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Long non-coding RNAs associated with oral squamous cell carcinoma

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Abstract. – Oral squamous cell carcinoma (OSCC) is one of the most common and fatal diseases in the head and neck region worldwide. To better understand the carcinogenesis and to find efficient biomarkers and therapeutic targets are still in urgent need. The studies of expression profile and mechanisms of long non-coding RNAs (lncRNAs) develop fast in recent years. This molecule form is aberrantly expressing and influencing many cellular processes in different cancer types. But its expression pattern and association with oral squamous cell carcinoma are yet to be elucidated. Here we reviewed recently reported OSCC-related lncRNAs and their relationship with the clinical status of patients. Functional mechanisms were also discussed. With the emerging knowledge and techniques in this area, more efficient biomarkers and therapeutic targets are promising in the future.

Key Words:
Long non-coding RNA, LncRNA, Oral squamous cell carcinoma, Biomarker.

Introduction

Oral squamous cell carcinoma (OSCC), including oral cavity and oropharyngeal, is among the most common cancers arising from the head and neck region. About 600,000 new cases arise annually worldwide, two-thirds of which occur in developing countries, especially in Asia[1-3]. The estimated incidence rate is 48.1 per 100,000 population per year, and the estimated mortality rate in China is 22.1, according to recent data. The mortality of males doubled than that of female[4]. Oral cavity and oropharyngeal squamous cell carcinoma account for approximately 90% of all cases[3]. Approximately 75% of OSCCs are due to tobacco smoking and alcohol consumption. Other risk factors include: (1) the consumption of nitrosamine-rich foods, such as salted fish; (2) the chewing of betel quid with or without tobacco; (3) viruses infection such as Epstein-Barr virus (EBV), or high-risk human papillomavirus (HPV), i.e., HPV16 and HPV18[5]. In the past two-three decades, the overall prognosis for advanced-stage OSCC has barely improved, leaving the patients and their families with heavy disease burden[6,7]. Nowadays, it is still in urgent need of a better understanding of the mechanism of carcinogenesis and the looking for effective biomarkers and therapeutic targets.

It is estimated that a large portion (>70%) locus of the human genome can be transcribed, but only 2% could be translated into proteins. The majority of transcribed molecules couldn't serve as blueprints for proteins[8]. Statistics of the most recent GENCODE release reveal that only 34% of the human genome are protein-coding genes, the rest are non-coding genes, including long non-coding RNAs (lncRNA, >200 nt), small non-coding RNAs (<200 nt), and pseudogenes[9]. The human genome has more than 15,000 lncRNA genes, encoding for more than 28,000 lncRNA transcripts, which consist of nearly 14% of the total transcripts (GENCODE)[9]. Small ncRNAs, like microRNAs, are well studied and proved to have an important role in cancers, including oral cancer[10-13]. However, long non-coding RNAs have aroused researchers' interest over the last decade[14-16].

Unlike some highly conserved RNAs, such as microRNAs (miRNAs), or circular RNAs (circRNAs), lncRNAs lack conservation across species. Similar to mRNAs, most lncRNAs are transcribed by RNA polymerase II and have

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some mRNA signatures, such as 5′ capping and splicing. But they have much fewer functional open reading frames (ORFs) than that of mRNAs[17]. LncRNAs can be classified into five categories by their locations: sense lncRNAs, antisense lncRNAs, bidirectional lncRNAs, intronic lncRNAs, and intergenic lncRNAs[12,18-20].

Some lncRNAs are considered bifunctional, revealed recently by computational and high throughput methods. They have coding functions, as well as non-coding by either small ORFs or alternative splicing and/or allele-specific expression into different isoforms[21]. This discovery refreshed our understanding of the complexity of this molecule.

In this review, we focused on recently reported lncRNAs that are considered being associated with oral squamous carcinoma. We summarized the expression profile and their relationship with the clinical status of patients. Studies on head and neck squamous cell carcinomas, like laryngeal or pharyngeal, were not included. Functional mechanisms of lncRNAs in OSCCs were discussed.

