Int J Clin Exp Pathol 2019;12(9):3535-3541
www.ijcep.com /ISSN:1936-2625/IJCEP0098015

Original Article

The clinicopathologic significance of Notch3 expression in prostate cancer

Ae Ri Kim[1], Mi Jin Gu[2]

[1]*Department of Pathology, Daegu Fatima Hospital, Daegu, South Korea;* [2]*Department of Pathology, Yeungnam University College of Medicine, Daegu, South Korea*

Received June 5, 2019; Accepted July 22, 2019; Epub September 1, 2019; Published September 15, 2019

Abstract: The Notch3 signaling pathway plays an important role in oncogenesis, tumor maintenance, and resistance to chemotherapy in human cancers. However, its role in prostate cancer (PC) is less clear. In this study, we investigated a total of 142 PC patients who underwent radical prostatectomy and examined the expression of Notch3 in PC cells using immunohistochemistry on tissue microarrays and evaluated their clinicopathological significance. The overexpression of Notch3 was observed in 22 (15.5%) out of 142 PC cases. The overexpression of Notch3 was significantly associated with lymph node metastasis (P = 0.013), higher pT stages (P = 0.033), higher pathological tumor stages (P = 0.034), and higher grades groups (P = 0.025). However, the overexpression of Notch3 was not correlated with lympho-vascular invasion, neural invasion, extra-prostatic extension, or the serum prostate-specific antigen level. This study demonstrates that Notch3 plays an oncogenic function in PC and the overexpression of Notch3 is correlated with invasiveness, metastasis, and higher Gleason grades, reflecting the features of aggressive tumors in PC, and could be an important biomarker and a possible therapeutic target. Further studies evaluating the association between Notch3 expression and survival are required.

Keywords: Biomarker, Notch3, prognosis, prostate cancer

Introduction

Prostate cancer (PC) is the most common malignancy diagnosed in men and the second leading cause of cancer-related mortality in the US [1]. Most PCs are generally slow-growing, localized in the prostate gland, and many patients are managed by active surveillance rather than aggressive treatments [2-4]. Patients with PC are currently stratified as having a low-, intermediate-, or high-risk form of the disease based on the risk of post-treatment relapse, and these risk categories use clinicopathological factors, including the prostate-specific antigen (PSA) level, clinical T stage, and biopsy Gleason score (GS) [5, 6]. Patients with low-risk PC are generally advised to undergo active surveillance given the indolent clinical course the disease and the significant side effects of definitive therapy. Patients with intermediate- and particularly high-risk PC are at a significant risk for developing metastases and typically undergo radical prostatectomy (RT) and radiation therapy [7-9]. However, even within the different risk categories, the clinical outcomes after treatment are diverse. Furthermore, although treatment modalities for PC have improved, more than 30% of patients may eventually have their PC progress and metastasize/relapse and result in fatal outcomes [10]. Although the genomic and biomarkers for PC have been evaluated, there remains a lack of reliable, validated markers for use in the clinical setting [11, 12].

The Notch pathway is comprised of 4 transmembrane receptors (Notch1 to 4) and 5 cell-bound ligands (Jagged 1 and 2, Delta-like 1, 3, and 4) [13-15]. The ligands activate Notch receptors and initiate the Notch signaling pathway. This Notch signaling pathway plays a crucial role in cell differentiation, self-renewal, proliferation, apoptosis, and death, so they have been considered key targets for the diagnosis and treatment of various diseases [16]. In the prostate, Notch plays an important role in nor-

mal development, and its deregulation is a feature of tumorigenesis. Although most studies revealed that the Notch signaling pathway is upregulated in PC compared to benign prostatic tissue, it remains unclear whether the function of Notch signaling in PC is to stimulate or inhibit PC progression [16]. Drabick and Schell suggested that moderate activation of the Notch signaling pathway could support tumor growth, but high or mild activation of it might inhibit growth [17]. A meta-analysis using gene profiling data indicated that the Notch signaling pathway shows distinctive features of aggressive PC with high GS [18-20]. Jagged 1 and 2 are highly expressed in metastatic PCs [19, 21]. The expression of Notch1 in PC is controversial. Notch1 expression increased in PC with higher GS [22], but the other study revealed a decreased expression in PC compared to benign prostatic tissue [23]. Using gene expression analysis, Ross reported that Notch3 is highly expressed in PC and has a metastatic potential. However, the study was conducted in PC with only GS 6 (3 + 3) or 8 (4 + 4) [19]. The aim of this study is to evaluate Notch3 expression and its clinicopathological and prognostic significance in PC using immunohistochemistry.

