

# Identification of genes and signaling pathways associated with squamous cell carcinoma by bioinformatics analysis

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**Abstract.** The present study aimed to investigate the genes and signaling pathways associated with squamous cell carcinoma (SCC) by bioinformatics analysis. For this purpose, the GSE2503 was downloaded from the Gene Expression Omnibus database, and the differentially expressed genes (DEGs) between 6 normal skin and 5 SCC samples were analyzed using the Linear Models for Microarray Data package. Gene Ontology (GO) and pathway enrichment analysis of DEGs were performed, followed by functional annotation and construction of a protein-protein interaction (PPI) network. Subnetwork modules were subsequently identified and analyzed. A total of 181 DEGs, including 95 upregulated and 86 downregulated DEGs, were identified, in addition to 20 GO biological processes terms enriched by upregulated DEGs and 14 enriched by downregulated DEGs. The upregulated DEGs were enriched in 18 pathways, and the downregulated DEGs were enriched in 7 pathways. Following functional annotation, three upregulated transcription factors (TFs), including hypoxia inducible factor 1, alpha subunit (*HIF1A*), and six downregulated TFs were identified. In the PPI network and subnetwork, matrix metalloproteinase 1 (*MMP1*), also known as interstitial collagenase, and interleukin 8 (*IL8*) were the hub genes with the highest degree of connectivity (degree = 8). Integrin alpha (*ITGA*)6 and 2 were enriched in several pathways, including focal adhesion and extracellular matrix-receptor interaction. DEGs of SCC were primarily enriched in pathways associated with cancer and cell adhesion. Therefore, DEGs such as *IL8*, *MMP1*, *HIF1A*, *ITGA6* and *ITGA2* may be potential targets for the diagnosis and treatment of SCC.

## Introduction

Squamous cell carcinoma (SCC) is a histologically distinct type of cancer (1). It arises from the uncontrolled proliferation of epithelial cells or cells exhibiting cytological or tissue architectural characteristics of SC differentiation, including the presence of keratin, tonofilament bundles or desmosomes, which are structures involved in cell-cell adhesion (1). SCC occurs in numerous tissues, including the lips, mouth, esophagus, lungs, urinary bladder and prostate (2). Of all the cases of SCC, 2.5% become metastatic and lead to substantial morbidity, which constitutes a considerable economic burden to the healthcare system (3). The incidence of SCC has notably increased worldwide over the last decade (4). Thus, an improved understanding of the underlying molecular mechanisms and gene networks involved in the development and progression of skin SCC is required.

Numerous studies on the mechanisms and therapeutic strategies for the treatment of SCC have been reported to date (5-8). Exposure to ultraviolet radiation, a potent mutagen and DNA-damaging agent, is considered to be a significant risk factor for the development of SCC (5,6). Carcinogenesis is a multistep process. During tumor progression, multiple genes experience up- or downregulation (7). A number of genes and signaling pathways involved in the progression of SCC have been previously identified (3,6-9). Streit *et al* (8) reported that thrombospondin-1 (TSP-1) was an effective inhibitor of angiogenesis and tumor growth in carcinomas of the skin. In addition, the authors observed that the expression of TSP-1 was downregulated in patients with SCC. Previous studies have established that the transforming growth factor  $\beta$ -mothers against decapentaplegic homolog 4 (SMAD4) signaling pathway is required for effective epithelial wound healing, and a conditional deletion of SMAD4 in the epidermis causes defects in skin wound healing, which are accompanied by spontaneous skin inflammation and SCC (6). Additionally, talin 1 and laminin alpha 3, which participate in signaling pathways associated with adhesion and migration, have been previously observed to be over-expressed in SCC (9). Padilla *et al* (3) and Nindl *et al* (7) selected differentially expressed genes (DEGs) between SCC and normal skin (NO) samples using the microarray expression profile dataset GSE2503. However, the interactions

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**Key words:** squamous cell carcinoma, differentially expressed genes, pathway enrichment analysis, protein-protein interaction

between the carcinogenic genes that lead to SCC remain to be elucidated.

In the present study, GSE2503 was downloaded and used to identify the DEGs between SCC and NO samples, in order to investigate the underlying molecular mechanisms of SCC. Subsequently, functional enrichment analysis and functional annotation were performed. In addition, protein-protein interaction (PPI) networks and subnetworks were constructed and analyzed, in order to study and identify target genes for the diagnosis and treatment of SCC. The results of the present study may facilitate the understanding of the mechanisms responsible for the carcinogenesis and development of SCC. Furthermore, the genes identified in the present study may serve as biomarkers for the diagnosis and prognosis of SCC.

## Materials and methods

**Affymetrix microarray data.** The microarray expression profile dataset GSE2503 (3,7), which is based on the Gene Expression Omnibus (GEO) Platform 96 GeneChip® Human Genome U133A 2.0 Array (Affymetrix, Inc., Santa Clara, CA, USA), was downloaded from the National Center of Biotechnology Information GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). The dataset contained 15 samples, including six NO, four actinic keratosis and five SCC. In the present study, the SCC and NO samples were analyzed by bioinformatics methods.

