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Upregulated delta-like protein 3 expression is a diagnostic and prognostic marker in endometrial cancer

A retrospective study

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Abstract

Upregulated delta-like protein 3 (DLL3) functions as a Notch ligand and has been a target for cancer therapy. The present study assessed DLL3 expression as a tumor marker for endometrial cancer.

RNA-Seq expression data and clinicopathologic records from 545 patients with endometrial cancer were downloaded from The Cancer Genome Atlas database. Mann–Whitney *U* and logistic regression tests were applied to associate the level of DLL3 expression with clinical variables from the patients. Kaplan–Meier curves and log-rank tests were performed to compare overall survival of patients stratified by different levels of DLL3 expression. Multivariate Cox regression tests were used to analyze independent predictors for endometrial cancer. DLL3 expression was upregulated in endometrial cancer tissues compared to para-carcinoma tissues ($P = .0003$). High DLL3 expression was associated with the age of patients (odds ratio [OR] = 1.74), advanced stages of the International Federation of Gynecology and Obstetrics system (OR = 2.9), grade III/IV (OR = 5.1), myometrial invasion (OR = 2.2), pelvic involvement (OR = 12.9), and para-aortic lymph node metastasis (OR = 9.9) (all $P \leq .001$). Furthermore, upregulated DLL3 expression was also associated with a median overall survival of 112 months (HR = 1.85, confidence internal 1.202–2.846, $P = .005$). The multivariate analysis showed that DLL3 overexpression and advanced tumor stages, grades, and lymph node metastases were all independent prognostic predictors for endometrial cancer.

The DLL3 expression could be a potential and novel tumor marker for early diagnosis and an independent predictor of poor survival for patients with endometrial cancer.

Abbreviations: DLL3 = delta-like protein 3, DSL = Delta-Serrate-Lag2, EGF = epidermal growth factor, FIGO = International Federation of Gynecology and Obstetrics, TCGA = The Cancer Genome Atlas.

Keywords: biomarker, delta-like protein 3, endometrial cancer, prognosis, The Cancer Genome Atlas

1. Introduction

Endometrial cancer is a common female neoplasm, especially in postmenopausal women, and accounted for approximately 320,000 new cancer cases and 76,000 cancer-related deaths globally in 2014, which makes it the 6th most common cancer in women.[1] The incidence and mortality of endometrial cancer have been increasing over the past decades.[2–4] For example, in China alone, endometrial cancer accounts for approximately 7% of malignancies in females and up to 25% of female reproductive system cancers.[5] Endometrial cancer morbidity is 2nd only to cervical cancer in China, and it may be 1st in certain geographic areas such as Beijing or Shanghai, China. More recently, there has been a trend toward an increasing incidence in younger women.[5] To date, treatment of endometrial cancer usually consists of conventional surgery, radiotherapy, chemotherapy, and hormonal therapy based on the stage of disease. Overall, 90% of patients with endometrial cancer undergo some form of surgery followed by other types of add-on therapy.[6–8] Endometrial cancer, if diagnosed at an early stage, has a 5-year survival rate of up to 90%; however, approximately 15% of patients are diagnosed with advanced stages of disease or high-risk histopathology leading to worse survival rates.[9,10] To date, universal biomarkers to precisely diagnose endometrial cancer have not been identified, although CA-125 may be useful in advanced stages and serious endometrial cancer. Furthermore, there is no effective biomarker to predict treatment response currently (such as adjuvant radiation and/or chemotherapy) in endometrial cancer. In these regards, identification of novel biomarkers for early detection and prognostic indicators or treatment responses could greatly improve the management of

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endometrial cancer. Moreover, detection of altered gene expression in endometrial cancer could improve our understanding of endometrial cancer development and progression and therefore help to develop novel therapeutic strategies for the effective control of endometrial cancer.

Our present research focused on delta-like protein 3 (DLL3), which is an atypical member of the Notch receptor ligand family that is able to inhibit activation of the Notch receptors.[11,12] DLL3 contains different functional domains (such as a Delta-Serrate-Lag2 [DSL] domain, epidermal growth factor [EGF] repeats, and a transmembrane domain), and aberrant DLL3 expression has been found in neuroendocrine cancers and high-grade serous ovarian cancer.[13] A previous study targeted the DLL3 protein as a novel therapeutic strategy to control high-grade neuroendocrine carcinomas, including small-cell lung cancer.[13] In this study, we assessed DLL3 expression as a tumor marker for endometrial cancer and explored DDL3 expression as a prognostic predictor.

