

Original Research

NOTCH Pathway Genes in Ovarian Cancer: Clinical Significance and Associations with Immune Cell Infiltration

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Abstract

Background: Activation of the *NOTCH* signaling pathway is associated with tumorigenesis. The aim of this study was to investigate *NOTCH* pathway gene functions and regulatory mechanisms in ovarian cancer (OC). **Methods:** We conducted a bioinformatics analysis of publicly available datasets in order to identify potential *NOTCH*-related mechanisms, associated genes, biological pathways, and their relation to immune function. **Results:** Significant differential expression of the *NOTCH* pathway genes *DLL1*, *DLL3*, *DLL4*, *HES1*, *HEY1*, *JAG1*, *NOTCH2*, *NOTCH3*, and *NOTCH4* was observed between OC samples and normal controls. Low expression of *DLL4* and of *NOTCH4* in OC patients was associated with International Federation of Gynecology and Obstetrics (FIGO) stage ($p < 0.001$ and $p = 0.036$, respectively), while high expression of *NOTCH3* was associated with race ($p = 0.039$) and age ($p = 0.044$). *JAG2* and *NOTCH1* expression were significantly associated with progression-free interval (PFI) ($p = 0.011$ and $p = 0.039$, respectively). *DLL1* (Hazard Ratio (HR): 2.096; 95% CI: 1.522–2.886, $p < 0.001$) and *NOTCH1* (HR: 0.711; 95% CI: 0.514–0.983, $p = 0.039$) expression were independently associated with PFI in multivariate analysis. *DLL1*, *DLL3*, *JAG1*, *JAG2*, *NOTCH3* and *NOTCH4* expression could significantly differentiate OC from non-cancer samples. Genes associated with the *NOTCH* pathway were mainly enriched in five signaling pathways: the *NOTCH* signaling pathway, breast cancer, endocrine resistance, Th1 and Th2 cell differentiation, and oxidative phosphorylation. The expression of *NOTCH* pathway genes was significantly associated with immune cell infiltration. **Conclusions:** *NOTCH* pathway genes appear to play an important role in the progression of OC by regulating immune cells, endocrine resistance, Th1 and Th2 cell differentiation, and oxidative phosphorylation. *JAG2* and *NOTCH1* are potential biomarkers and therapeutic targets for the treatment of OC.

Keywords: *NOTCH* pathway; bioinformatics analysis; ovarian cancer; prognosis; immune infiltration

1. Introduction

Ovarian cancer (OC) has the highest mortality of all gynecologic cancers [1]. The median age of patients diagnosed with OC is approximately 60 years, with most women diagnosed at advanced stages and with metastases [2]. OC is characterized by high levels of recurrence and metastasis, drug resistance, and mortality [3]. Despite advances in surgical techniques and the application of combination chemotherapy, the 5-year survival rate for patients with advanced OC is only 40–45% [4]. Although a great deal of research has been carried out on the pathogenesis of OC, little is known about its regulation and the underlying mechanisms. Molecular and genomic aberrations in OC data has accumulated thanks to the advent of large, multi-cohort genomic mapping libraries like The Cancer Genome Atlas (TCGA) [5,6]. However, how the data relates to each other, OC disease state, body processes, and other factors has not been well characterized. This characterization can provide clues as to mechanisms behind OC, which can lead to devel-

oping better treatments. Also, this work can lead to development of biomarkers for early detection of OC. The lack of good techniques for early detection contributes significantly to the low survival rate of OC.

The *NOTCH* signaling pathway is a highly conserved ligand-receptor signaling pathway involved in various aspects of cancer biology, including cancer stemness, angiogenesis, epithelial-mesenchymal transition (EMT), tumor immunity, and drug resistance [7,8]. The most interesting members of the *NOTCH* pathway are the *NOTCH* receptors (*NOTCH1*, *NOTCH2*, *NOTCH3*, *NOTCH4*), the serrated typical *NOTCH* ligands Jagged 1 (*JAG1*) and Jagged 2 (*JAG2*), the Delta-like typical *NOTCH* ligands (*DLL1*, *DLL3*, *DLL4*), and the typical downstream genes known as hair and division enhancer 1 (*HES1*) and hair/division enhancer-associated YRPW patterned protein 1 (*HEY1*) [8,9]. There is evidence that *NOTCH* signaling plays a pleiotropic role in cancer, with dysregulation leading to reduced proliferation and apopto-



sis of cancer cells [10,11]. mRNA expression levels for the four types of *NOTCH* receptor (*NOTCH1-4*) have different prognostic significance in OC patients [12]. The *NOTCH2/NOTCH3/DLL3/MAML1/ADAM17* signaling network has also been associated with OC [13]. Genes that are associated with *NOTCH* pathway genes can be identified by bioinformatic analysis. Overall, the mechanism and function of the *NOTCH* pathway in OC remains unclear, and its mechanism of action has still to be clarified.

Cancer results from the co-evolution of malignant cells and the tumor microenvironment (TME) [14]. Bidirectional interactions between the TME and cancer cells regulate its progression, development, metastasis, invasion, and resistance to therapy [15]. Immune infiltration is a critical part of the TME and plays a central role in OC progression [16,17]. It is important to realize the immune system plays a significant role in the development and progression of OC, and that its dysregulation can result in immune escape and resistance to therapy [18]. Studies of immune cells in the TME of OC have focused on T cells, DCs, MDSCs, macrophages, NK cells, $\gamma\delta$ T cells, and B cells [19]. Therefore, identification of the genes that regulate the immune response is essential for understanding how OC cells evade or even suppress the antitumor immune response. To date, activated *NOTCH* signaling pathways in the TME have been widely reported in various cancer types [8,14,20]. However, so far there have been few studies on the correlations between *NOTCH* members and immune responses in OC, or on the associated mechanisms.

In the present study we conducted a comprehensive bioinformatics analysis of public datasets to investigate potential *NOTCH*-related mechanisms, genes, biological pathways, and their relation to immune function. This study looked to identify valuable candidate biomarkers or targets for the treatment of OC.

2. Materials and Methods

2.1 cBioPortal Analysis

Mutations in *NOTCH* pathway genes in OC were studied using the cBio Cancer Genomics Portal (<http://cbioportal.org>) [21–23]. We performed visualization and analysis queries based on references [23], with the studied proteins being *DLL1* [ENSG00000198719], *DLL3* [ENSG00000090932], *DLL4* [ENSG00000128917], *HES1* [ENSG00000114315], *HEY1* [ENSG00000164683], *JAG1* [ENSG00000101384], *JAG2* [ENSG00000184916], *NOTCH1* [ENSG00000148400], *NOTCH2* [ENSG00000134250], *NOTCH3* [ENSG00000074181], and *NOTCH4* [ENSG00000204301].

2.2 Dysregulation of *NOTCH* Pathway Genes in OC

R (version 3.6.3, statistical analysis and visualization, R Core Team, Vienna, Austria) and ggplot2 (version 3.3.3, for visualization, R Core Team, Vienna, Austria) were used [24]. Using the Toil process, UCSC XENA

(<https://xenabrowser.net/datapages/>) RNAseq data was uniformly processed into TPM format for TCGA and GTEx [22,24–28]. Data for OC was extracted from TCGA, while GTEx was used for the corresponding normal tissues [24]. RNAseq data were converted into TPM (transcripts per million reads) format by log2 transformation for the comparison of gene expression between samples [24].

