Secondary analysis of existing microarray data reveals potential gene drivers of cutaneous squamous cell carcinoma

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Abstract
Cutaneous squamous-cell carcinoma (cSCC) is the second most common skin cancer, with an increasing incidence in recent years. To define the molecular basis that drive cSCC development and progression, this study aimed at identifying potential novel molecular targets for the diagnosis and therapy of patients with cSCC. Two data sets with the accession number GSE45164 and GSE66359 were downloaded from Gene Expression Omnibus (GEO) database. After the identification of differentially expressed genes (DEGs) from these two data sets, respectively, between cSCC samples and controls, a combination of DEGs from these two data sets were subjected to the following analyses, including functional annotation, protein–protein interaction (PPI) network and module construction, transcription factor (TF)–target regulation prediction, and drug–gene interaction predictive analysis. A total of 204 upregulated genes and 213 downregulated genes were found in two data sets which were used for the follow-up analysis. Upregulated and downregulated genes were mainly involved in the functions such as cell division, mitotic nuclear division, cell cycle, and p53 signaling pathway. Interferon induced protein family members and proteasome subunit members were involved in the TF–target regulatory network, such as PSMB8, CXCL10, and IFIT3. Eight upregulated genes (TOP2A, CXCL8, RRM2, PSMB8, PSMB9, PBK, CXCL10, and ISG15) that were hub genes in the PPI network and significant modules were identified in the predicted drug–gene interaction. In conclusion, TOP2A, CXCL8, RRM2, PSMB8, PSMB9, PBK, CXCL10, and ISG15 may be potential targets for the diagnosis and therapy of patients with cSCC.

KEYWORDS
cutaneous squamous-cell carcinoma, differentially expressed genes, drug–gene interaction, module, transcription factor

1 | INTRODUCTION

Squamous-cell skin cancer, also known as cutaneous squamous-cell carcinoma (cSCC) is characterized by an aberrant proliferation of keratinocytes arises most commonly in sunlight-exposed skin and is the second most common skin cancer, leading to a considerable impact on the quality of life and a substantial proportion of deaths (Zhou et al., 2018). Whilst most patients are cured surgically and have a favorable prognosis, a small subset of patients, especially the immunosuppressed patients, are associated with the high-risk cSCC and a proportion will have a local recurrence, lymph node metastases, and poor prognosis (Karia et al., 2014; Navarrete-Dechent, Veness,
Droppelmann, & Uribe, 2018). Thus, defining the critical molecular basis that drive cSCC is an urgent research need for the improvements of treatment in the management of cSCC both in the general and high-risk population groups.

Gene expression analysis such as DNA microarray analysis provides an efficient method and has been a global approach to clarify gene expression changes in many cancer types, including skin cancers (Padilla, Sebastian, Jiang, Nindl, & Larson, 2010). Recently, though antitumor functions of IL-24 established in other cancer types, significant upregulation of IL-24 was found in the invading front of cSCC via enhancing focal expression of MMP7 (Mitsui et al., 2014). L. Zhang, Qin, Wu, Chen, & Zhang (2018) identified several genes as potential pathogenic genes contributing to the progression of actinic keratosis to cSCC. HOXB7 was identified as an upregulated gene in cSCC and silencing of the HOXB7 suppressed cSCC cell migration and invasion while induced cell apoptosis (Gao, Chen, & Yang, 2018). A recent study reported the expression of chitinase-3-like protein 1 (YKL-40) in cSCC (Salomon, Piotrowska, Matusiak, Dziegiel, & Szepietowski, 2018). In addition, Notch-effector CSL was shown to promote the tumorigenesis of cSCC by repressing histone demethylase KDM6B (Al Labban et al., 2018). However, our current knowledge about the gene driving cSCC is still limited and the mechanism of the pathogenesis of this cancer as well as the development of biomarkers for tumor detection, diagnosis, and prognosis remain underexplored.