2. Materials and methods

2.1. Data collection and analysis

The level of DLL3 mRNA and the corresponding clinicopathologic data from patients with endometrial cancer (545 tumor and 35 adjacent tumor tissues) were downloaded from the The Cancer Genome Atlas (TCGA database; https://gdc-portal.nci.nih.gov/) on July 10, 2017. The case data for this retrospective study was derived from the TCGA database, so no ethical approval is required. We then standardized DLL3 expression data in each patient using the Trimmed Mean of M-values method. The differential DLL3 expression between tumor and para-carcinoma tissues was subsequently analyzed using Limma, an R/Bioconductor software package, according to a previous study.[14] To guarantee the quality of the study, the data were collected and analyzed in a blinded fashion by 2 investigators.

2.2. Statistical analyses

The median level of DLL3 expression in tumor tissues was 5.086; this value was used as the cut-off point for upregulated DLL3 expression in each tumor case. We then performed a Mann–Whitney *U* test and a logistic regression test to associate DLL3 expression with different clinicopathologic data. The Cox regression test was used to analyze overall survival and association with clinicopathologic characteristics. The Kaplan–Meier curves and the log-rank test were used to analyze overall survival stratified by DLL3 expression in these patients with endometrial cancer. All statistical analyses were performed using SPSS version 23.0 (IBM Corp, Armonk, NY) and GraphPad Prim 7.0 (GraphPad Software, La Jolla, CA). A *P*-value $<.05$ was considered to be statistically significant.

3. Results

3.1. Characteristics of patients

From the TCGA data, we obtained 545 patients with primary endometrial cancer, and tissue specimens from these patients were profiled for differentially expressed genes in June 2017. Patients' clinicopathologic characteristics are shown in Table 1. Specifically, the median age of this patient cohort was 64 years old (ranged between 31 and 90 years old) with the following patient demographic breakdown: 70% of the patients were White, 21% of the patients were African-American, 4% of the patients were Asian, and <3% of the patients were Native American/Alaskan Native and Native Hawaiian/Pacific Islander patients. Histologically, the majority of patient tumors were endometrioid adenocarcinomas (74.9%, n=408), followed by serous endometrial adenocarcinomas (21.1%, n=115), with the remaining tumors being classified as "mixed" (4%, n=22). Moreover, patients with stages I, II, III, and IV cancer comprised 62.62%, 9.58%, 22.84%, and 4.96% of the cohort population, respectively, and patients with tumor grades 1, 2, and 3 comprised 18.5%, 22.7%, and 58.8% of the cohort population, respectively. Nearly half of all patients had deep myometrial invasion (49.6%), and 10% and 5% of these patients had tumor pelvic and para-aortic lymph node metastasis, respectively. The median follow-up period was 30.3 months (ranged between 0 and 228 months).

3.2. Upregulation of DLL3 expression in endometrial cancer

The level of DLL3 expression was assessed using a cDNA microarray in both endometrial cancer and the paracarcinoma tissues. In this study, we downloaded the data from the TCGA database and assess the level of differential DLL3 expression in endometrial cancer tissues, and found that the level of DLL3 expression was significantly upregulated in endometrial cancer tissue when compared to para-carcinoma tissue ($P<.0003$; Fig. 1).

3.3. Association of DLL3 expression with clinicopathologic variables from patients with endometrial cancer

We then associated DLL3 expression with clinicopathologic variables from patients with endometrial cancer using a cut-off point of the DLL3 level (the median expression value was 5.086). We found that the upregulated DLL3 expression was associated with older patients (≥64 years; odds ratio [OR]=1.74 for age ≥64 years vs <64 years) and higher tumor International Federation of Gynecology and Obstetrics (FIGO) stages (OR=2.9 for stage I/II vs stage III/IV), grade 3 (OR=5.1 for grade 1/2 vs grade 3), deep myometrial invasion (OR=2.2), pelvic (OR=12.9), and para-aortic lymph node (OR=9.9) metastases ($P \leq .001$; Fig. 2 and Tables 1 and 2).