Transcriptome Studies of LncRNAs in OSCC

To get the landscape of lncRNAs expression in the whole genome scale, several studies have been done using different techniques, such as microarray or RNA-sequencing. Gibb et al[22] first described the lncRNA expression profile in the human oral mucosa. They performed Serial Analysis of Gene Expression (SAGE) on 6 normal oral mucosa and 10 biopsies of oral premalignant lesions and reported the expression of 325 long non-coding RNAs in oral mucosa, suggesting lncRNAs contribute to the transcriptome of the oral mucosa. Up to 60% of the detected lncRNAs were found to be aberrantly expressed in oral premalignant lesions. Conway et al[23] compared 19 HPV negative patients' trios: normal oral mucosa, dysplasia, and OSCC using RNA-sequencing data. The results showed that several antisense RNA transcripts were dysregulated at different points in the pathological process and could be carcinogenic candidates, including a HOTAIR (HOX transcript antisense RNA) transcript and some antisense HOX genes. Gao et al[24] re-annotated microarray data using a web server called ncFANs, and found 8 differentially expressed lncRNAs in tongue squamous cell carcinoma (TSCC)[25]. Jia et al[26] analyzed lncRNA microarray in nine paired samples of TSCC *vs.* matched non-malignant tongue tissues, and miR-26a and lncRNA MEG3 expressions were found reduced in TSCC. Yang et al[27] analyzed the RNA-Seq data from NCBI, and the results showed that 52 lncRNAs were significantly differentially expressed compared to control. Then, they verified three highly expressed genes by RT-PCR. Zhu et al[28] compared oral cancer and paired normal oral mucosa from 6 patients using microarrays, and 934 lncRNAs were found upregulated, and 1119 were significantly downregulated in OSCC. Feng et al[29] performed transcriptome analysis between oral squamous cell carcinoma and healthy oral mucosa using four different microarray data sets, including more than 400 cases. Using the criteria of FDR < 0.01, they found that 658 lncRNA transcripts were significantly differentially expressed. Among them, 36 lncRNAs showed more than a 2-fold change. They also verified three lncRNAs, which showed the highest fold change (LOC441178, HCG22, and C5orf66-AS1) with RT-PCR.

Bioinformatical Analysis of LncRNAs

In the genome-wide screening of lncRNAs of interests, some online bioinformatical software was developed to accelerate our insight into the whole picture of this area. NcFANs is a web service for functional annotation of human and mouse lncRNAs using open-accessed microarray data in data banks like Gene Expression Omnibus (GEO)[24,30,31]. The re-annotation of the existing data provides researchers a new approach to look into different aspects of numerous high-throughput genomic experiments. Terai et al[32] reported a computational prediction interface of RNA-RNA interactions, which can be used in lncRNA functional prediction. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is another bioinformatics resource that can be used in the gene functional classification or clustering[33]. Co-lncRNA is a web-based server that allows users to identify pathways that may be affected by co-expressed protein-coding genes (CEGs) of a single or multiple lncRNAs[34]. TANRIC, an open-access tool for investigating the function and clinical relevance of lncRNAs in cancer, provides the expression profiles of lncRNAs in large patient cohorts of 20 cancer types. The source includes the cancer genome atlas (TCGA), cancer cell line encyclopedia (CCLE), and other independent datasets[35]. Along with those open-access, high-throughput, cancer-related resources like TCGA, GEO, etc., these tools have boosted the genome-wide research of lncRNAs a lot.

The Aberrant Expression of LncRNAs in OSCC and their Associations with Clinical Features

The expression pattern and functional mechanism of one certain lncRNA screened by high-throughput methods have drawn researchers' interests and been intensively studied. Some researchers have also assessed their association with clinical or pathological status. A series of lncRNAs were found to have the potential to serve as biomarkers for diagnosis or prognosis, and the prediction of treatment responses. Here we summarized individual aberrantly expressed lncRNAs in OSCC clinical cohort studies, and their links to clinical features (Table I).

There were more than 20 lncRNAs reported to be upregulated in OSCC, individually. But only a few were reportedly downregulated and correlated with patient outcomes. Overall survival (OS) is an important parameter that has been evaluated in cancer treatment, so most authors would assess it in their researches. In our summary, HOTAIR, MALAT1, H19, NEAT1, DLEU1, KCNQ10OT1, ANRIL, AFAP1-AS1, PDIA3P, CCAT2, and MIR31HG were all upregulated in OSCC, and the higher expression levels were related to a poorer OS rate. HOTAIR is among the most intensively studied lncRNAs, and its role as a biomarker has been assessed by different researchers. HOTAIR overexpression was not only related to unfavorable prognosis but also related to advanced tumor stage and the presence of metastases[36-39]. UCA1 overexpression was related to OSSC progression *via* WNT/β-catenin signaling pathway and is demonstrated to promote metastasis[40]. FOXC1 was a cancer-associated coding gene, and its overexpression contributes to poor survival in patients with breast cancer, OSCC, and hepatocellular carcinoma. LncRNA FOXCUT expression was positively correlated with FOXC1 in OSCC, and both of them were overexpressed in OSCC patients[41]. Some researchers have found a few lncRNAs that were associated with different therapeutic responses, such as chemotherapy. For instance, Arunkumar et al[42] checked the expression of lnc-RoR in patients based on their therapeutic response category, and the results showed that patients with stable disease (SD) and progressive disease (PD) expressed higher levels of lnc-RoR than the patients with complete response (CR) and partial response (PR). It reveals that lnc-RoR was overexpressed in the majority of samples resistant to chemotherapy and/or radiotherapy. UCA1 was found to be overexpressed in cisplatin (CDDP) chemo-resistance OSCC cells and the mechanism study found that UCA1 accelerated cell proliferation, increased CDDP chemo-resistance, and restrained apoptosis partly by interacting with miR-184[43].

