

Identification of critically carcinogenesis-related genes in basal cell carcinoma

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Background: Basal cell carcinoma (BCC) is a frequent malignant tumor of skin cancers with high morbidity. The objective of this study was to identify critical genes and pathways related to the carcinogenesis of BCC and gain more insights into the underlying molecular mechanisms of BCC.

Materials and methods: The gene expression profiles of GSE7553 and GSE103439 were downloaded from the Gene Expression Omnibus database with 19 tumors and 6 normal skin tissues. Differentially expressed genes (DEGs) were screened between BCC samples and normal tissues, followed by gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. Subsequently, protein-protein interaction (PPI) network was constructed for these DEGs, and module analysis was performed.

Results: A total of 313 DEGs were obtained. Among them, 222 genes were upregulated and 91 genes were downregulated. Enrichment analysis indicated that the upregulated genes were significantly enriched in cell cycle and mitosis, while the downregulated genes were mainly associated with unsaturated fatty acid metabolic process and cell differentiation. In addition, *TOP2A*, *CDK1*, and *CCN1* were identified as the top three hub genes ranked by degrees in the PPI network. Meanwhile, three subnetworks were derived, which indicated that these DEGs were significantly enriched in pathways, including “cell cycle”, “extracellular matrix-receptor interaction”, “basal cell carcinoma”, and “hedgehog signaling pathway”.

Conclusions: The novel critical DEGs and pathways identified in this study may serve pivotal roles in the carcinogenesis of BCC and indicate more molecular targets for the treatment of BCC.

Keywords: basal cell carcinoma, differentially expressed genes, enrichment analysis, bioinformatics analysis

Introduction

Cutaneous basal cell carcinoma (BCC) is recognized as a common subtype of nonmelanoma skin malignancies with high morbidity, which accounts for ~80% of newly diagnosed nonmelanoma skin carcinomas.¹ In the last decade, there has been a substantial increase in the incidence of BCC.² Due to the characteristics of slow-growing and locally aggressive, metastasis rarely occurred in patients with BCC, which resulted in a relatively good prognosis. As we all know, long-term exposure to sunlight, especially ultraviolet light, is considered as the main risk factor of skin cancers.³ However, the underlying molecular mechanisms for the development of BCC has not been completely illuminated. Meanwhile, the treatments of BCC are limited and drug resistance is ubiquitous in advanced or metastatic BCC patients. Therefore, an urgent need exists for further exploring the potential mechanisms of BCC and finding more effective molecular targets for the treatment of BCC.

To date, several signaling pathways and molecules have been demonstrated to be involved in the tumorigenesis and progression of BCC at the molecular level, such as the hedgehog signaling pathway.⁴ Genes included in this pathway, such as the hedgehog receptors patched (PTCH1) or smoothed (SMO), have been extensively studied.^{5,6} Mutations in these genes may cause constitutive hedgehog pathway activation, which promote the development of BCC. Recently, two new hedgehog pathway inhibitors, Vismodegib and Sonidegib, have been approved by the Food and Drug Administration for the targeted treatment of BCC.^{7,8} However, the response rate of advanced or metastatic BCC is not promising and the secondary drug resistance may also occur.

With the development of high-throughput technology, more and more new potential targets have been uncovered in BCC. In addition to canonical hedgehog pathway components, the transcription factor serum response factor was identified as a noncanonical hedgehog activator by multidimensional genomics analysis, which leads to the amplification of the hedgehog transcription factor glioma-associated oncogene family zinc finger-1 (GLI1).⁹ At the DNA level, Bonilla et al performed a genomic analysis of 293 BCC samples and revealed that mutations in other cancer-related genes also drove the initiation of BCC, including MYCN, PTPN14, and LATS1.¹⁰ Thus, much more molecular targets remain to be elucidated.

Bioinformatics analysis of gene expression profiles or other high-throughput data are now playing a critical role in investigating the mechanisms of human disease, particularly in tumors. Accordingly, in the present study, we first time integratively reanalyzed the gene expression profiles of 19 BCC and 6 normal tissues deposited in two datasets by differentially expressed genes (DEGs) screening and functional and pathway enrichment analysis. By protein–protein interaction (PPI) network analysis, we identified top three hub genes (TOP2A, CDK1, and CCNB1). Finally, module analysis revealed that several critical pathways were mainly associated with the carcinogenesis of BCC, which might be used as molecular targets for the treatment of BCC.

