Comprehensive analysis of lncRNA-associated competing endogenous RNA network in tongue squamous cell carcinoma

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Background. Increasing evidence has demonstrated that long non-coding RNAs (lncRNAs) play an important role in the competitive endogenous RNA (ceRNA) networks in that they regulate protein-coding gene expression by sponging microRNAs (miRNAs). However, the understanding of the ceRNA network in tongue squamous cell carcinoma (TSCC) remains limited.

Methods. Expression profile data regarding mRNAs, miRNAs and lncRNAs as well as clinical information on 122 TSCC tissues and 15 normal controls from The Cancer Genome Atlas (TCGA) database were collected. We used the edgR package to identify differentially expressed mRNAs (DEmRNAs), lncRNAs (DElncRNAs) and miRNAs (DEmiRNAs) between TSCC samples and normal samples. In order to explore the functions of DEmRNAs, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed. Subsequently, a ceRNA network was established based on the identified DElncRNAs–DEmiRNAs and DEmiRNAs–DEmRNAs interactions. The RNAs within the ceRNA network were analyzed for their correlation with overall disease survival. Finally, lncRNAs were specifically analyzed for their correlation with clinical features in the included TSCC patient samples.

Results. A total of 1867 mRNAs, 828 lncRNAs and 81 miRNAs were identified as differentially expressed in TSCC tissues ($|\log_2$ fold change$| \geq 2$; adjusted P value < 0.01). The resulting ceRNA network included 16 mRNAs, 56 lncRNAs and 6 miRNAs. Six out of the 56 lncRNAs were found to be associated with the overall survival in TSCC patients ($P < 0.05$); 10 lncRNAs were correlated with TSCC progression ($P < 0.05$).

Conclusion. Our study deepens the understanding of ceRNA network regulatory mechanisms in TSCC. Furthermore, we identified ten lncRNAs (PART1, LINC00261, AL163952.1, C2orf48, FAM87A, LINC00052, LINC00472, STEAP3-AS1, TSPEAR-AS1 and ERVH48-1) as novel, potential prognostic biomarkers and therapeutic targets for TSCC.

1   **Comprehensive analysis of lncRNA-associated competing endogenous RNA network in**

2   **tongue squamous cell carcinoma**

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22   **Abstract**

23   **Background.** Increasing evidence has demonstrated that long non-coding RNAs (lncRNAs) play

24   an important role in the competitive endogenous RNA (ceRNA) networks in that they regulate

25   protein-coding gene expression by sponging microRNAs (miRNAs). However, the understanding

26   of the ceRNA network in tongue squamous cell carcinoma (TSCC) remains limited.

27   **Methods.** Expression profile data regarding mRNAs, miRNAs and lncRNAs as well as clinical

28   information on 122 TSCC tissues and 15 normal controls from The Cancer Genome Atlas (TCGA)

29   database were collected. We used the edgR package to identify differentially expressed mRNAs

30   (DEmRNAs), lncRNAs (DElncRNAs) and miRNAs (DEmiRNAs) between TSCC samples and

31   normal samples. In order to explore the functions of DEmRNAs, Kyoto Encyclopedia of Genes

32   and Genomes (KEGG) pathway analysis was performed. Subsequently, a ceRNA network was

33   established based on the identified DElncRNAs–DEmiRNAs and DEmiRNAs–DEmRNAs

34   interactions. The RNAs within the ceRNA network were analyzed for their correlation with overall

35   disease survival. Finally, lncRNAs were specifically analyzed for their correlation with clinical

36   features in the included TSCC patient samples.

37   **Results.** A total of 1867 mRNAs, 828 lncRNAs and 81 miRNAs were identified as differentially

38   expressed in TSCC tissues ($|\log_2\text{fold change}| \geq 2$; adjusted P value < 0.01). The resulting ceRNA

39   network included 16 mRNAs, 56 lncRNAs and 6 miRNAs. Six out of the 56 lncRNAs were found

40   to be associated with the overall survival in TSCC patients ($P < 0.05$); 10 lncRNAs were correlated

41   with TSCC progression ($P < 0.05$).

42   **Conclusion.** Our study deepens the understanding of ceRNA network regulatory mechanisms in

43 TSCC. Furthermore, we identified ten lncRNAs (PART1, LINC00261, AL163952.1, C2orf48,

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45 potential prognostic biomarkers and therapeutic targets for TSCC.

46

47 **Introduction**

48 Tongue squamous cell carcinoma (TSCC) is the most common type of oral squamous cell

49 carcinoma (OSCC) with remarkable invasiveness, early lymph node metastasis and a poor

50 prognosis (Sano & Myers 2007; Zhou et al. 2015). The quality of life for TSCC survivors is often

51 reduced due to speech disfunction, mastication, and deglutition. Recently, the incidence and

52 mortality of TSCC have steadily risen in the United States (Siegel et al. 2014; Siegel et al. 2015;

53 Siegel et al. 2016; Siegel et al. 2017; Siegel et al. 2018). Despite significant advancements in

54 surgical excision, radiotherapy and chemotherapy, mortality rates and recurrence rates for this

55 cancer remain high (Adeel & Suhail 2016; Chen et al. 2018). For these reasons, the molecular

56 mechanisms of TSCC tumorigenesis urgently require further study; potential biomarkers as well

57 as therapeutic targets in this cancer should be identified in order to improve clinical outcomes.

58

59 Long noncoding RNAs (lncRNAs) are a subclass of noncoding RNAs longer than 200 nucleotides

60 (Ponting et al. 2009). Of late, lncRNAs have been a new focus of cancer research and were found

61 to be involved in tumorigenesis and metastasis (Chen et al. 2017; Gutschner et al. 2013; Yang et

62 al. 2017; Yang et al. 2016b; Yuan et al. 2014). In TSCC, lncRNAs were reported to act as

63 oncogenes or tumor suppressors and affect patient prognosis. For example, knockdown of AFAP1-

64   AS1 could suppress cell proliferation, migration and invasion in TSCC (Wang et al. 2018).

65   Additionally, the overexpression of lncRNA MEG3 inhibited cell proliferation and induced

66   apoptosis in TSCC (Jia et al. 2014). The current literature has demonstrated that lncRNAs regulate

67   gene expression via genetic imprinting, splicing regulation, chromatin remodeling, mRNA decay,

68   and translational regulation (Zhu et al. 2013). However, the formation and development of tumors

69   is a complex pathophysiological process. The mechanisms by which lncRNAs affect TSCC

70   biology remain unelucidated.

71

72   Salmena *et al*. proposed the competing endogenous RNA (ceRNA) hypothesis, which stated that

73   lncRNA could crosstalk with mRNA by sharing common microRNA response elements (MREs)

74   with miRNA (Salmena et al. 2011). More and more studies have validated the involvement of

75   ceRNA crosstalk in the development and progression of various tumors, such as those of breast

76   cancer, hepatocellular cancer and pancreatic cancer. Some of the few relevant studies on such

77   crosstalk in TSCC have been verified, such as LINC00511/ miR-765/ LAMC2 (Ding et al. 2018)

78   and H19/ let-7a/ HMGA2 (Kou et al. 2018). Furthermore, comprehensive analysis of TSCC-

79   associated lncRNAs and miRNAs in a whole genome wide context is lacking, especially based on

80   high-throughput sequencing with a large-scale sample size.