Maternally expressed gene 3 (MEG3) encodes a lncRNA produced by various normal tissues, which plays the role of a tumor suppressor. The loss of this RNA expression causes cell growth and proliferation in human cancers. It was found to be downregulated in OSCC compared with paired normal tissue in two independent studies, and Jia et al[26] also reported the high expression level was associated with poor prognosis. GAS5 was also reported to be downregulated in OSCC, both *in vivo* and *in vitro*[27], but the clinical outcomes were not assessed in the study.

Except for tissue biopsy, body fluid detection techniques have been emerging recently. Liquid biopsy is a test of material, such as plasma, serum, and saliva that supposed to contain circulating tumor cells or circulating cell-free tumor DNA (ctDNA) coming from tumor apoptosis or necrosis. It has some advantages compared with traditional biopsy, and that makes it a promising test in clinical usage. For example, the access of samples is continuous and easier, making it possible to monitor a patient's status dynamically[44,45]. Tang et al[46] tested lncRNAs in tissue and saliva samples from OSCC patients, and found subsets of lncRNAs expressed across non-tumor, tumor, and metastatic tissue samples. Some lncRNAs, such as MALAT1 and HOTAIR, can be detected in the saliva samples and could be a potential diagnose markers for OSCC. Three lncRNAs (lincRNA-p21, GAS5, and HOTAIR) in plasma were studied in head and neck cancer patients, including OSCC, and GAS5 was found to be a marker for treatment response. GAS5 levels in patients with PR or PD status were significantly higher compared with patients with CR status[47].

Functional Mechanisms of LncRNAs in OSCCs

LncRNAs are reported to be regulators in many cellular processes in variate cancer types. They act at epigenetic, transcriptional, and post-transcriptional levels. Most lncRNAs are found located in the cell nucleus, exhibiting comparatively lower expression levels to coding mRNAs. The functional mechanisms of lncRNAs partially depend on their locations within the cells. For example, nuclear-located lncRNAs act as scaffolds for chromatin-modify-

Table I. Aberrantly expressed lncRNAs in OSCC and their association with clinical features.

LncRNA	Tumor site	Comparing group	Status in literature (tumor *vs.* control)	Related clinical features	Ref.
HOTAIR	OSCC OSCC	50 OSCC and adjacent normal control 76 OSCC and adjacent normal tissue	Upregulated Upregulated	OS Metastasis, OS, DFS, histological differentiation	68 39
MALAT1	TSCC OSCC	127 TSCC/adjacent normal control 54 OSCC/10 non-tumor control	Upregulated Upregulated	Metastasis OS	69 70
MEG3	TSCC OSCC OSCC	76 TSCC/adjacent normal control 83 OSCC tumor samples, as well as the corresponding normal tissues 45 OSCC and normal control	Downregulated Downregulated Downregulated	Tumor size, cancer progression, OS NA TNM stage	26 71 56
H19	OSCC	42 OSCC and matched control	Upregulated	TNM stage, nodal invasion, OS	66
NEAT1	OSCC	30 OSCC and adjacent non-tumor tissues	Upregulated	TNM stage, OS	54
UCA1	TSCC TSCC OSCC	124 TSCC and adjacent normal mucosal tissues 94 TSCC/adjacent normal control 30 OSCC tumor tissues and their corresponding normal tissues	Upregulated Upregulated Upregulated	Metastasis, TNM stage metastasis Tumor progression and chemo-resistance	40 72 43
FOXCUT	OSCC	23 OSCC/adjacent normal control	Upregulated	NA	41
DLEU1	OSCC	29 samples of primary OSCC tissue and 17 adjacent normal tissue	Upregulated	OS	73
Lnc-p23154	OSCC	49 OSCC and adjacent non- tumor tissue	Upregulated	Metastasis	74
KCNQ1OT1	TSCC	3 chemo-sensitive tissues and 3 chemo-insensitive tissues from TSCC patients, 95 adjacent normal tissues and 102 TSCC tissues	Upregulated	Chemo-sensitivity, Gleason score, T stage, lymph node status, OS	75
AC007271.3	OSCC	80 OSCC cases and 70 controls	Upregulated	Clinical stage	76
CILA1	TSCC	155 TSCC and adjacent normal	Upregulated	Metastasis, chemo-sensitivity, disease stage, poor prognosis	77
FTH1P3	OSCC	134 OSCC tissues and adjacent non-cancer tissues	Upregulated	TNM stage, OS	78
ANRIL	OSCC	130 Oral cancer tissues and corresponding normal tissues	Upregulated	Pathological node, TNM stage, OS	79
LINC00511	TSCC	20 TSCC tissues and corresponding adjacent tissues	Upregulated	NA	80
AFAP1-AS1	TSCC	103 pairs of human TSCC tissues and corresponding adjacent normal tongue mucous tissues	Upregulated	OS	81

Table Continued

Table I *(Continued)*. Aberrantly expressed lncRNAs in OSCC and their association with clinical features.