**Data preprocessing and differential expression analysis.** The original array data were converted into expression measures. Background correction, quartile data normalization and probe summarization were performed using the Robust Multi-array Average (10) algorithm in the R affy package (<https://www.bioconductor.org/packages/release/bioc/html/affy.html>). Paired t-test based on the Linear Models for Microarray Data package (<https://bioconductor.org/packages/release/bioc/html/limma.html>) (11) in R (<https://www.r-project.org/>) was used to identify DEGs between SCC and NO samples. Multiple testing correction was performed with the Benjamini-Hochberg method (12) to obtain the adjusted P-value. Subsequently,  $\log_2$ -fold change ( $\log_2FC$ ) was calculated. Only those genes exhibiting  $|\log_2FC| > 1.0$  and adjusted  $P < 0.05$  were regarded as DEGs.

**Gene ontology and pathway enrichment analysis.** Gene Ontology (GO; <http://geneontology.org/>) (13) is a tool for unification of biology that collects structured, defined and controlled vocabulary for large-scale gene annotation. The Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/>) (14) knowledge database is a collection of online databases regarding genomes, enzymatic pathways and biological chemicals. The Database for Annotation, Visualization and Integrated Discovery (DAVID; <https://david.ncifcrf.gov/>) (15) contains a comprehensive set of functional annotation tools that have been developed for associating functional terms with lists of genes via clustering algorithms. In order to analyze the identified DEGs at the functional level, GO enrichment and KEGG pathway analysis were performed using the DAVID online tool.  $P < 0.05$  was set as the threshold value.

**Functional annotation of DEGs.** Functional annotation analysis was performed to determine whether the identified DEGs functioned as transcription factors (TFs). The Tumor Suppressor Gene Database (TSGene; <http://bioinfo.mc.vanderbilt.edu/TSGene/>) (16) integrates TSGs with large-scale experimental evidence to provide a comprehensive resource for the investigation of TSGs and their implication in the molecular mechanisms of cancer. The Tumor Associated Gene (TAG; <http://www.binfo.ncku.edu.tw/TAG/GeneDoc.php>) database (17) contains information regarding genes involved in carcinogenesis. In the present study, all the oncogenes and TSGs known to date were extracted from the TAG and TSGene databases.

**PPI network construction.** The Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org/>) database (18) is a precomputed global resource designed to evaluate PPI information. In the present study, the STRING online tool was used to analyze the PPI of DEGs, and those experimentally validated interactions with a combined score  $> 0.4$  were selected as significant.

The majority of the PPI networks in the biological network constructed were observed to obey the scale-free attribution (19). Thus, the degree of connectivity was statistically analyzed in networks using cytoscape ([www.cytoscape.org](http://www.cytoscape.org)) (20), to obtain the significant nodes or hub proteins (21) in the PPI networks.

**Subnetwork identification and functional enrichment analysis.** The BioNet package (<https://www.bioconductor.org/packages/release/bioc/html/BioNet.html>) (22) provides a comprehensive set of methods for the integrated analysis of gene expression data and biological networks. The GeneAnswers (23) package (<https://www.bioconductor.org/packages/release/bioc/html/GeneAnswers.html>) facilitates the understanding of the associations between a list of genes and any relevant annotations.

In the present study, the BioNet package was used to identify the subnetworks in the constructed PPI networks, with a threshold false discovery rate (FDR)  $< 0.001$ . Subsequently, the GeneAnswers package based on Entrez Gene ID (<http://www.ncbi.nlm.nih.gov/gene>) was used to identify over-represented GO terms with an FDR  $< 0.05$ , and significantly enriched pathways with  $P < 0.05$ . Subsequently, data integration and network visualization were performed to obtain heat maps and association networks of the results derived from the enrichment analysis and the corresponding genes.

## Results

**Identification of 181 DEGs.** Following data preprocessing, a total of 181 genes that were differentially expressed in SCC compared with NO were identified. These DEGs included 95 upregulated and 86 downregulated genes.

**GO and pathway enrichment analysis.** A total of 20 GO biological processes (BPs) terms enriched by the upregulated DEGs (including cell adhesion) and 14 GO BPs terms enriched by the downregulated DEGs (including oxidation-reduction processes)

Table I. GO functional and KEGG pathway enrichment analyses for the most significantly up- and downregulated DEGs.

Category	Term	Description	Degree of connectivity	P-value
Upregulated DEGs				
BP	GO:0007155	Cell adhesion	18	1.53x10 <sup>-5</sup>
BP	GO:0006954	Inflammatory response	12	5.69x10 <sup>-5</sup>
BP	GO:0050900	Leukocyte migration	10	4.37x10 <sup>-6</sup>
BP	GO:0030216	Keratinocyte differentiation	7	3.74x10 <sup>-6</sup>
BP	GO:0032602	Chemokine production	5	2.27x10 <sup>-5</sup>
Downregulated DEGs				
BP	GO:0055114	Oxidation-reduction	10	4.33x10 <sup>-4</sup>
BP	GO:0042391	Regulation of membrane potential	7	1.23x10 <sup>-3</sup>
BP	GO:0006898	Receptor-mediated endocytosis	4	5.09x10 <sup>-3</sup>
BP	GO:0006805	Metabolism of xenobiotics	4	9.58x10 <sup>-3</sup>
BP	GO:0003215	Cardiac right ventricle morphogenesis	3	7.73x10 <sup>-5</sup>
Upregulated DEGs				
KEGG	5200	Signaling pathways in cancer	8	9.37x10 <sup>-4</sup>
KEGG	4810	Regulation of actin cytoskeleton	5	1.14x10 <sup>-2</sup>
KEGG	4640	Hematopoietic cell lineage	4	2.36x10 <sup>-3</sup>
KEGG	5219	Bladder cancer	3	2.41x10 <sup>-3</sup>
KEGG	5412	Arrhythmogenic right ventricular cardiomyopathy	3	1.18x10 <sup>-2</sup>
Downregulated DEGs				
KEGG	480	Metabolism of glutathione	3	3.14x10 <sup>-3</sup>
KEGG	330	Metabolism of arginine and proline	3	3.91x10 <sup>-3</sup>
KEGG	980	Metabolism of xenobiotics by cytochrome P450	3	8.41x10 <sup>-3</sup>
KEGG	982	Metabolism of drugs by cytochrome P450	3	9.08x10 <sup>-3</sup>
KEGG	310	Lysine degradation	2	2.79x10 <sup>-2</sup>

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; BP, biological process.

were identified by GO and pathway enrichment analysis. The most significant GO BPs terms are presented in Table I.

