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 < .001). Furthermore, upregulated DLL3 expression was also associated with a median overall survival of 112 months (HR = 1.86, confidence interval 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
In this study, we assessed DLL3 expression as a grade neuroendocrine carcinomas, including small-cell lung cancer. 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. A previous study targeted the DLL3 protein as a novel therapeutic strategy to control high-expression as a prognostic predictor.

In 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. DLL3 expression was significantly upregulated in endometrial cancer tissue when compared to para-carcinoma tissue. 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 ≤ .001; Fig. 2 and Tables 1 and 2).

### 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 assessed 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 ≤ .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...
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.\cite{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.\cite{20–22} Similar to other cancers, we evaluated the distant lymphoma nodes and organ metastasis using whole body computed tomography (CT) in clinic.\cite{23,24} Despite the high accuracy of assessing the preoperative staging 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 staging 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.

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.

Table 1

| Characteristic                  | Total | %     | High | Low | P-value |
|--------------------------------|-------|-------|------|-----|---------|
| Age at diagnosis, yr           |       |       |      |     |         |
| Median age (ranges)            | 64 (31–90) | 53.2  | 126  | 164 | .001    |
| <64                            | 290   | 47.8  | 145  | 107 |         |
| >64                            | 250   |       |      |     |         |
| BMI                            |       |       |      |     | P-value |
| Median BMI (ranges)            | 32.2 (17.4–81.6) |      |      |     |         |
| Race                           |       |       |      |     | <.05    |
| 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                      |       |       |      |     | P-value |
| 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 | .000    |
| II/IV                          | 151   | 27.8  | 100  | 53  |         |
| Grade                          |       |       |      |     | .000    |
| 1/2                            | 220   | 41.2  | 58   | 162 |         |
| 3                              | 314   | 58.8  | 205  | 109 |         |
| Myometrial invasion            |       |       |      |     | .000    |
| Superficial (<50%)             | 236   | 50.42 | 83   | 153 |         |
| Deep (≥50%)                    | 232   | 49.58 | 126  | 106 |         |
| Pelvic lymph node              |       |       |      |     | .000    |
| Negative                       | 487   | 89.4  | 219  | 268 |         |
| Positive                       | 58    | 10.6  | 53   | 5   |         |
| Para-aortic lymph node         |       |       |      |     | .000    |
| Negative                       | 515   | 94.5  | 245  | 271 |         |
| Positive                       | 30    | 5.5   | 27   | 273 |         |

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).
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. 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. Notch signaling can promote cell proliferation during neurogenesis and cell-fate determination during embryonic development and in stem cells. 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. 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. Another previous study speculated that the Notch pathway could differentially regulate neuroendocrine versus epithelial cell fate in lung development. 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. 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

| 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   |
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.\(^{13,33}\) 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

| 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 (III vs II)                              | 543 | 2.91 (1.95–4.35)        | <.001   | 4.08 (2.69–6.19)          | .000    |
| Grade (grade 1 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    |

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 \(P = .0045\).
Author contributions

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