LncRNA	Tumor site	Comparing group	Status in literature (tumor *vs.* control)	Related clinical features	Ref.
CEBPA-AS1	OSCC	60 pairs of OSCC tissues and matched paraneoplastic normal tissues	Upregulated	Poor differentiation, lymph node metastasis and high clinical stage	82
PDIA3P	OSCC	58 OSCC and non-tumor adjacent tissues or from patients with reshaping of gingival tissues	Upregulated	OS	83
AC132217.4	OSCC	30 OSCC tissues and normal tissues	Upregulated	Metastasis	84
CCAT2	OSCC	62 OSCC tissues and adjacent normal tissue	Upregulated	Poor differentiation, higher T stage, and clinical stage, OS	85
LncHIFCAR/ MIR31HG	OSCC	15 OSCC tumors and the surrounding non-cancerous mucosa tissues	Upregulated	Age, advanced tumor grade, OS, RFS	86
TUG1	TSCC	27 TSCC tissues and adjacent normal tongue tissues	Upregulated	NA	87
LINC00668	OSCC	50 OSCC and normal oral mucosal tissue	Upregulated	NA	88
HAS2-AS1	OSCC	96 OSCC tissues and paired normal mucosa	Upregulated	Lymph node metastasis	28
LINC-RoR	OSCC	60 OSCC and 8 adjacent normal tissues	Upregulated	Tumor recurrence and poor therapeutic response	42
LACAT1	OSCC	78 OSCC tumor tissues and adjacent tissues	Upregulated	Pathology stage, OS	89

OSCC: oral squamous cell carcinoma; TSCC: tongue squamous cell carcinoma; NA: not available in the literature; OS: overall survival; DFS: disease free survival; RFS: recurrence free survival.

ing complexes and cause chromatin reprogram, imprinting, and histone modification. They can also combine with target genes and their promoters, upregulate transcription of enhancers, and influence epigenetic events *via* transcription-dependent mechanisms, such as DNA methylation, along with directly influencing the transcription machinery. Aside from regulating all aspects of gene expression, lncRNAs are also involved in the regulation of mRNA splicing, degradation, protein activity, and post-transcriptional control. They can function as scaffolds for higher-order complexes, signaling molecules *via* exosomes, and vehicles for increased genetic diversity[48-51][11]. The expression patterns of them are highly tissue-specific[9].

Here are some examples of lncRNAs' functional mechanisms. Li et al[52] found that lncRNA ROR would promote tumor chemo-resistance by suppressing the P53 signaling pathway. LOC401317 was reported to affect cell cycle by increasing p21, as well as decreasing cyclin D1 and cyclin E1 expression, and promote apoptosis through the induction of poly ADP-ribose polymerase (PARP) and caspase-3 cleavage[53]. MiRNA sponging is a major route of lncRNA functional mechanism, which is repeatedly reported. LncRNA NEAT1 was found to promote cancers by regulating miR-107/CDK6, or miR-365/RGS20 pathways[54,55]. MEG3 was reported to play an antitumor effect by inducing miR-26a or miR-21[26,56]. HOTAIR promotes cancer metastasis as a molecular scaffold, which targets the histone modification complexes PRC2 and LSD1, and reprograms chromatin states. It also represses E-cadherin expression by binding to EZH2 and H3K27me3 with the E-cadherin promoter[37,39].