2.3 Correlation Heat Map

Correlations between all pairs of genes in the *NOTCH* pathway were assessed using Pearson's correlation coefficient [24]. R (version 3.6.3) and ggplot2 (version 3.3.3) were used for the analysis [24].

2.4 Association of *NOTCH* Pathway Gene Expression with the Clinical Features of TCGA-OC

R (version 3.6.3) and Basic R package were used for this analysis [24]. The grouping condition was the median.

2.5 Survival Analysis

R (version 3.6.3), survminer package (version 0.4.9, for visualization, R Core Team, Vienna, Austria), and survival package (version 3.2-10, R Core Team, Vienna, Austria) were used for the statistical analysis of survival data [24]. The two subgroups studied were 0–50 and 50–100 [24]. Overall survival (OS) and progression-free interval (PFI) [24] were used for prognostic evaluation. Prognostic information for the controls was provided as supplemental data [20].

2.6 Univariate and Multivariate Cox Regression Analysis

R (version 3.6.3) and survival package (version 3.2-10) were used for regression analysis [24]. Cox regression was used as the statistical method [29,30]. PFI was used for the type of prognosis. Included variables were FIGO stage, primary therapy outcome, race, age, histologic grade, anatomic neoplasm subdivision, venous invasion, lymphatic invasion, tumor residual, tumor status, and 11 *NOTCH* pathway genes [23]. Prognostic information from the reference was provided as supplemental data [31].

2.7 Forest Plot

R (version 3.6.3) and ggplot2 package (version 3.3.3) were used for Forest plots [24,30].

2.8 ROC Curve Analysis

R (version 3.6.3), pROC package (version 1.17.0.1, R Core Team, Vienna, Austria) and ggplot2 package [version 3.3.3] were used for analysis [24]. The clinical variables were tumor and normal [24].

2.9 Correlation Analysis for Genes Associated with *NOTCH* Pathway Genes

R (version 3.6.3) and stat base package (version 3.6.3) were used for this analysis [24].

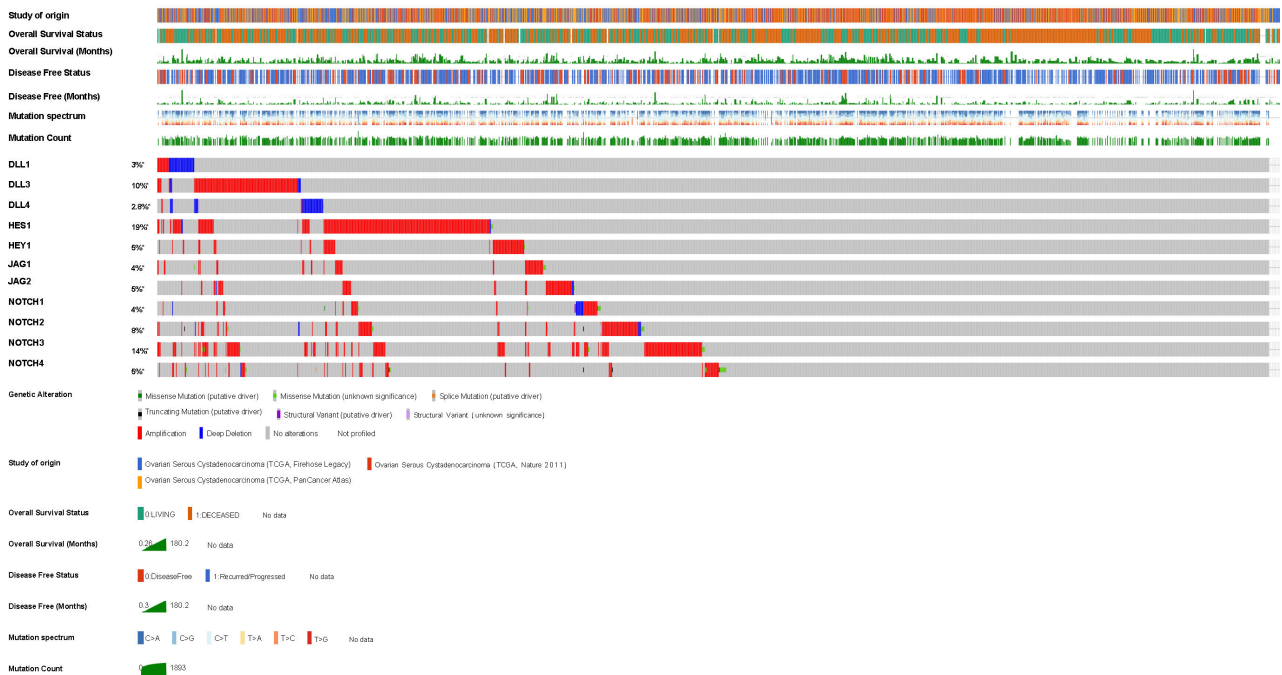


Fig. 1. Genetic alterations of *NOTCH* pathway genes in ovarian cancer (OC) as observed using cBioPortal (RNA Seq V2 RSEM).

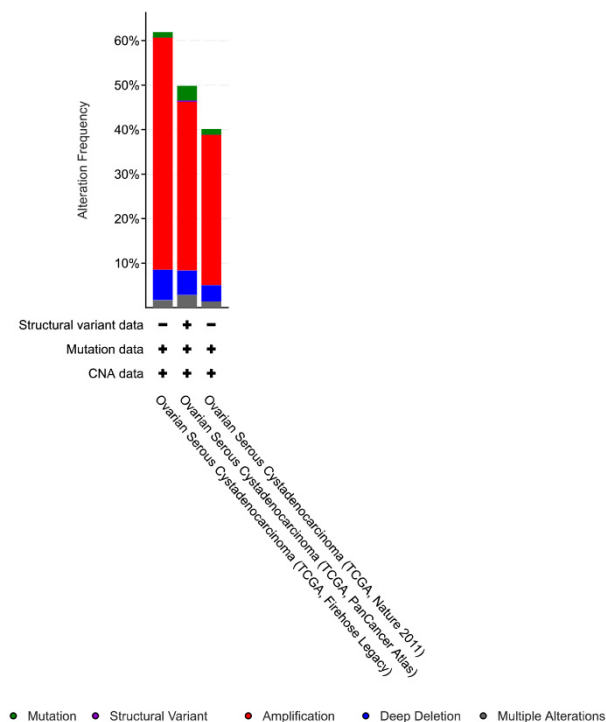


Fig. 2. The distribution of genetic alterations in *NOTCH* pathway genes in OC cases based on the cancer type summary in cBioPortal.

2.10 Functional Enrichment Analysis of Genes Associated with *NOTCH* Pathway Genes

R (version 3.6.3), ggplot2 package (version 3.3.3) and clusterProfiler package (version 3.14.3, R Core Team, Vienna, Austria) were used [24].

2.11 Correlations between *NOTCH* Pathway Gene Expression and Immune Cells

R (version 3.6.3) and GSVA package (version 1.34.0, R Core Team, Vienna, Austria) were used for this analysis [24,31]. The immune-infiltration algorithm used was ssGSEA (built-in algorithm in the GSVA package) [24]. The immune cell types studied here include aDC (activated DC), B cells, CD8 T cells, cytotoxic cells, DC, eosinophils, iDC (immature DC), macrophages, mast cells, neutrophils, NK CD56_bright cells, NK CD56_dim cells, NK cells, pDC (Plasmacytoid DC), T cells, T helper cells, Tcm (T central memory), Tem (T effector memory), Tfh (T follicular helper), Tgd (T gamma delta), Th1 cells, Th17 cells, Th2 cells, and Tregs [23,32]. Markers for the 24 immune cell types were obtained from a previous reference [33].

