Expression Profiles Reveal Involvement of *VEGF*, *IGF1*, *BIRC5*, and *MMP1* in Vulvar Carcinogenesis

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Abstract

Objective: The objective of this study was to identify key genes and shed light on the underlying molecular mechanisms of vulvar squamous cell carcinoma (VSCC). **Methods:** Bioinformatic software was utilized for the identification and characterization of key differentially expressed genes (DEGs) from microarrays GSE63678 and GSE38228, which contain VSCC and normal vulvar tissue data. These microarrays were obtained from Gene Expression Omnibus (GEO). Immunohistochemical assays (55 VSCC and 50 normal vulvar tissues) were utilized to validate the expression of *VEGF*, *IGF1*, *BIRC5*, and *MMP1* screened from the identified DEGs. SPSS 18.0 software was used for statistical analyses of the relationships between *IGF1*, *BIRC5*, *VEGF*, *MMP1* expression levels and patient clinicopathological characteristics. **Results:** A total of 141 DEGs were identified, among which 18 genes were closely correlated with the biological characteristics of VSCC. Four of the 18 genes (*VEGF*, *IGF1*, *BIRC5*, and *MMP1*) screened from the GEO database were markedly enriched in pathways in cancer ($P < 0.05$), and could be considered key genes in VSCC based on KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis in DAVID (Database for Annotation, Visualization and Integrated Discovery). The expression levels of these 4 hub genes, determined by immunohistochemical assays, were consistent with the bioinformatics results. Higher expression of *IGF1* showed significant association with well-differentiated carcinomas ($P = 0.017$). *BIRC5* expression levels showed a positive correlation with clinical stage ($P = 0.039$); compared with those in menopause for over 10 years, patients in menopause for less than 10 years at the time of diagnosis tended to have significantly higher expression of *BIRC5* ($P = 0.003$). *VEGF* and *MMP1* expression levels were not correlated with any of the tested clinicopathological characteristics. **Conclusion:** *VEGF*, *IGF1*, *BIRC5*, and *MMP1* were identified as being associated with VSCC using integrated bioinformatic methods, which may provide important insights into the pathogenesis of this disease and help to identify new biomarkers.

Keywords

vulvar squamous cell carcinoma, gene chip, *VEGF*, *IGF1*, *BIRC5*, *MMP1*

Introduction

Vulvar cancer is an uncommon disease that comprises 5% of all gynecological tumors.[1] The vast majority of vulvar cancers are of the squamous epithelial carcinoma type, which accounts for approximately 95% of vulvar malignant tumors.[2] The average age of onset is 50-60 years old. It is reported that the 5-year specific survival rates were 100% and 100% in patients with stage I and II vulvar cancer, which were higher than that in late-stage vulvar cancer (Stage III 86%, Stage IV 29%).[3] It seems that the prognosis of patients with early-stage vulvar cancer is better, which may be due to a slowly progression and a mature treatment strategy for patients. However, the driving factors in tumor progression and the underlying molecular mechanism are still unclear.

As a reliable and high-throughput research technique, gene chips based on microarray technology have been commonly used for more than ten years.[4] They enable the simultaneous detection of expression levels of numerous genes, and have

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become increasingly useful in understanding the genetic basis, prognosis and drug development of tumors. Only a few studies using this technique have focused on the identification of molecular targets in VSCC. One study,[5] which for the first time investigated the expression profile of VSCC using microarray technology, aimed to delineate the pathways involved in the pathogenesis of VSCC. Another study,[6] focused on genomic aberration patterns and expression profiles of VSCC, illustrated a concordant relationship between aCGH results and the level of gene expression found by microarray. Genes located in gained regions were generally overexpressed while those located in lost regions were downregulated. One fascinating study,[7] revealed common specific pathogenetic patterns and gene markers for cervical (CC), endometrial (EC) and vulvar cancer (VC). Altogether, previous studies[5-7] have laid the foundations for the present study to integrate, reanalyze and experimentally validate clinical samples. Herein, we performed data remining based on the GSE38228 and GSE63678 datasets.[6,7] Furthermore, 4 hub genes (*IGF1*, *BIRC5*, *VEGF* and *MMP1*) selected in the present research were verified with clinical samples, and the relationships between their expression levels and patient clinicopathological characteristics were analyzed.

