Bioinformatics analysis of microarray data to identify the candidate biomarkers of lung adenocarcinoma

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Background: Lung adenocarcinoma (LUAD) is the major subtype of lung cancer and the most lethal malignant disease worldwide. However, the molecular mechanisms underlying LUAD are not fully understood.

Methods: Four datasets GSE118370, GSE85841, GSE43458 and GSE32863 were obtained from the Gene Expression Omnibus (GEO). Identification of differentially expressed genes (DEGs) and functional enrichment analysis were performed using limma and clusterProfiler packages, respectively. Protein-protein interaction (PPI) network was constructed via STRING database and the module analysis was performed by Cytoscape. Then, overall survival analysis was performed using Kaplan-Meier curve, and prognostic candidate biomarkers were further analyzed using oncomine database.

Results: Totally, 349 DEGs were identified, including 275 downregulated and 74 upregulated genes which were significantly enriched in the biological process of extracellular structure organization, leukocyte migration and response to peptide. The mainly enriched pathways were complement and coagulation cascades, malaria and prion diseases. By extracting key modules from the PPI network, 11 hub genes were screened out. Survival analysis showed that except VSIG4, other hub genes may be involved in the development of LUAD, in which MYH10, METTL7A, FCER1G and TMOD1 have not been reported previously to correlated with LUAD. Briefly, novel hub genes identified in this study will help to deepen our understanding of the molecular mechanisms of LUAD carcinogenesis and progression, and to discover candidate targets for early detection and treatment of LUAD.

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15 Abstract

16 **Background:** Lung adenocarcinoma (LUAD) is the major subtype of lung cancer and the most

17 lethal malignant disease worldwide. However, the molecular mechanisms underlying LUAD are

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26 **Results:** Totally, 349 DEGs were identified, including 275 downregulated and 74 upregulated

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29 complement and coagulation cascades, malaria and prion diseases. By extracting key modules

30   from the PPI network, 11 hub genes were screened out. Survival analysis showed that except

31   VSIG4, other hub genes may be involved in the development of LUAD, in which MYH10,

32   METTL7A, FCER1G and TMOD1 have not been reported previously to correlated with LUAD.

33   Briefly, novel hub genes identified in this study will help to deepen our understanding of the

34   molecular mechanisms of LUAD carcinogenesis and progression, and to discover candidate targets

35   for early detection and treatment of LUAD.

36   **Introduction**

37   Lung cancer remains the one of leading healthy issues worldwide, with estimated 2.1 million new

38   cases and 1.8 million deaths in 2018(Bray et al. 2018). It has been ranked the first and second

39   cancer morbidity of male and female in China respectively, and has the highest mortality rate (Sun

40   et al. 2018). Lung adenocarcinoma (LUAD) is the most common subtype of lung cancer (Maemura

41   et al. 2018; Walters et al. 2013). More than 60% of LUAD patients were observed harboring

42   targetable gene alterations, which leading to remarkable responses in treating with tyrosine kinase

43   inhibitors (TKIs), and associated with improved survival rate (Kris et al. 2014). Despite the

44   substantial advance in combined therapies, the prognosis of LUAD is still dismal, the 5-year

45   survival rate is not over 20% (Chen et al. 2014b; Ettinger et al. 2013). Lacking sensitive and

46   specific early biomarkers, high possibility of drug resistance and metastasis, is considered to

47   contribute the high mortality of this disease. Therefore, there has a pressing need for identifying

48   the more sensitive and specific biomarkers or drug targets of LUAD for developing effective

49   diagnosis and treatment strategies.

50   Microarray technology provides an all-in-one system biology solution from hardware to

51   software systems. It can simultaneously scan the hybridization signals of tens of thousands of gene

52   probes in the chip and carry out quantitative analysis on the transcriptome profile of samples.

53   Recent advances especially in the algorithms of probe signal detection and analysis, such as the

54   introduction of artificial intelligence technologies, will make the results of microarray more

55   accurate and reliable(Gan et al. 2019a; Gan et al. 2019b; Peng 2006). The microarray technique

56   also provides a powerful tool for exploring the gene regulation pattern and molecular mechanisms

57 involved in oncogenesis and progression of LUAD. Recently, different types of biomarkers

58 including coding genes, miRNAs, long non-coding RNAs and circRNAs have been identified in

59 lung cancer. Dysregulation of these molecules is involved in the tumor progression or is associated

60 with the prognosis of patients (Di et al. 2019; Vargas & Harris 2016; Vencken et al. 2015; Wei &

61 Zhou 2016).   In view of the complexity of the molecular regulatory network of LUAD, current

62 studies on tumor biomarkers are not sufficient. Therefore, it is still necessary to identify novel

63 prognostic biomarkers, which will help us develop more sensitive and effective diagnostic and

64 therapeutic strategies. However, limited sample size and significant variability among different

65 projects make it hard to obtain credible results. In this study, four microarray datasets containing

66 mRNA expression data between LUAD and non-cancerous tissues were downloaded from GEO

67 and the DEGs were screened out. GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes

68 and Genomes) and PPI network analyses were performed to explore the key modules and hub

69 genes involved in LUAD progression. In sum, 349 DEGs and 10 hub genes were screened out,

70 which may be candidate biomarkers for LUAD.

