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Original Article

Expression of Notch1 and p53 in esophageal squamous cell carcinoma and their correlations with clinicopathological features

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Abstract: Background: The Notch1 gene plays an important role in tumorigenesis and tumor progression. Recently, it has also been shown that p53 is upstream effectors of the notch signal pathway. In this study, we explored Notch1 and p53 expression in esophageal squamous cell carcinomas (ESCC). The possible associations of Notch1 expression and p53 expression with clinicopathological factors, prognosis. Methods: Notch1 and p53 protein expressions were evaluated by immunohistochemistry (IHC) in 123 surgically resected ESCC and were correlated with clinicopathological. Kaplan-Meier plots and Cox proportional hazards regression model were used to analyze the prognostic value of Notch1 and p53 expression. Results: In the 123 patients, Notch1 showed high in 42.3% (52/123), and p53 was positive in 61.8% (76/123). In addition, high expression of p53 was significantly correlated with patient age, pathological stage and tumor location ($P<0.05$) in ESCC patients. Patients with higher level of Notch1 staining in ESCC had shorter overall survival, whereas we found no relationship between p53 expression and overall survival. Univariate and multivariate analyses revealed that high Notch1 protein expression and histological differentiation were independent prognostic factors for ESCC. Conclusions: our findings suggest that Notch1 can serve as a good predictor of prognosis in ESCC patients. However, the role of p53 on patient outcome needs further study.

Keywords: Notch1, p53, esophageal squamous cell carcinoma, prognosis

Introduction

Esophageal cancer, one of the most common malignancy of the digestive tract, is a highly lethal disease with an overall 5-year survival is less than 15% [1]. More than 90% of esophageal cancer are squamous cell carcinomas arising from the esophageal mucosa in Asian countries [2]. Despite tremendous advances in diagnosis and multimodality therapy, the prognosis of ESCC remains extremely poor [3]. Hence, the identification of prognostic factors is essential for predicting patients' survival and determining optimal therapeutic strategies.

In humans, the Notch family is composed of four receptors members: Notch1, Notch2, Notch3, Notch4, and five ligands members: JAG1, JAG2, DLL1, DLL3, and DLL4 [4]. Notch protein, transmembrane receptor, which participates in the regulation of cell fate, proliferation, differentiation, and apoptosis [5]. Notch1, a member of notch family, was originally found to be expressed by neuronal cell [6], then abundantly detected in a variety of human cancers [7-9]. Numerous studies have reported that Notch1 and its ligand could be used as useful biomarkers to predict prognostic outcomes in a wide variety of malignant tumors, such as breast cancer [10, 11], bladder cancer [12], and prostate cancer [13]. More interestingly, notch signaling pathway also plays a role in other types of leukemia [14]. Recent study reported that high expression of Notch1 was associated with decreased overall survival by RT-PCR in ESCC [15]. However, few studies to date have investigated Notch1 expression in ESCC by immunohistochemical staining.

P53, as a kind of tumor suppressor protein, is localized on chromosome 17q13.1, and which is upstream effectors of the notch signal path-

Table 1. Notch1 expression, p53 expression and clinicopathological in 123 ESCC specimens

Characteristic	Total N=123	Notch1 expression, n		*P*	P53 expresison, n		*P*
		Low	High		Low	High	
Gender							
Male	87	51	36		36	51	
Female	36	20	16	0.657	11	25	0.273
Age (years)							
<65	69	41	28		35	34	
≥65	54	30	24	0.678	12	42	0.008
Tumor size							
<5 cm	94	51	43		35	58	
≥5 cm	29	20	9	1.000	12	18	0.351
Tumor site							
Upper	7	3	4		1	6	
Middle	60	33	27		29	31	
Lower	56	35	21	0.423	17	39	0.033
Differentiation							
Well	16	11	5		7	9	
Moderate	75	45	30		33	42	
Poor	32	15	17	0.274	9	23	0.372
Pathological stage							
T1-T3	58	36	22		16	42	
T4	65	35	30	0.543	31	34	0.044
Lymph metastasis							
Negative	93	55	38		35	58	
Positive	30	16	14	0.647	12	18	0.819
Vessel invasion							
Negative	106	58	48		40	66	
Positive	17	13	4	0.227	7	10	1.000
Perineuronal invasion							
Negative	97	54	43		35	62	
Positive	26	17	9	0.614	12	14	0.465

way [16]. A large number of target genes regulated by p53 involve in cancer cell proliferation and apoptosis [17]. Mutation of p53 gene results in the loss of its ability to induce cell death, which leads to uncontrolled cell proliferation, survival, mobility and invasiveness. Several experimental studies demonstrated that p53 mutation is the most common aberration in human cancers including breast carcinomas [18], colorectal cancer [19], and endometrial carcinomas [20]. High expression of p53 protein correlates with poor prognostic in patients with ESCC [21, 22].