81

82   To better understand how lncRNAs regulate gene expression by sponging miRNAs in TSCC, we

83   build a ceRNA network based on the TCGA database, including 6 mRNAs, 56 lncRNAs and 6

84   miRNAs. In addition, we found 10 lncRNAs to be associated with survival and 10 lncRNAs having

85   an association with carcinogenesis. Results of these analyses are a starting point to analyze ceRNA

86   crosstalk and gain insight into the molecular mechanisms participating in the tumorigenesis and

87   progression of TSCC.

88

89   **Materials and Methods**

90   **Patients and samples**

91   RNA sequencing data and the corresponding clinical information for our TSCC dataset were

92   retrieved from the TCGA data portal. The inclusion criteria were set as follows: 1) patients with

93   follow-up survival times less than 2000 days; 2) patients with detailed clinicopathological

94   information including age, gender, survival time, survival status, pathological stage, TNM stage.

95   As most patients were missing data about their metastatic states, we did not analyze this

96   information. After filtering available data with our inclusion criteria, a total of 122 TSCC patients

97   and 15 normal controls were included in our analysis. The clinical and pathological characteristics

98   of the TSCC patients are summarized in Table 1. This study conformed with the publication

99   guidelines provided by TCGA (https://cancergenome.nih.gov/publications/publicationguidelines)

100   and as our data was obtained from TCGA database, approval by an ethics committee was not

101   required.

102

103   **RNA sequence data processing**

104   Level 3 RNASeq and miRNASeq data from TSCC samples up to June 30,2018, including 122

105   TSCC tissues and 15 normal controls, were downloaded from the TCGA data portal. The sequence

106 data originated from IlluminaHiSeq_RNASeq and IlluminaHiSeq_miRNASeq sequencing

107 platforms; all the data are publicly available.

108

109 **Analysis of differential expression profiles**

110 The Ensembl database (http://www.ensembl.org/index.html, version 89) (Aken et al. 2016) was

111 used to identify lncRNA genes from the raw expression data. We discarded previously identified

112 lncRNAs that were not included in this database. Differential expression analysis of mRNAs

113 (DEmRNAs), miRNAs (DEmiRNAs) and lncRNAs (DElncRNAs) between TSCC and normal

114 tissues was carried out using the edgeR package (Robinson et al. 2010). For all p values, false

115 discovery rate (FDR) was applied for multiple testing correction. Absolute $\log_2 FC \geqslant 2$ and the

116 FDR < 0.01 were used as cut-off criteria.

117

118 **Functional enrichment analysis**

119 In order to better understand the mechanisms involved in the tumorigenesis of TSCC, we

120 conducted Gene Ontology (GO) functional enrichment analysis using DAVID (the Database for

121 Annotation, Visualization and Integrated Discovery) with FDR < 0.01 as the cut-off value. KEGG

122 analysis was performed using the ClusterProfiler package in the R language with a cut-off value

123 of adjusted p value < 0.05.

124

125 **Construction of ceRNA network**

126 We used the miRcode database (Jeggari et al. 2012) to predict lncRNA-miRNA interactions, which

127   were then combined with selected miRNAs. Secondly, TargetScan (Fromm et al. 2015),

128   miRTarBase (Chou et al. 2016) and miRDB (Wong & Wang 2015) were used to retrieve and

129   predict the targeted mRNAs of miRNA. In order to enhance the validity of this ceRNA network,

130   we only included miRNA-targeted mRNAs present in all three databases and DEmRNAs. Finally,

131   the ceRNA network was visualized using Cytoscape 3.6.1 software. A flowchart of the ceRNA

132   network is presented in Fig 1. We also performed linear regression analysis to explore the

133   correlation between ceRNA expression levels.

134

135   **Statistical analysis**

136   For overall survival analysis, the log-rank test was employed to compare the difference between

137   TSCC and normal samples using Kaplan Meier survival curve. The cut-off point of expression was

138   identified using survminer package (Li et al. 2018b). The edgeR package was used to screen out

139   DElncRNAs associated with clinical features, by setting cut-off criteria of absolute $\log_2 FC \geqslant 1$

140   and the FDR < 0.05. Unless specifically stated, a p value < 0.05 was considered to represent

141   statistical significance. All statistical analyses were performed using R software (version: 3.3.2).

142

143   **Results**

144   **DEmRNAs in TSCC**

145   According to the cut-off threshold of $\log_2 FC \geqslant 2$ and FDR < 0.01, 717 (38.40%) up-regulated and

146   1150 (61.60%) down-regulated genes were identified in TSCC (Supplementary Table 1). Fig. 2

147   shows the distribution of DEmRNAs between TSCC and normal controls. The expression heat

148   map of DEmRNAs is shown in Supplementary Fig. 1. Red or green represents significantly

149   upregulated and downregulated genes, respectively.

150

151   A total of 45 significantly enriched GO terms are listed in Supplementary Table 2 that correspond

152   to DEmRNAs. For "biological processes (BP)", the top five terms were muscle filament sliding,

153   collagen catabolic process, extracellular matrix organization, muscle contraction and skeletal

154   system development; for the "cellular component (CC)" ontology the top five were, extracellular

155   region, extracellular space, proteinaceous extracellular matrix, Z disc and collagen trimer; finally,

156   the top five "molecular function (MF)" terms were, structural constituent of muscle, extracellular

157   matrix structural constituent, calcium ion binding, heparin binding and cytokine activity (Fig. 3).

158

159   Additionally, a total of 20 significantly enriched KEGG pathways for the identified DEmRNAs

160   are listed in Table 2, and the top 10 KEGG pathways are shown in Fig. 3D. The cytokine-cytokine

161   receptor interaction pathway was found to harbor the largest number of DEmRNAs (Fig. 3E).

162

163   **DElncRNAs in TSCC**

164   Based on the cut-off criteria (log2FC $\geq$ 2 and FDR < 0.01), we identified 828 lncRNAs aberrantly

165   expressed in TSCC compared to normal tissues, including 517 up-regulated (62.44%) and 311

166   down-regulated lncRNAs (37.56%) (Supplementary Table 3). The distribution of all the

167   DElncRNAs are presented as a volcano plot in Fig.4 and an expression heat map of DElncRNAs

168   is shown in Supplementary Fig. 2.

169

DEmiRNAs in TSCC

To build our lncRNA-miRNA-mRNA ceRNA network, we also compared miRNA expression

profiles in tumor tissues with normal tissues. In total, 81 DEmiRNAs were identified, including

42 up- and 39 down-regulated miRNAs (Supplementary Table 4). A volcano plot of the related

DEmiRNAs is shown in Fig. 5; a corresponding expression heat map is shown in Supplementary

Fig. 3.

ceRNA network in TSCC

A dysregulated ceRNA network of lncRNA-miRNA-mRNA in TSCC was established based on

the above data in order to better elucidate the role of DElncRNAs. First, the 828 DElncRNAs were

retrieved from the miRcode, and 102 pairs of interacting lncRNAs and miRNAs were identified

using the Perl language. Subsequently, we predicted that six DEmiRNAs could interact with 56

DElncRNAs. Then we found that these six DEmiRNAs targeted 221 mRNAs in all three databases

(TargetScan, miRTarBase and miRDB). Among the 221 targeted mRNAs, only 16 mRNAs were

found in the 1867 DEmRNAs (Supplementary Fig. 4). Finally, we constructed a ceRNA network

relating to TSCC by incorporating 56 DElncRNAs, 6 DEmiRNAs and 16 DEmRNAs, as shown

in Fig. 6. To confirm these findings, we performed smooth curve fitting between the expression

levels of the DElncRNAs and DEmRNAs included in the ceRNA network. Our results indicated a

positive correlation between ceRNA expression levels. For example, LINC00472 interacted with

GREM2 mediated by mir-503 and SFTAIP regulated IL11 levels by sponging mir-211 (Fig. 7).