Table I also contains the most significantly enriched pathways of the upregulated and downregulated DEGs revealed by KEGG analysis. The upregulated DEGs were observed to be enriched in 18 pathways, while the downregulated DEGs were enriched in 7 pathways.

**Functional annotation of DEGs.** Upon analyzing the differential expression pattern of TFs and TAGs in SCC and NO samples, the present study identified a number of TFs, including hypoxia inducible factor 1, alpha subunit (*HIF1A*), aryl hydrocarbon receptor nuclear translocator-like 2 and paired-like homeodomain 1, which were significantly upregulated, in addition to six TFs, including nuclear receptor subfamily 3, hepatic leukemia factor and zinc finger protein 83, which were significantly downregulated in SCC. Among the upregulated DEGs, 11 genes were identified as TAGs. Of these, four were oncogenes and five were TSGs. The function of the remaining two genes identified in the analysis remains to be elucidated. Among the downregulated DEGs, five genes were identified as TSGs, including low-density lipoprotein receptor-related protein 1B and proline dehydrogenase (oxidase) 1.

**PPI network construction.** Based on the information contained in the STRING database, 104 protein pairs were identified (Fig. 1). A total of 14 nodes were selected as hub proteins (degree  $\geq 5$ ) in the PPI network, including matrix metallo-peptidase 1 (MMP1) (also known as interstitial collagenase), keratin 6A (KRT6A) and interleukin 8 (IL8), which presented a degree of connectivity of 8 (Table II).

**Subnetwork identification and functional enrichment analysis.** As represented in Fig. 2, 43 nodes, 75 protein pairs and 9 hub proteins with a degree  $\geq 6$  were identified in the subnetwork. The hub proteins, including IL8, MMP1 and KRT6A, are summarized in Table II.

Using heat maps (Figs. 3 and 4), the association between DEGs and BPs/signaling pathways was evaluated. For example, integrin alpha (*ITGA*)6 and 2 were observed to be enriched in several BPs terms, including extracellular matrix (ECM)-receptor interaction and regulation of the KEGG signaling pathways of cell and focal adhesion.

## Discussion

SCC is characterized by a high rate of proliferation and nodal metastasis (24). Therefore, early detection or prevention of