But to our knowledge, little information has been available on the relationships among the expression of Notch1 and p53 in ESCC so far. The aim of our work was to investigate the immunohistochemical expression of Notch1 and p53 in 123 cases of primary ESCC and adjacent normal tissues to explore the correlation between Notch1, p53 and the clinicopathological feathers, including patient outcome of ESCC.

Materials and methods

Patients and tissue samples

A total of 123 consecutive ESCC patients who underwent curative surgery without neoadjuvant treatment at the first teaching hospital of Xinjiang medical university between January 2007 and July 2014 were selected retrospectively. Majority of them were males (70.7%) and females constituted 36 (29.3%) cases. The age of the patients ranged from 35 to 81 years (mean, 63.2). The tumor size of the patients ranged from 1 to 8 cm (mean, 3.86). Of the 123 tumors, 16 (13%) were Well differentiated, 75 (61%) were moderately differentiated, 32 (26%) were poorly differentiated. The main clinical and pathologic variables of the patients are summarized in **Table 1**. For histologic examination, all tissue portions were fixed in formalin and embedded in paraffin. Tumors histological grades were classified as well, moderate and poor. All human tissue was collected using protocols approved by the ethics committee of the first teaching hospital of Xinjiang medical university.

Immunohistochemistry

Samples were fixed in neutral 10% formalin and embedded in paraffin after resection. The sections were deparaffinized using a graded ethanol series, and endogenous peroxidase activity was blocked by soaking 3% hydrogen peroxide for 10 min. Microwave antigen retrieval was

