Trapnell C, Williams BA, Pertea G, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010;28:511–5.

[20] Consortium GO. Gene ontology annotations and resources. Nucleic Acids Res 2013;41:D530–5.

[21] Kanehisa M, Sato Y, Kawashima M, et al. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 2015;44:D457–62.

[22] Li C, Chen J, Zhang K, et al. Progress and prospects of long noncoding RNAs (lncRNAs) in hepatocellular carcinoma. Cell Physiol Biochem 2015;36:423–34.

[23] Tafer H, Hofacker IL. RNAplex: a fast tool for RNA–RNA interaction search. Bioinformatics 2008;24:2657–63.

[24] Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011;43:904–14.

[25] Li J, Fan S, Han D, et al. Microarray gene expression profiling and bioinformatics analysis of premature ovarian failure in a rat model. Exp Mol Pathol 2014;97:535–41.

[26] Fan S, Li X, Tie L, et al. KIAA0101 is associated with human renal cell carcinoma proliferation and migration induced by erythropoietin. Oncotarget 2015;7:13520–37.

[27] Baghaei F, Shojaei S, Afshar-Moghaddam N, et al. Study of P21 expression in oral lichen planus and oral squamous cell carcinoma by immunohistochemical technique. J Dent 2015;16:156.

[28] Schultz CP, Liu K-Z, Kerr PD, et al. In situ infrared histopathology of keratinization in human oral/oropharyngeal squamous cell carcinoma. Oncol Res 1997;10:277–86.

[29] Shalinsky D, Bischoff E, Gregory M, et al. Retinoid-induced suppression of squamous cell differentiation in human oral squamous cell carcinoma xenografts (line 1483) in athymic nude mice. Cancer Res 1995;55: 3183–91.

[30] Wei P, Tang H, Li D. Insights into pancreatic cancer etiology from pathway analysis of genome-wide association study data. PLoS One 2012;7:e46887.

[31] Sanz G, Leray I, Dewaele A, et al. Promotion of cancer cell invasiveness and metastasis emergence caused by olfactory receptor stimulation. PLoS One 2014;9:e85110.

[32] Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982–1997. Oral Surg Oral Med Oral Pathol Oral Radiol Endol 2001;91:622–35.

[33] Yan S-K, Wei B-J, Lin Z-Y, et al. A metabonomic approach to the diagnosis of oral squamous cell carcinoma, oral lichen planus and oral leukoplakia. Oral Oncol 2008;44:477–83.

[34] Seyhan M, Özcan H, Sahin I, et al. High prevalence of glucose metabolism disturbance in patients with lichen planus. Diabetes Res Clin Pract 2007;77:198–202.

[35] Bandoh N, Ogino T, Katayama A, et al. HLA class I antigen and transporter associated with antigen processing downregulation in metastatic lesions of head and neck squamous cell carcinoma as a marker of poor prognosis. Oncol Rep 2010;23:933.

[36] Tang Q, Zhang J, Qi B, et al. Downregulation of HLA class I molecules in primary oral squamous cell carcinomas and cell lines. Arch Med Res 2009;40:256–63.

[37] Chao L, Fangqiong S, Dan Y, et al. Natural killer and cytotoxic T lymphocyte-mediated cytotoxicity enhanced by genetic overexpression of MHC class I chain-related protein A in oral squamous cell carcinoma: An experimental study in vivo. West China J Stomatol 2012;30:32–5.