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***Basic Study***

**Construction of an oesophageal cancer-specific ceRNA network based on miRNA, lncRNA and mRNA expression data**

Xue WH *et al*. Construction of an oesophageal cancer-specific ceRNA network

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**Abstract**

***AIM***

To explore the expression profile of micro-RNA (miRNA), long non-coding RNA (lncRNA) and messenger RNA (mRNA) in oesophageal squamous cell carcinoma (ESCC) in order to construct an oesophageal cancer-specific competing endogenous RNA (ceRNA) network.

***METHODS***

In this work, the expression data of miRNA, lncRNA and mRNA of ESCC was obtained. An oesophageal cancer-specific ceRNA network was constructed and investigated.

***RESULTS***

CeRNAs have the ability to reduce miRNA's targeting activity, leading to the de-repression of specific mRNAs with common miRNA response elements. CeRNA interactions make a critical effect in gene regulation and cancer development.

***CONCLUSION***

This study suggests a novel perspective on potential oesophageal cancer mechanisms as well as novel pathways for modulating ceRNA networks for treating cancers.

**Keywords**: Competing endogenous RNA; micro-RNA; Long non-coding RNA; Messenger RNA, Oesophageal squamous cell carcinoma

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**Core tip:** Competing endogenous RNA (ceRNA) may play acritical role in tumourigenesis; perturbations to ceRNA networks would result in the progression of oesophageal squamous cell carcinoma (ESCC). However, the role of competing endogenous RNA (ceRNA) in ESCC has not been comprehensively explored. This study was designed to investigate the expression profile of micro-RNA, long non-coding RNA and messenger RNA in ESCC to elucidate an oesophageal cancer-specific ceRNA network. Our report revealspotential molecular mechanisms of oesophageal cancer progression and suggests a novel approach to cancer therapeutics in the regulation of ceRNA networks.

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**INTRODUCTION**

Oesophageal squamous cell carcinoma (ESCC) has beenthe sixth leading death reason of cancer[[1](#_ENREF_1)]. According to the official statistics in America, more than 18000 cases were newly diagnosed with 15000deaths from oesophageal cancer in 2014, representing 5% of all cancer death[[2](#_ENREF_2)]. Recently, the incidence and mortality rate of ESCC have decreased in North America and Europe[[3](#_ENREF_3)]. However, there is a significant ethnic and geographic distribution in ESCC and it has been highly prevalent in China and other Asia countries.The presence of familial aggregation suggest that the risk factors for ESCC include environmental and genetic factors[[4](#_ENREF_4)]. When ESCC is diagnosed, most patients have already progressed to be advanced or metastatic. Thus, as there is no longer an opportunity for radical surgery, radiation and chemotherapy become the major palliative treatments[[5](#_ENREF_5)].

ESCC has been a complicated cancer and the tumorgenesis and cancer development has been closely associated with the aberrant expressions of protein coding mRNAs and non-coding RNAs[[6](#_ENREF_6)]. Approximately 98% of the human genome has been the non-coding RNAs, suggesting their promising effects on physiological and pathological processes[[7](#_ENREF_7)]. Micro-RNA (miRNA) suppresses the translation and induces the degradation of mRNA, thus modulatingthe expression and function of gene[[8](#_ENREF_8)]. The miRNAs have been proved to make critical effects in tumorigenesis, and the role of miRNA has been relatively well understood[[9](#_ENREF_9)]. LncRNAsarenewlyfound non-coding RNAs which were proved to participate in many diseases[[10](#_ENREF_10)]. However, the functional role of the large number of lncRNAs in oesophageal squamous cell carcinoma remains unclear.

Many studies have confirmed that ceRNAs are able to act as a sponge for miRNAs. The activity of miRNAs could be modulated with the variation of ceRNA abundance from individual genes[[11](#_ENREF_11)].Interactions between ceRNAs through sharing miRNAs indicate a new pathways of gene regulation which makes key effects in the cancer progression[[12-14](#_ENREF_12)]. CeRNAs act as miRNA’s molecular sponges through binding with miRNA (also known as miRNA response elements, MRE), thus inhibiting miRNA targeted genes[[15](#_ENREF_15)].The discovery of ceRNAs requires reassessing our understanding of gene regulatory networks and raising the probability of proposing a new molecular mechanism. Both of which may be the potential targets for gene treatment[[16-18](#_ENREF_16)].