Secondary analysis of existing data is a cost-effectiveness way to enhance the overall efficiency of the primary results from the original study (Cheng & Phillips, 2014). In this study, we thus downloaded microarray data sets from a public database and performed data reanalysis. To identify more potentially genes associated with cSCC, differentially expressed genes (DEGs) were identified in cSCC samples in comparison with control samples and several significant genes were found as suggested by subsequent bioinformatics analyses. Our study may provide novel intervention targets for cSCC therapy.

**FIGURE 1** Heat maps of DEGs and bubble plot display of the most significant top 20 GO terms of upregulated and downregulated genes. (a) Heat maps of DEGs in GSE45164. (b) Heat maps of DEGs in GSE66359. (c) The most significant top 20 GO terms of upregulated genes. (d) The most significant top 20 GO terms of downregulated genes. DEG: differentially expressed gene; GO: Gene Ontology [Color figure can be viewed at wileyonlinelibrary.com]
2 | MATERIALS AND METHODS

2.1 | Microarray data

The microarray data of human SCC for the past 5 years, which contained both the cancer samples and control samples, were searched from the public archive and resource NCBI GEO (Barrett et al., 2012), accessible at http://www.ncbi.nlm.nih.gov/geo/. Finally, two data sets with the accession number GSE45164 (Brooks et al., 2014) and GSE66359 (Farshchian et al., 2015) were selected. As shown in the original studies, the gene expression profile GSE45164 contained 10 surgically excised cSCC samples and a set of three normal human epidermis controls (Brooks et al., 2014). The platform used for this data set is GPL571 [HG-U133A_2] Affymetrix Human Genome U133A 2.0 Array. The gene expression profile GSE66359 included normal human epidermal keratinocytes (n = 5) purchased from PromoCell (Heidelberg, Germany) or from normal skin of patients in surgery for mammoplasty and cSCC cell lines (n = 8) from surgically excised cSCCs (Farshchian et al., 2015). The platform used for GSE66359 is GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.

2.2 | Data preprocessing and DEG screening

The original raw data (CEL files) were downloaded from the GEO database. We applied the affy package (Gautier, Cope, Bolstad, & Irizarry, 2004; version 1.58.0, http://bioconductor.org/help/search/index.html?q=affy/) to perform the background correction and normalization of the data, including conversion of raw data formats, imputation of missing values, background correction using Affymetrix MicroArray Suite (MAS) method (Hochreiter, Clevert, & Obermayer, 2006), and quantile normalization. The median in the box plot lay in a straight line, indicating that the data normalization was reasonable (Supporting Information Figure 1). The probe was annotated with a platform annotation file to remove probes that did not match the gene symbol; for different probes mapped to the same gene, we took the mean of the different probes as the final expression of the gene. The samples were subjected to differential expression analysis using the classical Bayesian method provided by the Limma package (Ritchie et al., 2015; version 3.10.3, http://www.bioconductor.org/packages/2.9/bioc/html/limma.html). The genes with a p < 0.05 and an absolute value of log2 fold change (FC) ≥ 2 were considered differentially expressed genes (DEGs).