Materials and methods

Study samples

The study cohorts consisted of 142 PC patients who underwent RT from January 2005 to October 2017. Medical records were reviewed to determine their most recent follow-up visit, survival status, and pre- and post-operative PSA levels. Resected prostate specimens were fixed for 1-2 days in 10% neutral-buffered formalin, and subsequently, after removal of the apical and bladder neck margins, were sectioned transversely at 3- to 4-mm intervals from the apex to the base. Whole sections were stained with hematoxylin and eosin (H&E) and then evaluated with respect to tumor location, tumor size, lympho-vascular invasion, perineural invasion, lymph node metastasis, surgical margin status, pathological tumor stage, grade, and prognostic grouping, as previously reported [24]. The Gleason scoring system was applied based on documentation from the International Society of Urological Pathology published in 2014 and on the new patient-centric grading system [25].

Overall survival (OS) was calculated from the date of RT to the date of death or the last follow-up. Disease progression was defined as a persistent or rising PSA level, greater than 0.4 ng/mL, on at least 2 occasions, a biopsy-proven local recurrence, or evidence of distant metastasis by a bone scan or other tests. The follow-up period ended on July 31, 2018. The study was approved by the Human Ethics Review Board of Yeungnam University Hospital (2018-07-027).

Immunohistochemistry with tissue microarray block

Tissue microarray blocks (TMAs) were constructed as two, 2-mm tissue cores from the area containing the most dominant Gleason grade pattern, one core from the area with the secondary Gleason grade pattern, and one core from an adjacent benign glandular area, as previously described [24].

Staining was performed on the TMA sections (4 μm) from formalin-fixed and paraffin-embedded tumor tissue. The sections were deparaffinized, and antigen was retrieved with an autoclave for 10 min at 121°C. Endogenous peroxidase activity was blocked. A rabbit polyclonal anti-Notch3 antibody (1:80, clone sc-515825, Santa Cruz, Biotechnology, CA, USA) was incubated with the sections for 1 h at room temperature. Detection was performed using an Envision + System peroxidase kit (DAKO, Carpinteria, CA, USA) from 30 min.

Immunohistochemical analysis

The sections were assessed by investigators who were blinded to the patients' clinicopathological information. Immunostaining intensity was scored as negative, faint or weak, moderate to strong; granular cytoplasmic and nuclear staining in 50% or more of the PC cells. There was no immunostaining on the basal and luminal cells of the normal prostatic glands. Faint staining was observed in the stromal cells. The expression of Notch3 was determined to be one of the following: low expression, negative and faint; overexpression, moderate to strong expression.

Statistical analyses

The associations between the Notch3 overexpression and the clinicopathologic factors were