Materials and methods

Microarray data

Two datasets (GSE7553 and GSE103439) were respectively retrieved from Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo/>), including 19 BCC and 6 normal tissues (Table 1).¹¹ These gene expression profiles were generated by GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array) containing 54,675 probes. The latest

Table 1 The basal information of two datasets in this study

GEO datasets	Platform	Number of BCC	Number of NS
GSE7553	GPL570	15	4
GSE103439	GPL570	4	2

Abbreviations: BCC, basal cell carcinoma; GEO, Gene Expression Omnibus; NS, normal skin.

annotation file of GPL570 platform was downloaded from Affymetrix official website (<http://www.affymetrix.com/>), in which 54,675 probes now mapped to 21,297 genes.

Data preprocessing and DEGs screening

The raw data files (.CEL files) of these 25 samples were processed by the R package “affy”.¹² Background adjustment and normalization were performed using the Robust Multichip Average algorithm. Once multiple probes mapped to the same gene, the average value was finally selected to represent the gene expression value. DEGs were screened between BCC and normal tissues by the “limma” package in R.¹³ Then, hierarchical clustering analysis was applied to the DEGs by the “pheatmap” package in R based on the Euclidean distance. The criteria of DEGs was set as $|\log_2 \text{fold change}| > 1$ and false discovery rate (FDR) < 0.05 .

Functional and pathway enrichment analysis

Gene ontology (GO) analysis defines the functions of gene products covering three domains, including biological process, molecular function, and cellular component.^{14,15} The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database is widely used to map large-scale datasets to pathway maps for higher-order functional information.¹⁶ The Database for Annotation, Visualization and Integrated Discovery (DAVID version 6.8, <http://david.abcc.ncifcrf.gov/>) consists of an integrated biological knowledgebase and analytic tools, which can systematically extract biological meaning from large gene/protein lists.¹⁷ With the online DAVID tool, we performed functional and pathway enrichment analysis for these DEGs. *P*-value < 0.05 was considered as significant.

Construction of PPI network and module analysis

Given the large number of DEGs, the “STRINGdb” package in R was used to investigate the potential interactions that existed in these DEGs.¹⁸ Briefly, 313 DEGs were mapped

to their corresponding proteins in the Search Tool for the Retrieval of Interacting Genes/Proteins database. Only interactions with a combined score of >0.4 were imported into Cytoscape software to visualize the PPI network.¹⁹ Each node in the network represents one protein, and the degree of each protein was termed as the number of its interactions. Then, the Molecular Complex Detection (MCODE) plug-in was used to analyze the PPI network to identify significant modules.²⁰ In addition, the functional and pathway enrichment analysis of genes in the subnetworks were performed. P -value <0.05 was set as the threshold.

Results

Identification of DEGs

We screened DEGs in the two datasets (GSE7553 and GSE103439). Compared with normal skin tissues, 1,871 DEGs and 5,357 DEGs were obtained, respectively (Figure 1A). Finally, a total of 313 aberrantly expressed genes (222 upregulated genes and 91 downregulated genes) were identified by integrated analysis (Figure 1B and C). Strikingly, the number of upregulated genes were largely more than downregulated genes (Table S1). The heatmap of hierarchical clustering analysis showed that these DEGs could clearly distinguish BCC samples from the normal skin samples (Figure 1D and E).

GO and KEGG pathway enrichment analysis

To further investigate the potential functions of these 313 DEGs, GO and KEGG pathways enrichment analysis was performed by the online DAVID tool. The results of GO analysis indicated that upregulated genes enriched in biological process were mainly involved in cell cycle and mitosis, such as the cell division ($P=4.39\times 10^{-11}$) and the mitotic nuclear division ($P=5.90\times 10^{-8}$) (Table 2). Meanwhile, downregulated genes were significantly enriched in unsaturated fatty acid metabolic process ($P=2.10\times 10^{-3}$) and cell differentiation ($P=6.76\times 10^{-3}$) (Table 3). With regard to pathway enrichment analysis, the most significant pathway of upregulated genes was cell cycle ($P=4.75\times 10^{-9}$) containing 13 genes. Interestingly, another five genes (LEF1, PTCH1, GLI2, FZD7, and GLI1) were enriched in the pathway named “basal cell carcinoma” ($P=2.60\times 10^{-3}$) (Table 2), while downregulated genes were most significantly involved in the biosynthesis and metabolism of unsaturated fatty acids ($P=5.26\times 10^{-3}$) (Table 3).

