The emerging role of long noncoding RNAs in oral cancer

Carolina Cavalieri Gomes, PhD,§ Silvia Ferreira de Sousa, PhD,¶ George Adrian Calin, PhD,# and Ricardo Santiago Gomez, PhD

Although less than 3% of the genome encodes proteins, at least 75% of the genome is transcribed into RNAs with no protein-coding potential (noncoding RNAs [ncRNAs]). On the basis of their size and the arbitrary 200 nucleotides cutoff, ncRNAs are classified into long ncRNAs (lncRNAs) or small ncRNAs (including microRNAs). Over the last few years, the role of microRNAs in oral squamous cells carcinoma (OSCC) has been extensively addressed, but the possible role of lncRNAs in OSCC remains unclear. We aimed to explore and discuss the potential role of lncRNAs in OSCC. The detection of lncRNAs in saliva holds promise not only as a noninvasive diagnostic tool in OSCC but also in the early detection of oral cancer recurrence. lncRNAs are promising future therapeutic targets in the OSCC scenario, and research in this field may expand greatly in the next decade. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;123:235-241)

Cancer is one of the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8 million cancer-related deaths reported in 2012.1 The estimated age-standardized incidence and mortality rates for oral and lip cancers worldwide in 2012 were 5.5 and 2.7 per 100,000 men and 2.5 and 1.2 per 100,000 women, respectively.1 The most important etiologic factors associated with the development of all head and neck cancers are the use of tobacco and alcohol, which accounts for about 65% of oral cavity cancers globally.2 Thus, cancer initiation and progression is thought to result from the interaction of environmental factors with genetic alterations. Recently, The Cancer Genome Atlas has reported the results of a comprehensive multiplatform genomic analysis of 279 patients with head and neck cancer, including 172 oral cavity cancers,3 revealing important somatic mutations and copy number alterations in such tumors.

Although it is now clear that the genome does not exist or operate in isolation, cancer is mistakenly considered a disease of the genome. The Human Genome Project was completed in 2003 (https://www.genome.gov/10001772); however, with understanding of the genome, new questions have arisen.4 In cancer, the genome and the epigenome operate together, with epigenomic organization affecting the genomic location of the mutations that provoke cancer.5

Interestingly, whereas about only 2% of the genome encodes proteins, at least 75% is actively transcribed into RNAs with no protein coding potential.6,7 As these RNAs lack the potential to code proteins, they are referred to as noncoding RNAs (ncRNAs). On the basis of their size and the arbitrary 200 nucleotides cutoff, ncRNAs are classified either into long ncRNAs (lncRNAs) or small ncRNAs (including microRNAs [miRNA]). Over the last few years, the role of miRNA in oral squamous cells carcinomas (OSCC) has been extensively addressed,8-10 but the possible role of lncRNAs in such tumors remains unclear. On this basis, in this revision, we aim to explore and discuss the potential role of lncRNAs in OSCC.

CORE CHARACTERISTICS OF lncRNAs

LncRNAs are ncRNAs that surpass 200 nucleotides in length. They belong to evolutionary conserved gene families.11 According to the most recent estimate by the GENCODE12 (version 24, December 2015), the human genome contains about 16,000 lncRNA genes, encoding 28,000 lncRNA transcripts. More lncRNA genes are expected to be discovered in the near future, as a small part of the human genome remains to be annotated.

Although the largest catalog of human lncRNAs has been established by the GENCODE annotation project,12 uniform nomenclature for lncRNAs and small ncRNAs was introduced in 2011 by the HUGO Gene Nomenclature Committee.13 LncRNAs have

Statement of Clinical Relevance

Long noncoding RNAs are noncoding RNAs with greater than 200 nucleotides that regulate gene expression in cancer and hold promise as future cancer therapeutic targets. Long noncoding RNAs in oral cancer has been poorly addressed.
Biologic Functions and Molecular Mechanisms of IncRNAs in Human Cancer

As a result of the central dogma of molecular biology, which believes that the genetic information flow moves from DNA to RNA to protein, not much attention has been given to the noncoding regions of the genome. These regions of the genome that do not code proteins were previously considered “junk” and were initially considered to be transcriptional noise, rather than exhibiting any functionality. Over the last few years, studies of ncRNA focused mainly on the roles of miRNAs in human disease. Beside being the most common RNA species, IncRNAs are one of the most poorly understood RNAs. Although there are about 16,000 IncRNA genes in the human genome, there are less than 10,000 small ncRNA genes. Because of their abundant existence in the human genome, as well as their tissue-specific expression patterns, IncRNAs started to gain attention, and their functional relevance in health and in disease, including cancer, is starting to be clarified.

