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Prognostic significance of Notch 3 gene expression in ovarian serous carcinoma

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The Notch signaling pathway is an important cell signaling system, which regulates cell differentiation, proliferation, and apoptosis, and is aberrantly activated in a wide range of cancer, including ovarian cancers. However, it remains unclear as to whether Notch signaling plays a role in the progression and prognosis of ovarian cancer. We examined the mRNA and protein expression of Notch 3, Jagged 1, and Jagged 2 in 98 ovarian epithelial tumors via real-time PCR and in 175 tumors with immunohistochemical analysis, and then correlated their expression levels with clinicopathological parameters and patient survival. In this study, we detected high levels of Notch3 mRNA and protein expression especially in serous ovarian carcinomas compared to their benign counterparts, accompanied by a positive correlation with the expressions of Jagged 1 and Jagged 2. High levels of Notch 3 mRNA expression (>2-fold than that of benign tumor) were noted in 63% of the serous carcinomas (mean level: 17-fold, $P = 0.032$). Additionally, Notch 3 protein overexpression was significantly associated with advanced stage ($P = 0.0008$), lymph node ($P = 0.001$), and distant metastasis ($P = 0.003$). Notably, high Notch 3 mRNA and protein expressions were correlated with chemoresistance ($P = 0.033$) and poor overall survival ($P = 0.027$, $P = 0.042$) in these patients. Our results indicate that the Notch 3 signaling pathway is involved in the tumor progression of ovarian serous carcinoma, and higher Notch 3 expression may be an independent poor prognostic factor in this subset of tumors. (*Cancer Sci* 2010; 101: 1977–1983)

Ovarian cancer is the second most commonly diagnosed gynecologic malignancy, and is the fifth leading cause of cancer-related mortality in females.[1] However, the success rate of treatment has remained relatively unchanged, owing to its late diagnosis at advanced stages and its resistance to conventional chemotherapy.[2] Ovarian cancer is a complex and heterogeneous disease, and its underlying biomolecular mechanisms have, until now, been incompletely understood.

The Notch signaling pathway is a highly conserved cell signaling system present in most multicellular organisms, regulating cell differentiation, proliferation, apoptosis, and cell–cell communication. The Notch signaling pathway includes Notch receptors, ligands, negative and positive modifiers, and transcription factors. In vertebrates, four different Notch receptors, referred to as Notch 1–4, and two families of Notch ligands (delta-like 1, 3, 4 and Jagged 1, 2) have been identified. Notch receptors are large transmembrane proteins, consisting of an extracellular portion rich in epidermal growth factor (EGF)-like repeats, a transmembrane domain, and a large intracellular domain. During activation, the Notch receptor is initially proteolytically cleaved by furin-like convertases to form a heterodimer capable of binding to five different ligands. After binding with ligands, this complex is cleaved by metalloprotease, releasing the extracellular portion of Notch. Notch receptors are cleaved by gamma-secretase, thereby enabling the release of the active Notch intracellular domain (NICD), which is then translocated into the nucleus. After translocation to the nucleus, NICD interacts with transcriptional regulatory factors to form a complex, resulting in the activation of a group of basic-helix-loop-helix-orange (bHLH-O) proteins.[3] Faulty Notch signaling has been implicated in many developmental diseases and a variety of human cancers. With regard to cancer, Notch functions diversely as an oncogene or tumor suppressor, and also perhaps as a cancer stem cell factor. It has been reported to perform oncogenic functions in T-acute lymphocytic leukemia (ALL), Hodgkin's lymphoma, and breast cancer.[4,5] However, Notch also functions as a tumor suppressor in cervical and thyroid cancer, as well as neuroblastoma.[6–9] With regard to ovarian cancer, a few recent studies have identified Notch 3 as a candidate oncogene[10] since the first study of this kind, in which up-regulation of notch 3 in ovarian cancer was detected as compared to normal tissue using the microarray technique.[11] However, the implication of Notch pathway in the prognosis of ovarian cancers has yet to be elucidated. Therefore, we evaluated alterations in the expression of Notch 3 and its important ligands, Jagged 1 and 2, in ovarian epithelial tumors, and correlated their expression with clinicopathological parameters to elucidate the role of the Notch signaling pathway in ovarian carcinomas. We also attempted to determine whether the expression of Notch 3 and its ligands is associated with patients' outcomes.

Materials and Methods

Tissue samples. For RT-PCR, the fresh tissue samples of 98 ovarian epithelial tumors including 12 benign, 32 borderline, and 54 malignant epithelial tumors were obtained at the time of surgery from patients who had undergone oophorectomies for ovarian epithelial tumors at the CHA Bundang Medical Center. Samples were immediately frozen in liquid nitrogen and stored at −80°C. With frozen sections, the samples confirmed that the purity of tumor cells was nearly 80% of tissue.