Conclusions and Challenges

LncRNA expression contributes significantly to the whole transcriptome. Gibb et al[19] have shown that altered expression of lncRNAs can be used as biomarkers for diagnosis and prognosis, as well as potential therapeutic targets. It is reasonable to consider that lncRNAs may provide the new biomarkers and therapeutic targets in the clinic. Most of the listed lncRNAs in this article have some potential to serve as biomarkers in diagnosis, prognosis, or treatment response prediction. Moreover, the restoration of these aberrantly expressed lncRNAs is reportedly to affect cell proliferation, apoptosis, and cell cycle, e.g., HOTAIR, or MEG3 in OSCC cell lines[26,57]. It makes them potential therapeutic targets in the future. However, the challenge of the clinical utility of these lncRNAs still exists. Except for several well-studied lncRNAs like HOTAIR[58-60], NEAT1[54,55,61], UCA1[40,62,63], or H19[64-67], most of them lack validation from a second group. And the study cohorts are relatively small and in need of expansion. Besides, our understanding of lncRNAs' functions in various cellular processes is still in the beginning. More efforts should be taken to explore their fundamental functions and elucidate the actual role which lncRNAs play in OSCC carcinogenesis and development.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Ryerson AB, Eheman CR, Altekruse SF, Ward JW, Jemal A, Sherman RL, Henley SJ, Holtzman D, Lake A, Noone AM, Anderson RN, Ma J, Ly KN, Cronin KA, Penberthy L, Kohler BA. Annual Report to the Nation on the Status of Cancer, 1975-2012, featuring the increasing incidence of liver cancer. Cancer 2016; 122: 1312-1337.
2) Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011; 11: 9-22.
3) Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45: 309-316.
4) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
5) Shield K, Ferlay J, Jemal A, Sankaranarayanan R, Chaturvedi A, Bray F, Soerjomataram I. The global incidence of lip, oral cavity, and pharyngeal cancers by subsite in 2012. CA Cancer J Clin 2017; 67: 51-64.
6) Mendez E, Houck JR, Doody DR, Fan W, Lohavanichbutr P, Rue TC, Yueh B, Futran ND, Upton MP, Farwell DG, Heagerty PJ, Zhao LP, Schwartz SM, Chen C. A genetic expression profile associated with oral cancer identifies a group of patients at high risk of poor survival. Clin Cancer Res 2009; 15: 1353-1361.
7) Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP. Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer 2005; 114: 806-816.
8) Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol 2012; 9: 703-719.
9) Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012; 22: 1775-1789.
10) Gomes CC, de Sousa SF, Gomez RS. MicroRNAs: small molecules with a potentially role in oral squamous cell carcinoma. Curr Pharm Des 2013; 19: 1285-1291.
11) Cao MX, Jiang YP, Tang YL, Liang XH. The crosstalk between lncRNA and microRNA in cancer metastasis: orchestrating the epithelial-mesenchymal plasticity. Oncotarget 2017; 8: 12472-12483.
12) Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011; 12: 861-874.
13) Min A, Zhu C, Peng S, Rajthala S, Costea DE, Sapkota D. MicroRNAs as important players and biomarkers in oral carcinogenesis. Biomed Res Int 2015; 2015: 186904.
14) Zou AE, Zheng H, Saad MA, Rahimy M, Ku J, Kuo SZ, Honda TK, Wang-Rodriguez J, Xuan Y, Korrapati A, Yu V, Singh P, Grandis JR, King CC, Lippman SM, Wang XQ, Hinton A, Ongkeko WM. The non-coding landscape of head and neck squamous cell carcinoma. Oncotarget 2016; 7: 51211-51222.
15) Maruyama R, Suzuki H. Long noncoding RNA involvement in cancer. BMB Rep 2012; 45: 604-611.
16) Peng L, Yuan X, Jiang B, Tang Z, Li GC. LncRNAs: key players and novel insights into cervical cancer. Tumour Biol 2015; 37: 2779-2788.
17) Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev 2011; 25: 1915-1927.
18) Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. Nat Rev Cancer 2018; 18: 5-18.
19) Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011; 10: 38.

20) Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 2013; 20: 300-307.

21) Nam JW, Choi SW, You BH. Incredible RNA: dual functions of coding and noncoding. Mol Cells 2016; 39: 367-374.

22) Gibb EA, Enfield KS, Stewart GL, Lonergan KM, Chari R, Ng RT, Zhang L, MacAulay CE, Rosin MP, Lam WL. Long non-coding RNAs are expressed in oral mucosa and altered in oral premalignant lesions. Oral Oncol 2011; 47: 1055-1061.

23) Conway C, Graham JL, Chengot P, Daly C, Chalkley R, Ross L, Droop A, Rabbitts P, Stead LF. Elucidating drivers of oral epithelial dysplasia formation and malignant transformation to cancer using RNA-seq. Oncotarget 2015; 6: 40186-40201.

24) Gao W, Chan JY, Wong TS. Long non-coding RNA deregulation in tongue squamous cell carcinoma. Biomed Res Int 2014; 2014: 405860.

25) Liao Q, Xiao H, Bu D, Xie C, Miao R, Luo H, Zhao G, Yu K, Zhao H, Skogerbo G, Chen R, Wu Z, Liu C, Zhao Y. ncFANs: a web server for functional annotation of long non-coding RNAs. Nucleic Acids Res 2011; 39: W118-124.

26) Jia LF, Wei SB, Gan YH, Guo Y, Gong K, Mitchelson K, Cheng J, Yu GY. Expression, regulation and roles of miR-26a and MEG3 in tongue squamous cell carcinoma. Int J Cancer 2014; 135: 2282-2293.

27) Yang M, Xiong X, Chen L, Yang L, Li X. Identification and validation long non-coding RNAs of oral squamous cell carcinoma by bioinformatics method. Oncotarget 2017; 8: 107469-107476.

28) Zhu G, Wang S, Chen J, Wang Z, Liang X, Wang X, Jiang J, Lang J, Li L. Long noncoding RNA HAS2-AS1 mediates hypoxia-induced invasiveness of oral squamous cell carcinoma. Mol Carcinog 2017; 56: 2210-2222.

29) Feng L, Houck JR, Lohavanichbutr P, Chen C. Transcriptome analysis reveals differentially expressed lncRNAs between oral squamous cell carcinoma and healthy oral mucosa. Oncotarget 2017; 8: 31521-31531.

30) Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013; 41: D991-995.

31) Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002; 30: 207-210.

32) Terai G, Iwakiri J, Kameda T, Hamada M, Asai K. Comprehensive prediction of lncRNA-RNA interactions in human transcriptome. BMC Genomics 2016; 17 Suppl 1: 12.

33) Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.