In this research, the VSCC-associated gene expression datasets GSE63678 and GSE38228 were downloaded from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/). Then, differentially expressed genes between VSCC and normal vulvar tissues (DEGs) in the 2 datasets were identified using the GEO2R online tool and Venn diagram software. Subsequently, a protein-protein interaction (PPI) network was established, and Cytotype MCODE (Molecular Complex Detection) was used for additional analysis of the DEGs to identify the hub genes. In addition, these core DEGs were imported into the Kaplan-Meier Plotter online database to determine whether the core DEGs provided significant prognostic information for cervical squamous cell carcinoma. Finally, an immunohistochemical assay was conducted, and the relationships between the expression levels and patient clinicopathological characteristics were analyzed. In conclusion, the bioinformatic study and experimental verification of our research provide a number of key biomarkers that could be of vital consequence in the progression of VSCC.

Methods

Microarray Data Information

We searched NCBI-GEO, a public database[8] of microarray/gene maps, and acquired the gene expression profiles of GSE63678 and GSE38228 in VSCC and normal vulvar tissues. Microarray data of GSE63678 comprise 6 VSCC tumor and 13 normal vulvar tissue samples, while the GSE38228 profile consists of 7 VSCC tumor and 5 normal vulvar tissues samples.

Identification of DEGs

The relevant samples included in GSE63678 and GSE38228 were categorized into 2 groups: -VSCC and normal vulvar tissues. DEGs between the 2 groups were identified by using the GEO2R online tool[8] with statistical significance reached at |logFC| > 1 and adjusted *P* value < 0.05. GEO2R is an interactive web tool especially developed for the identification of differentially expressed genes among selected groups (https://www.ncbi.nlm.nih.gov/geo/geo2r/). Then, we input the raw data in TXT format in Venn software to determine the common DEGs among the 2 datasets (http://bioinformatics.psb.ugent.be/webtools/Venn/). The DEGs with logFC<-1 were considered downregulated genes, while the DEGs with logFC>1 were considered upregulated genes.

PPI Network and Module Analysis

STRING (Search Tool for the Retrieval of Interacting Genes), an online tool, helps us to evaluate PPI information (https://string-db.org/).[9] Next, Cytoscape [10] was applied to examine the underlying correlation between these DEGs (maximum number of interactions = 0 and confidence score≥0.4). Furthermore, the MCODE plug-in in Cytoscape was used to check modules of the PPI network to identify the hub genes with parameters as follows: degree cutoff = 2, maximum depth = 100, k-core = 2, and node score cutoff = 0.2.

KEGG Pathway Enrichment Analysis

KEGG is a collection of databases concerning genomes, diseases, biological pathways, drugs, and chemical materials.[11,12] As an online bioinformatic tool (https://david.ncifcrf.gov/), DAVID bioinformatics resources consist of an integrated biological knowledge base and analytic tools aimed at systematically extracting biological meaning from large gene/protein lists.[13] In this study, a gene list containing 18 gene identifiers was analyzed with the Kyoto Encyclopedia of Gene and Genome (KEGG) pathway using one or more text and pathway-mining tools, such as gene functional classification, functional annotation chart or clustering and functional annotation table. In addition, DAVID provided help to visualize the DEG pathway enrichment (*P* < 0.05).

Immunohistochemical Assay

In this study, 55 VSCC tumor and 50 normal vulvar tissue samples from June 2013 to October 2019 were collected from the institutional database of Women's Hospital, School of Medicine, Zhejiang University, China. Among these 55 VSCC tumor samples, 37 were well differentiated, 15 were moderately differentiated and 3 were poorly differentiated; 45 were at clinical stage I, 5 were at clinical stage II and 5 were at clinical stag III; 42 patients were in menopause for over ten years while the other 13 patients were in menopause for less ten years. The average age of all cases was 66.84 years. Each enrolled patient

signed an informed consent form to allow her samples and records to be used for scientific research.

Immunohistochemical assays were performed on the VSCC sections. Antibodies against *VEGF* (1:250 dilution; Abcam cat# ab32152), *IGF1* (1:2000 dilution; Abcam cat# ab40657), *BIRC5* (1:100 dilution; Abcam cat# ab76424), and *MMP1* (1:200 dilution; Abcam cat# ab52631) were incubated overnight at 4°C after deparaffinization, hydration and antigen retrieval. After a washing procedure, a broad-spectrum secondary antibody was incubated. The immunostaining was individually interpreted by the researchers (ZT and LQ).