For immunohistochemical analysis, we utilized formalin-fixed, paraffin-embedded ovarian epithelial tumor tissues from 175 patients who had been surgically treated at the CHA Bundang Medical Center from 1998 to 2006. The ovarian tumors included 75 malignant, 49 borderline, and 51 benign tumors. Clinical and pathological data were retrieved from clinical databases as well as from the pathology reports archives. The histologic type and staging of the tumors were classified according to the World Health Organization (WHO) classification[12] and International Federation of Gynecology and Obstetrics (FIGO) staging system.[13] Informed consent was obtained from each

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patient prior to surgery and this study was approved by the Ethical Committee of Bundang CHA Medical Center.

Clinicopathological characteristics. Ninety-eight women were enrolled for real-time RT-PCR, and their ages ranged from 15 to 80 years (mean, 46.8 years). The histologic types were as follows: 46 serous tumors (29 adenocarcinomas, 13 borderline tumors, four benign tumors), 39 mucinous tumors (12 adenocarcinomas, 19 borderline tumors, eight benign tumors), six endometrioid carcinomas, and seven clear cell carcinomas (Table 1). Histologic grading for ovarian carcinoma was classified into low grade (13 cases, 24%) and high grade (41 cases, 76%). The clinical stages of 54 ovarian carcinomas upon initial diagnosis were as follows: low stage (I, II) in 24 cases (44.4%) and high stage (III, IV) in 30 cases (55.6%). Lymph node involvement and distant metastasis were detected in 16 cases (29.6%) and 11 cases (20.4%). The mean follow-up interval was 32 months (range, 9–86 months).

A total of 175 were enrolled for immunohistochemical analysis, and their ages ranged from 19 to 86 years (mean, 47.9 years). The histologic types included 73 serous tumors (43 adenocarcinomas, 14 borderline tumors, 16 benign tumors), 63 mucinous tumors (nine adenocarcinomas, 35 borderline tumors, 19 benign tumors), 31 endometrioid tumors (15 carcinomas, 16 benign tumors), and eight clear cell carcinomas. The histologic grading for the ovarian carcinomas was classified into low grade (14 cases, 18.6%) and high grade (61 cases, 81.4%). The FIGO stage upon initial diagnosis of the 75 ovarian carcinomas was as follows: FIGO stage I and II in 27 cases (36%), stage III in 34 cases (45%), and stage IV in 14 cases (18%).

RNA extraction and real-time RT- PCR for Notch 3, Jagged 1, and Jagged 2. Total RNA of each sample was separately extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Approximately 1 μg of total RNA was converted to cDNA with a Superscript First-strand Synthesis System (Invitrogen).

Real-time PCR was conducted on a CFX96 real-time PCR system (Bio-Rad Laboratories, Hercules, CA, USA). The final volume of 20 μL included 0.5 μL of cDNA template, 10 μL of TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA), and 1 μL of a mix containing primers and probes. The amplification began with 2 min at 50°C and 10 min at 95°C, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. The CFX Manager software (version 1.0, Bio-Rad Laboratories, Hercules, CA, USA) determined the cycle threshold (CT) by default values. Individual PCRs were performed in triplicate and the gene expression levels of Notch3, Jagged-1, and Jagged-2 relative to GAPDH were calculated via the $2^{-\Delta\Delta CT}$ method.

Immunohistochemistry with tissue microarray. For immunohistochemical analysis, the hematoxylin–eosin (H&E) sections for the selected cases (175 ovarian epithelial tumors) were reviewed, and the representative areas were marked on the H&E-stained sections and the corresponding paraffin blocks. For each case, three tissue cores with diameter of 2 mm were punched out from the marked tissue areas of each donor tissue block. They were then arranged into recipient paraffin blocks using a manual microarray device (UNITMA, Quick-RAY; UNITech Science, Seoul, Korea). Tissue microarray paraffin sections were deparaffinized for 30 min in xylene and rehydrated in graded alcohols. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 30 min. For antigen retrieval, the sections were heated in 0.1 mol/L citrate buffer (pH 6.0) for 15 min in a microwave oven. The slides were incubated overnight at 4°C with the following primary antibodies at 1:500 dilution: anti-Notch3 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-Jagged 1 (Santa Cruz Biotechnology), and anti-Jagged 2 (Abcam, Cambridge, MA, USA). Thereafter, incubation with the secondary antibody was conducted using the Dako EnVision Rabbit/Mouse kit (Dako, Glostrup, Denmark) for 30 min at room temperature. The sections were developed with diaminobenzidine and counterstained with hematoxylin. Each tumor was assigned a score representing the percentage of positive nuclear or cytoplasmic staining: 0, <5%; 1, 5–25%; 2, 25–50%; 3, 50–100%.