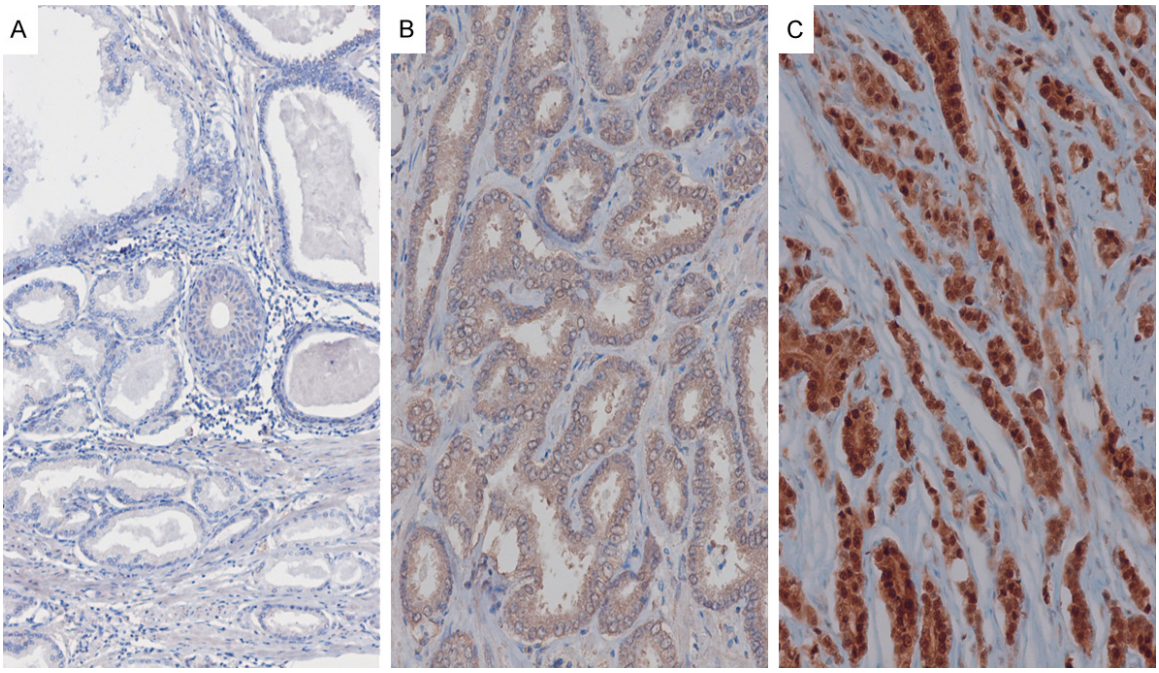

Figure 1. Examples of the expression patterns of Notch3 in cases of prostate cancer (A) No expression; (B) Weak expression; (C) Overexpression (immunohistochemical stain, ×200).

performed using a chi-square test or Fisher's exact test. OS was defined as described above. Disease-free survival (DFS) was defined as the postoperative interval without a known recurrence or metastasis. Survival curves were calculated using the Kaplan-Meier method. On the univariate and multivariate analyses, the Cox proportional hazard regression model was used to evaluate the potentially related prognostic factors for survival (IBM SPSS version 23; IBM Co., Armonk, NY, USA). A value of $P < 0.05$ indicated statistical significance.

Results

Patient characteristics

The patients ranged in age from 47 to 79 years (mean 66 years), and the patients were followed for a median of 31 months (range, 0-131 months). Their serum PSA levels at diagnosis ranged from 3.59 ng/mL to 175 ng/mL (mean 18 ng/mL). During their follow-up periods, 7 patients died; 6 patients died from another disease (e.g., stomach cancer, bladder cancer, lung cancer, and myocardial infarction) or from an unexpected event. Only 1 patient experienced metastasis to the sacrum (DFS, 12 months), with PC recurrence (urinary bladder), and died. The GS was 9 (4 + 5) and TNM (Tumor, Node, Metastases) was pT3aN0M0 at diagnosis.

Notch3 expression and its clinicopathological significance

Overall, 22 (15.5%) of 142 PC cases showed an overexpression on Notch3 (**Figure 1**). The overexpression of Notch3 was significantly associated with lymph node metastasis ($P = 0.013$), higher pT stages ($P = 0.033$), higher pathological tumor stages ($P = 0.034$), and high grade groups ($P = 0.025$). However, the overexpression of Notch3 was not correlated with lymphovascular invasion, neural invasion, or serum PSA level, as summarized in **Table 1**.

Survival results

No association was observed between Notch3 overexpression and OS. No independent prognostic factor was identified from the multivariate analysis.