Positive cells were indicated by the presence of yellow to brown DAB staining in the nucleus or cytoplasm. The number of positively stained cells out of 100 in 10 random fields (400× objective) was counted and represented as the percentage of positive cells. The semiquantitative immunoreactive score was based on the percentage of positive cells and the staining intensity. 0, <5% positive cells; 1, 5%-25% positive cells; 2, 26%-50% positive cells; 3, 51%-75%; 4, more than 76% positive cells. According to the DAB staining intensity, the cells were scored as follows: 0, no staining; 1, faint yellow; 2, brown yellow; and 3, dark brown. The expression categories were finally divided into a high expression group and a low expression group according to expression scores. Because the expression of *IGF1* is at a low level, 4 was set as the cutoff value, for which ≥4 is highly expressed and <4 is expressed at low levels. In the expression analysis of *VEGF*, *BIRC5*, and *MMP1*, cutoff value was set at 6: ≥6 indicates high expression; <6 indicates low expression. Five random fields (400× objective) of each slip were observed, and the average of the five fields obtained was the score of the cover flip immunostaining.

Statistical Analysis

The SPSS 18.0 software was used for statistical analyses. Fisher's Exact tests were used to evaluate protein expression.

Results

Identification of DEGs in VSCC

In total, there were 13 VSCC and 18 normal vulvar tissues samples in GSE63678 and GSE38228. Analysis with the GEO2R online tool, we identified 850 and 1012 DEGs in the 2 datasets, respectively. By screening with |logFC|>1 and adjusted *P* value < 0.05, we identified 195 upregulated genes and 655 downregulated genes in GSE63678, while 398 upregulated genes and 614 downregulated genes were identified from GSE38228. Next, Venn diagram software was used to identify the common DEGs between the 2 datasets. The results showed that a total of 141 common DEGs were filtered out, of which 39 were upregulated (logFC>1) and 102 were downregulated (logFC<-1) in the VSCC tissues (Table 1, Figure 1).

Table 1. Entire 141 Common DEGs Were Identified From 2 Profile Datasets, Including 39 Up-Regulated Genes and 102 Down-Regulated Genes in the Vulvar Cancer Tissues Compared to Normal Vulvar Tissues.

DEGs	Genes name
Up-regulated	CDH3, TNFAIP6, MYO1B, COL4A1, PLAU, SLC28A3, HOMER3, IL1A, MMP3, LPAR3, LAMC2, SUGCT, SCD, BASP1, POSTN, BIRC5, KIF20A, MYO5A, AIM2, GALNT6, UBE2C, NQO1, ITGA6, CCNB2, TDO2, PCDH7, IL36G, LIPG, VEGFA, TOP2A, CCNA2, NCAPG, PC, PRNP, MMP1, TYMP, MMP12, AURKA
Down-regulated	CHPT1, NR3C2, ZSCAN18, IGF1, VEZF1, ADD3, SLC27A6, SLC11A2, SASH1, MAOB, PTGDS, DHRS11, DIAPH2, SPAG16, ID4, ISL1, DACH1, TACC1, NFIB, ALDH3A2, ACKR1, NBEA, SLC25A4, NAAA, DDAH1, MEIS2, BCL2, SECISBP2L, PAMR1, CXCL12, EFHD1, GLUL, APOD, LDB2, CBX7, ARMCX1, ERMP1, NCOA1, ZBTB20, TLE2, ARMCX6, METTL7A, ITM2A, ZFP36L2, CBX6, MPC1, PLAGL1, FAM189A2, MSRA, ABCA8, DEPTOR, ISOC1, CYP3A5, JUND, FBLN1, NDRG2, SVEP1, PLPP3, PLLP, MEIS1, COBL, TGFBR3, EDN3, PLPP1, KLF2, P4HTM, SOSTDC1, NYNRIN, PIK3R1, CDKN2C, ACACB, CLDN8, FAM107A, GPX3, DDAH2, RRAGD, IL20RA, ABI3BP, DCN, PER2, SLIT2, CX3CR1, LONRF1, LDOC1, ATP6V0E2, MLPH, AR, GATM, KLHL3, GPD1L, OCA2, GULP1, CFD, SYNGR1, ALDH5A1, RNASE4, IGFBP5, EPHX2, ARMCX2, CYP4B1, NR2F2, NDN

Abbreviation: DEGs, differentially expressed genes.

Protein–Protein Interaction (PPI) Network and Modular Analysis

A total of 141 DEGs were imported into the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/) which then obtained 141 nodes and 196 edges, including 102 downregulated and 39 upregulated genes. Then, Cytoscape MCODE was applied to further analysis and 18 central nodes are highlighted in yellow: *NCAPG, BIRC5, AURKA, CCNA2, UBE2C, KIF20A, CCNB2, TOP2A, IL1A, PLAU, POSTN, IGF1, MMP1, CXCL12, VEGF, MMP3, DCN,* and *ANG* (Figure 2).