Table 1. Samples for mRNA and immunohistochemical analysis

mRNA expression analysis			Immunohistochemical analysis		
Benign (n = 12)	Serous	4	Benign (n = 51)	Serous	16
	Mucinous	8		Mucinous	19
				Endometrioid	16
Borderline (n = 32)	Serous	13	Borderline (n = 49)	Serous	14
	Mucinous	19		Mucinous	35
Malignant (n = 54)	Serous	29	Malignant (n = 75)	Serous	43
	Mucinous	12		Mucinous	9
	Endometrioid	6		Endometrioid	15
	Clear	7		Clear	8
Total		98	Total		175

Statistical analysis. Statistical analyses were conducted with the SAS statistics software package (SAS Enterprise Guide 4.1; SAS, Cary, NC, USA). The comparison and correlation between expressions of Notch 3, Jagged 1, and Jagged 2 were analyzed using the t-test and Pearson's correlation analysis. The associations between clinicopathologic factors and immunohistochemical markers were evaluated using the chi-squared test or Fisher's exact test. All tests were two-sided, and $P < 0.05$ was considered significant. For survival analysis, the Kaplan–Meier method and multivariate Cox regression analysis were performed and the resultant curves were compared via log-rank and Wilcoxon tests.

Results

Expression profiles of Notch 3, Jagged 1, and Jagged 2 by real-time RT-PCR in ovarian epithelial tumors. We compared the expression of Notch 3 and Jagged 1,2 mRNAs between ovarian carcinomas and benign/borderline tumors using real-time RT PCR. With regard to the stability of GAPDH, an endogenous control gene used for normalization, NormFinder showed that it was very stable with a stability value of 0.4 and a mean of 25.9 ± 0.3 cycles. The mean Notch 3 expression was significantly increased (17-fold, $P = 0.032$) in the serous carcinomas as compared to benign serous tumors (Fig. 1a). High levels of Notch 3 expression (>2-fold than that of benign tumor) were noted in 63% of the serous carcinomas. As compared with the borderline tumors, serous ovarian carcinomas exhibited higher levels of Notch 3 expression, but not statistically significantly higher. We also detected higher mean expression levels of Jagged 1 and Jagged 2 in serous carcinomas compared to benign tumors by 3-fold and 10-fold, respectively; however, these differences also were not statistically significant ($P = 0.211$, $P = 0.113$, respectively). Similarly, the clear cell carcinomas exhibited high levels (>2-fold than benign serous tumor) of Notch 3 and Jagged 1 expression in five of seven cases (71%). It is worth noting that the expression of Notch3 and Jagged 1 in mucinous adenocarcinomas tended to decrease by 0.5-fold as compared to their benign counterparts (Fig. 1b); however, this was not statistically significant. When correlating the expression of Notch 3 mRNA with its ligands – Jagged 1 and Jagged 2 mRNAs – Notch 3 expression was correlated positively with Jagged 1 ($R = 0.917$, $P = 0.01$) and Jagged 2 ($R = 0.918$, $P = 0.01$) expression regardless of histological subtypes (Fig. 2).