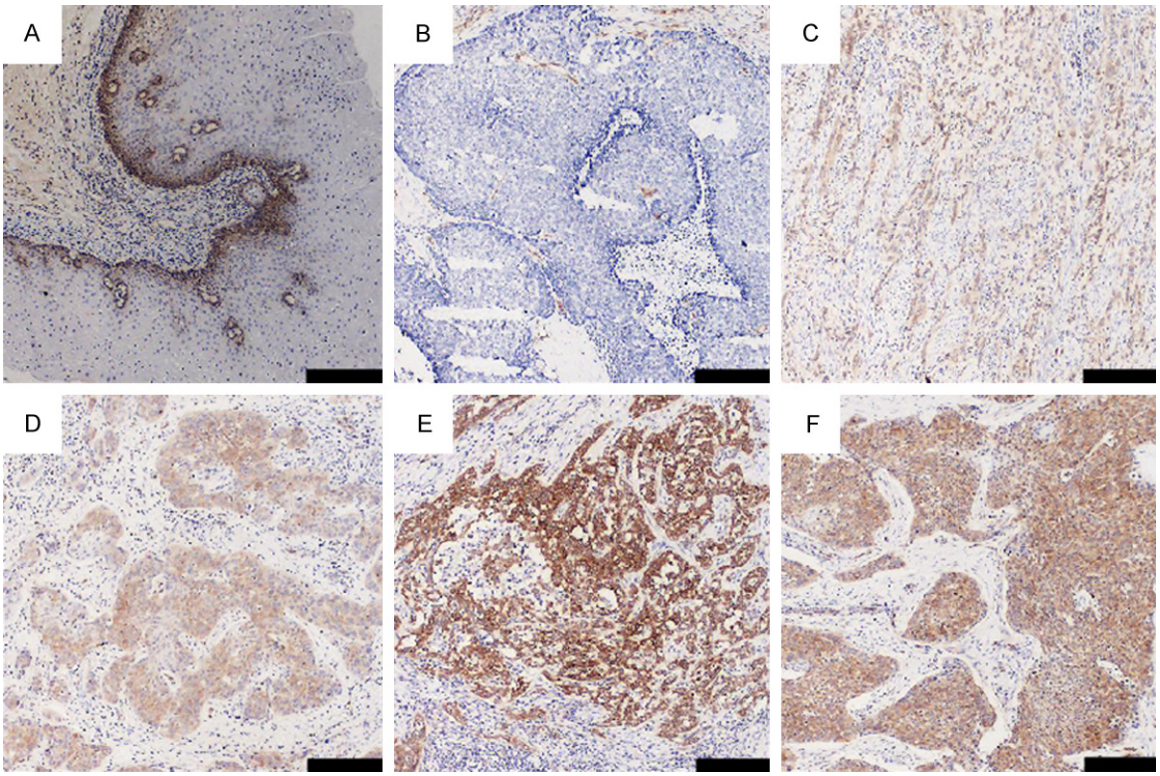

Figure 1. Immunohistochemical staining of Notch1 expression in ESCC and normal esophagus mucosa. A. In normal esophagus mucosa, Notch1 is localized to the cell membrane and cytoplasm. B. Negative expression of Notch1 in ESCC tumor tissue. C. Weak expression in ESCC tumor tissue. D. Moderate positive expression of Notch1 in ESCC tumor tissue. E, F. Strong positive expression of Notch1 in ESCC tumor tissue. Bar=200 μm.

carried out by placing the slides in 10 mM sodium citrate buffer (pH=6.0) at 100°C for 10 minutes and then allowed to cool. After rinsing in phosphate buffered saline (PBS), the sections were incubated with anti-Notch1 antibody (diluted 1:200, Abcam, USA) and anti-P53 antibody (diluted 1:100, Santa Cruz biotechnology, USA) for 2 h at room temperature and subsequently placed in secondary anti-mouse antibody for Notch1 or anti-rabbit antibody for p53 (Santa Cruz). The sections were finally incubated with DAB (DAKO). After rinsing in water, the sections were counterstained with hematoxylin, dehydrated, and cover slipped.

Notch1 and p53 expression was determined by 2 independent observers who were blinded to the clinicopathological data. If discordant interpretations were obtained, differences were resolved by a joint review or consultation, or both, with a third observer familiar with immunohistochemical pathology. When evaluating the p53 protein immunoreaction, staining was scored in semi-quantitative fashion. A cut-off value of 50% or more positively stained nuclei was defined as positive and the others were defined as low p53 expression. According to Liu et al. [23], a semi-quantitative evaluation of Notch1 reactivity was scored as 0 if there was no membranous or cytoplasm reactivity within the tumor, or as 1+, 2+, or 3+ depending on the intensity above the background level. The score of 2+ to 3+ was defined as positive and the score of 0 to 1+ was defined as negative.

Statistical analysis

Data analysis was performed using the SPSS 14.0 statistical program. The association between Notch1 and P53 expression and the clinicopathologic features was analyzed using chi-square test. For analysis of survival data, Kaplan-Meier curves were constructed, and the log-rank test was performed. Univariate and multivariate survival analyses ware performed using Cox proportional hazards model. $P<0.05$ was considered statistically significant.

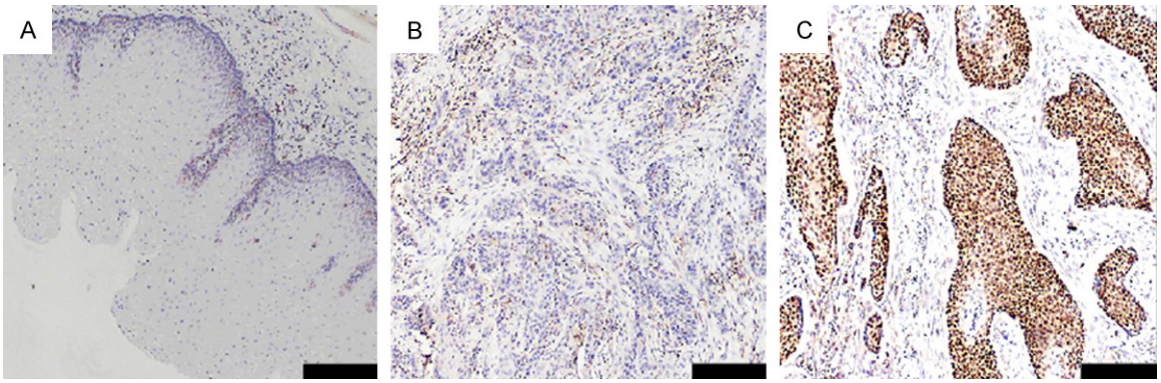

Figure 2. P53 protein was expressed significantly higher in ESCC tissues. A. In normal esophagus mucosa, p53 is localized to the cell basal membrane. B. P53 low expression in ESCC. C. P53 high expression in ESCC. Bar=200 μm.