3.4. Association of upregulated DLL3 expression with prognosis of endometrial cancer

The Kaplan–Meier curves and the log-rank test showed that upregulated DLL3 expression also associated with shorter overall survival of patients with endometrial cancer ($P=.0045$; Fig. 3). We then performed univariate and multivariate analyses to assess the association of overall survival with DLL3 expression and clinicopathologic characteristics. Our univariate analysis showed that high DLL3 expression, older age, advanced tumor stages, higher grade, and pelvic/para-aortic lymph node metastases were all associated with worse overall survival of patients (Tables 2 and 3). Moreover, our multivariate analysis showed that DLL3 overexpression and advanced tumor stages, grades, and lymph node metastases were all independent prognostic predictors for endometrial cancer (Table 3).

4. Discussion

Treatment and prognosis of endometrial cancer depends considerably on the tumor stage. Early stages of endometrial

Table 1

Characteristics of 545 patients with endometrial cancer from the The Cancer Genome Atlas cohort and association of delta-like protein 3 (DLL3) expression with clinicopathologic data.

Characteristic	Total	%	DLL3 expression		*P*-value
			High	Low	
Age at diagnosis, yr					
Median age (ranges)	64 (31–90)				
≤64	290	53.2	126	164	
>64	250	47.8	145	107	.001
BMI					
Median BMI (ranges)	32.2 (17.4–81.6)				
Race					
White	373	72.7	180	193	
African-American	107	20.9	58	49	
Asian	20	3.9	9	11	
Native American/Alaska Native	4	0.8	2	2	
Hawaiian/Pac Islander	9	1.8	4	5	>.05
Histology					
Serous endometrial adenocarcinoma	115	21.1	60	55	
Mixed serous and endometrioid	22	4.0	10	12	
Endometrioid endometrial adenocarcinoma	408	74.9	209	199	>.05
Stage					
I/II	392	72.2	172	220	
III/IV	151	27.8	100	53	.000
Grade					
1/2	220	41.2	58	162	
3	314	58.8	205	109	.000
Myometrial invasion					
Superficial (<50%)	236	50.42	83	153	
Deep (≥50%)	232	49.58	126	106	.000
Pelvic lymph node					
Negative	487	89.4	219	268	
Positive	58	10.6	53	5	.000
Para-aortic lymph node					
Negative	515	94.5	245	271	
Positive	30	5.5	27	273	.000

cancer can be cured by surgery followed by chemoradiotherapy, with favorable overall survival rates. Thus, early detection is the key to cure patients with endometrial cancer clinically, while the identification of biomarkers to predict prognosis or treatment responses could help medical oncologists to effectively control endometrial cancer. At the present time, the diagnosis and classification of endometrial cancer are according to the FIGO system. The magnetic resonance (MR) image is used to assess and evaluate the association with prognosis for many malignant tumors, such as breast cancer, nasopharyngeal carcinomas, retinoblastomas, and other cancers.[15–19] For endometrial cancer, routine MR imaging in combination with the T2-weighted imaging, diffusion weighted imaging, and dynamic contrast-enhanced MR, which are also used for preoperative assessment tumor staging.[20–22] Similar to other cancers, we evaluated the distant lymphoma nodes and organ metastasis using whole body computed tomography (CT) in clinic.[23,24] Despite the high accuracy of assessing the preoperative stageing and the prognosis of endometrial cancer with MR and CT images, we still prefer to have an earlier diagnosis and prognostic biomarkers that are not only used to assess the disease stageing but also to potentially transform therapeutic targets. Thus, we utilized data from TCGA to characterize expression of various genes in tissue samples for more than 30 types of human cancer. This analysis generates enormous amounts of comprehensive genetic, epigenetic, transcriptomic, and proteomic data to enable researchers to systematically analyze differential gene expression as biomarkers in these human cancers.

