Identification of key genes and pathways associated with cholangiocarcinoma development based on weighted gene correlation network analysis

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Background: As the most frequently occurred tumor in biliary tract, cholangiocarcinoma (CCA) is mainly characterized by its late diagnosis and poor outcome. It is therefore urgent to identify specific genes and pathways associated with its progression and prognosis.

Materials and Methods: The differentially expressed genes in The Cancer Genome Atlas (TCGA) were analysed to build the co-expression network by Weighted gene co-expression network analysis (WGCNA). Gene ontology (GO) as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were conducted for the selected genes. Module–clinical trait relationships were analyzed to explore the association with clinicopathological parameters. Log-rank tests and cox regression were used to identify the prognosis-related genes.

Results: The most related modules with CCA development were tan module containing 181 genes and salmon module with 148 genes. GO analysis suggested enrichment terms of digestion, hormone transport and secretion, epithelial cell proliferation, signal release, fibroblast activation, response to acid chemical, wnt, NADP metabolism. KEGG analysis demonstrated 15 significantly altered pathways including glutathione metabolism, wnt, central carbon metabolism, mTOR, pancreatic secretion, protein digestion, axon guidance, retinol metabolism, insulin secretion, salivary secretion, fat digestion. Key genes of SOX2, KIT, PRSS56, WNT9A, SLC4A4, PRRG4, PANX2, PIR, RASSF8, MFSD4A, INS, RNF39, IL1R2, CST1 and PPP3CA might be potential prognostic markers for CCA, of which RNF39 and PRSS56 also showed significant correlation with clinical stage.

Discussion: Differentially expressed genes and key modules contributing to CCA development were identified by WGCNA. Our results offer novel insights into the characteristics in the etiology, prognosis and treatment of CCA.

1 **Identification of key genes and pathways associated with cholangiocarcinoma**

2 **development based on weighted gene correlation network analysis**

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17 **Running title:** WGCNA analysis of cholangiocarcinoma

22 **Abstract**

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24 (CCA) is mainly characterized by its late diagnosis and poor outcome. It is therefore

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34 **Results:** The most related modules with CCA development were tan module containing

35 181 genes and salmon module with 148 genes. GO analysis suggested enrichment terms

36 of digestion, hormone transport and secretion, epithelial cell proliferation, signal release,

37 fibroblast activation, response to acid chemical, wnt, NADP metabolism. KEGG analysis

38 demonstrated 15 significantly altered pathways including glutathione metabolism, wnt,

39 central carbon metabolism, mTOR, pancreatic secretion, protein digestion, axon

40 guidance, retinol metabolism, insulin secretion, salivary secretion, fat digestion. Key

41 genes of SOX2, KIT, PRSS56, WNT9A, SLC4A4, PRRG4, PANX2, PIR, RASSF8,

42 MFSD4A, INS, RNF39, IL1R2, CST1 and PPP3CA might be potential prognostic markers

43   for CCA, of which RNF39 and PRSS56 also showed significant correlation with clinical

44   stage.

45   **Discussion:** Differentially expressed genes and key modules contributing to CCA

46   development were identified by WGCNA. Our results offer novel insights into the

47   characteristics in the etiology, prognosis and treatment of CCA.

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49   **Key words:** cholangiocarcinoma, weighted gene correlation network analysis,

50   progression, prognosis.

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Introduction

Cholangiocarcinoma (CCA), characterized by cholangiocyte differentiation, represents a type of aggressive tumor which derive form lesions in different parts of the biliary tree (Bergquist & von Seth 2015). As the most frequently occurred biliary tumor and the second after liver cancer, cholangiocarcinoma is mainly characterized by its late diagnosis and poor outcome(Blechacz 2017). Currently, it is estimated that overall 5-year survival rate of CCA is less than 10% and only approximately one third of the CCA patients are suitable for curative treatment at the time of diagnosis(Doherty et al. 2017). Therefore, it is urgently required to identify specific genes and pathways associated with its initiation, progression and prognosis.

The common category of CCA are on basis of different locations of intrahepatic, perihilar or distal cholangiocarcinoma(Oliveira et al. 2017). Most patients are diagnosed with non-resectable status and suffer an extremely poor prognosis(Rizvi & Gores 2017). A number of diseases have been reported to be related with the development of CCA including viral hepatitis B and C, choledocholithiasis, obesity, exposure to toxic agents, liver cirrhosis, diabetes mellitus, congenital hepatic fibrosis and primary sclerosing cholangitis(Erichsen et al. 2009). Every year, a larger number of cases occur in Southeast Asia, in which hepatobiliary flukes Opisthorchis viverrini infection or Clonorchis sinensis infection were considered to be implicated in the development of CCA(Razumilava & Gores 2014). Interleukin-6, a pro-inflammatory cytokine associated with downstream

85 activation of oncogenic pathways, has been linked with cholangiocarcinoma

86 development(Park et al. 1999). Frequent mutations of oncogenes such as KRAS, as well

87 as cancer suppressor genes of TP53 and SMAD4 were identified by next generation

88 sequencing in CCA(Chan-On et al. 2013). In addition, research from several case-

89 controlled studies has demonstrated multiple genetic polymorphisms that might be

90 implicated in cholangiocarcinoma carcinogenesis(Bridgewater et al. 2014).