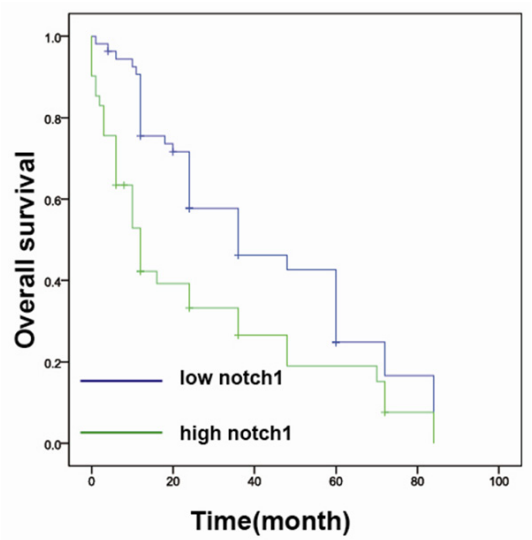

Figure 3. Kaplan-Meier curves for overall survival of ESCC with high or low Notch1 expression. Patients expressing high level of Notch1 have a significantly shorter survival.

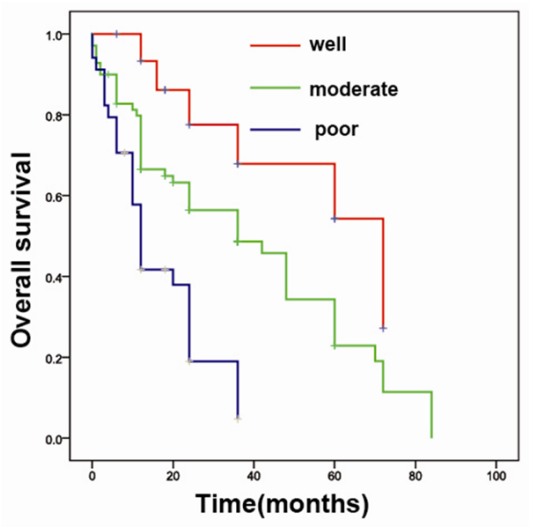

Figure 4. Correlation between tumor differentiation and patients' survival.

Results

Notch1 expression in ESCC and its relationship with clinicopathological characteristics

To investigate the relationship between Notch1 expression and clinicopathological parameters of ESCC patients, we evaluated Notch1 protein expression levels in 123 primary ESCC tissues using immunohistochemistry. As shown in **Figure 1**, the Notch1 staining was localized mainly in the membrane and cytoplasm, and the ratio of Notch1 high expression was 42.3% (52/123) in ESCC tissues. In the normal epithelia positive reaction was mainly localized in the membrane and cytoplasm of the basal cells (**Figure 1A**). We also analyzed the associations between expression levels of Notch1 and a series of clinicopathological characteristics, including age, gender, tumor size, differentiation, pathological stage, lymph metastasis, vessel metastasis, perineuronal invasion in ESCC patients (**Table 1**). However, there was no significant association between Notch1 expression level and these factors ($P>0.05$).

P53 expression in ESCC and its relationship with clinicopathological characteristics

The expression of p53 protein was also examined by IHC staining. As shown in **Figure 2**, positive staining of P53 was located in the nucleus

Table 2. Univariate and multivariate analysis of different prognostic features in 123 patients with ESCC

Factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender						
Male/female	1.063	0.456-2.480	0.887	1.061	0.589-1.912	0.843
Age						
≥60/<60	1.099	0.519-2.328	0.806	1.001	0.600-1.668	0.998
Tumor size						
≥5/<5	1.536	0.634-3.723	0.342	1.285	0.685-2.410	0.434
Location						
Upper/middle/lower	1.014	0.528-1.847	0.966	1.056	0.678-1.646	0.809
Differentiation						
Poor/moderate/well	2.601	1.423-4.754	0.002	2.563	1.658-3.961	0.001
T stage						
T1-3/T4	0.520	0.232-1.161	0.111	1.085	0.645-1.826	0.758
Lymph node metastasis						
Present/absent	1.049	0.435-2.532	0.915	1.049	0.555-1.983	0.882
Vessel invasion						
Present/absent	2.120	0.599-7.496	0.244	0.923	0.367-2.321	0.864
Perineuronal invasion						
Present/absent	2.022	0.855-4.779	0.109	1.134	0.611-2.103	0.691
P53						
High/low	0.996	0.464-2.138	0.991	1.280	0.706-2.319	0.416
Notch1						
High/low	3.754	1.761-8.004	0.001	2.430	1.359-4.347	0.003