As IncRNAs are greater than 200 nucleotides in length, they can fold into more complex three-dimensional structures, distinct from miRNAs. These three-dimensional structures are likely to determine the specific interactions of IncRNAs with transcription factors and histones as well as with other chromatin-modifying proteins, which, in turn, can affect the expression level of a broad spectrum of genes. In addition, IncRNAs can interact with DNA or RNA in a sequence-specific manner, forming duplex of triplex structures. Interestingly, although a large number of IncRNAs regulate transcription via repression, an activating function has been increasingly reported for several IncRNAs.

IncRNAs can act in a cis mode of regulation, that is, when neighboring genes and IncRNA are located on the same chromosomal regions. Conversely, IncRNAs can affect the expression of genes located on different chromosomes, acting in a trans regulation manner.

Although there is great variability in IncRNAs’ functions, these functions can be grouped as shown in Figure 1 and have been elegantly addressed by Prensner and Chinnaian and Kunej et al.

IncRNAs have important roles in gene regulation and can affect cell proliferation, survival, migration, or genomic stability; thus, they are differentially expressed in tumors. The highly aberrant IncRNA expression in human cancers was reported in the first-generation atlas for IncRNA profiling in cancer in 2011, including 19 different human cancers. Dysregulation in IncRNA expression is implicated in cancer progression and has been reported as an independent predictor of patient outcome. LncRNA deregulation can play a pivotal role in cancer initiation, progression, and metastasis.

On the basis of the effect of a given IncRNA on cancer phenotype, it can exert either tumor suppressor or oncogenic function. The tumor suppressor or oncogenic IncRNA function cannot be determined merely by the differential expression of IncRNAs in cancer but also depends on in silico predictions and functional studies. HOTAIR (Hox antisense intergenic RNA) and GAS5 (growth arrest-specific transcript 5) are well-characterized oncogenic and tumor suppressor IncRNAs, respectively. HOTAIR promotes metastasis, being overexpressed in different human cancers. In contrast, GAS5 induces cell arrest, being downregulated in breast cancer.

IncRNAs Expression Profiles in OSCC and Oral Dysplasia

IncRNAs have just begun to emerge as important molecules in oral carcinogenesis. Differential IncRNA expression comparing matched normal oral mucosa, oral dysplasia, and OSCC demonstrated by whole transcriptome analyses, such as Serial Analysis of Gene Expression, RNA sequencing, and microarray, have revealed several oral cancer—associated IncRNAs. Some of these results of expression analyses have been associated with in silico and functional studies to provide a deep understanding of the role of IncRNAs in oral carcinogenesis.

Differential IncRNA expression between OSCC and noncancerous oral mucosa has been evaluated by different authors. Table I provides a list of the published papers that have evaluated the IncRNA expressions in OSCC, as well as the main result of each publication regarding these RNA expressions. The publications in which OSCC were evaluated in the head and neck cancer group with no separate results by tumor site are not listed in the table.

As OSCC may be preceded by oral leukoplakia, the understanding of IncRNAs role in oral dysplasia is
equally important. Gibb et al.\textsuperscript{37} analyzed the Serial Analysis of Gene Expression libraries constructed from normal oral mucosa and oral dysplasia and provided a primary IncRNA expression profile for the oral mucosa. These authors identified 325 IncRNAs expressed in human oral tissue, 298 being novel and uncharacterized. From 325 IncRNAs expressed in the human oral mucosa, 164 were differently expressed between the normal mucosa group and the dysplastic mucosa group, with a group of IncRNAs strictly expressed in oral dysplasia and others expressed strictly in the normal oral mucosa.\textsuperscript{37} None of these differentially expressed transcripts in dysplasias or in normal mucosa is a well-characterized IncRNA. In the paper published by Han et al.,\textsuperscript{39} 108 IncRNAs were differentially expressed in normal oral mucosa versus oral dysplasia, 87 being upregulated and 21 downregulated.\textsuperscript{39}

Recently, RNA sequencing was performed in 19 trios of normal oral mucosa, dysplasia, and associated OSCC.\textsuperscript{38} By principal component analysis, these authors showed that expressed IncRNAs genes demonstrated a clear separation into two groups—normal and tumor samples—which contrasts with the negative results with coding genes, suggesting that noncoding are new actors in the oral carcinogenesis process.\textsuperscript{38} HOTAIRM1, an antisense noncoding to \textit{HOX} genes, was differentially expressed in the normal mucosa versus oral dysplasia, and several antisense \textit{HOX} transcripts had significant aberrant expression in normal tissue versus tumor, pointing to \textit{HOX} genes as potential drivers of OSCC progression.\textsuperscript{38}

**IncRNAs ASSOCIATED WITH CLINICOPATHOLOGIC PARAMETERS IN OSCC**

Some studies have addressed the association of IncRNA expression levels with OSCC clinicopathologic parameters, such as overall survival time and presence
of metastatic disease. We revised the main associations as follows:

Higher expression levels of HOTAIR, NEAT1, and UCA1 were reported in metastatic OSCC compared with nonmetastatic OSCC. In addition, HOTAIR expression significantly correlated with tumor size greater than 0.9 cm and with lower cumulative survival, and UCA1 showed elevated expression in cases with lymph node metastasis and demonstrated correlation with TNM stage, strengthening the potential role of lncRNA as a promoter of cell invasion.