190   We also contract GO and KEGG analysis to reveal the functions of the 16 DEmRNAs that were

191   involved in the ceRNA network. Only two GO terms were significantly enriched ($P < 0.05$) (Table

192   3).

193

194   **RNAs in the ceRNA network are related to survival**

195   LncRNAs, miRNAs and mRNAs associated with prognosis were identified using the expression

196   profiles of 59 lncRNAs, 6 miRNAs and 16 mRNAs in the ceRNA network using Kaplan Meier

197   Survival Curve. As a result, ten lncRNAs (PART1, LINC00261, AL163952.1, C2orf48, FAM87A,

198   LINC00052, LINC00472, STEAR-AS1, TSPEAR-AS1 and ERVH48-1) were observed to be

199   significantly related to overall survival rate (P < 0.05) (Fig. 8).

200

201   **lncRNAs in the ceRNA network are related to clinical features**

202   The 64 DElncRNAs from the ceRNA network were further analyzed to identify their correlations

203   with clinical features. TSCC patients were divided into subgroups according to pathological stage

204   (Stage III + IV vs. stage I + II) and TNM stage (T3 + T4 vs. T1 + T2, N2 + N3 vs. N0 + N1). We

205   found six lncRNAs with a high expression level (LINC00355, PSORS1C3, LINC00520,

206   AC112721.1, AL139147.1, SFTA1P) and four lncRNAs with a low expression level (HCG22,

207   LINC00492, AL035696.1, ERVH48-1) were significantly associated with the progression of

208   TSCC (Table 4).

209

210   **Discussion**

211 TSCC is the most common form of oral cancer. Dysregulated genes are considered a major cause

212 of oncogenesis and the development of TSCC. Recently, the crucial role of lncRNA in gene

213 expression regulation at three levels including transcription, post-transcription and translation has

214 attracted considerable interest. Accordingly, the ceRNA hypothesis was proposed, postulating that

215 lncRNAs could act as part of post-transcriptional gene expression control. This conclusion

216 generated new insights into the biology of cancer.

217

218 To better understand how lncRNA-associated ceRNA crosstalk affects TSCC, we exploited a

219 large-scale TSCC data from the TCGA database and successfully established a dysregulated

220 lncRNA-associated ceRNA network. In addition, growing evidence has indicated that lncRNAs

221 have greater potential as prognostic biomarkers than protein-coding genes due to their stronger

222 correlation with tumor status (Hauptman & Glavac 2013). Thus, we also identified ten lncRNAs

223 (PART1, LINC00261, AL163952.1, C2orf48, FAM87A, LINC00052, LINC00472, STEAR-AS1,

224 TSPEAR-AS1 and ERVH48-1) as prognostic biomarkers for TSCC. However, there is no research

225 to clearly explain the function of AL163952.1, C2orf48, FAM87A, STEAR-AS1, TSPEAR-AS1

226 and ERVH48-1.

227

228 PART1 is upregulated and its higher expression is associated with poor prognosis in prostate

229 cancer and non-small cell lung cancer (Li et al. 2017b; Sun et al. 2018). Elevated PART1 promotes

230 prostate cancer cell proliferation and inhibits cell apoptosis (Sun et al. 2018). On the contrary, we

231 found that PART1 had lower expression and was negatively correlated to survival rate in TSCC,

232 which was consistent with findings in OSCC (Li et al. 2017c). This may be because PART1 is

233 located on chromosome 5q12, a region that is usually lost in oral squamous cell carcinoma (OSCC)

234 and head and neck squamous cell carcinoma (HNSCC) (Abou-Elhamd & Habib 2008; Noutomi et

235 al. 2006). In our ceRNA network, low PART1 expression reduced levels of NR3C2 mediated by

236 mir-301b, and reduced expression of NR3C2 promotes tumor cell proliferation, metastasis and

237 epithelial-to-mesenchymal transition (Yang et al. 2018; Yang et al. 2016a; Zhang et al. 2017; Zhao

238 et al. 2018c). In addition, patients with low levels of NR3C2 have a poor prognosis in pancreatic

239 cancer and renal cell carcinoma (Yang et al. 2016a; Zhao et al. 2018c). The relationship between

240 mir-301b and NR3C2 has also been validated in pancreatic cancer (Yang et al. 2016a). Therefore,

241 PART1/mir-301b/NR3C2 axis may be an important mechanism that involves in TSCC

242 development.

243

244 LINC00261 is a tumor suppressor positively associated with prognosis in many tumors, such as

245 hepatocellular carcinoma (Zhang et al. 2018a), endometrial carcinoma (Fang et al. 2018) and non-

246 small cell lung (Liu et al. 2017). Its functions mainly include inhibiting tumor cell proliferation,

247 invasion and metastasis. LINC00261 was also down-regulated in our study. Notably, decreased

248 expression of LINC00261 indicated a better prognosis in TSCC. Our study found LINC00261

249 regulated the expression of ENPP4 and ENPP5, however, these two genes have not been

250 extensively studied. ENPP2 as their closely-related molecule has been widely reported to

251 participate in tumor development. Low expression of ENPP2 increases reactive oxygen species

252 (ROS) level, and high ROS level could promote tumor cell apoptosis (Cholia et al. 2018; Dawei

253 et al. 2018). The special function of LINC00261 still needs to be further investigated.

254

255 Several existing studies indicate that LINC00472 plays an important role in inhibiting tumor

256 development (Shen et al. 2015; Su et al. 2018; Ye et al. 2018). Our study also showed the similar

257 results that higher expression of LINC00472 was associated with better prognosis. In addition,

258 LINC00472 may regulate the expression of GREM2 by sponging mir-503. GREM2 is an

259 antagonist of bone morphogenetic proteins (BMP) and could activate Notch signaling pathway (Li

260 et al. 2018a) and Wnt/β-catenin signaling (Wu et al. 2015), which may be the reasons that GREM2

261 involves in the development of TSCC. Given that GREM2's functions have not been studied in

262 cancer, our results need to be verified by experiments.

263

264 The function of LINC00052 varies depending on the location of tumors. It acts as a tumor

265 suppressor through inhibiting cell proliferation, invasion and migration in hepatocellular

266 carcinoma (Xiong et al. 2016; Yan et al. 2018; Zhu et al. 2017), whereas it could promote breast

267 cancer growth (Salameh et al. 2017) and gastric cell metastasis and proliferation (Shan et al. 2017).

268 In our study, it may act as an oncogene because it is significantly up-regulated and its high

269 expression indicates a poor prognosis in TSCC. Unfortunately, our ceRNA network failed to find

270 miRNAs that could interact with LINC00052. The function of LINC00052 is needed to be further

271 investigated.