### TABLE 1

| Expression change | KEGG pathway | Count | p Value | Genes |
|-------------------|--------------|-------|---------|-------|
| **Up**            | **hsa04110**: Cell cycle | 14    | 4.09E-09 | CCNB1, CCNE2, CDK1, CDC6, MCM7, CDKN2C, TTK, BUB1B, CDC20, MCM2, MCM3, CCNA2, MYC, MCM5 |
|                   | **hsa03030**: DNA replication | 6     | 8.25E-05 | PRIM1, MCM7, MCM2, MCM3, MCM5, FEN1 |
|                   | **hsa05133**: Pertussis | 7     | 3.58E-04 | IL6, C3, IRF1, CXCL8, C1R, CXCL6, C1S |
|                   | **hsa05146**: Amoebiasis | 8     | 3.75E-04 | COL4A2, IL6, LAMA3, CXCL8, SERPINB1, SERPINB4, SERPINB3, COL5A1 |
|                   | **hsa05150**: Staphylococcus aureus infection | 6     | 5.78E-04 | C3, DSG1, CFH, C1R, CFI, C1S |
|                   | **hsa04115**: p53 signaling pathway | 5     | 1.02E-02 | CCNB1, CCNE2, CDK1, RR2M, IGBP3 |
|                   | **hsa00240**: Pyrimidine metabolism | 6     | 1.02E-02 | PRIM1, RR2M, UPP1, PNP, CMPK2, TK1 |
|                   | **hsa04610**: Complement and coagulation cascades | 5     | 1.13E-02 | C3, CFH, C1R, CFI, C1S |
|                   | **hsa05202**: Transcriptional misregulation in cancer | 7     | 1.98E-02 | IL6, ID2, CDKN2C, CXCL8, MMP3, IGBP3, MYC |
|                   | **hsa05222**: Small cell lung cancer | 5     | 2.28E-02 | CCNE2, COL4A2, LAMA3, ITGA6, MYC |
|                   | **hsa04512**: ECM-receptor interaction | 5     | 2.46E-02 | COL4A2, LAMA3, ITGA6, COL5A1, HMRR |
|                   | **hsa05323**: Rheumatoid arthritis | 5     | 2.55E-02 | IL6, CXCL8, CXCL6, MMP3, MMP1 |
|                   | **hsa05134**: Legionellosis | 4     | 3.11E-02 | CXCL1, IL6, C3, CXCL8 |
| **Down**          | **hsa00830**: Retinol metabolism | 5     | 4.54E-03 | RDH12, LRAT, DHR59, ADH1B, SDR16C5 |
|                   | **hsa04060**: Cytokine-cytokine receptor interaction | 8     | 9.69E-03 | IL1R2, IL20RB, IL1B, EDAR, IFNK, IL11RA, CCL27, GHR |
|                   | **hsa04630**: Jak-STAT signaling pathway | 6     | 1.72E-02 | IL20RB, CCND2, IFNK, IL11RA, GHR, IL13RA2 |
|                   | **hsa00590**: Arachidonic acid metabolism | 4     | 2.65E-02 | PTGS2, ALOX15B, GPX3, ALOX12B |

Note. ECM: extracellular matrix; KEGG: Kyoto Encyclopedia of Genes and Genomes.

The Kyoto Encyclopedia of Genes and Genomes (KEGG; Ogata et al., 1999) pathway and Gene Ontology (GO) Biological Process (BP) enrichment analysis of DEGs was analyzed using the commonly used enrichment analysis tool DAVID (Huang, Sherman, & Lempicki, 2008; version 6.8, https://david-d.ncifcrf.gov/). The number of enriched pathways and genes is presented in Table 1. The upregulated genes were significantly enriched in pathways related to cell cycle, DNA replication, and retinol metabolism. The downregulated genes were significantly enriched in pathways related to retinol metabolism, cytokine-cytokine receptor interaction, and Jak-STAT signaling pathway.
genes count \( \geq 2 \) and the significance threshold \( p < 0.05 \) were considered as the threshold for a significant enrichment result.

### 2.4 PPI network and module analysis of DEGs

The interaction between DEGs-encoded proteins was analyzed by STRING (Szklarczyk et al., 2014; version: 10.0, http://www.string-db.org/) database. The input gene set was all DEGs, and the species was set as human. The parameter PPI score was set to 0.7 (indicating high confidence). The network software was built by Cytoscape (version: 3.2.0, http://www.cytoscape.org/; Shannon et al., 2003).

The CytoNCA plug-in (Tang, Li, Wang, Pan, & Wu, 2015; version 2.1.6, http://apps.cytoscape.org/apps/cytonca) was used to analyze the topology properties of nodes in the PPI network, and the parameter was set to without weight. Through the score ranking of each node, the important nodes participating protein interaction

**FIGURE 2** PPI network. The yellow circle is the gene whose expression is upregulated, and the green prism indicates the gene downregulated. The node size is based on the degree value. The higher the degree, the larger the node. PPI: protein–protein interaction [Color figure can be viewed at wileyonlinelibrary.com]
relationship in the PPI network were obtained. Cytoscape's plugin MCODE (Saito et al., 2012; version: 1.4.2, http://apps.cytoscape.org/apps/MCODE) was applied to analyze the most significant clustering modules in the PPI network. The threshold score was selected as $\geq 10$. In addition, the genes in the significant modules were subjected to KEGG pathway and GO BP enrichment analyses using DAVID (Huang et al., 2008).