91 Although a number of genes and mechanisms have been proved to be closely

92 implicated in the development of CCA, the comprehensive picture of the whole genes and

93 regulations of CCA is still unclear. In recent years, bioinformatic methods become

94 increasingly effective in exploration and analysis of multiple genes or proteins of

95 complicated diseases. Weighted gene co-expression network analysis (WGCNA), a new

96 gene co-expression analysis method, has been successfully used to screen biomarkers

97 and pathways that could be applied in susceptibility genes, diagnose and treatment of

98 cancer. In this study, WGCNA was conducted to analyse data of TCGA data repository

99 of CCA to screen modules and core genes in pathogenesis, progression and survival of

100 CCA.

101 **Materials and Methods**

102 **Publically available data sets**

103 RNA expression as well as clinical parameters of CCA patients were obtained from

104 The Cancer Genome Atlas (TCGA) database (cancergenome.nih.gov). The level of gene

105 expression was tested as Transcripts Per Kilobase of exon model per Million mapped

106   reads (TPM). Clinical characteristics had the sample type, histology grade, recurrence,

107   histologic grade, and prognosis. Each sample must had complete pathology stage and

108   histology records. If the expression of genes showed limited variation, we regraded them

109   as noise and discard these ones because the results of these genes might come from

110   systematic error and have limited significance.

111   **Construction of co-expression network of genes**

112   In this study, we adopted WGCNA method to build a co-expression network for

113   certain genes using R language (Langfelder & Horvath 2008). We used WGCNA method

114   to calculate power number in order to construct modules through co-expression. WGCNA

115   method was also performed for construction of the co-expression network and extraction

116   of the genetic information in the most relevant module. "Heatmap tool" package of R

117   software was selected to analyze the correlation degree among modules. As a

118   representative of the gene expression profiles of a module, module eigengene (ME) was

119   used to evaluate the relationship between module and overall survival.

120   **Identification of clinical traits-related modules**

121   We then performed Pearson's correlation examination to explore the relation of MEs

122   with clinical traits of histologic grade, recurrence, pathologic M, pathologic N, pathologic

123   T. A p value less than 0.05 indicated statistical significance. The two most significant

124   modules are selected as hub modules.

125   **GO and KEGG analysis**

126   In the present study, we adopted the clusterprofiler package of R language to

127 investigate the possible biological procedures and signaling pathways of selected genes

128 in different module. Gene ontology (GO) analysis (Ashburner et al. 2000) as well as Kyoto

129 Encyclopedia of Genes and Genomes (KEGG) analysis were conducted(Kanehisa et al.

130 2004), which enable computational prediction of higher-level complexity of cellular

131 processes and organism behavior from genomic information.

Prognostic value of genes of hub modules

133   Prognosis investigation was conducted using the 'survival' package within R

134 software. Cox regression test was used to calculate the hazard ratio and its 95%

135 confidence interval while Kaplan-Meier method was adopted to draw survival curve. The

136 increased and decreased expression level of each gene in the candidate module was

137 decided according to median value.

Screening for candidate hub genes

139   We chose genes associated with prognosis as candidate genes. Next, we analyzed

140 the association between candidate genes and stage parameter. The genes associated

141 with both overall survival and clinical stage were selected as hub genes.

Results

Gene expression profile of CCA

144   Clinical characteristics and RNA expression value for 33 Cholangiocarcinoma

145 individuals were downloaded at TCGA. Altogether 6417 relatively more variant genes on

146 the basis of median absolute deviation value were considered in our subsequent

147 investigaiton. When soft thresholding power β came to 7, the connectivity amone genes

148 accord with a scale-free network distribution (Supplementary Figure 1). A total of 21

149 modules were screened using hierarchical clustering along with Dynamic branch Cutting

150 by WGCNA analysis. And each different color represent a sole module. Interaction

151 relationship analysis of co-expression genes was shown in Figure 1. The gene counts

152 within modules were from 37 to 909. In this study, the grey module collected a series of

153 genes which did not belong to any module.

154 **Association of module with clinical traits**

155 Identification of genes and modules which associate with clinical characteristic would

156 greatly help the understanding the underlying mechanisms of certain traits. In our

157 analysis, the clinical parameters of CCA of histologic grade, recurrence, pathologic M,

158 pathologic N, pathologic T were selected in the module–trait relationship (MTR) analysis.

159 As was suggested in Figure 2, the two relatively most significant modules of tan and

160 salmon were selected as hub modules because their significant relation with clinical

161 parameters including grade and T, N, M of CCA.

162 **Enrichment investigation of the hub modules**

163 We next proceeded GO and KEGG investigation of the genes within tan module and

164 salmon module. Altogether 50 terms showed significant difference in GO enrichment

165 analysis (Supplementary Table 1) and the most important terms were summarized in

166 Table 1. As was illustrated in Figure 3, the salmon module in GO analysis was related

167 with digestion, hormone transport, hormone secretion, epithelial cell proliferation,

168 regulation of hormone levels, signal release while the tan module was associated with

169 fibroblast activation, response to acid chemical, wnt signaling pathway, cell-cell signaling

170 by wnt, NADP metabolic process, negative regulation of neuron differentiation, negative

171 regulation of cell development. According to the KEGG analysis, 15 pathways were

172 significantly altered in hub modules including glutathione metabolism, wnt signaling

173 pathway, central carbon metabolism in cancer, mTOR signal, pancreas secretion, protein

174 digestion or absorption, axon guidance, retinol metabolism, insulin secretion, salivary

175 secretion, and fat digestion and absorption (Figure 4 and Table 2).