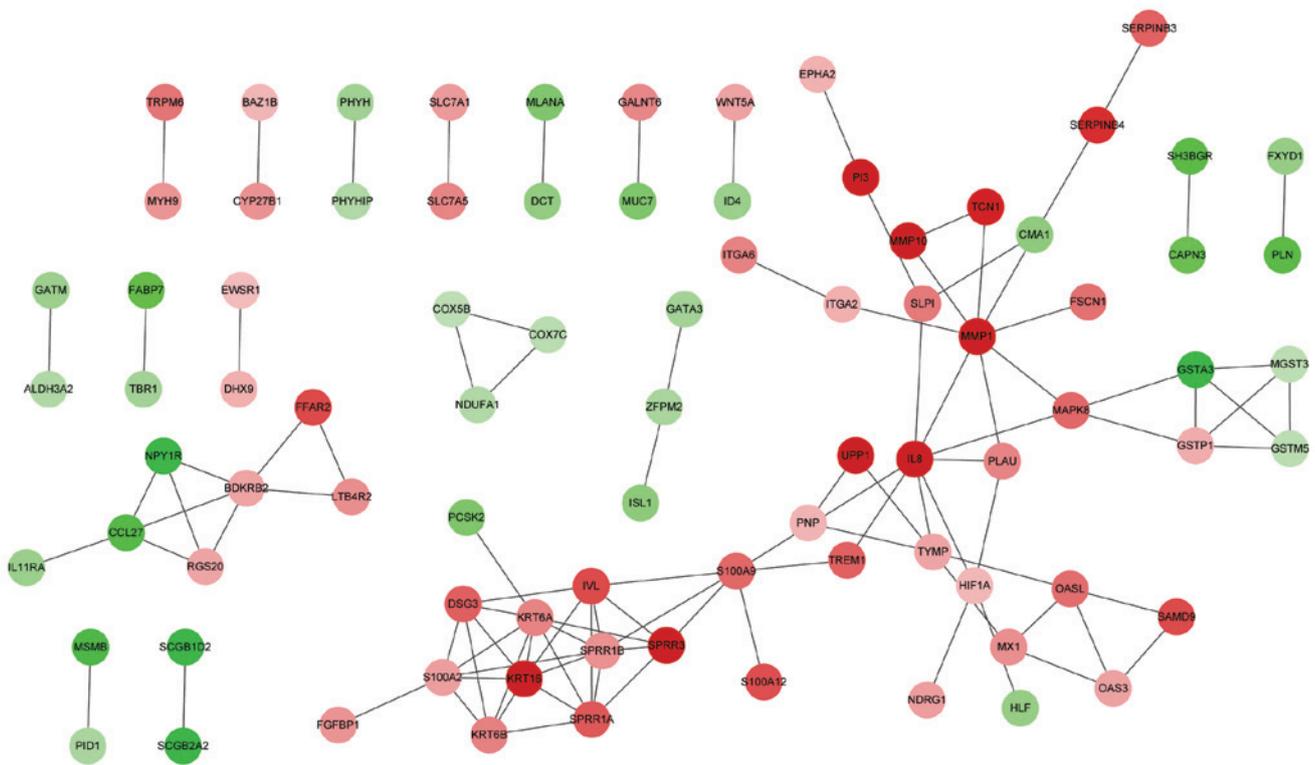


Figure 1. Constructed protein-protein interaction network of DEGs. Red, upregulated DEGs. Green, downregulated DEGs. DEGs, differentially expressed genes; ALDH3A2, aldehyde dehydrogenase 3 family, member A2; BAZ1B, bromodomain adjacent to zinc finger domain, 1B; BDKRB2, bradykinin receptor B2; CAPN3, calpain 3; CCL27, chemokine (C-C motif) ligand 27; CMA1, chymase 1, mast cell; COX5B, cytochrome *c* oxidase subunit 5B; COX7C, COX 7C; CYP27B1, cytochrome P450, family 27, subfamily B, polypeptide 1; DCT, dopachrome tautomerase; DSG3, desmoglein 3; DHX9, DEAH (Asp-Glu-Ala-His) box helicase 9; EPHA2, ephrin type-A receptor 2; EWSR1, Ewing sarcoma RNA-binding protein 1; FABP7, fatty acid binding protein 7, brain; FFAR2, free fatty acid receptor 2; FGFFBP1, fibroblast growth factor binding protein 1; FSCN1, fascin actin-bundling protein 1; FXDY1, FXYD domain containing ion transport regulator 1; GALNT6, polypeptide N-acetylgalactosaminyltransferase 6; GATA3, GATA binding protein 3; GATM, glycine amidinotransferase (L-arginine:glycine amidinotransferase); GSTA3, glutathione S-transferase alpha 3; GSTM5, GST Mu 5; GSTP1, GST Pi 1; HIF1A, hypoxia-inducible factor 1, alpha subunit; HLF, hepatic leukemia factor; ID4, inhibitor of DNA binding 4, dominant negative helix-loop-helix protein; IL8, interleukin 8; IL11RA, IL 11 receptor, alpha; ISL1, ISL LIM homeobox 1; ITGA2, integrin alpha 2; ITGA6, ITGA 6; IVL, involucrin; KRT6A, keratin 6A; KRT6B, KRT 6B; KRT16, KRT 16; LTB4R2, leukotriene B4 receptor 2; MAPK8, mitogen-activated protein kinase 8; MGST3, microsomal GST 3; MLANA, melan-A; MMP1, matrix metalloproteinase 1; MMP10, MMP 10; MSMB, microseminoprotein, beta-; MUC7, mucin 7, secreted; MX1, Mx dynamin-like guanosine triphosphate hydrolase 1; MYH9, myosin, heavy chain 9, non-muscle; NDRG1, N-myc downstream regulated 1; NDUFA1, nicotinamide adenine dinucleotide hydrogen dehydrogenase (ubiquinone) 1 alpha subcomplex; NPY1R, neuropeptide Y receptor Y1; OAS3, 2'-5'-oligoadenylate synthetase 3; OASL, OAS-like; PI3, peptidase inhibitor 3, skin derived; PID1, phosphotyrosine interaction domain containing 1; PLAU, plasminogen activator, urokinase; PLN, phospholamban; PNP, purine nucleoside phosphorylase; PHYH, phytanoyl-CoA 2-hydroxylase; PHYHIP, PHYH interacting protein; RGS20, regulator of G protein signaling 20; S100A2, S100 calcium-binding protein A2; S100A9, S100A 9; S100A12, S100A 12; SAMD9, sterile alpha motif domain containing 9; SCGB1D2, secretoglobulin, family 1D, member 2; SCGB2A2, SCGB, family 2A, member 2; SERPINB3, serpin peptidase inhibitor, clade B (ovalbumin), member 3; SERPINB4, SERPINB 4; SH3BGR, SH3 domain binding glutamate-rich protein; SLC7A1, solute carrier family 7 (cationic amino acid transporter, Y+ system), member 1; SLC7A5, SLC7A 5; SLPI, secretory leukocyte protease inhibitor; SPRR1A, small proline-rich protein 1A; SPRR1B, SPRR 1B; SPRR3, SPRR 3; TBR1, T-box, brain, 1; TCN1, transcobalamin I (vitamin B12 binding protein, R binder family); TREM1, triggering receptor expressed on myeloid cells 1; TRPM6, transient receptor potential cation channel, subfamily M, member 6; TYMP, thymidine phosphorylase; UPP1, uridine phosphorylase 1; WNT5A, wingless-type mouse mammary tumour virus integration site family, member 5A; ZFPM2, zinc finger protein, FOG family member 2.

this disease may be the most effective approach to improve patients' prognosis. In the present study, a total of 181 DEGs that were differently expressed in SCC vs. NO samples were identified via the gene expression profile contained in GSE2503. The upregulated DEGs were enriched in BPs terms associated with cell adhesion and cancer signaling pathways. In the constructed PPI network and subnetwork, the hub genes *MMP1* and *IL8* presented the highest degree of connectivity. Additionally, *ITGA6* and *ITGA2* were enriched in several pathways and GO BPs terms in the subnetworks. These results suggested that the above genes and signaling pathways may participate in the progression of SCC.