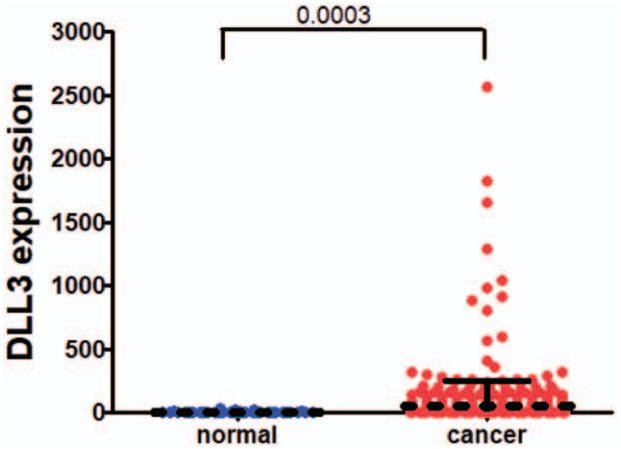

Figure 1. Upregulation of delta-like protein 3 (DLL3) expression in endometrial cancer tissue specimens. Level of DLL3 expression in endometrial cancer tissue specimens between cancerous and para-cancerous tissues (n=545 vs n=35; *P*=.0003).

In our present study, we assessed differential DLL3 expression in endometrial cancer tissues as a biomarker for early detection and prognosis of endometrial cancer using TCGA

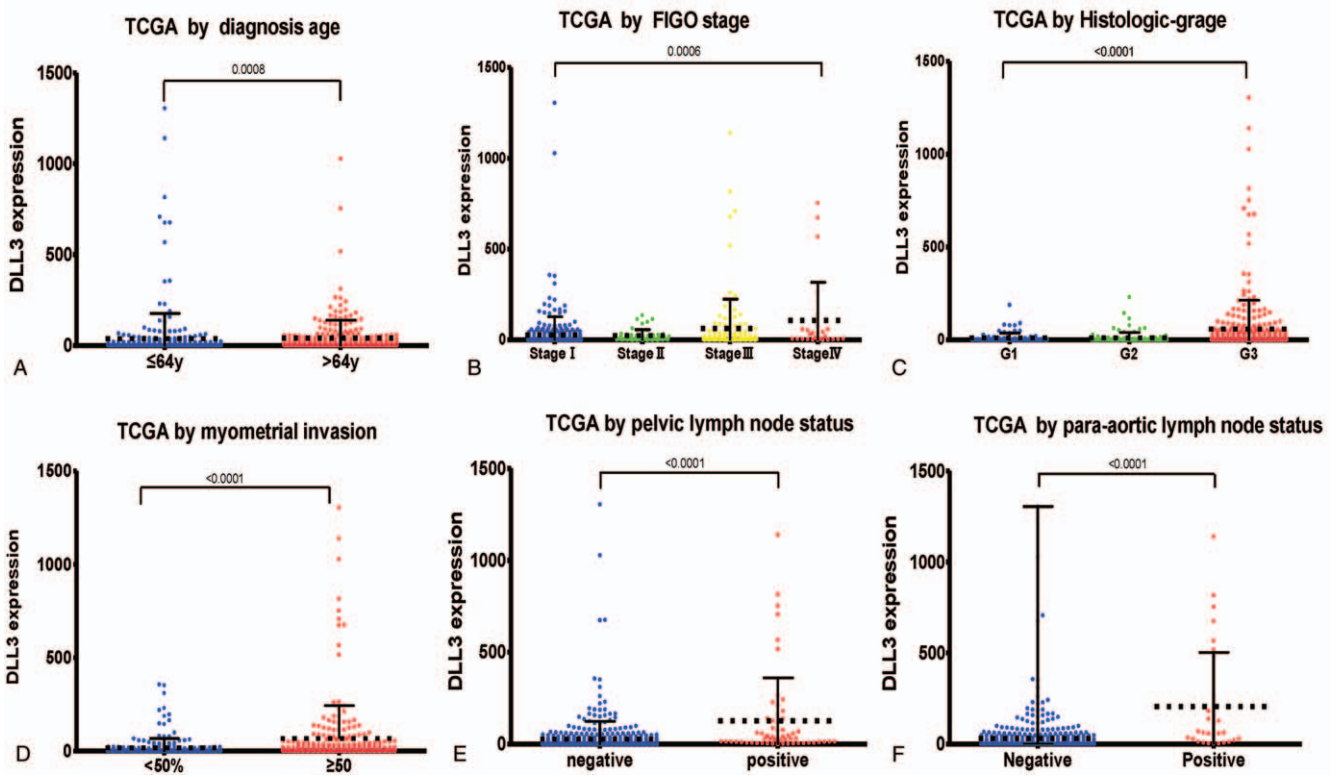

Figure 2. Association of delta-like protein 3 (DLL3) expression with clinicopathologic characteristics of endometrial cancer patients, including (A) patient age, (B) tumor stage, (C) grade, (D) depth of myometrial invasion, (E) pelvic lymph node, and (F) para-aortic lymph node metastases (all $P \leq .001$).