176 **Prognosis analysis**

177 Since the tan and salmon module were selected from as the hub modules, we are

178 interested in whether the genes with in the modules might predict the survival of CCA

179 patients. Of the 358 genes, a total of 15 genes (SOX2, KIT, PRSS56, WNT9A, SLC4A4,

180 PRRG4, PANX2, PIR, RASSF8, MFSD4A, INS, RNF39, IL1R2, CST1 and PPP3CA)

181 demonstrated significant association with the prognosis of CCA (Table 3). The survival

182 curve for the top 4 genes in salmon module (INS: HR=4.670, P= 0.006; RNF39:

183 HR=2.811, P= 0.032; CST1: HR=2.846, P=0.017; PPP3CA: HR=3.151, P=0.010) and the

184 top 4 gene in tan module (PRSS56: HR = 2.876, P = 0.018); SLC4A4: HR = 0.352, P =

185 0.025; PRRG4: HR = 0.339, P = 0.023; PIR: HR = 2.701, P = 0.027) were illustrated in

186 Figure 5. In addition, key genes of DNA repair and immune regulation were analysed in

187 relation to CCA prognosis, the results of which suggested that DNA repair gene XPC (HR

188 = 0.369, P = 0.048) and immune regulator CD28 (HR = 0.364, P = 0.045) were related

189 with favourable survival of CCA (Supplementary Table 2). We also analyzed the

190 prognosis of genes more frequently implicated in CCA including KRAS, TP53, BAP1. The

191 mutation frequency of KRAS, TP53, BAP1 were 5.71%(2), 8.57%(3), 5.71%(2) out of 33

192 CCA patients. And no significant association with prognosis was observed.

193 **Identification of hub genes associated with both stage and survival**

194   We then analyse the association between clinical stage and gene which showed

195 significant relation with prognosis. As was shown in Figure 6, two genes (RNF39 and

196 PRSS56) showed difference between two groups (Table 4).

197 **Discussion**

198   Although surgical therapy and liver transplantation might be possible options for

199 certain patients who suffer CCA, the five-year survival ratio of CCA remain extremely low.

200 Comprehensive exploration of the genetic and epigenetic profiles as well as their

201 complicated interactions with environment would greatly help the treatment and survival

202 for CCA patients. Until now, our understanding of the complex mechanisms of CCA is still

203 limited. One study using CCA data also analysed the genes implicated in CCA (Tian et

204 al. 2019). They dissected the genome network by other bioinformatic tool to explore the

205 protein-protein interaction. In this study, we provided our own gene lists with significance

206 by WGCNA. Besides, we adopted Kyoto Encyclopedia of Genes and Genomes analysis

207 to explore the biological functions for the tumor progression. A differentially expression

208 analysis followed by WGCNA to identify genes and pathways related were performed to

209 the clinical parameters and prognosis of CCA. In addition, enrichment analysis of the

210 genes in core modules suggested significant involvement of pathways such as

211 glutathione metabolism, wnt signaling, mTOR signaling, pancreatic secretion, protein

212 digestion and absorption, insulin secretion and salivary secretion.

213   In this present study, RNA sequencing data for CCA samples from TCGA were

214 systematically analyzed. According to the WGCNA analysis of most variant genes,

215 altogether 21 modules were identified and the two most significant modules of tan and

216 salmon were selected as hub modules. GO analysis of salmon module demonstrated

217 enrichment in digestion, hormone transport, hormone secretion, epithelial cell

218 proliferation, regulation of hormone levels, signal release. It can be concluded from our

219 analysis that abnormal hormone regulation might be closely implicated in CCA

220 progression. Previously, autocrine parathyroid hormone-like hormone was indicated to

221 promote intrahepatic CCA cell growth through enhanced ERK/JNK-ATF2-cyclinD1

222 signalling(Tang et al. 2017). There were also reports of CCA patients with aberrant levels

223 of parathyroid hormone-related protein(Matsumoto et al. 2014; Ozawa et al. 2017). From

224 this point of view, future investigations concerning the synthesis, transport and secretion

225 of relevant hormones might provide novel insight into CCA development.

226   In addition, the tan module was associated with fibroblast activation, response to acid

227 chemical, wnt signalling pathway, NADP metabolic process, negative modulation of

228 neuron differentiation, negative modulation of cell development. Wnt signalling pathway

229 has been proved to be involved in the growth and progression of various types of cancer

230 (Clevers & Nusse 2012). Previously, enhanced wnt signalling has been found to be a

231 characteristic of CCA (Boulter et al. 2015) which related with metastasis or

232 chemoresistance (Wang et al. 2015). Besides, lncRNA PCAT1 regulated the

233 development of extrahepatic CCA progression by Wnt and β-catenin signalling

234 pathway(Zhang et al. 2017a). It is worth noting that response to acid chemical was also

235 a hallmark in CCA development according to our study. Indeed, glycocholic acid and

236 taurochenodeoxycholic acid were found to be potential phenotypic biomarkers in

237 CCA(Song et al. 2018). Conjugated bile acids were suggested to promote invasive growth

238 of cholangiocarcinoma cells via activation of sphingosine 1-phosphate receptor 2(Liu et

239 al. 2014). The role of other biological processes such as NADP metabolic process and

240 neuron differentiation required further studies to elucidate.

241   According to the KEGG analysis, 15 pathways were significantly altered in hub

242 modules including glutathione metabolism, wnt signaling pathway, central carbon

243 metabolism in cancer, mTOR signaling pathway, pancreatic secretion, protein digestion

244 and absorption, axon guidance, retinol metabolism, insulin secretion, salivary secretion,

245 and fat digestion and absorption. Some of the identified pathways have been suggested

246 to play critical role in CCA progression and therapy. In intrahepatic cholangiocarcinoma

247 (ICC) mouse model, investigators proved that mTOR kinase inhibitors inducing strong

248 ICC cell apoptosis and may be beneficial for the treatment of ICC(Zhang et al. 2017b). It

249 has been reported that the contact inhibition of cholangiocarcinoma cells could be

250 overcome by c-Myc via the mTOR pathway(Luo et al. 2017). The identified significant

251 pathways of secretion and absorbance of multiple digestive juice remind us the

252 importance of digestive balance in the development of CCA, which required further

253 investigations to follow. The significant altered pathways of insulin secretion, salivary

254 secretion, and fat digestion and absorption confirmed the key role of digestive regulation

255 in CCA, as was indicated by a number of previous investigations(Chaiyarit et al. 2011;

256 Chen et al. 2013; Rizvi et al. 2014).

257   The five-year survival rate of CCA currently remains unsatisfactory despite recent

258 improvements in treatments of surgical resection and chemotherapy(Squadroni et al.