HR indicates hazards ratio; CI indicates confidence interval.

of the ESCC. In the group of normal adjacent tissue, positive expression of p53 was observed only in the nuclei of basal cells (**Figure 2A**). It was also observed that P53 protein was expressed significantly higher in ESCC when compared to normal esophagus mucosa. Based on the overall p53 expression, all the tumor specimens were divided into a p53 low expression group (n=47) and a p53 high expression group (n=76).

Table 1 summarized the correlation between expression of p53 and the clinicopathological findings. Statistical analysis showed that p53 expression was not correlated with any of the following clinicopathological characteristics: gender (*P*=0.273), tumor size (*P*=0.351), differentiation (*P*=0.372), lymph metastasis (*P*=0.819), vessel invasion (*P*=1.000), perineuronal invasion (*P*=0.465). However, we found that high p53 expression was significantly correlated with age (*P*=0.008), tumor site (*P*=0.033) and pathological stage (*P*=0.044). Our results suggest that high level of p53 expression is associated with advanced pathological stage.

Association between risk factors and survival time

To investigate the relationship between level of protein expression and survival, we used Kaplan-Meier methods. We found that the survival of patients with high Notch1 protein expression was significantly shorter than of protein with low Notch1 protein expression (**Figure 3**). Furthermore, the ESCC patients with well differentiation were associated with a survival benefit (**Figure 4**). As shown in **Table 2**, univariate analysis showed that overall survival was correlated with differentiation and Notch1 expression. However, gender, age, tumor size, location, T stage, lymph node, vessel invasion, perineuronal invasion and P53 were not significantly associated with overall survival (**Table 2**). Furthermore, Cox multivariate regression analysis indicated that differentiation (*P*=0.001) and Notch1 expression (*P*=0.003) were each recognized as independent prognostic factors for overall survival of ESCC patients. Taken together, these data suggest that Notch1 might represent a novel and potentially useful independent biomarker for the prognosis of patients with ESCC.

Discussion

ESCC remains one of the most malignant gastrointestinal cancers in China. Despite improvements in early detection, surgical techniques and chemoradiotherapy, the clinical outcome of ESCC patients remains unsatisfactory. Thus, it is urgently needed to investigate the effective molecular markers to improve the outcome of patients with ESCC.

Recent accumulating evidence suggests that the notch signaling pathway plays a critical role in carcinogenesis in many human malignancies [24]. In the current study, we found that Notch1 and p53 were up-regulated in ESCC tissue.

Notch signaling regulated squamous-cell differentiation to maintain epithelial integrity in the oral-esophageal squamous epithelia [25]. Notch1, one of the key receptors in the Notch signaling pathway, encodes an important member of Notch family proteins [26]. Notch1 has been shown to be abundantly expressed in a large variety of solid tumors promoting tumor growth and invasiveness. As previously described, high-levels coexpression of JAG1 and Notch1 is associated with poor prognosis in human breast cancer [27]. On the other hand, Huang et al. demonstrated that reduced expression of Notch-1 expression correlated to advanced clinical stage (P=0.001) and lymph node metastasis (P=0.026) associated in LAD patients. This reflects both the complexity of Notch1 regulation in cancers, and its tissue specificity.

In the current study, we found that Notch1 was highly expressed in 42.3% (52/123) of primary ESCC patients. After survival analysis, Notch1 was shown to attain a significantly poorer prognostic. Indeed, the Cox regression test showed that Notch1 protein was detected as an independent prognostic factor. These outcomes suggest that Notch1 is associated with ESCC. A previous report showed that significant positive correlation between abnormal tumor Notch1 expression and lymph node metastasis in human lung adenocarcinoma [28]. In this research, we also determined the expression of Notch1 in 123 ESCC specimens using immunohistochemistry and correlated this expression with clinicopathological parameters. No positive correlations were found between Notch1 expression in tumor and lymph node metastasis or vessel invasion, perineuronal invasion. Thus, the metastatic potential of cancer cells cannot be explained just by Notch1 expression.