database data. Our results showed that DLL3 expression was upregulated in endometrial cancer tissues, older patients (≥64 years), as well as in advanced FIGO stages (III/IV), grade 3, myometrial deep invasion, and patients with pelvic and para-aortic lymph node metastases. Furthermore, we found that upregulated DLL3 expression is also associated with shorter overall survival of patients with endometrial cancer. DLL3 overexpression and advanced tumor stages, grade, and lymph node metastases were all independent prognostic predictors for endometrial cancer. Our current data demonstrated that DLL3 expression could be a potential and novel tumor marker for early diagnosis and prediction of prognosis in patients with endometrial cancer. Future studies will also investigate targeting DLL3 expression or function as a novel anti-endometrial cancer therapy.

Indeed, DLL3 belongs to the Notch signaling family, which is a fundamental and conserved cell signaling system in multicellular organisms.[25,26] Notch signaling can promote cell proliferation during neurogenesis and cell-fate determination during embryo development and in stem cells.[15] To date, it is well documented in the literature that Notch signaling is frequently dysregulated in various human cancers and targeting Notch signaling pathway networks could be used as a novel cancer therapy strategy.[27–30] In contrast, although activation of the Notch pathway had a pro-oncogenic role in some cancers, its expression and activation were reduced in other cancers, including neuroendocrine tumors such as high-grade serous ovarian cancer, small-cell lung cancers, and large-cell neuroendocrine tumors.[31,32] Another previous study speculated that the Notch pathway could differentially regulate neuroendocrine versus epithelial cell fate in lung development.[33] In tumor biology, dysregulation of Notch signaling is crucial, but it remains unclear whether activation of the Notch signaling pathway exerts a tumor promoting or suppressing role in various cancers, which is dependent on different factors and cell responses, such as inflammation.[34] On the contrary, DLL3 is a member of the DSL ligands for Notch receptors. In contrast with other ligands of the Notch receptors, the DLL3 protein predominantly resides in the Golgi apparatus

Table 2

Univariate delta-like protein 3 (DLL3) expression association with clinicopathologic characteristics analyzed by logistic regression test.

Clinicopathologic variables	Total N	Odds ratio in DLL3 expression	*P*-value
Age (<64 yr vs ≥64 yr)	545	1.741 (1.237–2.450)	.001
Stage (I or II vs III or IV)	543	2.915 (1.952–4.353)	<.001
Grade (grade 1 or 2 vs grade 3)	534	5.054 (3.460–7.384)	<.001
Myometrial invasion (deep vs superficial)	468	2.191 (1.511–3.177)	<.001
Pelvic lymph nodes (positive vs negative)	545	12.972 (5.097–33.014)	<.001
Para-aortic lymph nodes (positive vs negative)	545	9.918 (2.972–33.104)	<.001