PPI network analysis and module analysis

After data of interactions imported into Cytoscape software, the PPI network with 202 nodes and 1,245 edges was constructed. Based on this network, TOP2A (degree =64),

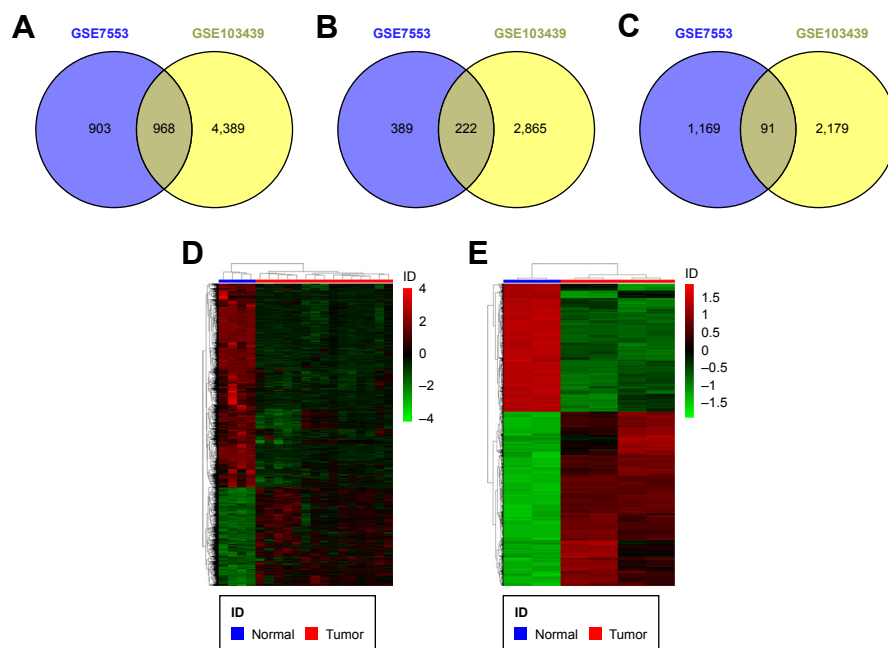


Figure 1 DEGs in the two datasets.

Notes: (A) Common DEGs between GSE7553 and GSE103439. (B) Common upregulated DEGs between GSE7553 and GSE103439. (C) Common downregulated DEGs between GSE7553 and GSE103439. (D, E) Hierarchical clustering analysis of the DEGs in GSE7553 and GSE103439, respectively. Red and green indicate higher expression and lower expression, respectively.

Abbreviation: DEGs, differentially expressed genes.

Table 2 The top 10 GO terms and KEGG pathways of upregulated genes

Term	Count	P-value	Genes
GO:0051301:cell division	24	4.39E-11	<i>KIF14, CDK1, KIF11, NEK2, NUF2, KIF18B, NDC80, BIRC5, CDC20, CDC25C, MCM5, CCNE2, CCNB1, SPC25, MAD2L1, CCNB2, HMCN1, SGO2, SPAG5, NCAPG, NCAPG2, ZWINT, CENPW, BUB1B</i>
GO:0007067:mitotic nuclear division	17	5.90E-08	<i>CDK1, KIF11, NEK2, KIF15, NUF2, BIRC5, NDC80, CDC20, PBK, CEP55, CDC25C, SPC25, CCNB2, NCAPG2, BUB1B, CENPW, ASPM</i>
GO:0000070:mitotic sister chromatid segregation	7	4.24E-07	<i>MAD2L1, NEK2, SPAG5, ZWINT, NUSAP1, KIF18B, NDC80</i>
GO:0007062:sister chromatid cohesion	11	4.75E-07	<i>SPC25, MAD2L1, SGO2, ZWINT, KIF18A, NUF2, BUB1B, NDC80, BIRC5, CDC20, CENPK</i>
GO:0007052:mitotic spindle organization	7	1.35E-06	<i>CCNB1, SPC25, KIF11, PCNT, TTK, NDC80, STMN1</i>
GO:0007019:microtubule depolymerization	5	4.11E-06	<i>KIF14, STMN3, KIF18A, KIF18B, STMN1</i>
GO:0045893:positive regulation of transcription, DNA templated	21	4.56E-06	<i>SOX11, PAX6, TGFB3, ATAD2, LEF1, TBX1, CREB5, SOX9, GLI2, MDK, FZD7, GLI1, MYCN, SMARCD3, LHX2, ZNF711, TFAP2B, CAND2, RFX3, PTCH1, SOX18</i>
GO:0030574:collagen catabolic process	8	1.18E-05	<i>MMP10, COL6A3, COL6A2, COL6A1, ADAMTS3, COL11A1, COL5A2, MMP12</i>
GO:0007059:chromosome segregation	8	1.77E-05	<i>SPC25, KIF11, NEK2, SPAG5, NUF2, CENPW, NDC80, TOP2A</i>
GO:0006260:DNA replication	11	1.92E-05	<i>CDK1, GINS2, POLE2, DTL, RRM2, BRIP1, CDC25C, MCM5, FEN1, MCM6, NFIB</i>
hsa04110:cell cycle	13	4.75E-09	<i>CCNE2, CCNB1, CDK1, MAD2L1, CCNB2, GADD45G, TGFB3, TTK, BUB1B, CDC20, CDC25C, MCM5, MCM6</i>
hsa04115:p53 signaling pathway	6	6.65E-04	<i>CCNB1, CCNE2, CDK1, CCNB2, RRM2, GADD45G</i>
hsa04974:protein digestion and absorption	6	0.002273	<i>COL6A3, COL6A2, COL6A1, COL11A1, COL5A2, DPP4</i>
hsa05217:basal cell carcinoma	5	0.002604	<i>LEF1, PTCH1, GLI2, FZD7, GLI1</i>
hsa03030:DNA replication	4	0.006291	<i>POLE2, MCM5, FEN1, MCM6</i>
hsa04512:ECM-receptor interaction	5	0.013198	<i>COL6A3, COL6A2, COL6A1, COL11A1, COL5A2</i>
hsa04914:progesterone-mediated oocyte maturation	5	0.013198	<i>CCNB1, CDK1, MAD2L1, CCNB2, CDC25C</i>
hsa05200:pathways in cancer	10	0.021829	<i>CCNE2, TGFB3, RUNX1T1, LEF1, BIRC5, PTCH1, GLI2, FZD7, GNG7, GLI1</i>
hsa04114:oocyte meiosis	5	0.027764	<i>CCNE2, CDK1, MAD2L1, CDC20, CDC25C</i>
hsa04340:hedgehog signaling pathway	3	0.032592	<i>PTCH1, GLI2, GLI1</i>