272

273 Considering the correlation between DElncRNAs and clinical characteristics, we found 10

274 DElncRNAs related to pathologic stage, T stage and N stage. Though the function of these

275 lncRNAs are not well investigated, they also may be as therapeutic targets and present a new road

276 to understand the pathogenesis of TSCC. HCG22 was negatively associated with tumor stage in

277 our study. Similarly, Zhao *et al*. finds a significant inverse correlation between HCG22 and tumor

278 size (Zhao et al. 2018a). Regarding OSCC, HCG22 was found to be correlated with poor survival

279 basing on TCGA database. However, Feng *et al*. did not find a similar association using their

280 clinical data (Feng et al. 2017). LINC00355 was positively correlated with distant metastasis,

281 lymphatic metastasis and tumor stage, and negatively correlated with prognosis in colon

282 adenocarcinoma (Zhang et al. 2018b). Upregulated LINC00355 was also associated with poor

283 prognosis in prostate cancer (Jiang et al. 2018). In our study, LINC00355 was positively associated

284 with tumor stage, however, it was not identified to be a prognosis biomarker.

285

286 Another DElncRNA, SFTA1P has been reported to be a tumor suppressor by inhibiting cell

287 proliferation, invasion and migration in gastric cancer (Ma et al. 2018). It also increases cisplatin

288 chemosensitivity in lung squamous cell carcinoma; similarly, elevated SFTA1P indicates a longer

289 life (Li et al. 2017a). However, we found SFTA1P was up-regulated in TSCC, especially in lymph

290 node metastasis tumor. Thus, we speculate that SFTA1P may act as an oncogene. Our ceRNA

291 network indicated that SFTA1P up-regulated the expressions of IL-11 or HOXC8 by binding mir-

292 211, and elevated IL-11 or HOXC8 contributes to development of cancer , such as breast cancer

293 (Cai et al. 2018; Li et al. 2014) and non-small cell lung cancer (Liu et al. 2018; Zhao et al. 2018b).

294 Therefore, it is reasonable that our results are not consistent with other studies. Though the

295   functions of IL-11 and HOXC8 are not validated in TSCC, our results also offer new ideas for the

296   development of TSCC.

297

298   Our study identified some valuable lncRNAs that are associated with carcinogenesis and survival.

299   Few of them have been validated in vitro and in vivo, however, none of the lncRNAs were

300   validated in TSCC. Hence, these valuable lncRNAs still need to be verified, and our ceRNA

301   network, which was build based on high-throughput sequencing, requires further verification.

302   **Conclusion**

303   Taken together, we identified aberrantly expressed mRNAs, lncRNAs, and miRNAs and then

304   successfully constructed a functional ceRNA network for TSCC tumorigenesis. Key lncRNAs

305   should be check closely for association with survival and clinical features in TSCC patients, which

306   provide novel lncRNAs as potential prognosis biomarkers and therapeutic targets.

307

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Clinicopathological characteristics of 122 patients with tongue squamous cell carcinoma

1 **Table 1**:

2 **Clinicopathological characteristics of 122 patients with tongue squamous cell carcinoma**

Characteristic	Subtype	No. of cases (%)
Age (years)	< 60	59 (48.4)
	⩾ 60	63 (51.6)
Gender	Male	85 (69.7%)
	Female	37 (30.3%)
Pathologic stage	Stage I	13 (10.7%)
	Stage II	19 (15.6%)
	Stage III	30 (24.6%)
	Stage IV	60 (49.1%)
Pathologic T	T1	19 (15.6%)
	T2	42 (34.4%)
	T3	40 (32.8%)
	T4	21 (17.2%)
Pathologic N	N0	49 (40.2%)
	N1	17 (13.9%)
	N2	51 (41.8%)
	N3	1 (0.8%)
	NX	4 (3.3%)
Vital status	Alive	72 (59%)
	Dead	50 (41%)

Significantly enriched KEGG pathways regulated by DEmRNAs in tongue squamous cell carcinoma

1 **Table 2**:

2 **Significantly enriched KEGG pathways regulated by DEmRNAs in tongue squamous cell**

3 **carcinoma**

ID	Description	pvalue	p.adjust	Count
hsa04970	Salivary secretion	2.93E-12	8.40E-10	31
hsa04974	Protein digestion and absorption	9.63E-11	1.38E-08	29
hsa04512	ECM-receptor interaction	3.42E-08	3.27E-06	24
hsa04060	Cytokine-cytokine receptor interaction	2.38E-06	0.000171	49
hsa04020	Calcium signaling pathway	8.26E-06	0.000402	34
hsa04510	Focal adhesion	8.41E-06	0.000402	36
hsa00500	Starch and sucrose metabolism	2.25E-05	0.000924	12
hsa04261	Adrenergic signaling in cardiomyocytes	5.94E-05	0.00213	27
hsa05414	Dilated cardiomyopathy (DCM)	0.000147	0.004373	19
hsa05410	Hypertrophic cardiomyopathy (HCM)	0.000152	0.004373	18
hsa04976	Bile secretion	0.00022	0.00573	16
hsa03320	PPAR signaling pathway	0.000363	0.008688	16
hsa04973	Carbohydrate digestion and absorption	0.000833	0.018399	11
hsa04971	Gastric acid secretion	0.001295	0.026541	15
hsa04260	Cardiac muscle contraction	0.001957	0.035163	15
hsa00910	Nitrogen metabolism	0.00196	0.035163	6
hsa04964	Proximal tubule bicarbonate reclamation	0.002199	0.035586	7
hsa04610	Complement and coagulation cascades	0.002232	0.035586	15
hsa05146	Amoebiasis	0.002566	0.038758	17
hsa00830	Retinol metabolism	0.003408	0.048901	13

GO terms enriched by 16 DEmRNAs that were involved in the ceRNA network

1   Table 3: GO terms enriched by 16 DEmRNAs that were involved in the ceRNA network

Category	Term	PValue	Genes
GOTERM_BP_FAT	GO:0007167~enzyme linked receptor protein signaling pathway	0.0161	CHRDL1, PTPRT, GREM2
GOTERM_BP_FAT	GO:0030509~BMP signaling pathway	0.0257	CHRDL1, GREM2

The correlations between DElncRNAs in the ceRNA network and clinical characteristics of tongue squamous cell carcinoma

1 **Table 4**

2 **The correlations between DElncRNAs in the ceRNA network and clinical characteristics of**

3 **tongue squamous cell carcinoma**

Comparisons	Downregulated	Upregulated
Pathologic Stage (Stage III + IV vs. stage I + II)	HCG22,LINC00492,AL035696.1	LINC00355,PSORS1C3
Pathologic_T (T3 + T4 vs. T1 + T2)	ERVH48-1	LINC00520, PSORS1C3
Pathologic_N (N2 + N3 vs. N0+ N1)	LINC00492, ERVH48-1	AC112721.1, AL139147.1, SFTA1P

Figure 1

Flow chart of the ceRNA network construction.