### 2.5 TF-target regulation prediction

TF prediction was performed using the WebGestalt GAST (http://www.webgestalt.org/option.php) analysis tool (B. Zhang, Kirov, & Snoddy, 2005). We used the overrepresentation enrichment analysis method to perform TF-target enrichment on DEGs in the PPI clustering module. The enrichment parameters were set as the minimum enrichment gene number 2, and the significant results of FDR <0.05 were displayed.

### 2.6 Drug–gene interaction predictive analysis

The Drug–Gene Interaction database (DGIdb) mines existing resources and generates assumptions about how genes are therapeutically targeted or prioritized for drug development (Griffith et al., 2013). In this study, DGIdb2.0 (Griffith et al., 2013; http://www.dgidb.org/) was used to predict drugs based on the module genes. The parameters were set as: preset filters: FDA approved; antineoplastic; all the default. All the

### TABLE 2 The KEGG pathways enriched by genes in the module-A and module-B

| Module    | Kyoto Encyclopedia of Genes And Genomes (KEGG) pathway | Count | p Value      | Genes                                      |
|-----------|--------------------------------------------------------|-------|--------------|--------------------------------------------|
| Module-A  | hsa04110:Cell cycle                                     | 6     | 9.21E-08     | CCNB1, CDK1, TTK, BUB1B, CDC20, CCNA2      |
|           | hsa04115:p53 signaling pathway                          | 3     | 2.50E-03     | CCNB1, CDK1, RRM2                         |
|           | hsa04914:Progesterone-mediated oocyte maturation        | 3     | 4.18E-03     | CCNB1, CDK1, CCNA2                        |
|           | hsa05203:Viral carcinogenesis                           | 3     | 2.18E-02     | CDK1, CDC20, CCNA2                        |
| Module-B  | hsa04062:Chemokine signaling pathway                   | 6     | 1.45E-05     | CXCL1, CXCL13, CXCL6, CXCL11, CCL27, CXCL10|
|           | hsa04060:Cytokine–cytokine receptor interaction         | 4     | 8.13E-03     | CXCL13, CXCL11, CCL27, CXCL10             |
|           | hsa05133:Pertussis                                     | 3     | 8.39E-03     | C3, IRF1, CXCL6                           |
drug–gene relationship pairs related to the module genes were predicted, and cytoscape was used to construct the network map.

2.7 Ethical statement

The data of cSCC patients we used in this study are all from the publicly accessible GEO database. Separate ethics committee approval is not required.

3 RESULTS

3.1 DEG screening

According to the screening criteria, a total of 116 DEGs were obtained from GSE45164, of which 63 were upregulated and 53 were downregulated. From GSE66359, we obtained 335 DEGs of which 160 were upregulated and 175 were downregulated. The clustering heat maps of DEGs is shown in Figure 1. To ensure that the next analysis does not lose the disease-associated DEGs, we took a collection of DEGs from two data sets, removing genes with opposite expression trends. A total of 204 upregulated genes and 213 downregulated genes were included in the next analysis.

3.2 Functional enrichment analysis of DEGs

As shown in Table 1, KEGG pathway enrichment analysis results showed that the upregulated gene were enriched in 13 pathways, such as cell cycle, DNA replication, and Staphylococcus aureus infection. The downregulated genes were involved in four pathways including retinol metabolism, cytokine–cytokine receptor interaction, Jak-STAT signaling pathway, and arachidonic acid metabolism.

The upregulated genes were significantly enriched in 63 GO BP terms, and the downregulated genes were significantly enriched in 35 GO BP terms. We selected the more significant top 20 terms for bubble plot display (Figure 1c,d). The results indicated the upregulated genes were mainly involved in the functions such as cell division and mitotic nuclear division, whereas the downregulated genes were mainly associated with the functions such as keratinocyte differentiation, epidermis development, and keratinization.