Lately, complex and multidimens molecular maps of large cancer crowd were uncovered by research alliance such as The Cancer Genome Atlas (TCGA). With these information, a synthetic analysis could be performed on the association between molecular alterations and certain cancer type [[19-21](#_ENREF_19)]. Many ceRNAs were revealed in various cancer types. Until now, few study has been performed on clarifying the association among lncRNAs, miRNAs and mRNAs in ESCC. Therefore, in this study, the ceRNA network in ESCC was constructed, which may help to elucidatethe specific biological mechanisms of ESCC progression.

**MATERIALS AND METHODS**

***Data sets and pre-processing***

The expression data of miRNA and mRNA in 101 oesophageal cancer patients was collected from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI) with login numbers of GSE45670[[22](#_ENREF_22)] (38 patients, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE45670), GSE26886[[23](#_ENREF_23)](28 patients,http://www.ncbi.nlm.nih.Gov/geo/query/acc.cgi?acc=GSE26886),GSE17351(10samples, http://www.Ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE17351),GSE55856[[24](#_ENREF_24)](216patients, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55856), and GSE66274[[25](#_ENREF_25)](60patients, http://www.ncbi.nlm.nih.Gov/geo/query/acc.cgi?acc=GSE66274). Various miRNA targets and oesophageal cancer data sets were also applied for assessing the reliability of this approach, aimed to constructing the ceRNA network. Under these circumstances, we also implanted the expression profiles of 170 matched miRNA and mRNA of oesophageal cancer patients from TCGA[[26](#_ENREF_26)].Annotation information of lncRNAs was obtained with Affymetrix Human Genome U133 Plus 2.0 arrays. The network of protein-protein interaction was constructed involving STRING database system.

***Functional analysis***

DAVID (Databases for Annotation, Visualization and Integrated Discovery) was included to determine the pathways of KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO Term biological processes were enriched with central genes recommunities in the ceRNA network. The p-values<0.05 indicated enriched gene sets[[27](#_ENREF_27)].

***Network visualisation and community detection***

The miRNA-LncRNA-mRNA interaction network was visualised by Cytoscape Software, and topology analysis was performed with network analyser plugin. MCODE plugin was also applied (with its default parameters) to figure out the communities (dense clusters) in network[[28](#_ENREF_28)].

***Bioinformatics analysis on the associated expressions of lncRNAs, miRNA, and mRNAs***

The single-stranded miRNAs would bind the mRNA transcripts, thus the post-transcriptional regulation of mRNA has been set up according to the relationships among miRNAs, lncRNAs and mRNAs[[29](#_ENREF_29),[30](#_ENREF_30)]. First, the miRNAs, lncRNAs and mRNAs which were differentially expressed were chosen from ESCC specimens or corresponding normal tissues. The differential expressions of miRNAs, lncRNAs and mRNAs were identified with standard selection criteria, which were set at *P <* 0.05 and fold change > 2. In addition, the co-expression network of miRNA, lncRNA and mRNA was constructed according to the connections among the differentially expressed miRNA, lncRNAs and mRNAs.

***Statistical analysis***

The data was expressed as the mean ± standard deviation (SD). Student’s t-test and ANOVA were applied in the statistical analysis for comparing two groups and multiple groups analysis results, respectively[[31](#_ENREF_31)]. The fold change and Student's t-test was applied to analyse the significance of microarray analysis. The *P <* 0.05 indicated statistically significant difference. The P-value was corrected with false discovery rate. The differentially expressed lncRNAs, miRNAs and mRNAs was expressed as fold change values (*P <* 0.05).

**RESULTS**

***Clustering analysis***

We used unsupervised hierarchical clustering analysis in this study. Cases were organized by clustering analysis on the basis of immunostaining profiles, and cases were placed together with similar immuneprofiles as neighbouring rows in a clustergram. The dendrogram is applied to demonstrate the relationship among cases and immunemarkers. The branch length of dendrogram indicated the correlations in immunostaining results. The unsupervised hierarchical cluster analysis demonstrated the correlation of expression maps between biological replicates and group conditions (Figure 1A, B and C).