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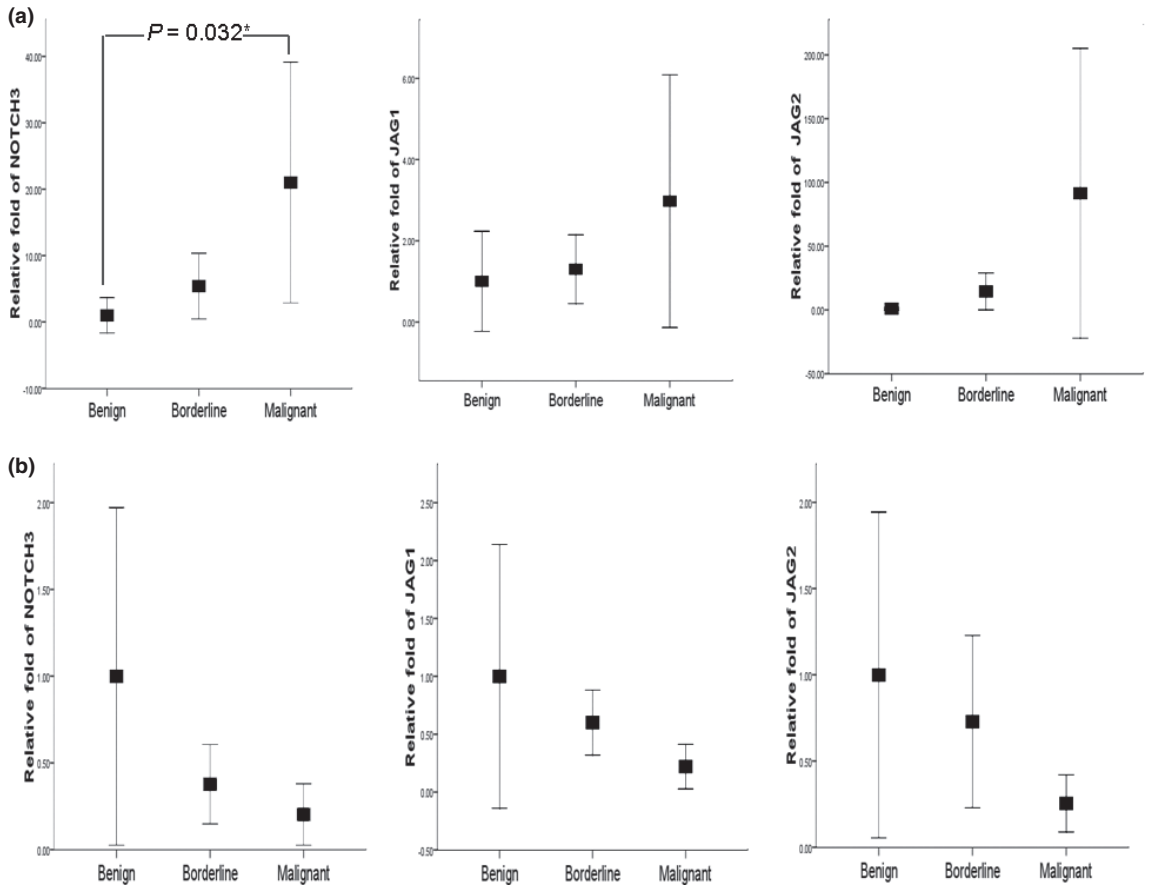

Fig. 1. Comparison of Notch 3, Jagged 1, and Jagged 2 mRNA expressions via quantitative real-time PCR. (a) Serous tumor. Notch 3 mRNA expression was significantly increased (17-fold, $P = 0.032$) in serous carcinomas as compared to benign serous tumors. (b) Mucinous tumor. The expression of Notch 3 and Jagged 1 in mucinous adenocarcinomas tended to decrease by 0.5-fold as compared to its benign counterparts.

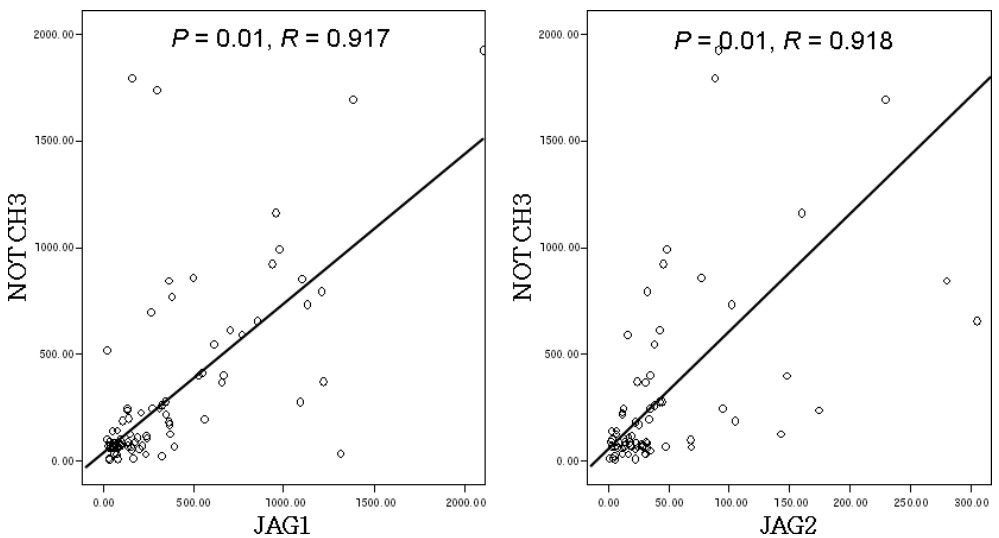

Fig. 2. Correlation of Notch 3, Jagged 1, and Jagged 2 mRNA expression. Notch 3 mRNA expression was correlated positively with Jagged 1 and Jagged 2 mRNA expression (Pearson's correlation, $P = 0.01$, 0.01).