3. Results

3.1 Alterations in the mRNA Expression of *NOTCH* Pathway Genes in OC

The online tool cBioPortal was used to examine *NOTCH* pathway gene alterations in OC. As shown in Fig. 1, the frequency of genetic alterations in OC was: *DLL1*, 3%; *DLL3*, 10%; *DLL4*, 2.8%; *HES1*, 19%; *HEY1*, 5%; *JAG1*, 4%; *JAG2*, 5%; *NOTCH1*, 4%; *NOTCH2*, 8%, *NOTCH3*, 14%; and *NOTCH4*, 5%. As shown in Fig. 2, the frequency of genetic alterations in *NOTCH* pathway genes in OC patients from TCGA, Firehose Legacy were: mutation, 1.2%; amplification, 52.05%; deep deletion, 6.85%; and multiple alterations, 1.71%. For TCGA, Pan-Cancer Atlas: mutation, 3.25%; structural variant, 0.34%; amplification, 37.84%; deep deletion, 5.48%; and multiple alter-

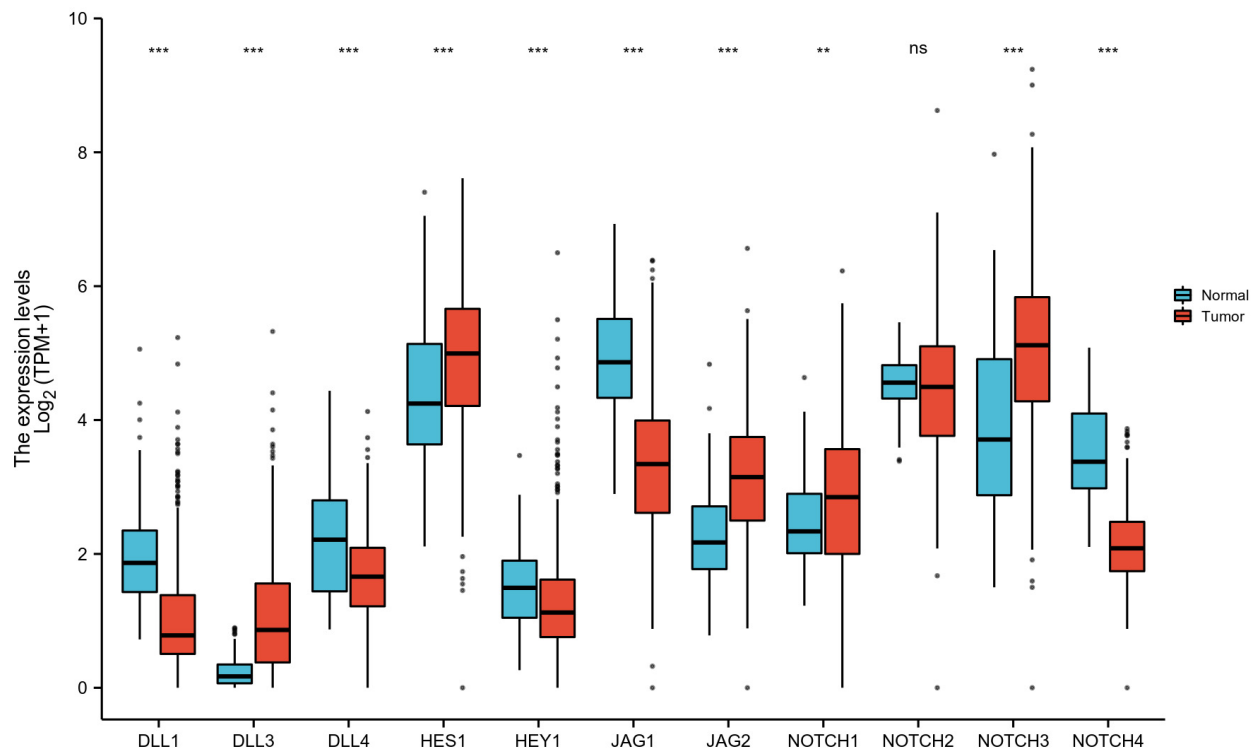


Fig. 3. TCGA mRNA levels for the *NOTCH* pathway genes in OC tissue and in unpaired normal ovarian tissue. ns, not significant ($p \geq 0.05$); **, $p < 0.01$; ***, $p < 0.001$.

ations 2.91%. For TCGA, Nature 2011: mutation, 1.23%; amplification, 33.74%; deep deletion, 3.68%; and multiple alterations, 1.43%. In general, amplifications accounted for most of the genetic alterations detected in OC.

A total of 427 OC tumors and 88 samples of normal ovarian tissue were used to assess *NOTCH* pathway gene expression (Fig. 3). The expression levels of *DLL1*, *DLL4*, *HEY1*, *JAG1* and *NOTCH4* were all significantly lower in OC samples than in normal ovarian tissues (1.064 ± 0.832 vs. 1.991 ± 0.827 ; 1.688 ± 0.631 vs. 2.206 ± 0.878 ; 1.33 ± 0.876 vs. 1.51 ± 0.632 ; 3.339 ± 1.027 vs. 4.916 ± 0.835 ; and 2.113 ± 0.568 vs. 3.514 ± 0.689 , respectively; all $p < 0.001$). In contrast, the expression levels of *DLL3*, *HES1*, *JAG2*, *NOTCH1* and *NOTCH3* were all significantly higher in OC tumor samples (1.075 ± 0.884 vs. 0.25 ± 0.246 ; 4.9 ± 1.119 vs. 4.383 ± 1.168 ; 3.121 ± 0.929 vs. 2.284 ± 0.731 ; 2.82 ± 1.063 vs. 2.487 ± 0.676 ; and 5.086 ± 1.253 vs. 3.933 ± 1.353 , respectively; all $p < 0.001$ except *NOTCH1*, $p = 0.002$). No significant difference was seen in the level of *NOTCH2* expression between OC tumor tissues and healthy ovarian tissues.

Pearson correlation analysis was used to test for relationships between genes involved in the *NOTCH* pathway. A significant negative correlation was found between the expression of *DLL3* and *NOTCH2*, while the expression levels of most other *NOTCH* pathway genes showed a significant positive correlation with each other (Fig. 4).

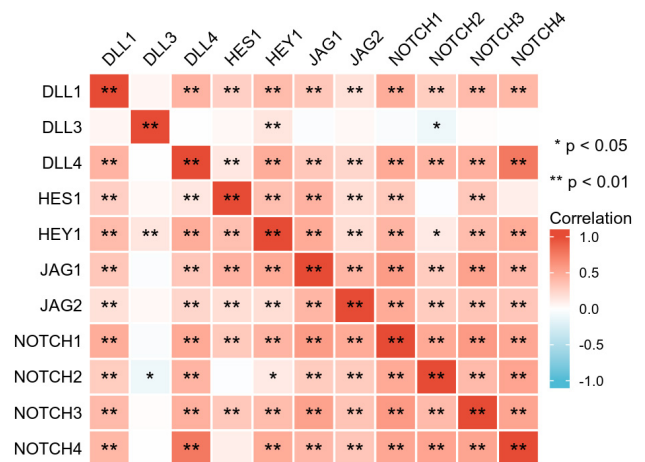


Fig. 4. Correlations between the expression level of genes in the *NOTCH* pathway.

