Differential expression and prognostic value of long non-coding RNA in HPV-negative head and neck squamous cell carcinoma

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ABSTRACT

BACKGROUND: Long non-coding RNA (lncRNA) has emerged as a new avenue of interest due to its various biological functions in cancer. Abnormal expression of lncRNA has been reported in other malignancies but has been understudied in head and neck squamous cell cancer (HNSCC).

METHODS: lncRNA expression was interrogated via qRT-PCR array for 19 HPV-negative HNSCC tumor-normal pairs. The Cancer Genome Atlas (TCGA) was used to validate these results. The association between differentially expressed lncRNA and survival outcomes was analyzed.

RESULTS: Differential expression was validated for 5 lncRNA (*SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*). There was also an inverse association between *MEG3* expression (not significantly differentially expressed in TCGA tumors but highly variable expression) and 3-year relapse-free survival (RFS).

CONCLUSION: We identified and validated differential expression of 5 lncRNA in HPV-negative HNSCC. Low *MEG3* expression was associated with favorable 3-year RFS, although the significance of this finding remains unclear.

Key words:
Head and neck cancer; HNSCC; lncRNA; ncRNA; TCGA; survival

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer type worldwide and accounts for approximately 350,000 deaths per year [1, 2]. Risk factors such as tobacco, alcohol use, and more recently, human papilloma virus (HPV) have been identified as etiological factors in the occurrence of HNSCC. Despite advances in the treatment of localized HNSCC, approximately half of patients will develop recurrent disease [3], which is a major contributor to patient mortality. In addition, HPV-positive and HPV-negative HNSCC are biologically distinct with the latter being associated with poorer prognosis. Unlike other malignancies, there are no tools in widespread use that can identify early disease and there is no systematic approach that has proven effective in monitoring for early evidence of recurrence. Hence, novel markers are needed that appropriately characterize those patients with early stage disease as well as identify and characterize the response of individual patient's treatment.

While only 1.2% of our DNA sequence encodes proteins, approximately 75% of the human genome is capable of being transcribed into RNA [4], and it has become increasingly apparent that RNA plays a diverse and important role in genome integrity through production of both proteins as well as non-coding RNA (ncRNA), including long non-coding RNA (lncRNA). While the microRNA (miRNA) class of ncRNA have been widely studied in the context of cancer, lncRNA, which are larger (> 200 bases and ~1-2 exons in length) [5] and have a more complex secondary/tertiary structure, have recently begun to garner increased attention [6]. This is largely due to their diverse biological functions [7], which can include inhibition of target gene transcription, initiation of alternative splicing, generation of protein scaffolding and chromatin organization, and alteration of transcription factor activity.

Even though 18,000 human long non-coding human transcripts have been catalogued in GENCODE v7 [4], the lncRNA Database (http://www.lncrnadb.org), a database of functionally annotated eukaryotic lncRNA, only contains information for 127 human lncRNA, highlighting the gap in our knowledge of lncRNA biology. Identification of lncRNA transcripts that are associated with human diseases and the corresponding pathobiology (e.g. aggressiveness or responsiveness to treatment) would therefore provide a welcome means for prioritizing functional studies.

Several lncRNA have recently been reported to be differentially expressed in various cancers and to play a role in cancer growth, invasion, epithelial-to-mesenchymal transition, motility, and metastatic potential [8, 9], and as such are increasingly being recognized as having a very strong potential as cancer biomarkers [10]. Several recent studies have reported that higher expression of lncRNA, such as *HOTAIR (HOX antisense intergenic RNA)* and *Malat-1 (metastasis associated lung adenocarcinoma transcript 1)*, is associated with invasion and metastasis in various epithelial cancers [11-17], although the prognostic significance of lncRNA in HNSCC has been understudied to date. The aim of this study was to identify differentially expressed lncRNA in HPV-negative HNSCC and assess its impact on outcomes in these groups of patients.

MATERIALS & METHODS

Tumor Samples

Initial identification of differentially expressed lncRNA in HPV-negative HNSCC was conducted using a quantitative real-time PCR (qRT-PCR) array consisting of 84 lncRNA with reported involvement in various cancers using paired archival formalin-fixed paraffin-embedded (FFPE) tumor tissue and paired adjacent normal squamous tissue from 19 patients treated for incident HNSCC at the University of Cincinnati Cancer Institute. Raw RNA-sequencing (RNA-seq) reads for all available HPV-negative head and neck cancer tumors (n = 444) and all available adjacent normal tissue (n = 44) was downloaded from The Cancer Genome Atlas (TCGA) for independent validation of significant results.

RT2 lncRNA qPCR Cancer Array

Total RNA was extracted from each sample using the RNeasy FFPE kit (Qiagen, *Valencia, CA*), and converted to cDNA using the RT2 First Strand kit (Qiagen), according to the manufacturer's respective suggested protocols. The RT2 Profiler lncRNA qPCR Cancer PathwayFinderArray (96-well format) was used to profile expression of 84 lncRNA transcripts that have been associated with various cancer types. The array also contains 3 reverse transcription controls, 3 positive PCR controls, a probe for human gDNA contamination, and 5 housekeeping genes for normalization: *beta actin (ACTB)*; *beta-2 microglobulin (B2M)*; *Ribosomal protein, large, P0 (RPLP0)*; *7SK small nuclear RNA (RN7SK)*; and *Small nucleolar RNA, H/ACA box 73A (SNORA73A)*. PCR mastermix was prepared with 250ng of total cDNA and dispensed in 24µl aliquots into each RT2 lncRNA PCR Array well. RT-qPCR was performed using an Applied Biosystems StepOnePlus™ Real-Time PCR System (ThermoFisher Scientific, *Waltham, MA*)

under the following conditions: 10 min at 95°C using HotStart Taq Polymerase followed by 40 cycles of 15s at 95°C and 1min at 60°C. A complete list of the 84 lncRNA included on the array can be found in **Supplemental Table S1**.

Differential Expression

Expression was normalized to the geometric mean of 5 housekeeping control genes: *beta actin (ACTB)*; *beta-2 microglobulin (B2M)*; *Ribosomal protein, large, P0 (RPLP0)*; *7SK small nuclear RNA (RN7SK)*; and *Small nucleolar RNA, H/ACA box 73A (SNORA73A)*. Differential expression of each lncRNA was described in terms of fold-change for tumor relative to adjacent normal tissue based on a one-sample t-test, adjusted for multiple testing by false discovery rate (FDR) estimation and *Q* values using the methods proposed by Benjamini & Hochberg (53). Expression was considered significantly differential where $Q \leq 0.10$.

Replication Using TCGA RNA-Sequencing Data

RNA-sequencing (RNA-seq) data in the form of bam files of aligned reads was obtained for head and neck cancers in TCGA project where downloaded from the Cancer Genomics Hub. Reads not mapped to human genome where aligned to HPV (NC_001526) E6 and E7 viral oncoproteins using *bowtie* aligner [18]. HPV-status was inferred by designating the sample to be from a HPV-positive tumor if more than 1,000 reads mapped to HPV oncoproteins and HPV-negative tumor otherwise. The harmonized read counts for head and neck TCGA samples aligned to lncRNA defined in the GDC.h38 GENCODE v22 GTF file were downloaded from NCI Genomic Data Commons [19] using the *TCGAbiolinks* Bioconductor package [20].