3.3 PPI network and module analysis

The PPI network is shown in Figure 2, consisting of 209 nodes and 1,127 interaction pairs. The nodes with higher topological score can be regarded as key nodes of the network. The top 10 nodes with higher topological score included cyclin dependent kinase 1 (CDK1, upregulated, degree = 57), cyclin B1 (CCNB1, upregulated, degree = 49), cell division cycle 20 (CDC20, upregulated, degree = 47), cyclin A2 (CCNA2, upregulated, degree = 47), DNA topoisomerase II α (TOP2A, upregulated, degree = 46), ubiquitin conjugating enzyme E2 C (UBE2C, upregulated, degree = 44), ribonucleotide reductase regulatory subunit M2 (RRM2, table 3: The top 10 nodes in PPI network, module-A and module-B

| Nodes PPI network | Change | Degree | Nodes Module-A | Change | Degree | Nodes Module-B | Change | Degree |
|-------------------|--------|--------|----------------|--------|--------|----------------|--------|--------|
| CDK1              | up     | 57     | CDK1           | up     | 57     | PSMB8          | up     | 21     |
| CCNB1             | up     | 49     | CCNB1          | up     | 49     | RSAD2          | up     | 18     |
| CDC20             | up     | 47     | CCNA2          | up     | 47     | CXCL10         | up     | 17     |
| CCNA2             | up     | 47     | CDC20          | up     | 47     | ISG15          | up     | 17     |
| TOP2A             | up     | 46     | TOP2A          | up     | 46     | IFIT3          | up     | 17     |
| UBE2C             | up     | 44     | RRM2           | up     | 44     | IFIT1          | up     | 17     |
| RRM2              | up     | 44     | UBE2C          | up     | 44     | IL8            | up     | 16     |
| KIF11             | up     | 43     | KIF11          | up     | 43     | IFIT2          | up     | 15     |
| NDC80             | up     | 42     | NDC80          | up     | 42     | IFI44          | up     | 14     |
| TTK               | up     | 42     | TTK            | up     | 42     | IFT35          | up     | 14     |
upregulated, degree = 44), kinesin family member 11 (KIF11, upregulated, degree = 43), NDC80, kinetochore complex component (NDC80, upregulated, degree = 42), and TTK protein kinase (TTK, upregulated, degree = 42).

Two modules were aggregated from the PPI network (Figure 3a). The module-A (score = 30.467) contained 31 nodes and 457 interaction pairs; the module-B (score = 10.48) contained 26 nodes and 131 pairs of interactions. We found that the genes involved in these two modules were almost upregulated. The top 10 nodes in the PPI network were also the top 10 in the module-A (Table 3). The hub genes in the module-B included proteasome subunit β 8 (PSMB8), C-X-C motif chemokine ligand 10 (CXCL10), ISG15 Ubiquitin-Like Modifier (ISG15), and Interferon Induced Protein with Tetratricopeptide Repeats 3 (IFIT3). Pathway enrichment analysis of genes in these two modules (Table 2) showed that these genes were mainly concerned with cell cycle, p53 signaling pathway, progesterone-mediated oocyte maturation, viral carcinogenesis, chemokine signaling pathway, chemokine signaling pathway, and pertussis. The top 20 enriched GO BP results are shown in Figure 3b,c. The genes in module-A were mainly involved in the functions such as cell division and mitotic nuclear division; the genes in module-B were associated with defense response to virus and type I interferon signaling pathway.

3.4 | TF-target regulatory network

According to webgestal, six TFs were predicted to regulate gene in the modules, resulting in 47 regulatory relationship pairs (Figure 4), including 13 upregulated module genes. The upregulated gene interferon regulatory factor 1 (IRF1) can act as a TF. Additionally, interferon induced protein family members and proteasome subunit members were involved in the TF-target regulatory network, such as PSMB8, CXCL10, and IFIT3.

3.5 | Drug–gene interaction prediction

Based on the DGIdb predictions of the module genes, we obtained 42 drug-gene interaction pairs, including eight upregulated genes (TOP2A, CXCL8, RRM2, PSMB8, PSMB9, PBK, CXCL10, and ISG15) and 39 drugs (FDA-listed + antitumor drugs), as shown in Figure 5.