259 2017). The selected genes hold great possibilities to become potential biomarkers for the

260 prediction of survival of CCA patients. We eventually screened 15 prognosis-related

261 genes in CAA after analyzing the hub modules of tan and salmon. Increased expression

262 of SOX2 in CCA cells lead to cell proliferation, decreased cell apoptosis, and increased

263 cell invasion and metastasis(Sun et al. 2014). Besides, SOX2 expression correlated with

264 aggressive behavior and worse overall survival in intrahepatic cholangiocarcinoma(Gu &

265 Jang 2014). Among the identified prognosis-related genes, RNF39 and PRSS56 also

266 showed significant correlation with clinical stage. Until now, little is known about the

267 functions of these two genes in cancer. RNF39 encoded a member of ring finger proteins,

268 which was studied in Behcet's disease, a chronic inflammatory autoimmune

269 disease(Kurata et al. 2010). In addition, RNF39 protein was suggested to participate in

270 the replication process according to both studies on HIV-1 individuals and cell-lines(Lin

271 et al. 2014). PRSS56 has been established as an effective marker of adult neurogenesis

272 in the brain of mouse(Jourdon et al. 2016). Variants in PRSS56 may lead to primary

273 angle-closure glaucoma and high hyperopia(Jiang et al. 2013). Whether RNF39 and

274   PRSS56 could be a robust biomarker for CCA and their underlying mechanisms still

275   required further studies to explore. In addition, DNA repair gene XPC and immune

276   regulator CD28 were related with favourable survival of CCA, which indicate probable

277   implication of DNA repair and immune response in CCA development. Because no distant

278   metastasis information was available In TCGA data of CCA, we did not perform

279   association analysis of genes with CCA metastasis, which require future studies to clarify.

280   **Conclusion**

281   In summary, differentially expressed genes and key modules contributing to

282   progression of cholangiocarcinoma were identified by means of WGCNA. Pathways

283   including glutathione metabolism, wnt signaling, central carbon metabolism in cancer,

284   mTOR signaling, pancreatic secretion, protein and fat digestion, insulin secretion, and

285   salivary secretion might be closely related with CCA development. Key genes such as

286   RNF39 and PRSS56 might be potential prognostic markers for CCA. The results offer

287   novel insights into the understanding of the development and prognosis of CAA.

288

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290

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410 **Figure legends**

411 **Figure 1.** Interaction relationship analysis of co-expression genes in cholangiocarcinoma.

412 **Figure 2.** The module–clinical trait relationships of genes involved in clinicopathological

413 parameters of cholangiocarcinoma patients.

414 **Figure 3.** Gene Ontology analysis for genes in the hub modules of tan module and salmon

415 module in cholangiocarcinoma.

416 **Figure 4.** KEGG pathway enrichment analysis for genes in the hub modules of tan module

417 and salmon module in cholangiocarcinoma.

418 **Figure 5.** The correlation between the expression levels of key genes of hub modules

419 and the survival of CAA patients. A, INS; B, PPP3CA; C, CST1; D, RNF39; E, PRSS56;

420 F, PRRG4; G, SLC4A4; H, PIR.

421 **Figure 6.** The differential expression of potential hub genes in different stages of CCA

422 patients. A, differential expression of INS, PPP3CA, CST1 and RNF39; B, differential

423 expression of PRSS56, PRRG4, SLC4A4 and PIR.

424 **Supplementary Figure 1.** Threshold selection of WGCNA analysis.

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Gene ontology (GO) analysis of the genes involved in hub modules.

Gene ontology (GO) analysis of the genes involved in hub modules.

1   Table 1. Gene ontology (GO) analysis of the genes involved in hub modules.

Module	ID	Description	GeneRatio	P value	Count
tan	GO:0003179	heart valve morphogenesis	5/181	3.36E-05	5
tan	GO:0006739	NADP metabolic process	5/181	3.36E-05	5
tan	GO:0003170	heart valve development	5/181	5.67E-05	5
tan	GO:0003281	ventricular septum development	6/181	5.83E-05	6
tan	GO:0060411	cardiac septum morphogenesis	6/181	6.92E-05	6
tan	GO:0003279	cardiac septum development	7/181	7.37E-05	7
tan	GO:0072537	fibroblast activation	3/181	0.00013	3
tan	GO:0001101	response to acid chemical	12/181	0.00015	12
tan	GO:0016055	Wnt signaling pathway	15/181	0.00016	15
tan	GO:0198738	cell-cell signaling by wnt	15/181	0.00017	15
salmon	GO:0007586	digestion	16/148	1.02E-12	16
salmon	GO:0010817	regulation of hormone levels	22/148	1.53E-10	22
salmon	GO:0009914	hormone transport	15/148	7.10E-08	15
salmon	GO:0046879	hormone secretion	14/148	2.85E-07	14
salmon	GO:0023061	signal release	16/148	6.22E-07	16
salmon	GO:0030072	peptide hormone secretion	12/148	1.16E-06	12
salmon	GO:0046883	regulation of hormone secretion	12/148	1.84E-06	12
salmon	GO:0050679	positive regulation of epithelial cell proliferation	10/148	2.07E-06	10
salmon	GO:0090276	regulation of peptide hormone secretion	10/148	8.73E-06	10
salmon	GO:0050673	epithelial cell proliferation	13/148	1.32E-05	13

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of the genes involved in hub modules.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of the genes involved in hub modules.