3.2 Relationship between *NOTCH* Pathway Gene Expression and the Clinical Characteristics of TCGA OC Patients

The clinical characteristics and gene expression data for 379 OC tumor samples were downloaded from the TCGA database (Supplementary Table 1). *DLL4* and *NOTCH4* expression were associated with FIGO stage ($p < 0.001$ and $p = 0.036$, respectively), while *NOTCH3* expression was associated with race ($p = 0.039$) and age ($p = 0.044$). The expression levels of other *NOTCH* pathway

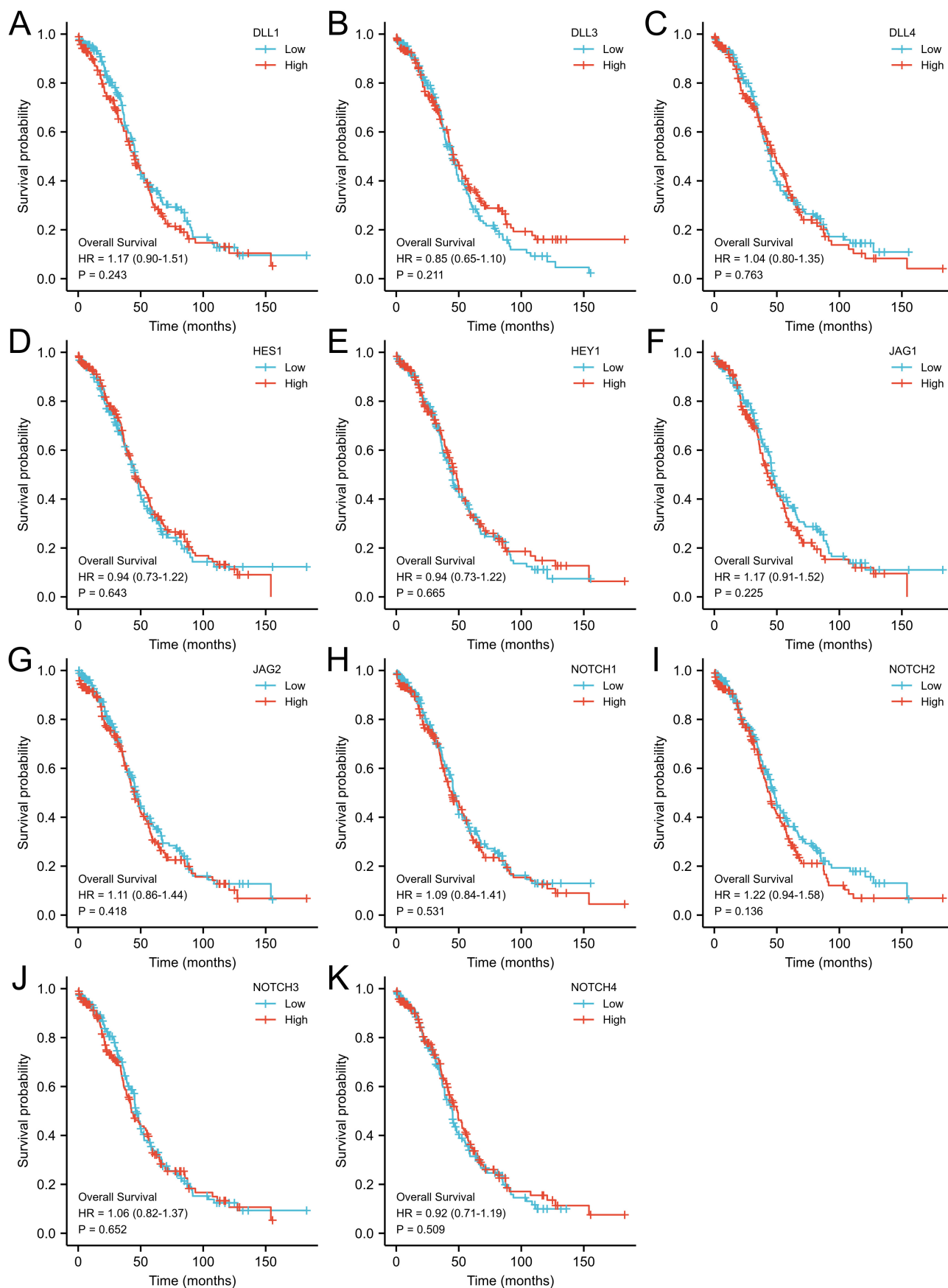


Fig. 5. Overall survival (OS) analysis for *NOTCH* pathway gene expression in OC patients using Kaplan Meir plot web tool. (A) *DLL1*, (B) *DLL3*, (C) *DLL4*, (D) *HES1*, (E) *HEY1*, (F) *JAG1*, (G) *JAG2*, (H) *NOTCH1*, (I) *NOTCH2*, (J) *NOTCH3*, and (K) *NOTCH4*.