Normalized counts (count per million) for each of the 7 lncRNA in each sample were calculated using the *cpm* function in the Bioconductor package *edgeR* [21]. Since the distributions of lncRNA expression were non-linear, differential expression was assessed non-parametrically, using the Wilcoxon rank sum test, comparing tumor expression (n = 444) to that of all available normal samples (n = 44), and was considered differential where $p \leq 0.05$. Median fold-change was determined for each tumor by comparing its expression to the median expression of the normal samples. A description of the distribution of expression values for the 7 lncRNA in the 44 adjacent normal samples is provided in **Supplemental Table S2**.

Survival Analysis Using TCGA Samples

To visualize 5-year overall survival (OS) and 3-year relapse-free survival (RFS), univariate Kaplan-Meier and cumulative incidence (to account for death as a competing risk [22, 23]) functions were generated, respectively, comparing curves for low, normal, and high expression levels. Discrete multivariable Cox proportional hazards and cumulative incidence models were fit to assess 5-year OS and 3-year RFS, respectively, for each of the significant lncRNA, adjusted for age, sex, race, smoking status, tumor site, and stage at diagnosis, as established *a priori*. Missing values of model covariates were imputed (m = 20) using multivariate normal regression, based on age, sex, stage, and primary tumor site. Log-log plots [i.e. -log(-log (S(t))) vs. log(t)] were generated for each model to verify that the proportional hazards assumption was met. Statistical analyses were conducted using Stata 13 (Stata Corp, *College Station, TX*). All statistical tests were two-sided, and significance was considered when the unadjusted $p \leq 0.05$.

RESULTS

The median age for the UC cases (n = 19 tumor-normal pairs) was 64 years, 74% of which were male. UC and TCGA sets differed in terms of smoking status (p = 0.01), with fewer non-smokers in the UC set, but were comparable in terms of age, sex, race, primary tumor site, AJCC stage group, and tumor grade (**Table 1**). The majority of tumors in both sets originated in the oral cavity (63% and 66%, respectively), and presented at an advanced stage (III or IV).

Eleven of the lncRNA included on the array were not detected in any of the tumor or normal samples (**Supplemental Table S1**). Twenty lncRNA were significantly differentially expressed at a nominal p-value $\leq$ 0.05 (8 upregulated, 12 downregulated). After FDR adjustment, 7 lncRNA remained significantly differential (Q $\leq$ 0.10: *SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*, *MEG3* and *TERC*; 4 upregulated, 3 downregulated; **Table 2**). Expression of these 7 lncRNA was also significantly differential for 5 of the 7 lncRNA in the TCGA validation set (*SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*). It should be noted, however, that although *MEG3* and *TERC* were not significantly differentially expressed in the TCGA validation set, there was wide variability in terms of expression in both directions (i.e. upregulation and downregulation) for individual tumors relative adjacent normal samples (**Figure 1B**). Interestingly, Black patients in the TCGA dataset were significantly more likely to exhibit high (> 2-fold) and low (< 0.5-fold) *MEG3* expression (**Table 3**).

The relationship between each of the 7 lncRNA and 5-year OS and 3-year RFS was assessed using the TCGA dataset. Amongst the 7 lncRNA identified, only differing levels of *Maternally Expressed 3* (*MEG3*) had an impact on 3 year RFS. Patients with low *MEG3* expression (<0.5 fold change) were found to have a significantly lower 3-year RFS while higher *MEG3* expression (> 2 fold change) appeared to have better 3-year RFS, although this did not

reach statistical significance **(Figure 2 and Table 4)**. We also analyzed the associations between the 7 lncRNA and clinical characteristics of HPV-negative HNSCC in the TCGA dataset and found that low *MEG3* expression was associated with locally advanced disease and low expression of *HANDS-2AS1* correlated with more locally advanced cancer, although this did not appear to have an impact on survival outcomes. Restriction of the differential expression analysis to the 44 tumor-normal pairs in the TCGA validation set yielded similar results (data not shown).

DISCUSSION

There is increasing evidence that aberrant expression of lncRNA plays a role in the genesis and progression of HNSCC [24, 25]. Through our present study, we were able to identify and validate 5 differentially expressed lncRNA in HPV-negative HNSCC (*SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*). Further, we found that low expression of *MEG3* was associated with more favorable 3-year RFS, although the significance of this finding is unclear.

MEG3 is an long non-coding RNA located at the *DLK-MEG3* locus on chromosome 14q32.3 and is reported to be a tumor suppressor gene, exerting its effect in part through interaction with tumor suppressor and master regulator p53 [26, 27]. *MEG3* has been reported to be downregulated in multiple solid tumor types [28], which is consistent with what we observed in our 19 HPV-negative HNSCC tumor-adjacent normal pairs. However, our observed association of low *MEG3* expression with better 3-year RFS is contrary to what has been reported for other solid tumor types [29] and therefore should be interpreted with caution. In particular, Jia and colleagues found that low expression of *MEG3* correlated with poorer outcomes in squamous cell carcinoma of the anterior tongue, and overexpression of *MEG3* inhibited cell proliferation and cell cycle progression in SCC-15 and Cal 27 tongue squamous cell carcinoma cell lines [30].

While the other differentially expressed lncRNA transcripts in our study were not associated with OS or RFS in the TCGA head and neck tumors, they have been identified as potential markers for poor prognosis in other cancer types. *SPRY4-IT1*, which is located on the *SPRY4* gene, has been implicated in cell growth invasion and increased apoptosis [31], and elevated expression of *SRPY4-IT1* has been associated with poorer outcomes in esophageal squamous cell carcinoma, which, like HPV-negative HNSCC, is also strongly associated with tobacco and alcohol [32]. Overexpression of *HEIH* has been reported in hepatocellular carcinoma,

where it is an independent predictor for recurrence and survival and interacts with the lysine methyltransferase and Polycomb Repressor Complex 2 member *Enhancer of Zeste Homolog 2* (*EZH2*)[33]. Upregulation of both *LUCAT1* and *LINC00152* have been associated with poorer outcomes for non-small cell lung cancer (NSCLC) [34, 35], and *HAND2-AS1* has been reported to be upregulated in stage IVS neuroblastoma [36] but downregulated in metastatic hepatocellular carcinoma [37], with the latter being more in-line with our narrative of lower expression of *HAND2-AS1* in HPV-negative HNSCC.

Strengths of our study include the comprehensive assessment of 84 cancer-associated lncRNA and our ability to access raw RNA-seq data from TCGA. This allowed the alignment of our data with annotated lncRNA sequences to validate our findings in an independent dataset and assess the impact of significantly differential lncRNA on overall and relapse-free survival, and to infer HPV-status by aligning to HPV16 E6/E7 viral mRNA transcripts. Our study also has several limitations, including the modest sample size of our initial discovery set, which may adversely impacted our power to detect smaller effect sizes, increasing the risk of false-negative results. However, our use of a stringent FDR-control and validation in an independent set of tumors using TCGA data yields high confidence in our significant results. Furthermore, since no adjustments were made for multiple comparisons in the survival analyses, we cannot rule out the spurious nature of the observed association between MEG3 and RFS. Additionally, use of archival FFPE tissue for our discovery set likely attenuated the lncRNA expression levels, which could reduce our sensitivity for detection of signal or more subtle differences in expression. Our use of the RNeasy FFPE kit, which is specifically engineered to maximize the integrity and downstream results for RNA extracted from FFPE [38], helps mitigate this issue; it is also notable

that 5 of the 7 differentially expressed lncRNA identified with the array were replicated using RNA-seq data from fresh tissue, supporting the validity of our findings.