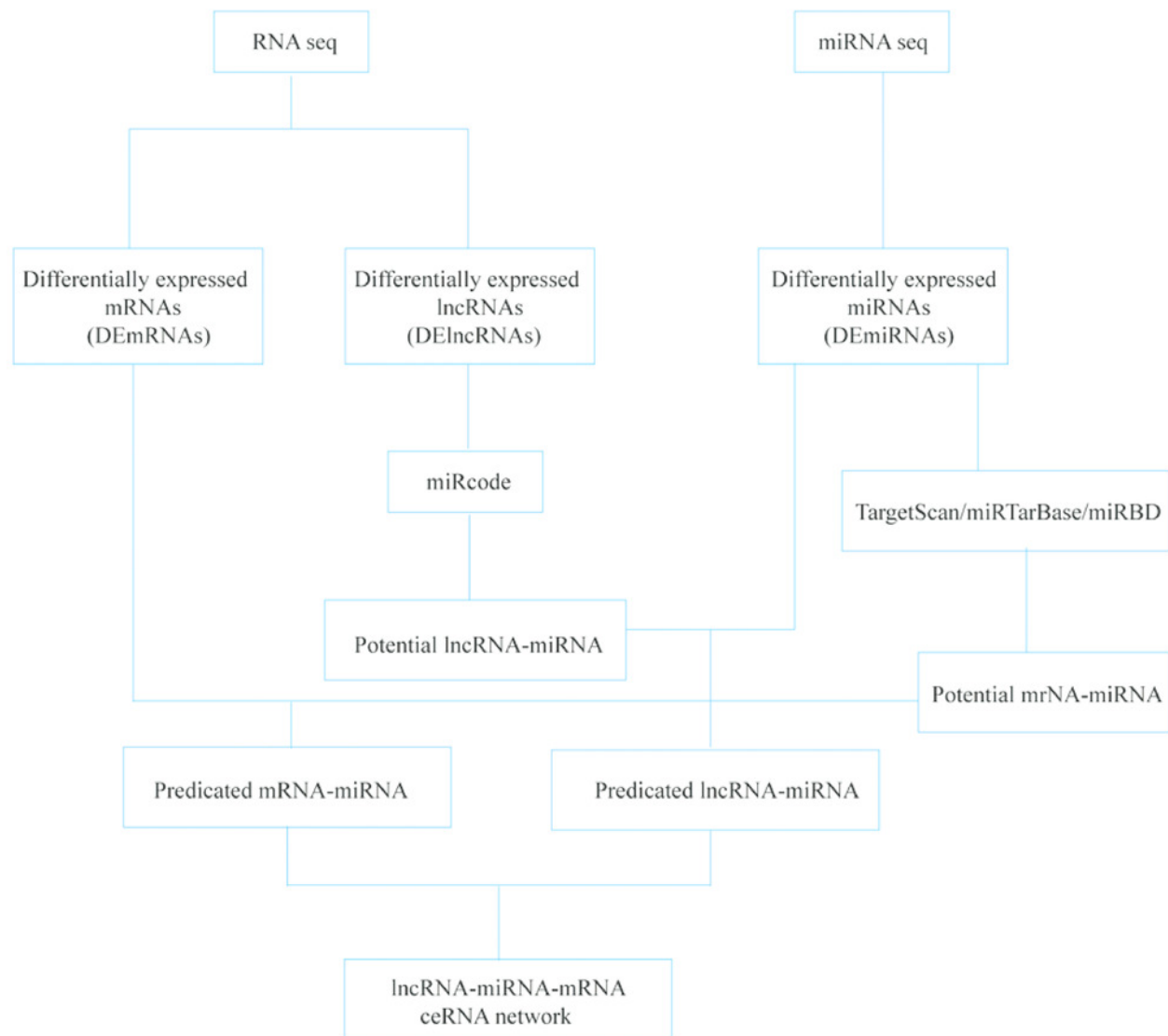
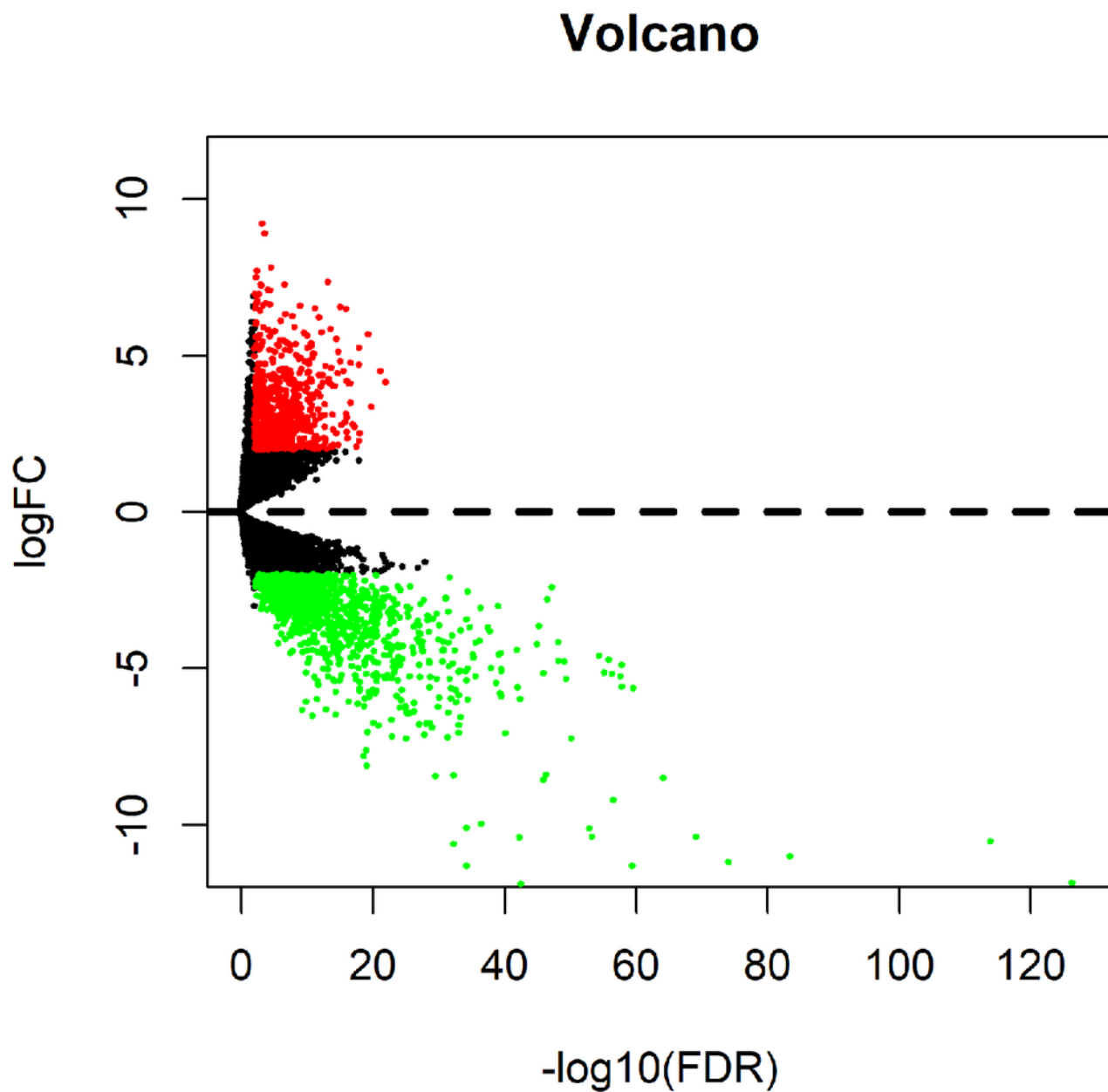



Figure 2

Volcano map of DEmRNAs.

Red spots represent up-regulated genes, and green spotsrepresent down regulated genes.