71

Materials & Methods

73 **Data download and pre-processing**

74 Four datasets GSE118370, GSE32863, GSE85841 and GSE43458, which contain the gene

75 expression data of LUAD and normal tissues, were downloaded from GEO

76 (https://www.ncbi.nlm.nih.gov/geo/) by getGEO function in package GEOquery (Davis & Meltzer

77 2007). The detail information of GEO datasets was listed in table 1. The raw expression files of

78 four microarray datasets were pre-processed according to the method described previously with

79 minor modifications (Giulietti et al. 2016). Briefly, the CEL format files were input and

80 background correction and normalization were conducted using the Robust Multichip Average

81 (RMA) function implemented in affy package in R environment (Bolstad et al. 2003; Irizarry et

82 al. 2003). Next, the array probes were converted into matched gene symbols according to

83 annotation information. In case of multiple probes corresponding to a single gene, the value of

84   gene expression was designated as the mean of the probes. Then, the batch effects among different

85   platforms were removed by ComBat function of sva package. Finally, the normalized microarray-

86   based data of four datasets were merged into a single global dataset which contained a total of

87   12,926 common genes in all four GEO datasets.

88   **Identification of DEGs**

89   Identifying differentially expressed genes in different disease states and investigating their

90   functions and interactions may help to unravel potential regulatory mechanisms for disease

91   occurrence and progression. In present study, the DEGs between LUAD and normal tissues were

92   identified by limma package (Ritchie et al. 2015). The Benjamini-Hochberg procedure was

93   introduced to reduce the false positive rate (FDR) in multiple comparisons (Benjamini & Hochberg

94   1995). Genes with | log2 Fold Change | $\geqslant$ 1 and FDR < 0.05 were considered as DEGs.

95   **GO and KEGG enrichment analysis**

96   GO and KEGG analyses were conducted using enrichGO and enrichKEGG functions of

97   clusterProfiler package, respectively (Yu et al. 2012). p.adjust (FDR)<0.05 was considered to be

98   statistically significant.

99   **Construction of PPI network and module analysis**

100   The network of proteins interaction provides valuable clues for understanding the molecular

101   mechanisms underlying the progress of carcinoma. The PPI network was constructed by Search

102   Tool for the Retrieval of Interacting Genes (STRING) (https://string-db.org/) with interaction

103   score of 0.9 as the threshold. Subsequently, the candidate modules were detected by Cytoscape

104   plugin molecular complex detection (MCODE) with default parameters: degree cut-off=2, node

105   score cut-off=0.2, k-core=2, and max depth=100.

106   **Hub gene analysis**

107   The seed genes in modules referred to hub genes. The overall survival analyses were performed

108   using online tool Kaplan-Meier Plotter (http://kmplot.com/)(Gyorffy et al. 2013). The logrank

109   P<0.05 was considered statistically significant. The association of expression level of hub genes

110   with clinical traits were analysis using Oncomine database(Rhodes et al. 2007).

111

Results

113   **Data preprocessing and DEG screening**

114 Four GEO datasets were downloaded, pre-processed and merged into a global dataset which

115 contained 112 LUAD and 102 normal samples. Totally, 349 DEGs were identified by limma

116 package (Ritchie et al. 2015), including 74 up-regulated genes and 275 down-regulated genes. The

117 most statistically significant up-regulated and down-regulated genes are listed in Table 2. The

118 distribution of DEGs was presented by volcano plot (Fig. 1).

119 **GO and KEGG analysis**

120 The biological functions and pathways analyses were conducted using R package clusterProfiler

121 (Yu et al. 2012). The GO categories of biological process (BP), cellular component (CC) and

122 molecular function (MF) were enriched respectively (Fig.2A-C) and the top 15 GO terms of the

123 up-regulated and down-regulated DEGs were listed in supplemental table 1 ,2 respectively. The

124 up-regulated DEGs were mainly associated with extracellular matrix processing, such as

125 extracellular matrix organization (BP, GO:0030198), extracellular matrix (CC, GO:0031012) and

126 serine-type endopeptidase activity (MF, GO:0004252). The down-regulated DEGs were most

127 significantly related to response to corticosteroid (BP, GO:0031960), extracellular matrix (CC,

128 GO:0031012) and growth factor binding (MF, GO:0019838). The DEGs were mainly enriched in

129 pathways of complement and coagulation cascades(hsa04610) , malaria (hsa05144), prion

130 diseases(hsa05020), fluid shear stress and atherosclerosis(hsa05418) , AGE-RAGE signaling

131 pathway in diabetic complications(hsa04933) , vascular smooth muscle contraction (hsa04270),

132 IL-17 signaling pathway (hsa04657), leukocyte transendothelial migration (hsa04670), protein

133 digestion and absorption (hsa04974) and drug metabolism - cytochrome P450(hsa00982) (Table 3

134 and Fig. 2D).