Abbreviations: ECM, extracellular matrix; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

CDK1 (degree =59), and CCNB1 (degree =54) were screened as the top three hub genes due to the higher degrees (Figure 2). Subsequently, we performed module analysis of the whole network by the MCODE plug-in. Three modules were

identified and created as subnetworks. In addition, pathway enrichment analysis of genes included in each subnetwork was performed, which revealed that DEGs in modules 1–3 were mainly associated with “cell cycle”, “extracellular

Table 3 The top 10 GO terms and KEGG pathways of downregulated genes

Term	Count	P-value	Genes
GO:0048704:embryonic skeletal system morphogenesis	4	7.52E-04	<i>HOXB2, HOXB7, HOXA5, HOXA6</i>
GO:0036109:alpha-linolenic acid metabolic process	3	0.001566736	<i>ELOVL5, FADS1, FADS2</i>
GO:0006636:unsaturated fatty acid biosynthetic process	3	0.002096572	<i>ELOVL5, FADS1, FADS2</i>
GO:0043651:linoleic acid metabolic process	3	0.002699483	<i>ELOVL5, FADS1, FADS2</i>
GO:0001558:regulation of cell growth	4	0.005913176	<i>MELTF, BCAR1, NANOS1, CYR61</i>
GO:0009952:anterior/posterior pattern specification	4	0.005913176	<i>HOXB2, HOXB7, HOXA5, HOXA6</i>
GO:0007267:cell–cell signaling	6	0.006210487	<i>BMP2, ADRB2, FADS1, AREG, GDF15, CYR61</i>
GO:0055007:cardiac muscle cell differentiation	3	0.006763636	<i>BMP2, SIK1, NRG1</i>
GO:0060325:face morphogenesis	3	0.008308263	<i>DKK1, TIPARP, RRAS</i>
GO:2000726:negative regulation of cardiac muscle cell differentiation	2	0.013694378	<i>BMP2, DKK1</i>
hsa01040:biosynthesis of unsaturated fatty acids	3	0.005255803	<i>ELOVL5, FADS1, FADS2</i>
hsa01212:fatty acid metabolism	3	0.02175659	<i>ELOVL5, FADS1, FADS2</i>
hsa05230:central carbon metabolism in cancer	3	0.037090419	<i>SLC1A5, HKDC1, MYC</i>