GO and KEGG pathway analyses

(A) Top 5 cellular component terms of dysregulated genes in the GO analysis. (B) Top 5 biological processes terms of dysregulated genes in the GO analysis. (C) Top 5 molecular function terms of dysregulated genes in the GO analysis. (D) Top10 pathways of dysregulated genes in the pathway analysis. (E) Cytokine-cytokine receptor interaction map from KEGG analysis.

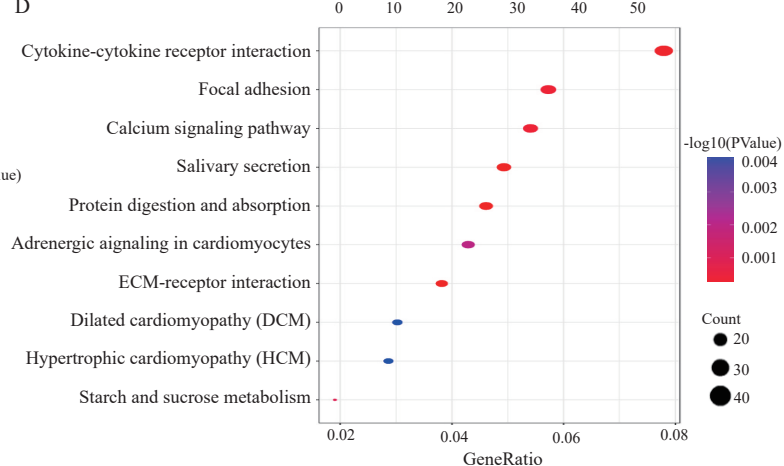
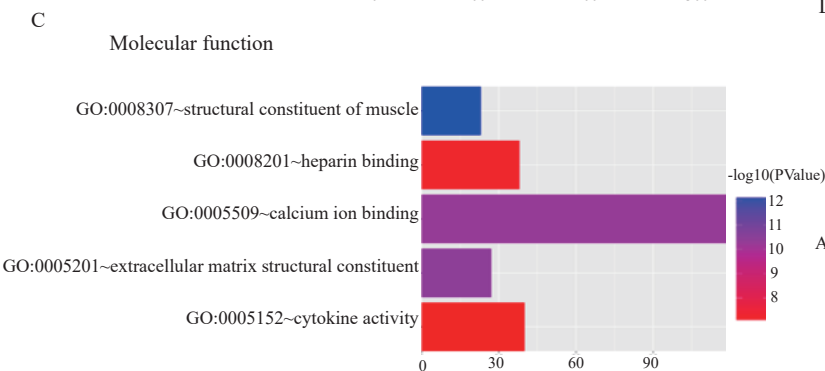
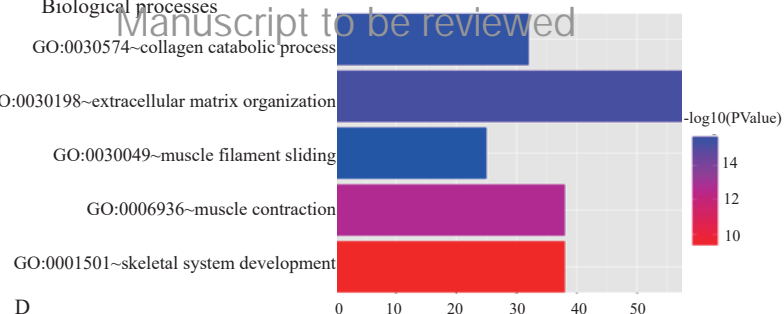
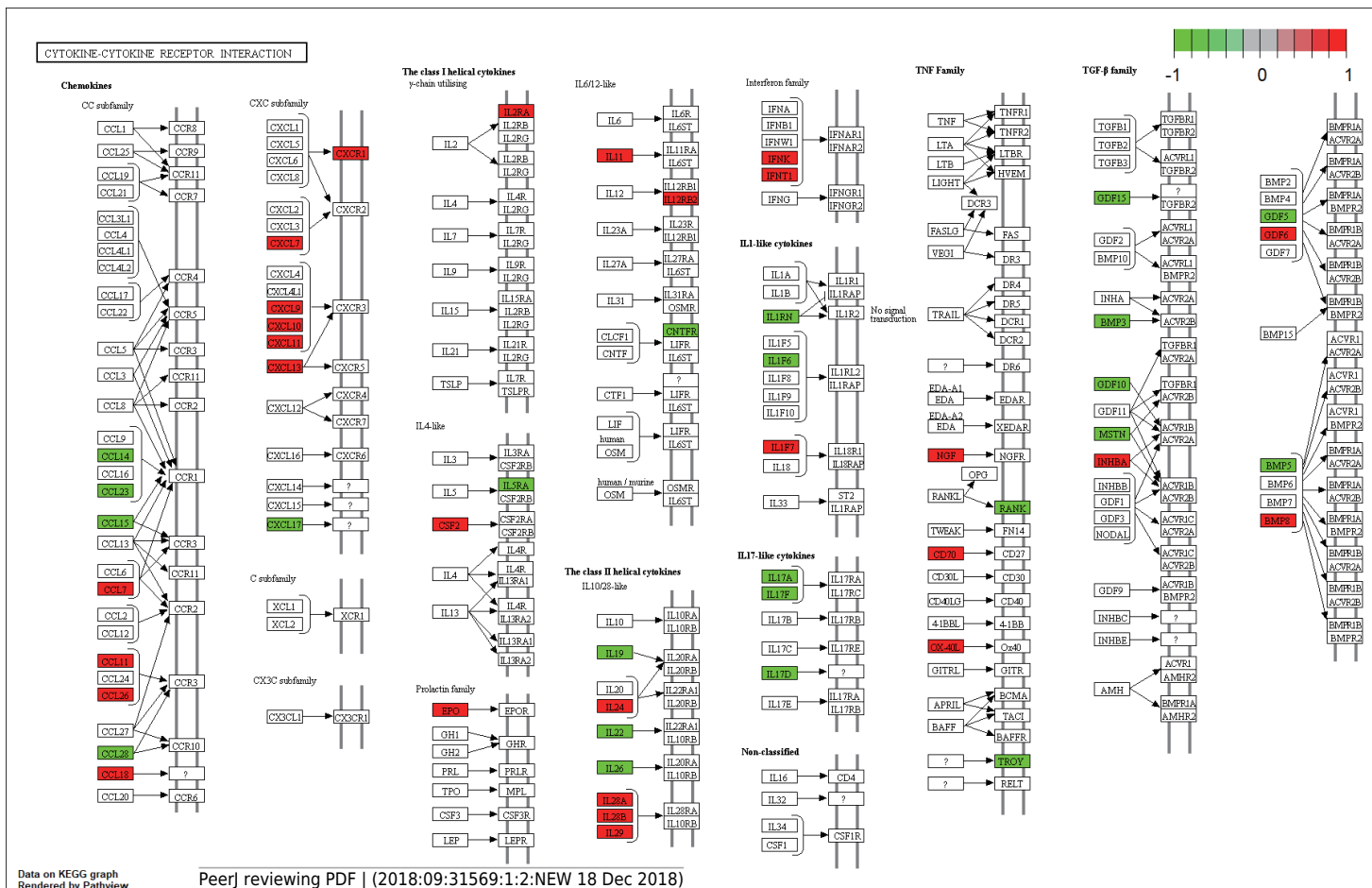



Figure 4

Volcano map of DElncRNAs.