Analysis of 18 Core Genes for KEGG Pathway Enrichment

Ultimately, 18 genes via DAVID were analyzed via DAVID for KEGG pathway enrichment, which indicated that 4 genes (*VEGF, IGF1, BIRC5, MMP1*) were markedly enriched in pathways in cancer (*P* < 0.05), and could be considered key genes in VSCC (Table 2).
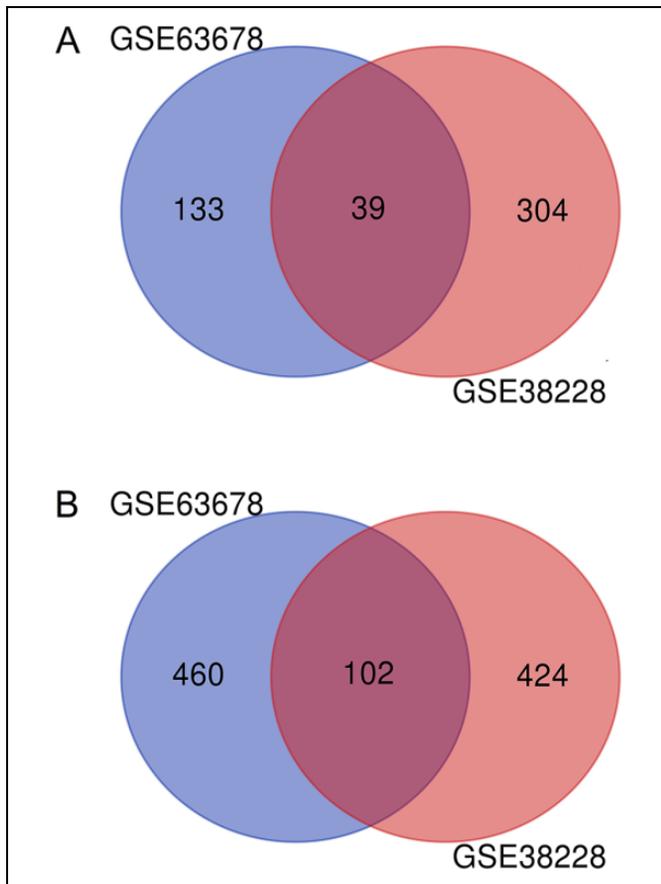
Figure 1. Identification of 141 common DEGs in the 2 datasets. A, 39 common up-regulated genes were searched by GSE63678 and GSE38228. B, 102 common down-regulated genes were searched by GSE63678 and GSE38228.

Validation of VEGF, IGF1, BIRC5, and MMP1 Expression With Immunohistochemical Assay in Normal Vulvar Tissues Versus Vulvar Cancer Samples

IHC assays of 55 VSCC and 50 normal vulvar tissue sections were conducted to validate the expression levels of *VEGF*, *IGF1*, *BIRC5*, and *MMP1* (Table 3). The *VEGF*, *IGF1* and *MMP1* proteins were mainly located in the cytoplasm, while *BIRC5* was mainly located in the nucleus. The IHC analysis showed consistent results with the bioinformatic analysis (Figure 3).

Relationships Between IGF1, BIRC5, VEGF, MMP1 Expression Levels and Patients' Clinicopathological Characteristics

Clinicopathological data of 55 VSCC patients were collected including age, tumor dimension, tumor differentiation, clinical stage and menopausal duration. The 55 VSCC patients were divided into a high expression group and a low expression group according to the median value of the corresponding protein expression level (Table 4). The *IGF1* expression status was not correlated with age ($P = 0.662$), tumor dimension ($P =$

0.575), clinical stage ($P = 0.86$) or menopausal duration ($P = 0.141$). However, tumor differentiation ($P = 0.017$) was significantly associated with *IGF1* expression, and well-differentiated carcinomas frequently presented higher expression of *IGF1*. *BIRC5* expression was not related to age ($P = 0.147$), tumor dimension ($P = 0.182$) or tumor differentiation ($P = 0.309$). However, *BIRC5* expression levels showed a positive correlation with clinical stage ($P = 0.039$) and menopause duration ($P = 0.003$). *VEGF* and *MMP1* expression levels were not correlated with any of the tested clinicopathological characteristics.

Discussion

In this study, we first utilized bioinformatic methods to identify differentially expressed genes (DEGs) in VSCC patients. Next, we investigated the clinical significance and prognostic value of *IGF1*, *BIRC5*, *VEGF*, and *MMP1* in VSCC. The results showed a significant upregulation of *BIRC5*, *VEGF*, and *MMP1* expression and a distinct downregulation of *IGF1* expression in VSCC compared to normal vulvar tissues.