34) Zhao Z, Bai J, Wu A, Wang Y, Zhang J, Wang Z, Li Y, Xu J, Li X. Co-lncRNA: investigating the lncRNA combinatorial effects in GO annotations and KEGG pathways based on human RNA-Seq data. Database (Oxford) 2015; 2015. pii: bav082.

35) Li J, Han L, Roebuck P, Diao L, Liu L, Yuan Y, Weinstein J, Liang H. TANRIC: an interactive open platform to explore the function of lncRNAs in cancer. Cancer Res 2015; 75: 3728-3737.

36) Lu MY, Liao YW, Chen PY, Hsieh PL, Fang CY, Wu CY, Yen ML, Peng BY, Wang DP, Cheng HC, Wu CZ, Shih YH, Wang DJ, Yu CC, Tsai LL. Targeting LncRNA HOTAIR suppresses cancer stemness and metastasis in oral carcinomas stem cells through modulation of EMT. Oncotarget 2017; 8: 98542-98552.

37) Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.

38) Wu J, Xie H. Expression of long noncoding RNA-HOX transcript antisense intergenic RNA in oral squamous cell carcinoma and effect on cell growth. Tumour Biol 2015; 36: 8573-8578.

39) Wu Y, Zhang L, Zhang L, Wang Y, Li H, Ren X, Wei F, Yu W, Liu T, Wang X, Zhou X, Yu J, Hao X. Long non-coding RNA HOTAIR promotes tumor cell invasion and metastasis by recruiting EZH2 and repressing E-cadherin in oral squamous cell carcinoma. Int J Oncol 2015; 46: 2586-2594.

40) Yang YT, Wang YF, Lai JY, Shen SY, Wang F, Kong J, Zhang W, Yang HY. Long non-coding RNA UCA1 contributes to the progression of oral squamous cell carcinoma via regulating WNT/beta-catenin signaling pathway. Cancer Sci 2016; 107: 1581-1589.

41) Kong XP, Yao J, Luo W, Feng FK, Ma JT, Ren YP, Wang DL, Bu RF. The expression and functional role of a FOXC1 related mRNA-lncRNA pair in oral squamous cell carcinoma. Mol Cell Biochem 2014; 394: 177-186.

42) Arunkumar G, Deva Magendhra Rao AK, Manikandan M, Arun K, Vinothkumar V, Revathidevi S, Rajkumar KS, Rajaraman R, Munirajan AK. Expression profiling of long non-coding RNA identifies linc-RoR as a prognostic biomarker in oral cancer. Tumour Biol 2017; 39: 1010428317698366.

43) Fang Z, Zhao J, Xie W, Sun Q, Wang H, Qiao B. LncRNA UCA1 promotes proliferation and cisplatin resistance of oral squamous cell carcinoma by sunppressing miR-184 expression. Cancer Med 2017; 6: 2897-2908.

44) Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Kaczor T, Tu M, Wei F, Garcia-Godoy F, Wong DT. Emerging technologies for salivaomics in cancer detection. J Cell Mol Med 2017; 21: 640-647.

45) Gualtero DF, Suarez Castillo A. Biomarkers in saliva for the detection of oral squamous cell carci-

noma and their potential use for early diagnosis: a systematic review. Acta Odontol Scand 2016; 74: 170-177.

46) Tang H, Wu Z, Zhang J, Su B. Salivary lncRNA as a potential marker for oral squamous cell carcinoma diagnosis. Mol Med Rep 2013; 7: 761-766.

47) Fayda M, Isin M, Tambas M, Guveli M, Meral R, Altun M, Sahin D, Ozkan G, Sanli Y, Isin H, Ozgur E, Gezer U. Do circulating long non-coding RNAs (lncRNAs) (LincRNA-p21, GAS 5, HOTAIR) predict the treatment response in patients with head and neck cancer treated with chemoradiotherapy? Tumour Biol 2016; 37: 3969-3978.

48) Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. Sci Signal 2010; 3: ra8.

49) Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, Tempst P, Rosenfeld MG, Glass CK, Kurokawa R. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. Nature 2008; 454: 126-130.

50) Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129: 1311-1323.

51) Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, Willard HF. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 1991; 349: 38-44.

52) Li L, Gu M, You B, Shi S, Shan Y, Bao L, You Y. Long non-coding RNA ROR promotes proliferation, migration and chemoresistance of nasopharyngeal carcinoma. Cancer Sci 2016; 107: 1215-1222.

53) Gong Z, Zhang S, Zeng Z, Wu H, Yang Q, Xiong F, Shi L, Yang J, Zhang W, Zhou Y, Zeng Y, Li X, Xiang B, Peng S, Zhou M, Li X, Tan M, Li Y, Xiong W, Li G. LOC401317, a p53-regulated long non-coding RNA, inhibits cell proliferation and induces apoptosis in the nasopharyngeal carcinoma cell line HNE2. PLoS One 2014; 9: e110674.

54) Huang G, He X, Wei XL. LncRNA NEAT1 promotes cell proliferation and invasion by regulating miR365/RGS20 in oral squamous cell carcinoma. Oncol Rep 2018; 39: 1948-1956.