***Cancer-specific lncRNAs, miRNA and mRNAs in oesophageal squamous cell carcinoma***

The inter-connected complexity of physiological, cellular and molecular functions has increasingly grown, thus novel approaches are required to simultaneously demonstrate multiple datasets[[32](#_ENREF_32)]. There are multiple intersecting regions (generally as circles)in Venn diagram, which enable the description of all logical relations among various data sets[[33](#_ENREF_33)].Here, we selected 21 miRNAs from GSE66274 and GSE55856;228 mRNAs from GSE26886, GSE17351 and GSE45670; and 31 lncRNAs from GSE26886, GSE17351, and GSE45670 (Figure 2A, B and C).

***mRNA GO analysis in oesophageal squamous cell carcinoma***

In the Gene Ontology (GO) database, there are structured, controlled vocabularies and classifications covering several molecular and cellular biology domains. GO has been applied for the annotation of genes and sequences[[34](#_ENREF_34)].

The 228 genes with differential expression were analysed with the GO database. The enrichment of these genes was analysed in specific pathways. Enrichment analysis is used to evaluate the significance of the function, which helps provide GO terms with a more definitive function demonstration[[35](#_ENREF_35)]. As shown in Table 1, the most highly enriched GO path was ‘extracellular matrix organization’. The genes in extra cellular matrix organization’ were MMP3, MMP10, LAMA3, MMP9, MMP13, COL11A1, BMP7, MMP12, LAMC2, COL27A1, ITGB4, PDGFRA, ADAMTS2, IBSP, COL10A1, COL7A1, MMP11, MFAP2, MMP1 and COL1A1. The second most highly enriched GO path was **‘**collagen catabolic process**’** (Figure 3).

***mRNA pathway analysis in oesophageal squamous cell carcinoma***

Kyoto Encyclopedia of Genes and Genomes (KEGG) systematically interpreted sequence data by computerizing biochemical pathways and other types of molecular interactions[[36-38](#_ENREF_36)]. The results showed that the most highly enriched pathway was Transcriptional misregulation in cancer (Table 2). The genes in transcriptional misregulation in cancer’ were TCF3, CXCL8, SIX1, IGFBP3, MLF1, PLAU, MEIS1, HOXA10, MMP9, SIX4, HPGD, and MMP3. The second most highly enriched pathway was ‘ECM-receptor interaction’ (Figure 4).The genes in ECM-receptor interaction’ were ITGB4, COL1A1, COL11A1, ITGA3, LAMC2, SPP1, LAMB3, and IBSP.

***mRNA proteinre gulation network analysis***

Protein-protein interactions have been not only direct binding, but also indirectactions[[39](#_ENREF_39)]. Genomic associations between protein-coding genes are provide for interring functional links between proteins. Genes that have the same function are often located in close to each other and tend to participate in gene-fusion events[[40-42](#_ENREF_40)]. The database STRING has been used to analyse these associations[[43](#_ENREF_43)].We input the shared differential mRNAs from GSE45670, GSE26886 and GSE17351into the STRING database. Several nodes with high degrees were COL27A1, COL7A1, COL1A1, ITGB4, ITGA3, SERPINE1, MMP1, MMP9, and MMP10 (Figure 5).

***ceRNA network analysis***

Competing endogenous RNAs (ceRNAs) share common MRE and hence regulate RNA transcripts by competedly binding with general microRNA molecules[[44](#_ENREF_44)]. The ceRNAs could be relieved from microRNA-mediated repression and their expression levels could be positively modulated [[45](#_ENREF_45)].The discovery of ceRNAs provides many implications for cancer, which have already been extensively discussed[[46](#_ENREF_46)].

Based on the expression profiles of specific miRNA, lncRNA and mRNA in patients with oesophageal cancer, the ceRNA network was constructed with a computational method proposed for this study (Figure 6) and it was drawn with Cytoscape 3.0[[47](#_ENREF_47)]. The ceRNA network has integrated the interactions of miRNA-lncRNA-mRNA by negative regulation.