CONCLUSION

Expression of lncRNA is dysregulated in HPV-negative HNSCC. Specifically, we have identified and validated 5 differentially expressed lncRNA: *SPRY4-IT1*, *HEIH*, *LUCAT1*, *LINC00152*, and *HAND2-AS1*. Additional studies are needed to confirm the potential prognostic value of *MEG3* expression in HNSCC.

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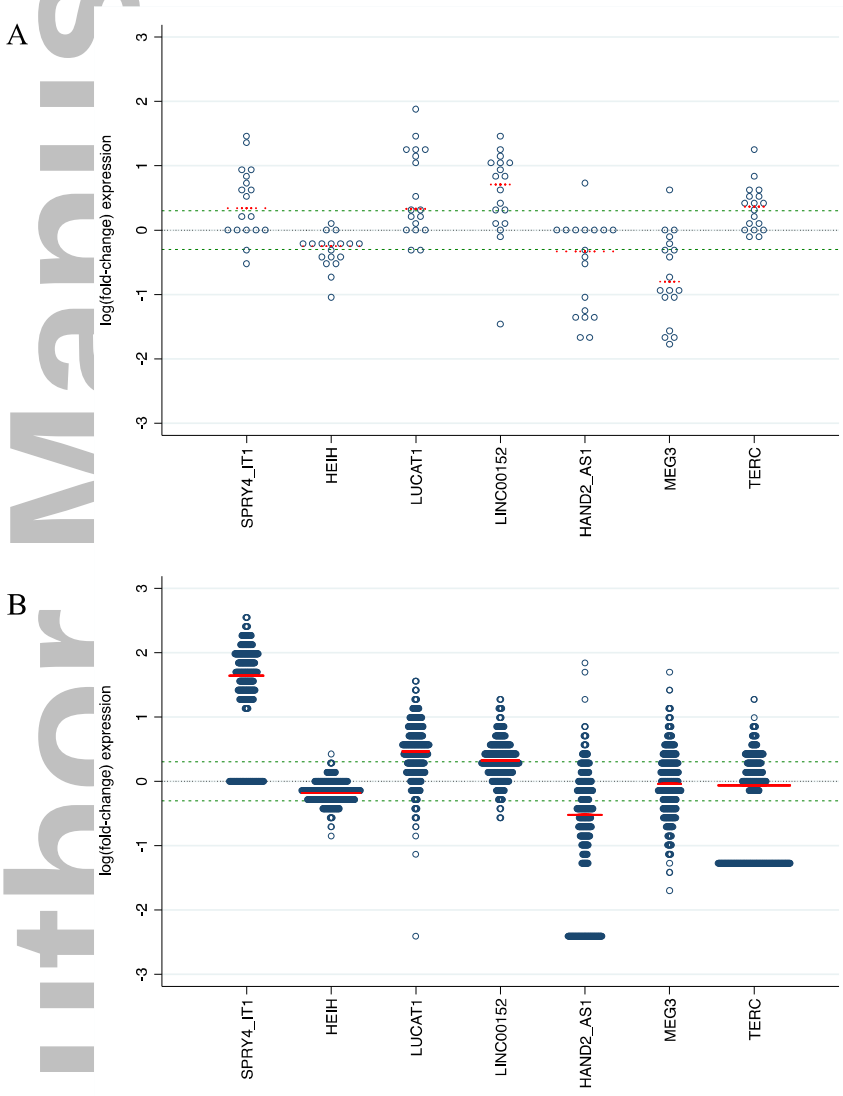
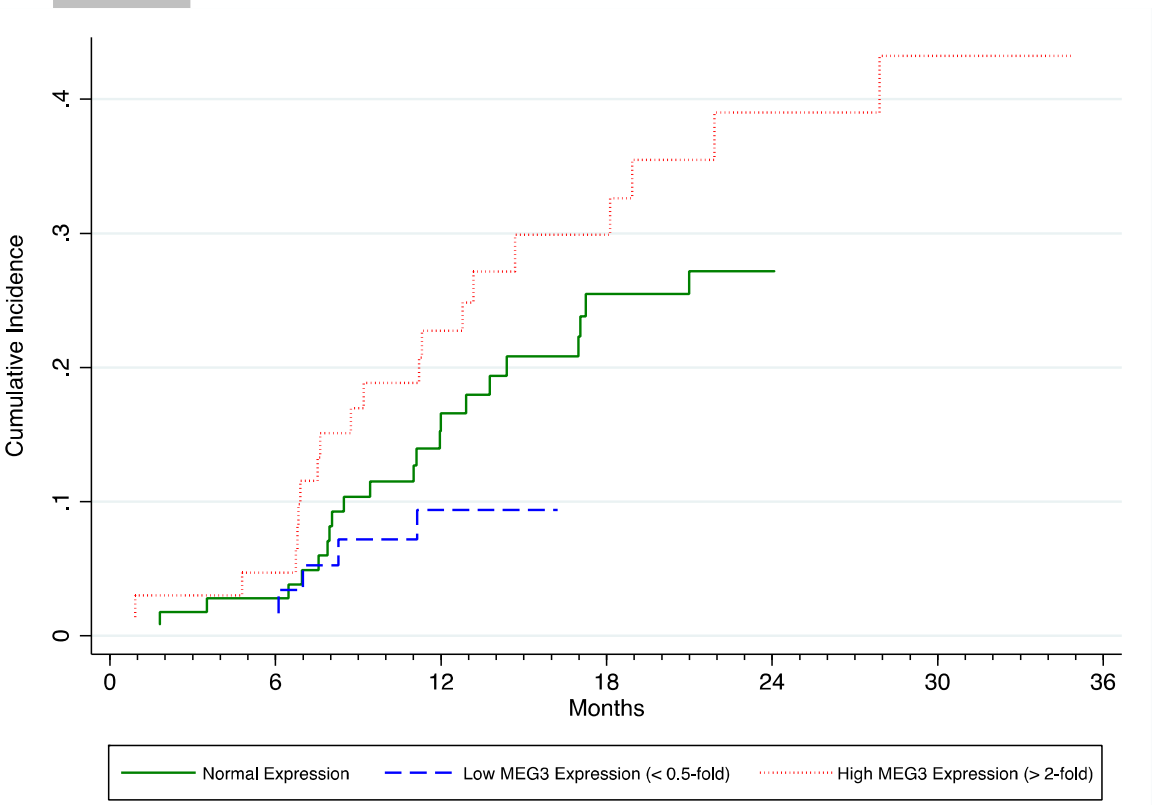
FIGURES

Figure 1. Distribution of log(fold-change) in HPV-negative head and neck squamous cell carcinoma (HNSCC) tumors relative to adjacent non-malignant squamous tissue for (A) University of Cincinnati patients (n = 19 tumor-normal pairs) and (B) HPV-negative tumors (n = 444) and adjacent normal tissue (n = 44) with RNA-sequencing data through The Cancer Genome Atlas (TCGA). The black dashed center line at 0 corresponds to neutral expression and blue dashed lines above and below the center line correspond to respective fold changes of 2.0 and 0.5 respectively; mean log(fold-change) for each lncRNA is denoted by a red dashed line.



Footnote:
Fold-change for the 19 tumors samples in panel A was calculated by comparing the expression of each tumor to its paired adjacent normal sample; fold-change for the 444 TCGA tumors in panel B was calculated by comparing the expression to the median expression of the 44 adjacent normal TCGA samples.

Figure 2. Cumulative incidence function for 3-year relapse-free survival of HPV-negative TCGA head and neck squamous cell carcinoma (HNSCC) tumors according to *MEG3* expression level.