P53 protein is encoded by the human gene p53, which is composed of 393 amino acids and is located at chromosome 17p13.1. Mutation of the p53 protein plays an important role in apoptosis and regulating cell growth and promoting tumorigenesis [17]. It has been reported that p53 may transactivate Notch1 directly, and p53 dysfunction may lead to the inactivation of Notch signaling [16]. The immunohistochemical results revealed that p53 protein expression was detected in the nucleus of cancer cells. In the current study, we found that p53 was highly expressed in 61.8% (76/123) of primary ESCC patients. Our finding is consistent with the studies published by Kate et al. [22]. Our results showed that high expression of p53 in ESCC had a significant correlation with elderly (P=0.008), lower tumor site (P=0.033), and advanced pathological stage (P=0.044). Positive p53 expression was significantly more frequently found in T4 stage, which was not reported in other studies. Although p53 expression in T1-3 and T4 are different, the survival of different pathological stage and p53 different expression was not significantly correlated. These findings differed from other studies in that overexpression of p53 was associated with decreased survival [22, 29]. Discrepancies in the findings between previous reports and our results may result from the differences in patients' characteristics between these studies.

In conclusion, high expression level of Notch1 was associated with poor prognosis in ESCC. We propose that the evaluation of Notch1 expression using an IHC assay is important and useful in routine practice. However, as IHC analysis for p53 showed no association with prognosis, further investigation is needed to verify its prognostic role in ESCC.

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Disclosure of conflict of interest

None.

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References

[1] Lin Y, Totsuka Y, He Y, Kikuchi S, Qiao Y, Ueda J, Wei W, Inoue M, Tanaka H. Epidemiology of