Cell adhesion is a common event in BPs (23). Alterations in the expression levels of cell-cell adhesion molecules have

been previously proposed to contribute to the progression of malignant tumors (25). In the present study, *IL8*, one of the hub genes with the highest degree of connectivity, was observed to be enriched in the BP of cell adhesion. *IL8* is a proinflammatory cytokine that promotes chemotaxis and degranulation in neutrophils (26). Overexpression of *IL8* or its receptors has been previously observed in cancer cells, endothelial cells and tumor-associated macrophages, suggesting a regulatory function for *IL8* within the tumor microenvironment (26). Notably, *IL8* has been previously reported to be able to stimulate cellular proliferation and angiogenesis in head and neck SCC by acting in an autocrine or paracrine manner (27). Additionally, a previous study on cultured oropharyngeal SCC lines has suggested that the overexpression of *IL8* may enhance

Table II. Statistical analysis of the degrees of connectivity corresponding to the most significant hub genes identified in the protein-protein interaction network and subnetwork.

Gene	Degree of connectivity	Adjusted P-value
<i>MMP1</i>	8	0.012
<i>KRT6A</i>	8	0.028
<i>IL8</i>	8	0.005
<i>SPRR1B</i>	7	0.031
<i>KRT16</i>	7	0.001
<i>SPRR1A</i>	6	0.012
<i>IVL</i>	6	0.015
<i>S100A9</i>	6	0.028
<i>S100A2</i>	6	0.014

IL8, interleukin 8; IVL, involucrin; KRT6A, keratin 6A; KRT16, KRT 16; MMP1, matrix metalloproteinase 1; S100A2, S100 calcium-binding protein A2; S100A9, S100A 9; SPRR1A, small proline-rich protein 1A; SPRR1B, SPRR B.