4 | DISCUSSION

In this study, a total of 204 upregulated genes and 213 downregulated genes were identified from two microarray data sets, which were then subjected to the comprehensive analyses. Several hub genes were found from the PPI network and modules as well as the regulatory network. Interestingly, they were also found in the predicted drug-gene interaction, including TOP2A, CXCL8, RRM2, PSMB8, PSMB9, PBK, CXCL10, and ISG15.

TOP2A encodes a DNA topoisomerase, which is an enzyme that cleaves DNA to elicit topological changes. Amplification of TOP2A has been demonstrated in many types of cancer, such as colorectal cancer and breast cancer (Al-Kuraya et al., 2007; Olsen et al., 2004).
However, there is no direct evidence showing the correlation between this gene and cSCC. CXCL8 affects multiple processes involved in the progression of SCC (Christofakis, Miyazaki, Rubink, & Yeudall, 2008). CXCL10 is highly expressed in a variety of human diseases and may act as a novel therapeutic target in cancer (Liu, Guo, & Stiles, 2011). Overexpression of RRM2 significantly enhances cellular invasiveness (Duxbury & Whang, 2007), playing a critical role in determining tumor malignancy (K. Zhang et al., 2009). We suggest potentially important roles of these genes in the development and occurrence of cSCC.

PSMB8 and PSMB9 encode the proteasome subunit (Agarwal et al., 2010). Inhibition of proteasome has been a novel therapeutic approach in human cancers (Orlowski & Kuhn, 2008). A recent study provide evidence that proteasome inhibitors prime cSCC cells for rapid BAK-dependent death (McHugh et al., 2018). Few studies have investigated the roles of PSMB8 and PSMB9 in the development of cSCC. PBK, also known as PDZ Binding Kinase, is regulated by cell-cycle-specific transcription factors CREB/ATF and E2F and its expression has been found in human urinary bladder transitional cell carcinoma and colon cancer (Singh et al., 2014; Yang et al., 2016). Studies has shown that PBK promote cancer cell proliferation by regulation of the DNA damage and interacting with tumor suppressor p53 (Ayllon & O’connor, 2007; Hu et al., 2010). The ubiquitin-like protein ISG15 play an important role in antiviral immunity and defense (Skau & Chen, 2010). In addition, evidence indicates the association of human papillomavirus infection and cSCC (Masini et al., 2003). Altogether, these data indicate that these genes may play critical roles in the pathogenesis of cSCC and further investigation towards their functions is needed.

However, it should be noted that the main limitation of this study is the lack of experimental verifications which will be the focus in our future study.

In conclusion, the present study identified several candidate genes in cSCC. It would be of interest to further identify the downstream mechanisms of these genes involved in this cancer. This would aid in the development of novel targets for cSCC therapy.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

H. L. conceived of the project and wrote the manuscript. D. C., P. L., S. X., X. L., and R. Z. performed the computational analyses and confirmed the results. All authors read and approved the final manuscript.

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REFERENCES

Agarwal, A. K., Xing, C., DeMartino, G. N., Mizrachi, D., Hernandez, M. D., Sousa, A. B., ... Garg, A. (2010). PSMB8 encoding the β5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy syndrome. The American Journal of Human Genetics, 87(6), 866–872.

Al-Kuraya, K., Novotny, H., Bavi, P., Siraj, A. K., Uddin, S., Ezzat, A., ... Tornillo, L. (2007). HER2, TOP2A, CCND1, EGFR and C-MYC oncogene amplification in colorectal cancer. Journal of Clinical Pathology, 60(7), 768–772.

Al Labban, D., Jo, S. H., Ostano, P., Saglietti, C., Bongiovanni, M., Panizzon, R., & Dotto, G. P. (2018). Notch-effector CSL promotes squamous cell carcinoma by repressing histone demethylase KDM6B. Journal of Clinical Investigation, 128(6), 2581–2599.

Aylón, V., & O’connor, R. (2007). PBK/TOPK promotes tumour cell proliferation through p38 MAPK activity and regulation of the DNA damage response. Oncogene, 26(24), 3451–3461.

Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., ... Soboleva, A. (2012). NCBI GEO: Archive for functional genomics data sets—Update. Nucleic Acids Research, 41(D1), D991–D995.