135 **Construction of PPI network and module analysis**

136 PPI network reflect the spatiotemporal relationship of macromolecules within the cell which will

137 provide valuable information about molecular mechanisms in physiological and pathological

138   process. To explore the molecular mechanisms underlying LUAD progression, online database

139   STRING was applied to construct the PPI network. The interaction score of 0.9 (highest

140   confidence) was set as threshold, and nodes without connections were removed from network.

141   Finally, the PPI network consisted of 349 nodes with 277 edges, and average local clustering

142   coefficient was 0.337(PPI enrichment p-value< 1.0E-16) (Fig. 3A). Then, the key modules were

143   identified via MCODE plugin. Eleven functional clusters of modules and related hub genes were

144   detected. The top 3 significant modules were presented in Fig. 3C-E. The KEGG analysis of

145   module genes revealed that the top 3 modules were mainly associated with chemokine signaling

146   pathway(hsa04062), complement and coagulation cascades(hsa04610), human cytomegalovirus

147   infection(hsa05163) and vascular smooth muscle contraction(hsa04270) (Fig. 3B).

148   **Hub genes analysis**

149   A total of 11 genes were identified as hub genes. The overall survival analysis of the hub genes

150   was performed using Kaplan-Meier curve. Except VSIG4, LUAD patients with downregulated

151   ADAMTS8, AOX1, EFEMP1, METTL7A, MYH10, PTGER4, TMOD1, CDH13 and upregulated

152   PRC1 showed worse overall survival (Fig. 4A-I). It is worth noting that FCER1G is downregulated

153   in LUAD patients, but the low expression level is associated with better overall survival (HR =

154   1.87) (Fig. 4J). Subsequently, the expression status of hub genes with HR<0.5 or HR>2 were

155   further validated using oncomine database. The result showed that ADAMTS8, METTL7A and

156   MYH10 were significantly downregulated and PRC1 was markedly overexpressed in LUAD in

157   the different datasets (Fig. 5). In the Okayama Lung dataset, the alternation of ADAMTS8,

158   METTL7A, MYH10 and PRC1 were associated with tumor grade (Fig. 6), implicating vital roles

159   of these genes in the carcinogenesis or progression of LUAD.

160

161   **Discussion**

162   In this study, four GEO datasets were analyzed and 349 DEGs were identified, including 74 up-

163   regulated and 275 down-regulated genes. The KEGG analysis revealed the top three enriched

164   pathways were complement and coagulation cascades, malaria and prion diseases. These

165 annotation results provided valuable clues to reveal molecular interactions in the development of

166 LUAD. Indeed, the complement system has been reported to play critical roles in tumor

167 progression (Zhao et al. 2019). The overexpressed C3 in blood and downregulated C3 in tumor

168 tissues were observed in lung cancer patients, and related to poorer prognosis (Ajona et al. 2013;

169 Lin et al. 2014; Mehan et al. 2012). The confusing experimental results suggest that the

170 complement system may be involved in complex tumor regulatory processes by reshaping tumor

171 microenvironment, which is worthy of further study. In recent years, malaria and prion disease,

172 which had not previously attracted much attention, have also been found to be associated with

173 tumors. Epidemiological study has shown that the incidence of malaria is negatively correlated

174 with the mortality of colorectal cancer, breast cancer and lung cancer (Qin et al. 2017). The

175 proposed anti-tumor mechanisms included systemly stimulating the immune responses (Chen et

176 al. 2011) and inhibiting key pathways in tumor progress (Deng et al. 2018). Previously, prion

177 protein (PrPc) was thought to play a role only in the central nervous system. However,

178 accumulating evidence shows that PrPc has wider biological functions that were not previously

179 expected (Mehrpour & Codogno 2010). PrPc may be associated with the biology of many cancers,

180 and overexpression of PrPc promotes the proliferation, invasion and metastasis of the gastric

181 cancer cell(Liang et al. 2009; Mehrpour & Codogno 2010; Pan et al. 2006). These data may

182 provide new ideas and directions for the mechanism research and therapeutic strategy of lung

183 adenocarcinoma. The GO analyses indicated that DEGs were significantly related to biological

184 process of extracellular matrix organization and process. Previous studies have reported that

185 extracellular matrix (ECM) remodeling promotes cancer progression and is associated with a poor

186 prognosis in lung cancer patients (Kopparam et al. 2017; Xia et al. 2012). The up- and down-

187 regulated DEGs were simultaneously enriched into extracellular matrix process, which is

188 consistent with the propensity for metastasis and highly invasive characteristics of LUAD.