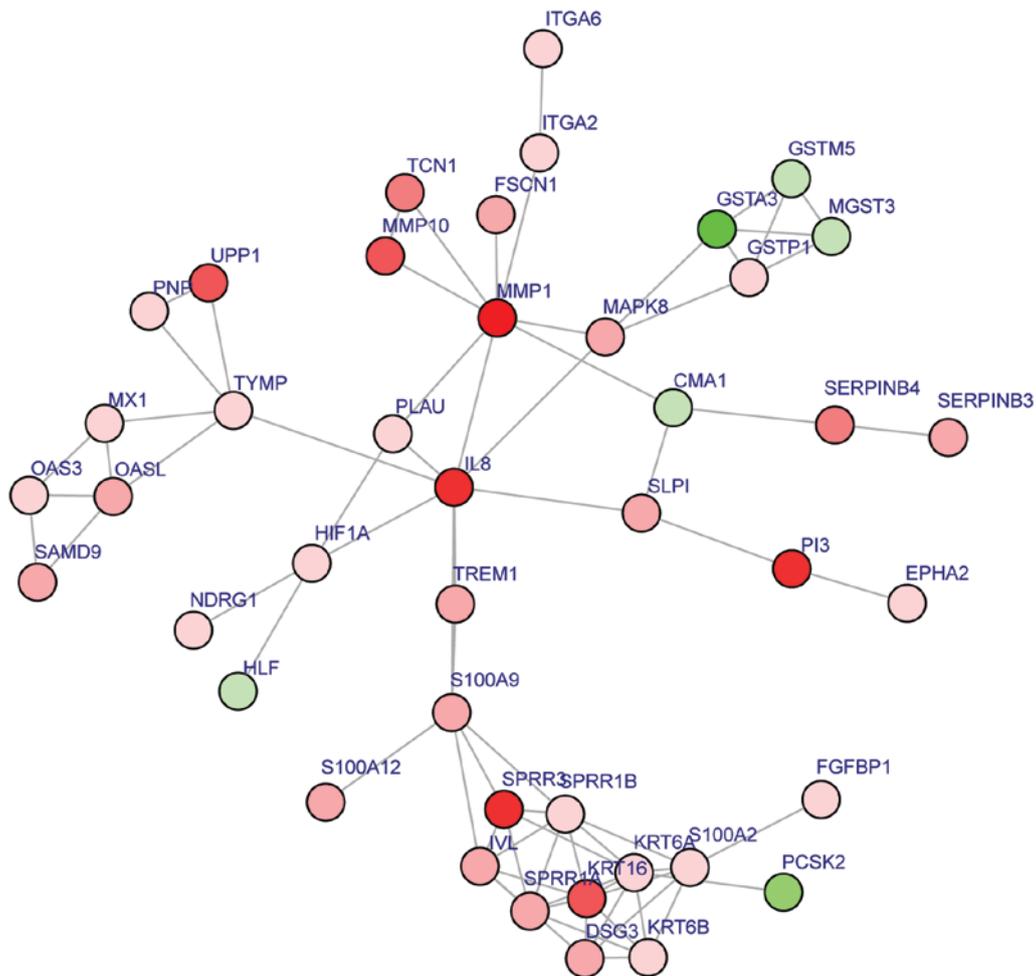


Figure 2. Constructed subnetwork of DEGs. Red, upregulated DEGs. Green, downregulated DEGs. DEGs, differentially expressed genes; CMA1, chymase 1, mast cell; DSG3, desmoglein 3; EPHA2, ephrin type-A receptor 2; FGFBP1, fibroblast growth factor binding protein 1; FSCN1, fascin actin-bundling protein 1; GSTA3, glutathione S-transferase alpha 3; GSTM5, GST Mu 5; GSTP1, GST Pi 1; HIF1A, hypoxia-inducible factor 1, alpha subunit; HLF, hepatic leukemia factor; IL8, interleukin 8; ITGA2, integrin alpha 2; ITGA6, ITG 6; IVL, involucrin; KRT6A, keratin 6A; KRT6B, KRT 6B; KRT16, KRT 16; MAPK8, mitogen-activated protein kinase 8; MGST3, microsomal GST 3; MMP1, matrix metalloproteinase 1; MMP10, MMP 10; MX1, Mx dynamin-like guanosine triphosphate hydrolase 1; NDRG1, N-myc downstream regulated 1; PCSK2, proprotein convertase subtilisin/kexin type 2; PI3, peptidase inhibitor 3, skin derived; OAS3, 2'-5'-oligoadenylate synthetase 3; OASL, OAS-like; PLAU, plasminogen activator, urokinase; PNP, purine nucleoside phosphorylase; S100A2, S100 calcium-binding protein A2; S100A9, S100A 9; S100A12, S100A 12; SAMD9, sterile alpha motif domain containing 9; SERPINB3, serpin peptidase inhibitor, clade B (ovalbumin), member 3; SERPINB4, SERPIN 4; SLPI, secretory leukocyte protease inhibitor; SPRR1A, small proline-rich protein 1A; SPRR1B, SPRR 1B; SPRR3, SPRR 3; TCN1, transcobalamin I (vitamin B12 binding protein, R binder family); TREM1, triggering receptor expressed on myeloid cells 1; TYMP, thymidine phosphorylase; UPP1, uridine phosphorylase 1.