Discussion

Early detection with advances in screening have made it possible to detect PC in the localized stage in most patients, thus providing a

Table 1. Correlation between pathological factors and the expression of Notch3 in prostate cancer

Variable	Notch3		P
	Negative	Positive	
Vascular invasion			0.700
Present	23 (82.1)	5 (17.9)	
Absent	97 (85.1)	17 (14.9)	
Neural invasion			0.301
Present	62 (81.6)	14 (18.4)	
Absent	58 (87.9)	8 (12.1)	
pN stage			0.013
N0	75 (86.2)	12 (13.8)	
N1	1 (25.0)	3 (75.0)	
Extra-prostatic extension			0.134
Present	45 (78.9)	12 (21.1)	
Absent	75 (88.2)	10 (11.8)	
Capsule invasion			0.079
Present	63 (79.7)	16 (20.3)	
Absent	57 (90.5)	6 (9.5)	
pT stage			0.033
pT2	67 (88.2)	9 (11.8)	
pT3a	40 (87.0)	6 (13.0)	
pT3b	13 (65.0)	7 (35.0)	
Stage			0.034
I	1 (100)	0 (0)	
II	67 (88.2)	9 (11.8)	
III	50 (83.3)	10 (16.7)	
IV	2 (40.0)	3 (60.0)	
Serum PSA (ng/mL)			0.363
< 10	68 (88.3)	9 (11.7)	
≥ 10 & < 20	27 (81.8)	6 (18.2)	
≥ 20	25 (78.1)	7 (21.9)	
Grade groups			0.025
Grade group 1 + 2	64 (91.4)	6 (8.6)	
Grade group 3 + 4 + 5	56 (77.8)	16 (22.2)	

Values are presented as number (%).

relatively favorable prognosis and long-term survival by using standard therapeutics [26]. However, despite early detection, between 9% and 22% of newly diagnosed patients have a GS of 8-10 [19]. Moreover, even some patients with early stage PC show dismal clinical outcomes with surgery or radiation therapy alone [27]. GS is still the most potent predictor of PC mortality following therapy [19, 28]. Thus, the identification of molecular markers that are associated with aggressiveness and metastasis is essential for predicting the clinical outcomes and deciding which PC treatment should be used.

The Notch signaling pathway regulates a wide variety of cellular processes, including cellular differentiation, survival, self-renewal, proliferation, and the cell cycle during development, and plays an important role in human malignancies [14, 29]. Although the Notch signaling pathway works as a tumor suppressor in some human malignancies, more recent studies support the theory that the Notch3 signaling pathway plays an oncogenic function by promoting proliferation, blocking differentiation, and inhibiting apoptosis [30].

In ovarian cancer, Notch3 overexpression is related to recurrence, advanced stages, lymph node metastasis, or chemo-resistance [31, 32]. Moreover, a higher Notch3 expression is associated with a poor prognosis in lung adenocarcinoma [33] and is a prognostic biomarker in pancreatic adenocarcinoma, with its expression being related to tumor stage and lymph node metastasis [34]. Notch3 overexpression also enhances tumor growth and chemo-resistance in urothelial carcinoma [35]. However, there are limited studies on the importance of the Notch pathway in PC, with some in vitro studies indicating a role for Notch1 and the Jagged 1 ligand.

Regarding Notch3, Long suggested that the Notch3 gene is one of the biomarkers that could predict the clinical recurrence of PC after RT [2]. Furthermore, PC with metastatic potential reveals an upregulation of the Notch3 receptor. However, this study was conducted in PC with only GS 6 (3 + 3) and 8 (4 + 4). Higher GS 8 (4 + 4) revealed more metastatic potential [19]. Danza et al. reported that hypoxia triggers the activation of Notch3, and Notch3 overexpression has a significant correlation with Gleason grade, so PC with Notch3 overexpression requires a more aggressive therapy [36]. Zhang et al. suggested that Notch3 overexpression was associated with chemoresistance [35]. In this study, Notch3 was highly correlated with tumor invasion, especially in PCs with seminal vesicle invasion as compared with PC within the prostate or with capsular invasion. Moreover, lymph node metastases were more evident in Notch3 overexpressing PC. As the stage of PC increases, Notch3 tends to become more overexpressed. Notch3 is also more expressed in higher grade groups (> 3) than in lower grade groups (1 and 2). Our study supports the hypothesis that Notch3 overexpres-

sion in PC is associated with tumor aggressiveness and may serve as a useful poor prognosis biomarker in PC.