Brooks, Y. S., Ostano, P., Jo, S.-H., Dai, J., Getsios, S., Dziunycz, P., ... Dotto, G. P. (2014). Multifactorial ERα and NOTCH1 control of squamous differentiation and cancer. The Journal of Clinical Investigation, 124(5), 2260–2276.

Cheng, H. G., & Phillips, M. R. (2014). Secondary analysis of existing data: Opportunities and implementation. Shanghai Archives of Psychiatry, 26(6), 371–375.

Christofakis, E. P., Miyazaki, H., Rubink, D. S., & Yeudall, W. A. (2008). Roles of CXCL8 in squamous cell carcinoma proliferation and migration. Oral Oncology, 44(10), 920–926.

Duxbury, M. S., & Whang, E. E. (2007). RRM2 induces NF-κB dependent MMP-9 activation and enhances cellular invasiveness. Biochemical and Biophysical Research Communications, 354(1), 190–196.

Farshchian, M., Nissinen, L., Siljamäki, E., Riihila, P., Toriseva, M., Kivisaari, A., ... Kähäri, V. M. (2015). EphB2 promotes progression of cutaneous squamous cell carcinoma. Journal of Investigative Dermatology, 135(7), 1882–1892.

Gao, D., Chen, H. Q., & Yang, Y. M. (2018). Specific knockdown of HOXB7 inhibits cutaneous squamous cell carcinoma cell migration and invasion while inducing apoptosis via Wnt/β-catenin signaling pathway. American Journal of Physiology Cell Physiology

Gautier, L., Cope, L., Bolstad, B. M., & Irizarry, R. A. (2004). Affy—Analysis of Affymetrix GeneChip data at the probe level. Bioinformatics, 20(3), 307–315.

Griffith, M., Griffith, O. L., Coffman, A. C., Weible, J. V., McMichael, J. F., Spies, N. C., ... Wilson, R. K. (2013). DGIdb: Mining the druggable genome. Nature Methods, 10(12), 1209–1210.

Hochreiter, S., Clevert, D.-A., & Obermayer, K. (2006). A new summarization method for Affymetrix probe level data. Bioinformatics, 22(8), 943–949.

Hu, F., Gartenhaus, R. B., Eichberg, D., Liu, Z., Fang, H. B., & Rapoport, A. P. (2010). PBK/TOPK interacts with the DBD domain of tumor suppressor p53 and modulates expression of transcriptional targets including p21. Oncogene, 29(40), 5464–5474.

Huang, D. W., Sherman, B. T., & Lempicki, R. A. (2008). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols, 4(1), 44–57.
Karia, P. S., Jambusaria-Pahajani, A., Harrington, D. P., Murphy, G. F., Qureshi, A. A., & Schmuits, C. D. (2014). Evaluation of American Joint Committee on Cancer, International Union Against Cancer, and Brigham and Women's Hospital tumor staging for cutaneous squamous cell carcinoma. Journal of Clinical Oncology, 32(4), 327–334.

Liu, M., Guo, S., & Stiles, J. K. (2011). The emerging role of CXCL10 in cancer. Oncology Letters, 2(4), 583–589.

Masini, C., Fuchs, P. G., Gabrielli, F., Stark, S., Sera, F., Ploner, M., & Abeni, D. (2003). Evidence for the association of human papillomavirus infection and cutaneous squamous cell carcinoma in immunocompetent individuals. Archives of Dermatology, 139(7), 890–894.

McHugh, A., Fernandes, K., South, A. P., Mellerio, J. E., Salas-Alanis, J. C., Proby, C. M., & Saville, M. K. (2018). Preclinical comparison of proteasome and ubiquitin E1 enzyme inhibitors in cutaneous squamous cell carcinoma: The identification of mechanisms of differential sensitivity. Oncotarget, 9(29), 20265–20281.

Mitsui, H., Suárez-Fariñas, M., Gualti, N., Shah, K. R., Cannizzaro, M. V., Coats, I., & Carucci, J. A. (2014). Gene expression profiling of the leading edge of cutaneous squamous cell carcinoma: IL-24-driven MMP-7. The Journal of Investigative Dermatology, 134(5), 1418–1427.