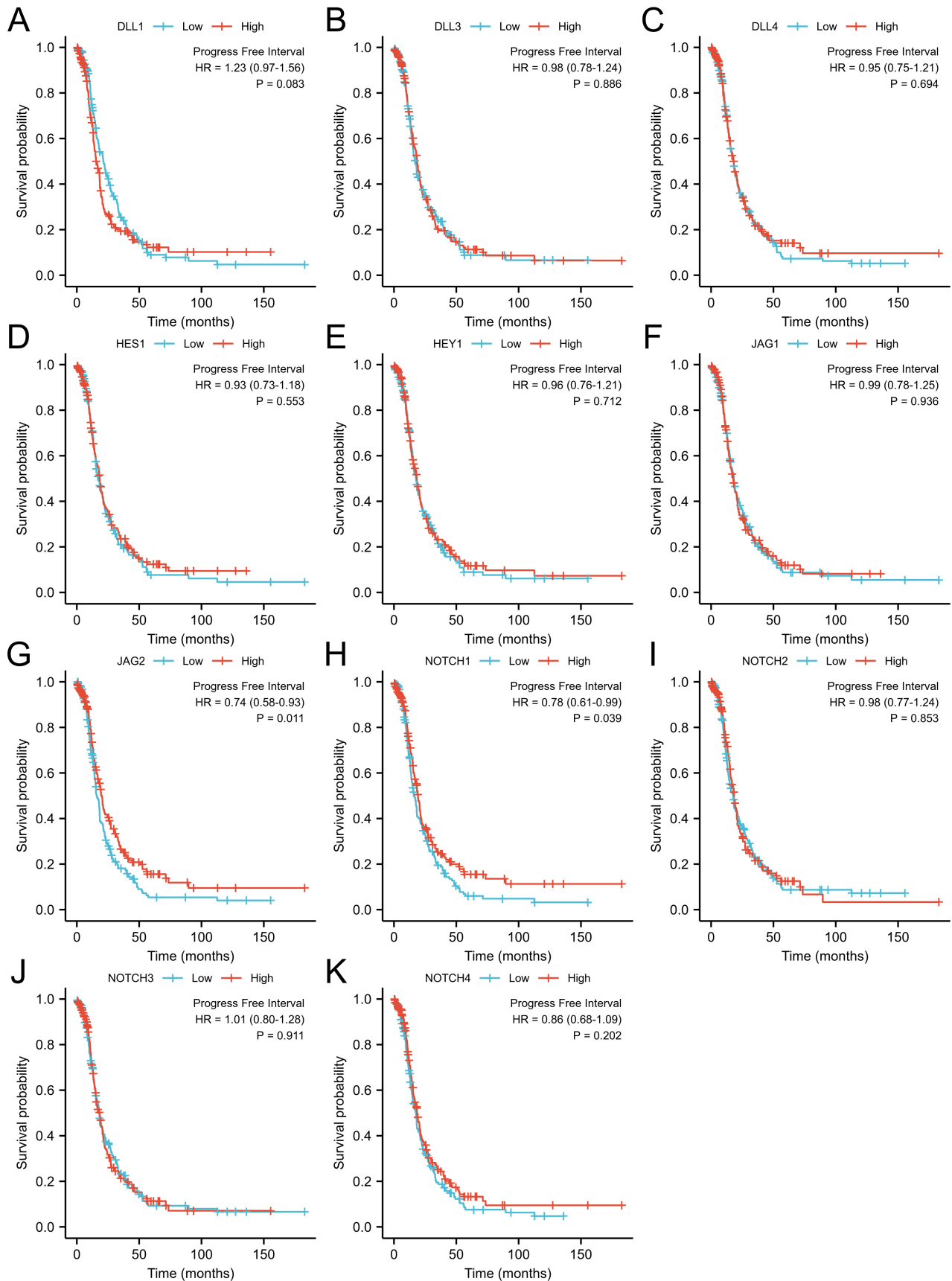


Fig. 6. Progression-free interval (PFI) analysis for *NOTCH* pathway gene expression in OC patients using Kaplan Meier plot web tool. (A) *DLL1*, (B) *DLL3*, (C) *DLL4*, (D) *HES1*, (E) *HEY1*, (F) *JAG1*, (G) *JAG2*, (H) *NOTCH1*, (I) *NOTCH2*, (J) *NOTCH3*, and (K) *NOTCH4*.

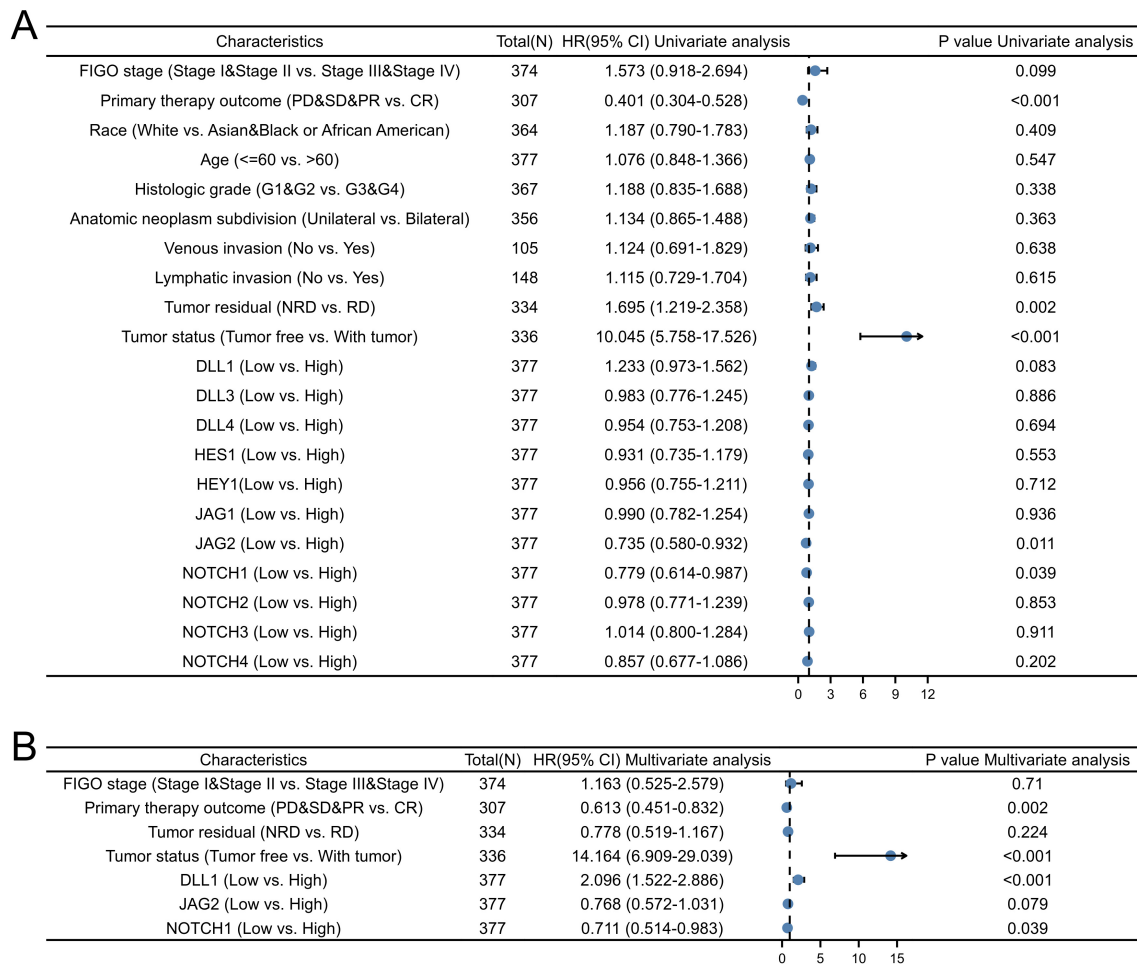


Fig. 7. Forest plots showing the results of Cox regression analysis for *NOTCH* pathway genes and other clinical features as predictors of progression free interval (PFI) in OC patients. (A) Univariate regression analysis. (B) Multivariate regression analysis.

genes (*DLL1*, *DLL3*, *HES1*, *HEY1*, *JAG1*, *JAG2*, *NOTCH1*, and *NOTCH2*) did not correlate significantly with the clinical data of OC patients.

3.3 Prognostic Value of *NOTCH* Pathway Genes in OC

The expression level of 11 *NOTCH* pathway genes (*DLL1*, *DLL3*, *DLL4*, *HES1*, *HEY1*, *JAG1*, *JAG2*, *NOTCH1*, *NOTCH2*, *NOTCH3*, and *NOTCH4*) showed no significant correlation with the overall survival of OC patients (Fig. 5). The expression of *JAG2* ($p = 0.011$) and *NOTCH1* ($p = 0.039$) were found to be significantly associated with the PFI of OC patients, whereas the remaining 9 genes (*DLL1*, *DLL3*, *DLL4*, *HES1*, *HEY1*, *JAG1*, *NOTCH2*, *NOTCH3* and *NOTCH4*) showed no significant correlation with the PFI (Fig. 6). Higher expression of *JAG2* and *NOTCH1* were correlated with significantly better PFI.

3.4 Univariate and Multivariate Cox Regression Analysis

Univariate Cox regression analysis (Fig. 7A) revealed that primary therapy outcome (HR: 0.401; 95% CI: 0.304–0.528, $p < 0.001$), *JAG2* expression (HR: 0.735; 95% CI: 0.580–0.932, $p = 0.011$), and *NOTCH1* expression (HR:

0.779; 95% CI: 0.614–0.987, $p = 0.039$) were predictors of good PFI in OC patients. In contrast, tumor residual (HR: 1.695; 95% CI: 1.219–2.358, $p = 0.002$) and tumor status (HR: 10.045; 95% CI: 5.758–17.526, $p < 0.001$) were predictors of worse PFI in OC patients. Multivariate analysis revealed that primary therapy outcome (HR: 0.613; 95% CI: 0.451–0.832, $p = 0.002$), tumor status (HR: 14.164; 95% CI: 6.909–29.039, $p < 0.001$), *DLL1* expression (HR: 2.096; 95% CI: 1.522–2.886, $p < 0.001$), and *NOTCH1* expression (HR: 0.711; 95% CI: 0.514–0.983, $p = 0.039$) were independent predictors of PFI.