Abbreviations: GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

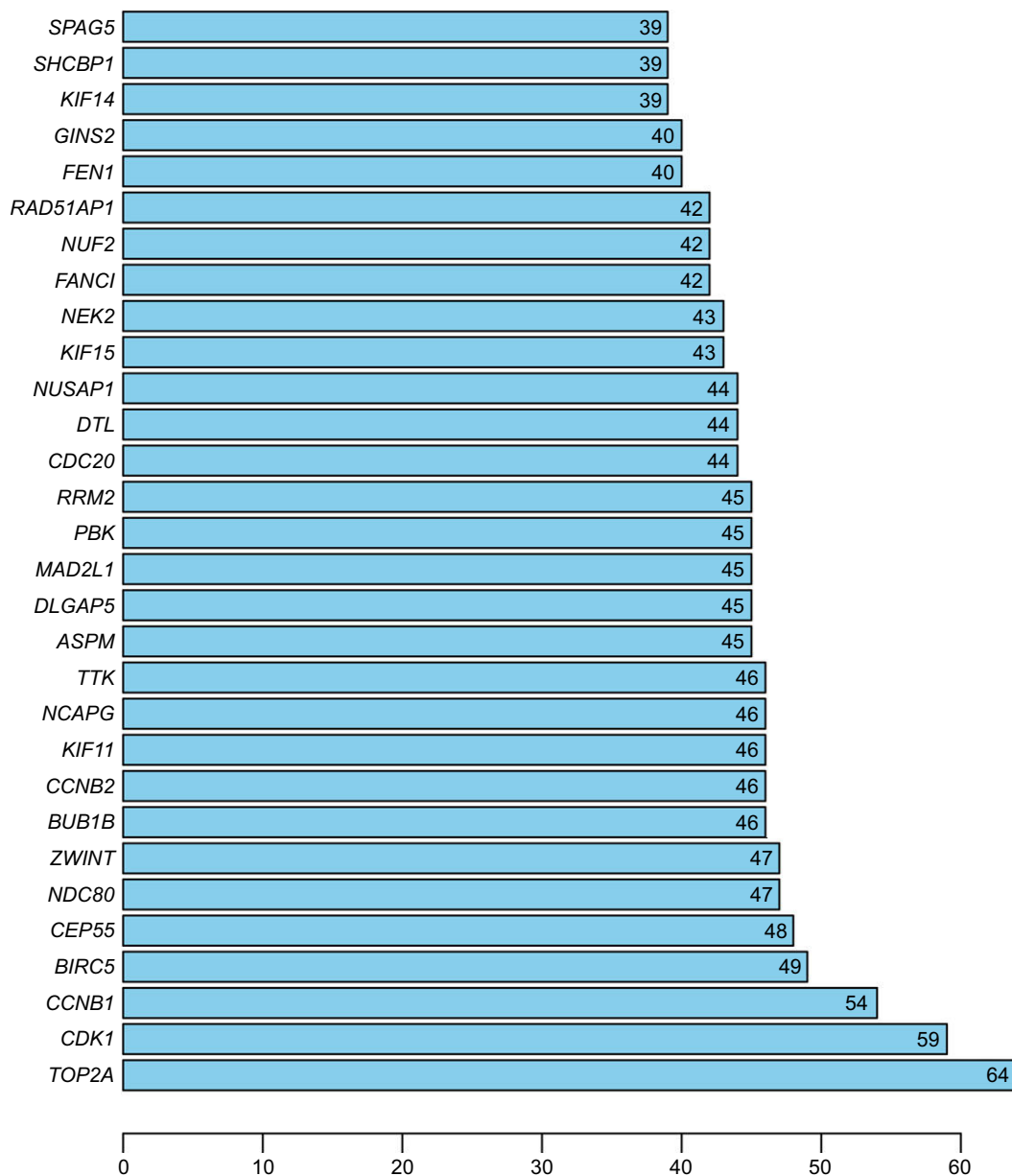


Figure 2 Histogram of degrees of the top 30 genes in the protein–protein interaction network.

Note: The number displayed on each column is the degree of each gene.

matrix (ECM)-receptor interaction”, “basal cell carcinoma”, and “hedgehog signaling pathway” (Figure 3).

Discussion

BCC, with low malignancy, is the most common skin cancer worldwide. Although rarely metastasize, BCC can cause substantial local tissue damage along with disfigurement and involve other adjacent areas of soft tissue, cartilage, and bone.⁷ Currently, the targeted treatments of BCC implicated in clinical practice mainly focus on the hedgehog signaling pathway.²¹ However, the issue of drug resistance and poor response rate cannot be ignored. In order to explore

more potential therapeutic targets, the gene expression profiles of BCC need to be comprehensively studied. In our present study, a bioinformatics approach was conducted to reanalyze the gene expression profiles of 19 BCC and 6 normal skin tissues. A total of 313 DEGs were identified with 222 upregulated genes and 91 downregulated genes. Functional and pathway enrichment analysis indicated that these DEGs were significantly associated with mitosis, cell cycle, and unsaturated fatty acid metabolic process. By PPI network and module analysis, three critical genes and four pathways were finally identified, which may play a key role in the carcinogenesis of BCC.