TABLES

Table 1. Clinicodemographic characteristics of HPV-negative head and neck squamous cell carcinoma (HNSCC) cases from the University of Cincinnati and TCGA validation set.

	University of Cincinnati (n = 19)	TCGA Validation Set (n = 444)	$p_{difference}$
Age			0.28[a]
Years, median (range)	64 (51-86)	61.5 (19-90)	
Sex			0.83[b]
Male	14 (74%)	317 (71%)	
Female	5 (26%)	127 (29%)	
Race			0.44[b]
White	16 (84%)	355 (83%)	
Black	3 (16%)	44 (10%)	
Other	0 (0%)	30 (7%)	
Smoking Status			0.01[b]
Never	1 (5%)	93 (21%)	
Former	15 (79%)	181 (42%)	
Current	3 (16%)	160 (37%)	
Primary Tumor Site			0.75[b]
Oral cavity	12 (63%)	294 (66%)	
Pharynx	1 (5%)	41 (9%)	
Larynx	6 (32%)	109 (25%)	
AJCC Stage Group			0.68[b]
I	0 (0%)	23 (5%)	
II	2 (11%)	74 (17%)	
III	5 (28%)	82 (18%)	
IV	11 (61%)	265 (60%)	
Tumor Grade			0.25[b]
Well differentiated	6 (32%)	59 (13%)	
Moderately differentiated	9 (47%)	276 (63%)	
Poorly differentiated	4 (21%)	96 (22%)	
Undifferentiated	0 (0%)	2 (<1%)	
Undetermined	0 (0%)	8 (2%)	

[a] Wilcoxon Rank Sum test
[b] Fisher's Exact test

Abbreviations:
TCGA = The Cancer Genome Atlas; AJCC = Americal Joint Committee on Cancer
hrHPV = high-risk human papillomavirus

Table 2. Differentially expressed lncRNA in HPV-negative head and neck squamous cell carcinoma (HNSCC).

		Human lncRNA qPCR Cancer Array ($n_{T\text{-}N\ pairs}$ = 19)			TCGA RNA-seq Replication Set (n = 444)	
lncRNA	Ensembl ID	Median Fold-Change	p-value	Q-value	Median Fold-Change	p-value
SPRY4-IT1	ENSG00000281881	2.19	0.006	**0.07**	3.52	< 0.0001
HEIH	ENSG00000278970	0.56	0.0001	**0.01**	0.64	< 0.0001
LUCAT1	ENSG00000248323	2.14	0.002	**0.04**	1.76	< 0.0001
LINC00152	ENSG00000222041	5.11	0.003	**0.06**	1.56	< 0.0001
HAND2-AS1	ENSG00000237125	0.47	0.005	**0.06**	0.13	< 0.0001
MEG3	ENSG00000214548	0.16	0.0003	**0.01**	0.33	0.50
TERC	ENSG00000270141	2.32	0.0007	**0.02**	0.64	0.12

Table 3. Association between expression of significantly differential lncRNA and patients characteristics and clinical features in the TCGA HPV-negative head and neck squamous cell carcinoma (HNSCC) samples (n = 444).

	SPRY4-IT1		*HEIH*		*LUCAT1*		*LINC00152*		*HAND2-AS1*		*MEG3*		*TERC*	
N = 444	Low (n = 89)	High (n = 0)	Low (n = 92)	High (n = 2)	Low (n = 19)	High (n = 291)	Low (n = 6)	High (n = 291)	Low (n = 275)	High (n = 43)	Low (n = 134)	High (n = 107)	Low (n = 189)	High (n = 99)
Age, per decade	1.08 (0.87-1.34)	---	**0.75 (0.60-0.94)**	---	1.11 (0.65-1.88)	0.99 (0.82-1.21)	---	1.09 (0.83-1.43)	0.87 (0.70-1.08)	**0.67 (0.48-0.92)**	0.84 (0.68-1.04)	0.93 (0.74-1.16)	1.05 (0.86-1.28)	**1.40 (1.08-1.81)**
Sex														
Male	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Female	0.72 (0.41-1.26)	---	0.56 (0.30-1.06)	---	0.60 (0.15-2.44)	1.07 (0.64-1.78)	---	0.90 (0.46-1.77)	0.60 (0.35-1.01)	0.62 (0.26-1.47)	1.45 (0.84-2.50)	1.00 (0.55-1.81)	1.36 (0.81-2.30)	0.65 (0.33-1.28)
Race														
White	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Black	1.26 (0.49-3.23)	---	1.15 (0.51-2.58)	---	0.49 (0.06-4.40)	0.74 (0.36-1.50)	---	0.94 (0.35-2.54)	1.16 (0.51-2.64)	1.57 (0.47-5.30)	**3.34(1.43-7.80)**	**2.85 (1.18-6.90)**	0.90 (0.41-1.99)	1.05 (0.43-2.57)
Other	0.97 (0.37-2.58)	---	**3.50 (1.47-8.35)**	---	0.88 (0.09-8.95)	1.41 (0.54-3.68)	---	0.81 (0.28-2.38)	0.90 (0.37-2.23)	---	0.74 (0.29-1.86)	0.41 (0.13-1.30)	0.85 (0.35-2.09)	1.00 (0.34-2.98)
Smoking status														
Never	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Former	1.27 (0.65-2.45)	---	1.12 (0.53-2.38)	---	0.73 (0.19-2.83)	0.67 (0.36-1.26)	---	0.60 (0.25-1.43)	0.59 (0.45-1.72)	0.54 (0.20-1.47)	1.64 (0.84-3.22)	0.68 (0.35-1.34)	1.57 (0.84-2.90)	1.59 (0.73-3.50)
Current	1.59 (0.77-3.30)	---	1.37 (0.65-2.91)	---	0.39 (0.07-2.20)	0.79 (0.40-1.54)	---	0.78 (0.30-2.00)	0.59 (0.29-1.20)	0.45 (0.16-1.24)	0.90 (0.44-1.85)	0.53 (0.26-1.08)	1.48 (0.77-2.84)	1.30 (0.56-3.03)
Primary tumor site														
Oral cavity	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Pharynx	1.34 (0.52-3.48)	---	0.53 (0.19-1.47)	---	3.20 (0.83-12.40)	0.61 (0.28-1.29)	---	**0.36 (0.14-0.92)**	1.08 (0.49-2.40)	0.52 (0.13-2.09)	1.29 (0.58-2.90)	0.92 (0.36-2.32)	1.15 (0.50-2.64)	1.73 (0.67-4.49)
Larynx	0.82 (0.43-1.54)	---	0.93 (0.50-1.74)	---	1.25 (0.31-5.08)	0.96 (0.55-1.66)	---	0.53 (0.26-1.06)	1.33 (0.74-2.41)	0.48 (0.16-1.47)	0.71 (0.39-1.30)	0.86 (0.46-1.62)	0.56 (0.31-1.01)	1.49 (0.77-2.88)
AJCC Stage Group														
Early (I or II)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Late (III or IV)	1.09 (0.52-2.28)	---	0.62 (0.28-1.37)	---	2.06 (0.42-10.13)	1.48 (0.78-2.82)	---	1.49 (0.65-3.42)	**2.86 (0.74-2.41)**	0.75 (0.20-2.81)	1.11 (0.56-2.18)	1.01 (0.48-2.14)	1.84 (0.94-3.60)	**0.39 (0.17-0.91)**
N classification														
N0	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
N1-3	0.88 (0.47-1.65)	---	1.65 (0.86-3.19)	---	0.47 (0.13-1.71)	0.82 (0.47-1.44)	---	1.25 (0.59-2.63)	**0.28 (0.15-0.54)**	1.31 (0.39-4.44)	**0.49 (0.28-0.88)**	0.72 (0.38-1.34)	0.97 (0.56-1.69)	**2.68 (1.26-5.69)**
Tumor grade														
Low	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Moderate	1.39 (0.66-2.92)	---	1.22 (0.56-2.64)	---	1.87 (0.20-17.04)	1.38 (0.73-2.58)	---	1.34 (0.58-3.13)	1.07 (0.52-2.19)	0.74 (0.25-2.19)	0.83 (0.41-1.66)	1.01 (0.48-2.19)	0.94 (0.48-1.85)	1.17 (0.49-2.81)
High/undifferentiated	0.64 (0.28-1.46)	---	0.67 (0.26-1.72)	---	7013 (0.78-64.74)	1.22 (0.58-2.58)	---	1.21 (0.45-3.28)	0.87 (0.38-1.96)	0.94 (0.28-3.21)	0.65 (0.29-1.47)	1.33 (0.31-1.85)	0.62 (0.28-1.35)	0.71 (0.26-1.90)