3.5 Diagnostic Value of *NOTCH* Pathway Gene Expression in OC

As shown in Fig. 8, the expression of several *NOTCH* pathway genes showed some tendency for differentiating normal and OC tumor tissues (*DLL1*: AUC = 0.823, CI = 0.785–0.861; *DLL3*: AUC = 0.839, CI = 0.801–0.876; *JAG1*: AUC = 0.883, CI = 0.849–0.916; *JAG2*: AUC = 0.769, CI = 0.718–0.820; *NOTCH3*: AUC = 0.739, CI = 0.679–0.799). Other genes showed low tendency (*DLL4*: AUC = 0.668, CI = 0.599–0.737; *HES1*: AUC = 0.642, CI

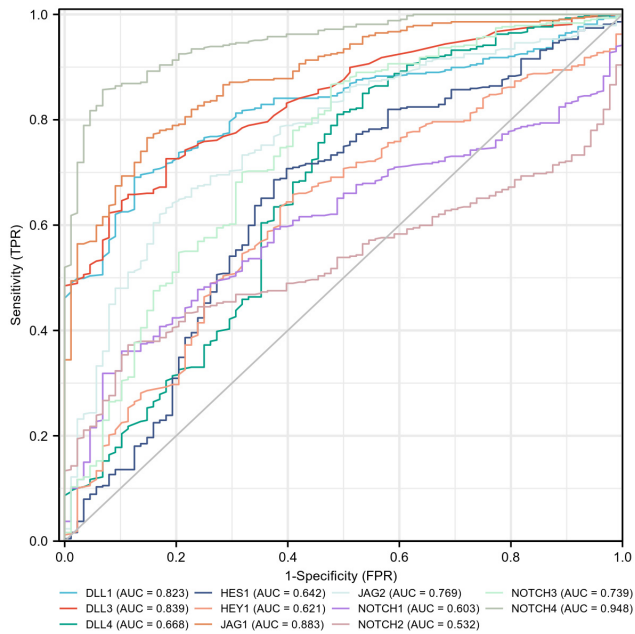


Fig. 8. ROC curves for *NOTCH* pathway genes in OC and normal ovarian tissues. The area under the curve (AUC) ranged from 0.5 to 1. When the AUC is closer to 1, diagnosis is more accurate for the differentiation of OC. An AUC between 0.5 and 0.7 has low accuracy, an AUC between 0.7 and 0.9 has some accuracy, while an AUC above 0.9 has high accuracy.

= 0.576–0.709; HEY1: AUC = 0.621, CI = 0.560–0.683; *NOTCH1*: AUC = 0.603, CI = 0.549–0.657; *NOTCH2*: AUC = 0.532, CI = 0.482–0.583), while high accuracy was shown by *NOTCH4* (AUC = 0.948, CI = 0.928–0.968).

3.6 Functions Associated with *NOTCH* Pathway Genes

The top five negatively associated genes with each *NOTCH* pathway gene are shown in Fig. 9, together with the top 5 positively associated genes. The genes most positively associated with *DLL1* were *ADAMTS7*, *DENND2A*, *ZNF697*, *RHOBTB3* and *DCHS1*, while those most negatively associated with this gene were *RTP4*, *EVA1C*, *EIF4EBP3*, *PDZK1IP1*, and *PLAAT4*. The genes most positively associated with *DLL3* were *SELENNOV*, *TIMM50*, *ECH1*, *RYR1* and *MRPS12*, while those most negatively associated were *VNN1*, *MVP*, *KCTD12*, *SERPINB1*, and *TMEM87A*. The genes most positively associated with *DLL4* were *NOTCH4*, *CDH5*, *VWF*, *PCDH12* and *ROBO4*, while those most negatively associated were *RPL39*, *HIGD2A*, *ATP5MD*, *RPL36A*, and *RPS27*. The genes most positively associated with *HES1* were *SMOX*, *DACT2*, *JAG1*, *ITM2C*, and *PDE9A*, while those most negatively associated were *HPSE*, *LTC4S*, *NPRI*, *CYP4B1*, and *EVA1C*. The genes most positively associated with *HEY1* were *PGF*, *PEAR1*, *TSPAN11*, *HSPA12B*, and *SIX2*, while those most negatively associated were *KLK7*, *PDZK1IP1*, *C19orf33*, *FOLR1*, and *MSLN*. The genes most positively associated with *JAG1* were *NOTCH1*, *BTBD3*, *NOTCH3*,

ATRN, and *CDS2*, while those most negatively associated were *COX5B*, *ATP5MC2*, *LAMTOR4*, *RPL32*, *NDUFA1*. The genes most positively associated with *JAG2* were *CEP170B*, *BRF1*, *AKT1*, *PACS2*, and *PLCB3*, while those most negatively associated were *OST4*, *COPS9*, *BLOC1S1*, *COX7C*, and *PFDN5*. The genes most positively associated with *NOTCH1* were *SEC16A*, *CAMSAP1*, *PLXNA1*, *RXRA* and *PRRC2B*, while those most negatively associated were *ATP5MF*, *UQCRCQ*, *NDUFA1*, *FAU* and *COX5B*. The genes most positively associated with *NOTCH2* were *CTDSP2*, *HIPK1*, *GIT2*, *ATXN1L* and *BAZ2A*, while those most negatively associated were *RNF181*, *ATP5MD*, *COX5B*, *ATP5F1E* and *RPS21*. The genes most positively associated with *NOTCH3* were *BRD4*, *WIZ*, *MYO9B*, *TET3* and *CEP250*, while those most negatively associated were *VAMP8*, *UQCRI1*, *PLAAT4*, *COX7B*, and *NDUFA1*. The genes most positively associated with *NOTCH4* were *CDH5*, *ROBO4*, *PCDH12*, *DIPK2B*, and *DLL4*, while those most negatively associated were *RPL12*, *RPL36A*, *RPL31*, *RPS21* and *RPS27*.

GO and KEGG enrichment analyses were conducted on the *NOTCH* pathway and related genes (102 genes in total) (Supplementary Table 2). As shown in Fig. 10 and Supplementary Table 3, the top five biological processes identified were the regulation of *NOTCH* signaling pathway, *NOTCH* signaling pathway, negative regulation of *NOTCH* signaling pathway, *NOTCH* signaling involved in heart development, and positive regulation of *NOTCH* signaling pathway. The top five cytological components were the mitochondrial inner membrane, polysomal ribosome, cytosolic ribosome, mitochondrial proton-transporting ATP synthase complex, and cytosolic part. The top five molecular functions were *NOTCH* binding, structural constituent of ribosome, electron transfer activity, cytochrome-c oxidase activity, and heme-copper terminal oxidase activity. As shown in Fig. 11 and Supplementary Table 3, the top five pathways identified were the *NOTCH* signaling pathway, breast cancer, endocrine resistance, Th1 and Th2 cell differentiation, and oxidative phosphorylation.