1 Table 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of the genes involved in hub modules.

Module	ID	Description	GeneRatio	P value	Count
tan	hsa05224	Breast cancer	10/81	3.65E-06	10
tan	hsa00480	Glutathione metabolism	6/81	2.84E-05	6
tan	hsa04310	Wnt signaling pathway	8/81	0.00017	8
tan	hsa05230	Central carbon metabolism in cancer	5/81	0.00066	5
tan	hsa05226	Gastric cancer	7/81	0.00111	7
tan	hsa04150	mTOR signaling pathway	7/81	0.0012	7
salmon	hsa04972	Pancreatic secretion	11/64	3.74E-10	11
salmon	hsa04974	Protein digestion and absorption	10/64	3.45E-09	10
salmon	hsa05217	Basal cell carcinoma	3/64	0.01654	3
salmon	hsa04360	Axon guidance	5/64	0.0167	5
salmon	hsa00830	Retinol metabolism	3/64	0.01948	3
salmon	hsa04380	Osteoclast differentiation	4/64	0.02372	4
salmon	hsa04911	Insulin secretion	3/64	0.03605	3
salmon	hsa04970	Salivary secretion	3/64	0.04162	3
salmon	hsa04975	Fat digestion and absorption	2/64	0.04786	2

Key genes associated with prognosis of cholangiocarcinoma patients in hub modules.

Key genes associated with prognosis of cholangiocarcinoma patients in hub modules.

1   Table 3. Key genes associated with prognosis of cholangiocarcinoma patients in hub modules.

Gene	Full name	Module	HR	95%CI	P value
SOX2	SRY-box 2	tan	0.382	0.145-1.002	0.043
KIT	KIT proto-oncogene receptor tyrosine kinase	tan	0.341	0.132-0.883	0.048
PRSS56	serine protease 56	tan	2.876	1.048-7.892	0.018
WNT9A	Wnt family member 9A	tan	0.389	0.144-1.050	0.041
SLC4A4	solute carrier family 4 member 4	tan	0.352	0.133-0.931	0.025
PRRG4	proline rich and Gla domain 4	tan	0.339	0.127-0.900	0.023
PANX2	pannexin 2	tan	2.600	0.963-7.022	0.037
PIR	pirin	tan	2.701	0.994-7.337	0.027
RASSF8	Ras association domain family member 8	tan	0.396	0.147-1.065	0.040
MFSD4A	major facilitator superfamily domain containing 4A	salmon	0.371	0.143-0.962	0.043
INS	insulin	salmon	4.670	0.458-47.564	0.006
RNF39	ring finger protein 39	salmon	2.811	1.063-7.434	0.032
IL1R2	interleukin 1 receptor type 2	salmon	0.379	0.144-0.997	0.041
CST1	cystatin SN	salmon	2.846	1.039-7.797	0.017
PPP3CA	protein phosphatase 3 catalytic subunit alpha	salmon	3.151	1.129-8.793	0.010

The association of prognosis-related genes with clinical of stage of cholangiocarcinoma patients.

The association of prognosis-related genes with clinical of stage of cholangiocarcinoma patients.

1   Table 4. The association of prognosis-related genes with clinical of stage of cholangiocarcinoma patients.

Gene symbol	Gene title	Position	OR	P
MFSD4A	Major Facilitator Superfamily Domain Containing 4A	1q32.1	1.036(0.988-1.093)	0.155
SOX2	SRY-Box 2	3q26.33	1.037(0.986-1.115)	0.184
INS	Insulin	11p15.5	1.125(0.996-1.320)	0.059
KIT	KIT Proto-Oncogene Receptor Tyrosine Kinase	4q12	1.033(0.991-1.091)	0.146
PRSS56	Serine Protease 56	2q37.1	**1.162(1.002-1.431)**	**0.013**
RNF39	Ring Finger Protein 39	6p22.1	**1.069(1.006-1.167)**	**0.019**
WNT9A	Wnt Family Member 9A	1q42.13	0.965(0.904-1.018)	0.235
SLC4A4	Solute Carrier Family 4 Member 4	4q13.3	1.001(0.973-1.029)	0.945
IL1R2	Interleukin 1 Receptor Type 2	2q11.2	1.027(0.993-1.068)	0.131
CST1	Cystatin SN	20p11.21	1.007(0.988-1.026)	0.490
PRRG4	Proline Rich And Gla Domain 4	11p13	0.983(0.922-1.041)	0.574
PANX2	Pannexin 2	22q13.33	0.987(0.940-1.024)	0.517
PIR	Pirin	Xp22.2	0.983(0.930-1.027)	0.489
RASSF8	Serine Protease 8	12p12.1	0.942(0.863-1.002)	0.094
PPP3CA	Protein Phosphatase 3 Catalytic Subunit Alpha	4q24	1.021(0.960-1.086)	0.492

Figure 1

Interaction relationship analysis of co-expression genes in cholangiocarcinoma.

Interaction relationship analysis of co-expression genes in cholangiocarcinoma.