189 In line with the GO analysis, among 11 hub genes identified by PPI network and modules

190 analysis, EFEMP1, PTGER4, ADAMTS8, CDH13, MYH10 and METTL7A are associated with

191 extracellular matrix process, and survival analysis indicated that down-regulation of these hub

192   genes was associated with worse overall survival. EFEMP1 plays distinct biological functions in

193   different tumors. In osteosarcoma and gliomas, EFEMP1 is overexpressed and promotes the

194   invasion and metastasis of tumor cells in vitro and in vivo by activating the expression of MMP-2

195   and notch signaling, respectively(Hu et al. 2012; Hu et al. 2009; Wang et al. 2015), while in gastric

196   cancer, endometrial carcinoma, hepatocellular carcinoma and lung cancer, EFEMP1 is

197   downregulated, and is proposed as a prognosis biomarker(Chen et al. 2014a; Kim et al. 2014;

198   Nomoto et al. 2010; Yang et al. 2013; Yue et al. 2007; Zhu et al. 2014). Overexpression of

199   EFEMP1 has been reported to suppressed invasion and migration of LUAD cells via inhibiting the

200   epithelial-to-mesenchymal transition (EMT) pathway(Kim et al. 2014).PTGER4 is overexpressed

201   and proposed as a therapeutic target for LUAD and other cancers(Doherty et al. 2009; Fulton et

202   al. 2006; Heinrichs et al. 2018; Kim et al. 2010; Ma et al. 2006; Xin et al. 2012). The above report

203   is inconsistent with our results, which may be due to the heterogeneity of the tumor and the limited

204   number of samples. Therefore, subsequent large sample functional verification is required.

205   ADAMTS8 encodes an inactive proenzyme and forms mature active enzyme by proteolysis(Apte

206   2009). ADAMTS8 is identified as a secretory angiogenesis inhibitor that inhibits VEGF-mediated

207   angiogenesis by blocking the EGFR signaling pathway(Choi et al. 2014; Dunn et al. 2006;

208   Vazquez et al. 1999). Downregulated ADAMTS8 in some cancers including NSCLC (Heighway

209   et al. 2002; Huang et al. 2019; Masui et al. 2001; Porter et al. 2004; Rodriguez-Rodero et al. 2013;

210   Zhao et al. 2018) is associated with poorer prognosis (Drilon et al. 2014; Li et al. 2015; Porter et

211   al. 2006). CDH13 encodes a member of cadherin superfamily. The hypermethylation in promoter

212   region of CDH13 was frequently observed in lung cancer, and proposed to correlate to drug

213   sensitivity and poorer prognosis (Kontic et al. 2012; Toyooka et al. 2006; Zhai & Li 2014; Zhong

214   et al. 2015). As the only upregulated hub gene, PRC1 is involved in the process of cytokinesis and

215   is upregulated in breast cancer, hepatocellular carcinoma, and lung cancer(Jiang et al. 1998; Liu

216   et al. 2018; Shimo et al. 2007; Zhan et al. 2017a). Recent research has found that it promoted

217   proliferation and metastasis of lung adenocarcinoma cells via potentiating the Wnt/β-catenin

218 pathway(Zhan et al. 2017b), and inhibiting the expression of PRC1 in LUAD cells by miR-1-3p

219 was reported to suppress tumorigenesis (Li et al. 2019).

220   Literature retrieval showed that the relation of LUAD and hub genes MYH10, METTL7A,

221 FCER1G and TMOD1 has not been reported. MYH10 belongs to the myosin superfamily, which

222 can regulate ECM remodeling (Kim et al. 2018; Kim et al. 2015). Methyltransferase METTL7A

223 was found to facilitate Hepatitis C Virus (HCV) propagation via recruitment of NS4B (Park et al.

224 2015). FCER1G encodes a high affinity IgE receptor which is associated with prognosis of renal

225 clear cell carcinoma (Chen et al. 2017). As an aldehyde oxidase, TMOD1 is an actin-capping

226 protein involved in regulation of the length and depolymerization of actin filaments.

227 Overexpression of TMOD1 promotes cell proliferation of breast cancers and metastasis of oral

228 squamous cell carcinoma (Ito-Kureha et al. 2015; Suzuki et al. 2016). However, the molecular

229 mechanism of these newly identified hub genes in LUAD remains largely unknown, and further

230 functional studies were warranted.

231   In present study, we have identified a set of candidate biomarkers that may play an important

232 role in the progression of LUAD. These newly identified hub genes could be used as research

233 subjects for exploring their roles in the disease process, so as to further deepen our understanding

234 of the molecular mechanism of LUAD, and also as potential prognostic biomarkers for clinical

235 validation studies to clarify their prognostic effects. However, there are still some limitations in

236 this study. First, this study is based on bioinformatics analysis of published data and lacks

237 experimental verification, and the study cannot determine whether there is a causal relationship

238 between the differential expression of hub genes and disease progression. Finally, although we

239 combined four GEO datasets, the number of samples is still relatively small, which may lead to

240 potential unreliable results. Therefore, subsequent bioinformatics analysis and experimental

241 verification with larger samples are necessary.