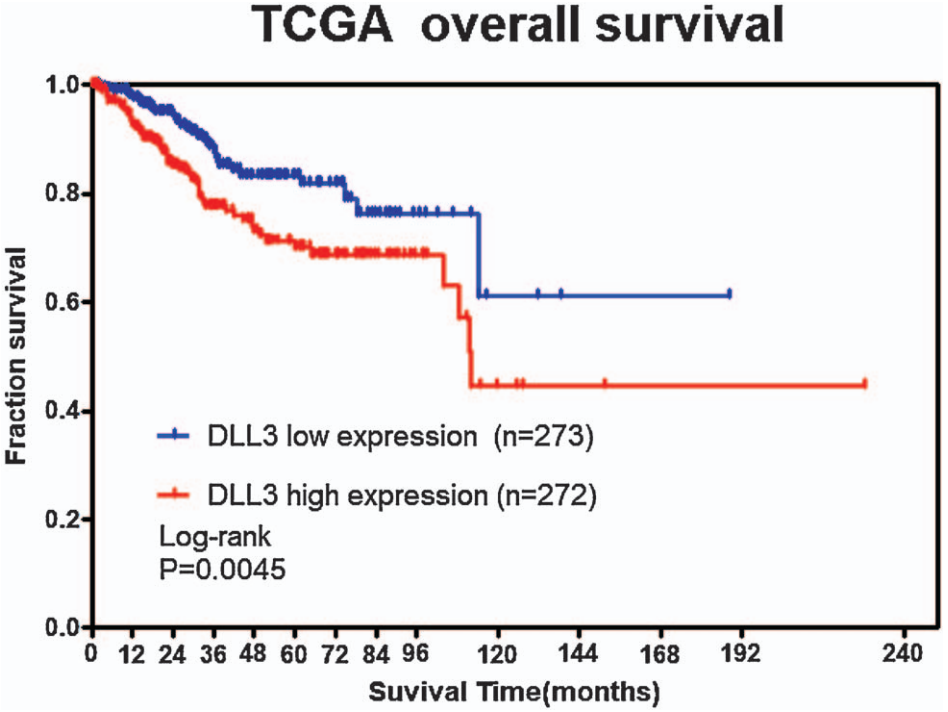

Figure 3. Kaplan–Meier curves and the log-rank analysis of overall survival stratified by delta-like protein 3 (DLL3) expression. These patients were followed up for the median period of time of 30.3 months (ranged between 0 and 228 months) and analyzed according to DLL3 expression for overall survival of these patients ($P = .0045$).

and inhibits Notch signaling.[35,36] DLL3 shares 36% homology with DLL1 but differs from other delta type DSL proteins.[37] Recently, DLL3 was shown to be expressed on the cell surface of high-grade neuroendocrine small cell lung cancer.[13] DLL3 was highly expressed in various human cancers, especially in lung cancer, although DLL3 expression was reduced in hepatocellular carcinoma.[38–41] A recent study reported that DLL3 expression promoted tumor growth and inhibited Notch signaling in lung cancer cells in mice.[42] Thus, a recent phase I clinical trial using a DLL3-targeted antibody-drug conjugate showed encouraging single-agent anti-tumor activity with a manageable safety profile.[43]

Thus, our present data on DLL3 overexpression in endometrial cancer are novel and consistent with the overexpression associated with lung cancer.[28,29] Furthermore, our literature search and review showed that DLL3 expression was not associated with any survival significance of patients with small-cell lung cancer but that elevated DLL3 expression was associated with poor overall survival of patients with lung adenocarcinoma, but not of patients with lung squamous cell carcinoma and with poor overall survival of patients with nonsmall-cell lung cancer.[39,44,45] Thus, our present data on the association of DLL3 overexpression with reduced overall survival of patients with endometrial cancer are consistent with that of the lung adenocarcinoma study.

There are several limitations in our present study. Our analysis only utilized RNA-seq expression data from the TCGA database and DNA mutation/methylation and protein data are not available to confirm these results. Moreover, the data treatment modality is not available and limits the clinical outcome analysis of this study. Thus, our future studies will assess DLL3 expression in a larger set of clinical samples to confirm the role of DLL3 in endometrial cancer.

In conclusion, our present study demonstrated that the upregulation of DLL3 expression in endometrial cancer tissues could be potentially useful as a diagnostic and prognostic marker in endometrial cancer and that DLL3 may represent a novel target for anticancer therapy.

Table 3

Univariate and multivariate analyses of prognostic association between delta-like protein 3 (DLL3) expression and clinicopathologic characteristics.