Low expression of MEG3 was considered a prognostic factor for poor clinical outcome in patients with tongue squamous cell carcinoma. MALAT1 expression correlated with positive nodal status and was increased in cases with poor overall survival, corroborating the possible role of this oncogenic lncRNA in the OSCC metastatic process, as reported in other human cancers. High tumor expression levels of IncMBL2-4:3 were associated with lymph node metastasis in patients with TSCC, and the reduction of IncAL355149.1-1 was associated with advanced T stage.

Altered expression of lncRNAs and their association with clinicopathologic factors, as reported above, enhance their use as biomarkers for diagnosis and prognosis. However, these results need to be further confirmed by multicenter studies with a larger cohort of samples.

### Table 1. List of the published papers that have evaluated the long noncoding RNA (lncRNA) expressions in oral cancer

<table>
<thead>
<tr>
<th>Study [Ref]</th>
<th>Cancer samples</th>
<th>Main finding</th>
<th>Method</th>
<th>Results validation or functional study*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al.,43 2016</td>
<td>TSCC</td>
<td>UCA1 upregulation in TSCC</td>
<td>qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Liang et al.,44 2016</td>
<td>TSCC</td>
<td>MALAT1 upregulation in TSCC</td>
<td>qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Zhang et al.,45 2015</td>
<td>TSCC</td>
<td>HOTTIP upregulation in TSCC</td>
<td>qPCR</td>
<td>No</td>
</tr>
<tr>
<td>Zhang et al.,41 2015</td>
<td>OSCC</td>
<td>41 upregulated and 119 downregulated lncRNAs in OSCC</td>
<td>Microarray (GSE2509)/Bioinformatics approaches</td>
<td>No</td>
</tr>
<tr>
<td>Zhou et al.,46 2015</td>
<td>OSCC</td>
<td>MALAT1 upregulation in OSCC</td>
<td>qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Zou et al.,47 2015</td>
<td>OSCC and TSCC</td>
<td>HOTAIR upregulation and MEG-3 and GAS5 downregulation in OSCC. Downregulation of GAS5 in TSCC</td>
<td>RNA sequencing</td>
<td>Yes</td>
</tr>
<tr>
<td>Wu et al.,48 2015</td>
<td>OSCC</td>
<td>HOTAIR upregulation in OSCC</td>
<td>qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Wu and Xie.,49 2015</td>
<td>OSCC</td>
<td>HOTAIR upregulation in OSCC</td>
<td>qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Fang et al.,50 2014</td>
<td>TSCC</td>
<td>UCA1 upregulation in TSCC</td>
<td>qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Gao et al.,46 2014</td>
<td>TSCC</td>
<td>Uptregulation of Inc-SPRR2 D-1, Inc-PPP2 R4-5, Inc-MBL2-4:3 in TSCC; Downregulation of Inc-AL355149.1-1</td>
<td>Microarray (GSE 9844) and functional reannotation</td>
<td>Yes</td>
</tr>
<tr>
<td>Jia et al.,42 2014</td>
<td>TSCC</td>
<td>MEG3 downregulation in TSCC</td>
<td>Microarray (GSE 51700) and qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Kong et al.,51 2014</td>
<td>OSCC</td>
<td>FOXCUT upregulation in OSCC</td>
<td>qPCR</td>
<td>Yes</td>
</tr>
<tr>
<td>Tang et al.,52 2013</td>
<td>OSCC</td>
<td>MALAT-1 and HOTAIR were detected at OSCC patient saliva</td>
<td>qPCR</td>
<td>No</td>
</tr>
</tbody>
</table>

TSCC, oral squamous cells carcinoma; qPCR, quantitative polymerase chain reaction; OSCC, tongue squamous cells carcinoma.

*In some of these papers, the authors associated lncRNAs expressions with clinical and prognostic parameters, but these data were not included in this table.
box C1 upstream transcript) by RNAi approaches in OSCC cells reduced the progression of the tumor in vitro and in vivo. Knockdown of these lncRNAs resulted in less proliferation, activation of cell apoptosis, and suppression of invasion and migration of OSCC cells. The suppression of invasion and migration of such cells occurred either by modulation of matrix metalloproteinase levels, which attenuates the epithelial–mesenchymal transition, suppresses vimentin expression, and induces E-cadherin, or by modulation of the WNT/β-catenin signaling pathway. Overexpression of UCA1 in human TSCC cell lines has also been shown to modulate the migration ability of cells, although with little effect in cell proliferation. In another study, changes in lncRNAs levels were observed in TSCC cell lines after treating the cells with cisplatin, indicating a possible link between lncRNAs expression and the development of resistance to chemotherapeutic drugs. Taken together, these in vitro findings strengthen the action of lncRNAs as candidates in OSCC tumorigenesis and provide emerging insights into lncRNA functions in oral cancer.