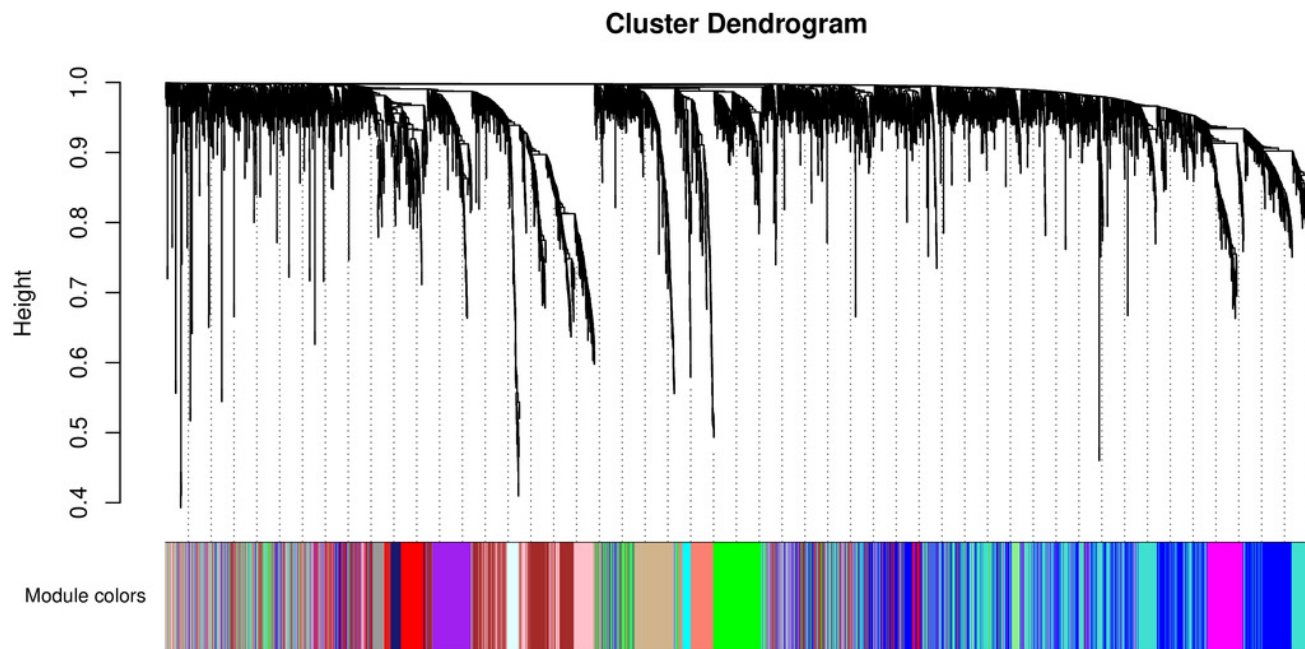



Figure 2

The module–clinical trait relationships of genes involved in clinicopathological parameters of cholangiocarcinoma patients.

The module–clinical trait relationships of genes involved in clinicopathological parameters of cholangiocarcinoma patients.

Module–trait relationships

	histologic_grade	recurrence	pathologicM	pathologicN	pathologicT	stage
MEroyalblue	−0.099 (0.6)	0.074 (0.7)	−0.13 (0.5)	−0.27 (0.1)	−0.09 (0.6)	−0.12 (0.5)
MEmagenta	−0.15 (0.4)	0.14 (0.5)	0.17 (0.3)	−0.15 (0.4)	−0.021 (0.9)	−0.014 (0.9)
MEblue	−0.19 (0.3)	0.083 (0.6)	0.056 (0.8)	−0.33 (0.06)	−0.1 (0.6)	−0.14 (0.4)
MEturquoise	0.025 (0.9)	0.14 (0.4)	0.13 (0.5)	−0.26 (0.1)	−0.063 (0.7)	−0.012 (0.9)
MEgreen	−0.045 (0.8)	0.092 (0.6)	−0.28 (0.1)	−0.35 (0.04)	−0.00036 (1)	−0.1 (0.6)
MElightgreen	0.00095 (1)	0.12 (0.5)	0.14 (0.4)	0.16 (0.4)	0.31 (0.08)	0.31 (0.08)
MElightyellow	−0.099 (0.6)	0.22 (0.2)	−0.12 (0.5)	−0.057 (0.8)	−0.18 (0.3)	−0.17 (0.3)
MEblack	0.032 (0.9)	−0.19 (0.3)	−0.096 (0.6)	−0.12 (0.5)	−0.13 (0.5)	−0.13 (0.5)
MEgreenyellow	0.42 (0.01)	0.23 (0.2)	0.091 (0.6)	0.1 (0.6)	−0.13 (0.5)	−0.0041 (1)
MEtan	0.35 (0.04)	−0.069 (0.7)	0.37 (0.03)	0.43 (0.01)	0.076 (0.7)	0.26 (0.1)
MEyellow	0.098 (0.6)	−0.23 (0.2)	−0.052 (0.8)	−0.28 (0.1)	−0.27 (0.1)	−0.2 (0.3)
MEpurple	−0.11 (0.6)	0.14 (0.4)	0.17 (0.3)	0.19 (0.3)	−0.064 (0.7)	−0.074 (0.7)
MEcyan	−0.27 (0.1)	0.038 (0.8)	0.13 (0.5)	0.15 (0.4)	−0.011 (1)	−0.0096 (1)
MEsalmon	−0.45 (0.009)	−0.11 (0.5)	0.38 (0.03)	0.41 (0.02)	0.31 (0.08)	0.27 (0.1)
MEgrey60	−0.1 (0.6)	−0.072 (0.7)	0.015 (0.9)	−0.23 (0.2)	−0.026 (0.9)	−0.096 (0.6)
MEmidnightblue	−0.32 (0.07)	−0.21 (0.2)	−0.0083 (1)	−0.0056 (1)	0.022 (0.9)	−0.064 (0.7)
MEred	−0.16 (0.4)	0.11 (0.6)	0.17 (0.4)	0.25 (0.2)	0.28 (0.1)	0.19 (0.3)
MElightcyan	−0.26 (0.1)	−0.22 (0.2)	−0.23 (0.2)	−0.24 (0.2)	−0.18 (0.3)	−0.25 (0.2)
MEbrown	−0.23 (0.2)	0.028 (0.9)	−0.23 (0.2)	−0.24 (0.2)	−0.31 (0.08)	−0.35 (0.05)
MEpink	−0.16 (0.4)	−0.17 (0.3)	−0.24 (0.2)	−0.25 (0.2)	−0.31 (0.08)	−0.34 (0.06)
MEgrey	0.24 (0.2)	0.17 (0.3)	−0.2 (0.3)	0.026 (0.9)	−0.11 (0.5)	−0.056 (0.8)

Figure 3

Gene Ontology analysis for genes in the hub modules of tan module and salmon module in cholangiocarcinoma.