Variables	N	Odds ratio (univariate)	*P*-value	Odds ratio (multivariate)	*P*-value
DLL3 expression	545	1.85 (1.202–2.846)	.005	1.002 (1.001–1.003)	.001
Age (<64 yrs vs ≥64 yrs)	545	1.74 (1.23–2.45)	.001	1.68 (1.10–2.55)	.15
Stage (I/II vs III/IV)	543	2.91 (1.95–4.35)	<.001	4.08 (2.69–6.19)	.000
Grade (grade 1/2 vs grade 3)	534	5.05 (3.46–7.38)	<.001	3.41 (1.95–5.96)	.000
Myometrial invasion (deep vs superficial)	468	2.19 (1.51–3.17)	<.001	0.93 (0.54–1.61)	.87
Pelvic lymph nodes (positive vs negative)	545	12.97 (5.09–33.01)	<.001	3.99 (2.45–6.50)	.000
Para-aortic lymph nodes (positive vs negative)	545	9.91 (2.97–33.104	<.001	3.46 (1.88–6.39)	.000

Author contributions

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References

[1] Shelley M. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr 2016;15:418–9.
[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.
[3] Morice P, Leary A, Creutzberg C, et al. Endometrial cancer. Lance 2016;387:1094–108.
[4] Dizon DS, Birrer MJ. Advances in the diagnosis and treatment of uterine sarcomas. Discov Med 2014;17:339–45.
[5] Wang Y, Wang Y, Duan X, et al. Interleukin-1 receptor-associated kinase 1 correlates with metastasis and invasion in endometrial carcinoma. J Cell Biochem 2018;119:2545–55.
[6] Vale CL, Tierney J, Bull SJ, et al. Chemotherapy for advanced, recurrent or metastatic endometrial carcinoma. Cochrane Database Syst Rev 2012;8:CD003915.
[7] Colombo N, Preti E, Landoni F, et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Suppl 2013;6:vi33–8.
[8] Galaal K, Al Moundhri M, Bryant A, et al. Adjuvant chemotherapy for advanced endometrial cancer. Cochrane Database Syst Rev 2014;5: CD010681.
[9] Li Q, Zhang C, Chen R, et al. Disrupting MALAT1/miR-200c sponge decreases invasion and migration in endometrioid endometrial carcinoma. Cancer Lett 2016;383:28–40.
[10] Ueda SM, Kapp DS, Cheung MK, et al. Trends in demographic and clinical characteristics in women diagnosed with corpus cancer and their potential impact on the increasing number of deaths. Am J Obstet Gynecol 2008;198:218.e1–6.
[11] Chapman G, Sparrow DB, Kremmer E, et al. Notch inhibition by the ligand DELTA-LIKE 3 defines the mechanism of abnormal vertebral segmentation in spondylocostal dysostosis. Hum Mol Genet 2011;20: 905–16.
[12] Dunwoodie SL, Henrique D, Harrison SM, et al. Mouse Dll3: a novel divergent Delta gene which may complement the function of other Delta homologues during early pattern formation in the mouse embryo. Development 1997;124:3065–76.
[13] Saunders LR, Bankovich AJ, Anderson WC, et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. Sci Transl Med 2015;7:302ra136.
[14] Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.
[15] Abdel Razek AA, Gaballa G, Denewer A, et al. Diffusion weighted MR imaging of the breast. Acad Radiol 2010;17:382–6.
[16] Razek AA, Lattif MA, Denewer A, et al. Assessment of axillary lymph nodes in patients with breast cancer with diffusion-weighted MR imaging in combination with routine and dynamic contrast MR imaging. Breast Cancer 2016;23:525–32.
[17] Razek AA, Kamal E. Nasopharyngeal carcinoma: correlation of apparent diffusion coefficient value with prognostic parameters. Radiol Med 2013;118:534–9.
[18] Razek AA, Elkhamary S, Al-Mesfer S, et al. Correlation of apparent diffusion coefficient at 3T with prognostic parameters of retinoblastoma. AJNR Am J Neuroradiol 2012;33:944–8.
[19] Razek AA, Gaballa G, Denewer A, et al. Invasive ductal carcinoma: correlation of apparent diffusion coefficient value with pathological prognostic factors. NMR Biomed 2010;23:619–23.
[20] Kinkel K, Forstner R, Danza FM, et al. Staging of endometrial cancer with MRI: guidelines of the European Society of Urogenital Imaging. Eur Radiol 2009;19:1565–74.
[21] Lalwani N, Dubinsky T, Javitt MC, et al. ACR appropriateness criteria (R) pre-treatment evaluation and follow-up of endometrial cancer. Ultrasound Q 2014;30:21–8.