Red spots represent up-regulated genes, and green spotsrepresent down regulated genes.

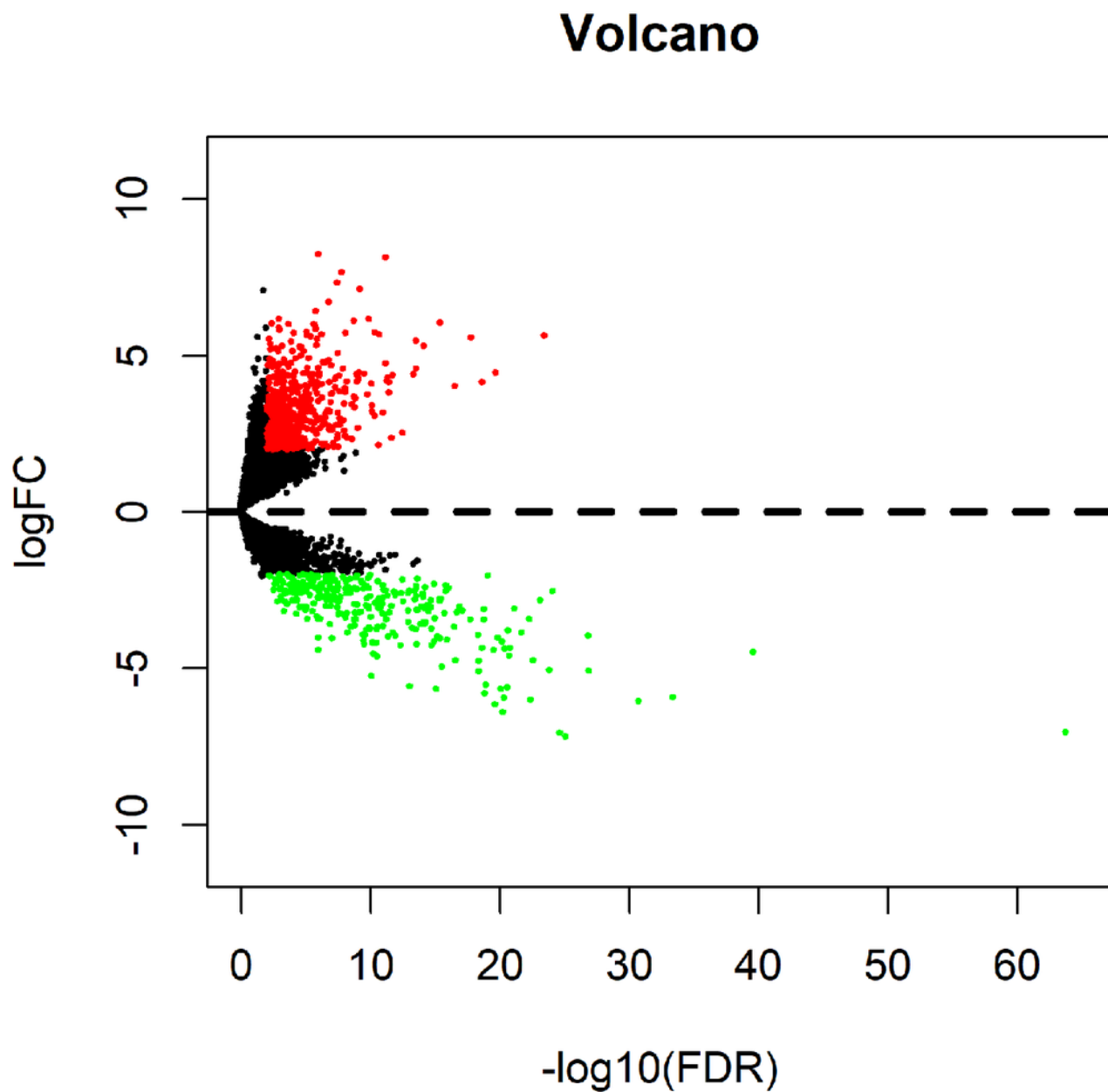



Figure 5

Volcano map of DEmiRNAs. Red spots represent up-regulated genes, and green spotsrepresent down regulated genes.

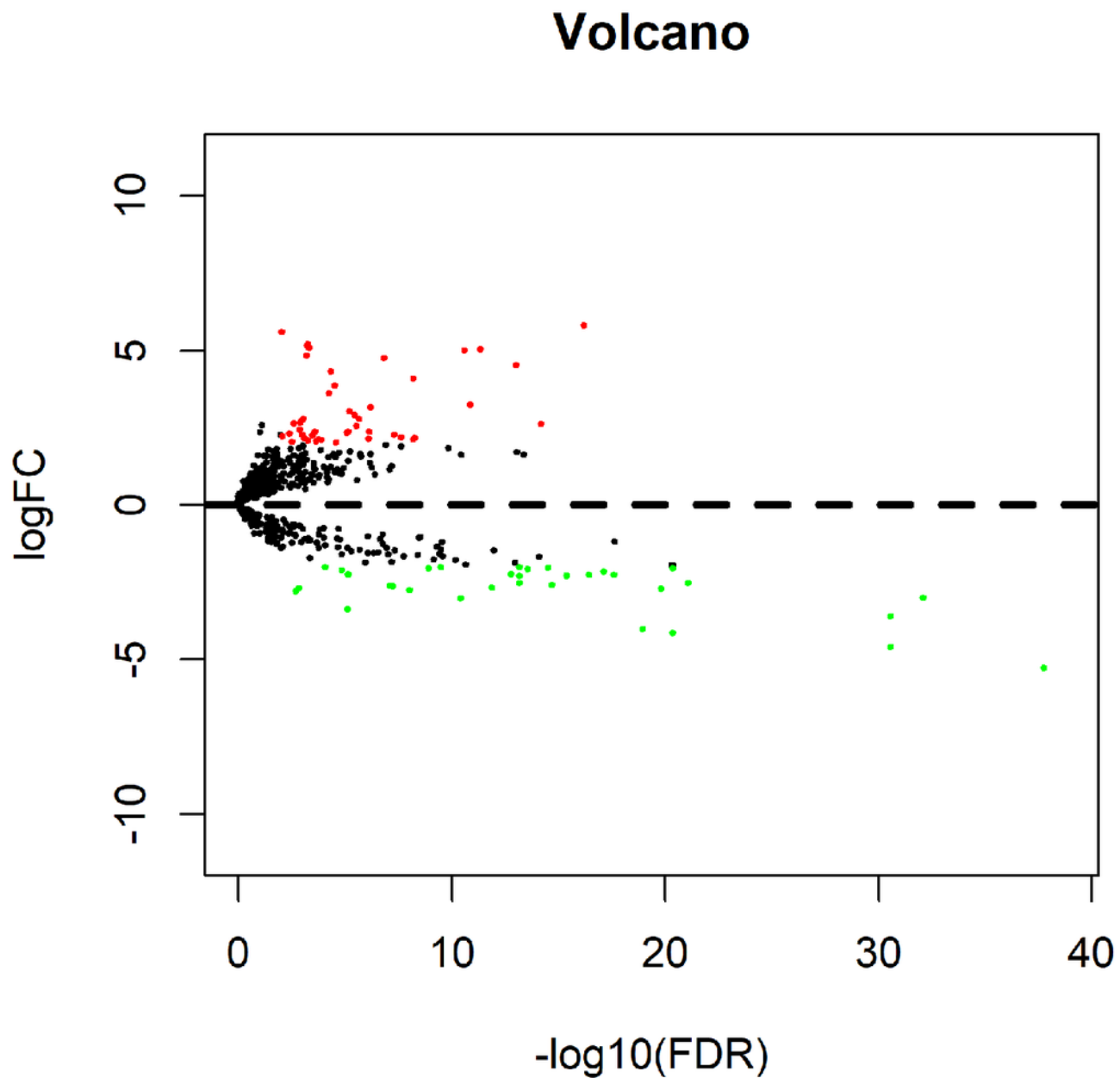



Figure 6

DElncRNAs mediated ceRNA regulatory network in TSCC. The red nodes indicate expression up-regulation, and blue nodes indicate expression down-regulation. LncRNAs, miRNAs and mRNAs are represented by diamond, rounded rectangle, and ellipse, respectively.