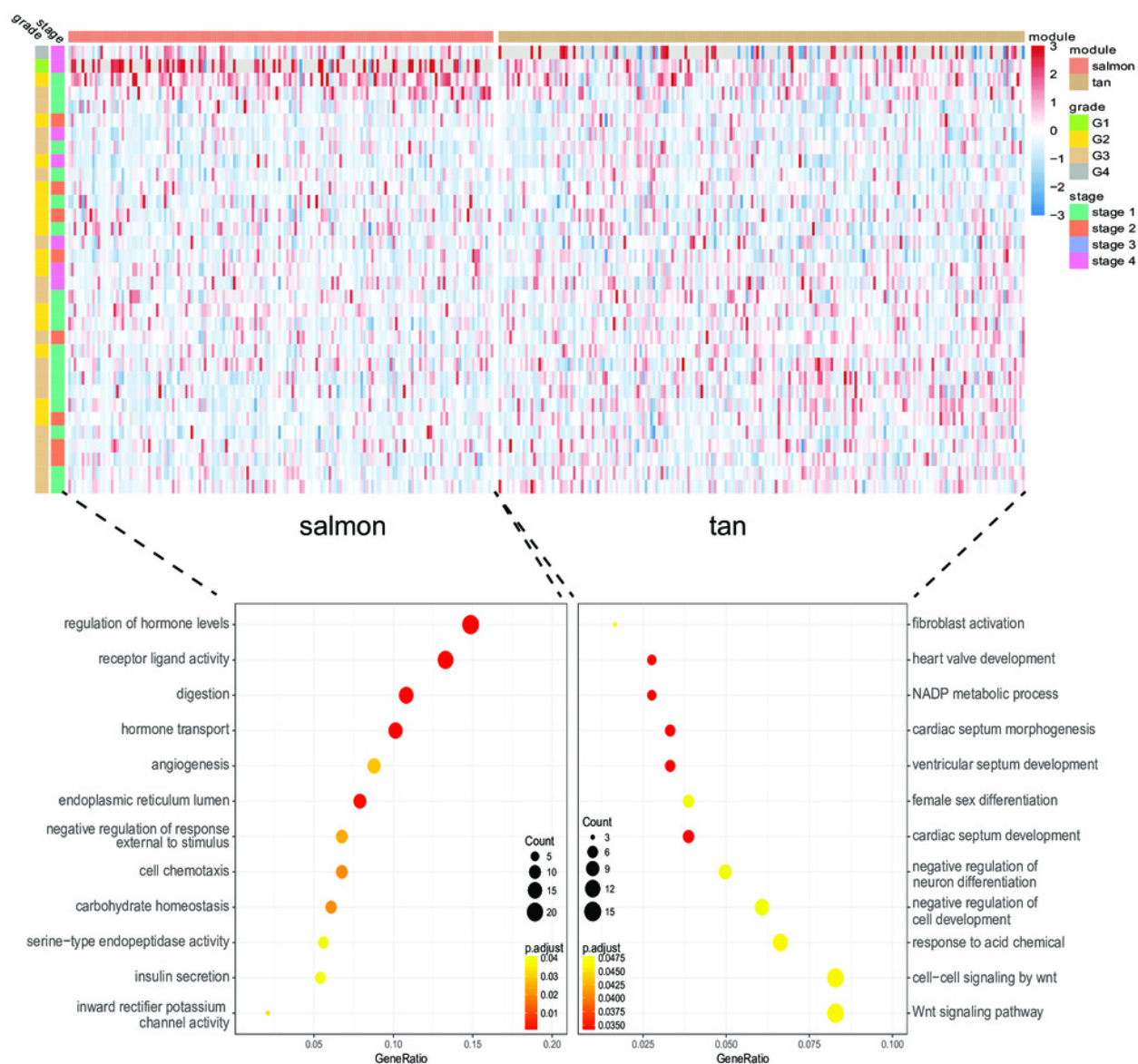
Gene Ontology analysis for genes in the hub modules of tan module and salmon module in cholangiocarcinoma.

![Figure 3]

Figure 4

The correlation between the expression levels of key genes of hub modules and the survival of CAA patients.

The correlation between the expression levels of key genes of hub modules and the survival of CAA patients.