Kaplan Meier Plotter was utilized to determine the prognostic values of *IGF1*, *BIRC5*, *VEGF*, and *MMP1*, which showed that increased *IGF1* or *BIRC5* expression correlated with better survival, while increased *VEGF* or *MMP1* expression correlated with poorer survival in cervical carcinoma patients (Supplementary Material). Previous studies have consistently reported[14-18] that *VEGF*, *MMP1* and *BIRC5 gene expression* is up-regulated and *IGF1* expression is down-regulated in both cervical and vulvar tumors. We also performed follow-up and survival analyses, which showed that the 4 genes had statistically insignificant prognostic value in VSCC, which may be due to the insufficient sample size and the short follow-up time of some cases. These results are presented in the Supplementary Material.

In addition, the *IGF1* expression status displayed in clinical samples was significantly related to tumor differentiation; well-differentiated carcinomas frequently presented a higher expression of *IGF1*. On the other hand, lower *BIRC5* expression was often shown in clinical stage I patients.

Studies investigating the carcinogenesis of VSCC have been conducted for decades. However, there are few studies about biomarkers that could help tailor conventional treatment and follow-up. Kalliopi[5] systematically investigated the expression profile of VSCC for the first time using the microarray technology and delineated the molecular parameters to reveal the cellular pathways involved in the pathogenesis of VSCC. Recently, second harmonic generation (SHG) microscopy has been used for the analysis of collagen fibers in VSCC and preneoplastic lesions and an evident decrease in the values of collagen fiber parameters in the VSCC was discovered by Leuridan *et al.*[19]

Previous research,[6] which mainly focused on genomic aberration patterns and expression profiles of VSCC, illustrated a concordant relationship between the imbalances scored by aCGH and the level of gene expression found by microarray.
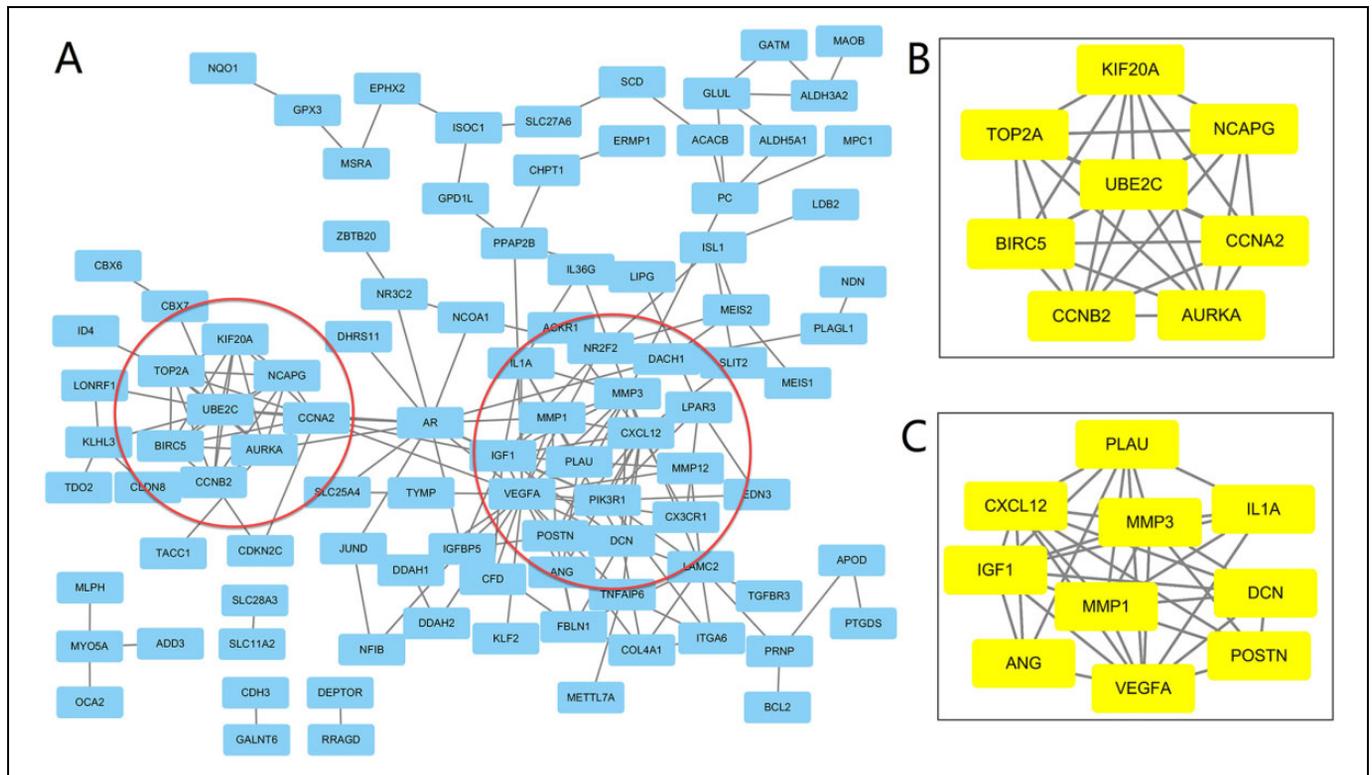
Figure 2. Common DEGs PPI network constructed by STRING online database and Module analysis. PPI network complex. The nodes meant proteins; the edges meant the interaction of proteins. The yellow part is the 19 hub nodes which was obtained by Module analysis via Cytoscape software (degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. Depth = 100).