There are 74 nodes in the oesophageal cancer-specific ceRNA network. The degrees of the hsa-miR-93-5p, hsa-miR-34c-5p and hsa-miR-18a-3p nodes were 14, 12 and 11, respectively. The density of our ceRNA network is confirmed with the high degree of nodes, suggesting the common competitions among RNAs for oesophageal cancer. The modes degree is also observed to follow power law distribution. For the miRNA, the expression of hsa-miR-196b-5p, has-miR-34c-5p and has-miR-18a-3p were up-regulated. However, the expression levels were down-regulated for has-miR-30a-3p, has-miR-150-5p and has-miR-133a-3p. All these analysis results suggest the scale-free ceRNA network in oesophageal cancer and the biological significance may be reflected by the topological structures including the hubs, nodes and communities.

***mRNA survival curves***

To further identify the key mRNAs that were associated with prognostic characteristics from 170ESCC patients, the overall survival has been profiled with the univariate Cox proportional hazards regression model (*P <* 0.05).Among the 6 significant mRNAs, the overall survival was negatively related to 5mRNA transcripts (STC2, SLC6A1, MMP12, EPCAM, and EPB411L4B) (*P <* 0.05) while positively associated with the remaining mRNA transcript (LAMC2) (*P <* 0.05) (Figure 7A-F).

**DISCUSSION**

It will be necessary to explore ceRNA cross-talk across multiple cancer types[[48](#_ENREF_48)]. TCGA was formed to meet these needs and its vast data set provides us with an unprecedented opportunity to systematically analyze the ceRNA network in cancer. These interesting findings led us to construct the oesophageal cancer-specific ceRNA network.

In this work, clustering analysis, mRNA GO analysis, mRNA pathway analysis, and mRNA protein regulation network analysis in oesophageal squamous-cell carcinoma were conducted to construct the ceRNA network. The results showed that the most highly enriched Gene Ontology path was ‘extracellular matrix organization’. The genes in ‘extracellular matrix organization’ were MMP3, MMP10, LAMA3, MMP9, MMP13, COL11A1, BMP7, MMP12, LAMC2, COL27A1, ITGB4, PDGFRA, ADAMTS2, IBSP, COL10A1, COL7A1, MMP11, MFAP2, MMP1 and COL1A1. Advances in structural genomics will make it possible to reveal the complete genome sequence of hundreds of organisms. The ceRNA network analysis indicated that the degree of has-miR-93-5p as an up-regulated gene was 14. All these results are relevant to the further development of treatment of oesophageal cancer.

Based on Kaplan-Meier analysis, overall survival was negatively related to 5 mRNA transcripts (STC2, SLC6A1, MMP12, EPCAM, and EPB41L4B) (*P <* 0.05) and it was positively associated with the remaining mRNA transcript (LAMC2) (*P <* 0.05). These mRNAs could be candidate and specific biomarkers for the diagnosis, prognosis and classification of ESCC.

In this research, a computational approach has been proposed for the construction of ceRNA network based on existing data of esophageal cancer. In this network, the junction nodes indicate paired gene pair in competing mRNA library. We observed that the ESCC-specific ceRNA network has been shown to be scale-free, and the dense clusters in the network are associated with promising markers. The results of mRNA pathway analysis showed that the most highly enriched pathway was transcriptional misregulation in cancer. In addition, overall survival was negatively related to the genesSTC2, SLC6A1, MMP12, EPCAM, and EPB41L4B,whileit was positively associated with LAMC2.These confirmed results suggested that the biological mechanism of ESCC could be discovered with the constructed ceRNA network. Importantly, a simple framework has been provided in our work for the construction of a ceRNAs network, which can be used to a variety of biological issues, such as ESCC and its biological processes. In short, cancer-specific miRNA, lncRNA and mRNA in ESCC can be successfully identified in present study with bioinformatics analysis from large scale samples. Moreover, understanding the ceRNA network in ESCC may reveal potential intended targets for cancer sub-populations or across cancers. This work suggests new approaches for studying the role and mechanism of ceRNA in human cancers using publicly available genomic data.