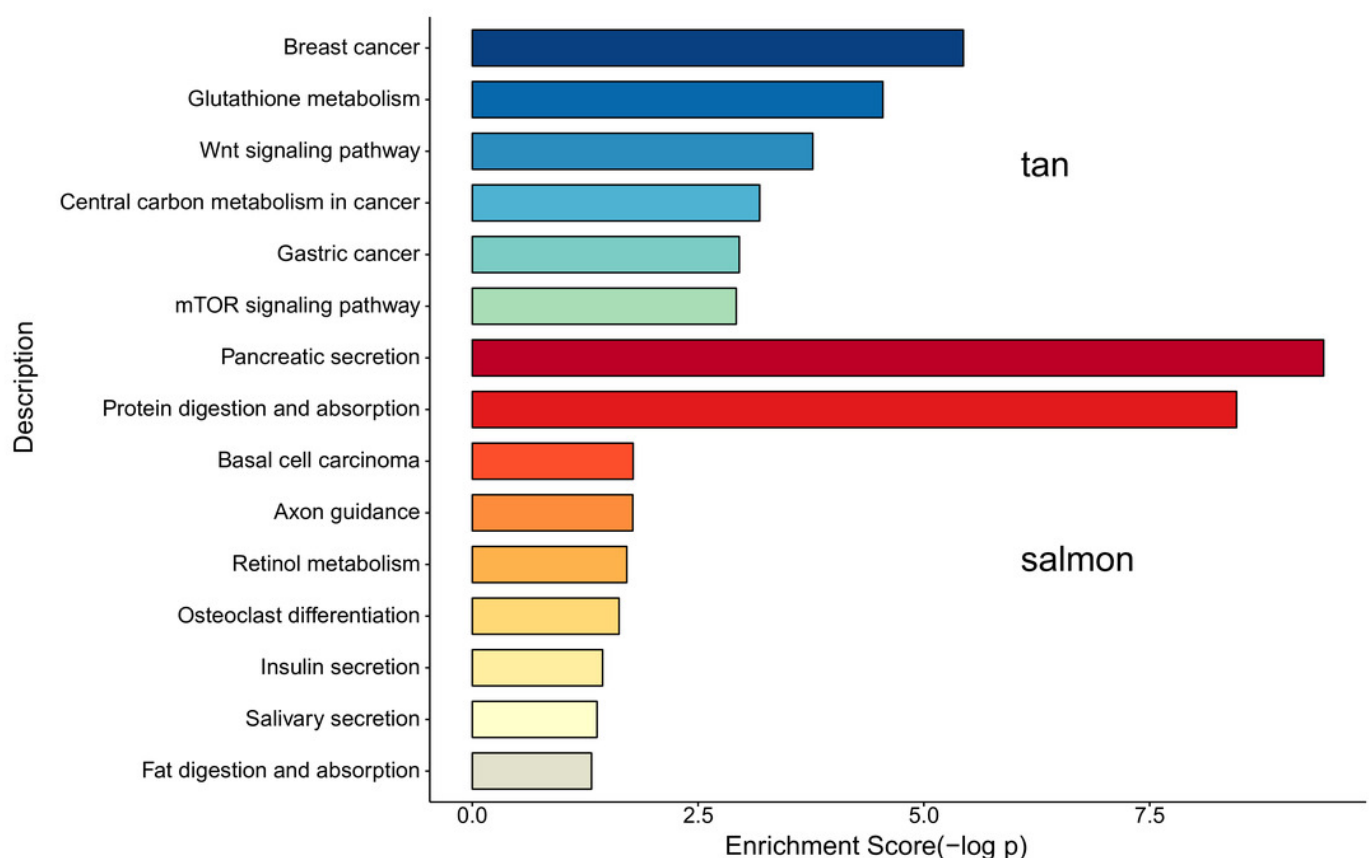



Figure 5

The correlation between the expression levels of key genes of hub modules and the survival of CAA patients.

The correlation between the expression levels of key genes of hub modules and the survival of CAA patients.

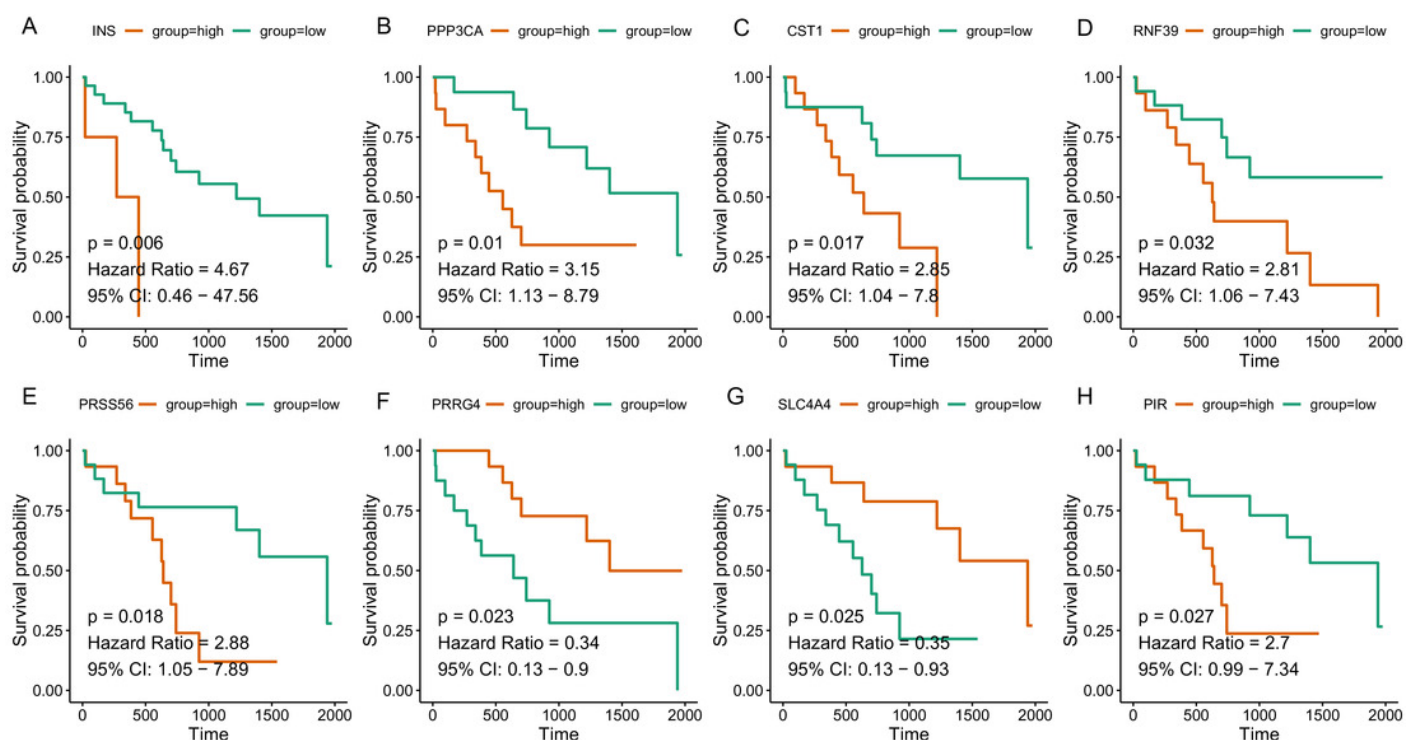



Figure 6

The differential expression of potential hub genes in different stages of CCA patients.

The differential expression of potential hub genes in different stages of CCA patients.