**POTENTIAL ROLE OF CIRCULATING AND SALIVARY lncRNAs AS OSCC BIOMARKERS**

The utility of circulating and salivary lncRNAs as potential biomarkers has gained interest in head and neck and oral cancers. Plasma levels of HOTAIR and other two lncRNAs (lincRNA-p21 and GAS5) were measured by quantitative polymerase chain reaction and associated with the treatment response of 41 patients with head and neck cancer who underwent radical chemoradiotherapy. The authors found higher expression of GAS5 in the patients with progressive disease compared with those with good clinical response. GAS5 is implicated in cell apoptosis, and the impact of such lncRNAs in the discrimination of patients with differential treatment responses may be further explored in patients with OSCC. Blood and saliva may provide novel insights into the establishment of new protocols for the detection and follow-up of patients with OSCC.

**FUTURE PERSPECTIVES OF lncRNAs IN OSCC RESEARCH AND THERAPY**

lncRNA research in OSCC is still incipient. Despite some promising results, the evidence of lncRNA expression and functional mechanisms in oral cancer remains very limited. lncRNAs hold promise as cancer biomarkers, promoting tumor formation, progression, and metastasis of prostate, bladder, and kidney cancers, among other cancer types. These molecules are promising candidates involved in the progression of oral potentially malignant lesions, and studies in this research field are just beginning. This is especially relevant because there is no reliable marker that distinguishes nonprogressive oral leukoplakia lesions from those that are prone to suffer malignant transformation.

The detection of PCA3 (prostate cancer associated 3) transcript in urine is already being used as a diagnostic assay for prostate cancer. As lncRNAs are identified in body fluids and have a site-specific expression profile, saliva can be useful for the early diagnosis of OSCC, salivary gland tumors, or metastatic diseases. It is also possible to use salivary lncRNAs’ specific expressions to monitor cancer recurrence. An important advantage of saliva is the noninvasive collection procedure.

Inhibition or restoration of lncRNAs that regulate cancer cell survival in a site-specific manner is a promising therapeutical approach. The upregulated and downregulated lncRNA profiles in oral cancer should be established, but the differentially expressed lncRNAs need to be functionally evaluated in the context of the cells investigated.

lncRNAs can be therapeutically targeted by different approaches to try to reestablish their homeostatic levels. Among the methods that aim to inhibit the upregulated oncogenic lncRNAs, the most explored is the delivery of small interfering RNAs, which are complementary to their target lncRNA. Other approaches include the use of other RNAi molecules, such as short hairpin RNAs and miRNAs, as well as small molecule inhibitors that act by preventing the interactions of lncRNAs with their protein partners, blocking the binding site of interaction or changing the secondary structure of lncRNAs. Silencing of MALAT1 in TSCC cells, lung adenocarcinoma cells, and cervical cancer cells by using short hairpin RNA reduced the migration and invasion abilities of the cells. In an in vitro colon cancer study, MYC-regulated miR-17-5 p and miR-20 a were shown to participate in the CCAT2-enhanced cell invasion. lncRNAs can also be degraded by the use of longer antisense oligonucleotides complementary to the target lncRNAs. These longer antisense nucleotides promote the lncRNA degradation by RNase H. This specific knockdown of potential oncogenic lncRNAs is being employed to inhibit their actions by silencing MALAT1, as demonstrated in human lung cancer cells in a mouse xenograft model. In contrast to the strategies to downregulate overexpressed lncRNAs, downregulated lncRNA expression can be induced by gene therapy strategies.

Despite the existence of several strategies to reestablish the homeostatic levels of lncRNAs, their in vivo delivery is still a challenge. As the studies
of lncRNAs in oral cancer have just started to emerge, the identification of key lncRNAs suitable for targeting in oral cancer is the first challenge to be overcome.

CONCLUSIONS

lncRNAs are more abundant than miRNAs, exhibiting a greater variety of functions in the regulation of gene expression. This abundance, along with the varied repertoire of functions and a site-specific expression pattern, makes lncRNA promising as future therapeutic targets in OSCC. lncRNA research in oral cancer is expected to undergo a vast expansion in the next decade.

R.S. Gomez, C.C. Gomes, and S.F. de Sousa are research fellows at the National Council for Scientific and Technological Development (CNPq), Brazil.

REFERENCES