Table 4. Crude and adjusted hazard ratios (HR) and subhazard ratios (SHR) for the association between expression of differentially expressed lncRNA and 5-year overall survival (OS) and 3-year relapse-free survival (RFS), respectively, in patients with HPV-negative head and neck squamous cell carcinoma (HNSCC).

	5-year Overall Survival (OS) (N = 439)			3-year Relapse-Free Survival (RFS) (N = 272)		
lncRNA expression	n	Crude HR	Adjusted HR[a]	n	Crude SHR	Adjusted SHR[a]
SPRY4-IT1						
Low (<0.5-fold)	0	---	---	0	---	---
Normal[b]	87	1.00 (reference)	1.00 (reference)	54	1.00 (reference)	1.00 (reference)
High (>2-fold)	352	0.93 (0.62-1.38)	0.89 (0.60-1.34)	218	1.40 (0.63-3.12)	1.30 (0.54-3.13)
HEIH						
Low (<0.5-fold)	92	0.82 (0.54-1.28)	0.88 (0.56-1.36)	59	0.91 (0.45-1.84)	1.14 (0.54-2.40)
Normal[b]	345	1.00 (reference)	1.00 (reference)	212	1.00 (reference)	1.00 (reference)
High (>2-fold)	2	---	---	1	---	---
LUCAT1						
Low (<0.5-fold)	18	1.49 (0.67-3.32)	1.61 (0.71-3.68)	12	3.07 (0.84-11.19)	2.58 (0.71-9.33)
Normal[b]	131	1.00 (reference)	1.00 (reference)	80	1.00 (reference)	1.00 (reference)
High (>2-fold)	290	0.92 (0.64-1.31)	0.85 (0.60-1.23)	180	1.50 (0.74-3.02)	1.54 (0.70-3.41)
LINC00152						
Low (<0.5-fold)	8	---	---	7	---	---
Normal[b]	68	1.00 (reference)	1.00 (reference)	41	1.00 (reference)	1.00 (reference)
High (>2-fold)	363	0.84 (0.55-1.28)	0.74 (0.48-1.14)	224	1.32 (0.52-3.34)	1.11 (0.41-3.04)
HAND2-AS1						
Low (<0.5-fold)	271	0.92 (0.64-1.31)	0.96 (0.66-1.39)	167	0.87 (0.47-1.64)	0.83 (0.43-1.60)
Normal[b]	126	1.00 (reference)	1.00 (reference)	80	1.00 (reference)	1.00 (reference)
High (>2-fold)	42	0.63 (0.30-1.34)	0.71 (0.33-1.51)	25	0.94 (0.31-2.83)	0.82 (0.25-2.65)
MEG3						
Low (<0.5-fold)	133	1.09 (0.75-1.57)	1.11 (0.76-1.62)	71	**0.38 (0.14-1.00)**	**0.28 (0.10-0.78)**
Normal[b]	202	1.00 (reference)	1.00 (reference)	127	1.00 (reference)	1.00 (reference)
High (>2-fold)	104	0.88 (0.56-1.40)	0.84 (0.53-1.34)	74	1.63 (0.89-2.98)	1.43 (0.75-2.74)
TERC						
Low (<0.5-fold)	187	1.01 (0.69-1.47)	0.90 (0.61-1.33)	115	1.25 (0.62-2.51)	1.30 (0.61-2.79)
Normal[b]	155	1.00 (reference)	1.00 (reference)	97	1.00 (reference)	1.00 (reference)
High (>2-fold)	97	0.98 (0.62-1.54)	0.97 (0.60-1.55)	60	1.75 (0.82-3.72)	2.24 (0.95-5.25)

[a] Adjusted for age, sex, race/ethnicity, smoking status, primary tumor site, and AJCC stage group

[b] Tumor expression >0.5-fold and < 2-fold was considered to be within normal range