Recent research into Notch pathway activity in cancer cells revealed that Notch signaling promotes the epithelial to mesenchymal transition [37, 38], enhances cancer stemness, and is resistant to conventional chemotherapies [39]. Recently, targeting the Notch pathway using gamma-secretase inhibitors (GSIs) to prevent cleavage of the oncogenic domain of Notch molecules and suppress Notch activity has garnered a great deal of attention [40]. The GSIs also blocked human prostate epithelial cell growth [41]. However, no benefits of using these GSIs have been reported for PC in clinical trials registered at the National Institutes of Health [16]. Future studies with extensive and consistent experimental data about the role of the Notch pathway are required to clarify the therapeutic effects of GSIs on PC.

Although we could not get meaningful results in relation to patient clinical outcomes because only 1 patient's death was PC-related, our study supports the theory that the Notch3 plays an oncogenic function in PC, and the overexpression of Notch3 is associated with tumor aggressiveness, lymph node metastasis, and higher grade groups of PCs.

In conclusion, we evaluated Notch3 expression using immunohistochemistry in PC. Notch3 plays oncogenic function in PC, and the overexpression of Notch3 is associated with tumor invasiveness, metastasis, and higher grade groups. Although larger scale studies with sufficient survival data are needed to confirm our results, Notch3 may be a biomarker for aggressive tumor features in PC and this study will inspire future research on Notch3-based targeted therapy.

Disclosure of conflict of interest

None.

Address correspondence to: Mi Jin Gu, Department of Pathology, Yeungnam University College of Medicine, 170, Hyeonchung-ro, Nam-gu, Daegu 42415, South Korea. Tel: +82-53-640-6756; E-mail: mjgu@yu.ac.kr

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.

[2] Long Q, Johnson BA, Osunkoya AO, Lai YH, Zhou W, Abramovitz M, Xia M, Bouzyk MB, Nam RK, Sugar L, Stanimirovic A, Williams DJ, Leyland-Jones BR, Seth AK, Petros JA, Moreno CS. Protein-coding and microRNA biomarkers of recurrence of prostate cancer following radical prostatectomy. Am J Pathol 2011; 179: 46-54.

[3] Kristiansen G. Diagnostic and prognostic molecular biomarkers for prostate cancer. Histopathology 2012; 60: 125-141.

[4] Moldovan PC, Van den Broeck T, Sylvester R, Marconi L, Bellmunt J, van den Bergh RCN, Bolla M, Briers E, Cumberbatch MG, Fossati N, Gross T, Henry AM, Joniau S, van der Kwast TH, Matveev VB, van der Poel HG, De Santis M, Schoots IG, Wiegel T, Yuan CY, Cornford P, Mottet N, Lam TB, Rouviere O. What is the negative predictive value of multiparametric magnetic resonance imaging in excluding prostate cancer at biopsy? a systematic review and meta-analysis from the european association of urology prostate cancer guidelines panel. Eur Urol 2017; 72: 250-266.

[5] Gonnissen A, Isebaert S, Perneel C, McKee CM, Van Utterbeeck F, Lerut E, Verrill C, Bryant RJ, Joniau S, Muschel RJ, Haustermans K. Patched 1 expression correlates with biochemical relapse in high-risk prostate cancer patients. Am J Pathol 2018; 188: 795-804.

[6] Grossman I, Alon A, Ilani T, Fass D. An inhibitory antibody blocks the first step in the dithiol/disulfide relay mechanism of the enzyme QSOX1. J Mol Biol 2013; 425: 4366-4378.

[7] Yang DD, Nguyen PL. Optimizing androgen deprivation therapy with radiation therapy for aggressive localized and locally advanced prostate cancer. Urol Oncol 2017; Epub ahead of print].

[8] Das P, Siegers GM, Postovit LM. Illuminating luminal B: QSOX1 as a subtype-specific biomarker. Breast Cancer Res 2013; 15: 104.