55) Wang P, Wu T, Zhou H, Jin Q, He G, Yu H, Xuan L, Wang X, Tian L, Sun Y, Liu M, Qu L. Long noncoding RNA NEAT1 promotes laryngeal squamous cell cancer through regulating miR-107/CDK6 pathway. J Exp Clin Cancer Res 2016; 35: 22.

56) Zhang L, Hu D, Zou L. Low expression of lncRNA MEG3 promotes the progression of oral squamous cell carcinoma by targeting miR-21. Eur Rev Med Pharmacol Sci 2018; 22: 8315-8323.

57) Li D, Feng J, Wu T, Wang Y, Sun Y, Ren J, Liu M. Long intergenic noncoding RNA HOTAIR is overexpressed and regulates PTEN methylation in laryngeal squamous cell carcinoma. Am J Pathol 2013; 182: 64-70.

58) Zheng J, Xiao X, Wu C, Huang J, Zhang Y, Xie M, Zhang M, Zhou L. The role of long non-coding RNA HOTAIR in the progression and development of laryngeal squamous cell carcinoma interacting with EZH2. Acta Otolaryngol 2016: 1-9.

59) Wu Y, Zhang L, Wang Y, Li H, Ren X, Wei F, Yu W, Wang X, Zhang L, Yu J, Hao X. Long noncoding RNA HOTAIR involvement in cancer. Tumour Biol 2014; 35: 9531-9538.

60) Kim HJ, Lee DW, Yim GW, Nam EJ, Kim S, Kim SW, Kim YT. Long non-coding RNA HOTAIR is associated with human cervical cancer progression. Int J Oncol 2015; 46: 521-530.

61) Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, Lawrence JB. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell 2009; 33: 717-726.

62) Gao J, Cao R, Mu H. Long non-coding RNA UCA1 may be a novel diagnostic and predictive biomarker in plasma for early gastric cancer. Int J Clin Exp Pathol 2015; 8: 12936-12942.

63) Nie W, Ge HJ, Yang XQ, Sun X, Huang H, Tao X, Chen WS, Li B. LncRNA-UCA1 exerts oncogenic functions in non-small cell lung cancer by targeting miR-193a-3p. Cancer Lett 2016; 371: 99-106.

64) Zhou J, Yang L, Zhong T, Mueller M, Men Y, Zhang N, Xie J, Giang K, Chung H, Sun X, Lu L, Carmichael GG, Taylor HS, Huang Y. H19 lncRNA alters DNA methylation genome wide by regulating S-adenosylhomocysteine hydrolase. Nat Commun 2015; 6: 10221.

65) Yang K, Hou Y, Li A, Li Z, Wang W, Xie H, Rong Z, Lou G, Li K. Identification of a six-lncRNA signature associated with recurrence of ovarian cancer. Sci Rep 2017; 7: 752.

66) Hong Y, He H, Sui W, Zhang J, Zhang S, Yang D. Long non-coding RNA H1 promotes cell proliferation and invasion by acting as a ceRNA of miR138 and releasing EZH2 in oral squamous cell carcinoma. Int J Oncol 2018; 52: 901-912.

67) Guo QY, Wang H, Wang Y. LncRNA H19 polymorphisms associated with the risk of OSCC in Chinese population. Eur Rev Med Pharmacol Sci 2017; 21: 3770-3774.

68) Song W, Sun Y, Lin J, Bi X. Current research on head and neck cancer-associated long noncoding RNAs. Oncotarget 2018; 9: 1403-1425.

69) Fang Z, Zhang S, Wang Y, Shen S, Wang F, Hao Y, Li Y, Zhang B, Zhou Y, Yang H. Long non-coding RNA MALAT-1 modulates metastatic potential of tongue squamous cell carcinomas partially through the regulation of small proline rich proteins. BMC Cancer 2016; 16: 706.

70) Zhou X, Liu S, Cai G, Kong L, Zhang T, Ren Y, Wu Y, Mei M, Zhang L, Wang X. Long non coding RNA MALAT1 promotes tumor growth and metastasis

by inducing epithelial-mesenchymal transition in oral squamous cell carcinoma. Sci Rep 2015; 5: 15972.

71) Liu Z, Wu C, Xie N, Wang P. Long non-coding RNA MEG3 inhibits the proliferation and metastasis of oral squamous cell carcinoma by regulating the WNT/beta-catenin signaling pathway. Oncol Lett 2017; 14: 4053-4058.

72) Fang Z, Wu L, Wang L, Yang Y, Meng Y, Yang H. Increased expression of the long non-coding RNA UCA1 in tongue squamous cell carcinomas: a possible correlation with cancer metastasis. Oral Surg Oral Med Oral Pathol Oral Radiol 2014; 117: 89-95.

73) Nishiyama K, Maruyama R, Niinuma T, Kai M, Kitajima H, Toyota M, Hatanaka Y, Igarashi T, Kobayashi JI, Ogi K, Dehari H, Miyazaki A, Yorozu A, Yamamoto E, Idogawa M. Screening for long noncoding RNAs associated with oral squamous cell carcinoma reveals the potentially oncogenic actions of DLEU1. Cell Death Dis 2018; 9: 826.