3.7 *NOTCH* Pathway Gene Expression in OC and Correlation with Immune Cells

As shown in Fig. 12, *NOTCH* pathway gene expression was correlated with tumor-infiltrating immune cells (TIIC) in OC. *DLL1* gene expression correlated positively with eosinophils, iDC, macrophages, mast cells, NK cells, Tcm, Tem, TFH and Tgd, and negatively with aDC, cytotoxic cells, T cells, Th1 cells, and Th17 cells. *DLL3* expression correlated positively with Th2 cells, and negatively with CD8 T cells, cytotoxic cells, DC, iDC, macrophages, neutrophils, NK CD56bright cells, NK CD56dim cells, pDC, T cells, Tcm, Tem, Th1 cells and Th17 cells. *DLL4* expression correlated positively with eosinophils, iDC, macrophages, mast cells, neutrophils, NK cells, pDC, T helper cells, Tcm, Tem, TFH and Tgd, and negatively with

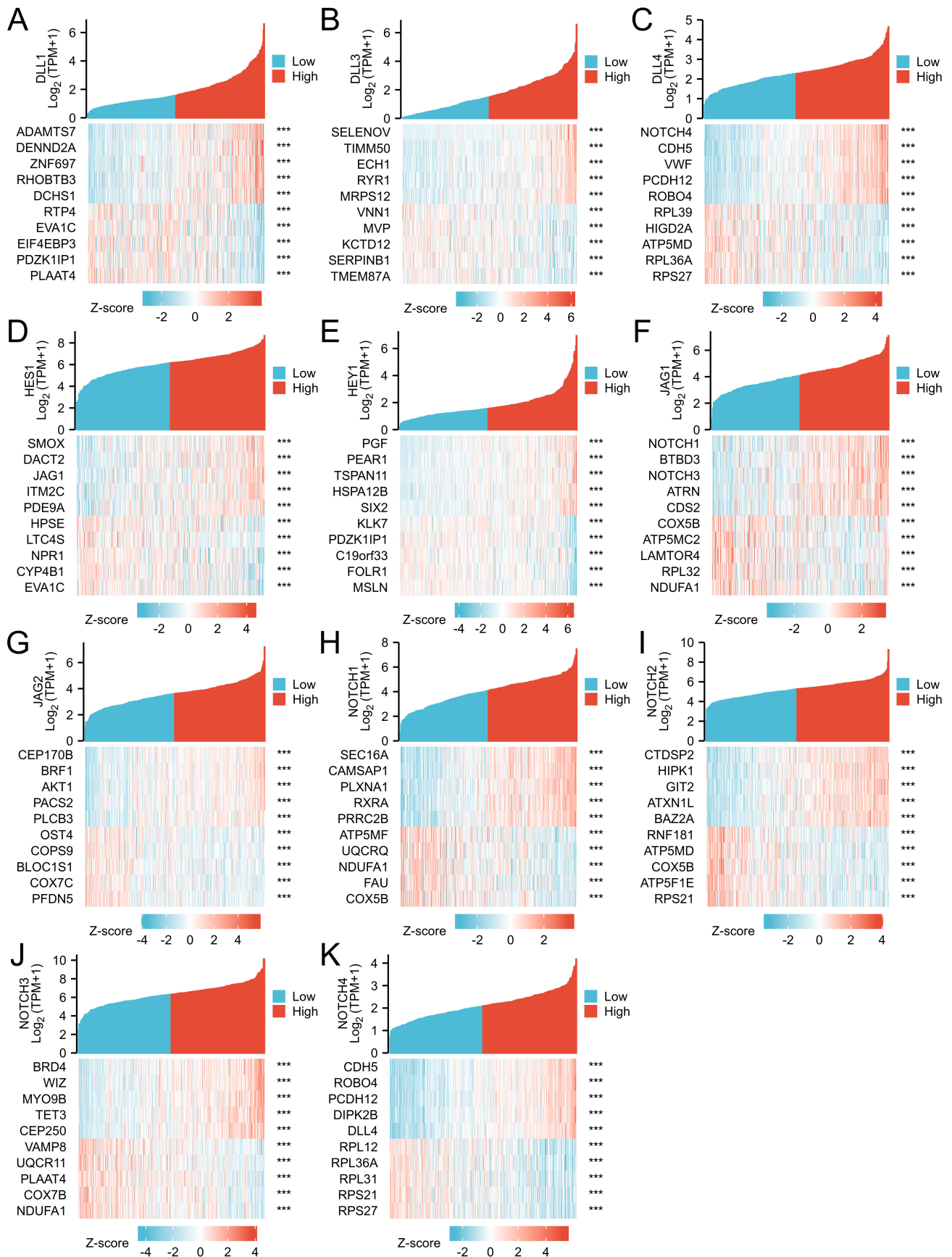


Fig. 9. Heatmap showing the top 10 genes that were correlated with *NOTCH* pathway genes. (A) *DLL1*, (B) *DLL3*, (C) *DLL4*, (D) *HES1*, (E) *HEY1*, (F) *JAG1*, (G) *JAG2*, (H) *NOTCH1*, (I) *NOTCH2*, (J) *NOTCH3*, and (K) *NOTCH4*.

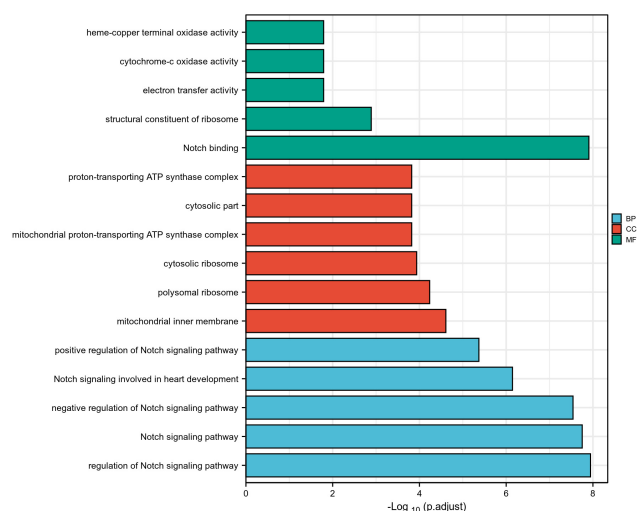


Fig. 10. GO analysis of genes involved in the *NOTCH* pathway.

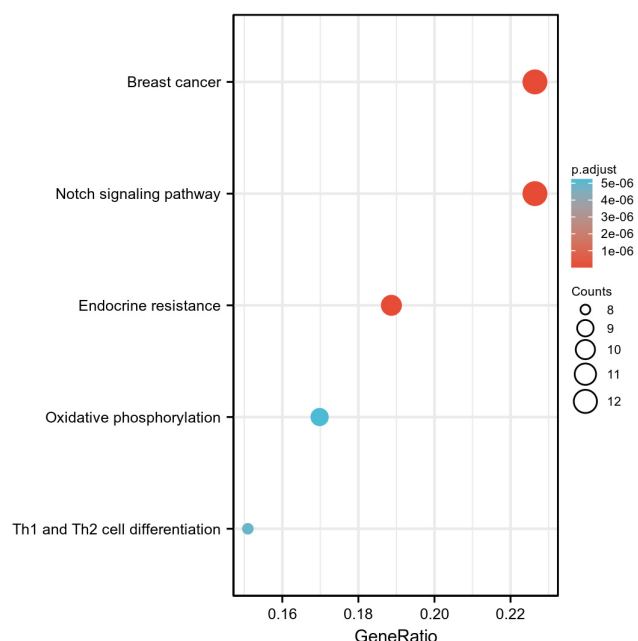


Fig. 11. KEGG analysis of genes associated with *NOTCH* pathway genes.