Table 2. KEGG Pathway Analysis of 18 Core Genes in VSCC.

Pathway ID	Term	Number of genes	%	*P*-value	Genes
hsa05200	Pathways in cancer	4	17.6	0.030	*VEGFA,IGF1, BIRC5,MMP1*
hsa04114	Oocyte meiosis	3	13.2	0.022	*CCNB2,IGF1, AURKA*
hsa04914	Progesterone-mediated oocyte maturation	3	13.2	0.014	*CCNB2,IGF1, CCNA2*

Abbreviation: KEGG, Kyoto Enclopaedia of Genes and Genomes.

Table 3. Protein Expression in Different Vulvar Tissue.[a]

Genes	Sample types	Number of samples	Number of samples		*P* value
			Low expression	High expression	
IGF1	vulvar cancer	55	16	39	0.000
	normal vulvar	50	0	50	
MMP1	vulvar cancer	55	6	49	0.000
	normal vulvar	50	50	0	
BIRC5	vulvar cancer	55	20	35	0.000
	normal vulvar	50	50	0	
VEGF	vulvar cancer	55	7	48	0.000
	normal vulvar	50	50	0	

[a]Fisher Exact test, normal vulvar versus vulvar cancer. A *P* value less than 0.05 was considered significant.

Genes located in gained regions were generally overexpressed while those located in lost regions were downregulated. In addition, the GSE63678 dataset contributor[7] revealed common specific pathogenetic patterns and gene markers for cervical (CC), endometrial (EC) and vulvar cancer (VC). We further investigated the possible pathogenesis of vulvar cancer and verified the results with clinical samples based on the afore-mentioned meaningful explorations. The relationship between
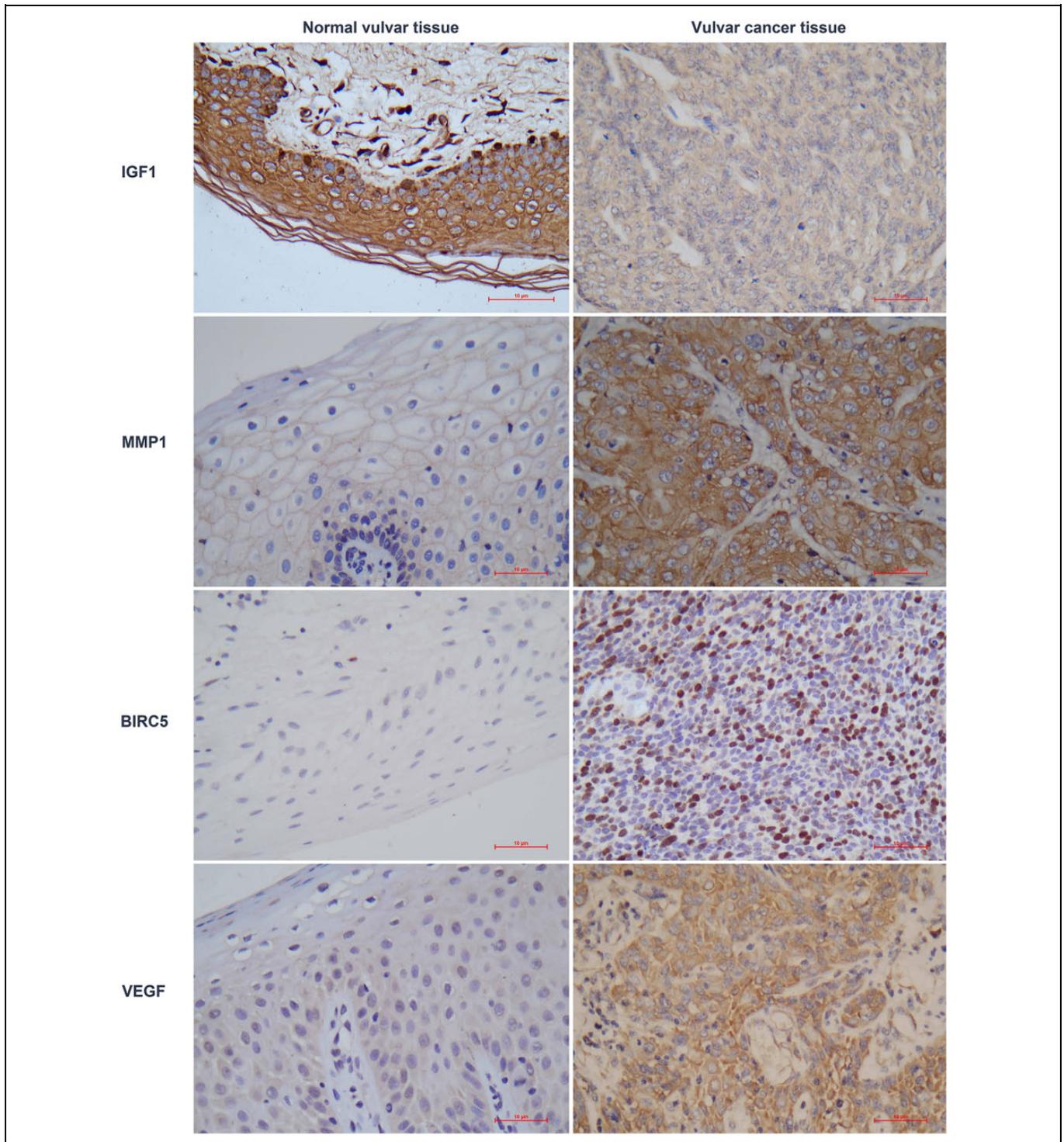
Figure 3. Validation of VEGF, IGF1, BIRC5, and MMP1 with immunohistochemical assay of normal vulvar tissues versus vulvar cancer tissues.