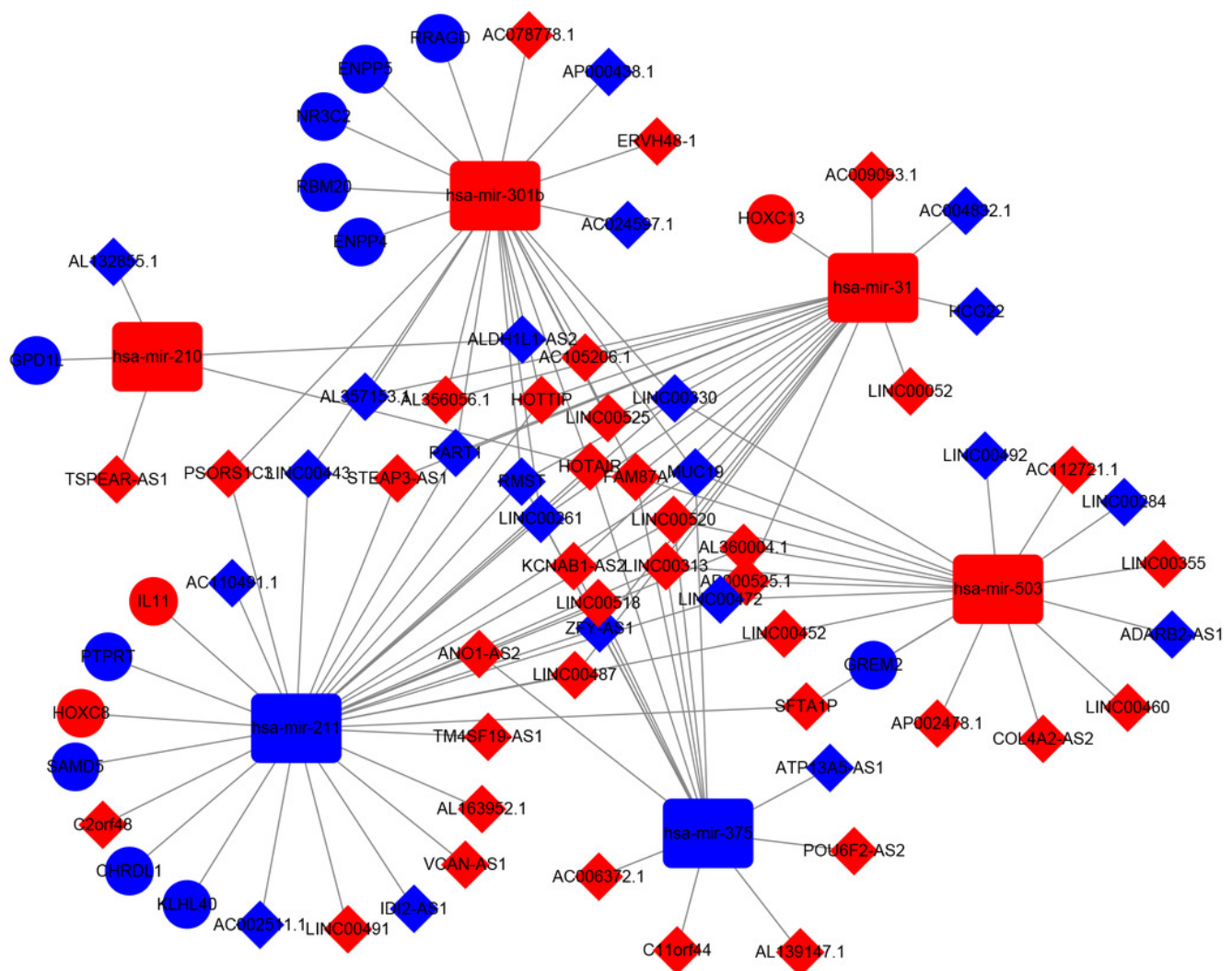
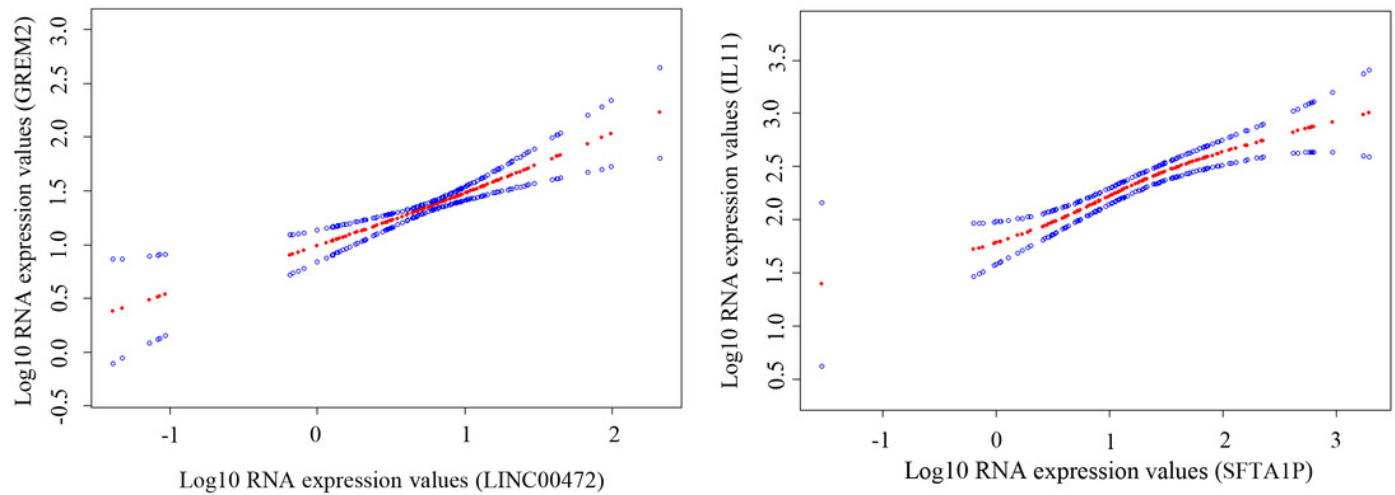



Figure 7

Linear regression of PART1 vs SAMD5 expression level.

Kaplan-Meiercurve analysis of DElncRNAs and overall survival rate in tongue squamous cellcarcinoma patients.