[9] Kotb AF, Elabbady AA. Prognostic factors for the development of biochemical recurrence after radical prostatectomy. Prostate Cancer 2011; 2011: 485189.

[10] Almén MS, Nordström KJ, Fredriksson R, Schiöth HB. Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. BMC Biol 2009; 7: 50.

[11] Kretschmer A, Tolkach Y, Ellinger J, Kristiansen G. Molecular biomarkers and prognostic factors for prostate cancer. Urologe A 2017; 56: 933-944.

[12] Bostrom PJ, Bjartell AS, Catto JW, Eggener SE, Lilja H, Loeb S, Schalken J, Schlomm T, Cooperberg MR. Genomic predictors of outcome in

prostate cancer. Eur Urol 2015; 68: 1033-1044.
[13] Pedrosa AR, Graca JL, Carvalho S, Peleteiro MC, Duarte A, Trindade A. Notch signaling dynamics in the adult healthy prostate and in prostatic tumor development. Prostate 2016; 76: 80-96.
[14] Bolos V, Grego-Bessa J, de la Pompa JL. Notch signaling in development and cancer. Endocr Rev 2007; 28: 339-363.
[15] Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999; 284: 770-776.
[16] Carvalho FL, Simons BW, Eberhart CG, Berman DM. Notch signaling in prostate cancer: a moving target. Prostate 2014; 74: 933-945.
[17] Drabick JJ, Schell TD. Poking CD40 for cancer therapy, another example of the goldilocks effect. Cancer Biol Ther 2010; 10: 994-996.
[18] Tomlins SA, Mehra R, Rhodes DR, Cao X, Wang L, Dhanasekaran SM, Kalyana-Sundaram S, Wei JT, Rubin MA, Pienta KJ, Shah RB, Chinnaiyan AM. Integrative molecular concept modeling of prostate cancer progression. Nat Genet 2007; 39: 41-51.
[19] Ross AE, Marchionni L, Vuica-Ross M, Cheadle C, Fan J, Berman DM, Schaeffer EM. Gene expression pathways of high grade localized prostate cancer. Prostate 2011; 71: 1568-1577.
[20] True L, Coleman I, Hawley S, Huang CY, Gifford D, Coleman R, Beer TM, Gelmann E, Datta M, Mostaghel E, Knudsen B, Lange P, Vessella R, Lin D, Hood L, Nelson PS. A molecular correlate to the Gleason grading system for prostate adenocarcinoma. Proc Natl Acad Sci U S A 2006; 103: 10991-10996.
[21] Santagata S, Demichelis F, Riva A, Varambally S, Hofer MD, Kutok JL, Kim R, Tang J, Montie JE, Chinnaiyan AM, Rubin MA, Aster JC. JAGGED 1 expression is associated with prostate cancer metastasis and recurrence. Cancer Res 2004; 64: 6854-6857.
[22] Bin Hafeez B, Adhami VM, Asim M, Siddiqui IA, Bhat KM, Zhong W, Saleem M, Din M, Setaluri V, Mukhtar H. Targeted knockdown of Notch1 inhibits invasion of human prostate cancer cells concomitant with inhibition of matrix metalloproteinase-9 and urokinase plasminogen activator. Clin Cancer Res 2009; 15: 452-459.
[23] Whelan JT, Kellogg A, Shewchuk BM, Hewan-Lowe K, Bertrand FE. Notch-1 signaling is lost in prostate adenocarcinoma and promotes PTEN gene expression. J Cell Biochem 2009; 107: 992-1001.
[24] Baek JA, Song PH, Ko Y, Gu MJ. High expression of QSOX1 is associated with tumor invasiveness and high grades groups in prostate cancer. Pathol Res Pract 2018; 214: 964-967.
[25] Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA, Grading C. The 2014 International Society of Urological Pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma: definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 2016; 40: 244-252.
[26] Liu X, Chen L, Huang H, Lv JM, Chen M, Qu FJ, Pan XW, Li L, Yin L, Cui XG, Gao Y, Xu DF. High expression of PDLIM5 facilitates cell tumorigenesis and migration by maintaining AMPK activation in prostate cancer. Oncotarget 2017; 8: 98117-98134.
[27] Lahdensuo K, Erickson A, Saarinen I, Seikkula H, Lundin J, Lundin M, Nordling S, Butzow A, Vasarainen H, Bostrom PJ, Taimen P, Rannikko A, Mirtti T. Loss of PTEN expression in ERG-negative prostate cancer predicts secondary therapies and leads to shorter disease-specific survival time after radical prostatectomy. Mod Pathol 2016; 29: 1565-1574.
[28] Shah RB. Current perspectives on the Gleason grading of prostate cancer. Arch Pathol Lab Med 2009; 133: 1810-1816.
[29] Egan SE, St-Pierre B, Leow CC. Notch receptors, partners and regulators: from conserved domains to powerful functions. Curr Top Microbiol Immunol 1998; 228: 273-324.
[30] Cui H, Kong YH, Xu M, Zhang H. Notch3 functions as a tumor suppressor by controlling cellular senescence. Cancer Res 2013; 73: 3451-3459.
[31] Park JT, Chen X, Trope CG, Davidson B, Shih IM, Wang TL. Notch3 overexpression is related to the recurrence of ovarian cancer and confers resistance to carboplatin. Am J Pathol 2010; 177: 1087-1094.
[32] Kim MJ, Kim AR, Jeong JY, Kim KI, Kim TH, Lee C, Chung K, Ko YH, An HJ. Correlation of ALDH1 and Notch3 expression: clinical implication in ovarian carcinomas. J Cancer 2017; 8: 3331-3342.
[33] Chen C, Wang X, Huang S, Wang L, Han L, Yu S. Prognostic roles of Notch receptor mRNA expression in human ovarian cancer. Oncotarget 2017; 8: 32731-32740.
[34] Mann CD, Bastianpillai C, Neal CP, Masood MM, Jones DJ, Teichert F, Singh R, Karpova E, Berry DP, Manson MM. Notch3 and Hey-1 as prognostic biomarkers in pancreatic adenocarcinoma. PLoS One 2012; 7: e51119.
[35] Zhang H, Liu L, Liu C, Pan J, Lu G, Zhou Z, Chen Z, Qian C. Notch3 overexpression enhances progression and chemoresistance of urothelial carcinoma. Oncotarget 2017; 8: 34362-34373.