242

243 **Conclusions**

244 In summary, this study identified several differentially expressed genes by integrating four GEO

245 datasets and extracted 11 hub genes from PPI network, among which 10 hub genes were shown to

246 be related to the occurrence and development of lung adenocarcinoma and 4 hub genes have not

247 been previously reported but may play an important role in LUAD. The molecular mechanism of

248 these novel hub genes in LUAD is worthy of further study, and relevant prognostic model can also

249 be constructed based on these genes for risk assessment, classification and prognostic judgment of

250 patients with LUAD.

251

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487
488

The detail information of four GEO datasets

Table 1 The detail information of four GEO datasets

ID	Tissue	Platform	Normal	Tumor
GSE118370	LUAD	GPL570	6	6
GSE85841	LUAD	GPL20115	8	8
GSE43458	LUAD	GPL6244	30 (never-smoker)	40 (never-smoker)
GSE32863	LUAD	GPL6884	58	58

GEO: Gene Expression Omnibus; LUAD: Lung Adenocarcinoma

Top ten up- and down-regulated DEGs

Table 2 Top ten up- and down-regulated DEGs

Up-regulated DEGs				Down-regulated DEGs		
Gene symbol	**Log2FC**	**FDR**		**Gene symbol**	**Log2FC**	**FDR**
SPP1	3.08474	1.74E-33		FABP4	-3.37555	1.25E-47
OCIAD2	1.477746	1.13E-29		STX11	-2.01766	1.51E-44
ETV4	1.366623	5.38E-27		CAV1	-2.70539	6.04E-43
TOP2A	1.777989	3.24E-26		FHL1	-2.3099	5.77E-42
COL10A1	1.364488	3.74E-26		TEK	-2.30698	1.09E-40
PROM2	1.570558	7.77E-26		AGER	-2.69928	6.05E-40
MMP11	1.929847	1.03E-23		FMO2	-2.44791	1.42E-39
UBE2T	1.154061	5.78E-23		CRYAB	-1.98382	1.77E-39
ABCC3	1.444218	3.71E-22		GRK5	-1.4854	1.53E-38
BAIAP2L1	1.067336	4.15E-22		TMEM100	-2.928	4.79E-38

1   DEGs: Differentially Expressed Genes; FDR: False Discovery Rate; Log2FC: Log2(Fold Change)

KEGG enriched pathways of DEGs

Table 3 KEGG enriched pathways of DEGs

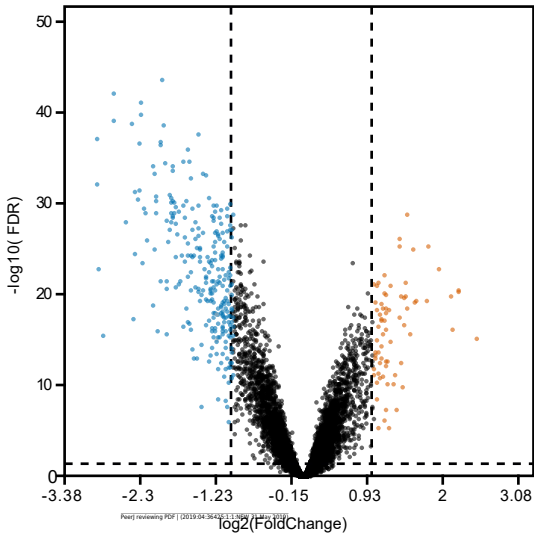
ID	Description	Count	p.adjust(FDR)
hsa04610	Complement and coagulation cascades	13	1.12E-05
hsa05144	Malaria	9	0.000271
hsa05020	Prion diseases	7	0.001406
hsa05418	Fluid shear stress and atherosclerosis	13	0.001921
hsa04933	AGE-RAGE signaling pathway in diabetic complications	10	0.007362
hsa04270	Vascular smooth muscle contraction	11	0.014433
hsa04657	IL-17 signaling pathway	9	0.014433
hsa04974	Protein digestion and absorption	8	0.039299
hsa04670	Leukocyte transendothelial migration	9	0.039299
hsa00982	Drug metabolism - cytochrome P450	7	0.039299