esophageal cancer in Japan and China. J Epidemiol 2013; 23: 233-242.
[2] Kato H, Fukuchi M, Miyazaki T, Nakajima M, Tanaka N, Inose T, Kimura H, Faried A, Saito K, Sohda M, Fukai Y, Masuda N, Manda R, Ojima H, Tsukada K, Kuwano H. Surgical treatment for esophageal cancer. Current issues. Dig Surg 2007; 24: 88-95.
[3] Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
[4] Lai EC. Notch signaling: control of cell communication and cell fate. Development 2004; 131: 965-973.
[5] Weijzen S, Rizzo P, Braid M, Vaishnav R, Jonkheer SM, Zlobin A, Osborne BA, Gottipati S, Aster JC, Hahn WC, Rudolf M, Siziopikou K, Kast WM, Miele L. Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. Nat Med 2002; 8: 979-986.
[6] Artavanis-Tsakonas S, Matsuno K, Fortini ME. Notch signaling. Science 1995; 268: 225-232.
[7] Radtke F, Raj K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? Nat Rev Cancer 2003; 3: 756-767.
[8] Nickoloff BJ, Osborne BA, Miele L. Notch signaling as a therapeutic target in cancer: a new approach to the development of cell fate modifying agents. Oncogene 2003; 22: 6598-6608.
[9] Donnem T, Andersen S, Al-Shibli K, Al-Saad S, Busund LT, Bremnes RM. Prognostic impact of Notch ligands and receptors in nonsmall cell lung cancer: coexpression of Notch-1 and vascular endothelial growth factor-A predicts poor survival. Cancer 2010; 116: 5676-5685.
[10] Reedijk M, Pinnaduwage D, Dickson BC, Mulligan AM, Zhang H, Bull SB, O'Malley FP, Egan SE, Andrulis IL. JAG1 expression is associated with a basal phenotype and recurrence in lymph node-negative breast cancer. Breast Cancer Res Treat 2008; 111: 439-448.
[11] Chen J, Imanaka N, Chen J, Griffin JD. Hypoxia potentiates Notch signaling in breast cancer leading to decreased E-cadherin expression and increased cell migration and invasion. Br J Cancer 2010; 102: 351-360.
[12] Shi TP, Xu H, Wei JF, Ai X, Ma X, Wang BJ, Ju ZH, Zhang GX, Wang C, Wu ZQ, Zhang X. Association of low expression of notch-1 and jagged-1 in human papillary bladder cancer and shorter survival. J Urol 2008; 180: 361-366.
[13] 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.
[14] Bellavia D, Campese AF, Vacca A, Gulino A, Screpanti I. Notch3, another Notch in T cell development. Semin Immunol 2003; 15: 107-112.
[15] Ogawa R, Ishiguro H, Kimura M, Funahashi H, Wakasugi T, Ando T, Shiozaki M, Takeyama H. NOTCH1 expression predicts patient prognosis in esophageal squamous cell cancer. Eur Surg Res 2013; 51: 101-107.
[16] Yugawa T, Handa K, Narisawa-Saito M, Ohno S, Fujita M, Kiyono T. Regulation of Notch1 gene expression by p53 in epithelial cells. Mol Cell Biol 2007; 27: 3732-3742.
[17] Di Agostino S, Strano S, Blandino G. Gender, mutant p53 and PML: a growing "affaire" in tumor suppression and oncogenesis. Cell Cycle 2013; 12: 1824-1825.
[18] Jung SY, Jeong J, Shin SH, Kwon Y, Kim EA, Ko KL, Shin KH, Ro J, Lee KS, Park IH, Lee S, Kim SW, Kang HS. Accumulation of p53 determined by immunohistochemistry as a prognostic marker in node negative breast cancer; analysis according to St Gallen consensus and intrinsic subtypes. J Surg Oncol 2011; 103: 207-211.
[19] Huerta S, Gao X, Dineen S, Kapur P, Saha D, Meyer J. Role of p53, Bax, p21, and DNA-PKcs in radiation sensitivity of HCT-116 cells and xenografts. Surgery 2013; 154: 143-151.
[20] Koshiyama M, Konishi I, Wang DP, Mandai M, Komatsu T, Yamamoto S, Nanbu K, Naito MF, Mori T. Immunohistochemical analysis of p53 protein over-expression in endometrial carcinomas: inverse correlation with sex steroid receptor status. Virchows Archiv A Pathol Anat Histopathol 1993; 423: 265-271.
[21] Murata A, Baba Y, Watanabe M, Shigaki H, Miyake K, Karashima R, Imamura Y, Ida S, Ishimoto T, Iwagami S, Sakamoto Y, Miyamoto Y, Yoshida N, Baba H. P53 immunohistochemical expression and patient prognosis in esophageal squamous cell carcinoma. Med Oncol 2013; 30: 728.
[22] Huang K, Chen L, Zhang J, Wu Z, Lan L, Wang L, Lu B, Liu Y. Elevated p53 expression levels correlate with tumor progression and poor prognosis in patients exhibiting esophageal squamous cell carcinoma. Oncology Lett 2014; 8: 1441-1446.
[23] Liu J, Fan H, Ma Y, Liang D, Huang R, Wang J, Zhou F, Kan Q, Ming L, Li H, Giercksky KE, Nesland JM, Suo Z. Notch1 is a 5-fluorouracil resistant and poor survival marker in human esophagus squamous cell carcinomas. PLoS One 2013; 8: e56141.
[24] Grishina IB. Mini-review: Does Notch promote or suppress cancer? New findings and old controversies. Am J Clin Exp Urol 2015; 3: 24-27.
[25] Naganuma S, Whelan KA, Natsuizaka M, Kagawa S, Kinugasa H, Chang S, Subramanian H,

Rhoades B, Ohashi S, Itoh H, Herlyn M, Diehl JA, Gimotty PA, Klein-Szanto AJ, Nakagawa H. Notch receptor inhibition reveals the importance of cyclin D1 and Wnt signaling in invasive esophageal squamous cell carcinoma. Am J Cancer Res 2012; 2: 459-475.

[26] Karamboulas C, Ailles L. Developmental signaling pathways in cancer stem cells of solid tumors. Biochim Biophys Acta 2013; 1830: 2481-2495.

[27] Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR, Lockwood G, Egan SE. High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res 2005; 65: 8530-8537.

[28] Huang J, Song H, Liu B, Yu B, Wang R, Chen L. Expression of Notch-1 and its clinical significance in different histological subtypes of human lung adenocarcinoma. J Exp Clin Cancer Res 2013; 32: 84.

[29] Yao W, Qin X, Qi B, Lu J, Guo L, Liu F, Liu S, Zhao B. Association of p53 expression with prognosis in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol 2014; 7: 7158-7163.