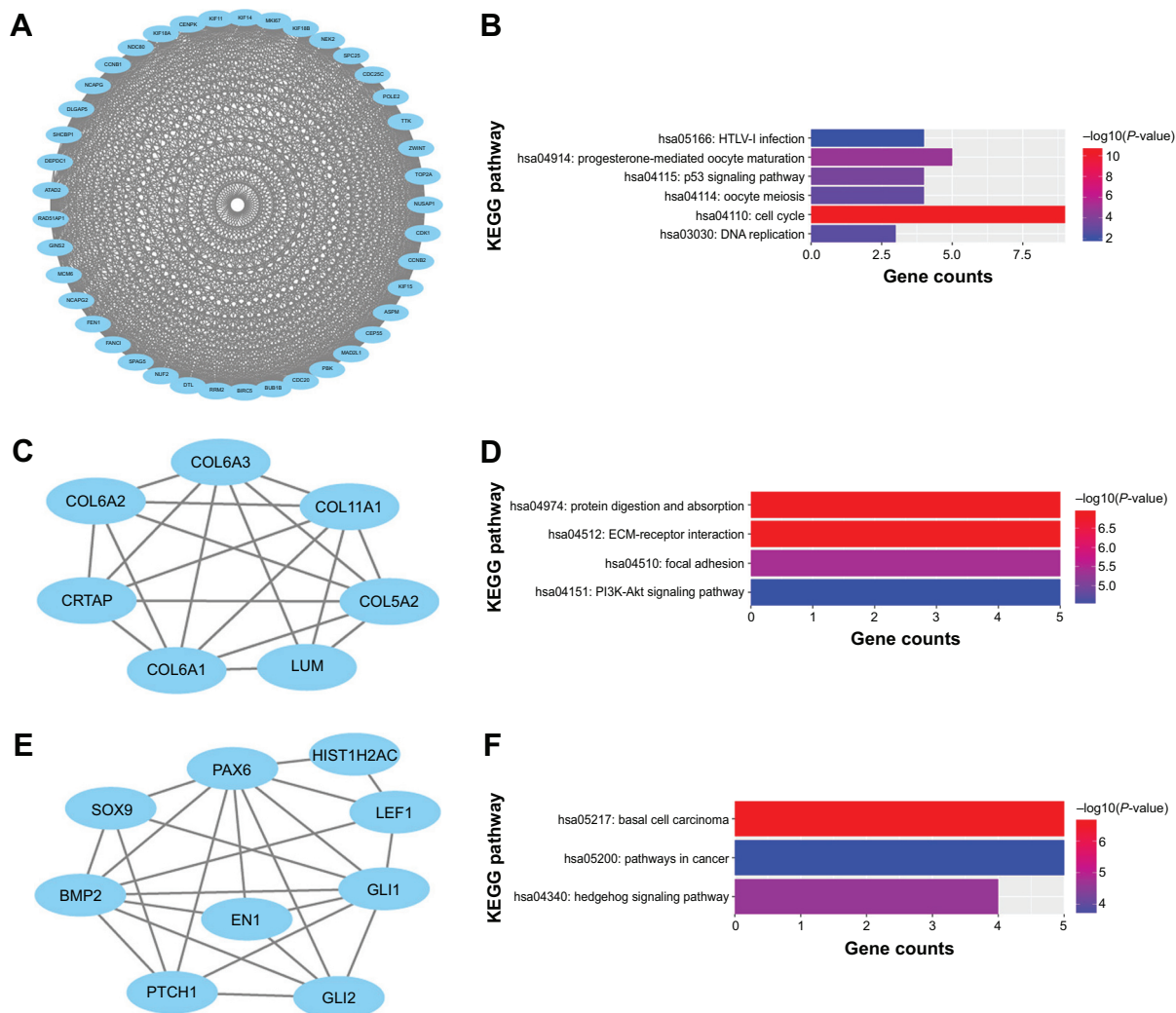


Figure 3 Three subnetworks obtained from the whole protein–protein interaction network. **Notes:** (A, B) Module 1 and the pathway enrichment analysis of genes in module 1. (C, D) Module 2 and the pathway enrichment analysis of genes in module 2. (E, F) Module 3 and the pathway enrichment analysis of genes in module 3. Vertical axis represents GO or pathway terms. P-values are displayed by gradient colors. **Abbreviations:** ECM, extracellular matrix; KEGG, Kyoto Encyclopedia of Genes and Genomes.