74) Wang Y, Zhang X, Wang Z, Hu Q, Wu J, Li Y, Ren X, Wu T, Tao X, Chen X, Li X, Xia J, Cheng B. LncRNA-p23154 promotes the invasion-metastasis potential of oral squamous cell carcinoma by regulating Glut1-mediated glycolysis. Cancer Lett 2018; 434: 172-183.

75) Zhang S, Ma H, Zhang D, Xie S, Wang W, Li Q, Lin Z, Wang Y. LncRNA KCNQ1OT1 regulates proliferation and cisplatin resistance in tongue cancer via miR-211-5p mediated Ezrin/Fak/Src signaling. Cell Death Dis 2018; 9: 742.

76) Shao T, Huang J, Zheng Z, Wu Q, Liu T, Lv X. SCCA, TSGF, and the long non-coding RNA AC007271.3 are effective biomarkers for diagnosing oral squamous cell carcinoma. Cell Physiol Biochem 2018; 47: 26-38.

77) Lin Z, Sun L, Xie S, Zhang S, Fan S, Li Q, Chen W, Pan G, Wang W, Weng B, Zhang Z, Liu B, Li J. Chemotherapy-induced long non-coding RNA 1 promotes metastasis and chemo-resistance of TSCC via the Wnt/beta-Catenin signaling pathway. Mol Ther 2018; 26: 1494-1508.

78) Liu M, Gao X, Liu C. Increased expression of lncRNA FTH1P3 promotes oral squamous cell carcinoma cells migration and invasion by enhancing PI3K/Akt/GSK3b/Wnt/β-catenin signaling. Eur Rev Med Pharmacol Sci 2018; 22: 8306-8314.

79) Chai L, Yuan Y, Chen C, Zhou J, Wu Y. The role of long non-coding RNA ANRIL in the carcinogenesis of oral cancer by targeting miR-125a. Biomed Pharmacother 2018; 103: 38-45.

80) Ding J, Yang C, Yang S. LINC00511 interacts with miR-765 and modulates tongue squamous cell carcinoma progression by targeting LAMC2. J Oral Pathol Med 2018; 47: 468-476.

81) Wang ZY, Hu M, Dai MH, Xiong J, Zhang S, Wu HJ, Zhang SS, Gong ZJ. Upregulation of the long non-coding RNA AFAP1-AS1 affects the proliferation, invasion and survival of tongue squamous cell carcinoma via the Wnt/β-catenin signaling pathway. Mol Cancer 2018; 17: 3.

82) Guo Y, Ma Y, Hu X, Song R, Zhu L, Zhong M. Long non-coding RNA CEBPA-AS1 correlates with poor prognosis and promotes tumorigenesis via CEBPA/Bcl2 in oral squamous cell carcinoma. Cancer Biol Ther 2018; 19: 205-213.

83) Sun CC, Zhang L, Li G, Li SJ, Chen ZL, Fu YF, Gong FY, Bai T, Zhang DY, Wu QM, Li DJ. The lncRNA PDIA3P interacts with miR-185-5p to modulate oral squamous cell carcinoma progression by targeting cyclin D2. Mol Ther Nucleic Acids 2017; 9: 100-110.

84) Li X, Ma C, Zhang L, Li N, Zhang X, He J, He R, Shao M, Wang J, Kang L, Han C. LncRNAAC132217.4, a KLF8-regulated long non-coding RNA, facilitates oral squamous cell carcinoma metastasis by up-regulating IGF2 expression. Cancer Lett 2017; 407: 45-56.

85) Ma Y, Hu X, Shang C, Zhong M, Guo Y. Silencing of long non-coding RNA CCAT2 depressed malignancy of oral squamous cell carcinoma via Wnt/beta-catenin pathway. Tumour Biol 2017; 39: 1010428317717670.

86) Shih JW, Chiang WF, Wu ATH, Wu MH, Wang LY, Yu YL, Hung YW, Wang WC, Chu CY, Hung CL, Changou CA, Yen Y, Kung HJ. Long noncoding RNA LncHIFCAR/MIR31HG is a HIF-1α co-activator driving oral cancer progression. Nat Commun 2017; 8: 15874.

87) Li ZQ, Zou R, Ouyang KX, Ai WJ. An in vitro study of the long non-coding RNA TUG1 in tongue squamous cell carcinoma. J Oral Pathol Med 2017; 46: 956-960.

88) Zhang CZ. Long intergenic non-coding RNA 668 regulates VEGFA signaling through inhibition of miR-297 in oral squamous cell carcinoma. Biochem Biophys Res Commun 2017; 489: 404-412.

89) Wang J, Huo F, Wang X, Xu Y. LncRNA LACAT1 promotes proliferation of oral squamous cell carcinoma cells by inhibiting microRNA-4301. Eur Rev Med Pharmacol Sci 2019; 23: 2427-2435.