**ARTICLE HIGHLIGHTS**

***Research background***

Oesophageal squamous cell carcinoma (ESCC) has been one of the most prevalent oesophageal cancer, its development is closely related to the abnormal expression of not only protein-encoding mRNA, but also non-coding RNA. Competitive endogenous RNAs (ceRNAs)regulatory networks include mRNAs, miRNA, lncRNA and circular RNAs, resulted in the cancer pathogenesis by regulating each other’s expression. However, their function has not been clarified in ESCC. Therefore, construction of a ceRNA network for ESCC may help to study the biological mechanisms in oesophageal cancer.

***Research motivation***

It is necessary to explore the CeRNA cross-talk across multiple cancer types. These issues have been addressed by TCGA, which provides large data set enabled us with an unprecedented opportunity to synthetically explore the ceRNA network for various cancers. These findings led us to construct an oesophageal cancer-specific ceRNA network. The present study found that there were mRNAs, miRNAs, lncRNAs in the ceRNA regulatory network, which might play a critical role in ESCC, and the abnormality in ceRNA regulatory networks would lead to the initiation and progression of ESCC.

***Research objectives***

Clustering analysis, mRNA GO analysis, mRNA pathway analysis, and mRNA protein regulation network analysis in oesophageal squamous-cell carcinoma were conducted to construct the ceRNA network. These confirmed results suggested that the biological mechanisms in the development of ESCC may be indeed revealed with the ceRNA network. Importantly, a simple framework was proposed in this study for constructing ceRNA networks in various biological processes including the study on ESCC.

***Research methods***

The expression data of miRNA and mRNA of 101 patients with esophageal cancer were obtained from the National Center for Biotechnology Information Gene Expression (NCBI). The expression profiles of 170 matched miRNAs and mRNA in esophageal cancer patients were also obtained from TCGA (The Cancer Genome Atlas). The KEGG pathway (Kyoto Gene and Genome Encyclopedia) and Go Term biological processes were identified with DAVID (Annotation, visualization, and comprehensive discovery databases).The results were rich in Cytoscape software, and were topologically analyzed by Cytoscape's Network Analyzer Plugin. In addition, communities (dense clusters) in the network was found with Cycloscape, using the MCODE plug-in (the default). Based on the relationship between miRNAs, lncRNAs and mRNAs, strands of stranded miRNAs have been established following transcriptional regulation of single nucleotide sequence-associated mRNA transcripts.

***Research results***

The results showed that the most highly enriched Gene Ontology pathway was ‘extracellular matrix organization’. The genes in ‘extracellular matrix organization’ were MMP3, MMP10, LAMA3, MMP9, MMP13, COL11A1, BMP7, MMP12, LAMC2, COL27A1, ITGB4, PDGFRA, ADAMTS2, IBSP, COL10A1, COL7A1, MMP11, MFAP2, MMP1 and COL1A1. The advances in structural genomics may reveal the complete genomic sequence of thousands of organisms. Thece RNA network analysis indicated that the degree of has-miR-93-5p as an up-regulated gene was 14. All these results are meaningful for further development in treatment of oesophageal cancer.The overall survival was negatively associated with five mRNAs (STC2, SLC6A1, MMP12, EPCAM, and EPB41L4B) (*P <* 0.05), and it was positively related to the remaining mRNA (LAMC2) (*P <* 0.05). These mRNAs can be applied as promising specific biomarkers for ESCC. The significantly dysregulated mRNAs and miRNAs need to be validated in the future.

***Research conclusions***

A ceRNA network was identified in gene regulation and cancer progression in ESCC. The overall survival was negatively related to five mRNAs (STC2, SLC6A1, MMP12, EPCAM, and EPB41L4B). A ceRNA network makes a significant effect in gene regulation and cancer development in ESCC. This study provides potential mechanisms in the development of oesophageal cancer and suggests new methods to modulate ceRNA networks for cancer treatment. CeRNA networks are implicated in the development of ESCC.A relationship between lncRNAs, miRNAs and mRNAs in oesophageal squamous cell carcinoma was constructed by bioinformatics analysis. Cytoscape software shows the miRNA-lncRNA-mRNA interaction network and the Cytoscape network analyzer plug-in for topology analysis. In addition, the communities (dense clusters) in the network were found with the MCODE plug-in (with the default parameters).The bioinformatics analysis was performed on the co-expression of lncRNAs, miRNA, and mRNAs. The results showed that the most highly enriched GOpath was ‘extracellular matrix organization’, which was associated with ESCC. By examing the ceRNA network, the nodes degrees were observed to follow a power law distribution. The expression of hsa-miR-196b-5p, has-miR-34c-5p and has-miR-18a-3p were up-regulated. However, the levels of has-miR-30a-3p, has-miR-150-5p and has-miR-133a-3p were down-regulated. The ceRNA network is associated with cancer progression. The understanding of ceRNA networks in ESCC may help uncoverun expected potential therapeutic targets that would be available in cancer sub-populations or across cancers.