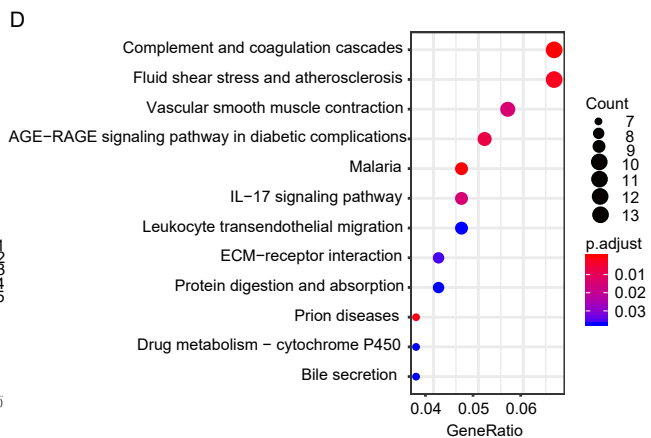
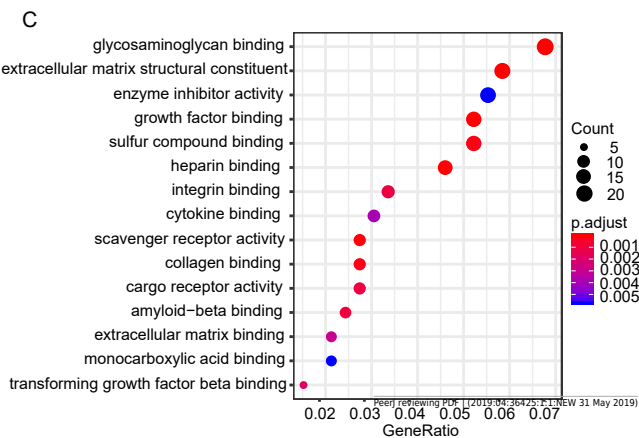
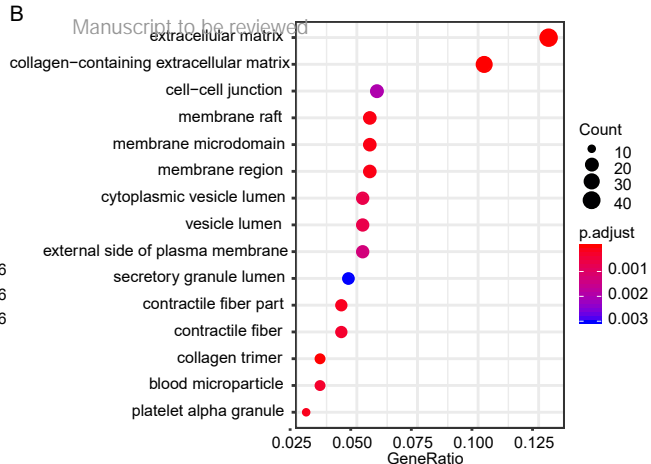
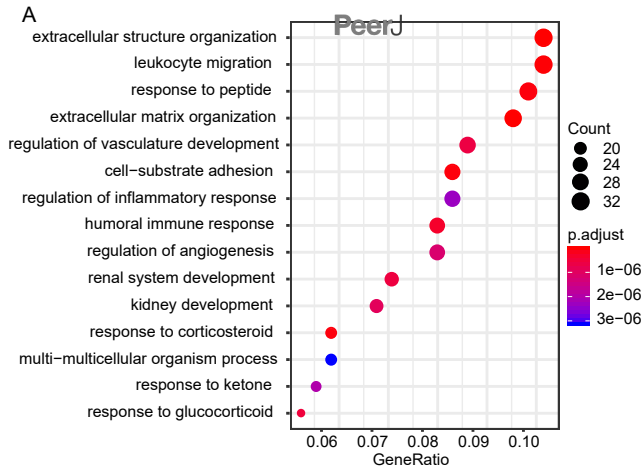
1 KEGG: Kyoto Encyclopedia of Genes and Genomes; DEGs: Differentially Expressed Genes; FDR: False