Navarrete-Dechent, C., Veness, M. J., Droppelmann, N., & Uribe, P. (2018). Cutaneous squamous cell carcinoma and the emerging role of sentinel lymph node biopsy. Giornale italiano di dermatologia e venereologia, 153(3), 403–418.

Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H., & Kanehisa, M. (1999). KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Research, 27(1), 29–34.

Olsen, K. E., Knudsen, H., Rasmussen, B. B., Balslev, E., Knoop, A., Ejlertsen, B., & Overgaard, J. (2004). Amplification of HER2 and TOP2A and deletion of TOP2A genes in breast cancer investigated by new FISH probes. Acta Oncologica, 43(1), 35–42.

Orlowski, R. Z., & Kuhn, D. J. (2008). Proteasome inhibitors in cancer therapy: Lessons from the first decade. Clinical Cancer Research, 14(6), 1649–1657.

Padilla, R. S., Sebastian, S., Jiang, Z., Nindl, I., & Larson, R. (2010). Gene expression patterns of normal human skin, actinic keratosis, and squamous cell carcinoma: A spectrum of disease progression. Archives of Dermatology, 146(3), 288–293.

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research, 43(7), e47.

Saito, R., Saito, M. E., Ono, K., Ruscheinski, J., Wang, P. -L., Lotia, S., & Ideker, T. (2012). A travel guide to cytoscape plugins. Nature Methods, 9(11), 1069–1076.

Salomon, J., Piotrowska, A., Matusiak, Ł., Dźigiel, P., & Szepietowski, J. C. (2018). Chitinase-3-like protein 1 (YKL-40) expression in squamous cell skin cancer. Anticancer Research, 38(8), 4753–4758.

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., & Ideker, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Research, 13(11), 2498–2504.

Singh, P. K., Srivastava, A. K., Daleza, D., Rath, S. K., Goel, M. M., & Bhatt, M. L. B. (2014). Expression of PDZ-binding kinase/T-LAK cell–originated protein kinase (PKB/TOPK) in human urinary bladder transitional cell carcinoma. Immunochemistry, 219(6), 469–474.

Skaug, B., & Chen, Z. J. (2010). Emerging role of ISG15 in antiviral immunity. Cell, 143(2), 187–190.

Słivaczuky, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., & von Mering, C. (2014). STRING v10: Protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Research, 43(D1), D447–D452.

Tang, Y., Li, M., Wang, J., Pan, Y., & Wu, F.-X. (2015). CytoNCA: A cytoscop plugin for centrality analysis and evaluation of protein interaction networks. Biosystems, 127, 67–72.

Yang, J., Yuan, D., Xing, T., Su, H., Zhang, S., Wen, J., & Dang, D. (2016). Ginsenoside Rh2 inhibiting HCT116 colon cancer cell proliferation through blocking PDZ-binding kinase/T-LAK cell–originated protein kinase. Journal of Ginseng Research, 40(4), 400–408.

Zhang, B., Kirov, S., & Snoddy, J. (2005). WebGestalt: An integrated system for exploring gene sets in various biological contexts. Nucleic Acids Research, 33(suppl_2), W741–W748.

Zhang, K., Hu, S., Wu, J., Chen, L., Lu, J., Wang, X., & Yen, Y. (2009). Overexpression of RRM2 decreases thrombospondin-1 and increases VEGF production in human cancer cells in vitro and in vivo: Implication of RRM2 in angiogenesis. Molecular Cancer, 8(1), 11.

Zhang, L., Qin, H., Wu, Z., Chen, W., & Zhang, G. (2018). Pathogenic genes related to the progression of actinic keratoses to cutaneous squamous cell carcinoma. International Journal of Dermatology, 57, 1208–1217.

Zhou, L., Xing, T., Su, H., Zhang, S., Wen, J., & Dang, D. (2016). HOXA9 inhibits HIF-α-mediated glycolysis through interacting with CRIP2 to repress cutaneous squamous cell carcinoma development. Nature Communications, 9(1), 1480.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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