cytotoxic cells. *HES1* expression correlated positively with eosinophils, NK cells and Th2 cells, and negatively with aDC, B cells, cytotoxic cells, DC, neutrophils, T cells, Tem, Th1 cells, Th17 cells and Tregs. *HEY1* expression correlated positively with eosinophils, iDC, macrophages, mast cells, neutrophils, NK cells, T helper cells, Tem, TFH, Tgd and Th2 cells, and negatively with cytotoxic cells, NK CD56bright cells and Th17 cells. *JAG1* expression correlated positively with eosinophils, iDC, NK cells, T helper cells, Tem, Tem, TFH and Th2 cells, and negatively with cytotoxic cells. *JAG2* expression correlated positively with eosinophils, NK cells, T helper cells, Tem and Tem. *NOTCH1* expression correlated positively with DC, eosinophils, iDC, macrophages, mast cells, neu-

trophils, NK CD56dim cells, NK cells, T helper cells, Tem, Tem, TFH and Th2 cells, and negatively with cytotoxic cells. *NOTCH2* expression correlated positively with DC, eosinophils, iDC, macrophages, mast cells, neutrophils, NK cells, T helper cells, Tem, Tem, TFH, Tgd and Th17 cells. *NOTCH3* expression correlated positively with eosinophils, NK cells, Tem and Tem, and negatively with aDC, CD8 T cells, cytotoxic cells, pDC, T cells, Th1 cells, Th17 cells, and T regs. *NOTCH4* gene expression correlated positively with eosinophils, iDC, macrophages, mast cells, neutrophils, NK cells, pDC, T helper cells, Tem, Tem, TFH and Tgd.

4. Discussion

OC patients with *NOTCH3* alterations, including up-regulation, experience poor survival, while the targeting of *NOTCH3* inhibits cell growth and induces apoptosis [34]. Associations have been reported between the expression of OCT4, *NOTCH1* and *DLL4* with OC differentiation, lymph node metastasis, clinical stage, and prognosis [35]. High expression of *NOTCH2* and *NOTCH3* mRNA in OC patients has been significantly associated with PFS, while high expression of *NOTCH4* mRNA has been significantly associated with OS [12]. Five genes (*NOTCH2*, *NOTCH3*, *DLL1*, *DLL3*, and *DLL4*) have also been associated with poorer survival in OC [35]. In the present study, significant differential expression between OC samples and controls was observed for the *NOTCH* pathway genes *DLL1*, *DLL3*, *DLL4*, *HES1*, *HEY1*, *JAG1*, *NOTCH2*, *NOTCH3*, and *NOTCH4*. *DLL4* expression was associated with FIGO stage ($p < 0.001$), while high *NOTCH3* expression was associated with race ($p = 0.039$) and age ($p = 0.044$). Low expression of *NOTCH4* was also associated with FIGO stage ($p = 0.036$). There were significant differences in PFI between patients with low and high expression of *JAG1* ($p = 0.011$) and *NOTCH1* ($p = 0.039$). Multivariate analysis revealed that *DLL1* (HR: 2.096; 95% CI: 1.522–2.886, $p < 0.001$) and *NOTCH1* (HR: 0.711; 95% CI: 0.514–0.983, $p = 0.039$) expression were independent predictors of PFI. Moreover, the expression of *DLL1*, *DLL3*, *JAG1*, *JAG2*, *NOTCH3* and *NOTCH4* could significantly differentiate between cancerous and non-cancerous tissue samples.

Estrogen promotes the differentiation of ovarian multi-ciliated cells (MCC) by reducing *DLL1* expression via the estrogen receptor β [36]. Sequential combination of cisplatin/eugenol targets the *NOTCH-Hes1* pathway and eliminates drug-resistant cancer stem cells [37]. OC progression and resistance have been linked to *JAG1*, an oncogene, and hence targeting the *GATA1/JAG1/NOTCH* pathway might represent a new treatment strategy [38]. *JAG1* inhibits OC cell growth and may act by suppressing the *NOTCH1* signaling pathway [39]. *JAG1/NOTCH3* interactions constitute a juxtaposed secretory loop that promotes the proliferation and dissemination of OC cells in the peritoneal cavity [40]. Thus, BMP9-*NOTCH1* signaling could

also be a new therapeutic target axis for OC treatment [41]. MiR-34b inhibits proliferation and epithelial-mesenchymal transition in OC by targeting *NOTCH2* [42]. Activation of *NOTCH3*-mediated signaling by IL-8 secreted from cancer-associated fibroblasts and cancer cells promotes stem cell sexuality in OC [43]. In the present study, genes associated with the *NOTCH* pathway were mainly enriched in five signaling pathways. These were the *NOTCH* signaling pathway, breast cancer, endocrine resistance, Th1 and Th2 cell differentiation, and oxidative phosphorylation. In conclusion, these findings suggest the *NOTCH* pathway could regulate OC progression by modulating endocrine resistance, Th1 and Th2 cell differentiation, and oxidative phosphorylation. Therefore, targeting of the cross-talk network rather than a single pathway may be a more effective treatment strategy.

The tumor infiltration of immune cells and the immune evasion by cancer cells play key roles in tumor progression [9]. JAG2+ TANs are closely associated with the IL-8-driven immune evasion microenvironment, thus providing a potential therapeutic target for enhancing the immunity against OC [44]. Glycolytic targeting of microRNA and EZH2 in OC has been reported to lead to effector T cell dysfunction [45]. In the present study, expression of the *DLL1*, *HES1* and *NOTCH3* genes was negatively associated with T cell infiltration, whereas expression of the *DLL4*, *HEY1*, *JAG1*, *JAG2*, *NOTCH1*, *NOTCH2* and *NOTCH4* genes was positively associated with T cells. In contrast, *DLL3* expression was positively correlated with Th2 cells and negatively correlated with other types of T cells. The use of adoptive T cells, such as chimeric antigen receptor T cell therapy, may therefore be a promising new paradigm for the treatment of OC. A better understanding of *NOTCH* signaling could improve therapeutic approaches.

In summary, we evaluated the expression levels, mutations, and immune correlations of the *NOTCH* pathway in OC in order to identify potential biomarkers and targets. In addition to improving clinical decision making, the results contribute to a deeper understanding of the complex involvement of the *NOTCH* pathway in OC. Future studies should investigate the role of *NOTCH* signaling in OC *in vitro* and *in vivo*, as none were performed in the present study.

5. Conclusions

Activation of *NOTCH* plays an important role in mediating the development and progression of OC through multiple pathways, including the regulation of immune cells, endocrine resistance, Th1 and Th2 cell differentiation, and oxidative phosphorylation. Downregulation of *JAG2* and *NOTCH1* expression were associated with significantly worse PFI in OC patients. *JAG2* and *NOTCH1* may be potentially useful biomarkers for the treatment of OC, or as therapeutic targets.

Availability of Data and Materials

The datasets for this study can be found in TCGA-OC (<https://portal.gdc.cancer.gov/>). All data generated or analyzed during this study are included in this article.

Author Contributions

BC, LJ, and XYL designed the research study. KJ, HW, and LM analyzed the data. XML and QC collected the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2809220>.

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