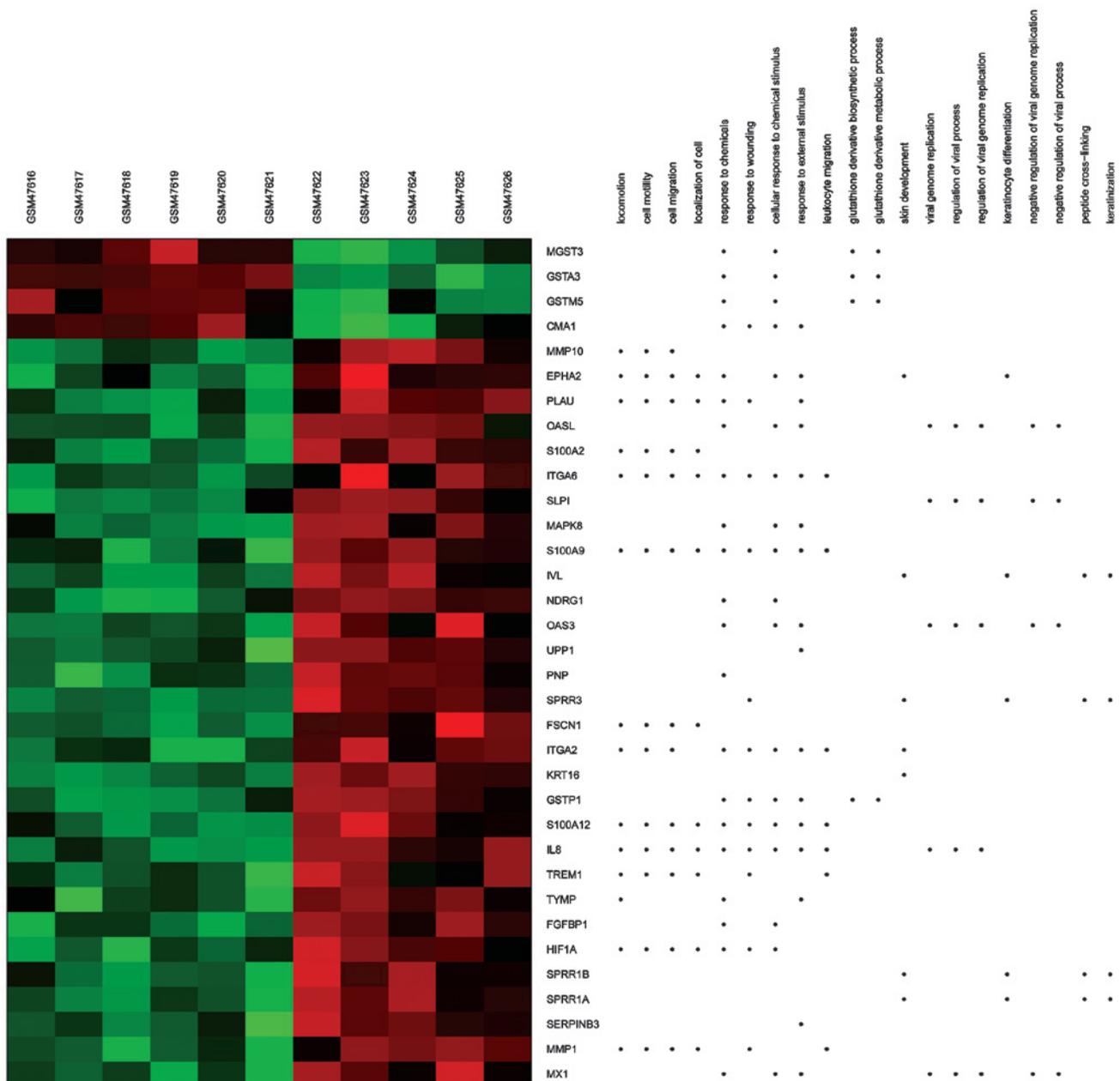


Figure 3. Heat map of Gene Ontology BPs generated via GeneAnswers. The dots indicate the BPs enriched by DEGs. Red, upregulated DEGs. Green, down-regulated DEGs. BPs, biological processes; DEGs, differentially expressed genes; GSM, genome-scale model; CMA1, chymase 1, mast cell; EPHA2, ephrin type-A receptor 2; FGFBP1, fibroblast growth factor binding protein 1; FSCN1, fascin actin-bundling protein 1; GSTA3, glutathione S-transferase alpha 3; GSTM5, GST Mu 5; GSTP1, GST Pi 1; HIF1A, hypoxia-inducible factor 1, alpha subunit; IL8, interleukin 8; ITGA2, integrin alpha 2; ITGA6, ITGA 6; IVL, involucrin; KRT16, keratin 16; MAPK8, mitogen-activated protein kinase 8; MGST3, microsomal GST 3; MMP1, matrix metalloproteinase 1; MMP10, MMP 10; MX1, Mx dynamin-like guanosine triphosphate hydrolase 1; NDRG1, N-myc downstream regulated 1; PLAU, plasminogen activator, urokinase; PNP, purine nucleoside phosphorylase; OAS3, 2'-5'-oligoadenylate synthetase 3; OASL, OAS-like; S100A2, S100 calcium-binding protein A2; S100A9, S100A 9; S100A12, S100A 12; SERPINB3, serpin peptidase inhibitor, clade B (ovalbumin), member 3; SLPI, secretory leukocyte protease inhibitor; SPRR1A, small proline-rich protein 1A; SPRR3, SPRR 3; TREM1, triggering receptor expressed on myeloid cells 1; TYMP, thymidine phosphorylase; UPP1, uridine phosphorylase 1.

the pathogenicity of oropharyngeal SCC by promoting cell growth (28). Therefore, *IL8* and the signaling pathways associated with cell adhesion appear to be closely connected with SCC, and they may be used as potential targets for the treatment of SCC.

Using the PPI networks and subnetworks constructed in the present study, *MMP1* was identified as one of the hub genes exhibiting the highest degree of connectivity. Additionally, *MMP1* was observed to be enriched in signaling pathways

associated with cancer. *MMP1* belongs to the MMP family, and participates in a variety of BPs, including cell proliferation, differentiation, migration, apoptosis and host defense (29). *MMP1* has been previously associated with cancer invasion and metastasis, since it degrades fibrillar collagens, thus enabling the tumor to traverse the extracellular space (29). Notably, *MMP1* is frequently detected in various types of cancer, and may be associated with advanced stages of the disease (30). For example, *MMP1* appears to be overexpressed