***Research perspectives***

Understanding the ceRNA network is of significance in identifyingpotential therapeutic targets for ESCC. Our study focuses on the function and mechanism of ceRNA in ESCC using publicly available genomic data.

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**Figure 1 Cluster analysis of differentially expressed profiles.** A: mRNAs; B: lncRNAs and C: miRNAs in tumour tissues *vs* adjacent non-tumour tissues. The result of hierarchical cluster analysis shows distinguishable expression profiles between samples. The rows show differentially expressed miRNAs, lncRNAs and mRNAs, while the columns show three paired samples. Red represents high expression and green represents low expression.

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**Figure 2 Venn diagram analysis of differentially expressed genes in comparison groups.** A: miRNAs; B: mRNAs; C:lncRNAs.

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**Figure 3 Top 23 GO enrichment terms for differentially expressed intersection mRNAs.** GO analysis of the common differentially expressed mRNAs.

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**Figure 4 Top 23 pathway enrichment terms for differentially expressed intersection mRNAs.** KEGG pathways of the common differentially expressed mRNAs.

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**Figure 5 mRNA protein regulation network analysis.** The protein-protein interaction networks constructed by Cytoscape software. Proteins are represented with colour nodes, and interactions are represented with edges.

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**Figure 6 The lncRNA-miRNA-mRNA ceRNA network.** The rectangles indicate miRNAs and circles represent mRNAs. The red indicates upregulation and green indicates downregulation.

C:\Users\admin\Desktop\FIGURE 7.tif

**Figure 7 Kaplan-Meier survival curves for eight mRNAs associated with overall survival.** Log-rank tests were performed to evaluate the survival differences between the two curves. Horizontal axis: Overall survival time, days; Vertical axis: Survival function.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Goid** | **Goname** | **Godiffgenecount** | **Gogenecount** | **enrichment** | **pvalue** | **FDR** |
| GO:0030198 | extracellular matrix organization | 20 | 210 | 25.11013216 | 1.54276E-21 | 1.68623E-18 |
| GO:0030574 | collagen catabolic process | 12 | 72 | 43.94273128 | 3.32314E-16 | 1.8161E-13 |
| GO:0022617 | extracellular matrix disassembly | 11 | 79 | 36.71164892 | 5.27621E-14 | 1.9223E-11 |
| GO:0045944 | positiveregulation of transcription | 18 | 708 | 6.7031285 | 1.3435E-09 | 3.6711E-07 |
| GO:0007155 | cell adhesion | 14 | 454 | 8.130373188 | 1.16162E-08 | 2.30356E-06 |
| GO:0044281 | smallmolecule metabolic process | 23 | 1363 | 4.449080643 | 1.26454E-08 | 2.30356E-06 |
| GO:0008285 | negativeregulation of cell prolifer | 12 | 358 | 8.837644279 | 6.42989E-08 | 1.00398E-05 |
| GO:0001501 | skeletal system development | 8 | 127 | 16.60827639 | 1.37682E-07 | 1.88108E-05 |
| GO:0048699 | generation of neurons | 4 | 11 | 95.87505006 | 2.60527E-07 | 3.16396E-05 |
| GO:0008284 | positiveregulation of cell prolifera | 12 | 411 | 7.69799672 | 2.89703E-07 | 3.16645E-05 |
| GO:0007165 | signal transduction | 18 | 1030 | 4.607587357 | 4.19668E-07 | 4.16997E-05 |
| GO:0055085 | transmembrane transport transmembrane transport | 13 | 538 | 6.370879256 | 7.27191E-07 | 6.6235E-05 |
| GO:0007566 | embryo implantation | 5 | 37 | 35.62924158 | 1.18601E-06 | 9.97159E-05 |
| GO:0048704 | embryonicskeletal system morph | 5 | 40 | 32.95704846 | 1.77383E-06 | 0.000131776 |
| GO:0006508 | proteolysis | 12 | 488 | 6.483353795 | 1.80845E-06 | 0.000131776 |
| GO:0008544 | epidermis development | 6 | 76 | 20.81497797 | 1.95203E-06 | 0.000133348 |
| GO:0048015 | phosphatidylinositol-mediated sign | 7 | 129 | 14.30693576 | 2.77867E-06 | 0.000178652 |
| GO:0043065 | positiveregulation of apoptotic pr | 8 | 197 | 10.70685838 | 3.9876E-06 | 0.000242136 |
| GO:0019369 | arachidonicacid metabolicproces | 5 | 50 | 26.36563877 | 5.53803E-06 | 0.000318583 |
| GO:0006979 | response to oxidative stress | 6 | 101 | 15.6627557 | 1.04573E-05 | 0.000571494 |