IGF1, *BIRC5*, *VEGF*, and *MMP1* expression levels and patient clinicopathological characteristics were analyzed in our study, which would be informative for mining new biomarkers involved in the carcinogenesis of VSCC.

Some studies have shown that *VEGF* plays a substantial role in driving the expansion of the tumor vascular bed.[20] One

study reported that the median serum *VEGF* concentration in 41 patients affected by VSCC was higher than that in 130 controls,[21] which is consistent with the present study. Other studies reported a significant correlation between microvessel density (MVD), *VEGF* IHC staining, and poorer OS in vulvar cancer patients.[22] Subsequently, Hantschmann[23]

Table 4. Association Between the Expression of *IGF1*, *BIRC5*, *VEGF*, and *MMP1* and Clinicopathological Characteristics.[a]

Patients characteristics	No. of patients	Samples of IGF1 expression			Samples of BIRC5 expression			Samples of VEGF expression			Samples of MMP1 expression		
		High level	Low level	P	High level	Low level	P	High level	Low level	P	High level	Low level	P
Age group (years)				0.662			0.147			0.508			0.452
<50	5	3	2		5	0		4	1		4	1	
≥50	50	36	14		30	20		44	6		45	5	
Tumor dimension (cm)		1.60 ± 1.23	1.91 ± 1.45	0.575	1.91 ± 1.38	1.30 ± 1.04	0.182	1.76 ± 1.27	1.19 ± 1.38	0.376	1.70 ± 1.30	1.62 ± 1.35	0.771
Tumor differentiation				0.017*			0.309			0.774			0.101
Well-differentiated	37	29	8		21	16		31	6		35	2	
Moderately-differentiated	15	10	5		11	4		14	1		12	3	
Poorly-differentiated	3	0	3		3	0		3	0		2	1	
Clinical stage				0.86			0.039*			0.776			1
I	45	32	13		26	19		39	6		39	6	
II	5	4	1		5	0		4	1		5	0	
III	5	3	2		5	0		5	0		5	0	
Menopause duration (years)				0.141			0.003*			0.265			0.069
0	4	1	3		4	0		4	0		2	2	
<10	9	7	2		9	0		8	1		9	0	
≥10	42	31	11		22	20		36	6		38	4	

[a]Fisher Exact test. A P value less than 0.05 was considered significant.