Position	Symbol	HN0096	HN0126	HN0527	HN0688	HN0709	HN0732	HN0793	HN0859	HN0868	HN0888
E02	MEG3	-0.8985966	0.63591582	-0.322393	-1.6401645	-1.5606673	-0.4019759	0	-0.9722428	-1.0118872	-0.9931063
C01	HEIH	-0.178421	-0.4128505	-0.2571178	-0.7597004	-0.4897232	-1.0457575	-0.2232988	0.00354669	-0.1805877	-0.2927706
G04	TERC	-0.0560605	0.40689769	0.59755259	0.43700497	0.2381967	0.52107267	0.46846549	0.12270725	0.32335525	0.83057584
D12	LUCAT1	1.063986	1.13244861	1.25295735	1.23620209	1.25458191	0.2071225	0	0.1369741	-0.3551677	-0.3182162
D03	LINC00152	1.04538166	-0.1304163	0.60255914	0.29194575	0.27091164	0.81406105	1.19848663	0.46178356	1.0320221	0
G02	SPRY4-IT1	1.4160394	0	0	0.80936441	0.6402528	0.62091646	0.9873668	-0.3120254	-0.5460704	0
B12	HAND2-AS1	-1.3957739	0	0	0	-1.3477537	-1.6307841	-0.4014283	-0.2636035	-1.6840297	-1.2644011
E05	MIR31HG	0.58746401	0	-0.9775716	1.80964387	1.3578034	-1.0030508	-0.1292451	0.12949654	0.49873774	0.42071469
C11	IPW	0.07758591	-0.5425723	1.08570077	-2.0222764	-1.8664611	-0.1730834	-1.3968556	-0.3969639	-1.79588	0.1144108
G03	SUMO1P3	-0.1696038	0.30601735	0.06130166	-0.0962591	0.00608078	1.04272117	0	0.82241655	1.0502792	-1.2684112
B03	DLEU2	-0.5476002	0.04824753	-0.6777807	-0.0852339	-0.0825995	0.127364	0.52592547	0.24689232	-0.562091	-0.8486301
G01	SNHG16	-0.012736	0.4501721	0.45235387	0.39830466	0.26023839	-0.0670192	0.26356506	0.09534373	0.04316572	0.47730941
B02	DGCR5	0.54150436	0.6552249	0	0	0.07386492	0.55094954	1.37548803	0	0.58517863	0
A08	CAHM	-1.1191864	0	-1.2090115	-0.0043648	0	-1.1752235	0	-0.473144	-1.2890369	0
G12	ZFAS1	0.12859311	0.12720202	0.2214664	0.14157518	0.05606593	-0.3223018	0.25662155	-0.1095231	0.29712556	0.40813817
G07	TUG1	0.12417806	0.4411765	0.29488489	0.46242795	0.00130093	-0.2091521	0.63805974	0.36574998	-0.2030096	0.74925694
D05	LINC00312	-0.733298	-0.3196645	-0.109579	0.18771808	-0.9538952	-0.3538905	-0.3101586	-0.1922621	0.2671013	0.03306169
A02	ADAMTS9-A	-0.7328283	-0.4610494	0	-1.6861328	0	-0.3301255	0	0	0	0
A12	CDKN2B-AS	0	0	0	0.22574852	0.41340036	0.51440164	0	0.46076257	0	0
F09	PVT1	0.41463915	0.60483062	0.42471834	0.59424887	-0.2406344	0.18483333	0.98182766	-0.3602148	0.53856117	0
B06	FTX	0.4039951	0.42745365	-0.0020896	-0.4550642	-0.7000571	-0.0618307	0	-0.2551596	-0.6655462	0.54749165
E07	MRPL23-AS1	-0.8693447	0.19393119	0	-0.6950788	-0.2777775	-0.1324147	0	0	-0.0115748	-0.4082678
G09	UCA1	-0.1087972	0.42673892	0.54501006	-1.015923	0.49000064	-1.6819367	0.96549333	-0.0443603	0.30085624	1.45453236
B11	H19	-0.6462761	1.03466455	-2.1739252	1.11555726	-1.3391345	-1.1965429	-0.3417023	0.27105126	-0.2895441	-0.7896815
C03	HIF1A-AS2	0.27982658	1.49426872	-0.202594	0.58901078	1.98140268	-0.2203314	-1.1129456	-0.1743792	-0.6912222	0.87400609
F04	POU5F1P5	0	0	0	1.35808149	0	0	0	0	0	0
F06	PTCSC1	0	0	0	-0.0862921	0	0	0	0	-0.8904215	-1.1118205
C05	HOTAIR	0	-0.2230084	-0.2047458	0	1.0009934	0.25348339	0	0.34869419	-0.3305901	0
D10	LINC01234	0	0	1.19207936	0	0.19412566	0	0.63480967	0	0	1.09029333
E12	PANDAR	0	0.68313713	0	0	0	0	0	0	0	0
E01	MALAT1	0.22757822	-0.225629	-0.0570499	-0.4765137	0.17548282	-0.1433315	-0.0084195	-0.2268721	-0.7057543	-0.9385475
C02	HIF1A-AS1	0	1.15208644	0	-0.7817271	-0.3371478	0	0	0.30856441	0.30713219	0.53417846
C06	HOTAIRM1	0	-1.1911141	0.36233165	0.43544619	-0.4048346	0	1.05524068	0.55723042	0.64715794	0
D04	LINC00261	0	0	0	0	0	0	0	0	0	0
F01	PCA3	-0.292515	0	-0.5525319	-0.7554755	0.700894	0	0	0	-1.2975695	-0.7454519
F02	PCAT1	0	0	0.80806236	0	0	0	0	0	0	0
A04	AIRN	0	0.91146124	0	0	1.3055878	0	0	0	0	-0.105961
F03	PCGEM1	0	0	0	0	0	0	0	0	0	0
E03	MIR155HG	0	0	0	0.7287838	0	0	0	0	0	0
A05	BANCR	-0.6267201	0	0	0.34196841	0	0.38969755	0	0	0	1.88459884
A03	AFAP1-AS1	0	0	0.83165865	0.8912642	0	0	0	0	0	0
C08	HOXA11-AS	0	0	0	0	0	0	0	0	-0.0034883	0
E10	NEAT1	-0.0693566	0.15185996	-0.2640819	-0.3186683	0.12794664	-0.8548036	0.05637118	-0.1685142	-0.1156012	-0.3691655
D08	LINC00963	-0.5386516	0	0.13700578	0.29563306	-0.7493361	-1.5243288	0	0.00744904	0.42068172	0.48380118
C04	HNF1A-AS1	-0.2278984	-0.2227907	-0.4391373	-0.1490476	-0.0915686	-0.7801536	-0.0806604	0.37668643	-0.2356744	-0.22062
G11	XIST	0	0	-0.5728386	0	0	0	0	0	0	0
A11	CCAT2	0	0	0	0	0	0	0	0	0	0
C07	HOTTIP	0	0	0	0	0	0	0	0	0	0
C09	HOXA-AS2	0	0	0	0	0	0	0	0	0	0
C10	HULC	0	0	0	0	0	0	0	0	0	0
D06	LINC00538	0	0	0	0	0	0	0	0	0	0
D09	LINC01233	0	0	0	0	0	0	0	0	0	0
D11	LSINCT5	0	0	0	0	0	0	0	0	0	0
E06	MIR7-3HG	0	0	0	0	0	0	0	0	0	0
F08	PTENP1	0	0	0	0	0	0	0	0	0	0
G06	TSIX	0	0	0	0	0	0	0	0	0	0
G10	WT1-AS	0	0	0	0	0	0	0	0	0	0
A07	BLACAT1	0	0	-1.1372725	0.55749517	-0.0151128	-0.3679478	0	0.25909193	-0.0224136	0
B10	GNAS-AS1	0	0	0	0	0	0	0	0	-0.3256901	0
B01	CRNDE	0.8636975	0	-0.2048154	0.12648857	0.10527168	-0.3643152	0	0.42887959	0.42634857	0
E08	NAMA	0	0	0	-0.9168559	0	-1.251812	0	0	-1.0118872	0
B07	GACAT1	0	0	0	-0.145694	0	0	0	0	0	0
A10	CCAT1	-0.5304725	1.01721704	1.43177374	1.20201671	-1.3001623	-1.0736576	0	0	0.37930552	-1.5157002
F11	RMST	-1.2915791	0	0	0	0	0	0	-0.674895	0	0
D01	KCNQ1OT1	-0.2432118	-1.2218487	-1.094744	1.05772695	1.51055841	-1.39794	0	-0.3884891	-0.0981604	0
E09	NBR2	0.01686627	0.20698769	0.41954272	-0.2070483	0.00548081	0.15363197	-0.2735173	0.28436305	0.39234516	0.36070699
A09	CBR3-AS1	0	0	0	0.83562717	0	0	0	-0.1683861	0	0
G08	TUSC7	0	0	0	-1.4841262	0	0	0	0	0	0
G05	TRERNA1	0	0	0	0	0	0	0	0	0	0
B05	EMX2OS	0.12703997	0	0	-0.8389316	-0.5319482	-0.8288588	0	0	-0.1877553	0
B08	GAS5	-0.1726307	0.1813862	0.49073081	0.07316826	-0.384576	0.11143055	0.14847926	-0.3356401	0.19526296	0.60854743
C12	JADRR	0	0	0	0	0	0	0	0	0	-0.4060497
A01	ACTA2-AS1	0	0	0	0	0	0	0	0	0	0
D02	KRASP1	0	0	0	0.85780273	0	-1.1700533	0	0	0	0
D07	LINC00887	0.67267038	-1.0660068	0.46311624	0	0.34592238	-0.8761484	0	-0.6185239	0.77328899	-0.0255344
A06	BCAR4	-0.823041	0	-0.4043936	0	0.9723232	0	0	0	0	0
F07	PTCSC3	-0.1127767	0.44593097	-0.1770478	0	0	0	-0.1435732	-0.5678327	0	0
F05	PRNCR1	-0.1066267	0.17304051	0	0.21434041	-0.2261404	-0.0154277	-0.4189611	0.11650812	0.29307514	-0.8833923
B04	DLX6-AS1	0	0.02267576	0	0.29585287	0	-0.6094181	0.12011307	0	0.43724306	-0.2488749
F12	RPS6KA2-AS	0	0	0	0	-1.2814983	0	0	0	0	0
F10	RMRP	-0.0374886	-0.0191334	0.04477487	-0.0659563	0.0586158	-0.2809174	0.11190071	-0.0128702	-0.2714839	-0.0680847
E04	MIR17HG	-0.2193227	0.36080161	0.61974011	0.09677102	-1.5850267	0.19843714	0	0.07954301	0.17868924	0
E11	NRON	0	0	0	-1.139662	1.32197726	0	0	0	0	0
B09	GAS6-AS1	0	0	0	-1.0065638	0	0	0	0.84016257	0	0