[22] Sala E, Rockall AG, Freeman SJ, et al. The added role of MR imaging in treatment stratification of patients with gynecologic malignancies: what the radiologist needs to know. Radiology 2013;266:717–40.
[23] Razek AA, Ezzat A, Azmy E, et al. Role of whole-body 64-slice multidetector computed tomography in treatment planning for multiple myeloma. Radiol Med 2013;118:799–805.
[24] Razek AA, Shamaa S, Lattif MA, et al. Inter-observer agreement of whole-body computed tomography in staging and response assessment in lymphoma: the Lugano classification. Pol J Radiol 2017;82:441–7.
[25] Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999;284:770–6.
[26] Wahi K, Bochter MS, Cole SE. The many roles of Notch signaling during vertebrate Somitogenesis. Semi Cell Develop Biol 2016;49:68–75.
[27] Brzozowa-Zasada M, Piecuch A, Michalski M, et al. Notch and its oncogenic activity in human malignancies. Eur Surg 2017;49:199–209.
[28] Moellering RE, Cornejo M, Davis TN, et al. Direct inhibition of the NOTCH transcription factor complex. Nature 2009;462:182–8.
[29] Li L, Tang P, Li S, et al. Notch signaling pathway networks in cancermetastasis: a new target for cancer therapy. Med Oncol 2017; 34:180.
[30] Tamagnone L, Zacchigna S, Rehman M. Taming the Notch transcriptional regulator for cancer therapy. Molecules 2018;23: pii: E431.
[31] Espinoza I, Miele L. Notch inhibitors for cancer treatment. Pharmacol Ther 2013;139:95–110.
[32] Kunnimalaiyaan M, Chen H. Tumor suppressor role of Notch-1 signaling in neuroend-crine tumors. Oncologist 2007;12:535–42.
[33] Morimoto M, Nishinakamura R, Saga Y, et al. Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells. Development 2012;139:4365–73.
[34] Fazio C, Ricciardiello L. Inflammation and Notch signaling: a crosstalk with opposite effects on tumorigenesis. Cell Death Dis 2016;7: e2515.
[35] Chapman G, Sparrow DB, Kremmer E, et al. Notch inhibition by the ligand Delta-like 3 defines the mechanism of abnormal vertebral segmentation in spondylocostal dysostosis. Hum Mol Genet 2011; 20:905–16.
[36] Geffers I, Serth K, Chapman G, et al. Divergent functions and distinct localization of the Notch ligands DLL1 and DLL3 in vivo. J Cell Biol 2007;178:465–76.
[37] Fehon RG, Kooh PJ, Rebay I, et al. Molecular interactions between the protein prod-ucts of the neurogenic loci Notch and Delta, two EGF-homologous genes in *Drosophila*. Cell 1990;61:523–34.
[38] Sharma SK, Pourat J, Abdel-Atti D, et al. Noninvasive interrogation of DLL3 expressi-on in metastatic small cell lung cancer. Cancer Res 2017;77:3931–41.
[39] Tanaka K, Isse K, Fujihira T, et al. Prevalence of Delta-like protein 3 expression in pat-ients with small cell lung cancer. Lung Cancer 2018;115:116–20.
[40] Maemura K, Yoshikawa H, Yokoyama K, et al. Delta-like 3 is silenced by methylation and induces apoptosis in human hepatocellular carcinoma. Int J Oncol 2013;42:817–22.
[41] Mizuno Y, Maemura K, Tanaka Y, et al. Expression of delta-like 3 is downregulated by aberrant DNA methylation and histone modification in hepatocellular carcinoma. Oncol Rep 2018;39:2209–16.
[42] Deng SM, Yan XC, Liang L, et al. The Notch ligand delta-like 3 promotes tumor growth and inhibits Notch signaling in lung cancer cells in mice. Biochem Biophys Res Commun 2017;483:488–94.
[43] Rudin CM, Pietanza MC, Bauer TM, et al. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: a first-in-human, first-in-class, open-label, phase 1 study. Lancet Oncol 2017;18:42–51.
[44] Liu ZY, Wu T, Li Q, et al. Notch signaling components: diverging prognostic indicat-ors in lung adenocarcinoma. Medicine (Baltimore) 2016;95:e3715.
[45] Yuan X, Wu H, Xu H, et al. Meta-analysis reveals the correlation of Notch signalling with non-small cell lung cancer progression and prognosis. Sci Rep 2015;5:10338.