Immunohistochemical expression profiles and the correlation with clinicopathological parameters. Notch 3, Jagged 1, and Jagged 2 were stained in the cytoplasm and/or nucleus, and the representative results of the immunohistochemical staining are shown in Figure 3 and Table 2. Noting that an immunohistochemical score of >1 (>5% positive cells) was considered indicative of overexpression, Notch3 and Jagged 2 proteins were significantly more overexpressed in the malignant serous tumors

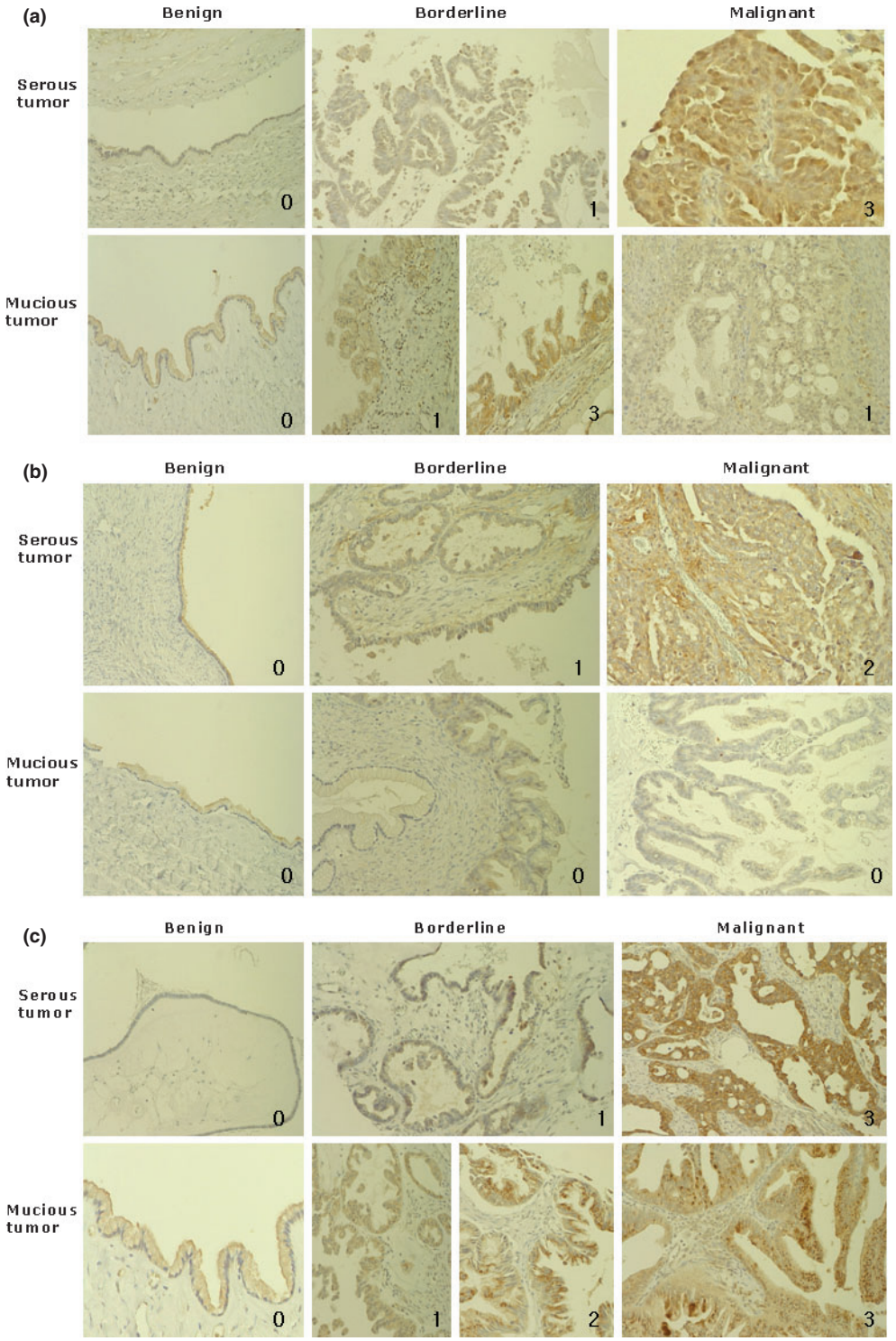

Fig. 3. Immunohistochemical stain of representative cases. The number of right lower corner is the immunohistochemical score. (a) The expression of Notch 3 was significantly higher in malignant serous tumors compared to benign serous tumors. The nuclear staining of carcinoma cells is noted. (b) The expression of Jagged 1 was not significantly different between benign, borderline, and malignant tumors in serous and mucinous tumors. (c) The expression of Jagged 2 was significantly higher in malignant tumors of serous and mucinous types compared to benign tumors.