Position	Symbol	HN1003	HN1028	HN1068	HN1129	HN1149	HN 1155	HN_1295	HN_1332	avg(log(FC))	avg(FC)	p-value
E02	MEG3	-0.3497896	-1.6326441	-0.1635493	-0.1335354	-0.9310724	0	-1.8268137	-0.7037737	-0.7170165	0.19185961	0.00026934
C01	HEIH	-0.047256	-0.4238891	0.10680263	-0.163486	-0.2398038	-0.5187007	-0.2082392	-0.3813245	-0.3173654	0.48154246	0.00086201
G04	TERC	-0.0890556	0.5263522	-0.0170507	-0.0475951	1.23042593	0.60702588	0.01836758	0.06759155	0.3436572	2.20626261	0.00167349
D12	LUCAT1	1.82856452	0.3018327	0.02600182	0.23050001	0.0322157	1.44351019	0.35958887	0.49084303	0.57355253	3.7458685	0.00258816
D03	LINC00152	0.89378395	1.41563431	0.10927474	1.083825	-1.4134127	0.81352094	0.10815895	1.24659459	0.54689528	3.52285915	0.00326028
G02	SPRY4-IT1	1.34362126	0.2046354	0.68812604	0.47629531	0	0.96465555	0	0.17831493	0.41508292	2.60065606	0.0040306
B12	HAND2-AS1	-0.5608253	0	0	0	0	0.77497656	-1.3448616	-1.0629839	-0.5656371	0.27187099	0.0041844
E05	MIR31HG	0.40844349	-0.3505678	0.16479882	1.06758411	2.04533276	1.17550312	1.07143276	1.66393638	0.55224758	3.56654397	0.00870931
C11	IPW	0.6008967	-0.489455	0	-0.9172146	-0.6968039	-1.2020404	0.34978245	-0.7726276	-0.5579921	0.27669921	0.01326837
G03	SUMO1P3	0.57649108	-0.053204	0.36070699	0.93753294	0.02681916	1.27795285	0.13289977	1.11005746	0.34021105	2.18882504	0.01524456
B03	DLEU2	-0.1755487	-0.316953	-1.5883803	-1.0329203	-0.1366177	0.13277183	-0.3592205	-0.6695862	-0.3334423	0.46404247	0.02026617
G01	SNHG16	-0.0272419	-0.0160131	-0.0025262	-0.1074046	-0.0975076	0.03634939	0.00389117	0.04828639	0.12214063	1.32477043	0.02340126
B02	DGCR5	0.15803061	1.20744047	0.02698244	0	-1.0644927	0	0.65267172	0	0.26460238	1.83908746	0.02366309
A08	CAHM	0	0	1.12507118	0.2751731	-1.3242217	-0.8738686	-1.79588	0	-0.4368718	0.36570269	0.02588886
G12	ZFAS1	0.01510816	0.34721289	-0.063788	0.08795879	0.12267451	0.08371756	0.0705181	0.06539296	0.107431	1.28065161	0.02626901
G07	TUG1	0.22857999	0.34949122	-0.1038046	-0.3365818	0.01038477	0.04016704	0.00086772	-0.1412223	0.15070863	1.41484424	0.02924743
D05	LINC00312	0.06205525	-0.3763373	0.57159238	-0.5310622	-1.2716462	0.0423392	-0.4404524	-0.7009287	-0.2849615	0.51884604	0.03001007
A02	ADAMTS9-A	-1.1668529	0	0	0	-1.2798407	0	0	0	-0.3142683	0.48498878	0.03476617
A12	CDKN2B-AS	0.6250036	1.05771554	0	-0.1887602	0	0.45806345	0	-0.6024076	0.16466266	1.46104186	0.03590647
F09	PVT1	-0.9168559	1.78815493	-0.163486	0.00380507	0.01774266	0.6705798	-0.1972263	0	0.24141802	1.7434842	0.04417059
B06	FTX	-0.3945872	-1.2865095	-0.0909256	-1.0385789	-1.0168249	0.3432904	-0.2700257	-0.7632109	-0.2932322	0.50905864	0.0512295
E07	MRPL23-AS1	0	0	-0.0449861	0	-0.7249191	0	-0.7442452	0	-0.206371	0.62176895	0.05637079
G09	UCA1	1.00561802	1.75059093	0.12398224	0.93823944	0.07144013	0.14466759	0.53304721	-0.3785121	0.30670488	2.02630529	0.05702183
B11	H19	1.03700794	0.35009289	-0.0290955	-1.924453	-0.3122927	-0.9965395	-1.7077439	-0.5331324	-0.4706494	0.33833784	0.05886015
C03	HIF1A-AS2	0.34966598	0.91298154	-0.041962	-1.1118205	1.89183172	0.40102123	0.13621307	0.05891926	0.30077182	1.99881143	0.07770507
F04	POU5F1P5	0.73070962	0	0	0	0	0	0	0	0.11604395	1.30630308	0.08236084
F06	PTCSC1	-0.859492	0	-0.059932	0	0	0	0	-0.7483618	-0.2086844	0.61846563	0.08423391
C05	HOTAIR	0	0.99091132	0.03850094	-0.6174427	0.29979049	0.35063561	0.21213431	0	0.11774203	1.3114207	0.10450357
D10	LINC01234	0	0	-0.8894103	0	0	0	0	0	0.12343876	1.32873619	0.12388892
E12	PANDAR	0	0.19700473	0	0	0	0	0	0	0.04889677	1.11917183	0.12539336
E01	MALAT1	-0.1252863	-0.0523703	0.52282228	-0.2236621	0.29959445	-0.8850556	-0.1051303	-0.4048346	-0.1862766	0.65121349	0.13472177
C02	HIF1A-AS1	0.65028809	0	-0.6439741	0	0.87896965	0.36938286	0	-0.1720495	0.12587242	1.33620293	0.13475073
C06	HOTAIRM1	0.53885011	-0.8138916	-0.2870979	-0.219035	0	0.38599928	0.81721488	0.85044405	0.15188567	1.41868399	0.14147893
D04	LINC00261	0	0.97969403	0	0	0	0	0	0	0.05442745	1.13351545	0.15284053
F01	PCA3	0	0.6381197	0.36011986	0	-0.9838026	0	-1.2564902	0	-0.2324835	0.58548598	0.15743448
F02	PCAT1	0	0	0	0	0	0	0	0	0.04489235	1.10889992	0.15794031
A04	AIRN	0	0	0	0	0	-0.9842212	1.01387744	0	0.11893024	1.31501357	0.15897492
F03	PCGEM1	0	0	0	0	0	0	0.745551	0	0.0414195	1.10006792	0.16096024