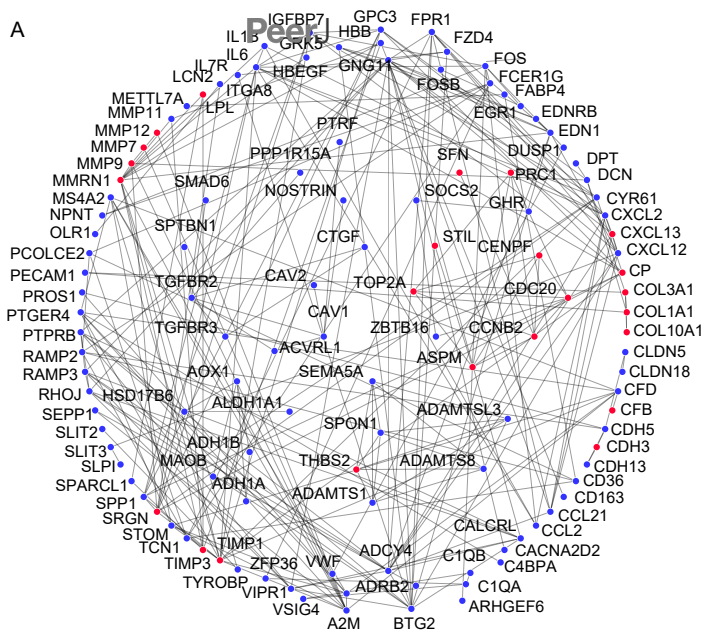
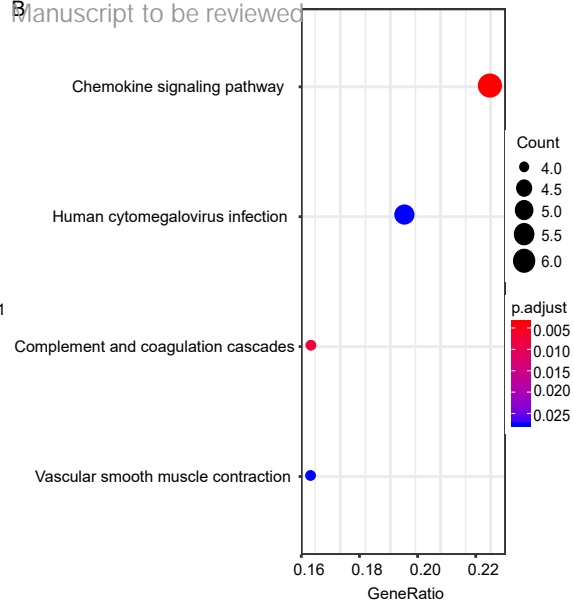
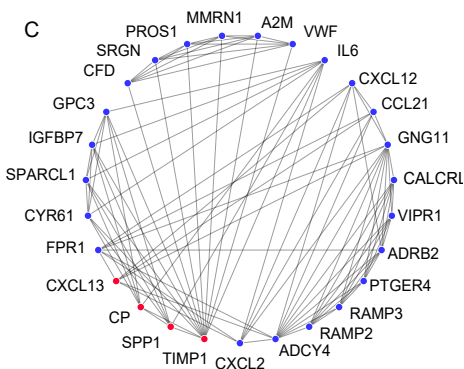
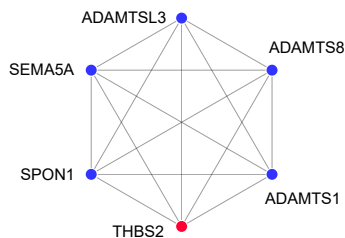
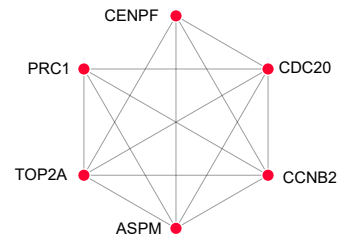
2 Discovery Rate

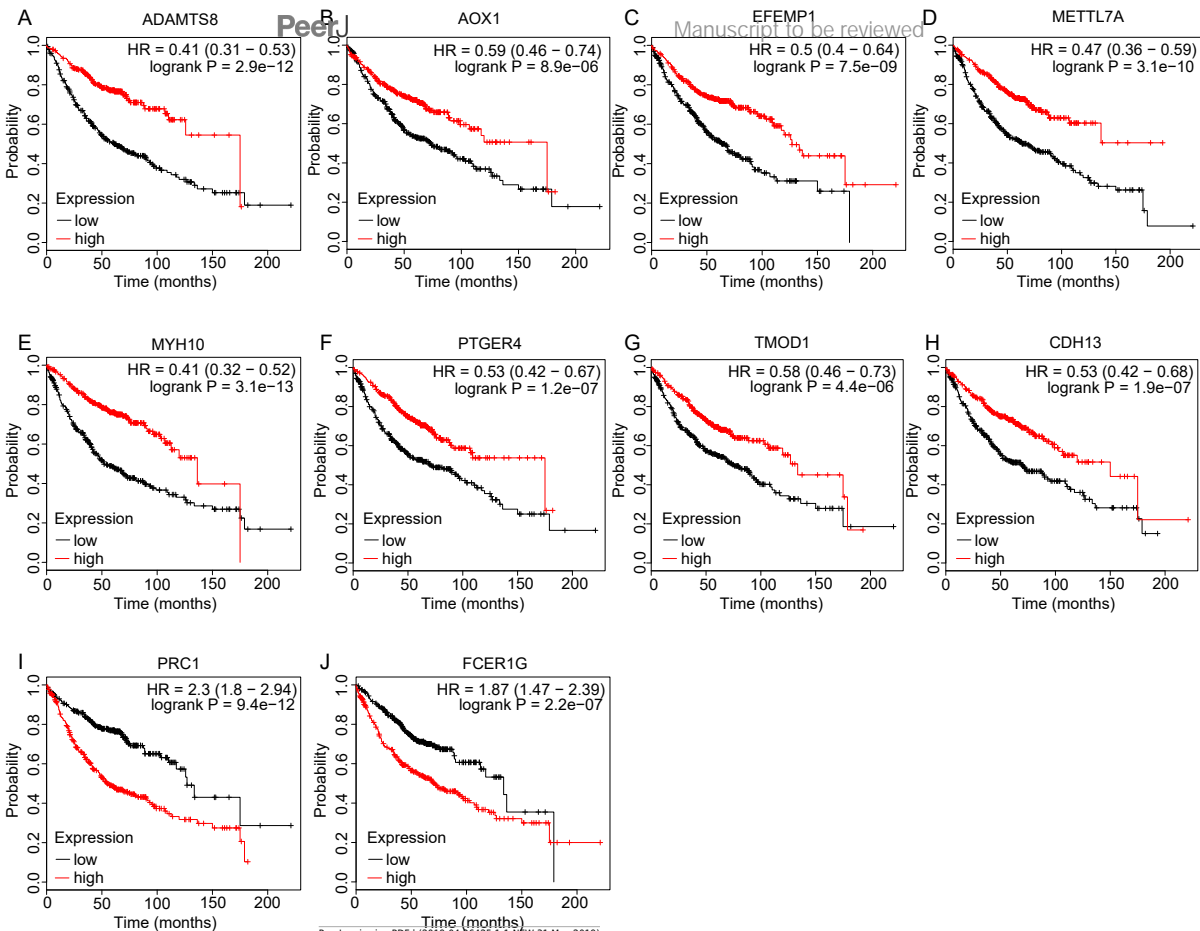
Volcano plot of the DEGs.