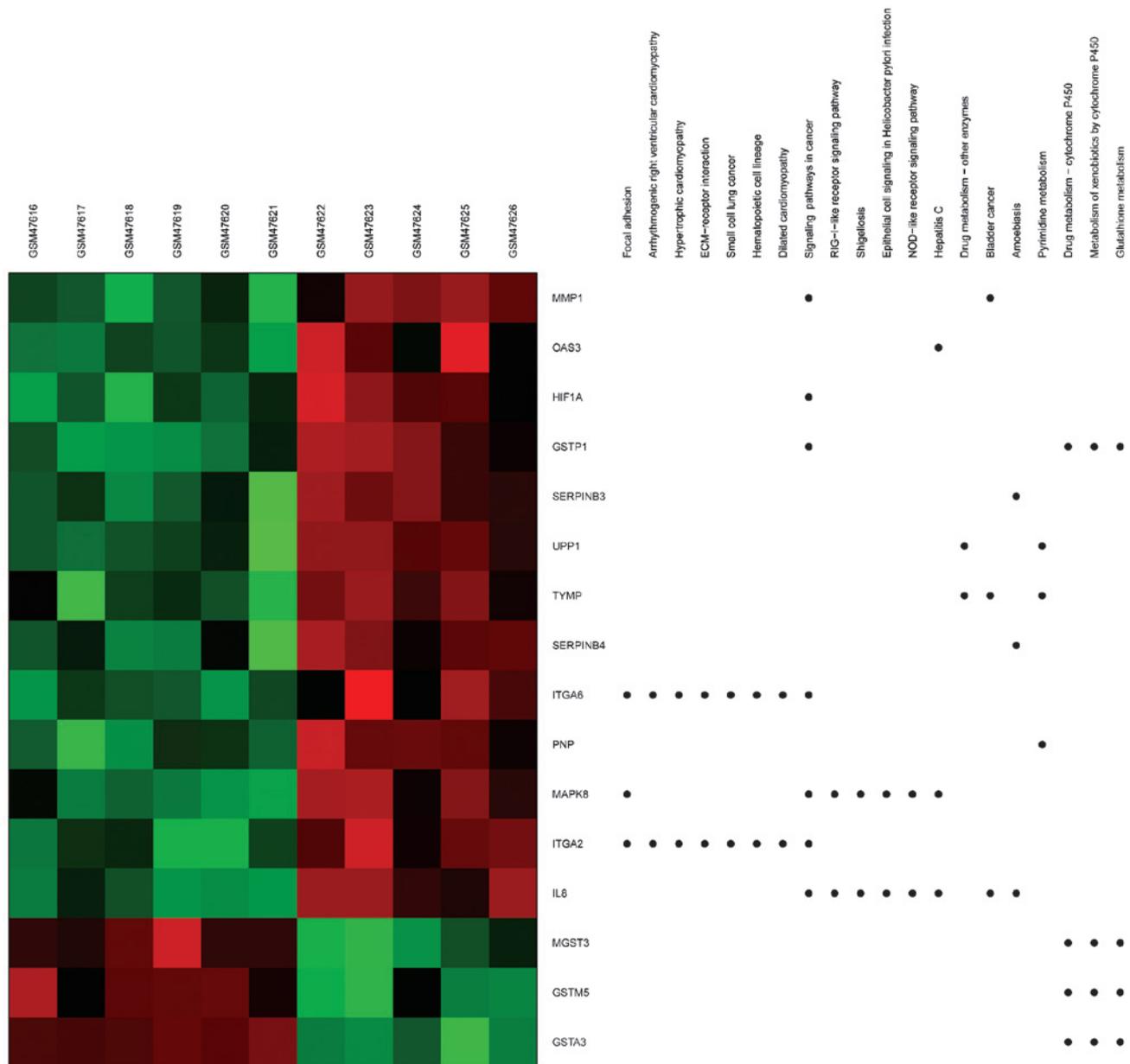


Figure 4. Heat map of Kyoto Encyclopedia of Genes and Genomes generated via GeneAnswers. The dots indicate the signaling pathways enriched by DEGs. Red, upregulated DEGs. Green, downregulated DEGs. DEGs, differentially expressed genes; ECM, extracellular matrix; GSM, genome-scale model; GSTA3, glutathione S-transferase alpha 3; GSTM5, GST Mu 5; GSTP1, GST Pi 1; HIF1A, hypoxia-inducible factor 1, alpha subunit; IL8, interleukin 8; ITGA2, integrin alpha 2; ITGA6, ITGA 6; MAPK8, mitogen-activated protein kinase 8; MGST3, microsomal GST 3; MMP1, matrix metalloproteinase 1; NOD, nucleotide-binding oligomerization; OAS3, 2'-5'-oligoadenylate synthetase 3; PNP, purine nucleoside phosphorylase; RIG-I, retinoic acid-inducible gene 1; SERPINB3, serpin peptidase inhibitor, clade B (ovalbumin), member 3; SERPINB4, SERPINB 4; TYMP, thymidine phosphorylase; UPP1, uridine phosphorylase 1.

in skin cancer, according to the studies by Nindl *et al* (7). Taken together, these data support the hypothesis that MMP1 is a candidate molecular marker associated with SCC.

In the present study, the TF *HIF1A* was identified to be overexpressed in SCC. *HIF1A* functions as a TF in response to cellular hypoxia, and participates in BPs associated with tumor angiogenesis and pathophysiology of ischemic disease (31). It has been previously reported that *HIF1A* may be a predictor of disease progression in esophageal SCC (32). Fillies *et al* (33) suggested that the overexpression of *HIF1A* may be an indicator of favorable prognosis in SCC of the oral cavity. Accordingly, *HIF1A* may be an important TF associated with SCC.

The present study also revealed that *ITGA6* and *ITGA2* were enriched in several GO BPs terms, including regulation of cell adhesion and migration, and KEGG signaling pathways of focal adhesion and ECM-receptor interaction. The protein product of the *ITGA6* gene is the integrin alpha chain alpha 6 (34). Integrins have a significant role in cell adhesion and migration (34), and different combinations of integrins act as receptors of certain ECM proteins (35). Integrins participate in a number of BPs (including cell adhesion, cell migration, blood clotting and tissue organization) and cancer processes (including cell migration, metastasis and invasion) (34). *ITGA6* interacts with the ECM protein laminin, and is involved in the regulation of cell adhesion, growth

and migration (36). The role of ITGA6 in cancer development has been widely documented. Friedrichs *et al* (37) observed that the overexpression of ITGA6 was associated with unfavorable prognosis in patients with breast cancer. In addition, previous studies have reported that ITGA6 is highly expressed in esophageal SCC tissues and participates in the tumorigenesis of esophageal SCC (38). Therefore, *ITGA6* may be a potential target gene for the treatment of SCC. Notably, *ITGA2* encodes a cell adhesion molecule termed  $\alpha 2\beta 1$  integrin receptor, which enables the interaction of the cells with the ECM and mediates the signaling events occurring within the ECM (39). Recent studies have indicated that the *ITGA2* gene is associated with various types of cancer, including colorectal (40) and breast cancer (41). In addition, Beaulieu (42) reported that *ITGA2* was expressed in colon cancer cell lines, and participated in the proliferation and migration of these cells. There are limited studies on the effects of *ITGA2* on SCC thus far (42). However, it may be speculated that *ITGA2* may be a key gene, along with *ITGA6*, in the progression of SCC.

In conclusion, the results of the present study provide a comprehensive bioinformatics analysis of DEGs that may be involved in SCC. The results of the current study may contribute to understand the underlying molecular mechanisms that lead to SCC. Furthermore, the DEGs identified in the present study, including *IL8*, *MMP1*, *HIF1A*, *ITGA6* and *ITGA2*, and certain signaling pathways associated with focal adhesion and ECM-receptor interaction, may be potential targets for the diagnosis and treatment of SCC.

However, the present study has a number of limitations. Thus, the size of the sample employed in the microarray analysis was small, which may generate a high number of false positive results. Additionally, the present study lacked experimental verification. Therefore, further genetic and experimental studies with larger sample sizes are required to confirm the findings of the present study.

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