E03	MIR155HG	0	0	0	0	0	0	0	0	0.04048799	1.09771093	0.16190372
A05	BANCR	0	0	0	0	0	0	0	0	0.11053026	1.28982343	0.16804508
A03	AFAP1-AS1	0	0	-0.5065419	0	0	0	0	0	0.06757672	1.1683601	0.17023522
C08	HOXA11-AS	0	0	0	0	0	0	0.50317761	0	0.02776052	1.06600813	0.18307651
E10	NEAT1	-0.0136762	0.15262464	0.06449543	-0.2709162	-0.2762161	0.13424088	-0.034328	-0.2663213	-0.1296728	0.74186897	0.22785187
D08	LINC00963	-0.2740067	0.09704897	-0.0680339	-0.1204454	-0.5442418	-0.6665527	-0.0041455	-0.189633	-0.1798753	0.66088315	0.22845897
C04	HNF1A-AS1	0.24956521	0.29150205	-0.2770372	-0.3469805	-0.2035644	-0.2265061	-0.7587026	0.64893544	-0.1496474	0.70852083	0.23800668
G11	XIST	-1.4710833	0	-0.1241866	-0.1847544	-0.2520447	0	0	0	-0.1447171	0.71661008	0.26160853
A11	CCAT2	0	0	0	0	0	0	0	0	0	1	0.3298768
C07	HOTTIP	0	0	0	0	0	0	0	0	0	1	0.3298768
C09	HOXA-AS2	0	0	0	0	0	0	0	0	0	1	0.3298768
C10	HULC	0	0	0	0	0	0	0	0	0	1	0.3298768
D06	LINC00538	0	0	0	0	0	0	0	0	0	1	0.3298768
D09	LINC01233	0	0	0	0	0	0	0	0	0	1	0.3298768
D11	LSINCT5	0	0	0	0	0	0	0	0	0	1	0.3298768
E06	MIR7-3HG	0	0	0	0	0	0	0	0	0	1	0.3298768
F08	PTENP1	0	0	0	0	0	0	0	0	0	1	0.3298768
G06	TSIX	0	0	0	0	0	0	0	0	0	1	0.3298768
G10	WT1-AS	0	0	0	0	0	0	0	0	0	1	0.3298768
A07	BLACAT1	1.18649299	0	-0.8560489	0.11501107	0	1.0029087	0.39283759	0	0.06194677	1.1533119	0.37249574
B10	GNAS-AS1	0	0.20836072	0	0	0	0.06829721	0	0	-0.002724	0.99374736	0.39171776
B01	CRNDE	-0.0879906	-1.623423	0.24350931	-1.4012095	-1.1765258	0.14292106	-0.3528127	-0.1299474	-0.1668846	0.68095022	0.39294213
E08	NAMA	0.4319585	0	0	0	0	0.35166105	0	0	-0.1331631	0.7359307	0.40081332
B07	GACAT1	0	0	0	0	0	0	0	0	-0.0080941	0.98153523	0.41819691
A10	CCAT1	-0.8961963	1.37696728	0	0.36960543	0	0	0	1.40264649	0.10351908	1.2691679	0.44006955
F11	RMST	0	0	0	0	0	0	0	0	-0.1092486	0.77759138	0.44708957
D01	KCNQ1OT1	-0.0472076	-0.3641143	1.00253754	-0.0745332	-0.276052	0.86492619	1.34307363	1.08852589	0.09228042	1.23674572	0.450613
E09	NBR2	0.01674093	-1.1157712	0.0953786	-0.4845236	0.2123208	-0.1793389	0.52704959	-0.0334828	0.02209625	1.05219504	0.45695212
A09	CBR3-AS1	0	0	0	-0.9197344	-1.4045038	0	0	0	-0.0920554	0.8089927	0.65849671
G08	TUSC7	0	0	0	0	0	0	0	0	-0.0824515	0.82708196	0.67507855
G05	TRERNA1	0	0	0	0	-1.4814861	0	0	0	-0.0823048	0.82736133	0.67596271
B05	EMX2OS	-0.638461	0	0	0	1.28437658	0	0	0	-0.0896966	0.8133986	0.68397551
B08	GAS5	-0.1066822	0.26738296	-0.1292451	-0.1385253	-0.5425723	-0.1117643	-0.2548471	-0.2261404	-0.0181242	0.95912634	0.70641475
C12	JADRR	0	0	0	0	-0.1557714	0	0	0	-0.0312123	0.93065286	0.7516704
A01	ACTA2-AS1	0	0	0	0	0	0	0	-0.554863	-0.0308257	0.93148159	0.75879464
D02	KRASP1	0	0	0	-0.0674755	0	0	0	0	-0.0210959	0.95258581	0.75964419
D07	LINC00887	-1.1674911	0	0.65502312	-0.7639669	0	0.38223323	1.1478772	0	-0.0043078	0.99013002	0.76402745
A06	BCAR4	0	-0.8124793	-0.18923	0	-0.0165541	-0.0388638	0	0	-0.0729021	0.84546933	0.77155105
F07	PTCSC3	0	0	0	0	0	0.08113119	-0.2726214	0.20346805	-0.0301845	0.93285784	0.7830549
F05	PRNCR1	-0.0026136	-0.1246493	-0.0584389	0.681196	-0.2632052	0.08206693	-0.1793389	-0.4243504	-0.0634954	0.8639818	0.84148615
B04	DLX6-AS1	0.10846354	-0.4952574	-0.1788141	0	0.080951	0	0	-0.1694755	-0.0353634	0.92179984	0.86157986
F12	RPS6KA2-AS	0	0.81584993	0	0	0	-0.5924392	0	0	-0.0587826	0.87340839	0.89927939
F10	RMRP	-0.1747687	-0.1228591	0.08461202	-0.077067	-0.0984327	0.14860265	-0.022688	0.06001754	-0.0412904	0.90930512	0.90905461
E04	MIR17HG	0.53137672	1.06565813	0.17897695	0.30756021	-0.2192508	-0.6505282	-0.9698052	-0.65738	-0.0379866	0.91624869	0.94512881
E11	NRON	0	0	0	0	0	-0.9150664	0	0	-0.0407084	0.91052443	0.95157688
B09	GAS6-AS1	0.57301093	0	0	0.14928071	-1.335358	0	0	0	-0.0433038	0.90509933	0.97077818

591x475mm (300 x 300 DPI)

lncRNA	Average Normalized Count	Standard Deviation	Median Normalized Count	Range	Inter-Quartile Range	$P_{skewness-kurtosis}$
SPRY4_IT1	0.11	0.018	0	0.00, 0.06	0, 0.018	0.0009
HEIH	20.36	8.47	19.59	8.56, 42.91	14.26, 23.49	0.007
LUCAT1	0.39	0.44	0.24	0.02, 1.92	0.08, 0.57	0.0001
LINC00152	7.07	7.52	5.26	0.90, 38.66	2.80, 7.89	< 0.0001
HAND2_AS1	0.67	0.95	0.28	0.00, 4.25	0.13, 0.73	< 0.0001
MEG3	15.71	23.84	5.67	0.49, 116.51	2.29, 19.23	< 0.0001
TERC	0.02	0.03	0.02	0.00, 0.12	0.00, 0.04	0.0007

230x101mm (300 x 300 DPI)