With regard to functional and pathway enrichment analysis, upregulated DEGs were mainly involved in the process of mitosis and cell cycle. Deregulation of cell cycle is a common feature in the initiation and progression of various cancers, which is often mediated by alterations in cyclin and cyclin-dependent kinase (CDK) activity.²² CDK1, as a mitotic CDK, is sufficient to drive the mammalian cell cycle without other interphase CDKs.²³ Accumulating evidences indicated that dysregulation of CDK1 activity was participated in a variety of tumors, including lung cancer,²⁴ prostate cancer,²⁵ and colorectal cancer.²⁶ Schmit et al also discovered that increased level of CDK1 and CCNB1 presented in nonmelanoma skin cancer cells (BCC and squamous cell carcinoma) compared with normal human epidermal keratinocytes growth.²⁷ Moreover, patched1, the BCC-related protein,

was found to be interacted with cyclin B1 to regulate cell-cycle progression in BCC.^{28,29} Recently, targeting cyclin-dependent kinases has become a promising approach in cancer therapy. AZD5438, as a highly specific inhibitor of CDK1, 2, and 9, was discovered to enhance the radiosensitivity of non-small-cell lung cancer.³⁰ In the present study, our results revealed that CDK1 was significantly upregulated in BCC samples and enriched in many cell cycle-related GO terms, which indicated the potential to be a therapeutic target in BCC.

Topoisomerases have been considered as important therapeutic targets for human malignancies. TOP2A, the major isoform of topoisomerase II, is capable of resolving catenanes and supercoils during DNA metabolic processes and plays a critical role in condensation and segregation of

chromosomes at mitosis. Accumulating studies highlighted that higher TOP2A expression level was correlated to advanced tumor stage and poor patients' survival in human cancers. At the protein level, increased expression of topoisomerase II α was demonstrated to be associated with elevated cell replication in BCC compared with squamous cell carcinoma.³¹ In our study, TOP2A was screened as the most significant gene with the highest degree and was up-regulated in BCC. Elevated expression of TOP2A was implicated in cell cycle, and targeting TOP2A was also considered as an important therapy for human cancers.³² Thus, TOP2A could be a critical target in BCC.

COL6A1, COL6A2, COL6A3, COL5A2, and COL11A1 are members of the collagen family, and these five genes are enriched in the pathway of "ECM–receptor interaction", which leads to a direct or indirect control of cellular activities such as adhesion, migration, differentiation, proliferation, and apoptosis. Accumulating evidence indicated that the "ECM–receptor interaction" pathway served as a critical role in the carcinogenesis and metastasis of human cancers, such as prostate cancer,³³ breast cancer,³⁴ and colorectal cancer.³⁵ In this study, we also screened "ECM–receptor interaction" as an important pathway by module analysis, which indicated the potential role in the pathogenesis of BCC.

Hedgehog signaling pathway, a highly conserved evolutionary pathway of signal transmission from the cell membrane to the nucleus, has been revealed to be associated with the development of cancers, especially in BCC.⁵ The main downstream genes of hedgehog signaling pathway include PTCH1, GLI1, and GLI2. In the module 3 analysis, these three genes were significantly enriched in "basal cell carcinoma", "hedgehog signaling pathway", and "pathways in cancer". Currently, targeting the hedgehog signaling pathway has been an important strategy for cancer therapy, which has achieved a promising success in BCC.²¹ However, the targeted genes were restricted to two genes (PTCH1 and SMO). Therefore, the other critical genes in this pathway are expected to be studied.

Of note, several limitations also existed in our work. First, the inclusive criteria for BCC patients and normal controls was not available due to lack of data from the public database. Second, the same as most previous studies, two relatively small patient cohorts were performed in this study. Third, there was a lack of validation in biological experiments or another dataset, which might increase the FDR in our results.

In conclusion, we performed a comprehensive bioinformatics analysis of DEGs obtained from 19 BCC and 6 normal skin tissues. Three hub genes and four pathways

were finally identified, which might play a critical role in BCC. Our results further revealed the potential molecular mechanisms during the initiation of BCC and laid the foundation of exploring effective molecular targets for the treatment of BCC. However, future biology experiments are required to confirm these findings.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Differentially expressed genes between basal cell carcinoma and normal skin tissues

Upregulated genes

ADAMTS3
LHX2
CHGA
LGR5
SOX11
S100A9
PTCH1
FBN3
FAT3
TMSB15A
KCNE1
MYCN
CRNN
COL11A1
MMP10
BNC2
GAS2
TOX2
SPON2
TFAP2B
GLI2
HEPH
LMO3
ADAMTS17
VASH2
LINGO1
DIO2
CHST2
PCDHB2
PCDH8
NPNT
SOX18
PITX2
UHRF1
TBX1
CREB5
ABI3BP
LINC00865
EDIL3
GPC4
SHCBP1
SLC6A1
SERPINB4
GJB6
APCDD1L
SOSTDC1
LRRN1
VCAN
BGN
FZD7
SFRP5
TNRC6C
MARCH1