than in the benign tumors ($P = 0.004$, 0.015). The nuclear expression of Notch 3, suggested as an activation marker of the Notch receptor gene by Choi *et al.*,[14] was found in 29 cases (67%) of serous carcinomas. The clear cell carcinomas also showed Notch 3, Jagged 1, and Jagged 2 overexpression in the majority of cases (88%, 62%, and 75%). However, the mucinous tumors showed no significant differences in Notch 3 expression between the benign and malignant tumors. As for Jagged 1, we noted no significant differences between benign, borderline, and malignant tumors in the serous and mucinous tumors. On the

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Table 2. Immunohistochemical expression profiles of Notch3, Jagged 1, and Jagged 2 according to tumor types

	Notch 3					Jagged 1					Jagged 2				
	0	1	2	3	*P*-value	0	1	2	3	*P*	0	1	2	3	*P*-value
Serous															
Benign	14 (78%)	2 (11%)	2 (11%)	0	0.004*	2 (11%)	16 (89%)	0	0	0.30	8 (44%)	7 (39%)	3 (17%)	0	0.015*
Borderline	3 (21%)	9 (64%)	1 (7%)	1 (7%)		3 (22%)	5 (36%)	6 (43%)	0		2 (14%)	4 (29%)	5 (36%)	3 (21%)	
Malignant	1 (2%)	10 (23%)	14 (33%)	18 (42%)		13 (30%)	16 (37%)	8 (19%)	3 (7%)		3 (7%)	8 (19%)	15 (35%)	17 (39%)	
Mucinous															
Benign	15 (79%)	2 (11%)	2 (11%)	0	0.12	17 (90%)	2 (10%)	0	0	0.14	18 (95%)	1 (5%)	0	0	<0.0001*
Borderline	18 (51%)	8 (23%)	7 (20%)	2 (6%)		35 (100%)	0	0	0		15 (42%)	10 (29%)	10 (29%)	0	
Malignant	5 (56%)	1 (11%)	1 (11%)	2 (22%)		9 (100%)	0	0	0		0 (0%)	0	4 (44%)	5 (56%)	
Endometrioid															
Benign	16 (100%)	0	0	0	0.12	14 (88%)	2 (12%)	0	0	0.004*	10 (63%)	4 (25%)	2 (12%)	0	0.033*
Malignant	12 (80%)	1 (7%)	2 (13%)	0		3 (20%)	9 (60%)	2 (13%)	1 (7%)		2 (13%)	4 (27%)	6 (40%)	3 (20%)	
Clear cell															
Malignant	1 (12%)	0	5 (63%)	2 (25%)		3 (38%)	1 (12%)	3 (38%)	1 (12%)		2 (25%)	1 (12%)	2 (25%)	3 (38%)	

*Fisher's exact test, $P < 0.05$.

other hand, Jagged 1 (13:87%, $P = 0.004$) and Jagged 2 (37:87%, $P = 0.033$) were found to be significantly overexpressed in endometrioid malignant tumors as compared to benign endometriotic cysts.

The correlations between the immunohistochemical expression of these proteins and clinicopathological prognostic parameters are shown in Figure 4. The overexpression of Notch 3 was significantly associated with advanced FIGO stage (III/IV) ($P = 0.0008$), lymph node metastasis ($P = 0.001$), and distant metastasis ($P = 0.003$). Additionally, the overexpression of Jagged 1 and Jagged 2 proteins was related significantly to lymph nodes ($P = 0.008$ and 0.05) and distant metastasis ($P = 0.04$ and 0.01).

Survival analysis. Follow-up was available for all 25 patients with serous carcinomas and the mean follow-up of the study population was 32 months (range, 9–86 months). Nine patients (36%) evidenced tumor recurrence or persistence after surgery, representing chemoresistance to the first-line chemotherapeutic regimen consisting of paclitaxel and cisplatin. Five patients (20%) died of disease during the follow-up period. The patients were divided into two groups, the higher-expressing group ($n = 12$) and the lower-expressing group ($n = 13$), by the cut-off value of 2-fold Notch 3 expression relative to that in the benign serous tumors. High Notch 3 expression (>2 fold) was associated more strongly with chemoresistant serous carcinomas than the low expression group (58.3% *vs* 15.4%, χ^2- test, $P = 0.033$), suggesting that Notch 3 might prove valuable as a predictive marker for chemoresistance. We also noted a statistically significant difference in overall survival between the higher-expression and lower-expression groups (overall survival 31.43% *vs* 100%, $P = 0.027$, Fig. 5).