The vermilion and blue dots represent DEGs filtered based on the cut-off values of |log2FoldChange| > 1.0 and adjusted P value(FDR) < 0.05, while the black dots represent genes that are not satisfied the cut-off values of differential expression. The horizon dotted line indicates the position of -log10(FDR) = 0.05, and the vertical dotted lines indicate the positions of |log2FoldChange|=1.0. Vermilion : upregulation; Blue: downregulation. DEGs: differentially expressed genes. FDR:False Discovery Rate.

Volcano Plot of DEGS

GO and KEGG analysis of DEGs.

(A-C) The top 15 terms of GO categories of biological process (BP), cellular component (CC) and molecular function MF, respectively. (D) KEGG pathway analysis of DEGs, p.adjust(FDR) <0.05 was considered significantly. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes;FDR:False Discovery Rate.

Protein-protein interaction network of DEGs and modules analysis.

(A) PPI network of DEGs; (B) The KEGG enrichment analysis of the genes in the top 3 modules. (C-E) the top 3 modules of PPI network. The red nodes represent the upregulated DEGs. The green nodes represent the downregulated DEGs. PPI: protein-protein interaction; DEG: differentially expressed gene; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Overall survival analyses of hub genes.

(A-J) The overall survival analyses of hub genes were performed using Kaplan- Meier Plotter online platform. Logrank P<0.05 was considered statistically significant.

Oncomine analysis of cancer vs. normal tissue of ADAMTS8 , METTL7A , MYH10 and PRC1.

Heat maps of ADAMTS8(A), METTL7A(B), MYH10(C) and PRC1(D) gene expression in lung adenocarcinoma samples vs. normal tissues. 1. Lung Adenocarcinoma vs. Normal, Hou Lung (Hou et al. 2010) ; 2. Lung Adenocarcinoma vs. Normal, Landi Lung (Landi et al. 2008) ; 3. Lung Adenocarcinoma vs. Normal, Selamat Lung (Selamat et al. 2012) ; 4. Lung Adenocarcinoma vs. Normal, Su Lung (Su et al. 2007) .

A

Median Rank	P- Value	Gene
79.5	7.34E-28	ADAMTS8

1 2 3 4

1. P-value=5.54E-27 FoldChange= -6.612
2. P-value=1.47E-27 FoldChange= -2.990
3. P-value=9.12E-38 FoldChange= -2.848
4. P-value=1.81E-11 FoldChange= -8.009

B

Median Rank	P- Value	Gene
547.5	1.13E-9	METTL7A

1 2 3 4

1. P-value=8.55E-13 FoldChange= -2.482
2. P-value=2.26E-28 FoldChange= -3.213
3. P-value=8.11E-17 FoldChange= -2.522
4. P-value=2.25E-9 FoldChange= -3.382

C

Median Rank	P- Value	Gene
235.5	1.07E-23	MYH10

1 2 3 4

1. P-value=4.97E-17 FoldChange= -2.673
2. P-value=2.15E-23 FoldChange= -2.649
3. P-value=1.79E-26 FoldChange= -2.797
4. P-value=1.72E-12 FoldChange= -3.491

D

Median Rank	P- Value	Gene
220.5	5.56E-7	PRC1

1 2 3 4

1. P-value=2.41E-17 FoldChange= 4.260
2. P-value=1.88E-14 FoldChange= 2.661
3. P-value=1.16E-19 FoldChange= 2.677
4. P-value=1.11E-6 FoldChange= 2.610

1 5 10 25   25 10 5 1   Not measured

%

The association between the expression of selected hub genes and tumor stage.

(A-D) The expressions of ADAMTS8, METTL7A, MYH10, PRC1 were correlated with tumor stage in the Okayama Lung dataset. 0: No value; 1: Stage I; 2: Stage II.