(Continued)

Table S1 (Continued)

MUM1L1
ZNF711
SHOX2
LOC101929122
F2RL2
DTL
TSPYL5
CASC15
PELI2
NRTN
GLI1
SETBP1
FNDC1
MEGF6
RAD51API
PAPPA
SOBP
HUNK
NINL
UCP2
HIST1H4C
ADGRL3
CHRDL2
NAP1L3
NTRK3
TOP2A
SOX9
TSPAN18
H2BFXP
DLGAP5
MAD2L1
S100A8
PLEKHG4B
NUF2
GMPR
NDC80
LRIG3
SLC7A2
CENPK
KRT85
ALDH1A3
MMP12
BIRC5
PCNT
KALRN
KCNS3
SDC2
CYFIP2
KIF11
COL5A2
CNTN4
GBP6
BACH2
HS3ST3A1
LEF1
SGO2
GINS1
CDH11
TM4SF1

(Continued)

Table S1 (Continued)

KIF14
 MARCKSL1
 STMN1
 LOC440173
 PCDHB10
 NEK2
 APOBEC3A
 SMARCD3
 IFI27
 SH3GL3
 SLC6A6
 NUDT10
 MDK
 CMPK2
 APELA
 SHANK2
 GADD45G
 RUNX1T1
 TTK
 CCNB1
 TET1
 LTBP1
 PBK
 KRT13
 CDC20
 ABCC12
 DENND2A
 BEND5
 ASPM
 NUSAP1
 RRM2
 CENPW
 DMD
 BRIP1
 SLAIN1
 SYNPO
 PTPRN2
 NRXN3
 STMN3
 MXRA5
 FANCD2
 DCHS1
 MCM6
 CDC25C
 CDH22
 COL6A2
 SORCS2
 DPP4
 TIGD1
 SPAG5
 MTFR2
 KIF15
 KIF18A
 RFX3
 PRIMA1
 CCNB2

(Continued)

Table S1 (Continued)

STON1
 CDC42EP4
 MCM5
 ZWINT
 SEMA6A
 ZNF367
 CEP55
 HMCN1
 TMTC1
 BUB1B
 PABPC4L
 FANCI
 ZNF566
 CAND2
 POLE2
 CDK1
 STC1
 ALMS1
 PLCE1
 NFIB
 PCSK5
 PLPPR1
 COL6A1
 NCAPG
 BHLHE41
 CCNE2
 PAX6
 MIR3682
 PRRT2
 GINS2
 NCAPG2
 CFAP44
 COL6A3
 FEN1
 TNFSF10
 PLEKHO1
 TNS3
 KIF18B
 ATAD2
 TMEM173
 ZNF853
 SLCO2A1
 TBC1D1
 GNG7
 DEPDC1
 SPC25
 OSBPL7
 TAGLN
 NTN1
 TGFB3
 BICC1
 IFI44
 MKI67
 LUM

(Continued)

Table S1 (Continued)**Downregulated genes**

CRTAP
 ID3
 CNTN1
 NRG1
 SLC22A15
 TIPARP
 MMP28
 IDH1-AS1
 KLHDC8B
 XG
 SMIM21
 HOXB2
 NEFL
 CRELD1
 HKDC1
 C11orf70
 MYADM
 LPAR3
 IL17RC
 FAMI10A
 BMP2
 CBS
 NUDT16P1
 PHLDB2
 IMPACT
 HOXB7
 RTN4RL1
 SLC1A5
 RRAS
 SNORA4
 GDF15
 C8orf88
 CDKN2AIPNL
 ESPN
 ADGRF4
 KLF6
 PTPN20
 BCAR1
 PRSS21
 MOCOS
 PLPP2
 LURAP1L
 MINDY2
 MAFF
 ERRFI1

Table S1 (Continued)

DLK2
 CTH
 GNAI1
 HOXA6
 CORO2A
 C1orf21
 MST1R
 EVPLL
 PYROXD2
 CYR61
 SYBU
 ELOVL5
 HOOK2
 SERPINB2
 FAS
 AREG
 FAM89A
 HIST1H2BD
 IL13RA2
 QPRT
 AP1S1
 BTBD16
 ACP5
 MFS2A
 ANTXR2
 RAB5C
 NANOS1
 CCGP1
 C11orf63
 FADS1
 HIST1H2AC
 EML2
 TSC22D3
 DKK1
 FKBP5
 MYC
 ATF3
 HOXA5
 EN1
 SIK1
 ECM2
 CHMP4C
 RNF128
 FADS2
 MELTF
 ADRB2

(Continued)

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