**Table 1 mRNA GO analysis in oesophageal squamous cell carcinoma**

**Table 2 mRNA pathway analysis in oesophageal squamous cell carcinoma**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **path\_id** | **path\_name** | **path\_diffgene\_cou** | **path\_gene\_count** | **enrichment** | **pvalue** | **FDR** |
| 05202 | Transcriptional misregulationinca | 12 | 179 | 17.67528856 | 2.34961E-11 | 3.94734E-09 |
| 04512 | ECM-receptor interaction | 9 | 87 | 27.27479872 | 2.19839E-10 | 1.84664E-08 |
| 04510 | Focal adhesion | 10 | 207 | 12.73702356 | 3.3619E-08 | 1.79605E-06 |
| 04151 | PI3K-Akt signalling pathway | 12 | 345 | 9.170656962 | 4.27632E-08 | 1.79605E-06 |
| 05146 | Amoebiasis | 7 | 108 | 17.08883994 | 8.29201E-07 | 2.78612E-05 |
| 01100 | Metabolic pathways | 19 | 1234 | 4.059539194 | 1.27125E-06 | 3.5595E-05 |
| 05200 | Pathways in cancer | 11 | 397 | 7.305340716 | 1.71545E-06 | 4.11707E-05 |
| 04810 | Regulation of actin cytoskeleton | 8 | 214 | 9.856313558 | 7.4028E-06 | 0.000155459 |
| 00590 | Arachidonic acid metabolism | 5 | 62 | 21.26261191 | 1.62994E-05 | 0.000304255 |
| 04115 | p53 signalling pathway | 5 | 68 | 19.38649909 | 2.57756E-05 | 0.00043303 |
| 04060 | Cytokine-cytokine receptorintera | 8 | 265 | 7.959438118 | 3.5607E-05 | 0.000543816 |
| 04974 | Protein digestion and absorption | 5 | 90 | 14.64757709 | 0.000101556 | 0.001421784 |
| 04666 | Fc gamma R-mediatedphagocyto | 5 | 92 | 14.3291515 | 0.000112939 | 0.001459525 |
| 05205 | Proteoglycans in cancer | 6 | 203 | 7.792799635 | 0.000540094 | 0.006142299 |
| 04610 | Complement and coagulationcasc | 4 | 69 | 15.28442827 | 0.000574387 | 0.006142299 |
| 04611 | Platelet activation | 5 | 130 | 10.14063029 | 0.000584981 | 0.006142299 |
| 05132 | Salmonella infection | 4 | 86 | 12.2630878 | 0.001341668 | 0.01252223 |
| 05222 | Small cell lung cancer | 4 | 86 | 12.2630878 | 0.001341668 | 0.01252223 |
| 05323 | Rheumatoid arthritis | 4 | 89 | 11.84972529 | 0.001529001 | 0.01351959 |
| 00564 | GlycerophospholipidmetabolismGlycerophospholipid metabolism | 4 | 95 | 11.10132159 | 0.001958567 | 0.016451962 |