described a high MVD in 29% of VSCCs and a correlation with TGF-α expression, outlining its role in promoting angiogenesis; tumors with both features tended toward having worse DFS, although this was not statistically significant. Not only a high vessel number but also increased vessel size and other vessel characteristics (shape and staining intensity) appeared to be related to prognosis.[24]

Insulin-like growth factor-1 (*IGF1*) is a pivotal regulator of normal tissue growth and development and is involved in the occurrence and progression of various cancers, including breast cancer.[25-27] Numerous studies confirmed that *IGF1* receptor (*IGF1*R) is overexpressed in approximately 90% of breast cancer cases and that *IGF1*R levels are higher in breast cancer cells than in normal breast tissues[28]; thus, the *IGF1* system appears to be a promising therapeutic target.[29] In addition, the *IGF1* signaling pathway has been implicated in HNSCC development and progression.[30] Furthermore, Mark[31] demonstrated the presence of an *IGF1* regulated *VEGF* autocrine loop in HNSCC. In oral squamous cell carcinoma (OSCC), Eik Schiegnitz[32] reported that serum levels of *IGF1* in OSCC patients were significantly lower than those in healthy subjects ($P < 0.001$), which is consistent this study's findings for VSCC. Additionally, they demonstrated that OSCC patients with a lower *IGF1* serum levels showed a significantly worse survival rate than the high expression group ($P = 0.049$). Little is known about *IGF1* tissue expression in VSCC.

BIRC5 is located at the crossroads of a number of cancer cell signaling networks and its functions are controlled and regulated by many upstream cellular signaling molecules[33] that make up the upstream signaling pathways of *BIRC5*. The upstream molecules[34,35] include binding proteins, protein regulators, various enzymes (protease, kinase, phosphatase), transcription factors, miRNAs, transporter and channel proteins, and receptors with or without kinase activity. By using *BIRC5* as a target, future translational research related to drug discovery and cancer therapeutics is flourishing.[36,37] It has been reported[38] that *BIRC5* expression increases significantly from normal squamous vulvar epithelia to high-grade classic vulvar intraepithelial neoplasia, and vulvar invasive keratinizing squamous cell carcinoma. In this study, the results that *BIRC5* is overexpressed in VSCC confirmed its involvement as an early event in vulvar carcinogenesis.

MMP1 is an interstitial collagenase that pertains to matrix metalloproteinases (MMPs), a family of zinc-dependent proteases that aim at the degradation and proteolytic process of components of the extracellular matrix.[39,40] Increasing evidence suggests that abnormal expression of *MMP1* is related to the progression of malignant tumors. It was reported that high expression levels of *MMP1* have significant prognostic value in bladder cancer,[41] prostate carcinoma[42] and gastric cancer.[43] Furthermore, *MMP1* has been shown to function as an oncogene.[44-46] High *MMP1* expression levels were significantly associated with lymph node metastasis, microvessel density and advanced TNM stage in esophageal cancer and head and neck squamous cell carcinoma (HNSCC). HNSCC of patients in the *MMP1* high expression group proved to have

worse disease-free survival and overall survival than those in the *MMP1* low expression group.[47] However, there have been no reports about *MMP1* expression in VSCC.

On the other hand, vulvar cancer is an uncommon gynecological malignancy primarily affecting postmenopausal women. Few data are available on hormonal receptor expression in VCSS tissues, but hormonal therapy is considered a valuable field of investigation.[48] It is worth noting in the present study, 2 of 55 VSCC patients were menopausal at an early age of 28; moreover, patients in menopause for less than 10 years at the time of diagnosis tended to have higher expression of *BIRC5*. This suggests that hormone levels may affect VSCC genesis and is worth further study.

Few studies have been reported about these 4 genes in VSCC thus far. Therefore, the current study could provide helpful information and directions for future studies in VSCC. The results demonstrated that these 4 genes could be involved in vulvar carcinogenesis. Subsequent experiments validated that the 4 hub DEGs (*VEGF, IGF1, BIRC5, MMP1*) participated in the molecular mechanisms of VSCC. The results may provide useful information and direction regarding the potential biomarkers and biological mechanisms of VSCC.

Abbreviations

DEGs, differentially expressed genes; GO, Gene Ontology; HR, hazard ratio; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCODE, molecular complex detection; MVD, microvessel density; PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes; VSCC, vulvar squamous cell carcinoma.

Authors' Note

Approval was obtained from the Human Ethics Committee of Women's Hospital, Zhejiang University School of Medicine (No. IRB-20200158-R). Each enrolled patient permitted and signed up informed consent to use her samples and records for scientific research.

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Supplementary Material

Supplemental material is available online for this article.

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