With regard to the protein expression, follow-up was available for all 75 carcinoma patients (mean, 30 months; range, 1–95 months). Fifty-three patients remained alive without disease, 20 patients died of ovarian carcinoma, and two died of unrelated causes. On multivariate Cox regression analysis, higher (>score 3) Notch 3 expression (hazard ratio [HR] = 9.3, $P = 0.013$) as well as advanced FIGO stage (HR = 53.1, $P = 0.010$) was identified as independent poor prognostic factors (Table 3).

Discussion

The Notch signaling pathway is known to be involved not only in the normal development of many tissues but also in a variety of diseases, including malignant neoplasms. With respect to the tumorigenesis of various malignant neoplasms, the Notch signaling may function as an oncogene or tumor suppressor.[15] In ovarian cancer, the Notch receptor and its Jagged ligand were considered as candidate oncogenes in the first microarray studies on this subject.[11,16] Since that time, the *Notch 3* gene was determined to function as an oncogene via gene amplification in serous epithelial ovarian cancer by single nucleotide polymorphism array, digital karyotyping, and RT-PCR assays conducted on 31 high grade ovarian serous carcinomas. Another previous report with 32 ovarian tumor samples[17] showed detectable levels of Notch 3 mRNA expression in 94% of ovarian carcinomas, whereas Notch 1 mRNA was reduced in ovarian carcinomas. By way of contrast with previous studies, our study included all types of epithelial ovarian carcinomas, but principally serous and mucinous ovarian cancers, which account for 90% of all epithelial ovarian cancers. In addition, we analyzed the correlation of the expression of Notch 3 and its ligands – Jagged 1 and 2 – in ovarian cancer with the relevant clinicopathological prognostic factors and patients' survival.

In the present study, we found that the expression of Notch 3 mRNA and protein in serous and clear cell adenocarcinomas were significantly higher than in their benign counterparts, via

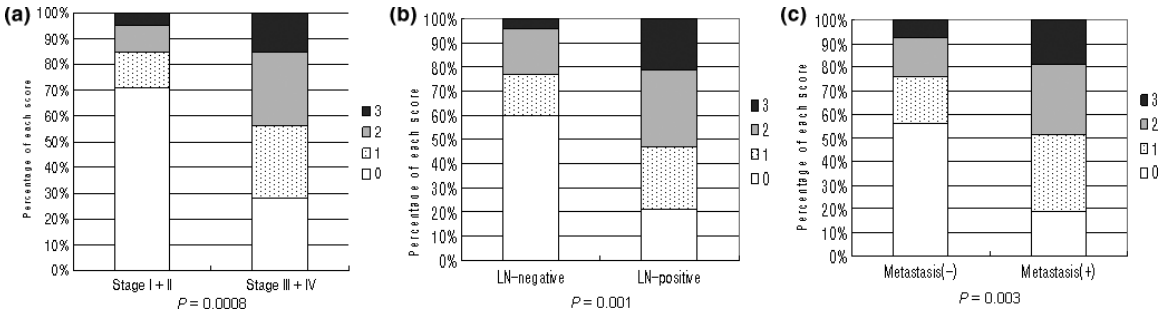

Fig. 4. Correlation of Notch 3 immunoreactive scores with clinicopathological parameters. Notch 3 overexpression was associated significantly with advanced International Federation of Gynecology and Obstetrics (FIGO) stage (a, $P = 0.0008$), lymph node metastasis (b, $P = 0.001$), and distant metastasis (c, $P = 0.003$) in serous carcinoma.

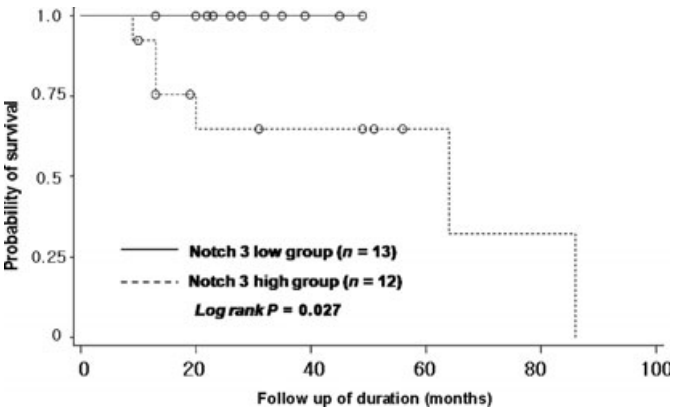

Fig. 5. Kaplan–Meier overall survival curve. The higher expression group of Notch 3 mRNA (>2-fold than benign tumor) demonstrated a significantly worse overall survival ($P = 0.027$) in ovarian serous carcinomas.