[36] Danza G, Di Serio C, Ambrosio MR, Sturli N, Lonetto G, Rosati F, Rocca BJ, Ventimiglia G, del Vecchio MT, Prudovsky I, Marchionni N, Tarantini F. Notch3 is activated by chronic hypoxia and contributes to the progression of human prostate cancer. Int J Cancer 2013; 133: 2577-2586.

[37] Talbot LJ, Bhattacharya SD, Kuo PC. Epithelial-mesenchymal transition, the tumor microenvironment, and metastatic behavior of epithelial malignancies. Int J Biochem Mol Biol 2012; 3: 117-136.

[38] Wang ZW, Li YW, Kong D, Banerjee S, Ahmad A, Azmi AS, Ali S, Abbruzzese JL, Gallick GE, Sarkar FH. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. Cancer Res 2009; 69: 2400-2407.

[39] Wang Z, Li Y, Ahmad A, Azmi AS, Banerjee S, Kong D, Sarkar FH. Targeting Notch signaling pathway to overcome drug resistance for cancer therapy. Biochim Biophys Acta 2010; 1806: 258-267.

[40] Mimae T, Okada M, Hagiyama M, Miyata Y, Tsutani Y, Inoue T, Murakami Y, Ito A. Upregulation of Notch2 and Six1 is associated with progression of early-stage lung adenocarcinoma and a more aggressive phenotype at advanced stages. Clin Cancer Res 2012; 18: 945-955.

[41] Ceder JA, Jansson L, Helczynski L, Abrahamsson PA. Delta-like 1 (Dlk-1), a novel marker of prostate basal and candidate epithelial stem cells, is downregulated by notch signalling in intermediate/transit amplifying cells of the human prostate. Eur Urol 2008; 54: 1344-1353.