Table 3. Multivariate Cox regression analysis for overall survival according to the clinicopathological parameters in ovarian cancer

Factors	Overall survival	
	Hazard ratio	*P*-value
Notch 3 immunoreactivity, Score ≥3 *vs* <3	9.356	0.013*
Jagged 1 immunoreactivity, negative *vs* positive	2.628	0.210
Jagged 2 immunoreactivity, negative *vs* positive	1.456	0.735
Grade, high *vs* Grade, low	3.198	0.363
Stage III+IV *vs* Stage I+II	53.147	0.010*
Lymph node metastasis, present *vs* absent	0.433	0.433
Distant metastasis, present *vs* absent	0.091	0.091

*Statistically significant ($P < 0.05$).

real-time RT-PCR. The immunohistochemical finding was in accordance with the results of real-time RT-PCR. Contrary to the results noted with serous carcinoma, the Notch 3 and Jagged 1 expressions in mucinous carcinomas exhibited no significant alterations. These results indicate that the Notch 3 pathway is involved in the development of serous and clear cell type ovarian carcinomas whereas it is not associated with the development of mucinous type ovarian carcinomas. Our findings also imply that the ovarian serous and mucinous types are molecular-pathogenetically different, which was recently suggested by other researchers.[18]

Two possible mechanisms responsible for the oncogenic functions of Notch signaling have been suggested: namely, ligand-dependent and ligand-independent pathways. In the ligand-dependent pathway, Notch signaling functions as an oncogene after ligand–Notch receptor binding followed by the activation of its pathway. Lymphoproliferative disorders, such as chronic lymphocytic leukemia (CLL) and Hodgkin's lymphoma are examples of this mechanism.[19,20] In the ligand-independent pathway, a variety of translocations and proviral insertions in the Notch receptor gene result in mutations of this gene, which function as oncogenes. This mechanism contributes to the oncogenesis of T-ALL and mouse mammary carcinomas.[4,5] Park *et al.*[21] carried out a functional study with the r-secretase inhibitor, which prevented the activation of Notch3 by inhibiting the proteolysis and translocation of the Notch 3 cytoplasmic domain to the nucleus in an ovarian cancer cell line. Further study using ovarian serous carcinoma cells showed that the interaction between the Notch 3 receptor and the ligand Jagged 1 might perform an important functional role in the development of ovarian cancer.[21] They also suggested that Jagged 1 is the primary Notch 3 ligand in ovarian carcinoma and serves to stimulate adjacent tumor cells in a juxtacrine manner through Notch 3 receptor. However, with regard to the results of another previous study, in which a significant overexpression of Jagged 2 in ovarian carcinoma was demonstrated,[15] and our results showing Jagged 2 overexpression and a positive correlation between Notch 3 and Jagged 2 mRNAs as well as Jagged 1, there is a possibility that both Jagged 1 and Jagged 2 are dominant ligands of Notch 3. Taken together with our results showing the overexpression of the Notch 3 mRNA and protein and nuclear expression of Notch 3 protein in more than 60% of serous carcinomas, the Notch 3 pathway is likely to play a key role in the oncogenesis of ovarian serous carcinomas. Considering that the Jagged 1 ligand was expressed at a high level in mesothelial cells,[21] which are the main cells in direct contact with ovarian cancer cells during the peritoneal seeding of the ovarian carcinomas, it may be surmised that the Notch 3–Jagged ligand interaction is involved in the progression and metastasis of serous carcinomas. Indeed, the results of this study showed that higher levels of expression of Notch 3 and its ligands, Jagged 1 and Jagged 2, were related significantly with advanced clinical stage, lymph nodes, and distant metastasis, thereby supporting our hypothesis.

Furthermore, our study also demonstrated that high levels of Notch 3 expression were associated with chemoresistance and poor patient survival. Notably, high Notch 3 expression in ovarian serous carcinoma was identified as an independent poor prognostic marker on multivariate Cox regression analysis. The poor survival rate of patients with high Notch 3 expression may be caused by chemoresistance to conventional first-line chemotherapy after primary operation, considering

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that seven (58.3%) of 12 patients exhibiting higher levels of Notch 3 expression (>2-fold) were resistant to first-line chemotherapy as compared to 15.4% who demonstrated lower levels of Notch 3 expression (<2-fold). To the best of our knowledge, our study is the first report to imply an association between higher Notch 3 expression and poor ovarian cancer prognosis, although such a correlation has been reported previously in other malignancies.[22,23]

According to our findings, namely the high expression of Notch 3 in ovarian serous carcinomas and its apparent association with poor prognosis, the Notch 3 pathway appears to be a promising prognostic marker and therapeutic target for improving patients' outcomes in this subset of ovarian carcinomas.

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Disclosure Statement

The authors have no conflict of interest.

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